2020

DISTRIBUTION OF NOVEL AND LEGACY ORGANIC POLLUTANTS IN SEABIRDS FROM CONTRASTING MARINE ENVIRONMENTS

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DISTRIBUTION OF NOVEL AND LEGACY ORGANIC
POLLUTANTS IN SEABIRDS FROM CONTRASTING
MARINE ENVIRONMENTS

BY

ANNA RUTH ROBUCK

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
OCEANOGRAPHY

UNIVERSITY OF RHODE ISLAND

2020

DOCTOR OF PHILOSOPHY

OF

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2020

ABSTRACT

Despite their ubiquity in the environment, large data gaps exist surrounding the distribution, biotic accumulation, and possible impacts of many high priority organic pollutants, particularly those considered emerging contaminants. Emerging pollutants, or contaminants of emerging concern (CECs), represent a dynamic and rapidly evolving group of substances, some of which have been extant for decades. Chapter 1 provides background about CECs covered in this dissertation, and highlights key data gaps explored within this work.

The first three manuscripts of this dissertation (Chapters 2 – 5) focus on per- and polyfluoroalkyl substances, or PFAS. PFAS are a family of CECs that demonstrate amphiphilic or hydrophilic environmental behaviors, and thus are ubiquitously distributed in aqueous and biological matrices. PFAS are associated with adverse effects in humans and wildlife at very low concentrations, and thus their biological behavior and impacts are under a great deal of scrutiny. Little work over the past two decades has evaluated these chemicals in marine food webs along the US East Coast, despite the proximity of this region to major human population centers and PFAS point sources. Tandem mass spectrometry and high-resolution mass spectrometry techniques were applied to tissue samples collected as part of a large-scale necropsy program to derive PFAS measurements in seabirds from the US East Coast. Names, Chemical Abstracts Service (CAS) number, and potential sources of each PFAS measured in this work are provided in Appendix B.

Chapter Two measured PFAS in seabird juveniles from three habitats along the US East Coast representing a range of exposure potential. Seabirds collected downstream from a major fluoropolymer production site contained the highest concentrations of legacy and novel PFAS, surpassing toxicity reference values established in controlled studies for avifauna. The novel PFAS Nafion by-product 2 (Nafion BP2) was detected in seabirds from all habitats, marking the first identification of this compound in biota beyond the industrially influenced Cape Fear region in North Carolina. Perfluorooctanesulfonic acid (PFOS) and perfluorononanoic acid (PFNA) were associated with decreased phospholipid levels, marking the first time this trend has been observed in a wild population. Chapter Three examined novel and legacy PFAS in Great Shearwaters from Massachusetts Bay over 2010 – 2019. PFOS and perfluorooctanesulfonamide (FOSA) decreased over the time series, while perfluoroalkyl carboxylic acids (PFCAs) and the novel PFAS 7:3 fluorotelomer carboxylic acid (7:3 FTCA) showed no clear trends over time. Multiple PFAS were significantly associated with morphometric variables, with increased PFAS levels associated with reduced organ weights and reduced fat depth in Great Shearwaters. Perfluorohexanesulfonic acid (PFHxS) and PFNA were negatively associated with mass of ingested plastic, marking the first time any relationship has been identified between plastic consumption and PFAS levels in a seabird. This relationship likely reflects the amphiphilic nature of PFAS that reduces the likelihood of sorption to plastic items in aqueous matrices, as well as the divergent exposure pathways for these two pollutant groups, as seabirds are primarily exposed to PFAS via diet as opposed to direct ingestion of plastics via discovery in the environment. Chapter Four assessed PFAS in eight

tissues obtained from juvenile seabirds collected from three habitats along the US East Coast, the same habitats assessed in Chapter Two. These tissue-specific measurements suggested blood, liver, kidney, and lungs are primary reservoirs of PFAS in seabirds. Novel per- and polyfluoroalkyl ether acids (PFEAs) preferred blood compared to liver, suggesting reduced compatibility with the binding environment of liver fatty acid binding protein that drives PFAS uptake in liver. These results were also the first to measure novel PFEAs in brain tissue, indicating these compounds are capable of surpassing the highly selective blood-brain barrier in vertebrate wildlife.

Chapter Five focuses on microplastics, another type of CEC. Microplastics are a pervasive pollutant in aquatic ecosystems and ingestion of plastics is associated with an increased risk or mortality in seabirds. Chapter Five examined plastic ingestion in 175 Great Shearwaters from 2010 – 2019, collected at multiple locations relevant to their annual cycle across the North and South Atlantic Oceans. Each bird contained an average of 8 plastic fragments, and more than 50% of individuals contained greater than 0.1g plastic, significantly surpassing the ecological threshold suggesting no more than 10% of a given population should contain greater than 0.1g ingested plastic. Breeding adults from the South Atlantic ingested plastic less frequently (~60%), while 98% of juveniles from Massachusetts Bay were found to contain ingested plastics. Each plastic fragment was assessed using Fourier-transform ion spectroscopy to discern polymer identity. We found low- and high-density polyethylene fragments dominated within all years and locations. Breeding adults contained a higher proportion of larger polypropylene fragments compared to juveniles and non-breeding adults; breeding adults in Massachusetts Bay likewise ingested marginally larger plastic items compared

to Massachusetts Bay juveniles but this was outside the limits of significance defined within the study. The abundance of larger plastic items in breeding and non-breeding adults is likely indicative of an increased use of remote, pelagic habitats subject to reduced inputs of smaller, land-derived plastics due to size-mediated distribution in the pelagic environment. Different signatures of polymer type and size between breeding and non-breeding mature birds likewise suggests rapid turnover of ingested plastics commensurate with migratory stage and location.

Chapter Six of this study leverages the vast amount of necropsy data derived in support of the included contaminant studies to correct the record regarding plumage and age characteristics of Great Shearwaters. Necropsy efforts demonstrated Massachusetts Bay is dominated by juvenile Great Shearwaters, with juveniles making up over 90% of collected birds considering the entire necropsy sample set. Nape plumage has been previously associated with individual age, with the suggestion that mature individuals possessed a completely white nape while the nape area of younger individuals was interrupted by variable amounts of brown or gray. Necropsy data collected herein suggest nape plumage is not a reliable indicator of age. Instead this data suggests nape plumage may be associated with sexual behaviors, as completely white napes were more abundant in females of all ages.

These results highlight the utility of necropsy efforts to explore multiple complimentary scientific questions within a given species, and raise further questions about Great Shearwater demographics, PFAS, and plastic ingestion. The abundance of juvenile birds in the bycatch sample set and overall Massachusetts Bay region raises questions about the impact of commercial fishing activities on Great Shearwater

population, as fishing activities remove hundreds to thousands of pre-reproductive individuals from the population each year. This work also demonstrates the ability of several novel PFAS to bioaccumulate in seabirds, and indicates the need for significantly more research describing the bioaccumulation potential of new PFAS formulations in a wider range of wildlife. Additionally, these data suggest potential impacts of PFAS at environmentally relevant, ongoing exposure levels, with uncertain population-scale impacts of reduced organ weights, altered fat storage or expression, and morphological alterations. Further research should evaluate the effects of legacy and novel PFAS in marine biota with a focus on potential implications for individual and population health considering the importance of body condition and lipid stores on bird migratory and reproductive ability. Plastic ingestion in Great Shearwaters likewise merits additional investigation; over 50% of individuals evaluated in the study contained more than 0.1 g of ingested plastic, surpassing a threshold established as problematic for seabird plastic ingestion. Further work should evaluate trends of plastic ingestion over time using harmonized methods to leverage the Great Shearwater as a sentinel for specific Atlantic habitats and monitor for potential impacts.

Within a management context, these results provide justification for continued investigation and potential phase-out or regulation of novel PFAS akin to some legacy formulations, considering their potential for long-range transport and bioaccumulation within offshore ocean biota demonstrated within this work. Additionally, the demonstrated abundance of long-chain PFCAs in seabirds demonstrates the importance of considering the downstream environmental transformations and impacts of PFAS currently in use. Long-chain PFCAs are rarely manufactured purposefully, but may

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ACKNOWLEDGMENTS

Funding for this dissertation research was provided by the University of Rhode Island (2015 – 2017) and the U.S. National Oceanic and Atmospheric Administration (NOAA) Dr. Nancy Foster Scholarship program (2017 – 2020) (NA17NOS4290028). Support was also provided by the NIEHS Superfund Research Program (P42ES027706), the Robert and Patricia Switzer Foundation (2019), and the Oak Ridge Institute for Science and Education Program (2019 – 2020).

My biggest thanks go to my Mom and Dad. This would have been impossible without you, and there aren't really words to describe what you mean to me. I'm also very grateful to my siblings and nephew Markie for all the support they provided before and during this process. Likewise, I'm so thankful for my lovely best girl Gypsy, who was the primary source of any sanity retained during this process. Thank you to my dear friends Shelby, Carolyn, Cherie, Brigid, Tiffany, Debbi, and Landis, for unwavering love and listening skills, despite me often taking months to return calls sometimes. Thank you to Mike, Paula, and Dennis for saving the day so many times personally and professionally, your love and support has made the last few years so much brighter.

I want to thank my major professor Rainer Lohmann for the tremendous amount of support provided over our tenure together. You provided a unique blend of personal and scientific independence, mentorship, and goal-setting, which allowed me to learn so much during this experience. Thank you for all you have done and continue to do to support my career, even at times when I had doubts about my own path and abilities.

I also want to offer immense thanks to Mark Cantwell, aka "Lab Dad". Your mentorship and unwavering support have made such a difference in the last few years.

You saved my morale and experiments essentially every day along the way, and I can't imagine getting through this process without your support 🐼.

Thank you to Dave Wiley, who indelibly changed my path by betting on my scientific star and supporting me in pursuit of the Nancy Foster Scholarship, while barely knowing me nonetheless. I'm so glad you were impressed instead of freaked out when I gave a bird mouth to mouth. Thank you for the inspiration, support, humor, and creativity you offered throughout my degree progress, and for always being on "Team Anna". I will steal glasses for you any time.

I would also like to thank my committee members Candace Oviatt and Gavino Puggioni – your classes were formative in my development as a well-rounded environmental scientist, and I value the time and perspective you have invested to help me achieve the PhD despite how much my dissertation has changed over the course of this process. I'd also like to thank my Defense Chair Angela Slitt, who has supported my interests in toxicology and lipidomics at every step of the way, while modeling good humor and kindness.

Thank you to the NOAA Nancy Foster Scholarship and all those who make the opportunity a reality, including Kate Thompson, Seaberry Nachbar, Claire Fackler, Liz Weinberg, Kathy Broughton, Steve Gittings, and so many others. This opportunity changed my personal and professional trajectory in every way imaginable, and I am thankful for the time and energy you all put into the program to provide financial support, professional development, and personal support to the Scholars. Also thanks and huzzah to my fellow Scholars! I am so proud and thankful to count you as friends and colleagues.

Resounding thanks to all the folks at Stellwagen Bank National Marine Sanctuary for supporting me and helping me grow as a professional and person during my years as a Scholar – Pete DeCola, Alice Stratton, Bibi Stokes, Ben Haskell, Anne-Marie Runfola, and Anne Smrcina. Pete, particular thanks to you for supporting the Massachusetts Bay water column study, it was a highlight of my PhD career to plan and execute “my own” water quality cruise with your aid. Also at SBNMS, special thanks to Amy Meloski, Dave Slocum, Peter Hong, and Tammy Silva, for lots of field work help and friendship over the years. Also, a special thanks to the most dedicated SBNMS volunteer in existence, Kevin Powers – one of the highlights of my dissertation journey was meeting you every day at WHOI for almost three weeks (!) to cut up birds and shoot the breeze. I would never have the comprehensive sample set that I was able to amass without your dedication at every step.

Beyond the folks above, the success of this work was facilitated by a veritable village. I am enormously grateful to the many collaborators, administrators, and friends who made this work possible in big and small ways. Thank you to David Smith and Meredith Clark for abundant help navigating the PhD process at GSO. Thank you to Michaela Cashman, Julia Sullivan, Melanie Hedgespeth, and Dave Katz, my EPA PFAS PFurious team– I could never have done this without your thoughtful support and good humor. Also thanks to Christine Gardiner, who was a source of assistance and friendship at some of the most challenging points of this process. Thank you as well to Gwen Emery, Lindsay Agvent, Tom Garrow, the entire Lohmann lab past and present, Heidi Pickard, Charlotte Wagner, the Sunderland lab, as well as the entire STEEP team. I

would particularly like to thank Judith Swift within URI STEEP for her continued support and coaching on public engagement and interview skills.

Another round of thanks to the NC folks who taught me so much and also reminded me that hobbies were a thing at a really bleak point in this process -- James McCord, Mark Strynar, Theresa Guillette, Kim McKeever, and Hannah Liberatore.

Last but far from least, thank you to Northeast Fisheries Science Center, Woods Hole Oceanographic Institute, NC Audubon, the Wildlife Clinic of Rhode Island, and University of Cape Town folks -- Gina Shields, Johanna Pedersen, Stephanie DePasquale, Michael Moore, Peter Ryan, Vonica Perold, Maelle Connan, Lindsay Addison, Marissa Sprouls, and the entire WRARI staff – who were so generous with their time and support to help me find, store, and necropsy the birds used in this work. Particular thanks to Michael Moore, who went above and beyond during the COVID-19 pandemic to secure access to WHOI MRF for me so I could finish one more year of necropsies.

DEDICATION

I wish to dedicate this effort to all those dear to me and my family that we lost during my PhD journey – Big R, Gramma Anne, Aunt Patti, Uncle Joey, Aunt Jeannie, Aunt Beenie, and Ben Privott. Each of these folks shaped me into the person I am today. I strive each day, while “vertical and ventilating”, to professionally and personally emulate a fraction of the grace, boldness, humility, humor, and kindness you each so artfully modeled to me and so many others.

I also dedicate this to my wider family, past and present. We are a family of immigrants, coal miners, bootleggers, engineers, mill workers, mechanics, highway construction workers, typists, lumberjacks, stay-at-home moms, nurses, teachers, farmers, and many other industrious enterprises. So many brilliant folks in my family worked hard in any way they could to make things a little better and more stable for our family. I am here today because of all those sacrifices along the way.

PREFACE

This dissertation is written and organized in the manuscript format according to URI Graduate School guidelines for dissertation preparation. It is a compilation of five manuscripts, one of which has been published, one of which is in review, and two of which are in some stage of submission. The first manuscript (Chapter 2) was published in *Environmental Science and Technology* in August 2020. The second manuscript (Chapter 3) is formatted for *Environmental Science and Technology*. The third manuscript (Chapter 4) is in review at *Environmental Science and Technology Letters*. The abstract of the fourth manuscript (Chapter 5) has been accepted to a special issue on plastic ingestion in *Frontiers in Marine Science* and will be submitted for full review in March 2021. The fifth manuscript (Chapter 6) is intended for *Avian Biology* and pending immediate submission. Appendix A contains co-author and collaborative works completed over the course of the dissertation.

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CHAPTER 1

INTRODUCTION

If we are going to live so intimately with these chemicals eating and drinking them, taking them into the very marrow of our bones - we had better know something about their nature and their power.” – Rachel Carson, Silent Spring

The environmental distribution and persistence of human-created pollutants represents a pervasive global problem, with various anthropogenic pollutants found in the deepest depths of the ocean to the highest polar latitudes¹⁻⁵. Despite their ubiquity in the environment, large data gaps exist surrounding the distribution, biotic accumulation, and possible impacts of many high priority organic pollutants, particularly those considered emerging contaminants. Emerging pollutants, or contaminants of emerging concern (CECs), represent a dynamic and rapidly evolving group of substances. The definition of what constitutes an emerging pollutant is somewhat tenuous as it relies heavily on human perspective and knowledge; today we understand emerging pollutants as those substances that are newly discovered or measurable, or lack data describing basic elements of their behavior, distribution, or effects^{6,7}.

The emerging pollutants explored herein have been extant for decades, but have only recently become widely measurable due to advances in analytical instrumentation or observational techniques, and have thus gained research traction due to increased awareness of their environmental ubiquity and potential impacts.

This dissertation contributes data and interpretation to describe the environmental distribution and biological occurrence of emerging contaminants in biotic and abiotic systems from the US Atlantic Coast, focusing on seabirds as sentinels of emerging contaminant dynamics in marine food webs. Seabirds serve as effective environmental indicators representing the health and condition of coastal or oceanic habitats in which they live, as their upper trophic level position, fast metabolism, and life history choices intimately connect them to the conditions of a given habitat⁸⁻¹⁰. Previous research indicates seabirds are particularly vulnerable to bioaccumulation of organic contaminants; polychlorinated biphenyls, pesticides, and flame retardants have all been documented in seabirds worldwide¹¹⁻¹⁴.

The dissertation specifically focuses on per- and polyfluoroalkyl substances (PFAS), while also providing data on active pharmaceutical ingredients (APIs) and microplastics (MPs) in lesser detail. Each of these pollutant families have demonstrated environmental persistence, bioaccumulative capacity, and toxic potential in humans and wildlife, yet are considered emerging contaminants due to a limited knowledge or understanding¹⁴⁻²¹.

PFAS. Per- and polyfluoroalkyl substances (PFAS) are a group of over 9,000 human-created chemicals or byproducts that have been manufactured since the 1940s^{22,23}. Various sub-groups of PFAS exist under the large PFAS umbrella, including perfluoroalkyl sulfonic acids (PFSAs), perfluoroalkyl carboxylic acid (PFCAs), perfluoroalkyl ether acids (PFEAs), and the so-called “precursor” compounds that readily transform into PFCAs, PFSAs, or other stable PFAS under environmental conditions²³. Perfluoroalkyl acids, or PFAAs, refers to both PFSAs and PFCAs. These

sub-groups within the wider PFAS class vary based on molecular structure and functional group but all are considered PFAS as they include at least one least one perfluoroalkyl moiety ($C_nF_{2n+1}-$) associated with a minimum of three carbon – fluorine (C-F) bonds^{22,23}. PFAS are often referred to by chain length, or how many carbons make up the backbone of the molecule. PFCAs are considered long-chain with seven or more alkyl carbons, while PFSA's are considered long-chain with six or more alkyl carbons²³.

Reliance on the C-F bond defines PFAS while also making them remarkably persistent in the environment^{24,25}. This bond is one of the strongest bonds in organic chemistry, and its prolific use in PFAS molecules renders powerless an arsenal of chemical and environmental mechanisms that degrade many other pollutants (^{24,26}). Some PFAS are capable of breaking down to some extent via in situ or in vivo oxidation, but because of the stability of the C-F bond, they often degrade into other, more stable PFAS structures^{27,28}. For example, a sub-group of PFAS called fluorotelomer alcohols (FTOHs) transform in the environment into the more persistent and toxic PFCA sub-group^{28,29}. PFAS are also chemically unique in that they are amphiphilic or “both loving”, allowing many PFAS to associate with both water and oil²⁶. This property means PFAS readily migrate within and beyond the water cycle^{30–32} while also accumulating in living organisms in ways distinct from hydrophobic organic contaminants^{33–35}.

Sources and environmental occurrence of PFAS. PFAS are solely created by human industrial processes^{23,26,36}. Major primary sources of PFAS include creation of PFAS via industrial activity^{37,38}, and use of aqueous firefighting foams (AFFFs) at airports,

military bases, and oil and gas operations^{31,39,40}, and the intermediate use of PFAS during production of consumer and industrial goods⁴¹. Due to their unique and attractive chemical qualities, PFAS can be found in hundreds of household or consumer goods including food packaging, take-out food containers, non-stick cookware, paper goods, cleaning products, cosmetics, fire-fighting foams, carpets, ski wax, textiles, waterproof clothing and coatings, paints, and many more^{41,42}. PFAS also play a central role in many industrial and workplace processes including electroplating, electronics creation, production of medical equipment, oil recovery, textile manufacture, plastics manufacturing, specialty chemical production, fuel cell production, and membrane manufacture⁴³.

PFAS do not remain confined to products or sites where they are used or discharged – their persistence coupled to high mobility allows them to leach into the environment readily, where they can be subsequently transported via water, air, or solid materials^{44,45}. PFAS display a wide range of environmental behaviors based on their unique chemical characteristics and subsequent speciation in the environment as zwitterionic, cationic, or anionic compounds^{46,47}. The most well-studied compounds are PFAAs which are primarily anionic at environmentally relevant pHs and therefore amphiphilic or hydrophilic in behavior⁴⁷.

Once in the environment, PFAS travel in regional and global biogeochemical cycles based on their individual physico-chemical characteristics. PFAS have been detected in air³², water³², ice from the polar regions⁴⁸, mountaintop glaciers⁴⁹, river water^{37,50}, lake water³², ocean water²⁵, sediments⁵¹ and soils⁵². PFAS measurements in remote polar regions indicate their ability for long-range transport via both

atmospheric and water-borne pathways, the two primary routes of environmental transport for these compounds^{28,29}.

Regardless of specific entry point into the environment, aquatic systems possess the greatest exposure potential for these compounds; the ocean is the ultimate sink for PFAS, as atmospherically transported PFAS oxidize or wash out of the gas phase into the water cycle, while water-borne PFAS in rain, rivers, and streams flow downstream to the ocean^{44,45,53}. PFAS are a suitable tracer indicating the age of ocean water -- any water mass that entered Atlantic Meridional Overturning Circulation after PFAS proliferation now carries PFAS, while older water masses possess significantly lower PFAS concentrations²⁵.

Human Exposure. Their environmental ubiquity also means PFAS are distributed pervasively in the biosphere. Humans are primarily exposed to PFAS via food, drinking water, and dust; dermal and inhalation exposure are more minor contributors to average adult PFAS exposure⁵⁴. Occupational PFAS exposure may exist for individuals working in an industry that produces or uses PFAS, or for those who work with AFFF, where inhalation is likely a significant source of exposure for those occupationally exposed⁵⁴. Seafood is a significant contributor of PFAS in human diet, and the European Food Safety Administration has previously suggested up to 86% of human dietary PFAS stems from seafood sources⁵⁴⁻⁵⁶. PFAS contamination of drinking water is associated with proximity to industrial sources, AFFF-impacted sites, airports, and wastewater treatment plants, though ambient levels of PFAS are detected in drinking water across the country^{31,57,58}. Children may be more exposed to PFAS via maternal transfer of PFAS in utero, through breast milk, as well as through

dermal and inhalation exposure through increased proximity to and contact with PFAS-containing textiles and carpets⁵⁴.

Wildlife Exposure. Exposure of wildlife to PFAS has materialized as a pervasive and poorly understood global challenge. In parallel to humans, wildlife exposure is an iterative series of events, whereby multiple human sources introduce PFAS into surrounding abiotic matrices, on a site- and use-specific basis. Wildlife are externally exposed to PFAS based on specific life histories and behaviors that dictate their interactions with ambient environmental exposures via air, water, soil/sediment use, and diet ⁵⁹. Wildlife may also be particularly exposed to PFAS via use of industrial or AFFF-impacted habitat ^{34,60,61}.

Since the first report on the global distribution and related exposure of perfluorooctane sulfonic acid (PFOS) in wildlife in 2001, concentrations of PFOS and other PFAAs have been measured in fish⁶², invertebrates like insects or crustaceans⁶⁰, shellfish, marine mammals⁶³, birds⁶⁴, reptiles⁶⁵, domestic animals⁶⁶, animal food products⁶⁶, plants⁶⁷, plant food products⁶⁸, and humans⁶⁹. These studies include investigations in wildlife from landscapes and aquatic habitats adjacent to human population centers to remote polar regions, including the Arctic^{70–73} Great Lakes^{64,74–76}, North Atlantic Ocean^{30,77–79}, Pacific Ocean^{11,80,81}, Antarctica^{82,83} well as in coastal regions and riverine systems across the globe^{50,84–86}.

Trends in PFAS occurrence and exposure over time and space. In recent years, a growing number of field-based studies have assessed geographic variation and temporal trends of PFAS concentrations in wildlife from multiple taxa^{64,65,70,71,87–90}. While the reported spatiotemporal trends of PFAS concentrations in wildlife vary

among compounds, species, and geographic location, the available data indicate that decreases for the majority of PFAAs in exposed wildlife has been relatively slow, if present at all. Some decreases in PFOS and FOSA have been documented following phase out of their production and use in the early 2000s^{45,79,90}. Data from other species or locations suggest sustained or increased PFOS in wildlife, as well as increased concentrations of PFCAs over time, which has been attributed to continued emissions of long-chain PFCAs, PFCA precursors, or PFOS precursors^{70,89}.

PFAS in seabirds. PFAS were first documented in seabirds from around the globe in the late 1990s^{91–93}, and continued research over the past thirty years has established PFAS in multiple species of seabirds from divergent environments, including guillemots from the Arctic^{72,94}, herring gulls from the Great Lakes^{64,95–97}, albatross from Midway Atoll⁸⁰, great skuas from the UK⁹⁸, cormorants from San Francisco Bay⁸¹, coastal and pelagic seabirds from British Columbia¹¹, and multiple species of petrels from the South Atlantic and Indian Oceans⁹⁹. The range of extant studies have provided discrete “snapshots” of PFAS levels in seabirds, as well as assessment of temporal trends dating back as far as 1968⁷². Trends identified in seabirds to date align with PFAS trends observed in other environmental matrices, including some decreases in PFOS and FOSA⁶⁴ and increases of long-chain PFCAs over time¹¹.

Wildlife exposure at contaminated sites. Increased public interest in PFAS and improved analytical capacity have resulted in identification of a growing number of PFAS-impacted sites, with reports of numerous contaminated sites across the United States, Europe, Australia and Asia. Localized PFAS contamination may be related to releases from fire-fighting activities involving the use of AFFF, releases from

industrial sites manufacturing or using PFAS, or application of sewage sludge containing high levels of PFAS^{52,100–103}. Habitat subject to high levels of concerted PFAS inputs may pose a risk to wildlife using the contaminated area, but abundant data gaps exist regarding the magnitude of wildlife exposure related to contaminated sites and potentially related individual or population level impacts.

Manufacturing activities are increasingly associated with concerted areas of PFAS contamination. Regulation and phase-outs have caused a geographical shift in production beyond North America and Europe, alongside a departure from long-chain aliphatic chemistries to replacements with fewer than seven fluorinated carbons, polyfluorinated structures, and/or modified iterations of legacy PFAS, such as perfluoroalkyl ether acids (PFEAs) incorporating different numbers of ether linkages, or legacy PFAS analogues substituting chlorine in previously fluorinated positions^{36,104,105}. This has resulted in an evolving and poorly understood landscape of PFAS exposure for wildlife surrounding production facilities, including both legacy PFAAs and novel compounds.

PFAS manufacturing and use sites in China, Europe and the US have been documented and observed to cause elevated levels of PFAS contamination in adjacent habitats, downstream environments, and regional wildlife^{36,38,52,106,107}. Zhou et al. found PFOS was the predominant compound in fish from Lake Tangxun, adjacent to a major fluorochemical production facility in Wuhan, China, with a mean PFOS value of 263 ng/g ww in crucian carp muscle and a mean value of 348 ng/g ww in sharpbelly muscle¹⁰⁷. Suspect and non-target screening subsequently revealed a suite of 330 novel fluorinated structures belonging to 10 different chemical classes in fish liver

from the same region¹⁰⁸. Songbirds near a fluorochemical production site in Antwerp, Belgium contain some of the highest PFOS concentrations identified to date in wildlife, with up to 48,056 ng/g in eggs of great tits nesting proximate to the production site^{109–111}. Songbirds nesting adjacent to manufacturing facilities and disposal sites in Minnesota contained significant concentrations of PFOS (geo. mean = 270 ng/g egg), and were found to be impacted by PFOS at lower levels than suggested by lab-based studies, with decreased hatching success estimated to occur at 150-250 ng/g egg^{112,113}. Chlorinated polyfluoroalkyl ether sulfonic acids (Cl-PFESAs, often referred to under the trade name F53B) are PFOS alternatives widely used in China, and have been identified beyond point sources in coastal biota, with the compound 6:2 Cl-PFESA displaying a trophic magnification factor (TMF) > 1 in a study involving marine biota samples from the Bohai Sea¹⁰⁶. In the US, production of PFEAs in North Carolina has resulted in downstream PFEA contamination in fish and humans, along with identification of associations between immune and liver function and elevated PFAS levels in fish^{34,114}.

Health Effects in Humans. PFAS are not innocuous, and the environmental ubiquity of this group of emerging pollutants does not translate to a lack of harm or risk. Potential human health and ecosystem impacts were first identified by industry in the 1960s, but it took almost four decades before that information was communicated to the public and action was taken to reduce, minimize, or eliminate some PFAS. To date, most identified human health impacts fall into four general categories: developmental effects, hormonal and immune disruption, potential carcinogenicity, and changes in blood lipid levels^{54,115}.

Many identified effects are associated with environmentally relevant levels of exposure, while some, like increased risk for some cancers, are associated only with high levels of PFAS exposure¹¹⁵. Animal studies offer additional insight about potential associations between PFAS and certain health effects such as liver degeneration, decreased immune response, and impaired mammary gland development, but should be interpreted with caution beyond the study species as adverse effects may be taxa-specific^{115,116}. Research detailing the effects of some PFAS on human health is rapidly expanding, yet uncertainties remain regarding how mixtures of PFAS or novel PFAS like Gen X and other PFEAs may impact human health. Existing studies suggest novel PFAS may impart similar impacts to the long-chain PFAAs they seek to replace^{117,118}.

Health Effects in Wildlife. Most wildlife research to date focuses on the occurrence of PFOA and PFOS in wildlife, with some attention to fluorotelomer-based substances, long-chain PFCAs or emerging PFAS⁵⁹. Existing data suggest these PFAS are widely abundant in wildlife at every level of biological organization and readily bioaccumulate and biomagnify in ways divergent from legacy persistent organic pollutants^{19,77,119}, due to their preference for aqueous and protein-based matrices like blood and liver¹²⁰. Traditional modeling based on the octanol-water partitioning coefficient, or K_{ow} , does not adequately reflect the internal behavior of PFAS¹²¹. Current modeling suggests a protein-partitioning model, in tandem with a phospholipid-based partitioning model may explain the observed distribution of PFAS, but more empirical measurements and mechanistic details are required to more fully apply these models within realistic food web scenarios^{33,122–124}. Long-chain

compounds have been identified as particularly bioaccumulative in multiple tissues of wildlife^{94,125–127}.

Laboratory data suggest PFAS may be associated with adverse impacts on growth, development, reproduction, and metabolism based on rodent, fish, and invertebrate studies^{128,129}. Most laboratory toxicity studies describing PFAS impacts rely upon freshwater receptors or other lab organisms that may poorly reflect marine wildlife endpoints and complex food webs¹²⁹. Additionally research identifying impacts of PFAS on wildlife species and food webs stands as a significant data gap and persistent management challenge. As of 2020, roughly 40 studies have investigated the impacts of PFAS in free-living wildlife including birds, mammals, reptiles, fish, and invertebrates¹²⁹. These studies have found impacts on reproductive success exposure^{112,113}, changes in a suite of enzymes and signaling pathways transmission^{130,131}, as well as associations between immune endpoints and morphometric measurements in diverse taxa¹³².

In birds, PFAS have been associated with altered metabolism, altered endocrine function, reproductive failure, decreased egg pipping ability, pathological changes in the liver, brain asymmetry, and other physiological changes indicative of altered immune function^{129,133–136}. These studies primarily rely on PFAS measurements in blood or egg paired with measurements of specific biomarkers in wildlife to arrive at these noted effects. Some of these studies note PFAS impacts at environmentally relevant levels of exposure. Significantly more research is required to more fully describe if or how PFAS may be chronically or acutely impacting wildlife or wildlife populations at ambient concentrations.

PFAS data gaps addressed in this work. While abundant data gaps exist related to the distribution and impacts of PFAS in natural and human environment, this dissertation builds upon existing knowledge by exploring specific and timely questions addressing the occurrence and bioaccumulation of legacy and novel PFAS in seabirds.

Manufacturing activities continue to introduce new PFAS formulations into the environment⁵², with little information provided to the public about their structures, human health risks, or environmental toxicity under protections as confidential business information. Continued research is vital to better describe the environmental and biological occurrence of PFAS adjacent to manufacturing activities, as well describe novel PFAS in the wider environment and biosphere. Additionally, exploration of legacy PFAS has focused on only a handful of PFAAs in select matrices and food webs^{23,36,137–139}, with scant investigation of PFAS levels in marine food webs along the US East Coast to date.

While some trends of PFAS have been identified over time, studies documenting changes in PFAA concentrations over the last decade (2010 – 2019) are limited; the existing data offer conflicting evidence about current trends^{11,88–90}. Additionally, studies exploring trends of PFAS beyond PFAAs are limited, and temporal and spatial understanding has not been achieved for compounds beyond PFAAs.

This work addresses these data gaps by measuring legacy PFAAs as well as five novel PFAS in seabirds across three US East Coast habitats including a habitat adjacent to a PFAS manufacturing site. This work further explores PFAS temporal trends by measuring legacy and novel PFAS over a ten-year time series in the Great Shearwater, a seabird from Massachusetts Bay.

Additionally, little work has assessed how novel PFAS partition between different tissues in homeothermic vertebrates, yet such data are vital to predict bioaccumulation and those tissues likely to be most impacted by PFAS. This work entails a tissue-specific study and subsequent commentary on bioaccumulation mechanisms for novel PFEAs and PFAAs to further expand our understanding of PFAS bioaccumulation potential.

Microplastics and seabirds. Plastic pollution in natural environments has emerged as a significant global problem over recent decades. Mismanaged plastic materials are transported to aquatic systems via point and non-point source pathways, and fragment into smaller and smaller pieces under continued exposure to environmental conditions, resulting in the proliferation of microplastics, or small plastic pieces generally >5mm in size^{140,141}. Seabirds have been documented to ingest small plastic fragments dating back to the 1960s – 1980s¹⁴², but are increasingly at risk of ingesting plastic items due to increasing concentrations of plastic pieces in ocean environments related to exponential growth in the production and use of plastics^{141,143,144}.

Seabirds in the order Procellariiformes are particularly vulnerable to plastic ingestion, as 91/144 Procellariiform species have been documented to ingest plastic, either directly from the environment or through the consumption of prey containing plastic items^{145–147}. This group, which includes Great Shearwaters, is thought to be especially prone to plastic ingestion due to reduced specialization of diet, wide-ranging foraging behaviors, and reliance on olfactory cues in prey location^{148–150}.

Accumulation of ingested plastics significantly increases risk of mortality in seabirds¹⁵¹, while the sublethal impacts of plastic ingestion are more poorly

understood due to the range of factors that might influence the magnitude of harm. These factors include the type and size of plastic ingested, the duration of time the item has already been in the environment, other diet items consumed, species-specific in vivo clearance rates, and exposure to other environmental stressors or co-contaminants^{152–156}.

Cohesive understanding of potential harm from plastic ingestion is also limited by patchy data describing the occurrence of ingested plastics over time, between different locations, and between life stages. Existing work often relies upon opportunistic samples of convenience from readily accessible species and therefore often depicts plastic ingestion at only a discrete time and place. Great Shearwaters have been previously assessed for plastic ingestion in such discrete studies across their breeding and non-breeding range^{149,157–159}. Additionally, existing work in Great Shearwaters has focused on the count and mass of ingested plastics, without further detail about the nature of the plastic items ingested or relationships to other physiological variables. With this work, we expand upon previous research in this species by offering a thorough characterization of the size of ingested plastic pieces, polymer types, temporal trends, and relationships to bird body condition by assessing Great Shearwaters across both their breeding and non-breeding range.

Summary. Overall, this dissertation contributes data that describes the occurrence of emerging contaminants including legacy and novel PFAS and microplastics in marine habitats over space and time, primarily using seabirds as sentinels of PFAS and plastic accumulation. We also contribute insight regarding the internal toxicokinetics of PFAS through tissue specific measurements in juvenile seabirds to better constrain the

internal behavior of PFAS in homeothermic organisms. We further used the large body of necropsy data derived in support of contaminant studies to improve plumage and demographic characterization of the Great Shearwater in the SW Gulf of Maine.

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CHAPTER 2

LEGACY AND NOVEL PER- AND POLYFLUOROALKYL SUBSTANCES IN JUVENILE SEABIRDS FROM THE U.S. ATLANTIC COAST

This manuscript has been published and is formatted for the journal

Environmental Science and Technology, DOI:

<https://dx.doi.org/10.1021/acs.est.0c01951>

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KEYWORDS: PFAS, PFEAS, PFESA, PFECA, PFOS, bioaccumulation, seabirds

ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic, globally distributed chemicals. Legacy PFAS, including perfluorooctane sulfonate (PFOS), have been regularly detected in marine fauna but little is known about their current levels or the presence of novel PFAS in seabirds. We measured 36 emerging and legacy PFAS in livers from 31 juvenile seabirds from Massachusetts Bay, Narragansett Bay, and the Cape Fear River Estuary (CFRE), USA. PFOS was the major legacy perfluoroalkyl acid present, making up 58% of concentrations observed across all habitats (range: 11 - 280 ng/g). Novel PFAS were confirmed in chicks hatched downstream of a fluoropolymer production site in the CFRE - a perfluorinated ether sulfonic acid (Nafion byproduct-2; range: 1 – 110 ng/g) and two perfluorinated ether carboxylic acids (PFO₄DA and PFO₅DoDA; PFO₅DoDA range: 5 – 30 ng/g). PFOS was inversely associated with phospholipid content in livers from CFRE and Massachusetts Bay individuals, while $\delta^{13}\text{C}$, an indicator of marine vs. terrestrial foraging, was

positively correlated with some long-chain PFAS in CFRE chick livers. These results detail concentrations of legacy and novel PFAS across different marine ecosystems along the US Atlantic East Coast. There is also an indication that seabird phospholipid dynamics are negatively impacted by PFAS, which should be further explored given the importance of lipids for seabirds.

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic organic contaminants used extensively in a variety of commercial, industrial, and military applications globally.¹ Continued, widespread use of PFAS with diverse formulae has resulted in the detection of multiple PFAS into the global environment²⁻⁴. Industrial production has shifted away from long-chain chemistries to replacements with fewer than seven fluorinated carbons, polyfluorinated structures, and/or structurally modified iterations of perfluoroalkyl acids (PFAAs) such as perfluoroalkyl ether acids (PFEAs) incorporating different numbers of ether linkages^{5,6}. PFEAs include both perfluoroalkyl ether carboxylic acids (PFECAs) and perfluoroalkyl ether sulfonic acids (PFESAs).

Both legacy PFAS and new formulations may be released to the environment via both direct or indirect discharges, or some interplay of the two^{1,7-9}. The continued inputs of PFAS act in tandem with their extreme environmental persistence to sustain the proliferation of these compounds within terrestrial¹⁰, marine¹¹, estuarine¹², freshwater¹³, cryosphere¹⁴, and atmospheric compartments^{14,15} worldwide. PFAS compounds can permeate biota within these

compartments, including marine invertebrates¹⁶, fish¹⁷, birds^{18–21}, and marine mammals²². This environmental and biological ubiquity presents challenges for ecological health, as substantial evidence suggests a variety of legacy and new PFAS have the potential for adverse effects across multiple taxa^{23,24}.

Toxicology and field studies suggest associations between PFAS concentrations and reproductive parameters, morphometric characteristics, and metabolic processes in avifauna^{25–31}. Controlled studies across multiple taxa, including birds, also suggest variable associations between PFAS and lipid production, metabolism, and storage pathways^{32–34}. Yet comprehensive assessments of PFAS exposure or potential toxicity in wild avifauna are stymied by a lack of foundational data detailing the environmental occurrence of diverse PFAS across multiple habitats and bird taxa. Transfer of PFAS via trophic interactions is likewise still under investigation, with habitat- and food web-specific trends apparent^{35–37}. Furthermore, there is a dearth of data detailing PFAS concentrations in avifauna near industrial point sources, or potential impacts related to chronic, elevated exposure from such direct or substantial discharges^{29,30,38}. This stands as a significant data gap considering that effective protection of endangered species and habitat surrounding contaminated sites relies on understanding exposure and impacts in bird species and other keystone wildlife.

The oceans are thought to be the final sink for legacy and novel PFAS compounds, receiving inputs via rivers/estuaries and atmospheric deposition².

Long-lived seabirds present an opportunity to assess and compare PFAS detection and trends across a range of coastal and oceanic habitats. In their given marine habitats, seabirds act as integrative sentinels due to their generally predictable life histories and foraging strategies, long life span, top predator trophic position, sensitivity to environmental stressors on observable time scales, and physiological interconnectivity to both air and water^{39,40}. They assimilate resources and related environmental conditions, and demonstrate organismal and population level responses. This responsiveness allows seabird population and individual condition to be utilized as indicators reflecting chemical contamination and/or overall ecosystem health or stress^{20,41–43}.

We used seabirds as sentinels to assess and contrast patterns and magnitude of PFAS exposure in three marine regions. We targeted 36 PFAS in the livers of 31 juvenile seabirds found dead in 2017 in three coastal and pelagic marine habitats with variable PFAS exposure potential. The main objectives of this study were to a) measure and compare legacy and emerging PFAS in different seabirds from marine habitats subject to variable direct or indirect PFAS discharges, and b) ascertain any association between stable isotope signatures approximating trophic habits, phospholipid levels, and PFAS concentrations.

MATERIALS AND METHODS

Chemicals and Reagents

A total of 36 PFAS were assessed in juvenile seabird livers using target and suspect screening, and 27 PFAS quantified (Table S1), including C₄–C₁₄

perfluorocarboxylates (PFCAs), C₄-C₁₀ perfluorosulfonates (PFSAs), three perfluoroalkyl ether carboxylic acids (PFECAs), one perfluoroalkyl ether sulfonate (PFESA), three fluorotelomer sulfonates (FTS), and three sulfonamide precursors (Tables S1,S4-S5). More details about target and suspect analytes, chemicals, and reagents can be found in the Supplementary Information (SI).

Sample Collection

Liver tissue was obtained from six species of deceased juvenile seabirds. Juvenile Great Shearwaters (*Ardenna gravis*) originated from Massachusetts Bay (n = 10) and Herring Gull chicks (*Larus argentatus smithsonianus*) from Narragansett Bay (n = 10). Royal Tern (*Thalasseus maximus*), Sandwich Tern (*Thalasseus sandvicensis*), Laughing Gull (*Leucophaeus atricilla*), and Brown Pelican (*Pelecanus occidentalis*) chicks originated from the Cape Fear River Estuary (n = 11) (CFRE) (Fig. S1). These birds, while unique species with nuanced life histories and food web roles, reflect broadly similar foraging preferences and strategies in coastal and pelagic habitats (Table S2). Literature also implies similar PFAS bioaccumulative capacity between different bird species, enabling a comparison of similar avifauna⁴⁴⁻⁴⁶.

The individuals analyzed here span several months in age, ranging from 2-4 week old chicks to ~6 month old juveniles. Based on the long tissue half-life of PFAS, these young birds predominantly reflect PFAS derived from maternal offloading, and thus the highest internal concentrations experienced by each individual over their lifetime⁴⁶⁻⁴⁸. More details supporting our use of a multi-species, variable age sample set can be found in the SI.

The selected habitats represent a continuum of potential PFAS exposure, with the highest likelihood of exposure in the CFRE downstream from a fluoropolymer production facility. Narragansett Bay represents an intermediate potential for PFAS exposure; it is a well-mixed coastal embayment adjacent to a large urban center, but lacks major PFAS production facilities. The lowest likelihood of exposure, based on increased distance from direct human sources of PFAS, is reflected by seabird juveniles collected from Massachusetts Bay. Massachusetts Bay is a productive offshore marine habitat encompassing Stellwagen Bank National Marine Sanctuary, and representative of the offshore pelagic environment of the North Atlantic (Fig. S1).

All individuals were necropsied in a standardized manner⁴⁹. Liver tissue was used for PFAS analysis and stable isotope analysis, while muscle was only used for stable isotope analysis. Additional details about sample condition, sample procurement, and individual sample details can be found in the SI.

Analysis of PFAS

Complete extraction methods are provided in the SI. Briefly, liver samples were lyophilized and solvent-extracted in methanol using sonication, centrifugation, and freezing, paired with graphitized non-porous carbon solid phase extraction. Measurement and quantification of 25 PFAS was achieved using liquid chromatography tandem-mass spectrometry (UPLC-MS/MS) experiments in negative electrospray ionization mode. Further details about quantification and instrumental parameters are provided in the SI. Estimates of PFAS concentrations were obtained by quantification using isotope dilution. Method recovery ranged

from 14 – 112% with a mean recovery of 62% across all compounds, similar to recoveries reported in other work using avian liver^{47,50,51} (Table S6).

Liver tissue was also assessed via suspect screening using high resolution mass spectrometry (HRMS). Fresh tissue aliquots were extracted for PFEAs of interest by means of protein precipitation and dilution^{19,52}. Sample extracts were measured using a ThermoFisher Orbitrap Fusion (Thermo Fisher Scientific, Waltham, MA, USA) operated in heated electrospray ionization in negative mode as previously described^{19,52}.

Concentrations of PFEAs were derived from these HRMS experiments. Nafion BP2 and PFO₅DoDA were assessed using native standards and reported here quantitatively while all other PFEAs were assessed based on previously determined accurate mass and spectral information using a semi-quantitative approach (Tables S1, S5)^{8,53}. PFO₄DA was the only PFEA detected in samples that lacked a native standard; PFO₄DA was reported as raw abundance and excluded from concentration calculations due to the lack of an authentic native standard. Mass-labelled surrogates of similar molecular weight and retention time were used in the absence of matched surrogates for quantitation of Nafion BP2 and PFO₅DoDA (Table S5).

For those compounds analyzed via both UPLC-MS/MS and HRMS, UPLC-MS/MS concentrations are reported here due to more rigorous quality assurance and increased sensitivity (Table S1). Further details about sample preparation,

analysis, and quality assurance for both UPLC-MS/MS and HRMS are provided in the SI.

Stable Isotope and Phospholipid Analysis

Stable isotope analysis was used to evaluate trophic transfer of PFAS, as well as to ensure trophic comparability of the sample set. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ were measured in muscle; $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were also measured in liver to facilitate comparison to a tissue with a faster isotopic turnover rate⁴², given the unknown rate of PFAS turnover in avian tissues.

Total lipid was extracted from liver tissue aliquots using a modified Folch method, and phospholipid content of liver tissue assessed colorimetrically using an EnzyChrom Phospholipid Assay Kit (BioAssay Systems, Hayward, CA)⁵⁴. More details about stable isotope and phospholipid analysis are available in the SI.

Statistical Analysis

All data manipulation and statistical analyses were performed in R version 3.6.1 (R Core Team, 2020)⁵⁵. Concentrations were converted to a wet weight basis for comparability to other literature values. Responses not detected or below the linear dynamic range of the curve were labeled as “nd” and assigned a value of zero. Observations below method reporting limits with a detection frequency higher than 50% were replaced with the half the method reporting limit for statistical analyses, and included as such in calculation of summed concentrations ($\sum_{19}\text{PFAS}$) and statistical analysis⁵⁶. Data were checked for normality and homoscedasticity using the Shapiro-Wilk test and Levene’s test. Concentration

data were non-normal despite log transformation and therefore treated non-parametrically for statistical analyses; habitat groups displayed no significant differences in variance. Differences between habitats were assessed using Kruskal-Wallis tests with post hoc application of Dunn's test with Bonferroni correction for multiple testing, or with the Wilcoxon rank sum test. Relationships between concentrations were assessed using Spearman rank correlation coefficients (R_s^2). R_s^2 presents the proportion of the rank variance explained by the correlation between variables with test assumptions more suitable for this dataset, providing insight about the relationship similar to the Pearson R^2 . Liver-water bioaccumulation factors (BAFs) were calculated by dividing geometric mean liver PFAS levels by measured or estimated surface water concentrations adjacent to nesting or collection locations, followed by log transformation; more details about BAF calculations can be found in the SI.

RESULTS AND DISCUSSION

Observed detection frequencies and patterns by habitat

Samples were screened for 36 analytes using target and suspect screening; 27 analytes were quantified using native standards. Only one semi-quantitative compound was detected via suspect screening, PFO₄DA. 19 of the 27 quantifiable analytes were measured above detection limits in at least one sample, and detection frequencies varied by habitat. PFOS, PFNA, PFDA, and PFUnDA were present in at least 97% of individuals (Table 1).

The highest $\sum_{19}$ PFAS measured was 390 ng/g w.w. liver comprised of 14 quantifiable analytes, found in a CFRE Royal Tern chick. The lowest $\sum_{19}$ PFAS concentration was observed in a juvenile Great Shearwater from Massachusetts Bay containing $\sum_{19}$ PFAS of 26 ng/g, comprised of 5 analytes above detection limits (Fig. 1). Chicks from the CFRE system contained significantly greater concentrations and number of PFAS than juveniles from Massachusetts Bay or Narragansett Bay (Dunn's test; $p < 0.001$) (Figs. 1a, 3a). There was no significant difference between mean $\sum_{19}$ PFAS levels observed in individuals from Narragansett Bay and Massachusetts Bay (Fig. 3a), though Great Shearwater individuals were older than Narragansett Bay chicks, and may underestimate levels found in chicks of this species. Within each habitat, concentrations were not significantly different between male and female chicks, though the sample size of sexed individuals was small (Table S17).

Multiple PFEAs were not detected, including novel PFECAs PMPA, PEPA, PFO_2HxA , and PFO_3OA , and novel PFESAs NVHOS, Nafion byproduct 4 and 1. No fluorotelomer sulfonates were detected above reporting limits, nor N-EtFOSAA or N-MeFOSAA. The lack of bioaccumulation of shorter-chain PFEAs may be analogous to the reduced bioaccumulation potential of short-chain PFCAs in upper trophic level homeotherms⁵⁷. The lack of detection of these compounds may also denote *in vivo* biotransformation. Research in other biota has shown fluorotelomer sulfonates are precursors to some PFCAs⁵⁸ while N-EtFOSAA and N-MeFOSAA are PFOS precursors⁷. Further work is required to deduce if the non-detects observed in this study are indicative of reduced bioavailability, rapid

biotransformation, or the true absence of a compound in these environments and food webs. FOSA, a perfluoroalkane sulfonamide, was only detectable in three birds from the CFRE. These detections may be the result of continuous, high levels of FOSA or FOSA-precursors related to production activities or legacy PFAS sources in the region⁵³. Such continuous inputs could exceed metabolic capacity and cause tissue residues of FOSA.

Continued dominance of PFOS in juvenile seabirds

PFOS was the most abundant compound in all sampled livers, making up 58% of total liver concentrations across the sample set. The proportion of PFOS measured in each individual varied by habitat, with the CFRE and Narragansett Bay individuals containing the highest geometric mean of 61% and 67% PFOS, respectively (Fig. 2).

These data align well with previous literature that determined a high proportion of PFOS in seabird livers, eggs, and serum in species from multiple ecological provinces^{20,21,59–63}. The highest PFOS concentration measured in this study was 280 ng/g w.w. liver in a Sandwich Tern chick from the CFRE, similar or higher than levels measured within the past ten years in other long-lived temperate seabirds^{20,21,60,61}. Average avian toxicity reference values (TRVs) for PFOS reported by Newsted et al. of 600 ng PFOS/g liver were approximately 2–30 times higher than PFOS levels reported in these young birds⁶⁴. Seven of eleven CFRE chicks exceeded the female-specific liver TRV of 140 ng PFOS/g liver; these birds were female or not yet able to be sexed visually⁶⁴. One female

from Mass. Bay and one unsexed bird from Narra. Bay also exceeded the female-specific TRV.

Our data indicate the continued occurrence of PFOS in juvenile avifauna at levels of toxicological concern despite a production phase-out of perfluorooctanesulfonyl fluoride (POSF), PFOS, and PFOS precursors in the US in the early 2000s¹. The phase-out in 2000-2002 resulted in decreased PFOS and/or precursor concentrations in select environmental matrices^{22,65}. Modeling and empirical results suggest decreased availability of volatile precursor compounds like FOSA, whose environmental occurrence responded quickly to the phase-out, likely caused any decreasing trends of PFOS in wildlife observed after 2002²². Yet biotic trends in PFOS vary based on spatial habits, proximity to local sources, and with trophic strategy, and there is no consistent global pattern of continually decreasing PFOS across multiple avian matrices^{18,60,63,66}. Current PFOS concentrations in wildlife reflect exposure to extant precursor compounds that may transform *in situ* or *in vivo* to PFOS, *in vivo* depuration of PFOS, and sustained transfer of PFOS itself via environmental or trophic interactions^{65,67,68}. Our results highlight the continued biological occurrence of PFOS as a function of these exposures.

Variable contribution of PFCAs by Habitat

Concentrations of PFCAs including PFDA, PFNA, and PFUnDA reported in this study were similar to or elevated compared to concentrations previously reported in temperate species, as well as in birds from Great Lakes and Arctic environments^{4,60-62,66,69}. In CFRE, mean concentrations of PFCAs increased up to

PFDA, where $\text{PFOA} < \text{PFNA} < \text{PFDA} > \text{PFUnDA}$, whereas in Massachusetts Bay $\text{PFNA} < \text{PFDA} < \text{PFUnDA}$ and in Narragansett Bay $\text{PFNA} < \text{PFDA} \approx \text{PFUnDA}$.

The proportion of $\sum\text{PFCAs}$ to $\sum\text{PFSAs}$ varied between habitats, with individuals from CFRE and Narragansett Bay dominated by PFSAs while individuals from Massachusetts Bay contained a significantly higher proportion of PFCAs (Dunn's test; $p < 0.001$) (Fig. 3b). The dominance of PFCAs in offshore Massachusetts Bay individuals was driven partly by significantly higher concentrations of the C_{11} PFCA, PFUnDA, in offshore individuals (Dunn's test; $p < 0.05$) (Fig. S2). Mean concentrations of other long-chain ($\text{C}_n\text{F}_{2n-1}\text{COOH}$, $n \geq 7$) PFCAs found in both Narragansett Bay and Massachusetts Bay were not significantly different.

The preferential dominance of PFUnDA seen here in seabirds from offshore Massachusetts Bay habitat has been observed in Arctic marine mammals as well as Arctic and temperate seabirds^{4,20,21,70}. These studies exemplify a broader pattern in which PFCAs with odd chain lengths are more abundant in biota than PFCAs with even chain lengths. This pattern is a result of preferential bioaccumulation of longer-chain homologues, coupled to substantial atmospheric and water-borne transport of PFCAs and PFCA precursors of variable chain length^{15,23,70–72}. Notably, our data suggests this pattern only applies to biota exposed to diffuse PFAS sources, as CFRE birds subject to localized point sources of PFAS actually contained greater concentrations of PFDA (C_{10}) compared to PFUnDA (C_{11}).

Limited data suggest environmentally relevant exposures of long-chain PFCAs are associated with changes in metabolic rate, oxidative stress, and reproductive behaviors in Arctic black-legged kittiwakes^{25,73}. We highlight the need for further research about the formation, long-range transport, occurrence, and effects of PFCAs and other long-chain PFCAs in marine systems supporting seabirds. Such a comprehensive understanding is necessary given the sustained or increasing occurrence of long-chain PFCAs in remote wildlife^{21,63}, the substantial suite of stressors currently impacting marine species globally, and the continued importance of marine food resources to human communities⁷⁴.

Potentially Confounding Factors

The opportunistic sample set of dead chicks and juveniles enabled us to measure PFAS in liver tissue, allowed the acquisition of unique samples from the CFRE before the cessation of certain industrial PFAS discharges, and allowed assessment of PFAS during a critical development window in immature individuals from data-deficient habitats. Yet this opportunistic sampling also introduced potentially confounding factors related to the variable species and ages of collected individuals.

The older Great Shearwater juveniles in this study were self-feeding for approximately two months, while the chicks from other habitats were still being provisioned by their parents. Great Shearwater PFAS profiles may therefore reflect increased input from dietary sources. Immature Shearwaters are thought to eat similar items as adult Great Shearwaters⁷⁵; hence – the PFAS profile should be conserved, allowing their comparison between habitats. More likely, the older

juveniles reflect growth dilution, and may underestimate PFAS concentrations present in chicks of this species.

Literature to date has yet to identify significant differences between uptake, metabolism, or elimination pathways and rates between similar bird species^{20,44–46}; bioaccumulation in birds appears driven by habitat and trophic exposures. However, species-specific toxicokinetics have been identified between multiple mammal species⁷⁶. We also have not investigated the possibility of developmental changes in molecular receptors of PFAS in birds or other wildlife, although PFAS have been found at higher levels in juveniles than adults across multiple taxa^{48,77,78}. We suggest additional research on the toxicokinetic behavior of PFAS in different bird species and across developmental stages, given their utility and importance as sentinel predators.

Association with phospholipid content

Lipid moieties play key roles in organismal metabolism, reproduction, migration, and other basic functions key to wildlife health and fitness. Phospholipid levels were significantly ($p < 0.05$) associated with PFOS in Massachusetts Bay and CFRE individuals ($R_s^2 = 0.52$ and 0.45 , respectively) (Fig. 4a). $\sum_{19}$ PFAS was more weakly associated with measured phospholipids ($R_s^2 = 0.14$ and 0.45 for Massachusetts Bay and CFRE individuals, respectively and $R_s^2 = 0.51$ when assessed as a total sample set, $n = 31$). There was also a statistically significant correlation between PFNA and phospholipid in CFRE chicks ($R_s^2 = 0.53$, $p < 0.05$).

The importance of phospholipids in the accumulation of long-chain PFAAs has been supported by both empirical observations from marine mammals as well as modeling results^{54,79–81}. Conversely, controlled studies in chickens suggest PFOS may impact lipid metabolism and production via gene suppression, suggesting PFOS may instead indirectly mediate lipid levels^{32,33,68,82}. Currently, our understanding of phospholipid-PFAS associations and relationships lacks substantial field-derived data beyond marine mammals.

These data, though derived from a small sample set, suggest an association between environmentally relevant concentrations of PFOS (and possibly PFNA) and decreased liver phospholipid content in wild seabirds, a previously unreported phenomenon (Fig. 4). Lipid levels in seabird livers may be influenced by a variety of physiological and nutritional constraints not measured within the scope of this study; we note the lack of comparative baseline data describing liver phospholipid levels in the species examined within this work as a possible limiting factor. However, lines of evidence from multiple disciplines suggest a high conservation of non-diet lipid composition in a given tissue between similar avian species^{83–86}. Controlled animal studies also point to the same relationship between PFOS exposure and changes in phospholipid content, lipid profiles, and lipid metabolism alongside altered expression of genes related to lipid dynamics in the livers of domestic chickens^{32,33,68,82}. Most relevant to the results seen here, subcutaneous delivery of 0.02 mg/ml and 0.1 mg/ml PFOS resulted in decreased phospholipid content in domestic chicken plasma after 28 days of exposure and 28 days of depuration⁶⁸.

Laboratory-based studies using model phospholipid bilayers, liposomes, and bacterial membranes exhibit an inverse relationship between PFAS levels and phospholipids, manifested via increased incorporation of PFAS into bilayers and subsequently decreased lipid content. These studies also found increased bilayer disruption by PFAS based on chain-length and functional group^{87,88}. The results from these controlled membrane studies are not easily translated to realistic biological conditions, yet in combination with other evidence (our data, previous work in pilot whales⁵⁴, and controlled animal studies referenced above) we highlight a potential and currently undefined relationship between PFOS and phospholipid responses in wild organismal systems at environmentally relevant exposure levels. Further research is warranted to better describe relationships between PFAS-driven lipid responses in combination with the role of lipids in PFAS partitioning.

Relationship to stable isotope data

$\delta^{15}\text{N}$ is frequently used as a proxy for trophic level across terrestrial and aquatic systems; while system and prey-base specific, enrichment of $\delta^{15}\text{N}$ typically indicates foraging at a higher trophic level⁴². Legacy persistent organic pollutants (POPs) like polychlorinated biphenyls or DDT frequently increase in concentration with trophic level as approximated by $\delta^{15}\text{N}$ values, due to bioaccumulation of hydrophobic POPs in lipid-rich consumer matrices⁸⁹. Here, we found $\delta^{15}\text{N}$ values and calculated trophic level were not significantly associated with concentrations of the majority of individual PFAS in each habitat

(Fig. S6, Table S23). PFOS and $\sum_{19}$ PFAS were positively associated with $\delta^{15}\text{N}$ only in Massachusetts Bay individuals (Table S23).

$\delta^{13}\text{C}$ values reflect basal sources of primary production supporting trophic networks⁴² and exhibit significantly less step-wise change with prey-consumer interactions. This allows bulk differentiation between inshore and offshore food chains based on characteristic enrichment or depletion of $\delta^{13}\text{C}$ associated with terrestrial vs marine primary production (Fig. S8)⁴². $\delta^{13}\text{C}$ ranged from -19.8 to -17.0 in CFRE individuals, and from -22.5 to -19.0 in Narragansett and Massachusetts Bay individuals. In contrast to previous studies, concentrations of C_9 , C_{10} , C_{11} , and C_{13} PFCAs, PFOS, and $\sum_{19}$ PFAS in CFRE birds were associated with enriched $\delta^{13}\text{C}$ values in seabird muscle and liver, likely reflecting the unique geomorphology of the CFRE system and energy-saving coastal foraging habits of CFRE seabird species (Fig. S7). PFTrDA and PFDA were negatively associated with $\delta^{13}\text{C}$ values in Mass. Bay individuals, which may reflect increased exposure to long-chain PFCAs in offshore environments via long-range transport and transformation of PFCA precursors. More details about relationships between PFAS and stable isotopes are available in the SI.

Unique occurrence of emerging compounds

HRMS analysis helped us confirm the presence of (previously identified) PFEAs in chick livers for the first time: Nafion BP2 and PFO5DoDA were found in livers of all CFRE individuals ($n = 11$) while PFO4DA was found in 7 of 11 CFRE individuals (Fig. 1b, Table S16).

The maximum Nafion BP2 concentration was 110 ng/g w.w. in a Laughing Gull chick and a maximum PFO5DoDA concentration of 30 ng/g w.w. in a Royal Tern chick (Fig. 1b). Nafion BP2 was also detected in two Great Shearwaters and one Herring Gull from outside the CFRE, or 15% of the non-CFRE sample set (Figs. 1b, S10).

The detection of these PFEAs in CFRE chicks is due to proximity to an industrial point source and the then-ongoing discharge of PFEAs to surface water. CFRE chicks were hatched in a well-mixed estuary ~145 km downstream from a fluoropolymer production facility in Fayetteville, NC. “Gen X” or HFPO-DA, along with other PFEAs were detected at high levels in Cape Fear River surface water and Wilmington, NC drinking water as a result of industrial wastewater discharges into the mainstem Cape Fear River^{53,90,91}. Increased research attention following the 2017 termination of the industrial discharge has revealed the presence of multiple PFEAs in downstream environments, fish, and humans^{52,92}.

Notably, concentrations of Nafion BP2, PFO5DoDA, and PFO4DA reported here are the highest biotic measurements of these PFEAs at the greatest distance from the production plant recorded to date, similar to measured PFOS concentrations and PFOS bioaccumulation factors in chick livers (Figs. 1, S9). Average PFEA concentrations reported here from seabird chick liver tissue (ppb, ng/g) are approximately 20 times higher than PFEA concentrations found in striped bass serum (ppb, ng/mL)⁵².

Observed PFEA concentrations in CFRE chicks are likely not a result of riverine foraging proximate to the plant discharge or incorporation of freshwater

prey items by seabird parents. The species sampled in this study are strictly marine, and do not inhabit or use freshwater, riverine habitats based on colony observations and their foraging preferences (marine and estuarine prey including forage fish, squid, and crustaceans)^{93–95}. PFEAs were previously measured in river water upstream from the breeding colony at very high concentrations while the industrial discharge was ongoing^{90,96}. Their detection in seabirds here suggests these emerging PFEAs are environmentally persistent and capable of significant downstream transport and biotic uptake via undefined water-borne, particulate, and/or atmospheric pathways.

The confirmed presence of Nafion BP2 in three individuals outside of the CFRE is the first identification of Nafion BP2 in biota outside of the CFRE region, reiterating the environmental persistence and mobility of Nafion BP2. These detections are difficult to explain because virtually no data exists describing Nafion BP2 environmental occurrence beyond the CFRE region. Nafion BP2 detections in non-CFRE chicks may be due to migratory proximity to the CFRE; we consider this unlikely due to the lack of evidence indicating use or reliance on the CFRE system by non-CFRE populations sampled in this study.

More likely, Great Shearwater juveniles and Herring Gull chicks accumulated Nafion BP2 as a result of exposure to indirect discharges contaminated with Nafion products or related degradation byproducts. Nafion is the brand name of a perfluorinated ionic polymer first discovered in the 1960s via modification of the Teflon polymer. The Fayetteville, NC production facility has

produced this ionic polymer since the 1980s. Nafion byproduct-2 is a side product from the reaction of the polymer precursors.⁹²

Little data exist describing how production, use, or disposal of this perfluorinated polymer may contribute to PFAS to the environment. Evidence from the CFRE suggests Nafion production waste streams may contribute substantial loads of Nafion byproducts to receiving environments^{52,53,90,92}. Feng et al. (2015) investigated the thermolysis products of Nafion 117, a typical Nafion membrane and suggested high-temperature uses or disposal of Nafion via incineration may produce multiple perfluorinated structures as a result of incomplete combustion⁹⁷; Nine groups of fluorinated analogues were identified as a result of thermal treatment of Nafion 117 membranes, including breakdown products structurally similar to Nafion BP2⁹⁷. Additional research documents chemical and mechanical degradation pathways relevant to Nafion membrane function and efficiency⁹⁸. The (albeit limited) detection of Nafion BP2 beyond CFRE individuals warrants additional screening of Nafion byproducts in diverse environmental matrices, investigation into the life-cycle of Nafion technologies, and potential environmental persistence of PFESAs.

Divergent sources of emerging and legacy PFAS in the CFRE system

Ratios of PFEAs to PFOS varied between individuals, with PFEA levels on the same order of magnitude as PFOS in select individuals (Fig. 1b inset). PFEA concentrations were not correlated with PFOS or long-chain PFCAs in CFRE chicks, while PFO5DoDA displayed a weak positive correlation with PFDA (Fig. S5). This lack of correlation suggests legacy PFAAs like PFOS were contributed

to the system via different pathways unassociated with the industrial facility producing PFEAs, in-line with their previous detection in surface waters from the region⁵³. Prior research indicates concentrations of PFCAs and PFSAAs in surface water are similar above and below the fluoropolymer facility in Fayetteville, NC while concentrations of PFEAs varied starkly upstream and downstream of the facility while active discharges from the facility were ongoing^{53,90}.

Implications for further research

Our results highlight the potential role of seabirds as key sentinels of marine environments, and confirm the sustained presence of legacy PFAS in marine systems along the US East Coast. We also document PFEAs in seabirds for the first time, reflecting the shifting suite of PFAS in production and in environmental matrices. As our current understanding of PFAS effects in wildlife is limited, future biomonitoring in seabirds and other wildlife should derive responses and effects related to ambient PFAS levels. Understanding PFAS levels and effects in marine food webs and biota ultimately stands to benefit public health and commerce as we continue to rely on marine food webs for economic, nutritional, and aesthetic services.

ASSOCIATED CONTENT

The following files are available free of charge:

Details about sample preparation, extraction methods, instrumental analysis, quality assurance and quality control, supplementary figures (pdf)

monitored ions, extraction performance, raw data, summary statistics, data inputs for comparison and baf calculations (excel workbook)

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The manuscript was primarily drafted by A. Robuck and finalized through contributions of all authors. Sample analysis and data interpretation were carried out by A. Robuck through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

A. Robuck acknowledges support from the National Oceanic and Atmospheric Administration Dr. Nancy Foster Scholarship program (NOAA Award Number NA17NOS4290028), the Robert and Patricia Switzer Foundation, the STEEP Superfund Research Program (NIEHS Award Number P42ES027706), and the Oak Ridge Institute for Science and Education (ORISE) program.

ACKNOWLEDGMENTS

The authors are indebted to the Northeast Fisheries Observer Program, Gina Shield, Johanna Pedersen, Michael Moore, Matthew McIver, Marissa Sprouls, Arianna Mouradjian, and the Wildlife Clinic of Rhode Island for assistance

obtaining and necropsying bird samples used in this study. The authors also acknowledge Christine Gardiner (URI) for laboratory assistance and Michael A. Mallin for context about the Cape Fear River and Estuary. We also acknowledge Melanie Hedgespeth, Andy Lindstrom, and Elsie Sunderland for providing pre-submission critical reviews. Although EPA and NOAA employees contributed to this article, this research was not funded by EPA and was conceived, designed, and implemented by URI. The primary author was not an ORISE participant at US EPA when the research was carried out. EPA's role was limited to advising PFAS and stable isotope analysis and therefore not subject to EPA's quality requirements. Consequently, the views, interpretations, and conclusions expressed in the article are solely those of the authors and do not necessarily reflect or represent NOAA or EPA's views or policies.

ABBREVIATIONS

PFAS: per- and polyfluoroalkyl substances

CFRE: Cape Fear River and Estuary

PFCAs: perfluoroalkyl carboxylate(s)

PFSAs: perfluoroalkyl sulfonates(s)

PFEAs: perfluoroalkyl ether acid(s)

PFECAs: perfluoroalkyl ether carboxylate(s)

PFESAs: perfluoroalkyl ether sulfonate(s)

BCF: bioconcentration factor

See SI Table S1 for the full names of all individual PFAS discussed in the scope of this analysis

EPA: US Environmental Protection Agency

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TABLES AND FIGURES

% Detection by Ecosystem						
Compound	Family	# Fluorinated Carbons	Mass. Bay	Narra. Bay	CFRE	All
N-MeFOSAA	FASAA	8	0	0	0	0
N- EtFOSAA	FASAA	8	0	0	0	0
FOSA ^a	FASA	8	0	0	27	10
4:2 FTS	FTS	4	0	0	0	0
6:2 FTS	FTS	6	0	0	0	0
8:2 FTS	FTS	8	0	0	0	0
PFBA	PFCA	3	100	80	73	84
PFPeA	PFCA	4	0	0	0	0
PFHxA	PFCA	5	0	30	27	19
PFHpA	PFCA	6	0	0	0	0
PFOA	PFCA	7	0	0	64	23
PFNA	PFCA	8	100	100	100	100
PFDA	PFCA	9	90	100	100	97
PFUnDA	PFCA	10	100	90	100	97
PFDoA	PFCA	11	0	10	27	13
PFTTrDA	PFCA	12	70	20	27	39
PFTeDA	PFCA	13	0	10	0	3
PMPA	PFECA	3	0	0	0	0
PFO ₂ HxA	PFECA	3	0	0	0	0
PEPA	PFECA	4	0	0	0	0
PFO ₃ OA	PFECA	4	0	0	0	0
HFPO-DA	PFECA	5	0	0	9	3
PFO ₄ DA ^b	PFECA	5	0	0	70	23
PFO ₅ DoDA	PFECA	6	0	0	100	36
Nafion BP4	PFESA	6	0	0	0	0
Nafion BP2	PFESA	7	20	10	100	45
Nafion BP1	PFESA	7	0	0	0	0
NVHOS	PFESA	4	0	0	0	0
PFBS	PFSA	4	0	10	0	3
PFPeS	PFSA	5	20	0	0	7
PFHxS	PFSA	6	10	10	55	26
PFHpS	PFSA	7	0	0	55	19
PFOS	PFSA	8	100	100	100	100
PFNS	PFSA	9	0	0	0	0
PFDS	PFSA	10	0	10	9	7
PFECHS	Cyclic PFSA	8	0	0	0	0

Table 2.1. Detection frequency of quantifiable and semi-quantitative analytes in seabird juveniles from each habitat and as a total sample set across all habitats combined. Mass. Bay = massachusetts bay, narra. Bay = narragansett bay, and cfre = cape fear river estuary. Family names are from buck et al. 2011. Compounds highlighted in gray are those compounds detected above reporting levels in at least 97% of individuals via lc-ms/ms. ^a detection based on raw abundances in comparison to blank raw abundances due to lack of authentic standards. ^b low recovery (14%) related to sample preparation.

Figure 2.1. Measured concentrations of A) PFAS in juvenile seabird livers measured via LC-MS/MS, B) two emerging PFAS measured via targeted HRMS, alongside B-inset) ratios of emerging PFEAs to PFOS in CFRE chicks. Nafion BP2 concentrations positively identified in non-CFRE chicks but below the linear dynamic curve range are graphed in panel B as half the reporting limit; grey arrows are used to distinguish these data points.

Figure 2.2. The composition of PFAS in liver tissue, presented by individual and grouped by habitat.

Figure 2.3. $\Sigma_{19}\text{PFAS}$ presented in A) as a boxplot, with the dark line representing the median, box limits representing the first and third quartiles, whiskers denoting 1.5 times the interquartile range, and crosses denoting outliers. The asterisk indicates a statistically significant difference between habitat mean $\Sigma_{19}\text{PFAS}$ compared via Wilcoxon rank sum test using the CFRE as the reference group, while B) presents ratios of ΣPFCA to ΣPFSA in each individual. Ratios above 1 indicate PFCA dominance, while ratios below 1 indicate PFSA dominance. (Small figure, 300 word equivalent)

Figure 2.4. A) presents log-transformed concentrations of PFOS vs phospholipid (PL) grouped by habitat, while B) displays log-transformed PFOS and phospholipid (PL), assessed as a total sample set ($n = 31$). Text annotation presents R , R^2 , and p -value derived from Spearman rank correlation analysis.

SUPPORTING INFORMATION

LEGACY AND NOVEL PER- AND POLYFLUOROALKYL SUBSTANCES IN JUVENILE SEABIRDS FROM THE U.S. ATLANTIC COAST

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This document contains 10 figures and 23 tables.

Chemicals, reagents, and materials

Analytical standards of greater than 99.9% purity, including mass-labeled surrogates, were purchased from Wellington Laboratories (Guelph, ON, Canada). Native Nafion BP2 and PFO5DoDA were provided by Chemours (Fayetteville, NC) in lieu of a commercial source. HPLC grade methanol was purchased from Fisher Scientific (Waltham, MA, USA). Ultrapure water for equipment cleaning was obtained from a Milli-Q system fit with an HPLC water polisher or via HPLC grade water purchased from Fisher Scientific (Waltham, MA, USA). ENVI Carb 2g cartridges were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate was purchased from Fisher Scientific (Waltham, MA, USA)

Sample collection details

All birds were obtained opportunistically for use in this study, and no birds were killed for the purposes of this study. Birds were collected and stored in accordance with URI Biosafety standards and the Migratory Bird Treaty Act.

Dead Great Shearwater juveniles (*Ardenna gravis*) were obtained as bycatch from the National Oceanic and Atmospheric Administration (NOAA) Northeast Fisheries Observer Program (Falmouth, MA). Dead Herring Gull (*Larus argentatus smithsonianus*) chicks were obtained from the Wildlife Clinic of Rhode Island (Narragansett, RI). Dead Royal Tern (*Thalasseus maximus*), Sandwich Tern (*Thalasseus sandvicensis*), Laughing Gull (*Leucophaeus atricilla*), and Brown Pelican (*Pelecanus occidentalis*) chicks were obtained as part of routine field and nest surveys conducted by North Carolina Audubon (Wilmington, NC).

Great Shearwaters in this study were self-feeding and had completed their first migration from their remote breeding site in the South Atlantic at approximately 5-6 months old, and are therefore considered juveniles. Great Shearwaters were confirmed to be juveniles based on molt status, presence of the bursa of Fabricius, and stage of gonad development. All birds from Rhode Island and North Carolina were pre-fledging birds under 8 weeks of age, still under parental care at time of death. All individuals were hatch-year juveniles, under approximately six months of age. Great Shearwaters were 5-6 years away from reproductive maturity, while gulls, terns, and pelicans, who mature more rapidly, were at least a year away from reproductive maturity (Tables S3-S4).

Support for a multi-species, variable age comparison

Species

The literature does not currently support the hypothesis of significantly different uptake, metabolism, or elimination rates between similar seabird species. The species included in this study are similar in trophic strategy and preferred prey items, facilitating an acceptable comparison.

a) Species-based differences in bioaccumulation capacity

We point to multiple studies across the literature that suggest foraging preferences, migratory strategies, and other life history details that determine exposure potential drive PFAS concentrations rather than innate differences in bioaccumulation capacity between seabird species^{1,2,11,3–10}.

The most valuable support of our sample design comes from studies investigating concentration trends in multiple species surrounding concerted PFAS sources. Lopez-Antia et al. (2017) examined PFOS levels in eggs from three species of bird surrounding a fluoropolymer production site in the Netherlands. The study focused on species that were unambiguously different from one another in terms of size and diet, unlike the similar seabirds included in our study. [Great tits](#) are a small passerine (songbird) species that primarily feeds on insects; [northern lapwings](#) are small-medium marsh wading birds that feed on small aquatic invertebrates or insects. [Mediterranean gulls](#) are opportunist seabirds that feed on fish, bivalves, or crustaceans. The study found no statistically significant differences in egg PFOS levels among the three species, suggesting the common exposure source drove PFOS levels in all birds without

clear species-level differences in adult uptake, metabolism, or elimination via maternal offloading. Similarly, Yoo et al. (2008) examined PFAS in eggs from three bird species nesting on Lake Shiwa in Korea, a large, industrially-influenced artificial seawater lake. The study found no significant differences in PFOS and Σ_5 PFCA between [little egret](#) (a large wading bird), [little ringed plover](#) (a small shorebird), and [parrot bill](#) (a small songbird-like bird) eggs.

Data from controlled studies indicate approximately equivalent accumulation efficiency in adult quails (a game bird) and mallards (a duck)⁹. Domestically reared adult birds from both species and sexes were exposed to the same PFOS levels via diet. Adult livers and serum were found to contain similar PFOS levels at the completion of exposure; larger differences were apparent between sexes of the same species than between different species. Egg yolk and 14-day old juvenile liver and serum also displayed comparable PFOS levels between both species, without the stark differences between sexes.

Roscales et al. 2019 compared PFAS levels in seabird serum from multiple habitats in or adjacent to the Southern Ocean¹¹. Two species were sampled within three colonies across different latitudes and ocean basins. Measurements show larger differences between the same species at different locations, compared to observed differences between different species at the same colony (Roscales et al. 2019, Table 1). This suggests location-based exposure factors, like proximity to PFAS sources or prey PFAS levels, are the primary determinant of PFAS in similar seabird species.

Beyond avifauna, we note that data from marine mammals suggests taxa with a shared phylogeny conserve cellular and tissue machinery driving internal kinetics of PFAS. For example, evidence in marine mammals demonstrates conservation of metabolic pathways at the family level rather than the species level; data to date suggests all odontocete cetaceans are unable to efficiently metabolize FOSA^{12–16}.

Species-specific toxicokinetic processes and rates are certainly possible and have been demonstrated across contrasting mammal species like rats and humans¹⁷. However, based on the available data showing similar tissue residues in seabirds and other types of birds subject to similar PFAS exposures, we suggest further data is required to rigorously support the claim of significantly different accumulation, metabolism, or elimination pathways or rates at a species level in similar birds. The data we have on hand suggests comparisons across multiple species are acceptable, and any major differences in PFAS levels are due to dietary and habitat factors. We highlight this as an important research gap considering the utility of birds as ecosystem sentinels.

b) Ontogenetic and diet differences that confound comparison between species

As the above evidence suggests, habitat-related exposure and dietary choices likely drive PFAS levels more than major innate differences in toxicokinetics between seabird species. We assert that the birds included in our study are roughly comparable in diet, all feeding primarily on forage fish and invertebrates (Table S2). All species demonstrate some reliance on Atlantic menhaden

Brevoortia tyrannus, a key forage fish found along the US Atlantic seaboard from Florida to Nova Scotia. All species are plungers and opportunists rather than pursuit divers. This broad similarity in trophic strategy and position allows our study to highlight and compare differences in PFAS levels due to habitat-driven factors like human inputs and proximity to production sources.

Age

Our study focused on how each habitat/lifestyle contributes PFAS to an associated sentinel predator, and by sampling juveniles and chicks we avoid life history complications associated with older birds to focus on this question. While chick and juvenile stages are distinct in many ways, we do not believe the differences in these life stages significantly compromise PFAS measurements in our study due to the influence of maternal offloading, and the long half-life of PFAS.

a) Both chick and juvenile birds primarily reflect maternal offloading

PFAS are very persistent in the environment and in biota. PFAS transferred from mother to offspring do not readily dissipate over the first few months of development. The lengthy plasma half-life of PFOS in birds (231 days) means that both chicks (~14 – 56 days old) and juveniles (~140 – 170 days old) within this study retain and reflect the influence of maternal offloading in liver measurements¹⁸. Short-term exposure trials in juvenile quail and mallard suggest the liver half-life of PFOS is roughly 2.5 times that of the serum half-life. Using the plasma half-life derived in Tarazona et al. 2015, this equates to an estimated liver half-life for PFOS of 578 days in birds, or 1.6 years. Liver elimination of

PFAS via biliary excretion has been demonstrated to be very slow (~1% of supplied dose)¹⁹, and liver metabolism of most PFAAs is thought to be negligible. Therefore we reiterate that all individuals of all ages used in this study primarily reflect the influence of maternal offloading. A longer duration of self-feeding by Great shearwaters is unlikely to offset or surpass the PFAS burden received *in ovo*; a study examining prey fish concentrations compared to egg and juvenile liver concentrations in guillemots suggested maternal offloading exposure drastically exceeded dietary inputs²⁰. Measurement of shearwater prey (sand lance) from Massachusetts Bay points to similar, low levels of PFAS exposure from offshore prey (Σ_{12} PFAAs: ~1 – 8 ng/g whole body, wet weight) (Robuck, unpublished data). We also point to seabird literature that suggests adults of these species provision their young with the same or similar species that they themselves consume, meaning the dietary additions provided to chicks were similar or identical to those prey items that contributed to PFAS burdens in mothers prior to maternal offloading (Table S2).

The duration of dietary exposure was longer for the older juveniles, who had been self-feeding for several months. More importantly, these birds also had a longer growth period, causing growth dilution of maternally offloaded PFAS in liver.

b) There is no evidence that specific cellular and tissue machinery known to drive PFAS accumulation differ with age.

PFAS accumulation is driven by associations with specific proteins in liver and blood, in tandem nonspecific associations with amphiphilic structural lipids^{21–}

²³. We are not aware of any data demonstrating age-based differences in the protein structures or binding efficiency of liver fatty acid binding protein and albumin, the two primary proteins identified to drive PFAS accumulation in liver and blood. Rather, data from *in ovo* studies demonstrates the ability of developing embryos to readily accumulate PFAS in liver, at levels on par with or exceeding maternal liver concentrations⁹. Data from in utero exposure studies in mice reiterate the same²⁴. Current, albeit limited, data suggest no appreciable changes in bulk liver phospholipid levels over chick development²⁵, though rapid changes in storage lipid content occur across all tissues during chick development²⁶. Our data in Figure 4 reiterates this – chicks from Narragansett Bay had phospholipid levels similar to older Great Shearwater juveniles (possibly due to similar PFOS levels) compared to chicks from the CFRE of the same age and from the same family. Elimination rates have been shown to be sex-dependent, but no data exist describing age-specific elimination differences in birds^{9,18,27}. Specific mechanisms of PFAS uptake may change on a bulk level with tissue growth as more tissue is available to interact with PFAS, but we believe this would not be apparent in our measurements normalized to ng PFAS/g tissue basis. More importantly, the maternally offloaded burden of PFAS would be diluted with tissue growth. Overall, more research is needed to explore changes in PFAS uptake with age, but data to date do not indicate substantial changes in tissue components driving bioaccumulation of PFAS.

c) *PFAS are not stored in fat*

PFAS are not stored in fat akin to legacy persistent organic pollutants like polychlorinated biphenyls or DDT^{28–31}, meaning the rapid development of adipose fat stores in juveniles that may impact hydrophobic pollutant levels is not likely to impact organ levels of PFAS in chicks or juveniles.

d) *PFAS demonstrate an inverse relationship with age*

Current literature suggests PFAS do not display the same positive relationships between age like some legacy POPs³². Data from several taxa indicate PFAS are significantly higher in juveniles compared to adults^{33–37}. Possible explanatory mechanisms for this negative relationship include the influence of maternal offloading combined with the long tissue half-lives of many PFAS, or growth-mediated biodilution. Research examining PFAS in maternal liver, egg, and chick liver have found the highest PFAS levels in egg, with subsequent chick liver containing PFOS on par with or higher than the mother's liver^{9,20}. This suggests that like other wildlife taxa, seabirds also display a negative relationship between PFAS and age. By measuring young birds, we are likely measuring the highest concentrations experienced by these individuals throughout their lifetimes, during a critical development window.

e) *Juveniles are not impacted by reproductive cycles which confound measurements in adults.*

Seabirds have been shown to offload significant concentrations of PFAS in eggs^{28,29,38}.

f) *Using juvenile or immature dead seabirds allows measurement of individuals of a known age and sex.*

Most adult seabirds (including all species included in this study) can't be aged or sexed by plumage or morphometric characteristics after their first year of age.

- g) *Juveniles demonstrate some of the most predictable ties to a given foraging habitat, helping to constrain dietary and habitat influences on PFAS levels.*

For chicks, this is caused by parents engaging in central-place foraging, meaning the adults find food for their chicks within a region adjacent to the nesting colony to facilitate continuous provisioning of chicks. For the Great Shearwater juveniles which were self-feeding, multiple years of tracking data suggest hatch year birds forage in a predictable home range across Massachusetts Bay, the Great South Channel off Cape Cod, and Georges Bank while adults may forage more widely across a larger pelagic region^{39,40}.

Figure S2.1. Map of the US Atlantic East Coast. Insets provide further detail about collection locations of from each habitat. Great shearwaters were collected as bycatch in Massachusetts Bay and off Cape Cod, designated as NOAA Fisheries Stat Areas 514 and 521. Stellwagen Bank National Marine Sanctuary, a key foraging area for Great Shearwaters, is outlined in white and indicated as “SB” in the top right inset. Herring Gull chicks came from nests located in Narragansett, RI and Newport, RI. The bottom inset shows the Cape Fear River and Estuary system, located in southeastern NC. A Chemours facility is located near Fayetteville, NC, designated on the map as a white star. The red box indicates the lower river and estuary portion of the system; CFRE chicks were hatched on dredge islands near the mouth of the estuary.

Sample condition, cause of death, and necropsy

Great Shearwaters and Herring Gulls were freshly or very recently dead, and frozen immediately with no decomposition apparent.

The procurement of tissues from juvenile Great Shearwaters caught via fishing bycatch is extremely unlikely to impact liver integrity. Juvenile Great Shearwaters were bycaught via gillnet. Lung hemorrhaging and edema was

apparent in some bycatch seabirds (indicative of drowning), but there was no evidence of seawater intrusion into the coelomic cavity housing the liver, and no perforations or damage to any other internal organs beyond lung hemorrhaging. As such we consider bycatch birds as excellent sample specimens, in good condition with no apparent liver degradation or trauma.

Tern, Laughing Gull, and Brown Pelican chicks were recently deceased; chicks spent a maximum of 24-48 hours exposed to the elements prior to collection due to the colony monitoring schedule. Some decomposition was apparent externally on a few individuals. This decomposition was entirely external, apparent via reduced eye integrity, mouth mucus membrane color, and skin pallor. No CFRE individuals displayed evidence of scavenging or substantial autolysis internally.

Phosphatidylcholines, the primary type of phospholipid measured with our bulk assay, have demonstrated stability up to 48 hours after death at 24.1 C, 50% humidity, and atmospheric pressure, suggesting the lipid measurements from CFRE chicks produced within our study are likely free from majorly compromising impacts related to post-death putrefaction, autolysis, or pH diminution^{41,42}.

Massachusetts and Narragansett Bay birds were otherwise healthy birds that met unfortunate ends. Narragansett Bay birds fell from their nests and broke one or more limbs, while Massachusetts Bay birds drowned or were strangled in fishing nets. There was no indication of other health problems impacting these individuals that could influence contaminant distributions observed.

Cape Fear birds died due to mostly uncertain causes – predation was not the cause of death because all deceased individuals were collected intact. Colony monitoring data from multiple locations suggests nest abandonment as the most common cause of chick death. Seabirds such as terns and pelicans are very sensitive to disturbance. They will readily abandon a nest due to human, predator, or insect disturbance ^{43–46}– the chick would then die of dehydration or cold stress. Chicks may also die from tidal flooding or starvation. Chilling, dehydration, and tidal flooding are unlikely to impact contaminant levels in chick livers. PFAS are minimally stored in fat, therefore any starvation-induced use of fat stores is unlikely to release PFAS to liver and overall circulation, should starvation have been the cause of death for any of these birds. No widespread starvation event was apparent in the CFRE region in 2017, making this final potential cause of death somewhat unlikely.

All birds were frozen following collection and tissues sampled within two months. Species identification was corroborated by both specimen collector and necropsy prosector. Each seabird individual was partially thawed and necropsied according to standard protocol, documenting morphometric features, organ weights, overall body condition, sex, and stomach contents after van Franeker 2004⁴⁷. Birds were sexed if gonads were visually identifiable, and aged using morphometric, gonad, and bursa characteristics along with nest observations. Multiple tissues including liver were collected from each bird, and all tissue samples were wrapped in solvent-cleaned aluminum foil, stored in polyethylene baggies, and frozen until analysis at -15C. More details about sample collection

locations, bird morphometric data, age, sex, and sample condition can be found in Table S3.

Sample preparation for UPLC-MS/MS

A modified extraction procedure was developed to maximize sample throughput while minimizing matrix effects and extraction losses, incorporating steps employed in several previously published extraction protocols^{28,48–50}. A tissue aliquot was weighed into a polypropylene tube and 4ml of methanol added, followed by 10 ng of isotopically labeled PFAS surrogate mix (1ng/μL). Samples were vortexed for 30 seconds, and allowed to equilibrate for 30 minutes. Samples were again vortexed for 30 seconds, sonicated for 20 minutes, and centrifuged at 4,000 rpm for ten minutes. The resulting supernatant was decanted into a fresh polypropylene tube, and the extraction procedure repeated with 4 mL of 2 mM ammonium acetate in methanol. The extract was frozen at -15C for at least four hours to encourage precipitation of additional biological material and then centrifuged for three minutes under refrigeration, directly followed by decanting the supernatant for clean-up.

The combined extract was cleaned up using Supelclean ENVI-Carb cartridges (2g, 12 mL, 100–400 mesh, Supelco, U.S.A.). The cartridges were cleaned with 8 mL methanol prior to sample introduction. After sample clean-up, each cartridge was rinsed with 2 mL of methanol. Clean-up was repeated if the final extract contained any hint of color. The extracts were dried to 250 ul at 32°C under 5-7 psi N₂, and reconstituted to 1 mL using 2mM ammonium acetate in water for a final sample makeup ratio of 3 parts aqueous: 1 part organic extract.

The 1 mL extracts were centrifuged at 10,000 rpm for fifteen minutes at 5°C to remove any remaining tissue residues; the final extract minus any pellet solids was transferred to an autosampler vial in preparation for instrumental analysis.

UPLC-MS/MS analysis

40 µl of sample extract was injected, and chromatographic separation achieved using a 50 mm BEH C-18 column interfaced to a Waters Acquity UPLC. The mobile phase was made up of methanol and HPLC-grade water modified with 2mM ammonium acetate, made fresh before each run. The applied gradient is detailed in Table S10, the flow rate was set at 0.4ml/min, and column temperature set at 45°C.

Detection of PFAS was carried out using UPLC-MS/MS in negative electrospray ionization mode. Multiple reaction monitoring (MRM) was employed, monitoring two transitions for each compound as available, detailed in Table S7 and S9. Desolvation temperature was set at 400°C, desolvation gas flow at 600 L/hr, source temperature was set at 150°C, and cone gas flow set at 30°C. Optimal transitions and analyte-specific energies were generated using Intellistart software in combination with manually performed direct infusion experiments.

Quantitation was carried out using an isotope-dilution approach; those analytes lacking a matched mass-labelled standard were quantified using a mass-labelled surrogate of similar molecular weight and retention time (Table S7).

Sample preparation for HRMS

A tissue aliquot was diluted with three parts water and homogenized to a uniform slurry. An aliquot of homogenate was diluted 4:1 with 0.1M formic acid

and vortexed for 30 seconds. The denatured extract was further diluted 5:1 with cold acetonitrile and centrifuged for 5 min at 10000 rpm. An aliquot of the supernatant was removed and combined with dilute ammonium formate buffer (2.5mM) to obtain a final sample extract ratio of 3 parts aqueous: 1 part organic extract.

HRMS analysis

100 ul of sample was injected, and chromatographic separation achieved using a Vanquish UPLC system equipped with an Accucore 100 mm reverse-phase C18 column, at a flow rate of 300 ul/min. Mobile phase constituents included Solvent A (95:5 water: acetonitrile) and Solvent B (95:5 acetonitrile:water). Mobile phase gradient is detailed in Table S11. Nafion byproduct 2 (Nafion BP2, or 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-ethanesulfonic acid) and PFO₅DoDA (2,2,4,4,6,6,8,8,10,10,12,12,12-Tridecafluoro-3,5,7,9,11-pentaoxadodecanoic acid) were identified with authentic native standards, while PFO₄DA (perfluoro-3,5,7,9-tetraoxadecanoic acid), PFMA (2,3,3,3-tetrafluoro-2-(trifluoromethoxy)-propanoic acid), PFO₂HxA (perfluoro-3,5-dioxahexanoic acid), PEPA (Perfluoro-2-ethoxypropanoic acid), PFO₃OA (Perfluoro(3,5,7-trioxaoctanoic) acid), NVHOS (1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoroethoxy)ethane sulfonic acid), Nafion byproduct 4 (2,2,3,3,4,5,5,5-4-(1,1,2,2-tetrafluoro-2- sulfoethoxy) pentanoic acid), Nafion byproduct 1 (2-[1-[difluoro[(1,2,2-trifluoroethenyl)oxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-ethanesulfonic acid) and PFECHS (perfluoro-4-

ethylcyclohexanesulfonate) were detected using previous accurate mass assessment information. PFAS were detected using a Thermo Orbitrap Fusion mass spectrometer using heated electrospray ionization in negative mode (Thermo Fisher Scientific, Waltham, MA, USA). Full scan accurate mass spectra were acquired from 70 to 700 Da with a resolving power of 120,000 Rs for MS1 and at 30,000 Rs for MS2, and a mass accuracy of ± 5 ppm. Data-dependent acquisition was carried out to acquire MS/MS of select features at a resolving power of 30000. The ion transfer tube was set at 250°C and vaporizer temp set at 30 °C. Interference from the tissue matrix prevented the use of the Fusion internal lock mass in this experimental method. Data acquisition and analysis was performed using Xcalibur and Compound Discoverer software (Thermo Fisher Scientific, Waltham, MA, USA).

Quality Assurance and Quality Control

Six-point, processed and matrix-matched calibration curves were prepared, one for each extraction method. Curve preparation entailed taking multiple aliquots of liver tissue (from the same batch of slurried tissue) through each extraction in its entirety. The matrix-matched approach is key to account for the influence or interference of biological co-elutents on PFAS response and derived concentrations^{50,51}.

The UPLC-MS/MS curve points were spiked directly before instrumental analysis with appropriate levels of native and mass-labelled standard, ranging from 0.25-100 ng/ml. The HRMS curve points were spiked before extraction, and therefore recovery-corrected all subsequent quantitation; these curve points

ranged from 0.05 -10 ng/400 μ l. The curves were used for quantification of samples prepared with the corresponding extraction method. Organic chicken liver, demonstrated to contain low concentrations of targeted PFAS, was used as the curve matrix. PFOS was consistently found in organic chicken liver samples, and thus the curve was corrected for background levels of PFOS by subtracting the average of measured background samples (n = 6) from PFOS responses measured in curve point samples. All calibration curves used for quantitation demonstrated an $R^2 \geq 0.98$, with most demonstrating an $R^2 \geq 0.99$.

During targeted analysis via UPLC-MS/MS, process blanks were prepared and analyzed with every 10 samples and found to be free of significant contamination; sample concentrations were not blank corrected as a result. Each sample was injected in duplicate, and duplicate injections monitored for stability. Mobile phase blanks (3:1 aqueous:organic) were analyzed between duplicate sample injections to monitor for analyte carryover or contamination.

Five samples consisting of chicken liver spiked with 2 ng of all native compounds were also prepared and used to calculate accuracy and precision metrics for UPLC-MS/MS. Accuracy ranged from 18-154% with a mean accuracy of 92%. Precision, calculated as percent relative standard deviation (% RSD), ranged from 7 – 150 %, with a mean % RSD of 28% (Table S13). Precision and accuracy were particularly variable for neutral sulfonamide acetic acids, those compounds lacking an identical mass-labelled surrogate like PFDS, and the C₁₃ and C₁₄ PFCAs.

Although the matrix-matched curves account for matrix effects in quantitation efforts, matrix effects were calculated for illustrative purposes following methods described by Chambers et al. 2008⁵¹. Matrix calculations indicated variable enhancement or suppression of each analyte, with an average ion suppression of -20% and an ionization enhancement of 31% (Table S14).

Equation 1:

$$\% \text{ Matrix Effects} = \left(\left(\frac{\text{Post-extraction spiked matrix}}{\text{Post-extraction spiked solvent}} \right) - 1 \right) \times 100$$

Method detection limits for UPLC-MS/MS ranged from 0.5 – 4.1 ng/ml based on spiked replicate samples multiplied by the Student's t-value appropriate for a single-tailed 99th percentile (Table S15). Method recovery ranged from 14 - 112%, with a mean recovery of 61% across all compounds (Table S6).

$$\text{Equation 2: } \% \text{ Recovery} = \frac{\text{Pre-extraction spiked sample}}{\text{Post-extraction spiked sample}} \times 100$$

During HRMS analysis, duplicate process blanks using formic acid and acetonitrile were prepared daily with each sample set, for a total of 8 process blanks. Process blanks were used to identify contamination introduced via sample preparation and instrument background signal. Mobile phase blanks were injected between different types of samples to monitor instrumental background noise and any carryover between samples. No contamination of emerging PFAS was apparent in process blanks; HFPO-DA and several other legacy PFCAs displayed

high levels of instrumental background noise as displayed in instrumental blanks and process blanks. No significant background noise was apparent for Nafion BP2, PFO5DoDA, or PFO4DA, the three PFEAs of interest reported here using HRMS analysis (Table S12). Method recoveries for HRMS were not tracked; each curve point sample was spiked with appropriate levels of native and internal standard and then taken through the extraction to create a recovery-corrected curve for quantification. Samples were analyzed in four batches, and curve stability monitored between runs. Curve responses at all levels varied less than 4% across all four runs for the three PFEAs reported using HRMS measurements in this analysis.

Quantification of emerging compounds via HRMS was limited to those samples above the linear range of the calibration curve; the lower detection limit was determined by comparison to blank values plus three times the standard deviation of blank responses (Table S12). Reporting of emerging compounds below the curve range or those without authentic standards is limited to raw abundances in comparison to process and instrumental blank values (Table S16).

PFOS concentrations measured via both HRMS and UPLC-MS/MS were compared for parity, and were generally found to be within 30% of UPLC-MS/MS results and all were within an order of magnitude. UPLC-MS/MS results were considered more precise due to lower levels of instrumental background noise along with higher levels of QA/QC and were therefore used for comparison and statistical analyses for all compounds excepting Nafion BP2 and PFO5DoDA.

Stable Isotope Analysis Sample Preparation and Analysis

Liver and muscle of seabird chicks were lyophilized, and ground to fineness using a mortar and pestle. 2-3 mg of tissue was weighed out into tin capsules for carbon and nitrogen stable isotope analysis, while 3-5 mg of muscle tissue were weighed out for sulfur isotope analysis.

Weighed samples were measured via IR-MS analysis, and results interpreted as parts per thousand relative to appropriate references. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were measured using an Isoprime 100 Isotope Ratio Mass Spectrometer coupled to a Micro Vario Elemental Analyzer (Elementar Americas, Mt.Laurel, NJ). $\delta^{34}\text{S}$ was measured by UC Davis Stable Isotope Laboratory using an Elementar Vario ISOTOPE cube interfaced to a SerCon 20-22 IRMS (Sercon Ltd., Cheshire, UK).

The nitrogen ($\delta^{15}\text{N}$) isotope composition was expressed as a part per thousand deviation (‰) from air. Carbon ($\delta^{13}\text{C}$) isotope composition was expressed relative to Vienna Pee Dee Belemnite where $\delta X = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 10^3$, where X is $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$, and R is the ratio of heavy to light isotope ($^{15}\text{N}: ^{14}\text{N}$, $^{13}\text{C}: ^{12}\text{C}$). Duplicates were analyzed every 10 samples, and a blue mussel reference material every 15 samples to ensure measurement quality.

Phospholipid analysis

50 mg of liver was homogenized at 4°C in 1 ml phosphate-buffered saline using a Beadruptor Elite bead mill homogenizer from Omni International. An aliquot of the homogenate was transferred to a 15ml polypropylene tube, and 3.75 ml of chloroform:methanol (2:1, v/v) was added. The solution was vortexed, and 0.5 ml deionized water added, followed by another 15 sec of vortexing. The

extract was then centrifuged at 3000 rpm for 5 min at room temperature, and the organic layer decanted into a pre-weighed glass vial. The organic extract was allowed to evaporate in a fume hood overnight, and the remaining lipid residue was then weighed to ascertain total lipid content. The residue was then re-suspended in 200 μ l of 1% Triton X-100 in 100% ethanol and shaken well for at least two hours. 20 μ l of the extract was then pipetted into a 96-well plate, inoculated with phospholipid kit working reagent, and assessed colorimetrically using a Spectramax M2 Multi-Mode microplate reader for phospholipid content via comparison to a 4-point curve containing 0-200 μ M phosphatidylcholine⁵².

More detail about stable isotope values and relationships

There is limited fine-scale comparability between stable isotope measurements between habitats included in this study, due to the species and food web specific nature of isotope fractionation factors, alongside inevitable and stark differences in isotopic composition at the base of each food web. As a result, here we focus on associations between PFAS concentrations and stable isotope measurements *within* each habitat, rather than considering isotope ratios across the sample set as a whole. Likewise, we apply previously described trophic level calculations to measurements within each habitat for illustrative purposes, noting that bulk trophic level calculations uncoupled from food-web specific data lack fine-scale insight.

Summary statistics associated with stable isotope analysis (SIA) of each tissue are presented in Table S20; a three-dimensional presentation of all SIA data can be found here [<http://rpubs.com/Arobuck/555350>].

Herring Gulls from Narragansett Bay evidenced the widest range of $\delta^{15}\text{N}$, while Shearwaters from Massachusetts Bay displayed the least variability in $\delta^{15}\text{N}$ (Table S21). Derived trophic level estimates reflected the same patterns of variability, with all birds evidencing an estimated trophic level between 3.24 – 4.59 based on liver $\delta^{15}\text{N}$ (Table S20, S21). Trophic level estimates assumed a calanoid copepod primary consumer, a realistic assumption for all habitats. $\delta^{15}\text{N}$ values and calculated trophic level were not significantly associated with concentrations of most individual PFAS or $\sum_{19}\text{PFAS}$ in each habitat. PFOS and $\sum_{19}\text{PFAS}$ were positively associated with $\delta^{15}\text{N}$ only in Massachusetts Bay individuals (Table S23). The lack of more significant associations with $\delta^{15}\text{N}$ may be related to similar trophic strategies and prey items utilized by birds included in this study, resulting in a limited range of $\delta^{15}\text{N}$ (Tables S21, S23). The lack of association likely also relates to the unique partitioning and accumulation pathways governing PFAS distribution in biota compared to legacy POPs^{11,53}.

Muscle $\delta^{34}\text{S}$ was also compared to concentrations of individual PFAS and $\sum\text{PFAS}$. Muscle $\delta^{34}\text{S}$ was only associated with PFUnDA in Narra. Bay individuals; no other statistically significant or observationally notable associations were present between $\delta^{34}\text{S}$ and PFAS concentrations. This association may be driven by variable coastal vs inland foraging strategies in herring gulls from Narra. Bay, coupled to the significance of PFUnDA as a PFAS common in marine food webs.

$\delta^{13}\text{C}$ and PFAS concentrations were most notably associated in birds from the CFRE. The association between $\delta^{13}\text{C}$ and PFAS levels in CFRE chicks is

likely a function of the marine and estuarine foraging habits of species included in this study, coupled to the local geomorphology of the CFRE system. Seabird parents foraging in the CFRE or in the adjacent coastal plume likely obtained resources comparatively enriched in $\delta^{13}\text{C}$ due to the abundance of *Sporobolus alterniflora* marshes in the CFRE lower estuary. These marshes make up a substantial portion of the undeveloped land area of the lower estuary and serve as habitat and nursery area for ecologically and commercially important fish and invertebrates^{54,55}. *Sporobolus alterniflora* typically reflects an enriched $\delta^{13}\text{C}$ value of -13.6‰⁵⁶, compared to more depleted $\delta^{13}\text{C}$ ratios observed in offshore food webs based on particulate organic matter and phytoplankton (-20‰ to -26‰^{57,58}) (Fig. S6). *Sporobolus alterniflora* marshes inhabit geomorphically protected inshore lagoon and barrier island environments in the CFRE, and were physically closer to upstream PFAS sources. Therefore, they were subject to less open-water dilution, likely increasing PFAS exposure potential for prey and predators reliant on these marsh systems. Surface water studies support this hypothesis, with predictable inverse relationships between salinity and surface water ΣPFAS apparent in estuarine⁵⁹, coastal shelf, and slope⁶⁰ environments. This suggests an increased potential for PFAS exposure in terrestrially-influenced environments like estuaries or brackish marshes. CFRE seabird parents also likely had an incentive to forage as close as possible to estuarine nesting colonies to conserve energy, further encouraging reliance on marsh and estuary resources. Seabird parents utilizing more offshore, marine systems for chick provisioning were likely foraging in environments with increased dilution and mixing,

obtaining larger prey with decreased PFAS levels and a more depleted $\delta^{13}\text{C}$ signature.

Bioaccumulation factors

Liver-water bioaccumulation factors (BAFs) were calculated by dividing liver geometric mean PFAS levels by measured or estimated surface water concentrations adjacent to nesting or collection locations, followed by log transformation. Water values for Massachusetts Bay and Narragansett Bay were derived from unpublished data from the Lohmann lab, while surface water estimates for the CFRE were estimated using data inputs from Zhang et al. 2019, assuming a conservation of mass dilution approach and estimating salinity near the collection site at M18 using salinity data from the Lower Cape Fear River Program (Tables S18-S19)^{61,62}.

BAFs reported here range from 0.5 – 3.7, with PFOS, PFNA, PFDA, PFO5DoDA, and Nafion BP2 displaying BAFs near or above 2 variably across the three habitats sampled in this study (Fig. S9). BAFs above 3.3 are considered “bioaccumulative” under regulatory protocol designed for persistent organic pollutants⁶³.

Miscellaneous Supporting Figures

Figure S2.2. Concentrations of PFUnDA by habitat. Habitat mean concentrations of PFUnDA presented as boxplots, with the dark line representing the median, box limits representing the first and third quartiles, whiskers denoting 1.5 times the interquartile range, and crosses denoting outliers. The points reflect measured observations contributing to the summary statistics presented by the boxplot. The asterisks indicate a statistically significant difference between habitat mean PFUnDA compared via the Wilcoxon rank sum test, using the CFRE group as the reference group. PFUnDA concentrations were displayed separately, as concentrations of this compound were significantly different between habitats and this was the second most abundant compound in many individuals. No toxicity reference thresholds exist for this compound in bird to date.

Figure S2.3. Observed concentrations of PFOS versus concentrations of PFNA, PFDA, and PFUnDA in A) all individuals across three habitats, and B) in only individuals from Narragansett Bay and Massachusetts Bay with two outlier individuals removed. All concentrations were measured via UPLC-MS/MS.

Figure S2.4. Concentrations of C8 – C14 PFCAs as a function of PFDA measured via LC-MS/MS in A) all individuals across three habitats, and B) in only individuals from Narragansett Bay and Massachusetts Bay. Associations between PFCAs as approximated by linear regression slightly decrease without CFRE data included, likely due to the truncation of the data range as a result of more similar concentrations observed in habitats removed from point sources and inclusion of MDL/2 values.

Figure S2.5. Observed concentrations of emerging PFEAs versus legacy PFAS in CFRE chicks with A) displaying concentrations of PFDA vs PFEAs and other long-chain PFCAs, and B) displaying concentrations of PFOS vs PFEAs. PFCA and PFSA concentrations were measured via UPLC-MS/MS while PFEAs were measured via HRMS.

Figure S2.6. $\delta^{15}\text{N}$ vs concentrations of PFAS. Measured $\delta^{15}\text{N}$ in seabird liver and muscle did not relate to concentrations of individual PFAS, or $\sum \text{PFAS}$ in seabird liver across any habitat. No correlation was found when the total sample set ($n = 31$) was assessed in the same way. Muscle $\delta^{15}\text{N}$ ratios are presented here; facet plots include only those compounds found in >40% of the sample set.

Figure S2.7. $\delta^{13}\text{C}$ vs concentrations of PFAS. Measured $\delta^{13}\text{C}$ in seabird liver and muscle did not relate to concentrations of individual PFAS, or $_{19}\Sigma\text{PFAS}$ in seabird liver from Massachusetts Bay or Narragansett Bay. No correlation was found when the total sample set ($n = 31$) was assessed in the same way. PFDA, PFNA, PFOS, PFUnDA, and $_{19}\Sigma\text{PFAS}$ were moderately associated ($p < 0.05$) with $\delta^{13}\text{C}$ ratios, only in CFRE chicks. See SI text for further discussion of this limited phenomenon. Muscle $\delta^{13}\text{C}$ ratios are presented here; facet plots include only those compounds found in $>40\%$ of the sample set.

Figure S2.8. $\delta^{13}\text{C}$ in key organic and inorganic matrices.

Figure S2.9. Log BAFs for 10 compounds across three habitats. BAFs were calculated by dividing mean liver concentrations observed in each habitat by observed or estimated surface water concentrations.

Supporting Tables

Compound	Family	# Fluorinated Carbons	Detected in biological samples above detection limits?	% Detection Mass. Bay	% Detection Nara Bay	% Detection CRE	% Detection All Habitats	Method of Instrumental Analysis	Reported concentrations derived from....	Quantified, using matched native standard or Semi-Quant?
NMFC5AA	FASAA	8	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
N-EPF5AA	FASAA	8	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
ROSA	FASA	8	Yes	0	0	0	27	10 UPLC-ASMS	UPLC-ASMS	Quantified, using matched native standard
42 FTS	FTS	4	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
62 FTS	FTS	6	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
82 FTS	FTS	8	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFBA	PFCA	3	Yes	100	80	73	84	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFPA	PFCA	4	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFHxA	PFCA	5	Yes	0	30	27	19	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFHxA	PFCA	6	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFDA	PFCA	7	Yes	0	0	0	64	23 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFNA	PFCA	8	Yes	100	100	100	100	100 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFDA	PFCA	9	Yes	90	100	100	100	97 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFUdA	PFCA	10	Yes	100	90	100	100	97 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFDoA	PFCA	11	Yes	0	10	27	13	13 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFTdA	PFCA	12	Yes	70	20	27	38	38 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFTeA	PFCA	13	Yes	0	10	0	3	3 UPLC-ASMS	UPLC-ASMS	Quantified, using matched native standard
PMPA	PFECA	3	No	0	0	0	0	0 HRMS	HRMS	Semi-quant
PFOPHA	PFECA	3	No	0	0	0	0	0 HRMS	HRMS	Semi-quant
PEPA	PFECA	4	No	0	0	0	0	0 HRMS	HRMS	Semi-quant
PFODCA	PFECA	4	No	0	0	0	0	0 HRMS	HRMS	Semi-quant
PFODCA	PFECA	5	Yes	0	0	9	3	3 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFODCA	PFECA	5	Yes	0	0	70	23	23 HRMS	HRMS	Semi-quant
PFODDCA	PFECA	6	Yes	0	0	100	38	38 HRMS	HRMS	Quantified, using matched native standard
Nalton BP1	PFECA	6	No	0	0	0	0	0 HRMS	HRMS	Semi-quant
Nalton BP2	PFECA	7	Yes	20	10	100	43	43 HRMS	HRMS	Quantified, using matched native standard
Nalton BP1	PFECA	7	No	0	0	0	0	0 HRMS	HRMS	Semi-quant
NHOS	PFECA	4	No	0	0	0	0	0 HRMS	HRMS	Semi-quant
PFBS	PFECA	4	Yes	0	10	0	3	3 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFPA6	PFECA	5	Yes	20	0	0	7	7 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFHxS	PFECA	6	Yes	10	10	55	28	28 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFHxS	PFECA	7	Yes	0	0	55	19	19 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFOS	PFECA	8	Yes	100	100	100	100	100 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFNS	PFECA	9	No	0	0	0	0	0 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFDS	PFECA	10	Yes	0	10	9	7	7 UPLC-ASMS; HRMS	UPLC-ASMS	Quantified, using matched native standard
PFCHS	Cyclic-PFECA	8	No	0	0	0	0	0 HRMS	HRMS	Semi-quant

Table S2.1. Table presenting all compounds assessed in this study, method of instrumental analysis applied, method used to report concentrations, quantification approach used based on availability of native standard, and summary of detections in liver extracts. Family designation based on Buck et al. 2011 and Strynar et al. 2015.

Common Name	Scientific Name	Family	Feeding guild after de Graaf et al. 1985 and Cornell Lab of Ornithology "Birds of the World"	Primary prey	Examples of Documented Prey Items	General habitat and breeding habits, including adult behavior prior to nesting that may influence maternal offloading	Likely pathways of PFAS exposure	Life-span***
Great Shearwater	<i>Ardeia gravis</i>	Procellariidae	Pelagic scavenger and opportunist; plunger	Habitat-specific forage fish, complex + invertebrates	DNA analysis indicates reliance on sand lance and menhaden. Mackerel, herring, Atlantic butterfish, and squid also identified prey items. (Powers et al. 2020)	Annually completes trans-equatorial migration to forage in Gulf of Maine over boreal summer. Juveniles preferentially forage in Massachusetts Bay and Georges Bank (Powers et al. 2017). Breeding adults leave the North Atlantic in September, and return to breeding colony in South Atlantic (St. Helena Island group). Adults stage in the area to replenish fat stores for about a month, then return to lay eggs in November. Once hatched (around February), parents engage in foraging trips of variable duration to provision chicks, using Southern Atlantic and Southern Ocean habitats to feed chicks (low PFAS exposure). Breeding adults and non-breeding individuals documented foraging in or near Antarctic Polar Front and Patagonian Shelf during the boreal winter.	<ul style="list-style-type: none"> • Atmospheric or water-borne PFAS or PFAS precursors transported to pelagic offshore environments. • Forage fish prey • Overall, low exposure potential due to preference for offshore pelagic environment • Most PFAS uptake due to use of North Atlantic; similar species that use Southern Ocean display lower PFAS levels, with different PFAS profiles • Southern hemisphere habitats display lower PFAS levels compared to North Atlantic counterparts. (Roscales et al. 2019) 	Likely >50 years based on data from similar Shearwater species
Herring gull	<i>Larus argentatus smithsonianus</i>	Laridae	Opportunistic generalist, coastal surface gleaner	Habitat-specific forage fish, complex + invertebrates	Observational studies at nesting sites and at sea suggest prey consists of fish, bivalves, and fishery discards. Natural fish prey can include menhaden, Atlantic silversides, mullet, and sand lance. Diet may vary based on human inputs and can include other birds, mammals, food of domestic animals, human refuse from beaches, landfills, or other available sources.	Ubiquitous across North America, present year-round in Southern New England and the greater Gulf of Maine. Herring gulls in Rhode Island are thought to be partial or non-migrants, migrating only when food is scarce. Breed in boreal summer along shorelines and on rooftops throughout North America, with adults provisioning chicks from coastal environments.	<ul style="list-style-type: none"> • Use of habitats/food associated with direct discharges of PFAS: Wastewater effluent, landfill leachate, ingestion of human refuse/food • Atmospheric or water-borne PFAS or PFAS precursors transported within coastal environments • Forage fish prey, bivalves • Overall, medium exposure potential due to proximity to human sources of PFAS, use of human resources for food 	Oldest observed: 29 years
Royal tern	<i>Thalasseus maximus maximus</i>	Laridae	Coastal plunger, opportunist	Habitat-specific forage fish, complex + invertebrates	Routine colony monitoring suggests NC colonies particularly rely on menhaden, mullet, and Atlantic silversides. Literature suggests insect and crustaceans also used as food by terns.	Distributed across North American Atlantic, Pacific, and Gulf Coasts. Breed in boreal summer on sandy and rocky shorelines. Breeding royal terns occupy the Atlantic coast north to Virginia, extending southerly to the coast of Texas. Royal terns winter from North Carolina south to Central America and the Caribbean. Highest PFAS exposure is breeding adults associated with staging and provisioning of chicks in CFRE systems, no known connectivity to other PFAS hotspots.	<ul style="list-style-type: none"> • Use of habitats/food resources downstream from industrial PFAS production site, demonstrated to contribute high levels of PFAS to environment 	Oldest observed: 30 years
Sandwich tern	<i>Thalasseus sandvicensis</i>	Laridae	Coastal plunger, opportunist	Habitat-specific forage fish, complex + invertebrates	Routine colony monitoring suggests NC colonies particularly rely on menhaden, mullet, and Atlantic silversides. Literature suggests insect and crustaceans also used as food by terns.	Globally distributed—Western hemisphere populations found across southeast US and Caribbean during boreal summer breeding season. Winter across Central America, the Caribbean, and northern South America. Highest PFAS exposure to breeding adults associated with staging and provisioning of chicks in CFRE systems, no known connectivity to other PFAS hotspots.	<ul style="list-style-type: none"> • Use of habitats/food associated with other direct discharges of PFAS: Wastewater effluent, landfill leachate, legacy PFAS sources from military sites using AFFF, textile production • Atmospheric or water-borne PFAS or PFAS precursors transported within coastal environments • Forage fish prey, bivalves • Overall, high exposure potential due to proximity to human sources of PFAS, proximity to industrial discharge of PFAS 	Oldest observed: 27 years
Laughing gull	<i>Leucophaea atricilla</i>	Laridae	Coastal plunger, opportunist	Habitat-specific forage fish, complex + invertebrates		Found across the Atlantic coast of North America, Caribbean, and northern South America. Breed in boreal summer along marine and estuarine sandy and rocky shorelines. Northern non-breeding populations migrate further south during the boreal winter. Highest PFAS exposure to breeding adults associated with staging and provisioning of chicks in CFRE systems, no known connectivity to other PFAS hotspots.		Oldest observed: 22 years
Brown pelican	<i>Pelecanus occidentalis</i>	Pelecanidae	Coastal plunger, opportunist	Habitat-specific forage fish, complex + invertebrates		Distributed across North American Atlantic, Pacific, and Gulf Coasts. Breed in boreal summer along marine and estuarine sandy shorelines. Populations are generally nomadic; they can be dispersive. Highest PFAS exposure to breeding adults associated with staging and provisioning of chicks in CFRE systems, no known connectivity to other PFAS hotspots.		Oldest observed: 27 years

Table S2.2. Table containing relevant life history details of seabird species included in this study. Diet and life history sources include species profiles on the Cornell Lab of Ornithology All About Birds data portal -- <https://www.allaboutbirds.org/guide/> -- and all references therein -- were referenced for diet information, in addition to personal communications from colony monitoring technicians. Age examples are from <https://www.pwrc.usgs.gov/BBL/longevity/longvrec.cfm>.

Sample Name	Habitat	Species	Tarsus (cm)	Wing chord (cm)	Culmen (cm)	Head-bill length (cm)	Total Length (cm)	Wet Weight (g)	Heart weight (g)	Liver weight (g)	Kidney weight (g)	Sex (M, F, CBD)	Estimated age
CF01	CRE	ROTE	3.49	12.6	3.44	8.06	28.8	321	2	14	NE	CBD ~ 2 - 4 weeks	
CF02	CRE	LAGU	4.33	10.1	2.29	6.5	23.9	124	0.9	5	NE	CBD ~ 2 - 4 weeks	
CF03	CRE	BRPE	8.66	23.8	15.4	24.2	82.5	1455	13	56.9	NE	CBD Hatch year, pre-fledge, less than 6 - 8 weeks	
CF04	CRE	BRPE	8.11	37	21.2	28.6	95.9	3205	22	125	26	F Hatch year, pre-fledge, less than 6 - 8 weeks	
CF05	CRE	SATE	2.77	17.4	2.98	7.07	31.8	168	2	7.1	NE	CBD Hatch year, pre-fledge, less than 6 - 8 weeks	
CF06	CRE	ROTE	3.26	10.1	3.29	8.21	24.4	225	2	13	3	F Hatch year, pre-fledge, less than 6 - 8 weeks	
CF07	CRE	ROTE	3.62	16	3.81	8.59	33.2	375	3	19	5	M Hatch year, pre-fledge, less than 6 - 8 weeks	
CF08	CRE	ROTE	3.52	34	1.76	10.46	42.2	203	1	6.8	CBD	CBD Hatch year, pre-fledge, less than 6 - 8 weeks	
CF10	CRE	ROTE	3.53	25	4.15	9.96	37.9	326	3	11.9	4	CBD Hatch year, pre-fledge, less than 6 - 8 weeks	
CF11	CRE	SATE	2.45	18	3.01	7.58	28.6	169	1	8	3	CBD Hatch year, pre-fledge, less than 6 - 8 weeks	
CF12	CRE	ROTE	2.5	3.49	2.2	5.78	17.5	89	1	4	2	CBD Hatch year, pre-fledge, less than 6 - 8 weeks	
GRSH P024	Mass Bay	GRSH	7.3	34.1	4.3	9.9	52.8	786.69	6.9	26.7	5.8	CBD Hatch year, ~5-6 months old	
GRSH P026	Mass Bay	GRSH	7.3	34.1	4.4	10.4	NE	800.1	9	26.7	5.7	F Hatch year, ~5-6 months old	
GRSH Q002	Mass Bay	GRSH	6.07	35.7	4.38	10.32	52.9	832	7	24	8	M Hatch year, ~5-6 months old	
GRSH Q011	Mass Bay	GRSH	6.01	30.2	4.45	10.36	51.9	1272	8	26	9	M Hatch year, ~5-6 months old	
GRSH Q013	Mass Bay	GRSH	5.8	34.6	4.5	10.4	51.8	737.2	6.9	26	4.5	F Hatch year, ~5-6 months old	
GRSH Q014	Mass Bay	GRSH	6.2	33.5	4.37	10.42	52.8	857	7	22	8	F Hatch year, ~5-6 months old	
GRSH Q026	Mass Bay	GRSH	5.99	33.6	4.6	10.65	51.9	863.2	6.8	30	5.6	M Hatch year, ~5-6 months old	
GRSH Q028	Mass Bay	GRSH	7.3	32.9	4.8	10.6	NE	830.9	10	28.8	9.4	M Hatch year, ~5-6 months old	
GRSH Q031	Mass Bay	GRSH	6.3	NE	4.85	10.79	52.4	1018.4	8.9	23	5.8	M Hatch year, ~5-6 months old	
GRSH Q033	Mass Bay	GRSH	6.5	NE	4.5	10.7	NE	800.3	6.7	24.3	7.4	F Hatch year, ~5-6 months old	
HG025	Narra Bay	HG	NE	NE	NE	NE	NE	NE	NE	NE	NE	CBD Hatch year, pre-fledge, likely 5 - 8 weeks	
HG034	Narra Bay	HG	NE	NE	NE	NE	NE	NE	NE	NE	NE	CBD Hatch year, pre-fledge, likely 5 - 8 weeks	
HG056	Narra Bay	HG	7.1	37	5.6	12.7	NE	800	7	28	6	M Hatch year, pre-fledge, likely 5 - 8 weeks	
HG057	Narra Bay	HG	6.9	36.9	5.2	12.3	NE	776	7	26	7	F Hatch year, pre-fledge, likely 5 - 8 weeks	
HG058	Narra Bay	HG	6.9	36.1	4.7	11.9	NE	826	6	16	5	CBD Hatch year, pre-fledge, likely 5 - 8 weeks	
HG059	Narra Bay	HG	6.3	39.4	5	11.6	NE	767	7	23	6	F Hatch year, pre-fledge, likely 5 - 8 weeks	
HG060	Narra Bay	HG	6.6	40	4.5	11.9	NE	770	6	26	7	F Hatch year, pre-fledge, likely 5 - 8 weeks	
HG061	Narra Bay	HG	7.4	37.5	5.32	12.39	59.9	1020	5	25	6	M Hatch year, pre-fledge, likely 5 - 8 weeks	
HG062	Narra Bay	HG	7.26	45	5.42	12.23	51.9	863	8	29	9	M Hatch year, pre-fledge, likely 5 - 8 weeks	
HG063	Narra Bay	HG	6.39	41.6	4.97	11.88	58.4	687	10	NE	NE	F Hatch year, pre-fledge, likely 5 - 8 weeks	

Table S2.3. Table containing relevant morphometric and collection details describing each individual included in this study. "NE" designates "Not Examined", "CBD" designates "Couldn't Be Determined"

Native Compounds	Abbreviation	CAS #	Stock Concentration	Stock Origin
Perfluoro-n-butanoic acid	PFBA	375-22-4	2000	Wellington
Perfluoro-n-pentanoic acid	PFPeA	2706-90-3	2000	Wellington
Potassium perfluoro-1-butanedisulfonate	PFBS	29420-49-3	1770	Wellington
Perfluoro-n-hexanoic acid	PFHxA	307-24-4	2000	Wellington
Sodium 1H, 1H, 2H, 2H-perfluoro-1-hexanesulfonate	4:2-FTS	N/A	1870	Wellington
Sodium perfluoro-1-pentanesulfonate	PFPeS	N/A	1880	Wellington
Perfluoro-n-heptanoic acid	PFHpA	375-85-9	2000	Wellington
Potassium perfluorohexanesulfonate	PFHxS	432-50-7	1824	Wellington
Perfluoro-n-octanoic acid	PFOA	335-67-1	2000	Wellington
Sodium 1H, 1H, 2H, 2H-perfluoro-1-octanesulfonate	6:2-FTS	N/A	1900	Wellington
Sodium perfluoro-1-heptanesulfonate	PFHpS	N/A	1900	Wellington
Perfluoro-n-nonanoic acid	PFNA	375-95-1	2000	Wellington
Perfluoro-1-octanesulfonamide	FOSA	754-91-6	2000	Wellington
Potassium perfluorooctanesulfonate	PFOS	402-147-0	1851	Wellington
Perfluoro-n-decanoic acid	PFDA	335-76-2	2000	Wellington
Sodium 1H, 1H, 2H, 2H-perfluoro-1-decanesulfonate	8:2-FTS	N/A	1920	Wellington
Sodium perfluoro-1-nonanesulfonate	PFNS	98789-57-2	1920	Wellington
Perfluoro-n-undecanoic acid	PFUnDA	2058-94-8	2000	Wellington
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	2000	Wellington
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	2991-50-6	2000	Wellington
Sodium perfluoro-1-decanesulfonate	PFDS	2806-15-7	1930	Wellington
Perfluoro-n-dodecanoic acid	PFDoA	307-55-1	2000	Wellington
Perfluoro-n-tridecanoic acid	PFTrDA	72629-94-8	2000	Wellington
Perfluoro-n-tetradecanoic acid	PFTeDA	376-06-7	2000	Wellington
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	HFPO-DA	13252-13-6	5000	Wellington

Table S2.4. Table presenting details about all native analytes measured in this study via LC-MS/MS.

Native Compound	Abbreviation	Chemical Formula	CAS #	Stock Concentration (ng/mL)	Assigned surrogate
Perfluoro-2-ethoxypropanoic acid	PEPA	C ₅ H ₉ F ₉ O ₃	267239-61-2	na	MPFBA-13C ₄
Perfluoro-2-methoxypropanoic acid	PFMA	C ₄ H ₇ F ₇ O ₃	13140-29-9	na	MPFBA-13C ₄
Perfluoro-3,5-dioxahexanoic acid	PFO2HxA	C ₄ H ₇ F ₇ O ₄	39492-88-1	na	MPFBA-13C ₄
Perfluoro(3,5,7-trioxaoctanoic) acid	PFO3OA	C ₅ H ₉ F ₉ O ₅	39492-89-2	na	M8PFOA-13C ₈
Perfluoro-3,5,7,9-tetraoxadecanoic acid	PFO4DA	C ₆ H ₁₁ F ₁₁ O ₆	39492-90-5	100000	M8PFOA-13C ₈
Perfluoro(3,5,7,9,11-pentaoxadodecanoic) acid	PFO5DoDA	C ₇ H ₁₃ F ₁₃ O ₇	39492-91-6	100000	M8PFOA-13C ₈
2-[1-[difluoro[(1,2,2-trifluoroethyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoroethanesulfonic acid,	Nafion BP1	C ₇ H ₇ F ₁₃ O ₅ S	29311-67-9	na	M8PFOS-13C ₈
2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoroethanesulfonic acid	Nafion BP2	C ₇ H ₇ F ₁₄ O ₅ S	749836-20-2	100000	M8PFOS-13C ₈
2,2,3,3,4,5,5,5,5,5,5,5-(1,1,2,2-tetrafluoro-2-sulfoethoxy)pentanoic acid	Nafion BP4	C ₇ H ₇ F ₁₂ O ₆ S	na	na	M8PFOS-13C ₈
1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoro-ethoxy)ethane sulfonate	NVHOS	C ₄ H ₂ F ₈ O ₄ S	801209-99-4 (free acid)	na	M8PFOS-13C ₈
1,2,2,3,3,4,5,5,5,6,6,6-decafluoro-4-(1,1,2,2,2-pentafluoroethyl)cyclohexanesulfonic acid,	PFECHS	C ₈ H ₇ F ₁₅ O ₃ S	646-83-3	na	na

Table S2.5. Table presenting details about all native analytes measured in this study via LC-HRMS.

Method % Recovery	
Compound	% Recovery
4:2 FTS_IS	55.5
6:2 FTS_IS	64.8
8:2-FTS_IS	46.9
EtFOSAA-IS	52.1
FOSA_IS	13.8
HFPODA_IS	78.7
MeFOSAA_IS	48.5
PFBA_IS	112.4
PFBS_IS	72.4
PFDA_IS	56.4
PFDoA_IS	31.4
PFHpA_IS	73.7
PFHxA_IS	86.0
PFHxS_IS	69.1
PFNA_IS	81.1
PFOA_IS	68.0
PFOS_IS	59.3
PFPeA_IS	72.6
PFTeDA_IS	34.5
PFUnDA_IS	46.1
Arithmetic Mean % Recovery	61.2
Arithmetic Mean % Recovery - FOSA (outlier)	63.7
Arithmetic Mean % Recovery of PFCAs	66.2
Arithmetic Mean % Recovery of PFSA	66.9

Table S2.6. LC-MS/MS Method recovery as a percentage, following: % Recovery=((Pre-extraction spiked sample)/(Post-extraction spiked sample)) × 100

Native PFASs	Predicted RT (min)	Quantification Transition (Da)	Dwell (s)	Cone (V)	Collision (eV)	Quantification Transition (Da)	Dwell (s)	Cone (V)	Collision (eV)	Quantification Reference
PFBA	1.1 213 > 169		0.017	20	11		—	—	—	M8FBA-13C4
PFPeA	2.6 263 > 219		0.007	20	10	—	—	—	—	M8PFPeA-13C5
PFBS	3.6 299 > 80		0.006	40	55 299 > 99		0.006	40	33	M3PFBS-13C3
PFHxA	4.3 313 > 269		0.005	20	10 313 > 119		0.005	20	22	M8PFHxA-13C5
4:2-FTS	4.2 327 > 307		0.005	35	20 327 > 81		0.005	35	20	M2-4:2-FTS-13C2
PFPeS	4.5 349 > 80		0.005	40	50 349 > 99		0.005	40	50	M3PFPeS-13C3
PFHpA	4.9 363 > 319		0.005	20	11 363 > 169		0.005	20	22	M4PFHpA-13C4
PFHxS	5 399 > 80		0.005	40	50 399 > 99		0.005	40	40	M8PFHxS-13C3
PFOA	5.5 413 > 369		0.005	20	11 413 > 169		0.005	20	22	M8PFOA-13C8
6:2-FTS	5.2 427 > 407		0.005	35	25 427 > 81		0.005	35	25	M2-6:2-FTS-13C2
PFHpS	5.5 449 > 80		0.005	40	50 449 > 99		0.005	40	50	M8PFHpS-13C8
PFNA	5.8 463 > 419		0.005	20	11 463 > 419		0.005	20	22	M9PFNA-13C9
FOSA	6.5 498 > 78		0.005	45	40 498 > 119		0.005	45	57	M8FOSA
PFOS	5.4 499 > 80		0.005	40	60 499 > 99		0.005	40	50	M8PFOS-13C8
PFDA	6.1 513 > 469		0.005	20	11 513 > 219		0.005	20	22	M8PFDA-13C6
8:2-FTS	5.9 527 > 507		0.005	40	30 527 > 81		0.005	40	30	M2-8:2-FTS-13C2
PFNS	6.1 549 > 80		0.005	40	60 549 > 99		0.005	40	50	M8PFNS-13C8
PFUnDA	6.4 563 > 519		0.005	20	11 563 > 269		0.005	20	22	M7PFUnDA-13C7
N-MeFOSAA	6.1 570 > 419		0.005	28	28 570 > 512		0.005	28	28	MeFOSAA-d3
N-EiFOSAA	6.2 584 > 419		0.005	31	30 584 > 526		0.005	31	30	EiFOSAA-d5
PFDS	6.4 599 > 80		0.005	40	60 599 > 99		0.005	40	50	M8PFOS-13C8
PFDoA	6.6 613 > 569		0.005	20	11 613 > 169		0.005	20	33	M2PFDoA-13C2
PFTeDA	6.8 663 > 619		0.005	20	11 663 > 169		0.005	20	33	M2PFTeDA-13C2
PFTeDA	6.9 713 > 669		0.005	20	11 713 > 169		0.005	20	33	M2PFTeDA-13C2
HFPO-DA	4.3 329 > 285		0.005		391 > 329		0.005			HFPODA-13C3
					285 > 169		0.005			

Table S2.7. Table of targeted native analytes, associated instrument parameters, and quantification parameters targeted with LC-MS/MS analysis.

Labeled Compounds	Abbreviation	Stock Concentration (ng/mL)
Perfluoro-n- ¹³ C ₄ butanoic acid	MPFBA- ¹³ C ₄	1000
Perfluoro-n- ¹³ C ₅ pentanoic acid	M5PFPeA- ¹³ C ₅	1000
Sodium perfluoro-1-[2,3,4- ¹³ C ₃]butanesulfonate	M3PFBS- ¹³ C ₃	929
Perfluoro-n-(1,2,3,4,6- ¹³ C ₅)hexanoic acid	M5PFHxA- ¹³ C ₅	1000
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- ¹³ C ₂]hexanesulfonate	M2-4:2-FTS- ¹³ C ₂	935
Perfluoro-n-(1,2,3,4- ¹³ C ₄)heptanoic acid	M4PFHpA- ¹³ C ₄	1000
Sodium perfluoro-1-[1,2,3- ¹³ C ₃]hexanesulfonate	M3PFHxS- ¹³ C ₃	946
Perfluoro-n-(13C ₈)octanoic acid	M8PFOA-13C ₈	1000
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- ¹³ C ₂]octanesulfonate	M2-6:2-FTS-13C ₂	949
Perfluoro-n-(13C ₉)nonanoic acid	M9PFNA-13C ₉	1000
Perfluoro-1-(13C ₈)octanesulfonamide	M8FOSA	1000
Sodium perfluoro-1-(13C ₈)octanesulfonate	M8PFOS-13C ₈	957
Perfluoro-n-(1,2,3,4,5,6- ¹³ C ₆)decanoic acid	M6PFDA-13C ₆	1000
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- ¹³ C ₂]decanesulfonate	M2-8:2-FTS-13C ₂	958
Perfluoro-n-(1,2,3,4,5,6,7- ¹³ C ₇)undecanoic acid	M7PFUnDA-13C ₇	1000
N-methyl-d ₃ -perfluoro-1-octanesulfonamid oacetic acid	MeFOSAA-d ₃	1000
N-ethyl-d ₅ -perfluoro-1-octanesulfonamid oacetic acid	EiFOSAA-d ₅	1000
Perfluoro-n-[1,2- ¹³ C ₂]dodecanoic acid	M2PFDoA-13C ₂	1000
Perfluoro-n-[1,2- ¹³ C ₂]tetradecanoic acid	M2PFTeDA-13C ₂	1000
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	HFPODA-13C ₃	50000

Table S2.8. Table of target mass-labeled compounds, associated abbreviations, and concentrations of purchased stock solutions used in both targeted and HRMS analysis.

Internal Standards	Predicted RT (min)	Quantification Transition (Da)	Dwell (s)	Cone (V)	Collision (eV)	Quantification Transition (Da)	Dwell (s)	Cone (V)	Collision (eV)
MPFBA-13C4	1.2	217 > 172	0.017	20	11	—	—	—	—
M5PFPeA-13C5	2.6	268 > 223	0.007	20	11	—	—	—	—
M3PFBS-13C3	3.6	302 > 80	0.006	40	55	302 > 99	0.006	40	33
M5PFHx-13C5	4.3	318 > 273	0.005	20	10	318 > 119	0.005	20	22
M2-4-2-FTS-13C2	4.2	329 > 309	0.005	35	20	329 > 81	0.005	35	20
M4PFHpA-13C4	4.9	367 > 322	0.005	20	11	367 > 171	0.005	20	22
M3PFHxS-13C3	5	402 > 80	0.005	40	50	402 > 99	0.005	40	40
M8PFOA-13C8	5.5	421 > 376	0.005	20	11	421 > 171	0.005	20	22
						421 > 223	0.005	20	22
M2-6-2-FTS-13C2	5.2	429 > 409	0.005	35	25	429 > 81	0.005	35	25
M9PFNA-13C9	5.8	472 > 427	0.005	20	11	472 > 423	0.005	20	22
						472 > 171	0.005	20	22
M8FOSA	6.5	506 > 78	0.005	45	40	506 > 172	0.005	45	57
M8PFOS-13C8	5.4	507 > 80	0.005	40	60	507 > 99	0.005	40	50
M6PFDA-13C6	6.1	519 > 474	0.005	20	11	519 > 219	0.005	20	22
						519 > 169	0.005	20	22
M2-8-2-FTS-13C2	5.9	529 > 509	0.005	40	30	529 > 81	0.005	40	30
M7PFUnDA-13C7	6.4	570 > 525	0.005	20	11	570 > 269	0.005	20	22
						570 > 219	0.005	20	33
MeFOSAA-d3	6.1	573 > 419	0.005	28	28	573 > 515	0.005	28	28
						573 > 483	0.005	28	28
EtFOSAA-d5	6.2	589 > 419	0.005	31	30	589 > 531	0.005	31	30
						589 > 483	0.005	31	30
M2PFDdA-13C2	6.6	615 > 570	0.005	20	11	615 > 169	0.005	20	33
						615 > 269	0.005	20	33
M2PFTeDA-13C2	6.9	715 > 670	0.005	20	11	715 > 169	0.005	20	33

Table S2.9. Table of target mass-labeled compounds, associated instrument parameters, and quantification parameters used for targeted LC-MS/MS analysis.

Time	Flow (ml/min)	% aqueous	% organic
0	0.4	75	25
0.5	0.4	75	25
5	0.4	15	85
5.1	0.4	5	95
5.6	0.4	5	95
7	0.4	5	95
9	0.4	75	25

Table S2.10. Table presenting mobile phase gradient ratios used during sample analysis via LC-MS/MS

Time	Flow (ml/min)	% aqueous	% organic
-3	0.3	80	20
0	0.3	80	20
0.5	0.3	80	20
3	0.3	50	50
3.5	0.3	50	50
5.5	0.3	40	60
6	0.3	40	60
7	0.3	0	100
11	0.3	0	100

Table S2.11. Table presenting mobile phase gradient ratios used during sample analysis via HRMS

Compound	Mean Instrumental Blank (n = 64)	Std. Dev. of Instrumental Blank	Mean Process Blank (n = 8)	Std. Dev. of Process Blank	MDL (in raw abundance)
HFPO-DA	6,558	7,848	50,584	12,527	88,164
Nafion BP2	708	127	712	37	822
PFO4DA	881	275	803	NA	1,707
PFO5DoDA	792	59	814	167	1,315

Table S2.12. Raw abundances of instrumental and process blanks observed in HRMS analysis. Estimated MDLs are calculated as the mean process blank area plus three times the standard deviation. The estimated MDL for PFO4DA was calculated using instrumental blank values due to limited detection in process blanks, precluding calculation of standard deviation using process blank values. HFPO-DA was analyzed via both HRMS and LC-MS/MS, but high instrumental and process blanks values associated with HRMS prevented quantification of HFPO-DA using this technique. Detections of HFPO-DA reported in this study are derived from LC-MS/MS measurements. Quantification limits for HRMS were constrained by the linear dynamic range of the curve.

Accuracy and Precision			
Compound	% Accuracy	SD % Accuracy	% RSD
4:2 FTS	118.0	9.2	7.8
6:2 FTS	114.3	16.0	14.0
8:2-FTS	111.6	28.5	25.6
EtFOSAA	101.3	26.4	26.1
FOSA	92.2	56.4	61.1
HFPODA	102.2	12.7	12.4
N-MeFOSAA	95.2	27.1	28.5
PFBA	154.0	10.8	7.0
PFBS	84.2	6.2	7.3
PFDA	101.8	16.2	15.9
PFDoA	89.1	28.8	32.3
PFDS	17.9	26.8	149.6
PFHpA	103.6	10.3	9.9
PFHpS	110.3	25.4	23.0
PFHxA	124.6	8.6	6.9
PFHxS	83.0	12.7	15.3
PFNA	93.1	6.4	6.9
PFNS	41.1	28.1	68.4
PFOA	99.3	7.7	7.8
PFOS	76.5	8.3	10.8
PFPeA	101.3	8.4	8.3
PFPeS	114.2	10.2	8.9
PFTeDA	67.4	20.3	30.1
PFTTrDA	19.6	16.7	85.5
PFUnDA	86.9	17.6	20.3

Table S2.13. Method accuracy and precision as relative standard deviation (RSD); both metrics were calculated using five spiked replicate samples consisting of organic chicken liver spiked with 2ng native stock and 10 ng of isotopically labeled surrogate stock.

% Matrix Effects compared to spiked solvent	
Compound	% Effect
4:2 FTS_IS	39.019
6:2 FTS_IS	68.15
8:2-FTS_IS	44.619
EtFOSAA-IS	-39.162
FOSA_IS	-37.001
HFPODA_IS	-5.387
MeFOSAA_IS	-20.812
PFBA_IS	-40.869
PFBS_IS	-5.92
PFDA_IS	-2.305
PFDaA_IS	-42.043
PFHpA_IS	-1.74
PFHxA_IS	-20.683
PFHxS_IS	-4.617
PFNA_IS	2.815
PFOA_IS	-3.067
PFOS_IS	1.688
PFPeA_IS	-10.996
PFTeDA_IS	-55.018
PFUnDA_IS	-16.539

Table S2.14. Table presenting the degree of ion enhancement or suppression associated with matrix interference calculated using: % Matrix Effects=((Post Extraction Spike in Chicken Matrix)/(Post Extraction Spike in Solvent)-1) × 100

Method Detection Limits, ng/ml	
Compound	MDL
4:2 FTS	0.548
6:2 FTS	1.24
8:2-FTS	2.379
EtFOSAA	4.139
FOSA	3.674
HFPODA	0.895
N-MeFOSAA	2.185
PFBA	0.69
PFBS	0.502
PFDA	0.983
PFDS	1.541
PFDoA	3.552
PFHpA	0.625
PFHpS	1.686
PFHxA	0.781
PFHxS	0.845
PFNA	0.459
PFNS	1.729
PFOA	0.539
PFOS	0.559
PFPeA	0.569
PFPeS	0.875
PFTeDA	2.197
PFUnDA	1.089
PFTTrDA	0.957

Table S2.15. Method reporting limits for LC-MS/MS analysis calculated using the standard deviation of spiked replicate samples multiplied by the Student's t-value appropriate for a single-tailed 99th percentile, according to: $[\text{MDL}]_{\text{s}} = t_{((n-1, 1-\alpha=0.99))} \times S_{\text{s}}$

Sample ID	Compound	Detect vs non-detect
CF10_G	Nafion BP2	Detect
CF01_G	Nafion BP2	Detect
CF02_G	Nafion BP2	Detect
CF03_G	Nafion BP2	Detect
CF04_G	Nafion BP2	Detect
CF05_G	Nafion BP2	Detect
CF06_G	Nafion BP2	Detect
CF07_G	Nafion BP2	Detect
CF08_G	Nafion BP2	Detect
CF09_G	Nafion BP2	Detect
CF11_G	Nafion BP2	Detect
CF12_G	Nafion BP2	Detect
GRSH_Q002_G	Nafion BP2	Detect
GRSH_Q026_G	Nafion BP2	Detect
HG_061_G	Nafion BP2	Detect
HG_062_G	Nafion BP2	Nondetect
P88026_G	Nafion BP2	Nondetect
CF010_G	PFO4DA	Nondetect
CF01_G	PFO4DA	Detect
CF05_G	PFO4DA	Detect
CF06_G	PFO4DA	Detect
CF07_G	PFO4DA	Detect
CF08_G	PFO4DA	Detect
CF11_G	PFO4DA	Detect
CF12_G	PFO4DA	Detect
CF010_G	PFO5DoDA	Detect
CF01_G	PFO5DoDA	Detect
CF02_G	PFO5DoDA	Detect
CF03_G	PFO5DoDA	Detect
CF04_G	PFO5DoDA	Detect
CF05_G	PFO5DoDA	Detect
CF06_G	PFO5DoDA	Detect
CF07_G	PFO5DoDA	Detect
CF08_G	PFO5DoDA	Detect
CF09_G	PFO5DoDA	Detect
CF11_G	PFO5DoDA	Detect
CF12_G	PFO5DoDA	Detect
HG_062_G	PFO5DoDA	Nondetect

Table S2.16. Detection of PFEAs, based on comparison to estimated method detection limit (mean process blank abundance + 3*std. dev.) or the lowest curve point.

Table S2.17. PFAS concentrations observed in this study. Responses not detected or outside the linear dynamic range of the curve were labeled as “nd” and assigned a value of zero. Observations below method detection limits but with clear signal, clear qualifier ions, and detected in at least 50% of samples in one habitat were replaced with the half the method detection limit for statistical analyses, and included as such in calculation of summed concentrations.

Compound	Habitat	Surface Water Conc. (ng/L)	Citation	Data notes	Mean liver concentration (ng/g ww)	Mean liver (ng/kg ww)	BAF = (Liver conc/water conc)	Log BAF
PFBA	Narra. Bay	0.42	Gardiner unpublished data	Average of surface water concentrations measured in September and October 2017 around Newport RI and URI Bay Campus, Narragansett, RI. HG chicks used in this study came from chicks collected from Newport and Narragansett RI close to these water sampling locations.	4.2	0.0042	9.94	1.00
PFDA	Narra. Bay	0.06	Gardiner unpublished data		2.2	0.0022	36.67	1.56
PFNA	Narra. Bay	0.18	Gardiner unpublished data		0.96	0.00096	5.37	0.73
PFOS	Narra. Bay	0.37	Gardiner unpublished data		24	0.024	65.75	1.82
PFUnDA	Narra. Bay	0.06	Gardiner unpublished data	Assumed chicks collected between M35-M18. Estimated estuarine dilution using conservation of mass, using volume estimates based on Ernsign et al. 2004. PFAS shown to linearly scale to salinity in other estuarine habitats (Munoz et al. 2017).	2.5	0.0025	45.45	1.66
PFTTrDA	Narra. Bay	0.04	Gardiner unpublished data		1.2	0.0012	34.29	1.54
PFHxA	Narra. Bay	0.34	Gardiner unpublished data		1.1	0.0011	3.28	0.52
PFTTeDA	Narra. Bay	0.25	Gardiner unpublished data		2.2	0.0022	8.93	0.95
PFBA	Mass Bay	0.22	Robuck unpublished data	1/2 LOD	10.6	0.0106	48.18	1.68
PFDA	Mass Bay	0.04	Robuck unpublished data		2.2	0.0022	55.00	1.74
PFNA	Mass Bay	0.10	Robuck unpublished data		1.4	0.0014	14.00	1.15
PFOS	Mass Bay	0.04	Robuck unpublished data		41	0.041	1025.00	3.01
PFTTrDA	Mass Bay	0.07	Robuck unpublished data	1/2 LOD	1.8	0.0018	27.69	1.44
PFUnDA	Mass Bay	0.06	Robuck unpublished data	1/2 LOD	5.8	0.0058	105.45	2.02
PFBA	CFRE	0.03	Calculations based on Lock and Dam 1 surface water concentrations measured in Zhang et al. 2019 with dilution down estuary calculated based on dilution in estuary at M18.	Assumed chicks collected between M35-M18. Estimated estuarine dilution using conservation of mass, using volume estimates based on Ernsign et al. 2004. PFAS shown to linearly scale to salinity in other estuarine habitats (Munoz et al. 2017).	1.5	0.0015	50.00	1.70
PFHxA	CFRE	0.03			2.7	0.0027	90.00	1.95
PFOA	CFRE	0.02			0.85	0.00085	36.96	1.57
PFNA	CFRE	0.01			6.6	0.0066	1100.00	3.04
PFDA	CFRE	0.01			21.5	0.0215	3583.33	3.55
PFHxS	CFRE	0.02			1.1	0.0011	50.00	1.70
PFOS	CFRE	0.03			150.1	0.1501	5003.33	3.70
Nafion BP2	CFRE	0.11			21.7	0.0217	197.27	2.30
PFO5DoDA	CFRE	0.24			16.43	0.01643	68.46	1.84

Table S2.18. Table providing surface water concentrations, mean liver concentrations, and calculation of bioconcentration factors (BCFs).

Compound	Surface water conc. at Lock and Dam 1 (Zhang et al. 2019)	Filling in below LOD values with half LOD	Estimated concentration at M23 based on conservation of mass down estuary (approximate)
PFBA	26	26	0.03
PFPeA	26	26	0.03
PFHxA	27	27	0.03
PFHpA	22	22	0.03
PFOA	19	19	0.02
PFNA	<10	5	0.01
PFDA	<10	5	0.01
PFBS	<11	5	0.01
PFHxS	18	18	0.02
PFOS	27	27	0.03
Nafion BP2	83	83	0.10
PFO5DoDA	200	200	0.24
PFO4DA	350	350	0.42
Mean Lock and Dam 1 salinity, based on conductivity conversion	0.06		
M18 June - Sept 2017 salinity, LCFRP data	24.825		
Freshwater river volume (mil m3) (Ensign et al. 2004)	127		
Estuarine volume (mil m3) (Ensign et al. 2004)	253		

Table S2.19. Worksheet containing surface water measurements from Lock and Dam 1 in the CFRE derived from Zhang et al. 2019, and subsequent estimation of estuarine water concentrations based on conservation of mass and salinity data. Water data from 10.1016/j.cageo.2004.03.015 and <https://doi.org/10.1021/acs.estlett.9b00525>.

Habitat	Tissue	Isotope/TL	Min (‰)	Median (‰)	Mean (‰)	Max (‰)	sd
CFRE	Liver	15N	12.05	13.39	13.43	14.86	0.75
Narra. Bay	Liver	15N	10.99	14.26	13.95	16.13	1.75
Mass. Bay	Liver	15N	13.11	13.76	13.80	14.53	
CFRE	Muscle	15N	11.59	12.62	12.57	13.75	0.74
Narra. Bay	Muscle	15N	10.86	12.93	12.84	14.51	1.23
Mass. Bay	Muscle	15N	11.43	12.43	12.30	12.78	0.46
CFRE	Liver	13C	-19.78	-18.39	-18.41	-17.04	0.89
Narra. Bay	Liver	13C	-22.53	-19.84	-20.07	-19.01	1.04
Mass. Bay	Liver	13C	-21.41	-20.43	-20.44	-19.63	
CFRE	Muscle	13C	-19.86	-18.26	-18.29	-16.93	0.96
Narra. Bay	Muscle	13C	-20.79	-19.74	-19.81	-18.83	0.76
Mass. Bay	Muscle	13C	-20.88	-20.05	-20.14	-19.41	0.47
CFRE	Muscle	34S	9.5	15.5	14.85	18.1	2.29
Narra. Bay	Muscle	34S	9.9	14.4	13.97	17.4	
Mass. Bay	Muscle	34S	19.4	19.9	19.88	20.4	0.4
CFRE	Liver	TL	3.51	3.87	3.88	4.25	0.2
Narra. Bay	Liver	TL	3.24	4.09	4.01	4.59	0.46
Mass. Bay	Liver	TL	3.79	3.96	3.97	4.17	
CFRE	Muscle	TL	3.39	3.66	3.65	3.96	0.19
Narra. Bay	Muscle	TL	3.2	3.75	3.72	4.16	0.32
Mass. Bay	Muscle	TL	3.35	3.61	3.58	3.71	0.11

Table S2.20. Summary statistics describing stable isotope measurements of bird liver and muscle. Trophic levels were calculated following methods of Fisk et al. 2001 and Hobson et al. 1994.

Sample	Habitat	N15 Liver (‰)	Liver TL	N15 Muscle (‰)	Muscle TL
CF01	CFRE	13.31	3.84	12.62	3.66
CF02	CFRE	12.05	3.51	11.92	3.48
CF03	CFRE	14.86	4.25	13.75	3.96
CF04	CFRE	12.68	3.68	11.69	3.42
CF05	CFRE	13.82	3.98	12.90	3.74
CF06	CFRE	13.53	3.90	12.77	3.70
CF07	CFRE	13.39	3.87	12.39	3.60
CF09	CFRE	13.28	3.84	11.59	3.39
CF10	CFRE	14.21	4.08	12.94	3.75
CF11	CFRE	12.93	3.74	12.03	3.51
CF12	CFRE	13.72	3.95	13.70	3.95
GRSH P024	Mass. Bay	13.71	3.95	11.89	3.47
GRSH P026	Mass. Bay	13.11	3.79	11.43	3.35
GRSH Q002	Mass. Bay	14.01	4.03	11.79	3.44
GRSH Q011	Mass. Bay	NA	NA	12.75	3.70
GRSH Q013	Mass. Bay	13.34	3.85	12.33	3.59
GRSH Q014	Mass. Bay	13.76	3.96	12.54	3.64
GRSH Q026	Mass. Bay	14.53	4.17	12.60	3.66
GRSH Q028	Mass. Bay	14.05	4.04	12.26	3.57
GRSH Q031	Mass. Bay	14.23	4.09	12.78	3.71
GRSH Q033	Mass. Bay	13.42	3.87	12.61	3.66
HG 025	Narra. Bay	12.93	3.74	11.75	3.44
HG 034	Narra. Bay	14.32	4.11	13.76	3.96
HG 056	Narra. Bay	12.44	3.62	11.82	3.45
HG 057	Narra. Bay	16.10	4.58	14.51	4.16
HG 058	Narra. Bay	12.24	3.56	12.90	3.74
HG 059	Narra. Bay	10.99	3.24	10.86	3.20
HG 060	Narra. Bay	14.20	4.08	12.96	3.75
HG 061	Narra. Bay	15.51	4.42	13.81	3.98
HG 062	Narra. Bay	16.13	4.59	14.19	4.08
HG 063	Narra. Bay	14.67	4.20	11.80	3.45
	Mean	13.72	3.95	12.57	3.65
	GeoMean	13.67	3.94	12.54	3.64
	SD	1.12	0.29	0.86	0.23
	Min	10.99	3.24	10.86	3.20
	Max	16.13	4.59	14.51	4.16

Table S2.21. Measured 15N ratios alongside estimates of trophic level (TL), assuming a copepod primary consumer after Fisk et al. 2001.

Correlation Coefficient		Dancey & Reidy (2007)
1	-1	Perfect
0.9	-0.9	Strong
0.8	-0.8	Strong
0.7	-0.7	Strong
0.6	-0.6	Moderate
0.5	-0.5	Moderate
0.4	-0.4	Moderate
0.3	-0.3	Weak
0.2	-0.2	Weak
0.1	-0.1	Weak
0	0	Zero

Table S2.22. Definition of correlation coefficient significance and descriptors, from <https://dx.doi.org/10.1016%2Fj.tjem.2018.08.001>.

Habitat	Compound	SI	Cor. Coeff	Estimate	Statistic	p.value	Correlation method	Alternative
CFRE	Nalton BP2	315N Liver	0.218	172.000	0.52	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP2	315C Liver	0.009	218.000	0.989	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP2	315N Muscle	0.373	138.000	0.261	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP2	313C Muscle	-0.073	236.000	0.830	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP2	334S	0.441	217.462	0.153	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP2	315N Liver	0.624	62	0.002	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP2	313C Liver	0.644	64	0.000	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP3	315N Muscle	0.164	139	0.651	Spearmen's rank correlation rho	two-sided	
CFRE	Nalton BP4	313C Muscle	0.115	146	0.759	Spearmen's rank correlation rho	two-sided	
Mass Bay	Nalton BP2	315N Liver	0.523	78.923	0.122	Spearmen's rank correlation rho	two-sided	
Mass Bay	Nalton BP2	313C Liver	0.058	155.426	0.877	Spearmen's rank correlation rho	two-sided	
Mass Bay	Nalton BP2	315N Muscle	0.174	136.277	0.631	Spearmen's rank correlation rho	two-sided	
Mass Bay	Nalton BP2	313C Muscle	0.058	174.574	0.873	Spearmen's rank correlation rho	two-sided	
Mass Bay	Nalton BP2	334S	-0.414	233.273	0.238	Spearmen's rank correlation rho	two-sided	
Narra Bay	Nalton BP2	315N Liver				Spearmen's rank correlation rho	two-sided	
Narra Bay	Nalton BP2	313C Liver				Spearmen's rank correlation rho	two-sided	
Narra Bay	Nalton BP2	315N Muscle				Spearmen's rank correlation rho	two-sided	
Narra Bay	Nalton BP2	334S				Spearmen's rank correlation rho	two-sided	
CFRE	PFBA	315N Liver	0.082	201.918	0.810	Spearmen's rank correlation rho	two-sided	
CFRE	PFBA	313C Liver	0.351	186.302	0.386	Spearmen's rank correlation rho	two-sided	
CFRE	PFBA	315N Muscle	0.219	171.780	0.517	Spearmen's rank correlation rho	two-sided	
CFRE	PFBA	313C Muscle	-0.361	259.362	0.276	Spearmen's rank correlation rho	two-sided	
CFRE	PFBA	334S	0.273	282.140	0.417	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFBA	315N Liver	-0.115	184.000	0.759	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFBA	313C Liver	0.584	72.000	0.098	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFBA	315N Muscle	0.273	120.000	0.445	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFBA	313C Muscle	0.115	146.000	0.759	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFBA	334S	0.433	236.398	0.213	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFBA	315N Liver	0.63	60.000	0.04	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFBA	313C Liver	0.297	116.000	0.407	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFBA	315N Muscle	0.442	92.000	0.204	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFBA	313C Muscle	0.200	132.000	0.584	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFBA	334S	0.152	140.000	0.682	Spearmen's rank correlation rho	two-sided	
CFRE	PFBA	315N Liver	0.050	232.000	0.881	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	313C Liver	0.691	68.000	0.02	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	315N Muscle	-0.593	240.000	0.19	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	313C Muscle	0.271	80.000	0.015	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	334S	-0.218	268.220	0.517	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	315N Liver	0.505	81.147	0.137	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	313C Liver	-0.832	269.317	0.002	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	315N Muscle	0.243	124.878	0.498	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	313C Muscle	0.511	249.256	0.132	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	334S	0.152	139.966	0.679	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	315N Liver	0.438	92.781	0.206	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	313C Liver	0.140	141.930	0.700	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	315N Muscle	0.207	130.898	0.567	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	313C Muscle	-0.209	177.018	0.620	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	334S	0.486	94.758	0.15	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	315N Liver	0.286	161.519	0.428	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	313C Liver	0.558	87.385	0.070	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	315N Muscle	0.154	186.143	0.651	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	313C Muscle	0.518	106.116	0.103	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	334S	-0.050	220.515	0.989	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	315N Liver				Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	313C Liver				Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	315N Muscle				Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	313C Muscle				Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	334S				Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	315N Liver				Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	313C Liver				Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	315N Muscle				Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	313C Muscle				Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	334S				Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	315N Liver	-0.048	230.048	0.894	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	313C Liver	0.636	81.988	0.038	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	315N Muscle	-0.001	242.091	0.995	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	313C Muscle	0.43	76.559	0.03	Spearmen's rank correlation rho	two-sided	
CFRE	PFDA	334S	-0.222	268.943	0.517	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	315N Liver	0.568	71.117	0.111	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	313C Liver	-0.579	280.289	0.080	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	315N Muscle	-0.176	194.089	0.628	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	313C Muscle	0.555	174.027	0.181	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFDA	334S	0.393	100.116	0.261	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	315N Liver	0.104	189.104	0.775	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	313C Liver	0.051	154.039	0.862	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	315N Muscle	0.088	150.914	0.818	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	313C Muscle	0.238	125.761	0.508	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFDA	334S	-0.018	168.018	0.960	Spearmen's rank correlation rho	two-sided	
CFRE	PFODhDA	315N Liver	0.336	146.000	0.313	Spearmen's rank correlation rho	two-sided	
CFRE	PFODhDA	313C Liver	0.091	200.000	0.797	Spearmen's rank correlation rho	two-sided	
CFRE	PFODhDA	315N Muscle	0.555	88.000	0.03	Spearmen's rank correlation rho	two-sided	
CFRE	PFODhDA	313C Muscle	0.246	168.000	0.488	Spearmen's rank correlation rho	two-sided	
CFRE	PFODhDA	334S	0.486	236.485	0.131	Spearmen's rank correlation rho	two-sided	
CFRE	PFOS	315N Liver	-0.045	230.000	0.900	Spearmen's rank correlation rho	two-sided	
CFRE	PFOS	313C Liver	0.216	80.000	0.010	Spearmen's rank correlation rho	two-sided	
CFRE	PFOS	315N Muscle	-0.138	250.000	0.694	Spearmen's rank correlation rho	two-sided	
CFRE	PFOS	313C Muscle	0.727	60.000	0.010	Spearmen's rank correlation rho	two-sided	
CFRE	PFOS	334S	-0.183	260.183	0.591	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFOS	315N Liver	0.603	68.000	0.077	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFOS	313C Liver	-0.188	196.000	0.608	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFOS	315N Muscle	0.872	54.000	0.001	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFOS	313C Muscle	-0.564	254.000	0.096	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFOS	334S	0.233	126.250	0.514	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFOS	315N Liver	-0.430	236.000	0.218	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFOS	313C Liver	-0.624	268.000	0.080	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFOS	315N Muscle	0.564	254.000	0.096	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFOS	313C Muscle	-0.273	210.000	0.444	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFOS	334S	-0.527	252.000	0.123	Spearmen's rank correlation rho	two-sided	
CFRE	PFTIDA	315N Liver	-0.284	282.144	0.381	Spearmen's rank correlation rho	two-sided	
CFRE	PFTIDA	313C Liver	0.589	138.928	0.269	Spearmen's rank correlation rho	two-sided	
CFRE	PFTIDA	315N Muscle	-0.400	268.024	0.223	Spearmen's rank correlation rho	two-sided	
CFRE	PFTIDA	313C Muscle	0.288	157.458	0.381	Spearmen's rank correlation rho	two-sided	
CFRE	PFTIDA	334S	0.148	187.422	0.684	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFTIDA	315N Liver	0.228	282.241	0.538	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFTIDA	313C Liver	-0.404	231.608	0.247	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFTIDA	315N Muscle	0.183	134.723	0.612	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFTIDA	313C Muscle	0.58	100.000	0.04	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFTIDA	334S	0.018	162.430	0.960	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFTIDA	315N Liver	0.068	153.734	0.851	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFTIDA	313C Liver	0.205	188.788	0.757	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFTIDA	315N Muscle	-0.083	178.919	0.822	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFTIDA	313C Muscle	0.048	187.114	0.896	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFTIDA	334S	0.005	153.734	0.851	Spearmen's rank correlation rho	two-sided	
CFRE	PFLUDA	315N Liver	0.009	217.995	0.878	Spearmen's rank correlation rho	two-sided	
CFRE	PFLUDA	313C Liver	0.706	64.447	0.010	Spearmen's rank correlation rho	two-sided	
CFRE	PFLUDA	315N Muscle	-0.146	252.073	0.689	Spearmen's rank correlation rho	two-sided	
CFRE	PFLUDA	313C Muscle	0.728	59.658	0.01	Spearmen's rank correlation rho	two-sided	
CFRE	PFLUDA	334S	0.052	199.862	0.769	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFLUDA	315N Liver	0.498	82.760	0.143	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFLUDA	313C Liver	-0.395	230.186	0.298	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFLUDA	315N Muscle	0.419	95.190	0.228	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFLUDA	313C Muscle	-0.468	242.234	0.172	Spearmen's rank correlation rho	two-sided	
Mass Bay	PFLUDA	334S	0.143	141.499	0.699	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFLUDA	315N Liver	0.459	83.753	0.148	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFLUDA	313C Liver	0.531	78.732	0.111	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFLUDA	315N Muscle	0.584	68.208	0.077	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFLUDA	313C Muscle	0.488	82.760	0.143	Spearmen's rank correlation rho	two-sided	
Narra Bay	PFLUDA	334S	0.693	50.603	0.028	Spearmen's rank correlation rho	two-sided	
CFRE	SumPFAS	315N Liver	0.164	234.000	0.634	Spearmen's rank correlation rho	two-sided	
CFRE	SumPFAS	313C Liver	0.818	84.000	0.044	Spearmen's rank correlation rho	two-sided	
CFRE	SumPFAS	315N Muscle	0.205	204.000	0.558	Spearmen's rank correlation rho	two-sided	
CFRE	SumPFAS	313C Muscle	0.541	100.000	0.08	Spearmen's rank correlation rho	two-sided	
CFRE	SumPFAS	334S	-0.338	294.338	0.308	Spearmen's rank correlation rho	two-sided	
Mass Bay	SumPFAS	315N Liver	0.439	93.781	0.208	Spearmen's rank correlation rho	two-sided	
Mass Bay	SumPFAS	313C Liver	-0.213	200.167	0.550	Spearmen's rank correlation rho	two-sided	
Mass Bay	SumPFAS	315N Muscle	0.688	54.669	0.030	Spearmen's rank correlation rho	two-sided	
Mass Bay	SumPFAS	313C Muscle	0.625	268.174	0.053	Spearmen's rank correlation rho	two-sided	
Mass Bay	SumPFAS	334S	-0.022	188.576	0.953	Spearmen's rank correlation rho	two-sided	
Narra Bay	SumPFAS	315N Liver	0.067	154.000	0.880	Spearmen's rank correlation rho	two-sided	
Narra Bay	SumPFAS	313C Liver	0.386	230.000	0.283	Spearmen's rank correlation rho	two-sided	
Narra Bay	SumPFAS	315N Muscle	-0.224	202.000	0.537	Spearmen's rank correlation rho	two-sided	
Narra Bay	SumPFAS	313C Muscle	-0.213	200.000	0.566	Spearmen's rank correlation rho	two-sided	
Narra Bay	SumPFAS	334S	0.164	192.000	0.657	Spearmen's rank correlation rho	two-sided	

Table S2.23. Results of Spearman correlation analysis assessing relationships between each PFAS and stable isotope ratios. Statistically significant ($p < 0.05$) relationships are highlighted in orange. Non-statistically significant relationships with $0.05 < p\text{-value} < 0.1$ are highlighted in yellow for illustrative purposes.

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CHAPTER 3

TIME TRENDS OF PER- AND POLYFLUOROALKYL SUBSTANCES FROM 2010 – 2019 IN A TRANS-EQUATORIAL MIGRATORY SEABIRD

This manuscript is formatted for submission to the journal *Environmental Science
and Technology*

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KEYWORDS: PFOS, PFAS, seabirds

ABSTRACT

Per- and polyfluoroalkyl substances, or PFAS, are industrial chemicals used widely in a range of consumer, industrial, and military applications. Increased regulatory scrutiny and research attention have brought about changes in the production and use of PFAS, affecting PFAS trends within environmental and biological matrices over the last two decades. Here we measured 27 PFAS in 116 Great Shearwaters collected in Massachusetts Bay, USA from 2010 – 2019. We found limited differences between sexes and no significant differences between age groups. Concentrations of PFOS and FOSA declined over the ten-year time series, while C8 – C11 PFCAs showed no significant trends over time. The lack of trends in long-chain PFCAs over time may be indicative of an ongoing change point, as long-chain PFCAs may no longer be increasing in the environment. Two less studied PFAS, Nafion BP2 and 7:3 FTCA, were detected in 36% and 64% of the sample set over time, respectively. Multiple PFAS were significantly associated with reduced liver and kidney weights and depth of body fat, while PFHxS and PFNA were negatively associated with mass and count of ingested plastics. Continued evaluation of long-chain PFCAs and emerging compounds is required to discern any changing trends and better describe the impacts of these compounds in biota subject to sustained concentrations.

INTRODUCTION

Per- and polyfluoroalkyl compounds, or PFAS, are a large family of anthropogenic chemicals first created in the 1940s¹. PFAS are highly persistent under a range of environmental and applied conditions¹. Many PFAS are also amphiphilic in nature which allows them to repel both oil and water due to the inclusion of hydrophobic carbon backbones coupled to a hydrophilic functional groups like -SO₄ or -COOH. These unique and attractive qualities have resulted in the incorporation of PFAS into a range of industrial, military, and consumer applications; a recent meta-analysis found over 200 distinct use categories involving over 1400 different PFAS².

Their prolific use within the built environment has resulted in their ubiquity in natural environments, including remote polar regions³ as well as environments closer to direct human sources^{4,5}. The spatial distributions of PFAS in the global environment are generally a function of their unique physico-chemical properties that facilitate long-range transport as well as their emissions during manufacturing and use. For example, emissions of perfluorooctane sulfonic acid (PFOS) are dominated by release from use of consumer and industrial products that previously incorporated PFOS directly or currently incorporate precursor compounds that transform into PFOS in vivo or in situ (like Me-FOSAA used in foot packaging products)^{4,6}. Local and regional “hot spots” of specific compounds or classes of PFAS may also be linked to manufacturing sites⁷ or surrounding locations where extensive product use or disposal has occurred (e.g., military

bases, wastewater treatment plants)^{8,9}. Population density can also serve as a proxy for relative exposure in fresh and coastal waters where emissions are more diffuse and driven predominantly by use of consumer products^{4,6}.

Regardless of specific emission source or compound, PFAS ultimately pose the most significant risk to marine environments, as the ocean is thought to be the ultimate reservoir for perfluorooctanoic acid (PFOA), PFOS, and other PFAS. PFAS have been identified in the surface ocean and at depth¹⁰, as well as in marine mammals¹¹, seabirds¹², fish¹³, crustacea and invertebrates^{14,15}. PFAS with more than 7 carbons have been documented to strongly bioaccumulate and biomagnify in marine food webs, particularly those supporting air-breathing organisms like seabirds and marine mammals^{15,16}. However, PFAS preferentially bind to protein¹⁷ or amphiphilic fatty acids¹⁸, unlike the preference for hydrophobic fatty matrices demonstrated by well-studied hydrophobic organic contaminants such as polychlorinated biphenyls. Therefore their occurrence and accumulation within different organisms, life stages, sexes, or food web scenarios is still under investigation.

Temporal trends of PFAS in marine environments are likewise of interest, as PFAS production and use has undergone significant changes with increased evidence of adverse effects within humans and wildlife¹⁹. For example, perfluoroalkyl acids (PFAAs) like PFOA and PFOS were phased out in the early 2000s, with increased use of replacements with fewer than seven fluorinated carbons, polyfluorinated structures, and/or structurally modified iterations of legacy PFAS, such as perfluoroalkyl ether acids (PFEAs) incorporating different

numbers of ether linkages^{20,21}. Changes to PFAS production and use patterns have previously been demonstrated to alter environmental concentrations of PFAS over time, as PFOS and FOSA rapidly decreased in wildlife and environmental matrices following the phase out of these compounds in the early 2000s¹¹. It is therefore expected that continued interventions aimed at curtailing the use of PFAS will result in further changes in the occurrence of PFAS over time and space.

In the present study we measured PFAS over a ten-year time series in a long-lived Atlantic seabird. In summary, the goals of the study were to (i) ascertain any temporal trends of PFAS over the last decade; (ii) assess the presence of novel and understudied PFAS in these birds, and (iii) explore PFAS dynamics between sexes, age groups, and in association with other biological and pollutant covariates.

MATERIALS AND METHODS

Study species

The Great Shearwater (*Ardenna gravis*) is a long-lived, trans-equatorial migratory seabird that inhabits the pelagic Gulf of Maine in the NW Atlantic during its non-breeding period over the boreal summer. Satellite tracking data and necropsy data suggest age-specific segregation of their non-breeding range, with preferential use of the SW Gulf of Maine and Massachusetts Bay by juveniles²². This species breeds during the boreal winter in the South Atlantic, on Gough Island, Inaccessible Island, and Nightingale Island in the St. Helena Island Group

and Kidney Island in the Falkland Islands²³. Contaminant levels measured in this species are primarily thought to reflect the influence of foraging in the Northern Hemisphere, due to the much lower levels of PFAS measured in other petrels at similar or higher trophic levels that solely rely on the Southern Atlantic Ocean²⁴.

Specimen Collection, Necropsy, and Sample Condition

Dead Great Shearwater juveniles and adults were obtained as bycatch from the National Oceanic and Atmospheric Administration (NOAA) Northeast Fisheries Observer Program (Falmouth, MA) from Massachusetts Bay. Bycatch collections spanned 2010 – 2019 and the majority of samples were collected in the months of August – November each year, after approximately 2 or more months of foraging in the NW Atlantic. Each individual was frozen individually as a whole specimen upon collection and stored at -20 °C until necropsy. All individuals were necropsied in a standardized manner²⁵, including measurements of morphometric characteristics, body condition, and organ weights. Age group was determined following the age characterization of Great Shearwaters as defined by Powers et al. (2020)²² using gonad size and the presence of the bursa of Fabricius, and sex based upon gonad presentation.

Chemicals, reagents, and materials

Analytical standards of greater than 99.9% purity, including mass-labeled surrogates, were purchased from Wellington Laboratories (Guelph, ON, Canada). Native Nafion BP2 was provided by Chemours (Fayetteville, NC) in lieu of a commercial source. HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Waltham, MA, USA). Ultrapure water for equipment cleaning

was obtained from a Milli-Q system fit with an HPLC water polisher or via HPLC grade water purchased from Fisher Scientific (Waltham, MA, USA). ENVI Carb bulk powder was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Ammonium acetate was purchased from Fisher Scientific (Waltham, MA, USA)

Extraction and analysis of PFAS

A total of 116 Great Shearwaters (*Ardenna gravis*) were analyzed for PFAS. 95 were juveniles and 21 were mature birds. The sample set was evenly split between males and females, with 55 females and 54 males examined. 7 birds could not be accurately sexed.

Aliquots of liver from each individual were homogenized and solvent-extracted in acetonitrile following addition of isotope-labelled surrogates, as previously described²⁶. Extracts were cleaned up using high-speed centrifugation paired with 25 mg of graphitized non-porous carbon and glacial acetic acid. Measurement and quantification of 27 PFAS was achieved using liquid chromatography tandem-mass spectrometry (UPLC-MS/MS) experiments in negative electrospray ionization mode. Estimates of PFAS concentrations were obtained by quantification using isotope dilution.

Matrix spikes and procedural blanks were included with the sample set, to monitor matrix effects, process recovery, and background contamination. Matrix effects were addressed using a 7-point matrix-matched curve, made up of chicken liver homogenate extracted in an identical fashion to GRSH liver samples, and spiked with native and isotope-labelled standards directly prior to analysis. The chicken liver matrix used for the curve contained trace levels of PFAS (namely L

- PFOS), and was corrected for background PFAS using the average of triplicate samples taken through the extraction. Data reported in this study were not blank corrected, due to low levels of process contamination identified in procedural blanks. PFBA and PFBS were not quantitatively reported here due to poor chromatography. Method detection limits were defined as procedural blank levels of a given analyte plus 3 times the standard deviation; in the absence of quantifiable blank concentrations, the lowest curve point (0.025 ng/mL) was deemed the method detection limit. Values below MDLs were considered zero for summation purposes. Method recovery ranged from 11 – 130% with a mean recovery of 76% across all compounds, similar to recoveries reported in other work using avian liver^{27–29}. FOSA, PFTeDA, and PFTrDA evidenced the lowest recoveries due to predictable loss of these analytes during sample preparation.

Analysis for total mercury

We used body feathers for analysis of total mercury (THg). The samples were washed with Milli-Q water and acetone in an ultrasonic bath respectively, and then rinsed with Milli-Q water 3 times. After freeze drying overnight, each sample was cut up and homogenized, and about 15 mg weighed into a nickel boat for analysis. All samples were analyzed using a direct thermal decomposition mercury analyzer (MA-3000, Nippon Instruments Corporation). Quality control methods included blanks, analytical duplicates and the certified reference materials (TORT-3 Lobster hepatopancreas and ERM-DB001 Human hairs, National Research Council of Canada) analyzed every 10 samples. Average

recoveries for the certified reference materials were $93.1 \pm 5.8\%$ (TORT-3) and $95.9 \pm 5.0\%$.

Stable isotope analysis

Body feathers were analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ to deduce any influence of trophic trends on results. Feathers were cleaned with acetone, and ground to fineness using a mortar and pestle. ~ 1 mg of feather was weighed out into tin capsules for carbon and nitrogen stable isotope analysis. Weighed samples were measured via IR-MS, and results interpreted as parts per thousand relative to appropriate references. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were measured using an Isoprime 100 Isotope Ratio Mass Spectrometer coupled to a Micro Vario Elemental Analyzer (Elementar Americas, Mt.Laurel, NJ).

The nitrogen ($\delta^{15}\text{N}$) isotope composition was expressed as a part per thousand deviation (‰) from air. Carbon ($\delta^{13}\text{C}$) isotope composition was expressed relative to Vienna Pee Dee Belemnite where $\delta X = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 10^3$, where X is $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$, and R is the ratio of heavy to light isotope ($^{15}\text{N}: ^{14}\text{N}$, $^{13}\text{C}: ^{12}\text{C}$). Duplicates were analyzed every 10 samples, and a blue mussel reference material every 15 samples to ensure measurement quality.

Statistical treatment

All data manipulation and statistical analyses were performed in R version 3.6.1 (R Core Team, 2020)³⁰. Quantitative data were checked for normality and homoscedasticity using the Shapiro-Wilk test and Levene's test. Concentrations were non-normal despite log transformation and therefore treated non-

parametrically as log-transformed variables for statistical analyses; year groups displayed no significant differences in variance. Summary statistics were derived using censored and uncensored data using the cenfit functions in the R package, NADA, version 1.6 - 1.1 (Lee 2020) to better account for left-censored data (Helsel 2012). Differences between years or groups were assessed using Kruskal-Wallis tests with post hoc application of Dunn's test with Bonferroni correction for multiple testing. Temporal trends were evaluated for compounds found in >80% of samples using generalized linear models (GLMs) with year as the sole predictor as a numeric variable to deduce any sustained temporal trends. Temporal trend assessment included all individuals with concentrations above detection limits given the lack of significant differences between mature and juvenile birds. 7:3 FTCA was also assessed for temporal trends despite its lower detection frequency (64%) using a generalized linear model, but these results were not reported quantitatively. Relationships between continuous, log-transformed numeric variables were assessed using Spearman rank correlation coefficients (R_s^2). Effects observed within all statistical tests were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Observed detection frequencies and concentrations

Samples were screened for 27 analytes, and 17/27 compounds were above detection limits in at least one sample. FOSA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFOS were detected in at least 80% of the sample set (Table 1).

PFOS was the most abundant compound in each individual and within each year, making up 49% of $\sum_{17}$ PFAS concentrations across all years with a mean concentration of 41 ng/g across all years and a maximum of 111 ng/g in a 2010 individual. After PFOS, PFUnDA or 7:3 FTCA generally alternated as the second most abundant compound in each sample. Concentrations of PFOS and PFUnDA were similar to concentrations previously observed in this species in 2017, as well as similar to concentrations observed in other temperate petrels^{12,31–33}.

The highest $\sum_{17}$ PFAS measured was 234 ng/g w.w. liver comprised of 15 quantifiable analytes, found in a 2010 juvenile GRSH. The lowest $\sum_{17}$ PFAS concentration was observed in a 2019 juvenile Great Shearwater from Massachusetts Bay containing $\sum_{17}$ PFAS of 9 ng/g, comprised of 6 analytes above detection limits.

Similarities and differences between age groups and sexes

Younger birds generally contained higher concentrations of individual and $\sum_{17}$ PFAS, but these results were not statistically significant in any given year or overall, possibly related to the low number of adults included in the sample set.

Considering solely mature individuals across all years ($n = 21$), mature females possessed lower concentrations of PFOA and PFHxS compared to mature males (Kruskal-Wallis, $p < 0.05$). Controlled studies in chickens and other animal models indicate differences in PFAS levels between sexes are related to differences in elimination efficiency mediated by sex hormone levels. For example, in a controlled study using adult mice, Kudo et al. (2002) experimentally castrated male rats which subsequently exhibited increased renal

clearance of PFOA approaching rates observed in females; reduced renal clearance rates were again apparent in the castrated males following testosterone treatment³⁴. We believe a similar sexually-mediated mechanism drives differences in PFOA and PFHxS levels in mature birds within our sample set, as higher concentrations in male individuals have likewise been identified in controlled studies involving mallards and quail^{35,36}.

However, concentration differences between sexes were not observed for any compound in juvenile birds within each year or overall. The lack of differences between sexes in young birds has been previously reported³⁷, and may be due to slow sexual maturation in long-lived seabirds in combination with the strong influence of maternal off-loading. The juveniles assessed within our study were primarily hatch year individuals less than 6 months in age, and therefore may be too young to exhibit the hormonally-mediated elimination differences apparent in older individuals due to their limited expression of sexual hormones. Like other Procellariiform seabirds, Great Shearwaters require an extended period of development (6 – 9 years) to reach sexual maturity. Changes in hormonal characteristics over this maturation period have been identified in other Procellariiforms (e.g. Wandering Albatross, *Diomedea exulans*) with very young males possessing significantly less testosterone compared to older males approaching or at sexual maturity³⁸; Great Shearwaters have not been specifically tested for such hormonal differences over maturation.

Additionally, young individuals likely reflect a disproportionate amount of PFAS derived from maternal offloading, which may obfuscate sex-based

concentration differences in the short-term. The tremendous persistence of PFAS in biological matrices means that PFAS transferred from mother to offspring are not likely to dissipate over the first months of development. Tarazona et al. (2015) established a plasma half-life estimate for PFOS in birds (231 days) using environmentally relevant doses of PFOS in chickens³⁹. Short-term exposure trials in juvenile quail and mallard suggest the liver half-life of PFOS is roughly 2.5 times that of the serum half-life³⁶. Using the plasma half-life derived in Tarazona et al. 2015 scaled using the exposure data relating plasma half-life and liver half-life, this equates to an estimated liver half-life for PFOS of 578 days in birds, or 1.6 years. Liver elimination of PFAS via biliary excretion has been demonstrated to be very slow (~1% of supplied dose)⁴⁰, and biological metabolism of most PFAAs is thought to be negligible. Therefore, the young birds (under about 6 months of age) assessed in this study likely reflect maternally-derived PFAS rather than unique PFAS profiles driven by hormonally-mediated processes. Few data sets exploring PFAS levels in birds over time or space report differences between sexes or lack thereof, and we encourage increased assessment of sex-based differences in other long-lived bird taxa to ascertain if juveniles of other species reflect the same apparent lack of sex-based dissimilarities early in development.

Detection of novel PFAS over time

Here we provide the first estimates describing the occurrence of two emerging PFAS within a sample set collected across a decade (Fig. 1). Nafion by-product 2, or Nafion BP2, and 7:3 FTCA were detected in 36% and 64% of our

sample set, respectively. Little or no data to date reports on the occurrence of these compounds in wildlife.

Nafion BP2 is an ether-based PFAS derived from the production of ionic polymers that are used across diverse industrial applications. Nafion BP2 has been previously reported in birds³⁷, fish⁴¹, and humans⁴² from the Cape Fear River watershed in southeast North Carolina, due to ongoing industrial activity in the region⁴³. Prior work from our group likewise identified this compound in a limited number of individuals from Massachusetts Bay and Narragansett Bay in 2017³⁷. Here we found Nafion BP2 in 36% (42/116) of Great Shearwaters dating back to 2010, with a maximum concentration of 0.230 ng/g in liver from a juvenile Great Shearwater bycaught in 2015. Temporal trends were not statistically assessed due to the limited detection frequency of this analyte. However, despite this limitation, our data is the first to measure this compound in biota before or after 2017-2018.

Additionally, we believe this is the first study to identify this compound over multiple years in a wildlife population with no significant connection to known industrial sources. Great Shearwaters have no migratory connectivity to the Cape Fear region, as only 15% of individuals use the offshore US East Coast for trans-equatorial migration, while 85% use the margins of the eastern Atlantic adjacent to the coasts of Africa and Europe. Those individuals that use the western Atlantic migration corridor stay well offshore, and have not been documented to use the riverine or estuarine habitats of the Cape Fear River region. This suggests the occurrence of Nafion BP2 in our sample set is a result of environmental

persistence facilitating long-range transport of this compound to pelagic environments; this work is some of the first to establish the long-range transport of Nafion BP2. Alternatively, our results also point to multiple additional source(s) beyond the Cape Fear region, which in turn suggests we may be underestimating exposure to this compound by assuming it may only be derived from one highly visible source. We recommend more comprehensive assessment of this ether-based sulfonic acid across a wider suite of environmental matrices, considering its demonstrated yet underexplored capacity for environmental occurrence

7:3 FTCA is a stable fluorotelomer carboxylic acid (FTCA), previously reported in a handful of studies examining marine mammals²⁶, waterbirds^{29,44}, birds of prey⁴⁵, Flesh-Footed and Wedge-tailed shearwaters from the Southern Hemisphere⁴⁶, as well as humans. Concentrations up to 1131 ng/g in a polar bear adult and 192 ng/g in an adult harbor seal were recently reported in marine mammals from the NW Atlantic²⁶. 7:3 FTCA was detected in 64% of our sample set at concentrations up to 62 ng/g. This compound alternated with PFUnDA as the second most abundant compound, after PFOS, within each given individual. To the best of our knowledge, this marks the first time this compound has been measured over time in a pelagic seabird. No significant differences in concentration were observed between sexes or ages considering the total sample set as a whole, and no temporal trends were apparent over time.

The lack of any temporal trend here is likely due to continued, widespread use and emissions of precursors that transform into FTCAs and PFCAs. 7:3

FTCA is derived from aerobic or anaerobic degradation of atmospherically-labile fluorotelomer alcohols, primarily 8:2 FTOH; degradation of FTOHs also results in formation of PFCAs at low yields⁴⁷. 7:3 FTCA may also be formed from *in vivo* degradation of polyfluoroalkyl phosphate surfactants (PAPs), nonpolymeric PFAS used in food contact paper products⁴⁸.

The documented source pathways of this compound suggest respiratory uptake from atmospheric reservoirs is likely driving 7:3 FTCA concentrations observed in seabirds within this study. Limited evidence from food web investigations underscores the assumed importance of atmospheric exposure; 27 forage fish from Massachusetts Bay were measured for 7:3 FTCA (Robuck et al. 2021, in prep), with no detections of 7:3 FTCA above limits of detection in fish. Loi et al. (2011) also did not find 7:3 FTCA in a range of marine fish and crustaceans⁴⁴. Any role of trophic transfer in 7:3 FTCA accumulation is made additionally unclear given its weak – moderate negative association with $\delta^{15}\text{N}$, suggesting birds feeding higher in the food web with higher ratios of $\delta^{15}\text{N}$ contain less 7:3 FTCA (Table 2), unlike the typical positive relationship between $\delta^{15}\text{N}$ and traditionally biomagnified pollutants⁴⁹. This negative relationship may instead suggest biotic metabolism of 7:3 FTCA into PFOA, previously documented in a range of biological systems⁴⁷. Biological metabolism may therefore limit the transfer of this compound between trophic levels, leaving respiratory uptake from atmospheric reservoirs as the primary route of exposure.

Neutral, volatile FTCA precursors such as 8:2 FTOH continue to enter the environment via use and disposal of consumer products⁵⁰, industrial activities, the

built environment⁵¹, and waste infrastructure⁵². As a result, 8:2 FTOH remains the dominant compound found in air at remote environments⁵³ and adjacent to urban centers⁵⁴, suggesting this precursor will continue to impart sustained biotic occurrence of downstream products like 7:3 FTCA. More research is warranted into local and global occurrence of 7:3 FTCA and other FTCAs, given limited but concerning toxicity data suggesting FTCAs may be significantly more toxic than PFCAs⁵⁵.

Trends over time

Temporal trends were assessed for FOSA, PFOS, PFOA, PFNA, PFDA, and PFUnDA using generalized linear models. FOSA and PFOS significantly decreased over 2010 – 2019 ($p < 0.05$). This aligns with trends observed in gannets from the United Kingdom over 1977 - 2014, white-tailed eagles from northern Europe measured over 2008 - 2017, several species of pelagic seabird from the Pacific coast of Canada from 1990 - 2011³¹, as well as multiple taxa from the Arctic across varying time scales³. Declining concentrations of these compounds here and across other environmental matrices are likely driven by the voluntary phase out of PFOS and its precursors in the early 2000s in the US and Europe.

Concentrations of PFCAs in Great Shearwaters did not significantly change over 2010 - 2019 within our study, although PFUnDA increased marginally but insignificantly ($p = 0.067$). This is in contrast to other datasets, as most temporal analyses of long-chain PFCAs in other biotic matrices have found increased concentrations of PFCAs over time. However, few currently published data sets

include data from the most recent decade. Assessing PFAS specifically over this period of time is crucial because of policy and industrial interventions that have altered PFAS use and management over the course of 2000 - 2020, many of which stand to alter ambient PFAS levels in the environment. For example, Prevedouros et al. (2006) suggested that long-chain PFCAs primarily enter the environment due to their use as processing aids in creation of fluoropolymers⁵⁶, or via *in situ* degradation of volatile precursors⁵⁷. Fluoropolymer production has instead increasingly shifted to rely upon ether-based PFEAs^{7,58} rather than the C8 or C9 PFCA formulations originally used; PFEAs are terminal endpoints themselves, and are not thought to further degrade to PFCAs⁵⁹.

We believe the sustained, rather than increasing, concentrations of PFCAs observed in our study may be suggestive of an ongoing change point where C8 – C11 PFCAs cease to increase in the environment and marine biota, due to shifts in production away from structures that degrade into long-chain PFCAs or process streams that produce long-chain PFCAs as impurities. A similar change point was observed for PFOS and FOSA following cessation of its use in the early 2000s¹¹. Limited data from other more recent datasets tenuously suggests the same for long-chain PFCAs. Jouanneau et al. assessed PFAS in plasma of white-tailed eagle nestlings from 2008 – 2017, and likewise found no significant trends in PFOA or PFNA, while PFUnDA and PFTrDA ceased to increase after approximately 2012⁶⁰. In the US, PFOA and PFUnDA levels in bald eagle nestlings' plasma was found to decline from 2006 to 2011⁶¹.

Further study is required to describe developing trends of PFCAs in different biological receptors, to truly discern any change in environmental and biological abundance; the lack of clear trends may also merely indicate sustained but level emissions or formation of PFCAs or PFCA precursors in recent years.

Additionally, continued investigation is warranted to describe potential adverse effects associated with long-chain PFCAs, given the paucity of data exploring PFCA levels in relation to acute or chronic impacts in wildlife populations and their sustained concentrations in the environment.

Correlation to morphometric, stable isotope, and mercury data

Morphometric measurements were collected for each individual as part of necropsy, in tandem with stable isotope ratios, plastic ingestion, and total mercury levels, which were collected in support of other datasets derived from this bird time series. Correlation analyses suggested a range of weak to moderate relationships between these covariates and specific PFAS concentrations (Table 2). Associations were considered weak with $\rho = |0.2 - 0.3|$, weak-moderate with $\rho = |0.3 - 0.4|$, and moderate with $\rho = |0.4 - 0.5|$.

Plastic mass and count were weakly to moderately negatively associated with PFHxS and PFNA levels. This is the first documented significant association between plastic ingestion and PFAS in seabirds, and suggests plastic pieces are not a significant vector of PFAS to wildlife that ingest plastic. This intuitively makes sense given the amphiphilic and hydrophilic chemical properties of most PFAS that reduce their proclivity for sorption to non-polar matrices. This relationship also reflects the divergent exposure pathways for these two pollutant

groups, as seabirds are primarily exposed to PFAS via diet as opposed to direct ingestion of plastics via discovery in the environment. While plastic ingestion is a growing and popular research topic, we emphasize the lack of relationship and inverse relationships between plastics and PFAS identified here, and caution that further research investigating PFAS and plastics should beware of spurious positive significant relationships in juxtaposition to the physicochemical tendencies of PFAS.

$\delta^{13}\text{C}$ ratios measured in body feathers ranged from -19.6‰ to -16.6‰; no consistent trends in $\delta^{13}\text{C}$ were apparent over time. However, PFOS and FOSA were positively associated with $\delta^{13}\text{C}$. This may suggest birds utilizing prey sources more reliant on terrestrial environments with higher (less negative) $\delta^{13}\text{C}$ influenced by C4 primary production are more exposed to PFOS and FOSA compared to individuals reliant on more pelagic environments and forage items reflective of a more negative, marine $\delta^{13}\text{C}$ signature⁶². These two compounds were likewise positively associated with total mercury, possibly indicative of similar biomagnification capacity among these highly bioaccumulative pollutants⁶³.

7:3 FTCA, PFDA, PFDoA, PFHxS, PFNA, PFOA, PFOS, PFTrDA, and PFUnDA were negatively associated with kidney and liver weights. Though a mechanism driving these associations is unclear, organ weight changes have been previously demonstrated as sensitive indicators of chemical influences. Decreased organ weights may impact bird health through reduced volume capacity to perform physiological functions. PFHpS was positively associated with wing

chord, along with PFTrDA although this association was only marginally significant ($p = 0.066$). These morphometric measurements were not significantly different between mature and juvenile individuals. Positive significant associations between wing chord and PFDA and PFTrDA have previously been reported in two species of Pacific shearwaters⁴⁶. PFDoA, PFOS, PFTrDA, and PFUnDA were also positively associated with body condition. The body condition index is a unitless measurement of bird condition based on the visual robustness of pectoral muscle, intestinal fat appearance, adipose fat layers, and prominence of the keel documented upon initiation of necropsy. Associations between morphometric characteristics and PFAS levels may be related to the demonstrated capacity of PFAS to impact hormonal dynamics that influence growth and development in birds and other taxa^{64–66}.

Additionally, PFDoA, PFHxS, PFTrDA, and PFUnDA were negatively associated with fat depth, measured adjacent to pectoral muscle. Unlike other hydrophobic pollutants, PFAS do not preferentially partition into adipose fat stores, therefore we believe this association reflects as-of-yet undetermined physiological or ecological dynamics rather than starvation-induced release of pollutants from fat stores. PFAS, specifically PFOS and PFNA, have been previously associated with changes in lipid dynamics in this species¹². Observations from other field studies and controlled research likewise demonstrate associations between PFAS and lipid dynamics in other taxa and molecular models^{18,67,68}. Reduced fat stores may impact organismal function and fitness, as seabirds rely on finely tuned fat reserves to successfully carry out

migration and reproduction. Given the importance of body condition and fat stores for individual and population health, we argue for more comprehensive evaluation of relationships between PFAS and sublethal endpoints like lipid and hormonal expression in seabirds and other wildlife, as detailed mechanistic or empirical explanations of the associations found in this study are lacking.

Implications

Our results confirm sustained concentrations of PFAS in multiple life stages of a pelagic seabird over a ten-year time series. PFOS and FOSA were found to decrease over time, while PFOA, PFNA, PFDA, and PFUnDA show no clear trends over time, indicating sustained concentrations. These results, in tandem with other emerging datasets, suggest a change point may be underway whereby PFCAs stop increasing in the environment and biota. Continued assessment of temporal trends is vital to ascertain the veracity of any such shifts, as well as to define environmental trends of understudied compounds like Nafion BP2 and 7:3 FTCA, two compounds that were identified in a significant portion of samples within this study. More focus should likewise be paid to deducing sublethal impacts of PFAS on wildlife, given the suite of relationships identified here between concentrations of specific PFAS and morphometric measurements. Continued attention to PFAS in wildlife over time ultimately stands to benefit humans and our own understanding of PFAS trends, as we continue to use air, water, and food resources subject to the same ambient environmental exposures as wildlife.

ASSOCIATED CONTENT

NA

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Author Contributions

The manuscript was primarily drafted by A. Robuck and finalized through contributions of all authors. Sample analysis and data interpretation were carried out by A. Robuck, alongside contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

A. Robuck acknowledges support from the National Oceanic and Atmospheric Administration Dr. Nancy Foster Scholarship program (NOAA Award Number NA17NOS4290028), the Robert and Patricia Switzer Foundation, the STEEP Superfund Research Program (NIEHS Award Number P42ES027706), and the Oak Ridge Institute for Science and Education (ORISE) program.

Competing Interests

The authors have no competing interests to declare.

ACKNOWLEDGMENTS

The authors are indebted to the Northeast Fisheries Observer Program, Gina Shield, and Stephanie DePasquale for their assistance obtaining samples used in this study. Although NOAA and EPA employees contributed to this article, this

research was conceived, designed, and implemented by URI. Consequently, the views, interpretations, and conclusions expressed in the article are solely those of the authors and do not necessarily reflect or represent NOAA views or policies.

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TABLES AND FIGURES

Compound	Family	# Fluorinated Carbons	% Detection	Mean $\pm$ SE	Range
N-MeFOSAA	FASAA	8	0	–	–
N-EtFOSAA	FASAA	8	0	–	–
FOSA	FASA	8	83	0.96 $\pm$ 0.08	0 - 3.7
4:2 FTS	FTS	4	0	–	–
6:2 FTS	FTS	6	0	–	–
8:2 FTS	FTS	8	0	–	–
PFBA	PFCA	3	nr	–	–
PFPeA	PFCA	4	0	–	–
PFHxA	PFCA	5	9	0.24 $\pm$ 0.03	0 - 1.3
PFHpA	PFCA	6	60	0.28 $\pm$ 0.04	0 - 2.2
PFOA	PFCA	7	95	0.30 $\pm$ 0.04	0 - 2.6
PFNA	PFCA	8	99	2.2 $\pm$ 0.13	0 - 6.8
PFDA	PFCA	9	99	2.4 $\pm$ 0.11	0 - 6.6
PFUnDA	PFCA	10	99	7.45 $\pm$ 0.38	0 - 24
PFDoA	PFCA	11	99	3.1 $\pm$ 0.18	0 - 10
PFTTrDA	PFCA	12	75	2.62 $\pm$ 0.17	0 - 8
PFTeDA	PFCA	13	60	1.73 $\pm$ 0.13	0 - 5
7:3 FTCA	FTCA	7	64	6.54 $\pm$ 1.01	0 - 62
HFPO-DA	PFECA	5	0	–	–
Nafion BP2	PFESA	7	36	0.04 $\pm$ 0.008	0 - 0.2
PFBS	PFSA	4	nr	–	–
PFPeS	PFSA	5	20	–	–
PFHxS	PFSA	6	73	1.32 $\pm$ 0.10	0 - 5
PFHpS	PFSA	7	43	0.52 $\pm$ 0.05	0 - 2
PFOS	PFSA	8	99	38 $\pm$ 2.6	0 - 111
PFNS	PFSA	9	57	0.18 $\pm$ 0.02	0 - 0.9
PFDS	PFSA	10	41	0.09 $\pm$ 0.03	0 - 0.3

Table 3.1. Detection frequency, arithmetic mean, standard error, and range of concentrations included in this study. PFBA and PFBS were not reported, or “nr” due to chromatographic imprecision. Family designations are after Buck et al. 2011.

Figure 3.1. Mean concentrations of A) Nafion BP2 and B) 7:3 FTCA in ng/g wet weight over time, with bars representing standard error of the mean.

Figure 3.2. Concentrations of six PFAS over 2010 – 2019; the blue line represents a linear model using year as the sole predictor to deduce any time trends. Only compounds with a detection frequency above 80% were meaningfully assessed for temporal trends.

Biological/chemical parameter	Associated compound(s)	Rho estimate	p-value	Strength of association
d15N	7:3 FTCA	-0.332	0.037	Weak - Moderate
	PFHxS	-0.244	0.050	Weak
Wet Weight (g)	7:3 FTCA	-0.309	0.018	Weak - Moderate
	FOSA	0.260	0.018	Weak
Liver Weight (g)	7:3 FTCA	-0.409	0.001	Moderate
	PFDA	-0.230	0.016	Weak
	PFDoA	-0.275	0.004	Weak
	PFHxS	-0.309	0.006	Weak - Moderate
	PFOS	-0.276	0.012	Weak
	PFtrDA	-0.263	0.077	Weak
	PFUdA	-0.274	0.004	Weak
Kidney Weight (g)	7:3 FTCA	-0.364	0.005	Weak - Moderate
	PFDA	-0.247	0.011	Weak
	PFDoA	-0.286	0.003	Weak
	PFNA	-0.210	0.033	Weak
	PFOA	-0.217	0.030	Weak
	PFUdA	-0.304	0.002	Weak - Moderate
d13C	FOSA	0.248	0.045	Weak
	PFOS	0.285	0.021	Weak
Hg	FOSA	0.339	0.014	Weak - Moderate
	PFOS	0.239	0.095	Weak
Tarsus	PFDoA	-0.318	0.001	Weak - Moderate
	PFHxS	-0.218	0.054	Weak
	PFOS	-0.266	0.016	Weak
	PFtrDA	-0.400	0.006	Moderate
	PFUdA	-0.294	0.002	Weak
Body Condition Index	PFDoA	0.210	0.030	Weak
	PFOS	0.255	0.022	Weak
	PFtrDA	0.418	0.004	Moderate
	PFUdA	0.260	0.007	Weak
Fat Depth (mm)	PFDoA	-0.301	0.003	Weak - Moderate
	PFHxS	-0.263	0.023	Weak
	PFtrDA	-0.397	0.008	Weak - Moderate
	PFUdA	-0.284	0.005	Weak
Wing Chord (cm)	PFHpS	0.292	0.079	Weak
	PFtrDA	0.279	0.066	Weak
Plastic Count	PFHxS	-0.376	0.001	Weak - Moderate
Mass of Plastic (mg)	PFHxS	-0.351	0.002	Weak - Moderate
	PFNA	-0.224	0.026	Weak
Head-Bill Length (cm)	PFHxS	-0.217	0.054	Weak

Table 3.2. Table presenting significant associations identified via Spearman rho correlation analysis between continuous biological covariates and PFAS concentrations. Associations above the pre-determined significance level ($p = 0.05$) but below $p = 0.1$ are highlighted in orange.

CHAPTER 4

TISSUE-SPECIFIC DISTRIBUTION OF LEGACY AND NOVEL PER- AND POLYFLUOROALKYL SUBSTANCES IN JUVENILE SEABIRDS

*This manuscript is in review at Environmental Science and Technology
Letters*

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KEYWORDS: PFOS, PFAS, seabirds, bioaccumulation, partitioning

ABSTRACT

Of the thousands of per- and polyfluoroalkyl substances (PFAS) potentially in the environment, only few have been investigated in detail. Little is also known about their partitioning among multiple wildlife tissues and organs, which is needed to better predict the ecological risk PFAS may pose. In this study, we analyzed 36 legacy and emerging PFAS in up to 8 juvenile seabird tissues collected from three habitats along the US Eastern Seaboard (Massachusetts Bay, Narragansett Bay and Cape Fear River Estuary). PFOS was the dominant compound across multiple tissues, while long-chain PFCAs dominated in brain (mean = 44% of total concentrations). Emerging perfluoroalkyl ether acids (PFEAs) Nafion byproduct-2 and PFO5DoDA were detected in greater than 90% of tissues from juveniles obtained from the Cape Fear River Estuary, a nesting region downstream from a major fluorochemical production site. This marks the first environmental detection of these compounds in multiple vertebrate organs, including the brain. PFEAs displayed a preference for partitioning into blood compared to liver, possibly due to the increased flexibility of albumin to accommodate multi-ether PFAS, while PFO5DoDA showed a reduced preference for brain. These results suggest future monitoring efforts and toxicological studies should focus on impacts of novel PFAS and long-chain PFCAs in multiple tissues beyond liver and blood, while exploring the unique binding mechanisms driving uptake of multi-ether PFEAs.

INTRODUCTION

Global biomonitoring studies document the pervasive accumulation of per- and polyfluoroalkyl substances (PFAS) in wildlife adjacent to human sources of PFAS¹⁻⁴ but also in animals from remote regions. The majority of these studies measured PFAS in select matrices only, such as whole body homogenate, serum, liver, feather, or egg, to evaluate hypotheses focused on spatial and temporal dynamics⁵⁻⁷. The most commonly measured matrices often encompass tissues collected using non-invasive methods. A smaller subset of research has investigated the distributions of PFAS within multiple tissues of wild organisms, including tissues such as brain, lungs, heart, muscle, kidney, gonads, and adipose tissue (fat)⁸⁻¹⁴. In this study, we analyzed PFAS in 8 different seabird tissues. Knowing the abundance of PFAS in multiple tissues and organs is key to better understand PFAS internal partitioning, identify tissue reservoirs, and predict PFAS physiological and ecological risks.

Existing tissue-specific work demonstrates the unique PFAS partitioning behavior compared to legacy persistent organic pollutants such as polychlorinated biphenyls (PCBs). Verreault et al. (2005) examined glaucous gull plasma, liver, egg, and brain and determined perfluorooctanesulfonic acid (PFOS) concentrations were greatest in plasma, followed by approximately equal concentrations in liver and egg, with the lowest concentrations in the brain⁹. Investigations in herring gull and guillemots subsequently found liver to contain higher concentrations of PFOS compared to plasma or muscle^{10,15}. Results from multiple taxa beyond avifauna demonstrate that blood, liver, and kidney contain

the highest concentrations of multiple perfluoroalkyl acids (PFAAs) normalized by tissue weight, with some indication of preferential uptake of long-chain perfluoroalkyl carboxylic acids (PFCAs, $C_nF_{2n+1}COOH$, $n \geq 7$) in the brains of marine mammals^{8,14,16}. This is in stark contrast to the behavior of hydrophobic organic chemicals like PCBs, which show a distinct preference for fatty tissue storage.

Beyond contrasting to legacy pollutants, tissue-specific measurements help constrain drivers of PFAS bioaccumulation. Empirical data from tissue-specific studies, modeling efforts, and data from controlled laboratory studies indicate interactions with specific proteins like albumin and liver fatty acid binding protein (L-FABP) determine the accumulation of various PFAS in liver, kidney, and blood matrices^{17,18}. For instance, the L-FABP interaction with perfluoroalkyl sulfonic acids (PFSAs, $C_nF_{2n+1}SO_3H$, $n \geq 6$) and PFCAs is driven by hydrophobic and hydrogen-bonding in the protein, mediated by the size and rigidity of the PFAS molecule¹⁹. PFCAs with an alkyl chain consisting of 4 -11 carbons and PFSAs with 6 -10 carbons favorably bind to L-FABP, with a decrease in binding observed beyond 11 carbons. Non-specific phospholipid-mediated pathways have also been hypothesized to drive PFAA uptake in brain and liver^{18,20,21}. Sizable data gaps exist describing these mechanisms empirically and mechanistically across different taxa and trophic web levels.

Notably, little information exists evaluating bioaccumulation mechanisms or tissue-specific behavior of novel and replacement PFAS^{11,12}, despite shifts towards production and use of PFAS with fewer than seven fluorinated carbons,

polyfluorinated structures, and/or structurally modified iterations of PFAAs, such as such as per- and polyfluoroalkyl ether acids (PFEAs) incorporating ether linkages²².

To address this knowledge gap, we present tissue-specific distributions of legacy and emerging PFAS using high resolution mass spectrometry (HRMS) in seabirds from the U.S. Atlantic Coast. We screened for the most widely assessed PFCAs and PFSAs along with 11 PFEAs. In summary, the aims of this study were to (i) derive the tissue distributions of 36 legacy and emerging PFAS in birds from three habitats and (ii) explore possible factors driving any differences in observed bioaccumulation between novel and legacy PFAS.

MATERIALS AND METHODS

Sample Collection

Deceased juvenile seabirds were obtained from three habitats reflecting different PFAS exposure potential. Habitats included the Cape Fear River Estuary (CFRE) downstream from a major fluoropolymer manufacturing site, Narragansett Bay adjacent to a major metropolitan center, and Massachusetts Bay, an offshore region removed from direct contact with PFAS point sources. The rationale supporting the evaluation of PFAS in similar seabird species from multiple habitats has been described previously²³. All individuals were necropsied according to standard protocol, and intact tissues wrapped in methanol-cleaned foil and stored in polyethylene bags or vials. Tissues collected included heart, brain, kidney, lungs, uropygial gland, adipose fat, liver, whole blood, pectoral

muscle, spleen, and feces; tissues were collected on an as-available basis, as some individuals were too small for accurate collection of all tissues. Tissues were frozen at -15°C until chemical analysis.

PFAS analysis via HRMS

A total of 36 PFAS were assessed in juvenile seabird livers via targeted and suspect screening analysis. A complete analyte list is available in Table S4. All tissues were extracted by means of protein precipitation in acetonitrile and dilution as previously described²⁴. PFAS were detected using with a Thermo Orbitrap Fusion mass spectrometer (ThermoFisher Scientific, Waltham, MA) with a heated electrospray ionization (HESI) source operated in negative mode, following a previously described HRMS workflow^{25–27}. Further details about sample preparation, analysis, quantification, and quality control are available in the Supplementary Information (SI).

RESULTS AND DISCUSSION

Detection of PFAS by tissue and region

17 of 36 analytes were detected in at least one tissue from juvenile seabirds using HRMS (Fig. 1). Nine PFAS (Nafion BP2, PFO5DoDA, PFO4DA, PFOS, PFNA, PFDA, PFUnDA, PFDoA, and PFTrDA) were found $\geq 50\%$ of at least two tissues from at least one habitat. The dominant five compounds (PFOS, PFDA, PFUnDA, PFDoA, and PFTrDA) were detected in at least two tissues in all three habitats. PFNA was only detected in livers from Massachusetts Bay and Narragansett Bay birds, but was detected across six tissues in CFRE individuals.

Nafion BP2 was detected in muscle, blood, and liver of birds outside of the CFRE, but levels in Narragansett Bay birds were below the linear dynamic range of the curve (< 0.5 ng/mL) and were not quantifiable. The largest number of analytes and the highest detection frequencies across each tissue type were observed in individuals from the Cape Fear sample set (Fig. 1).

Multiple short-chain PFCAs and PFEAs, fluorotelomer sulfonates, and acetic acid precursors like Et-FOSAA were not detected in any tissue (Table S5), suggesting reduced bioaccumulation potential compared to the detected long-chain structures, *in vivo* transformation of these compounds to other terminal endpoints, or simply levels below the detection limits applicable in this study.

PFOS and PFCA concentrations across multiple tissues

Concentrations of each analyte varied by tissue and habitat. PFOS was found at the highest mean concentration in most tissues where it was detected. It also made up the largest single percentage of the total sum concentration in most tissues (Fig. 2). The abundance of PFOS is noted across multiple wildlife taxa due to its highly persistence and bioaccumulative nature. Considering birds from all habitats, the highest mean concentrations of PFOS were observed in liver, kidney, lungs, and blood across all habitats (Fig. 3). High PFOS abundances in these relative to other tissues has been reported in wildlife from regions unimpacted by PFOS point sources^{9,10,15}. PFOS was found in all CFRE tissues examined, and at the highest concentrations (up to 480 ng/g in blood) (Table S2). When grouped by tissue type solely considering CFRE birds, PFOS decreased in concentration according to: Kidney = Liver > Blood = Lungs > Brain > Heart >

Muscle > Fat. The abundance of PFOS in CFRE samples is likely related to high ambient levels of PFOS across the wider Cape Fear region, and has likewise been noted in fish and human samples from the region²⁷⁻²⁹.

A total of 5 CFRE, 1 Massachusetts Bay, and 3 Narragansett Bay bird contained liver concentrations above the 140 ng/g female-specific avian toxicity references value (TRV)³⁰, though it is unclear if reproductive impacts are occurring in these seabird populations due to data deficiencies and potential differences in sensitivity to PFAS between avian species. No birds contained tissue residues above the overall avian TRV of 600 ng/g liver. TRVs are not available for specific avian tissues beyond liver, serum, and egg; this deficit stands as a large data gap as our dataset further demonstrates kidney, lungs, and brain act as reservoirs containing the highest mean concentrations of PFOS or other PFAS across multiple taxa (Fig. 3)^{17,31}.

CFRE birds displayed the largest number of PFCA detections across multiple tissues (Fig. S2). PFUnDA was the most abundant PFCA found in liver, with a mean concentration of 10, 10, and 5 ng/g in CFRE, Massachusetts Bay, and Narragansett Bay livers, respectively (Table S2). PFTrDA was the most abundant PFCA detected in the lungs and brain of Massachusetts Bay individuals, at a mean concentration of 19 ng/g in both tissues. Elevated levels of this C₁₃ PFCA solely in Massachusetts Bay individuals likely relates to their pelagic habits that entail disproportionate exposure to atmospherically transported precursor compounds that may oxidize *in situ* or *in vivo* to PFCAs³². Relatively high abundances of

long-chain PFCAs have been noted in other work investigating PFAS in remote wildlife^{3,33–35}, as well as in other tissue-specific research^{8,13,15}.

PFEA concentrations across multiple tissues of CFRE birds

PFEAs made up the largest single proportion of total PFAS in fat (54% in CFRE birds); PFEA levels in fat were dominated by Nafion BP2, but concentrations were overall low in fat compared to levels in other tissues (Fig. 3, Table S2). The highest PFEA levels were found in brain of CFRE individuals, with a maximum of 360 ng/g Nafion BP2 (Table S2). Mean concentrations of Nafion BP2 in blood from CFRE individuals (mean = 21 ng/ml) were approximately 70 times higher than Nafion BP2 levels observed in striped bass from the Cape Fear River (mean = 0.3 ng/ml)²⁷, and about 7 times higher than Nafion BP2 levels observed in human serum from Wilmington, NC (median = 2.7 ng/ml), a community where upstream industrial PFAS discharges in the Cape Fear River are known to impact finished drinking water³⁶. These concentrations in CFRE bird blood are also approximately two orders of magnitude higher than concentrations observed in humans surrounding a fluoropolymer production site in China (median = 0.097 ng/ml), which were found to be associated with altered glucose, cholesterol, and other enzymes and proteins in human serum³⁷.

Mean PFO5DoDA concentrations in blood of CFRE chicks (mean = 27 ng/ml) were about 90 times higher than concentrations measured in human serum from Wilmington residents (median = 0.3 ng/ml). Human populations in Cape Fear communities are exposed via air, drinking water, and any fish consumed from recreational fishing, while regional seabirds are wholly reliant on the

ecosystem for both food and water throughout the duration of their occupancy. Beyond differential reliance on the natural system, the higher observed concentrations of PFEAs in birds may be related to dose as a function of body weight and maternal offloading, with the smaller birds evidencing higher tissue concentrations when subjected to the same air/water dose.

Relative body burdens by tissue and body weight

We derived relative body burdens (RBBs) as the product of PFAS concentrations, organ weights, and total body weight (Eq. S1). The highest RBB of PFOS was found in liver, followed by blood and muscle (Fig. S2). In muscle, the high RBB of PFOS is driven by the large mass fraction of this component (~15 – 20% of total body weight) whereas in blood (~10% of total body weight) and liver (4 – 6% of total body weight) the large RBB is the product of the relatively large mass fraction of these compartments in tandem with the demonstrated affinity of PFOS for specific uptake mechanisms in these tissues including albumin, L-FABP, and membrane phospholipids^{17,19,38}.

RBBs observed in CFRE chicks suggest a chain-length dependence of PFCA accumulation in liver and brain (Fig. S2). C₉ PFNA had the highest relative mass in liver, with decreasing relative mass with increasing chain length (Fig. S2). C₁₂ PFDoDA and C₁₃ PFTrDA showed significantly decreased relative masses in liver and increased mass in brain compared to C₉ – C₁₁ PFCAs. Binding affinity of PFCAs with specific proteins in brain and blood-brain membrane transport pathways likely drives these trends. Binding displacement assays conducted using human liver fatty acid binding protein (L-FABP) suggest L-FABP interacts most

optimally with PFCAs that possess less than eleven carbon constituents, due to the rigidity of the fluorocarbon tail and the size of the L-FABP active site pocket¹⁹, while associations with phospholipid or pathways that act upon phospholipids have been shown to disproportionately prefer long-chain PFAAs, leading to increased concentrations of these in brain^{14,17}

The largest RBBs of Nafion BP2, PFO4DA, PFO5DoDA, and HFPO-DA were found in blood, driven by the large mass fraction of this compartment coupled to relatively high concentrations of PFEAs in whole blood (Fig. S2).

Legacy and novel PFAS in brain

10 compounds were detected in at least one brain sample and 8 detected in at least 50% of brain samples, demonstrating that multiple PFAS can migrate across the blood-brain barrier. PFOS displayed the highest mean concentration in brain, followed by Nafion BP2 >> PFUnDA > PFDA > PFTTrDA = PFO5DoDA > PFDaA > PFO4DA (Table S2). PFCAs made up the largest fraction observed in brain (44% of total concentration across all birds), with PFUnDA found at the highest mean concentration, with mean concentrations of 13, 9, and 3 ng/g in brains from CFRE, Massachusetts Bay, and Narragansett Bay individuals, respectively. The dominance of PFCAs was particularly notable in bird brains from Massachusetts Bay and Narragansett Bay individuals in which [PFOS] was lower, with PFCAs constituting a mean of 57% of total concentrations observed in brains (n = 6) (Table S5). This implies PFOS uptake into brain tissue is less consistent compared to PFCA uptake into brain tissue, which showed consistent brain tissue uptake across variable and distinct habitat exposure scenarios.

Pairwise concentration comparison suggests PFTrDA is most preferentially taken up into the brain compared to structurally analogous C₉ – C₁₂ PFCAs (Table S3). These observations are in agreement with results from marine mammals demonstrating the preference of long-chain PFCAs in brain¹⁴, hypothesized to be driven by phospholipid-mediated uptake.

Nafion byproduct-2, PFO4DA, and PFO5DoDA were all detected in brain tissue of individuals from the CFRE, with detection frequencies of 80%, 60%, and 100% of brain samples, respectively. Migration across the blood-brain barrier (BBB) is highly restricted for ionic species like PFAAs and PFEAs, and is thought to occur via active transport pathways³⁹. OATP1A2 is a key BBB influx transporter in mammals, and has been previously demonstrated to reject PFOA as a substrate⁴⁰. Instead, PFOA, PFNA, and PFDA inhibit uptake of the native substrate E3S by OATP1A2 when subjected to a mixture of C₄-C₁₂ PFCAs at a concentration of 100 μ M⁴⁰. However, empirical observations in marine mammals and birds demonstrate the presence of long-chain PFCAs, PFOS, and PFDS in brain tissue, suggesting BBB transport of PFCAs with C >9 or PFSAs with C $\geq$ 8 is possible, whether by OATP1A2 or another unspecified transport pathway related to or mediated by phospholipid interactions^{9,13,14}. Our data highlight that ether-based PFAS chemistries are also transported through this highly selective membrane.

Our results represent the first evidence that PFEAs are able to migrate across the blood-brain barrier in vertebrate wild animals subject to environmentally relevant PFEA exposures. We note that shifts in production towards ether-based

PFAS may not translate to reduced tissue accumulation in vertebrate wildlife receptors like seabirds, nor a reduction in any associated adverse effects of PFAS given ether-based chemicals are readily transported to multiple body compartments and sensitive tissues such as the brain. Data from polar bears¹⁶, frogs⁴¹, and mice⁴² suggest some legacy PFAS (PFOS, PFCAs) are associated with alterations of neurochemical signaling and proteins critical for brain development, with uncertain long-term implications. Modeling suggests ether-based PFAS have similar or higher toxic potency compared to PFAAs⁴³. Further research is required to examine PFEA impacts in the brain, given their proven ability to migrate into this tissue compartment.

Decreased preference of PFO5DoDA for brain

Concentration ratios facilitate the evaluation of compound partitioning between different tissues. While tissue concentrations are determined by exposure levels, distribution among organs probably reflects multiple pathways of varying importance. This leads to similar ratios between tissues subject to similar exposure and distribution pathways and divergent ratios between tissues subject to dissimilar PFAS exposure. Using ratios, we note that PFO5DoDA, a PFOA substitute with a backbone incorporating 6 carbons and 5 oxygens in ether linkages, partitions differently in brain than in other tissues when contrasted with PFUnDA, PFTrDA, PFOS, and Nafion BP2 (Fig. 4, Table S3).

This suggests a combination of structural features, such as the inclusion of multiple ether linkages (>2) that increase backbone flexibility, the molecular size of PFO5DoDA (11-member backbone), and/or the -COOH head group, reduce

transport across the BBB compared to other examined PFAAs and the PFESA Nafion BP2. We consider the ether-linkages, molecular size, and/or increased backbone flexibility of PFO5DoDA to be the most important features for its accumulation in brain, given that the -COOH functional group is also present in long-chain PFCAs that preferentially partition to brain. The ether-based chemicals distribute across most other tissues in a similar manner as long-chain PFAA analogues, with a preference for blood and kidney apparent in some comparisons (Table S3).

Relative sorption strength of PFEAs to proteins

The high PFAS concentrations observed in blood suggests albumin may offer increased binding opportunity for PFEAs via its multiple binding sites with distinct binding affinities that favor a range of PFAS chain lengths and functional groups^{44,45}. The flexible binding opportunities afforded by albumin is in contrast to the chain-length and charge-mediated interactions between PFAAs and the primary binding site of L-FABP¹⁹. Binding of PFCAs and PFSAs within the primary L-FABP cavity has been demonstrated to heavily rely on hydrophobic interactions, which may be altered with PFEAs that incorporate multiple ether linkages associated with alterations in dipole distribution⁴⁵.

Quantum chemical calculations performed via COSMOconf/TURBOMOLE confirm differences in surface charge density between the lowest energy PFEA conformations compared to their respective PFAA homologues (Table S7). Nafion BP2 possessed increased probabilities of positive surface charge density compared to PFOS, and reduced probabilities of neutral surface charge; given the

sensitivity of binding cavity interactions to charge, we hypothesize that reduced ability to engage in hydrophobic interactions may help explain the reduced presence of Nafion BP2 in liver compared to blood. However, PFO5DoDA possessed an increased probability of neutral surface charge compared to PFOA, but was found at higher concentrations in blood compared to liver. Steric effects may drive differences in binding of PFO5DoDA to L-FABP compared to C8 - C10 PFAAs, which were found at higher concentrations in liver compared to blood in CFRE individuals. Sheng et al. (2018) found that several ether- and chlorine-containing emerging PFAS were structurally distorted during the binding process in orientations distinct from the “head-out” binding observed for PFAAs; they also interacted with a different suite of amino acid residues⁴⁶.

Based on both calculated and observational results, we suggest multi-ether PFEAs engage with the L-FABP active site in a way distinct from the structural deformation and binding interactions observed between L-FABP and long-chain PFAAs. These differences may reduce the favorability of interaction in the primary L-FABP cavity, leading to reduced liver concentrations compared to blood (Figs. S2, S3). Alternatively, phospholipid binding is an additionally important mechanism driving PFAS accumulation in liver, in tandem with L-FABP interactions¹⁸. The multi-ether PFAS, with altered electron densities or conformational behavior compared to PFAAs, may be less likely to engage with uptake pathways that favor phospholipid-like species with a distinctly hydrophobic tail and a hydrophilic head group. Little research to date has examined the binding behavior of multi-ether PFEAs, which is needed

considering their burgeoning environmental relevance and continued incorporation in industrial processes.

Implications

This study documents the presence of PFAAs and PFEAs in multiple tissues and organs of chick and juvenile seabirds, including the first documentation of PFEAs in brain. Multi-ether PFEAs were most abundant in blood rather than liver, suggesting compounds containing multiple ether linkages may find more flexible binding sites within serum albumin compared to the primary active site of L-FABP. These important findings contribute to the prediction and testing of potential adverse effects and mechanisms related to PFAS in avian kidney, lungs, and brain. This work also provides the first estimates of PFEA internal distribution behavior in vertebrate wildlife with implications for other systems and receptors subject to similar environmental PFEA exposures due to ongoing production and use of these compounds. Overall, we caution that an industrial switch to PFEAs is unlikely to lead to reduced bioaccumulation in vertebrate wildlife (or humans for that matter), and is cause for concern and continued investigation. Our results reinforce the benefits of using birds as sentinels of bioaccumulation and their utility to better understand internal distributions of emerging and legacy PFAS, and identify target tissues of concern.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Additional summary data, analyte list (Excel workbook)

Additional text describing methods, quality assurance, and providing additional figures in support of the text (PDF)

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The manuscript was primarily drafted by A. Robuck and finalized through contributions of all authors. Sample analysis and data interpretation were carried out through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

A. Robuck acknowledges support from the National Oceanic and Atmospheric Administration Dr. Nancy Foster Scholarship program, the Robert and Patricia Switzer Foundation, the STEEP Superfund Research Program (NIEHS Award Number P42ES027706), and the Oak Ridge Institute for Science and Education (ORISE) program.

ACKNOWLEDGMENTS

The authors are indebted to the Northeast Fisheries Observer Program, Gina Shield, Johanna Pedersen, Michael Moore, Lindsay Addison, Matthew McIver, the Wildlife Clinic of Rhode Island, and Kevin Powers for assistance obtaining and necropsying bird samples used in this study. The authors also thank Detlef

Knappe for connecting members of this team to bring the study to fruition. The authors also acknowledge Michaela Cashman, Diane Nacci, and Steve Rego for critical feedback prior to peer review. The TOC art was created with BioRender.com, with the tern photo provided courtesy of Lindsay Addison. The authors also thank Andrea Ebert and Kai Uwe Goss for assistance with COSMOconf analysis and interpretation. Although EPA employees contributed to this article, the research presented was not funded by EPA and was conceived, designed, and implemented by URI. EPA's role was limited to advising PFAS analysis and therefore not subject to EPA's quality system requirements. Consequently, the views, interpretations, and conclusions expressed in the article are solely those of the authors and do not necessarily reflect or represent EPA's views or policies.

ABBREVIATIONS

PFOS – Perfluorooctanesulfonic acid

PFAAs – Perfluoroalkyl acids

PFEAs – Per- and polyfluoroalkyl ether acids

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TABLES AND FIGURES

Figure 4.1. Detection frequencies by habitat and tissue type, including 15 compounds that were found at least two samples. See SI for compound abbreviation definitions.

Figure 4.2. Mean percentages of three subclasses of PFAS observed in eight tissues, averaged across all habitats (left) and solely considering CFRE individuals (right). Percentages reported reflect the concentration of the given PFAS group, divided by the total concentration (in ng/g) observed in a given tissue. The proportion of PFSA in each tissue is majorly dominated by PFOS. PFEA proportions are driven by Nafion BP2. Proportions stratified by habitat and compound are presented in the SI, along with relative mass weighted proportions.

Figure 4.3. Mean concentrations and standard errors of each compound, observed in eight tissues, stratified by habitat as indicated by point and line color.

Figure 4.4. Ratios of PFAS concentrations in six tissues from CFRE individuals, with A) presenting concentrations of PFO5DoDA divided by PFUnDA and B) presenting concentrations of Nafion BP2 divided by concentrations of PFO5DoDA. The blue line within each box plot indicates the arithmetic mean while the black line indicates the median. The box hinges represent the first and third quartiles, and the whiskers indicate 1.5 times the interquartile range. Outliers are indicated by black dots. The red line indicates the mean of each concentration ratio including all tissues except brain. Common letters above each boxplot indicate tissues that share statistically indistinguishable concentration ratios as determined using Tukey's post-hoc test.

SUPPORTING INFORMATION

TISSUE-SPECIFIC DISTRIBUTION OF LEGACY AND NOVEL PER- AND POLYFLUOROALKYL SUBSTANCES IN JUVENILE SEABIRDS

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Wiley¹, and Rainer Lohmann[†]*

This document contains 3 figures and 7 tables

Chemicals, reagents, and materials. Analytical standards of greater than 99.9% purity, including mass-labeled surrogates, were purchased from Wellington Laboratories (Guelph, ON, Canada). Native Nafion BP2 and PFO5DoDA were provided by Chemours (Fayetteville, NC) in lieu of a commercial source. HPLC grade methanol, acetonitrile, and formic acid were purchased from Fisher Scientific (Waltham, MA, USA). Ultrapure water for equipment cleaning was obtained from a Milli-Q system fit with an HPLC water polisher or via HPLC grade water purchased from Fisher Scientific (Waltham, MA, USA). Ammonium formate was purchased from Fisher Scientific (Waltham, MA, USA).

Sample collection details. All birds were obtained opportunistically for use in this study, and no birds were killed for the purposes of this study. Birds were collected and stored in accordance with URI Biosafety standards and the Migratory Bird Treaty Act.

Dead Great Shearwater juveniles (*Ardenna gravis*) were obtained as bycatch from the National Oceanic and Atmospheric Administration (NOAA) Northeast Fisheries Observer Program (Falmouth, MA). Dead Herring Gull (*Larus argentatus smithsonianus*) chicks were obtained from the Wildlife Clinic of Rhode Island (Narragansett, RI). Dead Royal Tern (*Thalasseus maximus*), Sandwich Tern (*Thalasseus sandvicensis*), Laughing Gull (*Leucophaeus atricilla*), and Brown Pelican (*Pelecanus occidentalis*) chicks were obtained as part of routine field and nest surveys conducted by North Carolina Audubon (Wilmington, NC).

Figure S4.1. Map of the US Atlantic East Coast. Insets provide further detail about collection locations of from each habitat. Great shearwaters were collected as bycatch in Massachusetts Bay and off Cape Cod, designated as NOAA Fisheries Stat Areas 514 and 521. Stellwagen Bank National Marine Sanctuary, a key foraging area for Great shearwaters, is outlined in white and indicated as “SB” in the top right inset. Herring gull chicks came from nests located in Narragansett, RI and Newport, RI. The bottom inset shows the Cape Fear River and Estuary system, located in southeastern NC. A Chemours facility is located

near Fayetteville, NC, designated on the map as a white star. The red box indicates the lower river and estuary portion of the system; CFRE chicks were hatched on dredge islands near the mouth of the estuary.

The rationale supporting the use of similar seabird species to discuss bioaccumulation was presented elsewhere¹.

Sample preparation for HRMS. A tissue aliquot was diluted with three parts water and homogenized to a uniform slurry. An aliquot of homogenate was diluted 4:1 with 0.1M formic acid and vortexed for 30 seconds. The denatured extract was further diluted 5:1 with cold acetonitrile and centrifuged for 5 min at 10000 rpm. An aliquot of the supernatant was removed and combined with dilute ammonium formate buffer (2.5mM) to obtain a final sample extract ratio of 3 parts aqueous: 1 part organic extract.

HRMS analysis. 100 ul of sample was injected, and chromatographic separation achieved using a Vanquish UPLC system equipped with an Accucore 100 mm reverse-phase C18 column, at a flow rate of 300 ul/min. Mobile phase constituents included Solvent A (95:5 water: acetonitrile) and Solvent B (95:5 acetonitrile: water). Mobile phase gradient is detailed in Table S11. Nafion byproduct 2 (Nafion BP2, or 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-ethanesulfonic acid) and PFO₅DoDA (2,2,4,4,6,6,8,8,10,10,12,12,12-Tridecafluoro-3,5,7,9,11-pentaoxadodecanoic acid) were identified with authentic native standards, while PFO₄DA (perfluoro-3,5,7,9-tetraoxadecanoic acid), PFMA (2,3,3,3-tetrafluoro-2-(trifluoromethoxy)-propanoic acid), PFO₂HxA (perfluoro-3,5-dioxahexanoic acid), PEPA (Perfluoro-2-ethoxypropanoic acid), PFO₃OA (Perfluoro(3,5,7-

trioxaoctanoic) acid), NVHOS (1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoroethoxy)ethane sulfonic acid), Nafion byproduct 4 (2,2,3,3,4,5,5,5-4-(1,1,2,2-tetrafluoro-2- sulfoethoxy) pentanoic acid), Nafion byproduct 1 (2-[1-[difluoro[(1,2,2-trifluoroethenyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-ethanesulfonic acid) and PFECHS (perfluoro-4-ethylcyclohexanesulfonate) were detected using previous accurate mass assessment information¹. PFAS were detected using a Thermo Orbitrap Fusion mass spectrometer using heated electrospray ionization in negative mode (Thermo Fisher Scientific, Waltham, MA, USA). Full scan accurate mass spectra were acquired from 70 to 700 Da with a resolving power of 120,000 Rs for MS1 and at 30,000 Rs for MS2, and a mass accuracy of ± 5 ppm. Data-dependent acquisition was carried out to acquire MS/MS of select features at a resolving power of 30000. The ion transfer tube was set at 250°C and vaporizer temp set at 30 °C. Interference from the tissue matrix prevented the use of the Fusion internal lock mass in this experimental method. Data acquisition and analysis was performed using Xcalibur and Compound Discoverer software (Thermo Fisher Scientific, Waltham, MA, USA).

Quality Assurance and Quality Control

Six-point, processed and matrix-matched calibration curves were prepared. Curve preparation entailed taking multiple aliquots of “blank” liver tissue (from the same batch of slurried tissue confirmed to be mostly clean of PFAS by HRMS analysis) through each extraction in its entirety. The matrix-matched approach or another approach to account for matrix effects is vital to account for the influence

or interference of biological co-eluent on PFAS response and derived concentrations^{2,3}.

HRMS curve points were spiked before extraction, and therefore recovery-corrected all subsequent quantitation; these curve points ranged from 0.05 -10 ng/ml. PFOS was consistently found in organic chicken liver samples, and thus the curve was corrected for background levels of PFOS by subtracting the average of measured background samples (n = 6) from PFOS responses measured in curve point samples. All calibration curves used for quantitation demonstrated an $R^2 \geq 0.98$, with most demonstrating an $R^2 \geq 0.99$.

During HRMS analysis, duplicate process blanks using formic acid and acetonitrile were prepared daily with each sample set, for a total of 8 process blanks. Process blanks were used to identify contamination introduced via sample preparation and instrument background signal. Mobile phase blanks were injected between different types of samples and different tissue types to monitor instrumental background noise and any carryover between samples. No contamination of emerging PFAS was apparent in process blanks; HFPO-DA and several other legacy PFCAs displayed high levels of instrumental background noise as displayed in instrumental blanks and process blanks. No significant background noise was apparent for Nafion BP2, PFO₅DoDA, or PFO₄DA, the three PFEAs of interest reported here using HRMS analysis. Samples were analyzed in four batches, and curve stability monitored between runs. Curve responses at all levels varied less than 4% across all four runs for the three PFEAs reported using HRMS measurements in this analysis.

Quantification of emerging compounds via HRMS was limited to those samples above the linear range of the calibration curve; the method reporting limit was determined by comparison to blank values plus three times the standard deviation of blank responses or the lowest calibration curve point. Samples below method detection limits were assumed to be zero for summation purposes.

PFOS concentrations were previously measured in liver via both HRMS and LC-MS/MS for parity¹, and HRMS results were generally found to be within 30% of LC-MS/MS results and all were within an order of magnitude. Duplicate samples were processed for each tissue matrix, and were found to be within 40% of each other, with most samples within 15% of each other.

Relative body burdens

Relative body burdens (RBBs) were calculated, based on an equation modified from Shi et al. following Equation 1⁴:

$$\text{Equation 1: } RBB = \frac{C_{tissue} \times m_{tissue}}{\text{Total body weight}} \times 100$$

Tissue weights and body weights were measured for each organ in each individual. Organ weights that could not be completely measured (blood, fat, muscle) were approximated based on literature values or estimates for seabirds or other avifauna.

Figure S4.2. RBBs by habitat. Relative body burdens (RBBs) of each PFAS by tissue compartment, stratified by habitat.

Figure S4.3. TBRs by Habitat. Tissue-blood ratios of each PFAS by tissue compartment; point color denotes habitat, while the black bars delineate the standard error of the mean.

Figure S4.4. Proportions by Tissue. Mean percentages of three subclasses of PFAS observed in eight tissues, averaged across all habitats (left) and solely considering CFRE individuals (right). Percentages reported reflect the concentration of the given PFAS group, divided by the total concentration (in ng/g) observed in a given tissue. The proportion of PFSA in each tissue is majorly dominated by PFOS. PFEA proportions are driven by Nafion BP2. Proportions stratified by habitat and compound are presented in the SI, along with relative mass weighted proportions.

Tissue	Habitat	Mean	Std. Dev	Std. Error	Min	Max	Mean by Tissue
Blood	CFRE	8.9	41.5	2.6	0.0	479.3	4.2
Blood	Mass. Bay	0.7	2.8	0.3	0.0	17.8	
Blood	Narra. Bay	3.1	9.8	1.2	0.0	48.3	
Brain	CFRE	8.6	35.8	2.3	0.0	356.6	3.7
Brain	Mass. Bay	1.7	4.8	0.6	0.0	28.1	
Brain	Narra. Bay	0.6	2.1	0.2	0.0	10.3	
Fat	CFRE	2.3	13.3	1.2	0.0	139.1	0.8
Fat	Mass. Bay	0.1	0.5	0.1	0.0	4.0	
Fat	Narra. Bay	0.0	0.0	0.0	0.0	0.0	
Heart	CFRE	6.1	20.6	1.2	0.0	165.5	2.3
Heart	Mass. Bay	0.6	2.4	0.2	0.0	17.1	
Heart	Narra. Bay	0.1	1.0	0.1	0.0	8.6	
Kidney	CFRE	9.5	33.3	2.4	0.0	255.8	4.0
Kidney	Mass. Bay	1.5	4.4	0.5	0.0	21.9	
Kidney	Narra. Bay	1.0	4.4	0.5	0.0	32.3	
Liver	CFRE	9.1	36.6	1.9	0.0	384.0	6.3
Liver	Mass. Bay	2.5	8.1	0.5	0.0	62.7	
Liver	Narra. Bay	7.3	34.3	2.2	0.0	380.7	
Lungs	CFRE	7.3	26.4	2.0	0.0	258.5	3.8
Lungs	Mass. Bay	2.8	10.7	1.1	0.0	80.1	
Lungs	Narra. Bay	1.4	3.9	0.5	0.0	24.2	
Muscle	CFRE	4.6	15.7	0.9	0.0	136.1	2.3
Muscle	Mass. Bay	0.5	3.5	0.4	0.0	25.0	
Muscle	Narra. Bay	1.8	9.4	1.1	0.0	75.7	

Table S.4.1. Table presenting summary statistics of the sum PFAS in each tissue, stratified by habitat.

Table S4.2. Table presenting summary statistics of the each PFAS identified in at least one tissue in eight different tissues, stratified by habitat.

Habitat	Tissue	Ratio	Mean Ratio Value
CFRE	Brain	Nat BPSPFDA	4.24
CFRE	Kidney	Nat BPSPFDA	0.40
CFRE	Liver	Nat BPSPFDA	0.71
CFRE	Lungs	Nat BPSPFDA	0.27
CFRE	Liver	Nat BPSPFNA	1.96
CFRE	Blood	Nat BPSPFOSQDA	0.71
CFRE	Brain	Nat BPSPFOSQDA	5.11
CFRE	Heart	Nat BPSPFOSQDA	1.64
CFRE	Kidney	Nat BPSPFOSQDA	0.27
CFRE	Liver	Nat BPSPFOSQDA	1.20
CFRE	Lungs	Nat BPSPFOSQDA	0.20
CFRE	Muscle	Nat BPSPFOSQDA	1.70
CFRE	Blood	Nat BPSPFOS	0.27
CFRE	Brain	Nat BPSPFOS	0.40
CFRE	Heart	Nat BPSPFOS	0.40
CFRE	Kidney	Nat BPSPFOS	0.56
CFRE	Liver	Nat BPSPFOS	0.09
CFRE	Lungs	Nat BPSPFOS	0.60
CFRE	Muscle	Nat BPSPFOS	0.47
CFRE	Lungs	Nat BPSPFTGA	0.65
CFRE	Brain	Nat BPSPFUSA	3.40
CFRE	Kidney	Nat BPSPFUSA	0.58
CFRE	Liver	Nat BPSPFUSA	1.58
CFRE	Lungs	Nat BPSPFUSA	0.27
CFRE	Liver	PFDAFNA	2.42
CFRE	Blood	PFDAFFOSQDA	0.62
CFRE	Brain	PFDAFFOSQDA	1.68
CFRE	Heart	PFDAFFOSQDA	0.63
CFRE	Kidney	PFDAFFOSQDA	0.84
CFRE	Liver	PFDAFFOSQDA	1.55
CFRE	Lungs	PFDAFFOSQDA	0.84
CFRE	Muscle	PFDAFFOSQDA	0.98
CFRE	Blood	PFDAFOS	0.11
CFRE	Brain	PFDAFOS	0.12
CFRE	Heart	PFDAFOS	0.13
CFRE	Kidney	PFDAFOS	0.12
CFRE	Liver	PFDAFOS	0.15
CFRE	Lungs	PFDAFOS	0.12
CFRE	Muscle	PFDAFOS	0.11
CFRE	Lungs	PFDAFTGA	2.64
CFRE	Brain	PFDAFFUSA	0.74
CFRE	Kidney	PFDAFFUSA	1.32
CFRE	Liver	PFDAFFUSA	1.83
CFRE	Lungs	PFDAFFUSA	0.68
CFRE	Brain	PFDAFFDA	0.55
CFRE	Kidney	PFDAFFDA	0.19
CFRE	Liver	PFDAFFDA	0.11
CFRE	Lungs	PFDAFFDA	0.29
CFRE	Liver	PFDAFFNA	0.24
CFRE	Blood	PFDAFFOSQDA	0.10
CFRE	Brain	PFDAFFOSQDA	0.92
CFRE	Heart	PFDAFFOSQDA	0.96
CFRE	Kidney	PFDAFFOSQDA	0.16
CFRE	Liver	PFDAFFOSQDA	0.17
CFRE	Lungs	PFDAFFOSQDA	0.23
CFRE	Muscle	PFDAFFOSQDA	0.14
CFRE	Blood	PFDAFOS	0.02
CFRE	Brain	PFDAFOS	0.01
CFRE	Heart	PFDAFOS	0.06
CFRE	Kidney	PFDAFOS	0.02
CFRE	Liver	PFDAFOS	0.01
CFRE	Lungs	PFDAFOS	0.03
CFRE	Muscle	PFDAFOS	0.02
CFRE	Lungs	PFDAFTTGA	0.67
CFRE	Brain	PFDAFFUSA	0.36
CFRE	Kidney	PFDAFFUSA	0.23
CFRE	Liver	PFDAFFUSA	0.17
CFRE	Lungs	PFDAFFUSA	0.29
CFRE	Brain	PFNAFFDA	0.60
CFRE	Kidney	PFNAFFDA	0.26
CFRE	Liver	PFNAFFDA	0.46
CFRE	Lungs	PFNAFFDA	0.24
CFRE	Blood	PFNAFFOSQDA	0.04
CFRE	Brain	PFNAFFOSQDA	0.60
CFRE	Heart	PFNAFFOSQDA	0.07
CFRE	Kidney	PFNAFFOSQDA	0.19
CFRE	Liver	PFNAFFOSQDA	0.77
CFRE	Lungs	PFNAFFOSQDA	0.19
CFRE	Muscle	PFNAFFOSQDA	0.18
CFRE	Blood	PFNAFOS	0.01
CFRE	Brain	PFNAFOS	0.00
CFRE	Kidney	PFNAFOS	0.01
CFRE	Liver	PFNAFOS	0.03
CFRE	Lungs	PFNAFOS	0.03
CFRE	Muscle	PFNAFTTGA	0.62
CFRE	Brain	PFNAFFUSA	0.60
CFRE	Kidney	PFNAFFUSA	0.34
CFRE	Liver	PFNAFFUSA	0.84
CFRE	Lungs	PFNAFFUSA	0.26
CFRE	Brain	PFODAFFDA	0.04
CFRE	Kidney	PFODAFFDA	0.02
CFRE	Liver	PFODAFFDA	0.01
CFRE	Lungs	PFODAFFDA	0.03
CFRE	Liver	PFODAFFNA	0.03
CFRE	Blood	PFODAFPOSQDA	0.02
CFRE	Brain	PFODAFPOSQDA	0.05
CFRE	Heart	PFODAFPOSQDA	0.02
CFRE	Kidney	PFODAFPOSQDA	0.02
CFRE	Liver	PFODAFPOSQDA	0.01
CFRE	Lungs	PFODAFPOSQDA	0.02
CFRE	Muscle	PFODAFPOSQDA	0.02
CFRE	Blood	PFODAFPOS	0.01
CFRE	Brain	PFODAFPOS	0.00
CFRE	Heart	PFODAFPOS	0.01
CFRE	Kidney	PFODAFPOS	0.00
CFRE	Liver	PFODAFPOS	0.00
CFRE	Lungs	PFODAFPOS	0.00
CFRE	Muscle	PFODAFPOS	0.00
CFRE	Lungs	PFODAFPTGA	0.07
CFRE	Brain	PFODAFFFUSA	0.03
CFRE	Kidney	PFODAFFFUSA	0.03
CFRE	Liver	PFODAFFFUSA	0.02
CFRE	Lungs	PFODAFFFUSA	0.12
CFRE	Brain	PFODDAFFFDA	0.03
CFRE	Kidney	PFODDAFFFDA	1.48
CFRE	Liver	PFODDAFFFDA	1.63
CFRE	Lungs	PFODDAFFFDA	1.41
CFRE	Liver	PFODDAFFNA	2.05
CFRE	Blood	PFODDAFPOS	0.33
CFRE	Brain	PFODDAFPOS	0.08
CFRE	Heart	PFODDAFPOS	0.24
CFRE	Kidney	PFODDAFPOS	0.19
CFRE	Liver	PFODDAFPOS	0.10
CFRE	Lungs	PFODDAFPOS	0.18
CFRE	Muscle	PFODDAFPOS	0.20
CFRE	Lungs	PFODDAFFTGA	3.75
CFRE	Brain	PFODDAFFUSA	0.63
CFRE	Kidney	PFODDAFFUSA	1.97
CFRE	Liver	PFODDAFFUSA	1.47
CFRE	Lungs	PFODDAFFUSA	1.63
CFRE	Brain	PFODFFDA	8.34
CFRE	Kidney	PFODFFDA	8.69
CFRE	Liver	PFODFFDA	8.18
CFRE	Lungs	PFODFFDA	8.83
CFRE	Liver	PFODFFNA	19.41
CFRE	Blood	PFODSPFOSQDA	4.54
CFRE	Brain	PFODSPFOSQDA	16.01
CFRE	Heart	PFODSPFOSQDA	5.34
CFRE	Kidney	PFODSPFOSQDA	7.47
CFRE	Liver	PFODSPFOSQDA	12.04
CFRE	Lungs	PFODSPFOSQDA	7.05
CFRE	Muscle	PFODSPFOSQDA	9.16
CFRE	Lungs	PFODSPFTGA	22.48
CFRE	Brain	PFODSPFUSA	6.65
CFRE	Kidney	PFODSPFUSA	10.93
CFRE	Liver	PFODSPFUSA	14.97
CFRE	Lungs	PFODSPFUSA	9.38
CFRE	Brain	PFODAFFDA	0.92
CFRE	Kidney	PFODAFFDA	0.25
CFRE	Liver	PFODAFFDA	0.10
CFRE	Lungs	PFODAFFDA	0.41
CFRE	Liver	PFODAFFNA	0.22
CFRE	Blood	PFODAFFOSQDA	0.13
CFRE	Brain	PFODAFFOSQDA	1.61
CFRE	Heart	PFODAFFOSQDA	0.07
CFRE	Kidney	PFODAFFOSQDA	0.20
CFRE	Liver	PFODAFFOSQDA	0.16
CFRE	Lungs	PFODAFFOSQDA	0.35
CFRE	Muscle	PFODAFFOSQDA	0.25
CFRE	Blood	PFODAFPOS	0.02
CFRE	Brain	PFODAFPOS	0.10
CFRE	Heart	PFODAFPOS	0.01
CFRE	Kidney	PFODAFPOS	0.03
CFRE	Liver	PFODAFPOS	0.01
CFRE	Lungs	PFODAFPOS	0.05
CFRE	Muscle	PFODAFPOS	0.01
CFRE	Brain	PFODAFFUSA	0.61
CFRE	Kidney	PFODAFFUSA	0.30
CFRE	Liver	PFODAFFUSA	0.16
CFRE	Lungs	PFODAFFUSA	0.43
CFRE	Brain	PFUAFFDA	1.41
CFRE	Kidney	PFUAFFDA	0.78
CFRE	Liver	PFUAFFDA	0.68
CFRE	Lungs	PFUAFFDA	0.65
CFRE	Liver	PFUAFFNA	1.30
CFRE	Blood	PFUAFFOSQDA	0.42
CFRE	Brain	PFUAFFOSQDA	2.35
CFRE	Heart	PFUAFFOSQDA	0.46
CFRE	Kidney	PFUAFFOSQDA	0.66
CFRE	Liver	PFUAFFOSQDA	0.86
CFRE	Lungs	PFUAFFOSQDA	0.80
CFRE	Muscle	PFUAFFOSQDA	1.44
CFRE	Blood	PFUAFPOS	0.08
CFRE	Brain	PFUAFPOS	0.16
CFRE	Heart	PFUAFPOS	0.09
CFRE	Kidney	PFUAFPOS	0.09
CFRE	Liver	PFUAFPOS	0.07
CFRE	Lungs	PFUAFPOS	0.11
CFRE	Muscle	PFUAFPOS	0.11
CFRE	Lungs	PFUAFPTGA	2.42

Table S4.3. Table presenting non-zero and non-infinite pairwise ratios in each tissue, stratified by habitat.

Compound	CAS #	Family	# Fluorinated Carbons	Detected in at least one biological sample above detection limits?	Quantified, using matched native standard or Semi-Quant?
N-MeFOSAA	2355-31-9	FASAA	8	No	Quantified, using matched native standard
N-EtFOSAA	2991-50-6	FASAA	8	No	Quantified, using matched native standard
FOSA	754-91-6	FASA	8	No	Quantified, using matched native standard
4:2 FTS		FTS	4	No	Quantified, using matched native standard
6:2 FTS		FTS	6	No	Quantified, using matched native standard
8:2 FTS		FTS	8	No	Quantified, using matched native standard
PFBA	375-22-4	PFCA	3	Yes	Quantified, using matched native standard
PFPeA	2706-90-3	PFCA	4	Yes	Quantified, using matched native standard
PFHxA	307-24-4	PFCA	5	Yes	Quantified, using matched native standard
PFHpA	375-85-9	PFCA	6	No	Quantified, using matched native standard
PFOA	335-67-1	PFCA	7	Yes	Quantified, using matched native standard
PFNA	375-95-1	PFCA	8	Yes	Quantified, using matched native standard
PFDA	335-76-2	PFCA	9	Yes	Quantified, using matched native standard
PFUnDA	2058-94-8	PFCA	10	Yes	Quantified, using matched native standard
PFDoA	307-55-1	PFCA	11	Yes	Quantified, using matched native standard
PFTeDA	72629-94-8	PFCA	12	Yes	Quantified, using matched native standard
PFTeDA	376-06-7	PFCA	13	No	Quantified, using matched native standard
PMPA	13140-29-9	PFECA	3	No	Semi-quant
PFO2HxA	39492-88-1	PFECA	3	No	Semi-quant
PEPA	267239-61-2	PFECA	4	No	Semi-quant
PFO3OA	39492-89-2	PFECA	4	No	Semi-quant
HFPO-DA	13252-13-6	PFECA	5	Yes	Quantified, using matched native standard
PFO4DA	39492-90-5	PFECA	5	Yes	Semi-quant
PFO5DoDA	39492-91-6	PFECA	6	Yes	Quantified, using matched native standard
Nafion BP4	na	PFESA	6	No	Semi-quant
Nafion BP2	749836-20-2	PFESA	7	Yes	Quantified, using matched native standard
Nafion BP1	29311-67-9	PFESA	7	No	Semi-quant
NVHOS (free acid)	801209-99-4	PFESA	4	No	Semi-quant
PFBS	29420-49-3	PFSA	4	Yes	Quantified, using matched native standard
PFPeS		PFSA	5	Yes	Quantified, using matched native standard
PFHxS	432-50-7	PFSA	6	Yes	Quantified, using matched native standard
PFHpS		PFSA	7	No	Quantified, using matched native standard
PFOS	4021-47-0	PFSA	8	Yes	Quantified, using matched native standard
PFNS	98789-57-2	PFSA	9	No	Quantified, using matched native standard
PFDS	2806-15-7	PFSA	10	Yes	Quantified, using matched native standard
PFECBS	646-83-3	Cyclic PFSA	8	No	Semi-quant

Table S4.4. Table presenting all analytes included in this study. Semi-quantitative analytes lacked a matched native and internal standard. Quantitative analytes were measured using at least one exact matched standard.

Tissue	Habitat	PFOS			Nafion BP2			PFODA			PFOSdOA			PFDA			PFUNDA			PFDOA			PFTOA		
		Mean %	± SE	Range (%)	Mean %	± SE	Range (%)	Mean %	± SE	Range (%)	Mean %	± SE	Range (%)	Mean %	± SE	Range (%)	Mean %	± SE	Range (%)	Mean %	± SE	Range (%)	Mean %	± SE	Range (%)
Blood	CFRE	56 ± 4.9	27.69	0-85	10 ± 4.3	0-35	0-1	0 ± 0.1	nd	0-1	16 ± 2.4	7-27	0 ± 0.2	0-2	6 ± 0.2	0-10	5 ± 0.9	0-7	1 ± 0.4	0-2	1 ± 0.5	0-3	20 ± 1.8	18-22	0-3
Blood	Mass. Bay	50 ± 0.5	60.61	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	20 ± 2.3	17-22	nd	nd	20 ± 1.8	18-22	nd	nd	0-3
Blood	CFRE	53 ± 3.6	32.46	0-65	19 ± 6.1	0-65	0-1	0 ± 0.1	nd	0-1	4 ± 0.5	2-6	nd	nd	6 ± 0	3-9	8 ± 0.8	4-12	1 ± 0.3	0-4	5 ± 1.1	0-12	nd	nd	0-3
Brain	Mass. Bay	26 ± 1.8	23-29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	21 ± 1.7	18-24	8 ± 0.3	8-10	44 ± 3.9	40-52	nd	nd	0-3
Brain	Narrm. Bay	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	17 ± 0.7	0-34	nd	nd	69 ± 17.3	40-100	nd	nd	0-3
Fat	CFRE	43 ± 15.4	0-69	7-100	48 ± 20.2	7-100	nd	nd	nd	nd	5 ± 3.1	0-12	nd	nd	2 ± 0	0-7	2 ± 1.8	0-7	nd	nd	nd	nd	nd	nd	0-3
Fat	Mass. Bay	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	61	na	nd	nd	39	na	nd	nd	0-3
Fat	Narrm. Bay	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	na	nd	nd	nd	nd	nd	nd	0-3
Heart	CFRE	59 ± 4.7	22-78	0-43	42 ± 5.6	0-43	0-1	0 ± 0.1	nd	0-1	12 ± 1	7-18	1 ± 0.3	0-2	7 ± 0.3	0-10	5 ± 0.9	0-9	1 ± 0.3	0-2	1 ± 0.3	0-3	nd	nd	0-3
Heart	Mass. Bay	53 ± 16.9	0-78	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	17 ± 0.2	0-9	nd	nd	1 ± 0.3	0-3	nd	nd	0-3
Heart	Narrm. Bay	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0-3
Kidney	CFRE	65 ± 3.8	43-79	0-9	3 ± 1.1	0-9	nd	nd	nd	nd	11 ± 1.8	5-18	2 ± 0.3	0-3	8 ± 0.3	6-9	6 ± 0.3	5-7	1 ± 0.4	0-3	2 ± 0.4	0-3	nd	nd	0-3
Kidney	Mass. Bay	55 ± 2.3	50-61	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	19 ± 0.8	17-21	nd	nd	23 ± 2.9	17-29	nd	nd	0-3
Kidney	Narrm. Bay	65 ± 5.9	58-71	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	13 ± 1.9	11-15	nd	nd	22 ± 3.9	18-26	nd	nd	0-3
Liver	CFRE	67 ± 1.6	55-73	0-19	6 ± 1.8	0-19	nd	nd	nd	nd	7 ± 0.8	3-12	4 ± 0.5	2-9	9 ± 0.5	6-13	5 ± 0.4	2-7	1 ± 0.2	0-2	1 ± 0.3	0-2	nd	nd	0-3
Liver	Mass. Bay	58 ± 3.9	37-80	4 ± 4	4 ± 4	0-44	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	16 ± 1.3	10-25	1 ± 0.7	0-7	11 ± 0.9	7-16	nd	nd	0-3
Liver	Narrm. Bay	60 ± 10.0	0-100	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0-3
Lungs	CFRE	58 ± 0.9	45-70	0-3	2 ± 0.5	0-3	0-1	0 ± 0.1	nd	0-1	10 ± 1.3	6-15	2 ± 0.3	0-3	8 ± 0.3	6-11	7 ± 0.3	5-9	2 ± 0.4	0-3	9 ± 0.3	2-12	nd	nd	0-3
Lungs	Mass. Bay	50 ± 4.4	43-62	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	18 ± 0.9	17-21	3 ± 1.8	0-7	28 ± 3.5	16-34	nd	nd	0-3
Lungs	Narrm. Bay	62 ± 19.5	36-100	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10 ± 5.2	0-17	3 ± 2.6	0-8	14 ± 7	0-21	nd	nd	0-3
Muscle	CFRE	57 ± 4.4	21-71	0-70	14 ± 6.3	0-70	0-1	0 ± 0.1	nd	0-1	11 ± 1.5	1-18	1 ± 0.3	0-2	7 ± 0.3	0-8	7 ± 1.1	0-16	1 ± 0.5	0-6	1 ± 0.4	0-3	nd	nd	0-3
Muscle	Mass. Bay	nd	nd	nd	49	na	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	51	na	nd	nd	nd	nd	nd	nd	0-3
Muscle	Narrm. Bay	38 ± 19.8	18-57	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	14 ± 3.3	11-17	nd	nd	13 ± 12.5	0-25	nd	nd	0-3

Table S4.5. Table presenting proportions of each PFAS in each tissue, stratified by habitat.

Tissue	Fraction	Mean	Std. Dev. (%)	Median (%)	Range (%)	n	Habitat
Blood	% PFCAs	15	8	16	0-27	9	CFRE
Brain	% PFCAs	23	8	24	9-36	10	CFRE
Fat	% PFCAs	4	7	0	0-14	5	CFRE
Heart	% PFCAs	16	5	16	4-22	12	CFRE
Kidney	% PFCAs	19	3	21	14-23	8	CFRE
Liver	% PFCAs	19	4	19	13-28	12	CFRE
Lungs	% PFCAs	22	3	24	17-25	7	CFRE
Muscle	% PFCAs	17	8	16	0-28	12	CFRE
Blood	% PFEAs	28	20	21	10-73	9	CFRE
Brain	% PFEAs	23	20	17	5-60	10	CFRE
Fat	% PFEAs	54	35	49	16-100	5	CFRE
Heart	% PFEAs	25	20	18	7-74	12	CFRE
Kidney	% PFEAs	15	7	13	7-24	8	CFRE
Liver	% PFEAs	13	6	11	7-24	12	CFRE
Lungs	% PFEAs	12	4	10	9-18	7	CFRE
Muscle	% PFEAs	25	21	22	4-79	12	CFRE
Blood	% PFSAs	57	15	61	27-73	9	CFRE
Brain	% PFSAs	54	13	58	32-70	10	CFRE
Fat	% PFSAs	43	31	51	0-69	5	CFRE
Heart	% PFSAs	59	16	66	22-78	12	CFRE
Kidney	% PFSAs	66	9	65	53-79	8	CFRE
Liver	% PFSAs	68	5	69	55-73	12	CFRE
Lungs	% PFSAs	66	2	66	63-70	7	CFRE
Muscle	% PFSAs	58	15	63	21-71	12	CFRE
Blood	% PFCAs	21	13	18	0-42	15	All Habitats
Brain	% PFCAs	44	31	29	9-100	16	All Habitats
Fat	% PFCAs	23	44	0	0-100	9	All Habitats
Heart	% PFCAs	26	29	17	0-100	19	All Habitats
Kidney	% PFCAs	28	11	23	14-47	15	All Habitats
Liver	% PFCAs	24	12	21	0-55	33	All Habitats
Lungs	% PFCAs	33	18	25	0-57	14	All Habitats
Muscle	% PFCAs	21	13	17	0-51	19	All Habitats
Blood	% PFEAs	19	21	16	0-73	15	All Habitats
Brain	% PFEAs	15	19	7	0-60	16	All Habitats
Fat	% PFEAs	43	39	40	0-100	9	All Habitats
Heart	% PFEAs	19	20	16	0-74	19	All Habitats
Kidney	% PFEAs	9	9	8	0-24	15	All Habitats
Liver	% PFEAs	6	10	0	0-44	33	All Habitats
Lungs	% PFEAs	6	7	4	0-18	14	All Habitats
Muscle	% PFEAs	23	22	22	0-79	19	All Habitats
Blood	% PFSAs	60	16	61	27-92	15	All Habitats
Brain	% PFSAs	41	22	45	0-70	16	All Habitats
Fat	% PFSAs	34	33	42	0-69	9	All Habitats
Heart	% PFSAs	55	27	65	0-100	19	All Habitats
Kidney	% PFSAs	63	9	61	50-79	15	All Habitats
Liver	% PFSAs	70	13	70	37-100	33	All Habitats
Lungs	% PFSAs	61	14	63	43-100	14	All Habitats
Muscle	% PFSAs	56	22	62	0-84	19	All Habitats

Table S4.6. Table presenting proportions of each PFAS family in each tissue, stratified by CFRE and all habitats combined.

% Difference			
Sigma (Range)	PFOS - Nafion BP2	PFOA - PFO5DoDA	PFOA - PFO4DA
-0.03 - -0.021	0	0	0
-0.011 - -0.02	6.96	0	0
-0.01 - -0.006	4.174	4.43	3.607
0.001 - -0.005	-17.175	16.392	2.437
0	-22.757	1.888	-7.988
0.001 - 0.005	29.071	20.393	5.465
0.006 - 0.01	0.306	1.062	1.111
0.011 - 0.02	0.298	1.157	-0.348
0.021 - 0.03	0	-0.219	-0.155

Table S4.7. Percent difference of sigma probabilities between the lowest energy conformer of each PFEA anion, compared to an appropriate low energy PFAA anion analogue. Negative sigma values correspond to a positive surface charge density, while positive values correspond to a negative surface charge density

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CHAPTER 5

PLASTIC INGESTION IN THE GREAT SHEARWATER, AN ENDEMIC ATLANTIC SEABIRD

This manuscript has been formatted for Frontiers in Marine Science, and the abstract has been accepted to a special topic issue on plastic ingestion (March 2021)

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KEYWORDS: plastic ingestion, seabirds

ABSTRACT

Limited work to date has examined plastic ingestion in temperate, endemic Atlantic seabirds like Great Shearwaters (*Ardenna gravis*), although this species is particularly prone to plastic ingestion as wide-ranging petrels. We examined 175 Great Shearwaters obtained from 2010 - 2019 in the Northwest and South Atlantic to assess accumulation of ingested plastic as well as trends over time and between locations. Over 1500 plastic fragments were documented in the proventriculus portion of the gastrointestinal tract, with an average of 8 plastic fragments per bird. Juveniles accumulated plastic more frequently than adults, with an ingestion frequency of 98% compared to 58% in breeding adults. The frequency of plastic occurrence (FO) observed in juveniles was higher than in previous studies focused on this species in prior decades. Each fragment was assessed using

Fourier-transform ion spectroscopy to discern polymer identity. We found low- and high-density polyethylene fragments dominated within all years and locations. Breeding adults contained a higher proportion of larger polypropylene fragments compared to juveniles and non-breeding adults, likely indicative of an increased use of remote, pelagic habitats subject to reduced inputs of smaller, land-derived plastics. Different signatures of polymer type and size between breeding and non-breeding mature birds likewise suggests rapid turnover of ingested plastics commensurate with migratory stage and location. No fragments of the same size (4 – 10 mm in largest dimension) were found in the primary forage fish prey (sand lance, $n = 202$) that supports Great Shearwaters in Massachusetts Bay, suggesting this species directly ingest the bulk of their plastic loads rather than accumulating via trophic transfer. This work is the first to comprehensively measure the accumulation of ingested plastics by Great Shearwaters over the last decade and across multiple locations spanning their yearly migration cycle, and emphasizes their utility as sentinels of plastic pollution in Atlantic systems.

INTRODUCTION

Mismanaged plastic waste has emerged as a globally significant pollutant in aquatic ecosystems. Current estimates suggest 4.8 – 12.7 million metric tons of mismanaged plastic waste enter the global ocean each year as debris¹, with modeling efforts suggesting the amount of plastic debris entering marine environments will increase by an order of magnitude by 2025 without significant

policy and consumer interventions. The current amount of ocean plastic and projected additional releases are problematic due to potential for plastic debris to degrade habitat integrity, entangle marine life, act as a vector of invasive species transport, or be ingested by marine fauna². These impacts are predicted in marine life at every level of the food chain due to the wide spectrum of sizes and materials constituting ocean plastic loads^{2,3}.

Marine plastic debris originates from a variety of land-based human uses and activities, as well as from maritime sources such as shipping and fishing activities, leading to inputs of variable types and sizes of plastic materials to the marine environment⁴. Yet a key unifying characteristic of marine plastic debris is its inevitable degradation into progressively smaller pieces over time, leading to proliferation of meso-, micro- and nanoplastics in all compartments of the marine environment, even within the remotest marine habitats^{5,6}.

A significant portion of microplastics (<5mm) entering the ocean are thought to become fouled⁷ and settle out of the surface water compartment into intermediate depths or ocean sediments⁸, while plastic items of variable size and composition remain buoyant. An estimated 5 - 125 trillion pieces of buoyant plastic >100 um reside within the ocean surface layer, engaging in biophysical interactions and subject to oceanic scale circulation⁹⁻¹¹. Plastic pieces within coastal and pelagic surface environments are predominantly composed of polyethylene and polypropylene¹², two of the most highly produced olefin plastics used in an array of single-use consumer and industrial products.

Abundance of small plastic pieces within ocean surface environments results in an increased probability of encounter between euphotic marine fauna and plastic items, as well as increased opportunity for ingestion of plastic items. Ingestion of plastic may occur via direct ingestion of plastic pieces¹³, or ingestion of prey items containing plastic¹⁴. In vertebrate species, ingested plastics can result in blockages or tears within the digestive tract¹⁵, reduced feeding due to false satiation¹⁶, as well as potential uptake of adsorbed chemical pollutants or innate chemical additives¹⁷.

The accumulation of ingested plastics in seabirds has been documented since the 1960s; Wilcox et al. (2015) found that 59% of seabird species examined between 1962 and 2012 had ingested plastic, and, within those studies, on average 29% of individuals had plastic in their gastrointestinal tract⁴. Seabirds in the order Procellariiformes (e.g. albatrosses, shearwaters, petrels, and storm petrels) are thought to be most vulnerable to plastic ingestion^{18,19} related to their wide-ranging pelagic habits²⁰ and heavy use of olfactory cues²¹, yet the frequency of plastic ingestion varies widely between members of this order. Some albatross species have been shown to infrequently ingest plastics, while other tube-nosed seabirds such as short-tailed shearwaters²², Northern fulmars²³, and great shearwaters²⁴ have been found to ingest plastic at high rates (~80 – 100% frequency of occurrence). Modeling and review efforts suggest the incidence of plastic ingestion in seabirds increases with abundance of plastics in the environment²⁵. For example, Northern fulmars in the North Sea have been monitored for plastic ingestion at their breeding colonies since the 1970s; the ingestion of industrial

pellets, or nurdles, within this monitored population has been found to roughly track the measured abundance of industrial pellets in ocean surface water²⁵. Efforts to curb mismanagement and loss of industrial pellets in the 1980s and 1990s resulted in reduced concentrations of nurdles within the North Atlantic subtropical gyre, and declines in the geometric mean of industrial pellets in fulmar GITs. Seabird plastic ingestion also increases with use of plastic geographical hotspots^{4,26}; subtropical gyres and the southern boundary of the Atlantic, Pacific, and Indian Oceans are considered hotspots of plastic in the surface layer. Incidence of plastic ingestion also varies according to foraging strategy, with trophic generalists and surface seizing birds reliant on crustacean diets the most vulnerable²⁷.

However, data describing the occurrence of ingested plastic as well as trends over time and space is irregular. Existing studies often focus on a narrow subset of accessible species during the breeding season when chicks are immobile and adults are subject to area-restricted foraging behaviors, limiting understanding of plastic ingestion over the complete annual cycle and overall life cycle of a diversity of species. Likewise, few studies couple measurement of plastic ingestion with comprehensive necropsy data, description of plastic piece morphology, and identification of polymer type. Assessment of plastic polymer identity is particularly valuable, as it aids in identification of potential sources or source products. Those studies that incorporate polymer analysis indicate that polyethylene and polypropylene are typically the dominant polymers ingested in seabirds, and item composition is strongly correlated to item morphology²⁸. The

polymer composition of ingested plastics is related to spatial patterns of plastic abundance, rather than a preference for ingestion of any one polymer type.

We set out to investigate the accumulation of ingested plastics in Great Shearwaters (*Ardenna gravis*), an endemic Atlantic Procellariiform seabird. The US East Coast and the Gulf of Maine are more poorly described in terms of plastic ingestion in marine biota compared to other regions, despite the high density of human populations and potential plastic sources along this coastal margin. Great Shearwaters frequent the Gulf of Maine and Massachusetts Bay along the US East Coast, and have been previously assessed for plastic ingestion. However, no comprehensive efforts have harmonized methodology and pieced together trends describing occurrence over space and time in this species. Monitoring of plastic in this species is valuable due to its abundance²⁹, well-described habitat use^{30,31}, its colocation with key marine megafauna³², and its compact representation of divergent Atlantic habits, spanning developed coastal margins within the Gulf of Maine as well as vast pelagic regions of the South Atlantic.

Here we comprehensively describe plastic ingestion in this species, using bycatch and beach-cast samples obtained over 2010 - 2019 from multiple habitats included in the annual cycle of this trans-equatorial migrant. The goal of this study included a) evaluation of ingested plastics over time, to deduce any temporal trends, b) comparison of plastic categories and polymer types found between different locations and time periods, and c) assess the trophic or environmental origin of ingested plastics.

MATERIALS AND METHODS

Study species

The Great Shearwater (*Ardenna gravis*) is a long-lived, pelagic seabird in the *Procellariiformes* order of seabirds. Generally between 600 – 1100 g, this medium-sized “tube nose” is a trans-equatorial migrant that inhabits the Gulf of Maine in the NW Atlantic during its non-breeding period over the boreal summer. Tracking and necropsy data suggest age-specific segregation of their non-breeding range, with preferential use of the SW Gulf of Maine and Massachusetts Bay by juveniles³¹. This species breeds during the boreal winter in the South Atlantic, on Gough Island, Inaccessible Island, and Nightingale Island in the St. Helena Island Group and Kidney Island in the Falkland Islands²⁹. Great Shearwaters feed on squid, crustacea, and small forage fish including sand lance (*Ammodytes* spp.)³¹, and have regularly been observed attending fishing vessels seeking fisheries offal. Most foraging activities occur within the top 10 m of the water column via surface plunging and seizing behaviors, with most dives less than 2 m in depth³³.

The accumulation of ingested plastics has been previously assessed in this seabird within its breeding and non-breeding range over the past sixty years via studies covering a single year, or a span up to 14 years, including variable numbers of individuals (n = 1 – 84). The most recent published work examined 84 individuals collected between 2000 – 2011 from Sable Island, Nova Scotia, Canada in the NW Atlantic. These efforts have generally possessed low yearly

sample sizes from one geographic region, and have been unable to assess any temporal or spatial trends. *Specimen Collection, Necropsy, and Sample Condition*

A total of 175 Great Shearwaters were analyzed for plastics, from three habitats (Fig. 1). 135 dead Great Shearwater juveniles and adults were obtained as bycatch from the National Oceanic and Atmospheric Administration (NOAA) Northeast Fisheries Observer Program (Falmouth, MA) from Massachusetts Bay. Bycatch collections spanned 2010 – 2019 and the majority of samples were collected in the months of August – November each year, after approximately 2 or more months of foraging in the NW Atlantic. 15 Great Shearwaters were obtained in 2017 from the southeastern coast of North Carolina, USA, after having washed up dead on shorelines along the Outer Banks and the Crystal Coast region of North Carolina. 25 Great Shearwaters were obtained from long-lining activities around Gough Island in 2017 - 2018, in the St Helena Island group in the south Atlantic. Each individual was frozen individually as a whole specimen upon collection and stored at -20C until necropsy.

All individuals were necropsied in a standardized manner³⁴, including measurements of morphometric characteristics, body condition, and organ weights. Age was determined following the age characterization of Great Shearwaters as defined by Powers et al. (2020)³¹, and sex based upon gonad presentation. Multiple tissues, including the entire gastrointestinal tract (GIT), were collected and frozen at -15°C until analysis. Lung hemorrhaging and edema was apparent in some bycatch seabirds (indicative of drowning), but there was no

evidence of seawater intrusion into the coelomic cavity, and no perforations or damage to other internal organs excluding lung hemorrhaging.

Analysis of accumulated, ingested plastics

Food debris was commonly found in the esophagus and mouth of sampled bycatch individuals, likely due to regurgitation during entanglement and drowning. Given the abundance of bycatch samples used in this study, the esophagus and proventriculus were not assessed for plastic contents, assuming any assessment of plastic occurrence in these compartments may be biased by regurgitation. Plastics were instead assessed in the ventriculus, or “gizzard”, a muscular organ used to grind up squid beaks and other indigestible, hard diet materials. In *Procellariiform* seabirds, the isthmus connecting the proventriculus and ventriculus is angled and narrow, unlike the more linear junctions seen in other seabirds. This unique configuration is thought to restrict the passage of materials from the ventriculus back into the proventriculus, insulating evaluated organ contents from bias related to regurgitation. A small test ($n = 5$) assessing plastic in both the proventriculus and ventriculus demonstrated this approach was appropriate, as ~90% of accumulated plastics occurred in the ventriculus; this is further supported in the literature³⁵.

Plastics were obtained from the ventriculus as previously described^{25,34,36}. Briefly, the organ was cut open and flushed thoroughly using deionized water over a sieve with a mesh size of 1mm^2 . While plastics smaller than 1mm^2 may be present within the samples, we did not screen for this size fraction due to facility and equipment constraints. Plastic items were visually separated from natural

items with the aid of a dissection microscope, magnifying lenses, or density separation techniques as necessary. Natural and plastic materials were combined in plastic or glass petri dishes (respectively), and dried in a fume hood for ~ 2 days. Plastic material was further sorted into established qualitative categories outlined by van Franeker et al. (2015) and Provencher et al. (2017)^{36,37}. Each plastic item was first classified as an industrial (nurdle or pellet) or user plastic (non-industrial items likely sourced from consumer use) and further differentiated into five subcategories including sheet-like plastics (e.g., bag and film), thread-like plastics (e.g., microfibers, rope), foam (e.g., polystyrene), fragments (unidentifiable hard plastics), and other (e.g., balloon, rubber, melted

plastic). Each piece of plastic was weighed to the nearest 0.0001 g using an analytical balance, and length, width, and thickness measured to the nearest 0.01 mm using a calibrated electronic caliper. Soft plastics were weighed but not measured for morphology. Plastic pieces were also classified into a plastic size category (e.g. micro-, meso-, and macro-) after Barnes et al.⁵

Polymer analysis

1000 plastic pieces were analyzed for polymer identity using Fourier Transform Infrared (FTIR) spectroscopy. Prior to analysis, each individual fragment was cleaned with deionized water to remove biological debris and filed with a metal file to obtain a flat surface or further remove biological debris for the analysis. The fragments were scanned through a Thermo Scientific Nicolet TM iS5 FTIR spectrometer with an ATR accessory, using the Thermo Scientific Omnic software (Thermo Fisher Scientific, Waltham, MA). A diamond crystal

ATR plate was used for most analyses, however for analysis of black plastic a germanium crystal ATR plate was used due to the crystal's high refractive index and its capacity to accurately scan high absorbing substances such as carbon-black colored objects.

A spectrum was obtained for each plastic fragment and run through the spectral library search. The principal spectral libraries used were the Aldrich Polymers library, the Hummel Polymer and Additives library, and the Center for Coastal Studies (Provincetown, MA) private library. Each result from the spectral library was verified by identifying the spectrum's wavelength peaks and matching wavelength numbers to the known wavelength ranges of the corresponding polymer. Only fragments with greater than a 70% confidence of assignment were reported³⁸.

Each identified plastic fragment was categorized under one of 17 polymer groups, while the non-plastic fragments were categorized under four groups (biological, vegetation, rock, or natural fiber blend). Polyethylene was further divided into low density polyethylene (LDPE), high density polyethylene (HDPE), and polyethylene (inconclusive on the type of polyethylene). Additives ranged from poly(ethylene:propylene:diene) (EPDM rubber) to oil based additives. Mixed polymers were labeled under the primary polymer.

Statistical Analysis

All data manipulation and statistical analyses were performed in R version 3.6.1 (R Core Team, 2020) ³⁹. Quantitative data were checked for normality and homoscedasticity using the Shapiro-Wilk test and Levene's test. Plastic count and

mass data were non-normal despite log transformation and therefore treated non-parametrically for statistical analyses; year groups displayed no significant differences in variance. Differences between years or groups were assessed using Kruskal-Wallis tests with post hoc application of Dunn's test with Bonferroni correction for multiple testing. Relationships between continuous variables were assessed using Spearman rank correlation coefficients (R_s^2). Potential predictors of plastic ingestion mass or count were evaluated using generalized linear models (GLMs) considering year, age, sex, and morphometric measurements as potential predictors. Model evaluation was performed stepwise manually starting with the saturated model. Model significance and performance was further evaluated if needed using Tukey's Honest Significant Differences post hoc test for multiple comparisons and Akaike's Information Criterion (AIC) to rank models, with a lower AIC assumed to indicate the better model. Effects observed within all statistical tests were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Accumulation and frequency of ingested plastics

The mass and number of plastics varied by location and year. Considering all years of data, Massachusetts Bay birds contained an average of 8.5 plastic items and 151 mg plastic, Gough Island individuals contained an average of 9.6 plastic items and 236 mg plastic, and individuals found off the coast of North Carolina contained an average of 12 items and 181 mg plastic (Table 1). Breeding phase adults in the South Atlantic contained a significantly higher mass of plastic

compared to their North Atlantic counterparts (Kruskal-Wallis test, $p < 0.01$), but not a higher count of plastics. Count and mass data were highly correlated across all locations ($(R_s^2 = 0.88, p < 0.001)$). Wet weight, tarsus length, culmen length, wing chord, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, body condition index, liver weight, heart weight, kidney weight, and adipose fat depth were not significantly related to the count or mass of plastic individually or interactively, suggesting a lack of relationship between individual health and plastic occurrence/accumulation.

No statistically significant trends in count or mass were apparent over 2010 – 2019 considering all Massachusetts Bay individuals, likely related to low sample size and the resulting variability of plastic metrics apparent in some years (e.g. 2014, 2016, 2018). Only considering those years with sufficient sample size ($n > 15$ birds) from Massachusetts Bay, plastic mass increased over time between 2010 and 2019 ($(R_s^2 = 0.87, p = 0.044)$).

Count and mass averages found in birds from each location are similar to previously published estimates (mean number of plastics: 9.5 – 22.0, mean mass plastics: 0.17 – 2.52g)²⁴, but results are not directly comparable. Previous studies report mass and number of plastics in the total gastrointestinal tract rather than in specific compartments (i.e. the ventriculus) as we report here.

Frequency of occurrence, or FO, describes the number of individuals containing plastic as a function of the total sample set. FO varied by location, year, and age in this study. Young and hatch year birds possessed the highest FO, with bycatch individuals from Massachusetts Bay demonstrating an FO of 98% ($n = 103$) and young beach-cast birds from North Carolina evidencing an FO of

100% (n = 15). Mature birds from Massachusetts Bay possessed an FO of 87% (n = 23) while mature breeding birds from Gough Island possessed an FO of 58% (n = 58) (Table 2). Differential plastic ingestion between juvenile and adult birds has been previously found in short-tailed shearwaters from eastern Australia, where juveniles were found to ingest plastic more frequently than adults (85% vs. 62.7%, respectively), as well as a larger number of plastics compared to adults.

Our data adds nuance to age-specific plastic ingestion by suggesting adults during or directly after the chick-rearing phase have much lower rates of plastic ingestion compared to juveniles assessed during the non-breeding phase, while adults during the non-breeding phase have lower but more comparable rates to non-breeding juveniles. The substantially lower frequency of plastics in breeding adults may be due to offloading of plastic pieces to chicks prior to fragments traveling to the ventriculus and lower GIT beyond the influence of regurgitation, or this may be due to reduced plastic occurrence in habitats supporting adults during the chick-rearing phase. We consider the latter unlikely, as the South Atlantic subtropical gyre is thought to be a growing hotspot of plastic occurrence and seabird plastic ingestion^{9,10,26}. The lower rates of plastic ingestion in non-breeding adults may be due to use of more remote habitats within the NW Atlantic³¹, or increased foraging experience compared to naïve young birds⁴⁰.

The FO observed in juveniles is higher than previous literature estimates from studies of comparable sample size conducted within the same species. Moser and Lee (1992) estimated an FO of 64% based on assessment of 55 individuals from the NW Atlantic collected between 1975 – 1989¹⁸. More recently, Bond et

al. (2018) measured an FO of 88% based upon assessment of 84 Great Shearwaters beach-cast on Sable Island, Nova Scotia, Canada in the NW Atlantic from 2000 – 2011²⁴.

The increased FO in this study may suggest an increased prevalence of plastic ingestion in juveniles of this species compared to previous points in time, but more likely reflects artifacts of study design confounding direct comparison of reported results between studies³⁷. Prior work does not report FO as a function of age, and therefore previous literature estimates may be lower due to an increased proportion of mature birds with lower plastic ingestion rates. Additionally, prior work investigating plastics in Great Shearwaters incorporates a variety of sampling and reporting approaches that preclude direct comparability of reported metrics.

Plastic mass and count data were likewise not associated with any significant changes in morphological or body condition variables, similar to results observed in other work^{24,28}. We suggest future work evaluates the association between plastic ingestion and other biological endpoints related to nutritional condition or short-term biological responses like blood chemistry, considering plastic's potential interference with effective ingestion of natural food items and the uncertain but likely limited retention time of plastics in seabirds^{35,46}.

Plastic item morphology

Industrial plastics are defined as the raw plastic materials, often in the form of pellets or beads, that are melted down to create consumer plastic products; they are often called “nurdles”. These made up a small proportion of observed plastics

from each year and location, with no significant changes in the percent of industrial plastics over time or between locations. The percentage of industrial plastics within this sample set (mean = 8.2%) was equal to or lower than percent industrial metrics reported in previous studies from the North Atlantic ^{23,25,28}. The mean number of industrial pellets reported here is also consistent with measurements conducted between 1999 – 2006 using Great Shearwaters collected from the South Atlantic and western Indian Oceans, suggesting the ingestion of pellets from industrial sources remains consistent and lower than observed in prior decades ([Ryan, 2008](#)).

Each plastic item was classified into one of five morphological categories, including fragments, thread-like plastics, sheet-like plastics, foams, or “other”. Proportions of each plastic morphological category were assessed as a percentage of the total count of plastic items measured within a given year and location. Category ratios differed between years in Massachusetts Bay (Fisher’s exact test, two-sided, $p < 0.001$), but were consistently dominated by fragments (range: 70 – 93% fragments) (Fig. 2).

Category ratios differed between habitats (Fisher’s exact test, two-sided, $p = 0.004$) when considering all individuals from all habitats. However, the Gough Island sample set contained an apparent outlier, one individual with a count and mass of plastics much higher than all other sampled individuals. When this outlier was excluded, differences between the occurrence of plastic categories between habitats disappeared and all habitats were dominated by fragments (mean = 88%) (Fisher’s exact test, two-sided, $p = 0.669$). Excluding fragments from the analysis,

no differences existed between ratios of other morphologies between habitats ((Fisher's exact test, two-sided, $p = 0.6202$) (Fig. 2).

There was no difference between ratios of plastic categories between Massachusetts Bay and Gough Island mature birds, despite a slightly higher but statistically insignificant proportion of thread-like pieces in Massachusetts Bay mature birds. Likewise, there was no difference between category ratios observed in young and mature Massachusetts Bay birds.

We explored the occurrence of plastics found in surface waters and forage fish used by this species in the Gulf of Maine to deduce if birds inherited their consistent category signature from their environment or diet. Limited data details the occurrence of different plastic categories in surface water within the Gulf of Maine and South Atlantic habitats specifically used by Great Shearwaters, as most large-scale datasets present abundance by size rather than plastic piece category¹⁰. However, 2013 surveys conducted near Isles of Shoals in the SW Gulf of Maine found microfibers dominated (84%) in the surface layer (~1m) compared to fragments (14%)¹¹, suggesting Great Shearwaters in the Gulf of Maine may seek out plastic fragments as opposed to accumulating plastics based on likelihood of encounter. Additionally, stomach content analyses were performed on 202 *Ammodytes spp.* collected in 2019, the primary prey species of Great Shearwaters while in the Gulf of Maine. We found no plastic fragments of the same size or morphology as those found in Great Shearwater stomachs (Suca et al. 2020, in review). Only one thread-like item was found in one fish, suggesting Great Shearwaters in Massachusetts Bay are not deriving their accumulated plastic loads

from trophic transfer. This corroborates evidence from other seabirds suggesting the transfer of plastics between fish prey and seabird predators may be limited⁴¹.

The size of plastic items varied widely, yet the largest item measured from each location measured approximately 13mm in its widest dimension, suggesting a consistent limit on the size of plastic items based on bird gape size.

We compared the size of fragments between habitats and found mature birds from Gough Island contained the largest fragments compared to items found in individuals from Massachusetts Bay or SE North Carolina (Dunn's test, $p < 0.001$) (Fig. 3). This difference was likewise apparent when the comparison was restricted to mature birds from Massachusetts Bay and mature birds from Gough Island (Kruskal-Wallis test, $p = 0.0238$).

The abundance of larger plastic fragments in Gough Island adults is likely related to size-mediated distribution of plastic items in the surface ocean^{42,43} and maritime sources of intact plastic items⁴⁴, coupled to the highly pelagic foraging range of Great Shearwaters during their breeding and chick rearing phases. Smaller plastic items with higher surface area:volume ratios are thought to settle out of the water column rapidly moving away from coastal regions due to biofouling and other biophysical processes⁴², with larger, more buoyant pieces preferentially remaining in the surface ocean to circulate to more remote regions⁴³. During their breeding phase Great Shearwaters forage across highly pelagic regions along the boundary of the South Atlantic, South Pacific, and SW Indian Oceans⁴⁵, and therefore likely encounter a higher proportion of larger plastic items that have been retained through size-mediated dispersal processes to

date. Additionally, major ship-borne inputs of whole consumer items may likewise contribute to the abundance of larger plastic items in Great Shearwaters from Gough Island. The incidence of plastic debris derived from shipping and fishing activities is rapidly increasing in the South Atlantic around the breeding islands used by Great Shearwaters, providing inputs of intact consumer items to an otherwise remote region⁴⁴. These consumer products (e.g. beverage bottles) are often made of buoyant plastics, and would first fragment into larger pieces. An interplay of these two drivers is likely driving the size trends we see here. Regardless of specific cause, the increased abundance of larger plastic items in breeding birds is problematic, as larger plastic items are likely retained in the GIT longer and pose an increased risk of physical impairment or injury⁴⁶.

Polymer patterns and differences

1000 plastic pieces were assessed for polymer type using FTIR, and these results indicated the type of polymer ingested varied by age and habitat. Mature individuals from Gough Island contained higher proportions of polypropylene mixes (PP mix) compared to other locations and ages. Considering only fragments, mature birds from Massachusetts Bay possessed a significantly different polymer composition compared to their South Atlantic counterparts (Fisher's exact test, two-sided, $p = 0.002$), with North Atlantic birds possessing a lower proportion of PP mix, a higher proportion of polyethylene, and increased incidence of "Other" items comprised mostly of balloons (Fig. 3).

The different polymer signatures may indicate turnover of plastic pieces over time, allowing Great Shearwaters to reflect variable signatures of plastic

associated with divergent habitats. The retention time of plastic items in seabirds is generally unclear, but is thought to vary between different taxa based on other diet items present in the GIT as well as the size threshold required to pass through the lower GIT. Retention time is also based on initial item size, item composition, and rate of wear within the GIT³⁵. Fragments, the dominant morphology found in individuals within this study, may also be prone to reduced retention times due to increased exposure to UV and other environmental stressors that caused and continued their fragmentation from intact items³⁵.

van Franeker and Law (2015) suggested Northern fulmars excrete approximately 75% of their plastic load within one month based on assessment of plastic loads in fulmars between their breeding and non-breeding locations. However, their inference did not account for possible transfer from adults to chicks during chick-rearing, which may reduce the incidence of plastics in adults via offloading to chicks. Alternatively, direct studies of plastic pellet breakdown in seabirds suggest slow breakdown of plastics *in vivo*, as industrial pellets fed to white-chinned petrel fledglings lost only ~1% of their mass over 12 days within the GIT, making excretion within a month improbable ([Ryan and Jackson 1987](#)).

Our data strongly suggest that plastic fragments within the size ranges observed here are not be retained indefinitely by Great Shearwaters, but rather are replaced in less than 5-6 months to reflect changes associated with migration-related shifts in ambient environment. However, we acknowledge there is likely a lag of uncertain duration as birds excrete plastic from previous environments and assimilate new plastic items from current environments. We do not believe

changes in polymer signature between locations are due to dilution of signature by accumulation of more plastic items (e.g. apparent lower proportion of PP mixes in North Atlantic birds due to accumulation of more plastics overall), because birds from all locations contained similar counts and mass of plastic items (Table 1).

We also note that our inference is based on comparisons more removed from the influence of intergenerational transfer, as samples reflecting breeding phase adults were collected at the end of chick-rearing in March/April 2018, while adults and juveniles from Massachusetts Bay were collected in August – November after use of NW Atlantic regions for >2 months. *Recommendations for future monitoring*

Over 50% of Great Shearwaters sampled in this study surpass the EcoQ standard for plastic ingestion in seabirds, which suggests no more than 10% of a given seabird population should contain a mass of plastic greater than 0.1g^{24,25,34}.

We recommend increased standardization of approaches within studies focusing on plastic ingestion in Great Shearwaters to more fully leverage the utility of this species as an indicator of the subtropical and temperate Atlantic regions upon which it relies. Mass, count, and FO should be presented for each compartment of the GIT as a function of month, age, and sex³⁷, given the highly variable mass, count, and FO reported between different ages and locations across time. We particularly highlight the need for reporting plastic ingestion within each GIT compartment separately to facilitate increased use of bycatch samples within monitoring studies. Bycatch samples are prone to regurgitation impacting plastic loads in the proventriculus, but are more consistently available as Great Shearwaters are the most abundantly bycaught species in the Gulf of Maine^{48,49}

and are also bycaught in the South Atlantic along the Patagonian Shelf⁵⁰. Bycatch samples are also considered healthy prior to death and lack bias potentially associated with beach-cast samples which may have been sickly or compromised leading to death^{51,52}.

The subtropical North and South Atlantic stand to experience continued accumulation of plastic pieces in the coming decades⁴⁷; with increased parity of approach, Great Shearwaters offer an abundant, compact method to monitor future plastic dynamics in these regions, and assess the efficacy of any future interventions to address plastic pollution. We encourage transboundary cooperation to more fully develop monitoring of this species, using the body of work surrounding the Northern fulmar in the North Atlantic as a model.

1. Conclusions

Our results confirm sustained plastic ingestion in multiple life stages of a Procellariiform seabird reliant on both the North and South Atlantic. Juveniles and non-breeding adults accumulated plastics more frequently than mature adults in their breeding phase. Fragments were the dominant plastic item ingested across all life stages and locations, although breeding birds in the South Atlantic were found to ingest larger plastic fragments compared to North Atlantic individuals. Significantly different polymer types between North Atlantic and South Atlantic birds suggests plastics turnover within the GIT commensurate with migratory phase, allowing Great Shearwaters to reflect their ambient environment after an assumed acclimation period. Crucially, this suggests mitigation of plastic sources within both the North and South Atlantic are key to reduce the incidence of plastic

ingestion in this species, which occurs at levels well above EcoQ thresholds considered acceptable for ecological health. Understanding plastic ingestion levels and effects in marine food webs and biota such as seabirds remains an important priority, as ocean plastic pollution is predicted to exponentially increase over the coming decades

ASSOCIATED CONTENT

NA

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Author Contributions

The manuscript was primarily drafted by A. Robuck and finalized through contributions of all authors. Sample analysis and data interpretation were carried out by A. Robuck, C. Hudak, G. Emery, L. Agvent, and D. Wiley, alongside contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

A. Robuck acknowledges support from the National Oceanic and Atmospheric Administration Dr. Nancy Foster Scholarship program (NOAA Award Number NA17NOS4290028), the Robert and Patricia Switzer Foundation, the STEEP Superfund Research Program (NIEHS Award Number P42ES027706), and the Oak Ridge Institute for Science and Education (ORISE) program.

Competing Interests

The authors have no competing interests to declare.

ACKNOWLEDGMENTS

The authors are indebted to the Northeast Fisheries Observer Program, Gina Shield, and Stephanie DePasquale for their assistance obtaining samples used in this study. Although NOAA employees contributed to this article, this research was conceived, designed, and implemented by URI. Consequently, the views,

interpretations, and conclusions expressed in the article are solely those of the authors and do not necessarily reflect or represent NOAA views or policies.

ABBREVIATIONS

GIT – gastrointestinal tract

GRSH – Great Shearwater

FO – Frequency of occurrence

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TABLES AND FIGURES

Figure 5.1. Map of Great Shearwater migratory routes (black lines), and sample collection locations (marked by colored points). Surface plastic density is indicated by heatmap gradients in pink and blue. No samples were collected off the Patagonian Shelf (marked by the yellow dot), but this location is heavily used by both adult and juvenile Great Shearwaters as a staging location before and after trans-equatorial migration.

				Plastic Mass (mg)					Plastic Count (total # identified)				
Collection Location	Life Stage	Year	n	Mean	Median	Std. Dev.	Std. Err.	Range	Mean	Median	Std. Dev.	Std. Err.	Range
Mass. Bay	Non-breeding	2008	1	79.6	79.6	NA	NA	NA	5.0	5.0	NA	NA	NA
Mass. Bay	Non-breeding	2010	17	126.7	49.9	160.1	40.0	0.4 - 462.7	7.5	3.0	9.5	2.3	0 - 30
Mass. Bay	Non-breeding	2011	12	165.2	92.3	208.7	62.9	15.8 - 750.2	9.7	4.5	10.3	3.0	0 - 34
Mass. Bay	Non-breeding	2012	27	150.4	66.1	188.4	38.5	0 - 640.7	8.3	4.0	10.7	2.1	0 - 42
Mass. Bay	Non-breeding	2014	8	267.6	140.4	323.6	114.4	5.9 - 796.6	8.8	7.0	7.6	2.7	1 - 22
Mass. Bay	Non-breeding	2015	13	105.0	90.5	96.6	29.1	1.9 - 264.4	5.4	3.0	5.1	1.4	0 - 15
Mass. Bay	Non-breeding	2016	10	68.2	50.3	76.4	24.1	5.8 - 262.5	4.1	3.0	3.9	1.2	1 - 14
Mass. Bay	Non-breeding	2017	26	162.1	179.2	119.8	25.0	5.4 - 343.2	8.9	8.0	8.1	1.6	0 - 28
Mass. Bay	Non-breeding	2018	2	119.3	119.3	60.6	42.8	76.4 - 162.1	4.5	4.5	0.7	0.5	4 - 5
Mass. Bay	Non-breeding	2019	19	179.5	120.4	172.4	39.6	6.5 - 607.4	10.2	7.0	9.8	2.3	1 - 41
SENC	In migration	2017	15	180.8	136.6	139.7	36.1	18.5 - 445.4	12.0	12.0	7.2	1.8	3 - 28
Gough	Breeding	2012	1	1868.1	1868.1	NA	NA	NA	194.0	194.0	NA	NA	NA
Gough	Breeding	2018	24	220.3	38.9	375.3	96.9	1 - 1432	5.8	1.0	12.3	2.5	0 - 47

Table 5.1. Summary statistics describing accumulated ingested plastics by mass and count, in different regions and years.

Habitat	Life Stage	Year	Age	HY/AHY	n	FO (%)
Mass Bay	Non-breeding	All	Mature	na	23	87
Mass Bay	Non-breeding	All	Young	na	103	98
Mass Bay	Non-breeding	All	Young	HY	53	98
Mass Bay	Non-breeding	All	Young	AHY	16	94
Mass Bay	Non-breeding	2008	All	na	1	100
Mass Bay	Non-breeding	2010	All	na	17	94
Mass Bay	Non-breeding	2011	All	na	12	92
Mass Bay	Non-breeding	2012	All	na	27	89
Mass Bay	Non-breeding	2014	All	na	8	100
Mass Bay	Non-breeding	2015	All	na	13	85
Mass Bay	Non-breeding	2016	All	na	10	100
Mass Bay	Non-breeding	2017	All	na	26	92
Mass Bay	Non-breeding	2018	All	na	2	100
Mass Bay	Non-breeding	2019	All	na	19	100
SE NC	In migration	2017	Young	na	15	100
Gough	Breeding	2012	Mature	na	1	100
Gough	Breeding	2017 - 2018	Mature	na	24	58

Table 5.2. Frequency of occurrence (FO) of ingested plastic by location, year, and age.

Figure 5.2. Proportions of each plastic morphology, as a percentage of the total count of plastic items measured within a given year and location. The summary of the Gough Island dataset presented herein omits one clear outlier that possessed 194 plastic pieces of greater category diversity than observed within other mature birds from Gough Island.

Figure 5.3. A) Length and width and B) surface area and volume of plastic fragments, with colors indicating collection region. 95% of the data is displayed, with the upper 5% removed. Error bars present the standard error of the mean.

Figure 5.4. Polymer composition by A) year and B) age presented by location.

CHAPTER 6

NAPE COLORATION IS NOT A RELIABLE MARKER OF AGE IN GREAT SHEARWATERS *ARDENNIA GRAVIS*

This manuscript is formatted for submission to the journal Avian Biology

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ABSTRACT

Interpreting observations of petrels at sea is generally limited by a lack of differentiating plumage characteristics indicating individual sex or age. Exceptions exist, including great shearwaters (*Ardenna gravis*), which have been previously proposed to display age-based nape coloration. Pyle (2008) suggested after hatch year and after second year individuals possess a more complete white collar compared to hatch year or second year individuals. Here we combine observations of known age great shearwaters across their breeding and non-breeding range to evaluate nape as an honest indicator of age. Data from necropsies, at-sea photos, and in-colony photos (n = 333) were scored for nape aspect and compared to true age as ascertained via presentation of gonads and bursa of Fabricius or known breeding status. Pooling all data, nape presentation was not significantly different between age classes, and the accuracy of the nape ageing method was only 19%. Nape was significantly different between sexes in non-breeding individuals, with a higher proportion of white, “C” napes in females observed or necropsied in Massachusetts Bay. There was no evidence of the same sexual dimorphism in breeding individuals bycaught or observed at South Atlantic breeding colonies. We suggest nape is not a reliable indicator of age in this petrel species and suggest further evaluation of sex or trophic drivers as causes of plumage polymorphism in great shearwaters.

INTRODUCTION

The order Procellariiformes (hereafter petrels) includes many of the world’s most abundant pelagic seabird species, as well as many of its most threatened (Brooke

2004, Croxall et al. 2012). Petrels are long-lived compared to most other birds, with maturation taking from 3 to 12 years (Warham 1990). Recent interest in the early life history of petrels has been stimulated by rapid advances in bird-borne tracking technologies, which have revealed sometimes marked differences in both distribution and behavior between adults and immatures (Riotte-Lambert and Weimerskirch 2013, Campioni et al. 2020). However, notwithstanding technological advances, it remains challenging to track petrels during immaturity because most species are very wide-ranging and return rarely to land during this life history stage (Rodríguez et al. 2019). Direct observational study of seabirds at sea could provide additional data on age-related distribution, habitat use, and behavior. However, plumage coloration is largely uniform across age classes in most petrels (Onley and Scofield 2007), making it impractical to differentiate between adult and immatures observed visually at sea.

Some exceptions to this rule do occur. For example, plumage coloration changes systematically with age in albatrosses (especially *Diomedea* spp.) and giant petrels (*Macronectes* spp.) (Hyrenbach 2002, Carlos and Voisin 2008, Prince et al. 1997, Weimerskirch et al. 1989). However, plumage coloration can also vary due to sex, breeding status, diet, predation pressure, molt, and feather wear (Arkos 2007, Le Corre 1999, Meunier et al. 2011, Leal et al. 2019, Soravia et al. 2020). In some cases, these factors can confound attempts to age birds based on plumage alone. For example, among *Diomedea* spp. there is considerable overlap in the plumage aspect of immatures and females (Onley and Scofield 2007).

Great shearwaters (*Ardenna gravis*) are abundant (world population approx. 15 million individuals (Brooke 2004)) medium-sized petrels that breed in the Tristan da

Cunha group of islands, South Atlantic. In the austral summer adults forage in temperate to polar waters, including the Patagonian shelf, Benguela upwelling and intervening oceanic areas (Ronconi et al. 2018). In the boreal summer, great shearwaters migrate into the Northwest Atlantic, with high concentrations in coastal and pelagic waters of the Gulf of Maine, before continuing clockwise through the North Atlantic and back to breeding colonies in the fall (Voous and J. 1963, Powers et al. 2020). Despite recent at-sea study and tracking (Bugoni et al. 2015, Powers et al. 2017, Ronconi et al. 2018, Hong et al. 2019), there remains considerable uncertainty about the movements of immature great shearwaters, so the ability to discriminate between adults and immatures at sea would be very useful as age-related differences in life histories (Powers et al. 2020).

Great shearwaters have a dark brown cap and mantle, separated by a partial or complete collar of cream-colored feathers (Fig. 1). Pyle (2008) suggested that juvenile to hatching year/second (HY/SY) great shearwaters have a 'Nape with [an] indistinct to incomplete white collar', whilst after hatching year/after second year (AHY/ASY) they have a 'Nape with [an] indistinct to distinct white collar'. Pyle's Figure 201 illustrates three distinct nape classes, A, B or C forming a spectrum from dark, through partial, to light coloration (inset, Fig. 1). The caption adds that 'it is possible that AHY/ASYs resembling C can reliably be aged to ASY/ATY [after second/third year] or older' but notes that more study is required to confirm these patterns.

Here we assess nape plumage scores of known-status great shearwaters sampled in their colonies and bycaught in the South and North Atlantic in order to test whether

nape coloration is a reliable marker of age class. In addition, we test for variation in nape coloration by sex, region, and over time.

METHODS

Bycaught birds

From 2010 – 2017 the National Oceanic and Atmospheric Administration Northeast Fisheries Observer Program (Falmouth, MA) supplied us with dead great shearwaters bycaught in fisheries in Massachusetts Bay, USA ($n = 174$). Bycatch birds were obtained between May and December, with most birds collected in August, September, and November across all years. Additional dead great shearwaters were obtained in March and April 2018 during the chick-rearing phase adjacent to the breeding colony at Gough Island in the South Atlantic as longline bycatch in collaboration with Tristan Conservation ($n = 25$). Each individual was partially thawed and necropsied following van Franeker (2004) in order to obtain the following data: plumage characteristics, external morphometrics, organ weights, body condition, sex and stomach contents. Individuals were aged based on molt status, presence of the bursa of Fabricius, and stage of gonad development as previously described (Table 1) (Powers et al. 2020, Broughton 1994). Napes were classified following Pyle (2008) (Fig. 1) as A, B or C by ARR and/or KDP prior to necropsy.

Birds in the colony

The napes of adult and fledgling great shearwaters were photographed on Gough Island during the breeding season. Adults were photographed between colony arrival and early chick-rearing (September 22, 2017 to January 16, 2018) and fledglings just

prior to leaving the colony (May 3 to 8, 2018). Birds were removed by hand from burrows during the daytime, photographed and then returned to their burrows. Adults were either known breeders, previously marked as part of a long-term demographic and tracking studies, or unmarked non-breeders. Fledglings were taken from active breeding burrows. At the time, they had attained their first plumage, but some retained traces of down.

Napes of adults and unknown age great shearwaters were also photographed on Gough Island and Inaccessible Island during the 2009 breeding season. On Gough, birds were photographed during initial colony arrival and courtship period (September 22 to October 02), including individuals ($n = 2$) and pairs outside of burrows at night ($n = 5$ pairs, 10 individuals), individuals in flight at dusk ($n = 9$), and individuals handled in tagging study which were removed from burrows during the daytime ($n = 2$). Though these birds were attending nests and some pairs were observed copulating, age was not known and some may not have been mature, but all were likely after second year. On Inaccessible Island, during early incubation (November 10 to December 01) birds were photographed during tagging studies ($n = 4$ males and $n = 5$ females; Ronconi et al. 2018) or opportunistically at burrow entrances ($n = 3$). Due to opportunistic nature of photographs, nape scoring was not possible for all images.

Napes were scored A, B, or C from these photographs independently by the two coauthors who classified the necropsied birds. To ensure that this was done double-blind, EDW supplied ARR and KDP with a pdf file of the photographs, which ARR and KDP had not seen previously. The pdf was generated using an R script which arranged the images in a random order. The script also generated a file containing the

true ages of the birds in each image. This file was not consulted until after the napes had been classified. Nape classifications were highly consistent between both observers, with little or no disagreement between nape designations. Nape designations where disagreement occurred were removed from the sample set.

Statistical analyses

Birds assessed from the South Atlantic were designated as within the breeding/chick-rearing phase while birds from Massachusetts Bay were considered non-breeding. Chi-squared Tests of Independence or Fisher's exact tests were used to compare ratios of nape classes between age groups, sexes, locations and sampling years. Confusion matrices using Pyle's age classes (HY/SY, AHY/ASY) were used to assess classification accuracy of known-age individuals (Fielding and Bell 1997). Necropsied birds classified as "AHY/Not Mature" were omitted from this part of the analysis, as this category could include both HY/SY and AHY/ASY classes. All analyses were performed in R statistical software, version 1.3.959 (R Core Team, 2020)

RESULTS

We assessed the nape plumage class of 333 birds, comprising 135 photographed in colonies and 198 necropsied birds. Pooling all data, the ratio of nape classes (A:B:C) did not differ significantly between age classes, defined following either Pyle (2008) ($\chi^2_{4, 333} = 3.43, p = 0.489$), or Powers et al. (2020), ($\chi^2_{4, 333} = 4.01, p = 0.405$) (Fig. 2).

In our dataset, 218/333 individuals were assigned a B nape. This means that 65% of the individuals we observed could not be reliably aged using Pyle's suggested nape plumage method alone (according to the latter, B napes could be any age from HY to ASY). Based on breeding/chick-rearing status observed in the colony or on criteria assessed during necropsies, we were able to unambiguously classify 254 birds as HY or mature. As in the wider dataset, the majority of individuals (66%) in this subset had type B nape, and therefore could not be assigned to either HY/SY or AHY/ASY according to the Pyle method. Of the remaining 86 birds designated as A or C, 55% of HY birds were appropriately assigned as HY/SY based on nape aspect, while 43% of mature birds were correctly assigned to AHY/ASY. The resulting overall accuracy of the nape method, with consideration of the B napes, was therefore only 19% (Table 2).

Considering only birds bycaught in Massachusetts Bay from 2010 – 2017 (Fig. 3), the ratio of nape classes (A:B:C) differed significantly among some years (Fisher's exact test, two-sided, $p < 0.001$).

Nape class ratios also varied with location and sex. At all locations, some mature birds had type A napes, while some immatures from both Gough and Massachusetts Bay possessed type C napes. Nape presentation differed in Massachusetts Bay HY and mature birds compared to individuals from the same life history stages in the South Atlantic, with an increased frequency of Type C napes in Massachusetts Bay ($\chi^2_{2, 41} = 74.95, p < 0.001$). Nape presentation was not different between HY and Mature birds at Gough Island ($\chi^2_{2, 147} = 4.04, p = 0.133$), nor between birds at Gough and Inaccessible (Fisher's exact test, two-sided, $p = 0.112$), but nape presentation was

different between age classes in Massachusetts Bay ($X^2_{2, 174} = 10.88, p = 0.028$) (Fig. 4).

Further stratification by sex reveals that the relatively high proportion of type C napes in Mass. Bay is driven by females, which have a higher proportion of type C napes than males in that location ($X^2_{2, 174} = 12.16, p = 0.002$) (Fig. 5). This variable presentation of nape aspect and the increased proportion of C napes in Massachusetts Bay females occurs in each age class observed in this area (HY: $X^2_{2, 70} = 6.63, p = 0.036$, AHY/Not Mature: $X^2_{2, 74} = 8.93, p = 0.011$, Mature: $X^2_{2, 30} = 46.42, p < 0.001$).

However, this is not apparent among birds necropsied from bycatch in the South Atlantic adjacent to Gough Island where mature males have patterns of nape presentation statistically indistinguishable from mature females ($X^2_{2, 28} = 4.26, p = 0.119$). Though limited in size, lack of C nape scored in mature males ($n = ?$) and females ($n = ?$) from Inaccessible further suggests lack of sexual differences during the breeding/chick-rearing period. We could not ascertain if this also applies in immature birds from the breeding colony because the sexes of these birds were unknown.

DISCUSSION

Our results show that while a slightly higher proportion of fledgling and immature great shearwaters have all-dark napes compared to adults, this effect is small and not a reliable way of discriminating between HY/SY and AHY/ASY or older birds as hypothesized by Pyle (2008). The occurrence of known hatch-year great shearwaters with type C (light collared) napes and known mature, breeding individuals with type A (all dark) napes reinforces this point. Indeed, most great shearwaters

(65%), regardless of age, have nape coloration intermediate between all dark and a complete light collar. Thus, we suggest there is no evidence to suggest a lifetime progression from dark to light collars as suggested by Pyle (2008).

Given the lack of systematic variation in nape aspect with age, remaining causes of the differences that we observed potentially include environmental, sexual, or genetic factors. Specifically, our data suggests that nape plumage varies subtly but systematically with sex and location, as females in the NW Atlantic displayed a higher proportion of type C napes, with most samples informing this observation collected from August – November directly preceding or overlapping the timing of courtship, burrow staging, or breeding activities (September – January). Moreover, among females in the NW Atlantic, mature birds had a higher a proportion of type C napes than either HY and young birds. However, during the breeding/chick-rearing period at the colony, mature males and females displaying a statistically similar proportion of C napes (17% and 10%, respectively). The fact that this sex-specific shift of C nape presentation between locations occurs among mature birds suggests that nape plumage may have a sexual display function. Mature birds included in this study were not aged specifically using banding or colony monitoring, and therefore we are unable to comment on how nape characteristics may change over the long reproductive period of mature birds spanning decades of breeding seasons, as seen in other petrels (Hyrenbach 2002).

Evidence from seabirds and other bird taxa provides context regarding potential sexual drivers of nape characteristics in great shearwaters. Allopreening, especially of the head and neck, is a common pair formation/maintenance behavior among birds

(Kenny et al. 2017) and in some species more robust neck biting occurs. In Northern gannets, both male and females start the breeding season with yellow heads, yet the female head and nape become increasingly speckled with white plumage over the course of the breeding season (Redman et al. 2002). Males of this species engage in neck biting, which may damage feathers on the females' neck and prompt their replacement with white feathers. However, Nelson (2001) suggested the occurrence of white speckling on female birds cannot be related to courtship or sexual behaviors, due to the presence of white speckling in immature females and some males (Nelson 2001). Great shearwaters have also been observed to engage in allopreening during courtship, but it is unlikely that any white feathers on female napes lost during these behaviors would be replaced with energetically more costly brown plumage. . Additionally, this dataset lacks data detailing sex of immature birds in the South Atlantic to ascertain any transition (or lack thereof) to darker napes in non-breeding females not subject to courtship behaviors.

Nelson (2001) also suggested that gannet chicks have dark plumage because white plumage elicits an aggressive reaction from adults. In the case of great shearwaters, a white nape may be preferred for females only during pre-breeding and courtship stages, thereafter the white collar is replaced with darker nape plumage to avoid aggression from males. This may also explain the slightly higher incidence of dark, type A napes in immature individuals.

Additionally, great shearwaters are unusual among the small-medium petrels in that they engage in courtship display in the daylight, usually in the evening. They are also more patterned than other shearwaters and have less pronounced sexual

differences in vocalizations (Brooke 1988). These lines of evidence suggest this species may rely more on visual cues about conspecifics and potential mates. A higher proportion of white napes in mature females may therefore play a role in visual courtship displays and mate identification. In multiple taxa including and beyond petrels, plumage coloration and brightness indicate nutritional condition and quality of the individual with implications for reproductive behaviors (Hill 1991, McGraw et al. 2002, Meunier et al. 2011, Soravia et al. 2020). For example, nocturnal Mediterranean Storm Petrels (*Hydrobates pelagicus melitensis*) show condition-dependent presentation of chest and tail plumage in breeding males based on UV spectrometry measurements (Soravia et al. 2020). Differences in presentation were solely apparent in colonies subject to unfavorable environmental conditions. Soravia et al. hypothesized this sexual dimorphism in achromatic plumage may function as a signal of male quality during mate selection by females, or as an indicator of body condition to rival males during defense of burrow territories. Within our study species, disparity in plumage presentation between sexes during the pre-breeding period in Massachusetts Bay may likewise function in mate selection or other sexually-mediated roles not explored within the scope of this dataset.

In summary, our findings show that nape color is not a reliable signal of age as previously suggested and that underlying causes and patterns of sex-specific nape polymorphs in great shearwaters warrant further investigation across their breeding and non-breeding range. Despite the long-standing challenges associated with sexing and aging petrels at sea, the development of methods based on plumage and other external characteristics remains a very worthwhile goal. This is not least because this

group includes some of the world's most threatened birds (Croxall et al. 2012) and understanding and mitigating threats such as bycatch requires an understanding of if and how they affect different demographic groups asymmetrically at sea (Dias et al. 2019). Beak scaling, hormonal differences, telomere length, and pre-maxillary gape width have proven useful in age determination in other seabird taxa, and merit further exploration as ageing tools in great shearwaters and other petrels lacking age-based plumage characteristics.

ACKNOWLEDGEMENTS

We are grateful to Michael J. Moore for necropsy facility use and to Vonica A. Perold and Maëlle Connan for necropsy assistance in South Africa; to Johanna Pedersen for necropsy and data entry assistance in the US; to Fabrice le Bouard, Jaimie Cleeland and Kate Lawrence for collecting data on Gough and to the Royal Society for the Protection of Birds for facilitating fieldwork there and to the Tristan da Cunha Government for permitting this work. Funding was provided by the UK Natural Environmental Research Council (grant NE/M017990/1, awarded to EW). A. Robuck acknowledges support from the National Oceanic and Atmospheric Administration Dr. Nancy Foster Scholarship program, the Robert and Patricia Switzer Foundation, the STEEP Superfund Research Program (NIEHS Award Number P42ES027706), and the Oak Ridge Institute for Science and Education (ORISE) program. During the 2009 work on Gough and Inaccessible Islands, R. Ronconi was supported by a scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Killam Trusts, Dalhousie University. Funding for Massachusetts Bay work was

provided by the Volgenau Foundation and Stellwagen Bank National Marine
Sanctuary

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TABLES AND FIGURES

Figure 6.1: Examples of each nape class as observed in live birds. The diagram inset is reproduced from Pyle 2008.

Classification scheme	Age Class	Bursa present	Molt score = 50 ^a	Molt score < 50 ^a	Gonad class = 1 or 2	Gonad class = 3 or 4	Gonad size overlap with known breeder
Pyle	HY/SY	X	X	X	X		
Pyle	AHY/ASY			X	X	X	X
Powers et al.	HY	X	X		X		
Powers et al.	AHY/Not Mature ^b		X	X	X		
Powers et al.	Mature		X	X		X	X

Table 6.1: Age classes and associated criteria from Pyle (2008) and Powers et al. (2020).

Figure 6.2. Variation in nape class (as defined in Fig. 1) by age class defined following either (A) Pyle (2008) or (B) Powers et al. (2020). Both panels include observations from all locations.

	Inferred from nape		
True	AHY/ASY	HY/SY	Unk
AHY/ASY	20	26	107
HY/SY	12	28	59

Table 6.2. Confusion matrix presenting counts of known age (HY or Mature) birds, compared to the age class as predicted according to Pyle's nape classification system, under the "Inferred from nape" heading.

Figure 6.3. Nape classification of Massachusetts Bay bycatch individuals by year

Figure 6.4. Nape classification by age and location.

Figure 6.5. Nape classification by sex, age, and location.

CHAPTER 7

CONCLUSION

Improved characterization of Great Shearwater demographics and contributions to Stellwagen Bank National Marine Sanctuary research priorities. Execution of the contaminant studies that were the primary focus of this dissertation required a large-scale necropsy program that entailed collection of comprehensive demographic and morphometric data. To date, 366 Great Shearwaters and 22 individuals from other species collected from 2009 – 2019 were necropsied in support of various studies presented herein. Biological data from this necropsy work was used to collaboratively answer several questions about Great Shearwaters, a key sentinel species in Massachusetts Bay¹. Primarily, demographic data paired with plumage observations were used to correct guidance from previous work suggesting plumage is an indicator of age in Great Shearwaters (Chapter 5). Pyle (2008) previously suggested that nape coloration is an indicator of maturity, with completely dark napes associated with hatch year or second year birds while completely white napes were associated with older individuals. Here we found nape is not an honest signal of age in juvenile or adult Great Shearwaters, at any point in their life cycle. This finding enables future at-sea observational studies to avoid misleading age classifications based on plumage coloration. However, we noted a higher abundance of complete white napes in females, suggesting nape coloration may potentially act in mate selection or other reproductive processes. Further work is required to determine any significance of nape coloration in reproductive behaviors for this species; observations of nape aspect over

the duration of the breeding phase may be helpful to parse out sexual drivers of nape plumage.

Great Shearwaters have been previously documented to be the most commonly bycaught species in Massachusetts Bay^{2,3}; necropsy data obtained via this dissertation also revealed that ~90% of bycatch Great Shearwaters from Massachusetts Bay are juveniles, and males and females are equally represented in the bycatch sample set. This is the first quantitative description of Great Shearwater bycatch demography and leaves outstanding questions regarding how the preferential loss of pre-reproductive individuals may impact overall population dynamics, as bycatch of Great Shearwaters can remove thousands of individuals per year, though Great Shearwaters as a species are not considered threatened due to a previous population estimate around 15 million individuals in the early 2000s. A follow-up project exploring this question is underway using the demographic data collected within this dissertation and demographic modeling tools previously applied in other seabird-fishery interaction scenarios⁴.

Morphometric and demographic data combined with satellite tracking data of live birds also suggests that Massachusetts Bay and the SW Gulf of Maine are preferentially used by juvenile individuals as a “nursery” area upon their arrival to the Gulf of Maine, likely due to consistent supplies of sand lance (*Ammodytes spp.*), a key forage fish that favors sandy bottom substrates and shallow environments (co-author work, Powers et al. 2020)⁵. This work has implications for protected area management and resource conservation, due to potential sand mining and wind lease exploration within the SW Gulf of Maine. Additionally, time series data of bulk $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope ratios were collected to ascertain if pollutant dynamics were related to

shifts in trophic level or primary productivity (Chapter 2). These data suggest slight shifts in Great Shearwater trophic level based on variable $\delta^{15}\text{N}$ ratios over 2010 – 2019. While the changes over time are slight and are not thought to meaningfully impact pollutant dynamics seen here, they may indicate potential fluctuations in prey use, such as variable reliance on sand lance, menhaden, or squid each given year. Temperatures in the Gulf of Maine and Massachusetts Bay are rapidly changing due to climate change, with commensurate changes in regional fish assemblages that may be playing a role in the shifts we see here^{6,7}. Further work will continue analyzing stable isotope ratios in Great Shearwaters over time to better quantify any long-term shifts in trophic level, and incorporate baseline measurements from a wider suite of forage fish.

Additionally, involvement in Stellwagen Bank National Marine Sanctuary's Great Shearwater research team facilitated collaboration with sand lance research ongoing in the Sanctuary. In collaboration with another PhD candidate, bulk lipid measurements were carried out in 202 sand lance collected over the 2019 growing season, and results indicated that fish lipid content varied by up to 30% over growth and reproductive phases spanning February to November. Sand lance from Stellwagen Bank National Marine Sanctuary reached their peak lipid content commensurate with the arrival of Great Shearwaters in June - August. This work also predicted tremendous changes to sand lance abundance due to climate-related drivers (Co-author work, Suca et al., in review), and further research is required to understand how this may impact seabirds heavily reliant on the abundance of this lipid-rich species at a specific time and location. Ongoing work is also exploring PFAS and hydrophobic contaminants in the same sand lance previously measured for lipid content.

Distributions of PFAS in seabirds across space and time. PFAS were last assessed in seabirds from the US East Coast in the late 1990s and early 2000s. This dissertation provides an updated description of PFAS in select US East Coast seabirds by measuring legacy and novel PFAS in livers from several species of seabird from habitats representative of low, medium, and high PFAS exposure potential. We also measured PFAS in Great Shearwaters from Massachusetts Bay, an offshore environment associated with low PFAS exposure potential, from 2010 – 2019 (Chapters 1 & 2).

We found seabirds reared downstream from a major fluoropolymer production site in SE North Carolina contained elevated concentrations of PFAS, including novel PFEAs Nafion BP2, PFO5DoDA, and PFO4DA. Nafion BP2 was also detected in seabirds with no known connection to the region downstream from the industrial region, dating back to 2010 in Great Shearwaters. Our measurements represent the first identification of these PFEAs in seabirds, and at the furthest distances from the known production site to date. Our results also indicate the capacity for long-range transport of Nafion BP2, and raise the possibility of as-of-yet unidentified additional sources of PFEAs to the environment. Future work should prioritize measurements of PFEAs, specifically Nafion BP2, in multiple environmental and biological receptors to better constrain sources, distributions, and bioaccumulation potential of these emerging compounds.

We also found significant concentrations of legacy PFAAs including PFOS and C8-C13 PFCAs in seabirds from all three habitats, and in Great Shearwaters over 2010

– 2019. Individuals reared downstream from the industrial site in North Carolina possessed the highest total concentration of legacy PFAAs, while $\sum_{19}\text{PFAS}$ was statistically similar between Massachusetts Bay and Narragansett Bay birds despite their differential proximity to point sources of PFAS.

Despite being phased out in the early 2000s, PFOS was the most abundant compound identified in most individuals, at levels associated with toxicological significance (> 140 ng/g wet wt. in liver) in other bird taxa⁸. Measurements in Great Shearwater livers from 2010 - 2019 suggest PFOS and FOSA levels are slowly decreasing over time in this species due to the voluntary phase-out of PFOS and its salts in the early 2000s; this decline is in line with observations from other taxa and locations^{9,10}.

C8 – C13 PFCAs were also found in most individuals measured in this work; the C11 PFUnDA was generally the most abundant PFCA identified. PFCAs made up a larger proportion of $\sum_{19}\text{PFAS}$ in Massachusetts Bay birds compared to Narragansett Bay or Cape Fear individuals, and no consistent trends in PFCAs were identified in Massachusetts Bay Great Shearwaters over time. The lack of a clear temporal trend is in contrast to prior work that has identified increasing PFCA levels over time in birds and other taxa^{11,12}, and may indicate an ongoing change point whereby long-chain PFCAs are ceasing to increase in the environment¹³.

This work also described concentrations of 7:3 FTCA, a fluorotelomer carboxylic acid formed through degradation of volatile PFAS, in Great Shearwaters from Massachusetts Bay from 2010 - 2019. 7:3 FTCA likewise demonstrated no clear linear trend over time, suggesting sustained emissions over the duration of our time series,

but alternated with PFUnDA as the second most abundant compound found in each individual. This compound has been previously identified in marine mammals from the NW Atlantic, and our work marks the first identification of this compound in a seabird from the Atlantic Basin. This compound has demonstrated toxicity thresholds up to 10,000 times lower than PFCAs, and requires further research to constrain possible impacts or trends.

These results lead to further questions regarding current PFAS levels in other marine fauna. Further work should prioritize monitoring of PFAS in US East Coast environments and biota, considering the high population density along the US Eastern Seaboard, the abundance of PFAS sources present within this region¹⁴, and the importance of commercial fisheries to regional economies. Future efforts would benefit from inclusion of a broad compound list to evaluate trends in emerging PFAS like PFEAs and 7:3 FTCA, given the noted paucity of data describing their environmental and biological occurrence.

In particular, PFAS and other emerging contaminants should remain a monitoring focus in Massachusetts Bay, given the demonstrated ubiquity of these compounds in seabirds from the region, sustained pollutant inputs from regional urban sources, associations between PFAS and bird individual condition (see below), and the importance of this productive offshore region for commercial fisheries and critically endangered megafauna like the North Atlantic Right Whale.

Follow-up work based on the findings presented within this dissertation will measure novel and emerging PFAS in baleen, liver, fat, and skin from several species of whale that inhabit the Gulf of Maine and Massachusetts Bay, to provide first-of-its-

kind information and improve our understanding of PFAS levels in apex marine biota from this region. Additionally, a food web study incorporating sediment, water, plankton, fish, and Great Shearwaters from Massachusetts Bay is nearing completion (delayed due to COVID-19) and will further develop understanding of PFAS in the region.

Further work should also continue to monitor trends in PFAS levels over time in wildlife populations across the broader US Atlantic Coast, in tandem with characterization of PFAS levels in ambient natural waters using water grab sampling or passive sampling techniques. We expect changes in emissions or production of PFAS to be rapidly reflected in US East Coast biota and environments, due to the region's embedded population centers and PFAS sources. An increased body of time series data from the US East Coast would therefore allow evaluation of fluctuations or trend responses that may indicate the efficacy (or lack thereof) of future policy and production interventions; such data would also provide an indication of potential trend changes expected in other, more remote regions around the globe (i.e. the Arctic).

Associations between PFAS and bird condition. This work assessed relationships between PFAS levels measured in seabird livers and a suite of biological and morphometric variables measured in each bird, using Spearman correlation analyses. We found negative relationships between liver phospholipid levels and PFOS and PFNA concentrations in birds from Massachusetts Bay and the Cape Fear River Estuary, as well as negative relationships between fat depth and several PFAS (Chapters 1, 2). Negative associations between PFAS levels and lipid expression have been previously identified in controlled studies¹⁵, yet this marks the first

documentation of this phenomena in a wildlife population. The range of exposure associated with lipid changes in the offshore birds (11 – 44 ng/g) was much lower than thresholds identified by controlled studies and field-based studies (140 ng/g). Further work evaluating associations between PFAS and lipids in wildlife is crucial, given the importance of finely-tuned lipid dynamics for growth, reproduction, migration, and other key developmental milestones, and the potential for individual adverse effects to scale to population-level impacts. To date, population level impacts of PFAS in birds or other wildlife is completely unexplored. This finding has also led to a follow-up study involving measurement of 1,300 lipid moieties in wild birds and hepatocyte cultures of bird cells, to specifically identify the types of phospholipid most related to PFAS levels. Further exploration of this phenomena could be conducted using serum samples collected in live birds with commensurate measurement of blood chemical parameters using inexpensive veterinary testing kits; this would allow measurement of PFAS levels and serum lipids alongside other biomarkers indicative of physiological changes.

We also found varied associations between kidney weight, liver weight, wing chord, body condition of Great Shearwaters, and various PFAS levels (Chapter 2). Some of the same relationships between morphometric measurements and PFAS levels were recently identified in two species of Pacific shearwater¹⁶. These results are challenging to frame, because scant data exists describing the mechanism through which PFAS may be associated with or driving these relationships, but these changes may be related to PFAS-driven hormonal disruptions that impact structural growth. Additionally, it is unclear how synergistic or additive effects from unidentified co-

contaminants or trophic factors may be influencing these results. More controlled and field study investigating sublethal outcomes in avifauna exposed to environmentally relevant levels of PFAS and other pollutants would be helpful to better clarify if or how PFAS may be related to these morphometric outcomes. Again, it is unclear what, if any, population level impacts may result from the associations between PFAS and bird condition and size observed within this study.

Internal behavior of PFAS. PFAS were measured in eight tissues of seabirds from Massachusetts Bay, Narragansett Bay, and the Cape Fear River Estuary (Chapter 3). These tissue-specific measurements were particularly valuable, because they facilitated exploration of mechanisms driving partitioning of legacy and novel PFAS through comparative means.

This study found PFAAs and PFEAs throughout multiple tissues of birds from each habitat (Chapter 3). This is the first work to document PFO4DA, PFO5DoDA, and Nafion BP2 in multiples tissues of environmentally exposed vertebrates. Blood, liver, kidney, lungs, and brain were found to possess the highest PFAS concentrations. PFOS and PFNA were most abundant in liver, while PFEAs were more abundant in blood. Quantum chemistry calculations suggest the preferred partitioning of PFEAs into blood may be due to different surface charge potential and/or folding conformations that reduce the ability of PFEAs to bind to liver proteins in the same way as PFAAs. Long-chain PFCAs preferentially partitioned into brain tissue; PFEAs were also identified in brain, marking the first time these emerging compounds have been documented to cross the highly selective blood-brain barrier.

Further work should examine the tissue-specific distributions of PFEAs in other biota, given the increasing use of these emerging PFAS in fluoropolymer production and the lack of research parameterizing internal distributions of these ether-based compounds. Additionally, more research is required to determine levels of toxicological concern associated with potential impacts in multiple tissues. Existing thresholds of concern focus on liver, egg, or blood, yet this data and a limited number of other studies^{17–19} investigating PFAAs in a tissue-specific manner confirm that kidney, lungs, and brain are also important PFAS reservoirs.

The totality of PFAS research and associated expertise documented within this dissertation likewise contributed to development of a large critical review, as part of a wider team effort seeking to review human and wildlife exposure to PFAS (Co-author work, De Silva et al. 2021, Accepted).

Accumulation of ingested plastics in seabirds. The accumulation of ingested plastics was assessed in 175 adult and juvenile Great Shearwaters necropsied as part of this dissertation (Chapter 4). An average of 8 plastic pieces were found in each individual. Juveniles accumulated plastic more frequently than adults, with an ingestion frequency of 98% compared to 58% in breeding adults. The frequency of plastic occurrence (FO) observed in juveniles was higher than in previous studies focused on this species in prior decades^{20,21}. The physical dimensions, morphological category, and polymer makeup of each plastic piece were assessed, and revealed that breeding phase adults from Gough Island in the South Atlantic ingested larger plastic fragments compared to mature and juvenile Great Shearwaters in the NW Atlantic, likely due to size-mediated transport of larger plastic pieces to more remote pelagic environments²². While

polyethylene was the primary polymer ingested across all habitats, Gough Island adults ingested a higher proportion of polypropylene mixes compared to a higher incidence of polyethylene and “other” type plastics, composed primarily of balloons. These results suggest Great Shearwaters turn over plastics rapidly enough to reflect different plastic signatures associated with contrasting ambient environments. This contribution adds evidence to the otherwise still-uncertain retention time of ingested plastics in seabirds²³. Future work monitoring plastics in Great Shearwaters should prioritize use of standard approaches²⁴, while incorporating measurements from multiple age groups and locations to further refine the retention of plastic pieces in this sentinel species.

Overall Policy and Management Implications. The seabird demographic data produced within this work provide the first quantitative description of Great Shearwaters using the Massachusetts Bay region, and may be useful for future resource management. By demonstrating the importance of Massachusetts Bay for juvenile Great Shearwaters, this finding suggesting the need to balance the timing or location of compatible uses with use of the region by young, naïve seabird individuals reliant on consistent food resources and environmental conditions. These results also highlight the importance of bycatch practices that reduce potential for seabird-fishery interactions, considering bycatch is preferentially removing potentially thousands of juveniles per year with uncertain implications for the population.

The descriptions of PFAS occurrence and possible impacts described in this work may also be useful to inform policy and management initiatives. Primarily, this work documented widespread occurrence of PFOS, C9 – C13 PFCAs, Nafion BP2, and 7:3

FTCA in seabirds from several habitats. Only PFOS has been vetted for effects in controlled and field settings, highlighting the need for science and policy to expand to understand and potentially mitigate the proliferation of other PFAS beyond PFOS in biota.

The decreasing trends of PFOS and FOSA identified in this dissertation and elsewhere indicate that phase-out or regulation of specific, well-studied PFAS leads to decreases in environmental matrices on observable time scales. As more PFAS are deemed harmful, policies mandating cessation of use are essential to protect human and ecological health. More logically, this work suggests PFAS and other human-created chemicals should be vetted for the totality of environmental behaviors before production and introduction to the environment to prevent regrettable, unintended, and irreversible environmental outcomes. For example, long-chain PFCAs, rarely produced intentionally, along with a degradation compound 7:3 FTCA, were some of the most abundant compounds found in seabirds from multiple habitats. The downstream bioaccumulation of these inadvertently created compounds underscores the importance of understanding and assessing the risk of the total potential life-cycle of a given PFAS prior to use and release to prevent the environmental formation of bioaccumulative and ubiquitous terminal end products like long-chain PFCAs or 7:3 FTCA.

Given this proactive approach is not currently formalized in policy or practice within the US, results obtained within this dissertation indicate the need to avoid policy or scientific assumptions that assume novel PFAS are innocuous or environmentally irrelevant. For example, the use of novel PFEAs should better

account for the newly discovered bioaccumulation potential and environmental mobility of these novel PFAS. PFEAs are currently in use as replacements for some legacy PFAS, and these results demonstrate these new compounds spread in the environment and accumulate across a range of tissues akin to the compounds PFEAs are intended to safely replace, and therefore should receive the same scientific and regulatory scrutiny as their legacy counterparts. The preference of PFEAs to partition to blood compared to other tissues suggests blood monitoring may be an important tool to understand PFEA distributions in other species or locations, yet further research is required to parametrize accumulation and potential impacts of PFEAs in multiple tissues considering the abundant concentrations of these compounds found across eight tissues included in this study.

The sustained occurrence of both legacy and novel PFAS in seabirds and their association with a range of body condition changes at environmentally relevant concentrations demonstrated herein suggests sublethal impacts may be ongoing, yet little work meaningfully explores the implications of PFAS in marine food webs despite the importance of these environments for seafood production and human economies. Funding mechanisms should prioritize exploration of PFAS in marine food webs to better parameterize the underexplored range of individual and population-scale impacts of PFAS in marine biota at ambient exposure levels. Chemical production and clean-up policies should then follow suit to ensure marine food webs and seafood resources are safe for human and wildlife consumption.

This work additionally underscores the importance of policies and practices that support waste management and effective recycling, considering upwards of 90% of

plastic items found within seabirds were recyclable polymers that likely ended up in the environment due to waste mismanagement.

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APPENDIX A

COLLABORATIVE CONTRIBUTIONS

This appendix contains co-author and collaborative works undertaken during the PhD

Candidacy, beyond the primary research aims.

Contribution 1: Authorship of Stellwagen Bank National Marine Sanctuary's Condition Report sections on water quality and contaminant monitoring (pages 323 – 348)

Contribution 2: Co-author in Markham et al. 2018; contributed analysis of PBDE-209 in seal milk samples via GC/ECNI-MS (pages 349 – 358)

Contribution 3: Co-author in Powers et al. 2020; contributions including participation in field work, provision of all necropsy data, drafting and editing of text detailing necropsy/ageing methods and significance, and active role in manuscript editing and revision (pages 359 – 373).

Contribution 4: Co-author in Suca et al. 2020, in review; contributions included extraction and analysis of 202 fish for lipid content with primary author, active role in manuscript revisions related to lipid content hypotheses (pages 374 – 418).

Contribution 5: Co-author in De Silva et al. 2021, accepted; contributions included authorship of wildlife exposure and bioaccumulation section in collaboration with two co-authors (pages 419 – 476)

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
Office of National Marine Sanctuaries
Stellwagen Bank National Marine Sanctuary
175 Edward Foster Rd Scituate, Massachusetts 02066

August 28th, 2020

Dear Dr Lohmann and the Dissertation Committee of Anna Robuck,

I am writing to attest to the phenomenal work PhD candidate Anna Robuck contributed to the development of the Stellwagen Bank National Marine Sanctuary (SBNMS) 2020 Condition Report. The selected portions contained here reflect text and figures Anna contributed to this effort.

The Condition Report is a way for the sanctuary to evaluate and document the changing conditions of the resources protected and managed by the Sanctuary. It is a precursor to the planned revision to the SBNMS management plan, and as such provides data and information which will guide and inform any revisions that may be needed to more effectively manage these resources. To prepare the Condition Report, SBNMS staff (including Anna, as our Nancy Foster Scholar) developed background papers describing the current status of resources, and then presented their data and preliminary status report to an external expert panel, who made recommendations on the conditions and trends of these resources. Topic authors were further involved in multiple rounds of review and copyediting to arrive at the final resource management tool.

Anna was asked to provide write-ups on the following topics:

- *What is the eutrophic condition of sanctuary waters and how is it changing?*
- *Do sanctuary waters pose risks to human health and how are they changing?*
- *Have recent changes in climate altered water conditions and how are they changing?*
- *Are other stressors, individually or in combination, affecting water quality, and how are they changing?*
- *What are the levels of human activities that may adversely influence water quality and how are they changing?*
- *What are contaminant concentrations in sanctuary habitats and how are they changing?*

Anna's efforts in this regard vastly exceeded the contributions of a typical Nancy Foster scholar and fully attest to her consummate professionalism. She did an extraordinary job preparing her write-ups, demonstrating thorough research and comprehensive understanding of each topic. Her initial literature review provided to our Expert Panel included 28 pages of literature review and figures. At our Expert Workshop in February 2019, Anna presented her sections, and meaningfully discussed her assessment of the literature with experts who were advocating

contrary viewpoints, and overall defended her work with strong and well-supported arguments. She further worked with expert reviewers and NOAA staff to narrow the literature review down to the sections in print within the final 2020 Condition Report. Anna's contributions to this effort were significant and illustrate her unlimited potential as a research scientist should she decide to pursue this career.

Sincerely,

Captain Peter DeCola
US Coast Guard (retired)
Superintendent
Stellwagen Bank National Marine Sanctuary

Enclosures: As stated.

Office of National Marine Sanctuaries
National Oceanic and Atmospheric Administration

STELLWAGEN BANK NATIONAL MARINE SANCTUARY

2020 CONDITION REPORT

FINDINGS OF STATUS AND TRENDS

2007-2018

STELLWAGEN BANK

U.S. Secretary of Commerce
Wilbur Ross

Under Secretary of National Oceanic and Atmospheric Administration (NOAA)
Dr. Neil Jacobs (acting)

Assistant Administrator for National Ocean Service
Nicole LeBoeuf (acting)

Director, Office of National Marine Sanctuaries
John Armor

Cover photo: Two American lobsters (*Homarus americanus*) and a school of cunner (*Tautoglabrus adspersus*) use a historic shipwreck as home and habitat. The underwater photographer, Keith Ellenbogen, is the recipient of a Ernest F. Hollings Ocean Awareness Award to use the art of underwater photography to raise awareness about Stellwagen Bank National Marine Sanctuary. Photo: Keith Ellenbogen

Suggested Citation: Office of National Marine Sanctuaries. 2020. 2020 Condition Report: Findings of Status and Trends for 2007-2018. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries, Silver Spring, MD. 263 pp.

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¹ Tammy Silva was supported by the NOAA Dr. Nancy Foster Scholarship to conduct dissertation research on toothed whale occurrence in SBNMS relevant to this report.

² Peter Auster was supported by a grant from the National Marine Sanctuary Foundation to conduct analyses and synthesize information related to habitat, living marine resources, and biodiversity presented in this report.

³ Anna Robuck is supported by the NOAA Dr. Nancy Foster Scholarship in pursuit of Ph.D. research relevant to SBNMS water and habitat quality.

Water Quality

Despite several potential stressors, sanctuary water quality is good/fair and does not appear to be adversely impacted by human activities. The largest effluent contributor in the region and the primary potential human source of nutrients is the Massachusetts Water Resources Authority (MWRA) outfall, which is located approximately 12 nautical miles from the western boundary of SBNMS. Ongoing monitoring suggests that the MWRA outfall is currently not adversely influencing monitored water quality parameters in SBNMS, and no evidence suggests that eutrophication is occurring. The Massachusetts Bay Disposal Site (MBDS) is directly adjacent to the sanctuary's western boundary and receives dredged material deemed suitable for open water disposal. This site incorporates the areas of two historic disposal sites containing toxic materials, though deposited toxic materials show limited mobility, and assessments have not shown any associated contamination of SBNMS. Maintenance dredging and expansion of Boston Harbor will generate 12 million cubic yards of dredged material for disposal in MBDS, but this is not expected to impact sanctuary water quality. Limited data exist to thoroughly evaluate potential impacts to water quality from vessel discharge and sediment perturbation by mobile fishing gear.

Monitoring suggests that concentrations of bacteria and toxigenic phytoplankton species rarely reach or exceed levels of concern in SBNMS and likely pose low risk to human health. However, the presence of toxigenic phytoplankton species indicates the potential for harmful algal blooms (HABs). HAB dynamics in the Gulf of Maine may be related to climate change, but current data show no explicit association between HAB occurrence and increasing temperatures in SBNMS.

Climate change impacts in SBNMS are measurable, and the threat of climate change to ecological integrity is increasing. The Gulf of Maine is warming faster than 99% of the global ocean; increases in both surface and bottom temperatures in SBNMS reflect these trends. Recent work suggests changes in seasonal temperature dynamics, longer summer seasons, and changes to primary production in and around SBNMS. Climate change is causing shifts in phenology and distributions of plankton, fish, whales, and other organisms in the Gulf of Maine. Impacts of climate change on important prey (foundation) species like sand lance and the copepod *Calanus finmarchicus* are particularly concerning, as these changes have the potential to drive cascading ecosystem effects and impact abundance, distribution, and health of top predators. In addition, climate change is causing impacts on commercial and recreational fisheries, local businesses, and communities. More robust monitoring of climate change effects and ocean acidification conditions in SBNMS is necessary to understand trends, seasonal fluctuations, and the possible ramifications for water quality, shell-forming invertebrates, and the larger ecosystem.

Habitat

The sanctuary's diverse underwater landscape is a patchwork of habitats composed of both geologic and biogenic components. Benthic substrate types are generally correlated with seafloor communities and constitute important geologic habitat components. Data suggest measurable degradation of habitat quality

over the past ten years, primarily due to direct impacts of bottom-contact gear used in commercial fishing, which occurs extensively throughout SBNMS. Mobile, bottom-tending fishing gear can alter or remove important structural characteristics and/or biological components of the seabed, which is a concern for maintaining habitat integrity. Lower levels of direct impact are evident in the Western Gulf of Maine Closure Area, and the closure led to recovery in some areas. Significant increases in scallop dredging may have impacted the northwest corner of Stellwagen Bank in 2017; this effort has been reduced, but is expected to continue. Overall fishing effort has declined by around 55% since 2009; however, it is unclear whether reductions in effort have resulted in improved sanctuary benthic habitat integrity. Fixed gear has less impact on the seabed, but poses an entanglement risk for protected species. Fishing effort reductions and gear modifications have been implemented to reduce bycatch of small marine mammals and seabirds and to attempt to reduce serious injury and mortality of large whales.

Localized disturbance of benthic habitats was observed after the installation of the sanctuary's only submerged cable, the Hibernia Atlantic cable, in the year 2000. Post-installation monitoring showed that impacts to benthic communities were not significantly different than those caused by commercial fishing. Other potential localized seabed impacts, including anchoring of recreational fishing vessels, should be evaluated.

Legacy contaminants and metals in benthic habitats have been reported; however, they do not appear to remobilize beyond sites where they have been identified, and no population effects have been documented. Limited data suggest emerging contaminants are present within Massachusetts Bay at low concentrations, with uncertain biological ramifications, and may be shifting or increasing with the continued introduction of new chemicals. No data exist regarding microplastics in SBNMS, though surface water concentrations of microplastics in the Gulf of Maine are far lower than in the North Atlantic. Abundance of marine debris in SBNMS depends on natural forces and human drivers, which may increase with increasing population growth and coastal development. Additional work to characterize and quantify emerging contaminants and microplastics in SBNMS habitats is needed.

SBNMS is an urban sanctuary, and impacts to its acoustic environment are a concern. Designated shipping lanes such as the Boston Traffic Separation Scheme (TSS) cut through the sanctuary and are used by most commercial vessels transiting to the port of Boston. Commercial fishing vessels use the sanctuary year-round. The whale watching industry has expanded, and the numbers of recreational boaters and whale watchers are rising. Human-generated underwater noise from vessels, particularly from large commercial ships, can degrade habitat quality and interrupt behavior and communication of many marine species. Increasing noise levels and impacts of noise to some marine mammal and fish species have been documented in the sanctuary and are expected to continue. SBNMS has been at the forefront of raising awareness of the potential threat of noise to organisms and has pioneered the use of several advanced passive acoustic monitoring methods and technologies to further the study of ocean noise and its impacts.

STATE OF SANCTUARY RESOURCES

Water Quality (Questions 6–9)

The following information provides an assessment of the status and trends of key water quality indicators in SBNMS for the period 2007–2018. Eutrophic conditions and their influence on primary production in sanctuary waters is the focus of Question 6. Eutrophication is the accelerated production of organic matter, particularly algae, usually caused by an increase in the amount of nutrients (primarily nitrogen and phosphorus) in surface waters. Eutrophication can impact the condition of sanctuary resources, for example, by promoting nuisance and toxic algal blooms or impacting dissolved oxygen levels.

Question 7 focuses on parameters affecting public health. Human health concerns can arise from water, beach, and/or seafood contamination (bacteria or chemical). Indications of health impacts may include fishery closures and seafood consumption advisories.

Question 8 focuses on shifts in water quality due to climate drivers. Climate indicators include indices of large-scale climate patterns, water temperature, acidity, upwelling intensity and timing, and dissolved oxygen. Shifts in water temperature can affect species growth rates, phenology, distribution, and susceptibility to disease. Acidification can affect organism survival, growth, and reproduction. Upwelling influences oxygen content and nutrient cycling.

Question 9 assesses other biotic and abiotic stressors, individually or in combination, that may influence sanctuary water quality, but were not addressed in other questions. Examples include non-point source contaminants, and hard-to-quantify stressors that influence the condition of habitats and living resources. Such inputs may include industry discharges and emissions, fertilizers, pesticides, heavy metals, and sewage.

6. What is the eutrophic condition of sanctuary waters and how is it changing?

Status Description: Eutrophication has not been documented, or does not appear to have the potential to negatively affect ecological integrity.

Rationale: MWRA hydrographic modelling (Zhao et al. 2017a) suggests that eutrophication is not occurring. Dissolved oxygen has not approached hypoxic or anoxic conditions over time. Background nitrogen may be decreasing regionally, which would decrease the probability of eutrophication.

The 2007 rating for this question was “good” (very high confidence) and “not changing” (very high confidence). Since 2007, regional monitoring suggests no sustained increases of key nutrient concentrations or nutrient ratios. Observed nutrient dynamics within the sanctuary and greater Massachusetts Bay are primarily driven by regional circulation and hydrodynamics of the Gulf of Maine (Townsend et al. 2015, 2010, McManus et al. 2014, Libby et al. 2017). Thus, the rating remains unchanged.

SBNMS receives nutrient and pollutant inputs from coastal point and non-point sources and via atmospheric deposition from proximate and distant inland regions. Anthropogenic nutrient sources of primary importance include wastewater treatment plants discharging into rivers or directly into Massachusetts Bay. Notably, the MWRA Deer Island Wastewater Treatment Plant sewage outfall discharges approximately 350 million gallons of effluent daily into the offshore Massachusetts Bay environment, roughly 12 nautical miles west of the SBNMS boundary (Werme et al. 2017).

MWRA conducts ongoing water quality monitoring across Boston Harbor and Massachusetts Bay to identify impacts of the Deer Island Wastewater Treatment Plant outfall within the coastal and offshore environment. The monitoring program assesses an extensive suite of physical and chemical parameters, such as temperature, salinity, dissolved oxygen, and nutrient concentrations, in addition to biological parameters, such as chlorophyll concentrations and phytoplankton community composition. Monitoring has been ongoing since 1992, with increased monitoring in SBNMS directly following outfall relocation in 2000 until 2010, when it was determined that a smaller subset of offshore stations adequately represented offshore conditions. MWRA currently maintains one monitoring station in the southwest corner of SBNMS (F29), one station slightly outside the northwest corner of SBNMS (F22), and two stations within Cape Cod Bay (F01 and F02). These stations are monitored nine months of the year. The Northeastern Regional Association of Coastal Ocean Observing Systems also maintains a monitoring buoy along the northwest boundary of the sanctuary, collecting data on water temperature, salinity, turbidity, chlorophyll concentration, density, dissolved oxygen, wind, waves, and currents on an hourly basis. These current and historical data sources provide an excellent platform from which to identify and infer long-term water quality trends within and around SBNMS.

The 2007 condition report and 2010 management plan both suggested that eutrophication was not a pressing problem in SBNMS waters. Nitrogen and phosphorus (in elevated concentrations) are the primary nutrients that may contribute to eutrophication. Ammonium and nitrate are two forms of nitrogen that may particularly contribute. Nutrient dynamics in the region are dominated by large-scale circulation of the Gulf of Maine and influx of Gulf of Maine water into Massachusetts Bay (Figure S.WQ.6.1,

HydroQual 2000). Nutrient availability in surface and bottom waters is influenced by stratification and circulation patterns that are regulated by long-term climate dynamics and seasonal forcing. Surface water nutrient concentrations have remained roughly constant or declined over the course of MWRA monitoring (Figure S.WQ.6.3). Bottom water nutrient trends roughly approximate surface water results, yet bottom water reservoirs display predictably higher nutrient concentrations compared to surface waters (Figure S.WQ.6.4). Specifically, total nitrogen and total phosphorus have decreased in concentration since the early 2000s, possibly related to circulation shifts driven by the North Atlantic Oscillation (Figure S.WQ.6.4). SBNMS waters contain sustained concentrations of ammonium and nitrate, consistent with seasonal nutrient cycles observed across the region (McManus et al. 2014, Costa et al. 2017, Libby et al. 2017). Concentrations of orthophosphate and silicate, two other types of nutrients important for primary productivity, have decreased in the offshore environment since roughly 2010–2011. These nutrients typically come from terrestrial environments, so changes may reflect larger-scale shifts in circulation or precipitation altering riverine inputs or connectivity to offshore habitats (Figure S.WQ.6.3, Townsend et al. 2010, 2015, Berton et al. 2017). Overall, nutrient data from 1994–2016 suggest no evidence of increased eutrophication in sanctuary waters (Townsend et al. 2010, 2015, Costa et al. 2017, Libby et al. 2017). Modeling results support a lack of eutrophication potential in surface and bottom waters of Massachusetts Bay and SBNMS due to effluent inputs (Zhao et al. 2016, 2017a). Bottom water dissolved oxygen concentrations also do not indicate hypoxic or anoxic conditions indicative of eutrophication (Libby et al. 2017).

Figure S.WQ.6.1. A mass balance of nitrogen inputs into Massachusetts Bay from 1992–1994 suggests approximately 3% of the nitrogen in Massachusetts Bay originates from MWRA sources, while 92% of nitrogen in the system comes from regional circulation beyond the boundary of Massachusetts Bay. This modeling underscores the low impact of the MWRA outfall tunnel on nutrient dynamics in SBNMS and the greater Massachusetts Bay. Image: Modified from HydroQual 2000.

S.WQ.6.2. Location of MWRA monitoring sites across Massachusetts Bay. Station F29 is at the southwest corner of the sanctuary, while F22 is slightly outside sanctuary boundaries to the west. These two stations were used as representations of sanctuary water quality as captured within this monitoring program. Image: Libby et al. 2017

Figure S.WQ.6.3. Intra- and interannual nutrient data from F29, an MWRA monitoring station within SBNMS. Figures present monthly and yearly average concentrations of nitrate + nitrite, ammonium, orthophosphate, and silicate (top row) along with total dissolved nitrogen (TDN, bottom row). These data suggest an apparent lack of eutrophication as indicated by concentrations of key nutrients. Quasi-predictable seasonal fluctuations coupled with regional biogeochemical and hydrological dynamics drive observed nutrient concentrations and subsequent primary productivity in SBNMS and Massachusetts Bay. Gaps in nitrogen data reflect gaps in measurement of these parameters at station F29. Image: MWRA yearly average nutrient data, stations F02, F06, F29, F22, and N20. MWRA data courtesy of Kenneth Keay and Douglas Hersh

Figure S.WQ.6.4. Data from F22, an MWRA monitoring station directly adjacent to SBNMS boundaries, also suggest that eutrophication is not a pressing problem in SBNMS surface or bottom waters, or the surrounding far-field environment. Dashed purple lines indicate mean parameter concentration across time series, while red vertical lines demarcate MWRA outfall introduction in 2000. Note: F22 was used to denote total dissolved nitrogen (TDN) and total dissolved phosphorus (TPP) in the absence of identical data at F29. Image: MWRA yearly average nutrient data, stations F02, F06, F29, F22, and N20. MWRA data courtesy of Kenneth Keay and Douglas Hersh

Further evidence against eutrophication includes constant or declining regional primary production (Figure S.WQ.6.5, NEFSC 2017b, Libby et al. 2017, Oviatt et al. 2007). Notably, phytoplankton and zooplankton abundance and community makeup show significant seasonal and annual variation since MWRA monitoring began in 1992; however, these changes are likely driven by regional, decadal scale processes in the Gulf of Maine rather than eutrophication-related drivers (Oviatt et al. 2007, McManus et al. 2014, Costa et al. 2017, Libby et al. 2017).

Figure S.WQ.6.5. Primary production in the Gulf of Maine, left, and Georges Bank, right, has fluctuated over time, with current conditions suggesting stable and average overall system production. Dashed lines indicate +/- 1 standard deviation. Image: NEFSC 2017b

7. Do sanctuary waters pose risks to human health and how are they changing?

Status Description: One or more water quality indicators suggest the potential for human health impacts, but human health impacts have not been reported.

Rationale: Toxicogenic algae are present but not observed to cause demonstrable threats to human health. Observed water quality changes over the last 12 years may be related to changes in the North Atlantic Oscillation.

In the 2007 condition report, this question was rated “good” and “not changing.” The status has now been downgraded to “good/fair.” Ongoing bacterial monitoring suggests bacteria are rarely detectable. The downgraded rating stems from the documented occurrence of several toxigenic phytoplankton species with varying degrees of predictability; this suggests the potential for human health impacts, though these impacts have not been reported. Harmful algal bloom dynamics in SBNMS and the wider Gulf of Maine are thought to be mediated by large-scale circulation and climatic dynamics.

At present, SBNMS waters likely pose low risk to human health. Bacterial monitoring has been ongoing across the region by MWRA related to the Deer Island Wastewater Treatment Plant outfall, and indicates that water column bacterial counts consistently remain below detection limits, with a very low likelihood of exceeding thresholds protective of public health and resource use (Codiga et al. 2016).

The most likely source of possible human health effects in the region stems from the presence of toxigenic phytoplankton species (Figure S.WQ.7.1). Identified species of note include *Pseudo-nitzschia* spp., *Alexandrium* spp. (Pittman 2019), and *Dinophysis* spp., which have all been documented in SBNMS, Massachusetts Bay, and the greater Gulf of Maine. *Pseudo-nitzschia* spp. are classified as diatoms, while the latter two species are dinoflagellates; all produce toxins that may bioaccumulate, particularly in shellfish, and can cause widespread economic loss or health effects in humans (Anderson et al. 2000, Etheridge 2010, Lefebvre and Robertson 2010).

There is no definitive understanding regarding the risk of harmful algal blooms (HABs) to humans or wildlife that utilize the sanctuary, although risk from these or other toxigenic algae may be changing due to evolving HAB dynamics in the greater Gulf of Maine. *Alexandrium* spp. and *Dinophysis* spp. have been irregularly identified at low numbers within SBNMS and surrounding waters as measured via MWRA whole water phytoplankton assessments and regional research (Figure S.WQ.7.1, Anderson et al. 2014a, Gobler et al. 2017, Libby et al. 2017). *Alexandrium* spp. blooms are frequently a health concern in waters further north of the sanctuary (Anderson et al. 2014a, 2014b). Literature review and regional monitoring suggest *Alexandrium* spp. concentrations in excess of 1000 cells/L require increased monitoring for shellfish toxicity; MWRA maintains a caution threshold of 100 cells/L for the same taxa. Concentrations of *Alexandrium* spp. have infrequently surpassed either threshold, with notable occurrence of *Alexandrium* in the sanctuary only in 2005 according to MWRA cell count data.

Over 14 *Pseudo-nitzschia* species have been identified in the Gulf of Maine, seven of which are known to produce domoic acid, a neurotoxin. *Pseudo-nitzschia* spp. has been regularly documented in SBNMS according to MWRA survey data, with notable occurrences in sanctuary waters in the autumns of 2012, 2013, and 2016 (Figure S.WQ.7.1). Literature review, regional monitoring, and safety thresholds established by states in the region suggest *Pseudo-nitzschia* cell counts in excess of 20,000 cells/L merit

increased monitoring for potential adverse effects in shellfish. Data exist regarding the relationship between cell count and domoic acid production or bioaccumulation specific to the offshore environment (Fernandes et al. 2014), which makes understanding risks associated with cell count thresholds challenging. It is also unclear if or to what extent the presence of this toxigenic diatom may translate to domoic acid exposure in the sanctuary food web. Blooms of toxigenic *Pseudo-nitzschia* spp. have been implicated in marine mammal and bird deaths around the globe, yet no SBNMS-specific mortality events have been documented (Lefebvre and Robertson 2010, D'Agostino et al. 2017).

Climate change likely plays a pivotal role in local and regional HAB dynamics. Recent research examining the relationship between sea surface temperature and HAB dynamics across the greater Northwest Atlantic from 1982–2016 found that bloom season and growth rate of *Alexandrium* spp. and *Dinophysis* spp. were positively correlated with increasing sea surface temperature, suggesting that climate change may have already exacerbated these or other HAB species dynamics in the region (O'Neil et al. 2012, Gobler et al. 2017). Modeling work considering the greater Gulf of Maine also suggests that climatically-mediated variables, such as river discharge, wind patterns, and cross-shore transport, are specifically associated with paralytic shellfish poisoning toxicity events caused by *Alexandrium fundyense* in a given year; it is uncertain if or how these variables may be shifting within SBNMS and how this may translate to increased risk for toxic HAB events (Nair et al. 2013). Phytoplankton cell counts obtained via MWRA monthly monitoring data from F22 and F29 do not indicate an explicit association between HAB species occurrence and warming SBNMS surface water temperatures or repositioning of the outfall. Although this may be a function of sampling frequency or a similar artifact, substantial regional evidence of decadal variation modulating HAB dynamics suggests the same factors are moderating HAB occurrence locally in SBNMS (Anderson et al. 2014a, 2014b, Libby et al. 2017, Nair et al. 2013). Note that MWRA cell count and monitoring data, taken monthly from February until December, can only be used to moderately comment on the occurrence or risk of quickly evolving HAB blooms, as blooms may develop and dissipate over the course of a few days or weeks. Further study of phytoplankton communities and toxigenic algae dynamics in the face of changing regional dynamics is warranted.

Figure S.WQ.7.1. Yearly average cell counts of toxicogenic algae genera at two MWRA ambient monitoring stations. Grey area indicates the species was not observed. While there is no clear trend associated with cell counts of toxicogenic algae species over time, several types of toxicogenic algae occur regularly or semi-regularly in cell count surveys conducted at MWRA stations F29 and F22, in or directly adjacent to SBNMS waters. Image: MWRA monthly surface phytoplankton count data, stations F29 and F22. MWRA data courtesy of Kenneth Keay and Douglas Hersh

8. Have recent changes in climate altered water conditions and how are they changing?

Status Description: Climate-related changes have caused measurable, but not severe, degradation in some attributes of ecological integrity.

Rationale: Climate change is influencing the primary production cycle in the region, and has the demonstrated capacity to produce cascading effects within the ecosystem. Additional changes in water temperature, dissolved oxygen, stratification, sea level, precipitation, and storm activity have been documented or modeled, with some suggestion of changes in pH, though more monitoring is needed to more robustly identify acidification trends and effects.

This question is new and was not assessed in the 2007 condition report. SBNMS, as part of the Gulf of Maine, is one of the fastest warming regions around the globe. The current rating is based on changes in the phenology and distribution of fish, plankton, and other organisms that are being observed and attributed to climate change (Staudinger et al. 2019, Pershing et al. 2015, Nye et al. 2009), all of which are affecting production and food web dynamics within the ecosystem.

Climate change impacts have manifested regionally in remarkable and dramatic ways. Overall, the Gulf of Maine is warming 99% faster than the global ocean. This 1.5°C/decade temperature increase is likely due to the influence of decadal-scale cycles and the northward shift of the Gulf Stream (Pershing et al.

2015, Thomas et al. 2017). The Gulf of Maine is experiencing areas of increasingly warmer surface water and decreases in areas of cold water since 1980 (Figure S.WQ.8.1, NEFSC 2017a). Using monitoring data from the MWRA monitoring site in SBNMS, linear regression of yearly average water temperature data suggests increases in both surface water and bottom water commensurate with regional trends (Figure S.WQ.8.2). Seasonal water temperature changes have also been documented in the Gulf of Maine, with recent research suggesting an earlier summer start date, a later summer end date, and longer summer length based on data from a 33-year period (Thomas et al. 2017). Recent national predictions also suggest average annual air temperatures in the northeast may increase between 3.98 and 5.09°F as early as 2036 (Wuebbles et al. 2017).

Figure S.WQ.8.1. Changes in sea surface temperature and thermal habitat area over time. Red dashed line indicates 2016 data. Image: NEFSC 2017b

Figure S.WQ.8.2. Plot displaying average yearly surface water (left panel) and bottom water (right panel) temperatures observed at F29, the MWRA monitoring station on the southern boundary of SBNMS. Image: MWRA yearly average nutrient data, stations F02, F06, F29, F22, and N20. MWRA data courtesy of Kenneth Keay and Douglas Hersh

Dissolved oxygen concentrations have decreased over time in SBNMS and Massachusetts Bay according to MWRA monitoring data. This may be due to sampling artifacts or related to the reduced capacity of warmer water for oxygen. Note that decreased oxygen concentrations are likely not yet ecologically relevant, as bottom water consistently maintains saturation levels above 80% based on MWRA monitoring data (Libby et al. 2017).

Figure S.WQ.8.3. Plot displaying average annual surface (left panel) and bottom water dissolved oxygen (DO) concentrations (right panel) observed at five MWRA monitoring stations in Massachusetts Bay, with the purple line depicting the mean dissolved oxygen level observed over the time series. Image: MWRA yearly average nutrient data, stations F02, F06, F29, F22, and N20. MWRA data courtesy of Kenneth Keay and Douglas Hersh

Climate change is also likely affecting primary production within SBNMS and the surrounding offshore environment, with uncertain cascading effects within the regional food web (McManus et al. 2014, Costa et al. 2017, Libby et al. 2017). Discharge from the Merrimack River has been demonstrated to strongly correlate with conditions associated with key climate indices, the Atlantic Multidecadal Oscillation, and the North Atlantic Oscillation, with greater than average river discharge anticipated in future (Berton et al. 2017). Climatically-mediated river discharge from the Merrimack and other coastal rivers, Western Maine Coastal Current intrusion, wind stress, and inflow contributions to the greater region from low-salinity Scotian Shelf waters act to freshen the surface layer and increase stratification, producing variable and dynamic levels of primary production and nutrients (McManus et al. 2014, Townsend et al. 2015).

The Northeast is expected to experience higher rates of sea level rise by 2100 compared to the global average (NEFSC 2017b, Wuebbles et al. 2017). Massachusetts coastlines are projected to experience an average sea level rise of 2.83 mm/year. Cape Cod and outlying islands are particularly at risk in terms of

sea level rise (NOAA 2019b). The offshore location of SBNMS insulates it from direct effects of sea level rise. It is unclear how inundation of coastal areas and changing sediment sources/transport may impact offshore environments in Massachusetts Bay.

The Gulf of Maine region is particularly susceptible to ocean acidification, due to poor buffering capacity and existing low calcium carbonate concentrations as a result of substantial freshwater and low-salinity inputs from coastal rivers and Scotian Shelf flow (Gledhill et al. 2015, Townsend et al. 2015, Wang et al. 2017). However, recent increases in the temperature and salinity of the Gulf of Maine as a result of the northward shift in the Gulf Stream appear to be buffering against the impacts of ocean acidification on pH and aragonite saturation state (Salisbury and Jönsson 2018). Regional work has demonstrated that inorganic carbon concentrations are determined by seasonal cycles driven by primary production-respiration dynamics, with apparent CaCO_3 dissolution in bottom water during fall and winter (Wang et al. 2017). A pilot study in SBNMS from December 2011–June 2012 indicated decoupling of surface and bottom water pCO_2 in response to the spring bloom, with corresponding decreases in bottom water pH and calcium carbonate saturation state, a derived index of carbonate ion availability (SBNMS 2013). Sustained ocean acidification may result in sustained subsurface aragonite undersaturation across the region in 30–40 years, with variable and uncertain consequences for water quality or shell-forming invertebrates in SBNMS and the greater Gulf of Maine (Gledhill et al. 2015, Ekstrom et al. 2015, Wang et al. 2017). More robust monitoring incorporating both surface and bottom water measurements is necessary across SBNMS and the wider region to understand acidification trends, seasonal fluctuations, and possible ramifications for shellfish and the larger ecosystem.

A warming atmosphere and ocean also translates to increased water vapor in the atmosphere, impacting precipitation and tropical cyclone activity. In the northeast, the annual maximum daily precipitation was 17% higher between 1981 and 2015 than it was in the years between 1901 and 1960, while extreme precipitation from 1996–2014 was 53% higher than 1901–1995 (Figure S.WQ.8.5, Huang et al. 2017, Wuebbles et al. 2017). Though numbers of tropical cyclones are predicted to remain the same or even decrease globally, tropical cyclone intensity has increased over the past 40 years as illustrated by the doubling of the number of category 4 and 5 tropical cyclones since the 1970s. Moreover, more storms are predicted to track poleward under current emission and temperature change scenarios, likely translating to increased tropical activity reaching SBNMS and the greater North Atlantic (Tamarin-Brodsky and Kaspi 2017, Wuebbles et al. 2017). It is uncertain how increased hurricane and tropical storm exposure may impact local resources and processes.

9: Are other stressors, individually or in combination, affecting water quality, and how are they changing?

Status Description: Undetermined

Rationale: Ongoing contaminant monitoring has focused on a handful of legacy contaminants, leaving the majority of emerging organic contaminants unmeasured. No data exists to determine changes over time, which is the primary factor driving the rating and trend. More monitoring is needed in this area.

In the 2007 condition report, this question was rated “good/fair” and “not changing.” Since that report, limited data has emerged to document other water quality stressors. Sediment monitoring has indicated concentrations of man-made organic contaminants and metals have decreased or remained steady in the SBNMS region. Both modeling and observational work indicate that dredge material and any associated contaminants remain confined within disposal sites, and are not likely to impact water quality. However, ongoing contaminant monitoring has focused on only a handful of legacy contaminants, leaving the majority of emerging organic contaminants unmeasured, with no indication of how they may be changing in the system, which is the primary uncertainty driving the “undetermined” rating for status and trend for this question.

As an offshore region adjacent to urbanized margins, SBNMS is subjected to a variety of stressors with variable influence on observed water quality. Anthropogenic contaminants, wastewater discharges, and vessel discharges are stressors of particular relevance that may impact water quality within the sanctuary.

Current organic pollutant monitoring by MWRA within the region entails sediment sampling and measuring legacy hydrophobic chemicals that readily adsorb to particles and prefer to remain particle-bound (versus dissolved). Few data exist on concentrations of dissolved or suspended, particulate-associated hydrophobic pollutants within SBNMS or greater Massachusetts Bay (Dahlen et al. 2006, Nestler et al. 2017). Based on existing sediment monitoring and well-established partitioning behavior of these hydrophobic chemicals, dissolved concentrations of legacy organic contaminants are likely very low and not a significant stressor of water quality within SBNMS (Nestler et al. 2015).

However, there are no data describing dissolved concentrations of emerging contaminants with water-loving or hydrophilic chemistries, such as water repellents (PFASs) or personal care products (Wang et al. 2011, 2017), whose partitioning behavior is poorly understood. Microplastics and nanomaterials are also poorly described in the region. These emerging pollutants have been identified in water samples and biotic tissues regionally and are likely present in SBNMS due to its proximity to urban source areas (Shaw et al. 2009b, Costa 2012, Cantwell et al. 2016a, 2016b). Dissolved concentrations of emerging contaminants are likely below levels associated with acute impacts due to vigorous flushing mechanisms within Massachusetts Bay or possible degradation, but this has yet to be demonstrated empirically (Jones et al. 2005, Coreoran et al. 2010, Zhao et al. 2016). Testing for emerging contaminants by the EPA and the University of Rhode Island will occur in 2019. There are also no data informing how dissolved emerging organic contaminants may be changing over time.

SBNMS receives continued inputs of dissolved trace metals via offshore circulation, the MWRA outfall and other wastewater streams, and seasonally-mediated resuspension from local sediments (Kalnejais et al. 2015). While sediment metal concentrations are characterized within Massachusetts Bay via ongoing

MWRA monitoring, dissolved concentrations are less clearly documented. Modeling work suggests differential distributions of dissolved metals between surface and bottom water, while existing sediment monitoring implies that dissolved concentrations are likely below biologically meaningful thresholds due to generally low sediment concentrations (Li et al. 2010, Nestler et al. 2017, Werme et al. 2015). However, it is unclear how seasonal and climatic cycles impacting relevant physicochemical water column parameters may change the availability of dissolved metals over time (Atkinson et al. 2007).

Effluent volume from the MWRA Deer Island Wastewater Treatment Plant is not significantly increasing over time. Discharge volume mirrors regional rainfall trends, with an increasing percentage of effluent receiving full secondary treatment (Figure S.WQ.9.1, Werme et al. 2017). Additionally, the Merrimack River brings significant and rain-dependent flow to the northern area of the sanctuary; its discharge has the capacity to carry point and non-point source pollution from the large Merrimack watershed, which could threaten SBNMS water quality.

Figure S.WQ.9.1. Average annual effluent discharge volume, in green and yellow, is associated with regional precipitation, tracked in blue. Effluent discharge from the Deer Island Wastewater Treatment Plant has not increased over time. Image: Werme et al. 2017

Disposal of dredge material likely does not pose a threat to water quality, as modeling indicates that disposed sediment remains confined in the disposal area, and only sediment deemed safe for offshore deposition is considered for disposal (USACE 2015). While large commercial and cruise ship discharges have the potential to adversely influence water quality in the sanctuary, there is no data available on the levels of discharges that may be occurring in the sanctuary.

Habitat (Questions 10–11)

The following information provides an assessment of the status and trends of key habitat indicators in SBNMS for the period 2007–2018. Question 10 focuses on the integrity of major habitats within the sanctuary, including biologically (biogenic) and abiotically (physical) structured habitats. Physical habitats are abiotic structures, such as sand waves, mud-draped gravel, and piled boulders. Biogenic habitats are structure-forming species, which create habitat structures used by other living marine resources, including hydroids, anemones, and sponges. Biogenic habitats are layered on top of, and are often determined by, the coincident physical habitat. Changes to both biotic and abiotic habitat can significantly alter the diversity of living marine resources and ecosystem services. Question 11 examines concentrations and variability of contaminants in major sanctuary habitats.

10. What is the integrity of major habitat types and how is it changing?

Status Description: Selected habitat loss or alteration has caused measurable but not severe degradation in some attributes of ecological integrity.

Rationale: Data suggest measurable changes in habitat quality, likely due to the use of bottom-contact commercial fishing gear. Some habitat attributes show degradation, while others show improvement. Significant habitat degradation is observed in isolated areas due to chronic disturbance. Use of bottom-contact gear is intensive in SBNMS, but diminishing due to regulatory controls and fleet consolidation. An increase in scallop dredging started in 2017 and will continue. Seabed disturbance from anchoring and other activities might also be locally important and should be evaluated.

11. What are contaminant concentrations in sanctuary habitats and how are they changing?

Undetermined ?

Status Description: Undetermined

Rationale: Legacy contaminants have been reported in benthic habitats. However, they infrequently exceed thresholds of concern, do not appear to remobilize beyond sites where they have been identified (e.g., MBDS), and no indications of acute life history or population effects have been observed. Compounds of emerging concern are present, but poorly documented or monitored; thus, their status and trends could not be assessed. More monitoring is needed in this area.

In the 2007 condition report, this question was rated “good/fair” and “not changing”. The current undetermined ratings primarily stem from the abundance of unknowns surrounding contaminant occurrence, trends, and biological effects. MWRA and other regional monitoring has demonstrated the continued, low-level presence of legacy contaminants in benthic habitats, occasionally at concentrations above established regulatory thresholds of low concern. Legacy contaminants in sediments do not appear to be subject to remobilization or transport beyond sites where they have been identified (e.g., MBDS), and there are currently no indications of acute life history or population effects related to legacy contaminant exposures despite demonstrated bioaccumulation in regional fauna. However, biological impacts of contaminants are particularly difficult to parse out in wildlife and marine systems given the abundance of other abiotic and biotic stressors, compounding uncertainty related to assessing contaminant degradation in the sanctuary habitat. Compounds of emerging concern are also present in sanctuary habitats and biota, but are not well-documented or effectively monitored; limited information suggests

that emerging contaminant concentrations may be shifting or increasing with continued introduction of new chemicals to replace regulated compounds. Therefore, status and trends of these emerging compounds could not be assessed with the limited information available.

Contaminant concentrations in SBNMS sedimentary habitats are dynamic. Observed concentrations reflect the influence of local, regional, and global processes that distribute pollutants via atmospheric, particulate, or water-based transport at different time scales. Compound-specific partitioning and degradation pathways also determine environmental distribution and possible biological activity.

MWRA monitors locations across the nearshore-offshore continuum for sediment condition and infauna community structure annually, and sediment contaminant levels are determined every three years (Rutecki et al. 2017). This ongoing program, in combination with additional regional monitoring of sediments and biota, provides a relevant dataset describing concentrations of metals and selected organic contaminants. Regional monitoring of sediments indicates low yet persistent concentrations of metals, such as mercury, and legacy persistent organic pollutants (POPs), such as PAHs, PCBs, and organochlorine pesticides (OCPs) (Figures S.H.11.1—S.H.11.2, Nestler et al. 2015, Sunderland et al. 2012, Kimbrough et al. 2009). Most metals and legacy POPs continue to decrease or remain steady within SBNMS, as documented at one current MWRA monitoring site within sanctuary boundaries. These local trends are commensurate with declines seen regionally and worldwide, as stringent regulation translates to decreased or negligible environmental inputs of many metals and legacy POPs (Zhao et al. 2017b, Werme et al. 2015, Nestler et al. 2015).

Figure S.H.11.1. Sum metal concentrations have decreased over time at the MWRA monitoring station in SBNMS (FF04), according to MWRA monitoring data. Images: MWRA sediment chemistry data, stations FF04, FF09, FF01A, NF12. MWRA data courtesy of Kenneth Keay and Douglas Hersh

Figure S.H.11.2. Sum concentrations of all chlorinated persistent organic pollutants (POPs), including select organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), in sediment at four MWRA benthic monitoring stations across Massachusetts Bay; FF04 is the station within SBNMS. Image: MWRA sediment chemistry data, stations FF04, FF09, FF01A, NF12. MWRA data courtesy of Kenneth Keay and Douglas Hersh

Biotic monitoring also indicates sustained concentrations of metals and legacy POPs. Some regional studies of mussels, fish, pinnipeds, and cetaceans indicate significant tissue burdens of metals and/or legacy POPs, suggesting these compounds remain relevant in the offshore food web, are capable of bioaccumulation, and, in some cases, exceed estimated immunotoxicity thresholds (Nestler et al. 2016, Shaw et al. 2014, 2009b, 2005, Sunderland et al. 2012, Elfes et al. 2010, Kimbrough et al. 2009).

Additionally, a plethora of emerging contaminants remain uncharacterized within the Massachusetts Bay environment and food web, including per- and polyfluorinated substances, novel flame retardants, plasticizers, pharmaceuticals and personal care products, endocrine disrupting compounds, currently used biocides, and more. Few studies have documented various compounds regionally in water, sediment, or biota, and there is no substantial understanding regarding their presence, risk, or trends in SBNMS (Cantwell et al. 2016a, Costa 2012, Kimbrough et al. 2009, Shaw et al. 2009a, 2009b, Kolpin et al. 2002). Limited existing data suggest emerging contaminants are probably present within the overall Massachusetts Bay habitat, likely at low concentrations, with uncertain biological ramifications.

No data were found detailing concentrations of microplastic particles or fibers in SBNMS sediments, or how plastic levels may be changing over time; this is a major data gap, considering marine sediments are a major sink for microplastic pieces (Woodall et al. 2014). Surface water concentrations confirm the presence of microplastic contamination in the region. Surface tows between 1986–2008 suggest Gulf of Maine surface water concentrations of 1534 $\pm$ 200 plastic pieces per square kilometer, an order of magnitude lower than concentrations observed in the North Atlantic subtropical gyre (Law et al. 2010, Eriksen et al. 2014).

State of Sanctuary Resources

Concentrations of *Clostridium perfringens*, an anaerobic bacterium that acts as a tracer of wastewater effluent influence in sediments, have remained low or declined within SBNMS and at other offshore locations since outfall diversion. Observed concentrations are comparable to baseline levels at MWRA monitoring sites in and around SBNMS following the relocation of the outfall in 2000, suggesting that discharge from the outfall has minimal impact on bacterial loads in SBNMS sediments (Nestler et al. 2015, 2017).

Time Trends of Polybrominated Diphenyl Ethers (PBDEs) in Antarctic Biota

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Supporting Information

ABSTRACT: Polybrominated diphenyl ethers (PBDEs) are “emerged” contaminants that were produced and used as flame retardants in numerous consumer and industrial applications for decades until banned. They remain ubiquitously present in the environment today. Here, a unique set of >200 biotic samples from the Antarctic was analyzed for PBDEs, including phytoplankton, krill, fish, and fur seal milk, spanning several sampling seasons over 14 years. PBDE-47 and -99 were the dominant congeners determined in all samples, constituting >60% of total PBDEs. A temporal trend was observed for Σ PBDE concentrations in fur seal milk, where concentrations significantly increased ($R^2 = 0.57$, $p < 0.05$) over time (2000–2014). Results for krill and phytoplankton also suggested increasing PBDE concentrations over time. Trends of PBDEs in fur seal milk of individual seals sampled 1 or more years apart showed no clear temporal trends. Overall, there was no indication of PBDEs decreasing in Antarctic biota yet, whereas numerous studies have reported decreasing trends in the northern hemisphere. Similar PBDE concentrations in perinatal versus nonperinatal milk implied the importance of local PBDE sources for bioaccumulation. These results indicate the need for continued assessment of contaminant trends, such as PBDEs, and their replacements, in Antarctica.

INTRODUCTION

Antarctica is one of the most pristine places on the planet. However, even in this remote region, anthropogenic effects are measurable. Scientific exploration in Antarctica has occurred for decades and in the summer season, the continent hosts over 100 active facilities operated by 30 different nations.¹ Although pollution in Antarctica has typically been orders of magnitude lower than concentrations reported elsewhere around the globe, organic contaminants, particularly (semi-) volatile ones, have reached the region via long-range environmental transport by processes of global fractionation and cold condensation.² Legacy contaminants, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), have been reported along with more recent contaminants, such as polybrominated diphenyl ethers (PBDEs) and perfluoroalkyl substances, in numerous environmental matrices from the region.^{3–9} Some of this contamination has also been found to originate from research stations themselves.^{10,11}

Polybrominated diphenyl ethers are “emerged” contaminants that have been used as additive flame retardants for decades in a wide range of consumer and industrial applications (e.g.,

upholstery, electronics) and are easily leached from these manufactured goods into the environment, food webs, and ultimately reaching humans.¹² PBDEs, like many legacy persistent organic pollutants (POPs) (e.g., PCBs), are hydrophobic and lipophilic. The height of PBDE production was dominated by three different commercial mixtures (penta-, octa-, and deca-BDE).¹³ In 2009, penta- and octa-PBDE mixtures were listed by the Stockholm Convention.¹⁵ However, production of the deca-BDE has persisted in many countries and a massive reserve of products that contain PBDEs exist around the globe and will continue to leach them into the environment.^{5,14–16}

Several studies have reported PBDE concentrations starting to decrease over the last 5–10 years. For example, time trends of PBDEs in samples collected from Swedish mothers indicate a decreasing trend for most PBDEs, except for BDEs 153 and 209, from 1996 to 2010.¹⁷ PBDEs also decreased in Baltic

Received: April 6, 2018

Accepted: June 1, 2018

Published: June 19, 2018

Figure 1. Map of sampling locations. Black squares denote the boundaries of the Long Term Ecological Research (LTER) grid. Created with ArcGIS Explorer.

herring over the last decade.¹⁸ Across the Great Lakes in North America, PBDEs in fish started to decline in 1999–2000.¹⁹ So far, no consistent set of Antarctic samples has been available to document time trends of PBDEs in Antarctic marine biota, although several previous studies have detected PBDEs in the Antarctic environment.^{3,4,9,10,20}

Kelly et al. in 2008 presented evidence from a Canadian Arctic marine food web in which many PBDEs appeared to exhibit negligible biomagnification, with the exception of BDE-47, which did demonstrate food web biomagnification, albeit at a much lower level than PCBs.²¹ Yet in the same study, PBDEs in macroalgae were excluded from the trophic magnification factor calculation as their concentrations exceeded those from other trophic levels by 5–10 times.

We obtained a unique set of biotic samples from West Antarctica (Figure 1), including phytoplankton, krill, fish, and fur seal milk, spanning several years (2000–2014). A previous paper reported generally declining trends of several but not all PCBs and legacy OCPs in these fur seal milk samples; PBDE concentrations were not measured at the time.⁵ We used these samples to assess the presence and trophic transfer of PBDEs in the West Antarctic food web and to identify the PBDE temporal trends in this region either from yearly averages or in the case of repeatedly sampled fur seals, from individual trends over time. The food web structure was assessed using stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$).

Specific goals in this research were to (i) determine which PBDEs are being detected in different Antarctic biota; (ii) establish temporal trends of common PBDE congeners over a time period where global regulations and restrictions on production had been implemented (i.e., 2000s); (iii) contrast trends of PBDEs in Antarctic fur seal milk to those from the Arctic; and (iv) assess the difference of milk sampled before and after fur seals begin foraging locally in waters off the Antarctic Peninsula.

MATERIALS AND METHODS

Sample Collection. Milk samples were collected from Antarctic fur seals (*Arctocephalus gazella*) approximately 100

km off the Antarctic Peninsula at Cape Shirreff, Livingston Island (62.47°S, 60.77°W) over the austral summers of 2000/2001, 2001/2002, 2004/2005, 2009/2010, 2010/2011, 2011/2012, 2012/2013, and 2013/2014 (Figure 1 and Tables S1–S8). Most seals were multiparous females in their perinatal stage (i.e., the seals had all bred prior to the year of sample collection and milk was collected during the perinatal period, 1–2 days postpartum, prior to initiation of offshore foraging trips), except for the 2011/2012, 2012/2013, and 2013/2014 samples, which consisted of both perinatal and nonperinatal (i.e., after initiation of foraging cycles) milk samples (Tables S6–S8). Seals were assumed to have had at least one pup prior to the breeding season sampled as all seals were age 5 or older, with the majority being over the age of 7. Seal capture was performed following methods described in Polito and Goebel and as reported in Brault et al.^{6,22} In brief, seals were captured with hoop nets, sedated with 5 mg of midazolam, and anesthetized with isoflurane. Milk was collected after an intramuscular injection of oxytocin (0.25 mL, 10 U/mL) in precleaned vials and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.^{6,22} Temperature loggers sampling every 10 min were kept with samples to confirm storage temperature.

Phytoplankton samples were collected in a region of Antarctica that spans from the West Antarctic Peninsula to the Ross Sea (64.78°S, 64.07°W to 78.64°S, 164.30°W, Figure 1) over the austral summers of 2007/2008, 2009/2010, and 2010/2011 using ring net tows (Table S9). Phytoplankton samples consisted largely of diatoms (Antarctic Peninsula) and *Phaeocystis* sp. (Amundsen and Ross Seas). Further specifics on sample collection can be referenced in Brault et al.^{6,23}

All krill and fish samples were collected from within the Palmer Long Term Ecological Research (LTER) grid survey region (approx. 66.99°S, 69.28°W to 61.94°S, 73.78°W, Figure 1) via 700 μm ring net tows (taken at oblique angles, Table S10). Krill samples consisted predominantly of *Euphausia superba* and were collected during the austral summers of 2007/2008 and 2010/2011 and split into three size classes (juveniles, adults (including mature females), and gravid females). Fish samples consisted of either silverfish (*Pleuragramma antarcticum*)

Table 1. Comparison of Average PBDE Concentrations in Antarctic Biota (ng/g lipid) $\pm$ 1 Standard Deviation from This and Previous Studies

	Σ PBDEs ^a	BDE-28	BDE-47	BDE-99	BDE-100
plankton ^a			23 $\pm$ 3.5	22 $\pm$ 3.4	4.5 $\pm$ 0.7
phytoplankton ^b	53 $\pm$ 76	1.9 $\pm$ 2.7	20 $\pm$ 27	19 $\pm$ 32	4.8 $\pm$ 8.1
juvenile krill ^a			570 $\pm$ 210	620 $\pm$ 250	130 $\pm$ 51
juvenile krill ^b	0.65 $\pm$ 0.27	0.07 $\pm$ 0.11	0.49 $\pm$ 0.20	0.05 $\pm$ 0.05	0.04
adult krill ^a			2.0 $\pm$ 0.5	2.5 $\pm$ 0.6	0.5 $\pm$ 0.1
adult krill ^b	0.51 $\pm$ 0.78	0.04 $\pm$ 0.05	0.28 $\pm$ 0.23	0.13 $\pm$ 0.43	0.01
gravid krill ^b	0.35 $\pm$ 0.19	0.06 $\pm$ 0.05	0.18 $\pm$ 0.12	0.04 $\pm$ 0.06	0.02
krill ^c	5.6 $\pm$ 1.1				
krill ^f	0.095	0.001	0.011	0.009	0.002
krill ^e	0.027				
adult krill ^d	0.94	0.03	0.17	0.2	0.05
rockcod muscle ^e	5.8 $\pm$ 2.3				
rockcod muscle ^e	7.5				
Antarctic silverfish ^b	<LOD		<1.5	<1.5	
myctophid ^b	<LOD		<0.20	<0.20	
Weddell seal ^h	2.0	<LOD	<LOD	2.0	<LOD
Weddell seal ⁱ	1.5	<LOD	1.5	<LOD	<LOD
fur seal milk ^b	2.3 $\pm$ 1.9	0.07 $\pm$ 0.09	1.3 $\pm$ 1.4	0.36 $\pm$ 0.36	0.14 $\pm$ 0.15

^aChiuchiolio et al.,⁵ 64.7°S, 64.0°W. ^bThis study, Σ BDEs, Ross Sea to Antarctic Peninsula; results were averaged over all sampling seasons. ^cCorsolini et al.,⁸ Ross Sea, approx. 74°04'S, 179°06'E. ^dBengtson Nash et al.,²⁵ arithmetic means, ~63–69°S, 30–80°E. ^eCincinelli et al.,⁷ assuming 1% lipid content. ^fGalván-Malagón et al.,³⁵ mean values; around the Antarctic peninsula. ^gCorsolini et al.,²⁷ mean values, Ross Sea, based on 3.6% lipid content. ^hCipro et al.,²⁸ Weddell seal blubber from King George Island (62°05'S, 58°23'W). ⁱTrumble et al.,²⁹ adult Weddell seal blubber near McMurdo Station, Antarctica (77°55'S, 166°39'E). ^jNote that the number of BDE congeners included in the Σ PBDEs varies between studies.

cum) or myctophids (*Electrona antarctica*) and were collected in the same manner as krill (Table S11).

Sample Extraction. Fur seal milk extraction was conducted in two batches. The first batch ($n = 59$), which consists of samples from the five austral summers spanning from 2000/2001 to 2010/2011, was extracted at the Virginia Institute of Marine Science (VIMS) following previously established POP procedures as reported in Geisz et al.²⁴ In short, fur seal milk was freeze-dried, homogenized, subsampled (1 g of dry weight), solvent-extracted (65:35 dichloromethane/acetone), and analyzed for several POPs (e.g., 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), PCBs, and chlordanes) as well as lipid content.⁶ Sample extracts were shipped to the University of Rhode Island's Graduate School of Oceanography (URI-GSO) for PBDE analysis.

The second batch of fur seal milk samples (samples from 2011/2012, 2012/2013, and 2013/2014, $n = 71$) was extracted at URI-GSO, as detailed in the Supporting Information (SI). Briefly, 2 mL of fur seal milk was spiked with PBDE surrogates, extracted three times with 20 mL each of *n*-hexane/acetone (2:1), treated with concentrated sulfuric acid to denature lipids, and cleaned on solid-phase extraction cartridges. Percent lipid was measured separately.

Phytoplankton, fish, and krill samples were also extracted at VIMS. Samples were manually homogenized with a Virtis "45" tissue homogenizer (Virtis Co. Inc.), freeze-dried at -80 °C for approximately 72 h, and solvent-extracted. Further details on sample preparation can be gathered from Brault.²³ Following analysis at VIMS for several legacy POPs,⁶ phytoplankton, fish, and krill, sample extracts were shipped to URI-GSO to be analyzed for PBDEs.

PBDE Analysis. All samples were analyzed for mono-through hepta-brominated congeners (BDE-2, -8, -15, -30, -28, -49, -47, -100, -99, -154, -153, and -183) via gas chromatography (GC) tandem mass spectrometry (MS) on

an Agilent 6890N GC coupled to a Waters Quattro Micro MS/MS under electron ionization/MS/MS in multiple reaction monitoring mode using a DB-5MS column (Agilent J&W GC Columns, 122-5532, length 30 m, i.d. 0.250 mm, film 0.25 μ m) and splitless injection (for more details, see SI). Analysis of BDE-209 was conducted separately, and procedures are detailed in the SI.

Sample extracts were spiked with 10 μ L of a 5 ng/ μ L ¹³C₁₂-labeled PBDE surrogate (¹³C₁₂ BDEs-28, 47, 99, 153, and 183, Cambridge Isotope Laboratories) for a total concentration of 50 ng and 5.0 μ L of a 5.0 ng/ μ L injection standard (*p*-terphenyl-*d*₁₄, AccuStandard) for a total concentration of 25 ng. To the samples originally extracted at VIMS, the surrogate was added postextraction. These samples were corrected for the average recoveries of previously analyzed POPs (e.g., DDT, PCBs, and chlordanes), which were 79 $\pm$ 3.7% for phytoplankton, 69 $\pm$ 1.8% for krill, and 78 $\pm$ 1.8% for fur seal milk. The second batch of fur seal milk samples (2011/2012, 2012/2013, and 2013/2014) was spiked prior to extraction at URI-GSO directly with 20 μ L of a 2.0 ng/ μ L PBDE surrogate standard in nonane. Results presented below are only for compounds that were detected >30% of the time.

Quality Control. Laboratory blanks of a hydromatrix material were initially extracted alongside real samples, and any blanks included in the vial files for shipment from VIMS to URI-GSO were analyzed for PBDEs. All samples were blank-corrected; main detected congeners were BDE-47 and -99 with concentrations averaging 0.18 $\pm$ 0.20 ng (standard deviation (SD)) and 0.18 $\pm$ 0.23 ng, respectively (Table S12, SI). For BDE-209, amounts in blanks were similar to fur seal milk results, so results are not reported here.

At URI-GSO, laboratory blanks, matrix spikes ($n = 8$), blank spikes ($n = 4$), and duplicates of fur seal milk ($n = 10$) were included. Limit of detection (LOD) was calculated as the average detected blank concentrations + 3 times the standard

Figure 2. (a) Average sum of PBDEs per breeding season for the eight nonconsecutive austral summers sampled. Bars represent standard deviation. The first five austral summers (2000/2001–2010/2011) have had a recovery correction of 77.86% applied. (b) Average percent composition of PBDEs in fur seal milk from 2000/2001 to 2013/2014.

deviation (Table S13). For congeners that were not detected, the noise was used to derive LODs (Table S14); LODs ranged from 0.011 ng/g lipid (BDE-2, 15) to 0.16 ng/g lipid (BDE-99). Recoveries of the surrogate standards ranged from $83 \pm 2.4\%$ (BDE-183) to $94 \pm 3.1\%$ (BDE-47). Recoveries of the congeners in the matrix and blank spikes generally ranged from 92% (BDE-2) to 102% (BDE-47), as shown in Table S15. Relative percent difference for the duplicates ranged from 6.2% (BDE-100) to 21% (BDE-2).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Analysis. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopes for the majority of samples were determined via an elemental analyzer isotopic ratio mass spectrometer at VIMS, as described elsewhere (Figure S1).⁶ Some plankton samples were analyzed at the University of California, Santa Cruz (UCSC) on a Carlo Erba EA 1108 elemental analyzer coupled to a Finnegan Delta-Plus isotopic ratio mass spectrometer. Average values were $-29 \pm 0.40\text{‰}$ for $\delta^{13}\text{C}$ and $1.6 \pm 0.50\text{‰}$ for $\delta^{15}\text{N}$.

Statistical Analysis. Data were tested for normality using the Shapiro–Wilks test in RStudio, IBM SPSS Statistics 22, and SigmaPlot 12 software packages. Concentrations were natural log transformed to make data have a normal or near-normal distribution. Linear regressions were performed for each congener with >30% detection against fur seal age, breeding season, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. Any difference between PBDE concentrations in perinatal and nonperinatal milk was tested using a student's two-sample *t* test assuming unequal variances.

A student's *t* test was used to compare between detected PBDE concentrations in the krill samples of 2007 and 2011. Similarly, the one-way repeated analysis of variance (ANOVA) test was used to compare between detected PBDE concentrations in (i) the different krill samples (juveniles, adults, and gravid); (ii) phytoplankton samples collected in 2007–2011; and (iii) between the calculated trophic levels for the different seal samples collected in 2000, 2001, 2004, 2009, and 2010.

RESULTS AND DISCUSSION

PBDE-47 and -99 were the dominant congeners determined in all samples, generally constituting >60% of total PBDEs. Phytoplankton samples displayed the highest overall concentrations, followed by fur seal milk, krill, and lastly fish, in which no PBDEs were detected (Table 1). The lower brominated congeners (BDE-2, -8, -15, and -30) and BDE-183 were not regularly detected until 2011/2012. These trends could, at least partially, be explained by the switch in extraction procedures between VIMS (2000/2001–2010/2011) and URI-GSO. To ensure consistency across the various samples, we focus on the seven most routinely detected congeners (BDE-28, -49, -47, -100, -99, -154, and -153), which were also summed ($\Sigma_7\text{PBDEs}$).

Fur Seal Milk. Lipids in fur seal milk were high with an average value of $64 \pm 9\%$ (SD; range 14–84%). PBDEs were detected in all fur seal milk samples ($n = 130$; Tables S15–

Figure 3. Σ_7 PBDE trends in Arctic fur seals (red dotted line), reported BDE concentrations in Arctic ringed seals (orange dashed line, both left axis), and ratios of BDE concentrations in fur seal milk sampled twice over several years (blue solid line).

S22). The Σ_7 PBDEs in all fur seal milk samples ranged from 0.14 to 17 ng/g lipid with a mean $\pm$ SD of 2.1 ± 1.9 ng/g lipid (median of 1.7 ng/g lipid). BDE-47 was the most dominant congener with a range of 0.14–12 ng/g lipid and a mean of 1.3 ± 1.4 ng/g lipid (median of 0.95 ng/g lipid). BDE-99 was the second most dominant congener but showed less variability with a range from <LOD to 2.7 ng/g lipid, mean of 0.36 ± 0.36 ng/g lipid (median of 0.27 ng/g lipid). Similar PBDE concentrations of around 1.5–2.0 ng/g lipid (Table 1) have been reported for Antarctic Weddell seal blubber.^{28,29} For comparison, ringed seal blubber in the Canadian Arctic contained more elevated Σ_{15} PBDEs at 11–14 ng/g lipid.³⁰

Stable isotope analysis was only available for fur seal milk collected during the first five austral summers (2000/2001–2010/2011, $n = 59$). Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ demonstrated variability: $\delta^{13}\text{C}$ ranged from -26 to -20‰ with a mean $\pm$ SD of $-23 \pm 1.4\text{‰}$; $\delta^{15}\text{N}$ ranged from 8.0 to 14‰, with a mean $\pm$ SD of $10 \pm 1.2\text{‰}$.

Fur Seal Milk Trends. In fur seal milk, the dominant congeners BDE-47 and -99 showed no significant correlation with age, similar to results for legacy POPs, implying that older animals did not display greater concentrations.⁶ No significant relationships were found between fur seal milk PBDE concentration and $\delta^{13}\text{C}$ value from regression analysis. Few significant trends were observed between PBDE concentration and $\delta^{15}\text{N}$ value; most notably in 2000/2001, BDE-47 and Σ_7 PBDEs versus $\delta^{15}\text{N}$ both demonstrate significantly negative trends (i.e., decreasing concentration with increasing $\delta^{15}\text{N}$ value or trophic level). We have no explanation for this trend, but note that it disappears when looking at all fur seal milk results from 2000/2001 to 2010/2011 combined.

Σ_7 PBDE concentrations for the 2011/2012 breeding season were significantly greater than for the other breeding seasons, except for 2009/2010 and 2013/2014 (one-way repeated ANOVA, $p < 0.001$). Additionally, a temporal trend was observed for Σ_7 PBDE concentrations (Figure 2), where concentrations significantly increased ($R^2 = 0.57$, $p = 0.03$) over time (from 2000 to 2014). We note that the (increasing) slope of PBDE concentrations over time was not significantly different when considering either all 8 sampling years or just the first 5 years (those extracted at the VIMS).

Fur Seal Milk Trends in the Same Individuals. The previous discussion was based on average values from randomly sampled female fur seals over time. Milk samples from 18 of these individuals were collected twice with at least 1 year between sampling times. From 11 seals, only perinatal milk was obtained, from 3 seals only nonperinatal milk was obtained, and from four seals both perinatal and nonperinatal milk was obtained. Ratios for individual BDE congeners were calculated as the more recent concentration divided by the previous concentrations (Figure S3). No clear trends of changes in PBDE concentrations over time were discernible across all paired milk samples. These results contrast with time trends observed for PBDEs in the northern hemisphere.

In the Great Lakes, PBDE concentrations in fish peaked mostly from 1999 to 2000.¹⁹ Further away from source regions, PBDE concentrations in ringed seals in East Greenland started to decrease in the early 2000s,³⁰ a trend reported for most Arctic biota.³¹ PBDEs also decreased in Canadian seabirds with a significant and rapid decline after 2003.³² An analysis of PBDE mass flows in the U.S. and Canada predicted penta and octa-BDEs stocks to peak in their use around 2004, whereas BDE-209 stock peaked in 2008.¹⁵ These diverging trends between the northern hemisphere and our results for the Western Antarctic suggest that transport of PBDEs to the remote Southern Hemisphere has been delayed by a decade or so relative to the Arctic region, which is closer to the primary source region (Figure 3). The vast majority of PBDEs were produced and used in the northern hemisphere;³³ while they are certainly present in the southern hemisphere, it is unclear whether PBDEs in Antarctica are predominantly reflecting southern or northern hemisphere sources.

Perinatal vs Nonperinatal Milk. After breeding, female fur seals from Cape Shirreff spend up to 8 months away at one of three foraging grounds (off the Chilean coast, Patagonian shelf break, or around South Georgia) during the austral winter.³⁴ Perinatal milk thus represents PBDEs accumulated during the winter migration. In contrast, nonperinatal milk reflects PBDEs accumulated while foraging offshore Cape Shirreff's breeding beaches and any mobilization of PBDEs from lipid reserves. Across all fur seal milk samples, there were few significant differences in PBDE concentrations between perinatal and nonperinatal milk. The exception was for the 2012/2013

Figure 4. Comparison of BDE concentrations in perinatal and nonperinatal milk sampled from fur seals sampled twice during the same season ($n = 10$). Error bars represent one standard deviation.

Figure 5. Average Σ PBDEs (ng/g lipid) in (a) phytoplankton and (b) krill per sampling season. Note the uneven interval between years.

sampling period, in which mean $\pm$ SD concentrations of BDE-49 (0.33 ± 0.34 ng/g lipid) and BDE-99 (0.28 ± 0.19 ng/g lipid) in nonperinatal milk were significantly higher than those in perinatal milk (0.19 ± 0.15 and 0.15 ± 0.10 ng/g lipid, respectively; Mann–Whitney rank sum test $p < 0.05$). This is consistent with expectations based on the maternal body burden of lipid-bound pollutants being passed onto the pup during lactation, particularly in the case of the first pup.³⁵

The previous discussion was based on average values from randomly milked fur seals both perinatally and nonperinatally. Again, milk from several fur seals was sampled twice during the same season ($n = 10$: nine from 2011/2012, one from 2012/2013). We thus compared PBDE concentrations in perinatal and nonperinatal milk collected from the same individuals, typically 6 weeks apart. There was no significant difference in individual PBDE congener concentrations between perinatal and nonperinatal milk (Figure 4). On average, fur seals

accumulated similar BDE concentrations during their winter migration and feeding away from Cape Shirreff as they did while foraging offshore Cape Shirreff's breeding beaches. This implies that within their breeding area and foraging region PBDEs are present in similar concentrations and/or the importance of mobilizing PBDEs from their lipid reserves while at Cape Shirreff.

The average values mask a wide range of individual changes in PBDE concentrations (note large standard deviations in Figure 4). For two fur seals, PBDEs decreased about 2-fold over the course of their foraging trips around Cape Shirreff, whereas it increased for two other seals about 2-fold over this period. Median ratios of nonperinatal divided by perinatal milk were greatest for BDE-183 and BDE-99 implying preferential accumulation of these congeners off Cape Shirreff and/or their less-efficient biodilution within that time period. Overall, profiles of PBDEs in perinatal and nonperinatal milk were very similar (Figure 4), suggesting similar uptake/metabolism of these chemicals in fur seals.

In summary, there is a wide range of trends of BDE concentrations in individual fur seal milk sampled approximately 9 weeks apart. On the contrary to expectations, there were no significant trends of decreasing concentrations once fur seals started feeding off Cape Shirreff (Figure 4). This might indicate that fur seals, in some years, forage locally prior to coming ashore to give birth.

Phytoplankton. Lipid values in phytoplankton were very low ranging from 0.1 to 6.7%; only samples with lipid values >0.5% are presented here to avoid biasing PBDE concentrations high.

Phytoplankton Σ_7 PBDEs ranged from 3.8 to 320 ng/g lipid with a mean $\pm$ SD of 53 ± 76 ng/g lipid (median of 26 ng/g lipid, Table S23). BDE-47 and -99 were the two most prevalent congeners (Figure 5), with detection 97 and 91% of the time, respectively. Previously reported PBDE concentrations in plankton in 2005 were 2–3 times lower (Table 1).⁵ Macroalgae in the Canadian Arctic similarly contained Σ_{13} PBDEs at 320 ng/g lipid.¹¹

Stable isotope analysis of $\delta^{15}\text{N}$ on phytoplankton samples resulted in a wide range of values from -1.1 to 6.1‰ . We note that although the dominant composition of the plankton samples were identified as phytoplankton (i.e., diatoms or *Phaeocystis* sp.), samples may have some microzooplankton present despite efforts to remove any nonphytoplankton species. The $\delta^{13}\text{C}$ values of phytoplankton also had a large range, spanning from -33 to -19‰ , with a mean of $-29 \pm 0.61\text{‰}$ (median of -31‰).

Phytoplankton Trends. Few significant correlations were detected between PBDE phytoplankton concentrations and $\delta^{15}\text{N}$ values except for the 2010/2011 season, where almost all congeners detected showed a negative trend of decreasing concentration with increasing $\delta^{15}\text{N}$ value. BDE-47, -100, -99, -154, -153, and Σ_7 PBDEs versus $\delta^{15}\text{N}$ all had significantly negative trends ($p < 0.05$).

Correlations of phytoplankton PBDE concentrations versus sampling time were only significant for the 2010/2011 sampling season, in which all congeners with >30% detection (BDE-28, -49, -47, -100, -99, -154, -153, and Σ_7 PBDEs) have significantly negative trends; i.e., PBDE concentrations decreased toward the end of austral summer ($p < 0.05$). The austral summer 2010/2011 sampling season, spanning the period from December to March, was longer than the 2007/2008 or 2009/2010 seasons. The temporal trend in the 2010/

2011 austral summer may reflect a spike in concentrations picked up from the snow/ice melt early in the austral summer, with either a fading signal or dilution occurring as the season progressed. Legacy organic contaminants (e.g., PCBs, DDT, polycyclic aromatic hydrocarbons) have been detected in snow packs and glacial ice from both Arctic and Antarctic environments, and it has been proposed that in colder regions, where the timing of the melt may be more concentrated as compared to a temperate environment, there is a stronger pulse of organic contaminants released to the surrounding water column.³⁶ Chiuchiolo et al. in 2004 detected various OCPs and BDEs (-47, -99, and -100) in phytoplankton and suggested that phytoplankton incorporated POPs from snow and ice melt. Furthermore, POPs may be removed from the water column via sedimentation and organic carbon particle export, which occurs in a relatively short time following phytoplankton blooms in this region (i.e., December and January).⁸ Additionally, Geisz et al. in 2008 present further evidence of glacial meltwater acting as a source of, at least, DDT to the Antarctic marine food web.²⁴

There was a significant difference ($p < 0.05$, one-tailed two-sample t test assuming unequal variances) between diatoms and *Phaeocystis* for BDE-28, -47, -100, -153, and Σ_7 PBDEs, with *Phaeocystis* sp. having greater mean concentrations than diatoms. Similarly, the mean PBDE concentrations in phytoplankton ($\delta^{15}\text{N}$ values $< 2\text{‰}$) were greater than the mean of phytoplankton with possibly greater microzooplankton contamination ($\delta^{15}\text{N}$ values $> 2\text{‰}$) for BDE-153 ($p = 0.036$) and with lower significance for Σ_7 PBDEs ($p = 0.059$).

Krill. Average lipid % in krill ranged from 14 to 33%, much greater than for phytoplankton. PBDEs were detected in all krill samples, with lower concentrations and less variation in contaminant concentrations than was observed for phytoplankton. The average Σ_7 PBDEs ranged from 0.14 to 3.5 ng/g lipid with a mean $\pm$ SD of 0.61 ± 0.57 ng/g lipid (median of 0.49 ng/g lipid). BDE-47 was the dominant congener present in all size classes of krill, averaging around 70% of the total composition (Table S24, SI), followed by BDE-28 and -99, respectively. For Σ_7 PBDEs, juvenile krill ($n = 9$) had the highest concentrations among *E. superba* age classes with a mean of 0.65 ± 0.27 ng/g lipid, followed by adult krill ($n = 18$) with a mean of 0.51 ± 0.78 ng/g lipid, and gravid krill ($n = 7$) with a mean of 0.35 ± 0.19 ng/g lipid (Figure S2). The two *Thysanoessa* sp. krill samples displayed fairly high concentrations at Σ_7 PBDEs of 1.2 and 0.66 ng/g lipid. Previously reported PBDE concentrations in krill in 2005 were at least 10 times greater than what we measured, possibly indicating contamination (Table 1).⁵

Stable isotope analysis was performed on a subset of each size class of krill, with the exception of *Thysanoessa* sp. The $\delta^{15}\text{N}$ values (mean $\pm$ SD) were similar among the different size classes: $4.0 \pm 0.58\text{‰}$ for juvenile krill, $4.2 \pm 0.19\text{‰}$ for adult krill, and $4.1 \pm 0.43\text{‰}$ for gravid krill. There was slightly more variability for $\delta^{13}\text{C}$ values among the krill age classes; $\delta^{13}\text{C}$ values (mean $\pm$ SD) were: -24 ± 1.41 , -25 ± 0.47 , and $-23 \pm 0.68\text{‰}$ for juvenile, adult, and gravid krill, respectively.

Trends of Krill. No significant relationships were found between krill concentration and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ value. No significant differences ($p < 0.05$) were found between krill concentration means from the two sampling years of 2007/2008 and 2010/2011 (Figure 5). When comparing different size classes of krill (i.e., juveniles vs adults, adults vs gravid, gravid vs *Thysanoessa* sp.), BDE-47 and Σ_7 PBDEs were both

found to be significantly higher in juveniles than adults ($p < 0.05$).

Fish. Lipid values in fish ranged from 22 to 52%. PBDEs were not detected in any of the five fish samples. Low masses of BDE-47 and -99 (0.1–0.2 ng/sample) were initially determined, however, after blank corrections, all PBDEs in fish samples were <LOD (Table 1). Previous studies have detected PBDEs in Antarctic fish samples, though they have generally been able to extract larger amounts of tissue.^{3,4,9,10,37,38} The total ranges of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for fish samples were 9.2 to 11 and –24 to –21‰, correspondingly. For the myctophid fish species, the mean $\pm$ SD of $\delta^{15}\text{N}$ values was 9.5 ± 0.42 and –24 ± 0.24 ‰ for $\delta^{13}\text{C}$. In comparison, the mean $\pm$ SD of Antarctic silverfish $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were both higher (11 ± 0.63 and –21 ± 1.1 ‰, respectively) than those of the myctophid fish species.

■ IMPLICATIONS

Biota from other parts of the world, primarily regions closer to industrialized areas, have started to see a reduction in PBDE concentrations as a reflection of their phase-out.^{17,18,30,32} Time trends of PBDEs in Antarctic biota strongly demonstrate that a decrease of PBDE concentrations in Antarctic biota over the last decade has not (yet) occurred. These surprising results indicate the need for further research to see if and when PBDE concentrations in the Antarctic will start declining as reported in the Arctic. Similarly, the phase-out of PBDEs has led to various novel flame retardants being detected in the environment.^{39,40} As of yet, it is unclear whether these new flame retardants have been transported to Antarctica and have been accumulating in Antarctic biota. This will be an important research area in the near future.

Comparing trends of PBDEs in milk from randomly selected fur seals over several years were by and large similar to trends of the same fur seals milked years apart. Results show that there are large variations in PBDE trends in individuals over time, highlighting the need for large sample sets to determine representative trends. A better understanding of time trends in individual seals is warranted but would require a concerted effort to resample the same individuals over several years, ideally collecting both perinatal and nonperinatal milk.

The high concentrations of PBDEs in phytoplankton compared to the upper trophic level Antarctic fur seal were unexpected but not unprecedented and illustrate the complexity of the Antarctic food web and sampling under challenging circumstances (Table S25). For the phytoplankton sample collections on board a ship, we cannot rule out the possibility of contamination by PBDEs during sampling.^{41,42} Trends of PBDEs in biota are further complicated by the presence of point sources, such as snow and ice melt, which can release pollutant pulses in the austral summer. Finally, the differences in geographic sampling of biota (which were logistically constrained) and the migratory nature and diverse diet of Antarctic fur seals further add complexity to understanding PBDE trends in biota. Monitoring emerging pollutants in remote regions, such as Antarctica, can highlight important global trends of contaminants of concern and further our understanding of long-range transport and global response times to pollutant dynamics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.omega.8b00440.

Additional details related to sample IDs, chemical analysis, and concentrations of PBDEs (PDF)

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Notes

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■ ACKNOWLEDGMENTS

R.L. acknowledges funding from the National Science Foundation (ANT 1332492) to understand emerging pollutant dynamics in the Antarctic. We acknowledge Hugh Ducklow (LDEO) and the National Science Foundation Palmer LTER (PLR 1440435) for intellectual and logistical support and thank Rebecca Dickhut (Virginia Institute of Marine Science) for her prior leadership on this grant. We thank Dr. Deborah Steinberg, Kate Ruck (Virginia Institute of Marine Science), and Dr. Heidi Geisz (Florida State University) for sample collection. We are grateful to the many field assistants that helped in capture and sample collection of fur seal milk, especially G.I. McDonald, Y. Tremblay, J. Lyons, R. Burner, R. Buchheit, N. Pussini, C. Bonin, and K. Pietrzak. We are also grateful for the support of Drs. Rennie S. Holt and George Watters, directors of the US-AMLR program, and to Dr. D.P. Costa, UCSC for their support of Pinniped research at Cape Shirreff. All Antarctic fur seal research and sample collection were conducted under Marine Mammal Protection Act Permit Nos. 1024, 774-1649, 774-1847, and 16472-01 granted by the Office of Protected Resources, National Marine Fisheries Service.

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SPATIOTEMPORAL CHARACTERIZATION OF NON-BREEDING GREAT SHEARWATERS *ARDENA GRAVIS* WITHIN THEIR WINTERING RANGE

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Received 22 January 2020, accepted 17 June 2020

ABSTRACT

POWERS, K.D., WILEY, D.N., ROBUCK, A.R., OLSON, Z.H., WELCH, L.J., THOMPSON, M.A. & KAUFMAN, L. 2020. Spatiotemporal characterization of non-breeding Great Shearwaters *Ardena gravis* within their wintering range. *Marine Ornithology* 48: 215–229.

Movements of Great Shearwaters *Ardena gravis* wintering in the Northwest Atlantic showed age-based spatial and temporal flexibility, with foraging tactics linked to a defined physical preference of their primary prey. From 2013 to 2018, we tracked 58 Great Shearwaters during their wintering season using platform terminal transmitters deployed in the same area of the southwest Gulf of Maine. Utilization distributions (UDs) for individual birds were created from convex hulls, which were then combined for spatial and temporal analyses. Of the 95% kernel UD, 55% were contained within the Gulf of Maine and the remainder extended to the Scotian Shelf off Nova Scotia and the Grand Banks off Newfoundland. Analysis of fecal DNA from tagged birds and others captured with them indicated that Northern sand lance *Ammodytes dubius* were the primary prey while in the Gulf of Maine. This relationship was supported by the overlap of UD and sand lance habitat. The spatial occurrence of sand lance from bottomfish trawl survey data demonstrated that these fish preferred shallow water (< 100 m deep) with substrates consisting of high sand content (> 50%) and grain sizes ranging from 0.35–2.00 mm in diameter. These same properties were associated and spatially aligned with the collective 25% kernel UD of Great Shearwaters. Necropsy of bycaught Great Shearwaters from an area that overlapped in space and time with tagged individuals and sand lance habitat demonstrated that most birds (89%) were young (0–2 years), based on gonadal development, molt score, and/or bursa of Fabricius. Coupling demographic information from necropsies with spatial habits and movement timing of tagged birds suggests this region serves as a winter “nursery” for Great Shearwaters.

Key words: Great Shearwaters, utilization distribution, wintering range, satellite tracking, fecal DNA analysis, sand lance, bycatch necropsies

INTRODUCTION

The distribution of marine vertebrates is the product of a hierarchical structure of processes driven at multiple spatiotemporal scales (Mannocci *et al.* 2017). Many of these driving processes are in flux due to human-induced or climatically mediated stress, resulting in changes in faunal distribution and abundance (Nye *et al.* 2009, Moore *et al.* 2018). In seabirds, shifting environmental conditions are implicated in precipitous declines across taxa and ecoregions (Croxall *et al.* 2012, Paleczny *et al.* 2015, Dias *et al.* 2019). Characterizing current distribution patterns of seabirds is critical to identifying and managing seabird and ecosystem responses to current and predicted environmental changes (González-Solís & Shaffer 2009, Paleczny *et al.* 2015), and there is a paucity of data for juvenile seabirds in particular (Hazen *et al.* 2012). However, in order to be rigorous tools for ecosystem management, descriptions of seabird distributions must be augmented with explanations of the physical and biological processes that contribute to the dynamic nature of such patterns (Fauchald *et al.* 2000, Fauchald 2009, Tremblay *et al.* 2009).

Seabirds may forage across vast coastal and pelagic areas during breeding phases (Weimerskirch *et al.* 1993, Weimerskirch *et al.* 1994, Jouventin *et al.* 1994), and many undertake trans-equatorial

migrations between non-breeding and breeding locations (Schaffer *et al.* 2006, González-Solís *et al.* 2007, Hedd *et al.* 2012). Great Shearwaters *Ardena gravis* breed on islands in the South Atlantic and spend their wintering season in the North Atlantic, arriving in the western half in May. They return to the Southern Hemisphere passing down the east-central Atlantic starting in September (Brown 1986). Juveniles and sub-adults arrive in the western North Atlantic later (July) and are thought to return south in November, after breeding adults (Brown 1986, Powers *et al.* 2017). The boreal summer distribution of Great Shearwaters off the northeastern United States and Atlantic Canada has been examined in part by a series of loosely connected and spatially overlapping studies using shipboard and aerial observations (Powers 1983, Brown 1986, Winship *et al.* 2018, Wong *et al.* 2018, Carvalho & Davoren 2019). These studies indicate foraging flexibility of wintering shearwaters based on biophysical drivers. For example, Great Shearwaters exhibited inter-annual variability and dietary partitioning with Sooty Shearwaters *A. grisea* as prey availability shifted within the Bay of Fundy (Ronconi *et al.* 2010). Great Shearwater movements within the Gulf of Maine, more recently characterized with satellite tracking data (Powers *et al.* 2017), showed that birds preferred to forage in shallower waters (< 100 m), primarily over sloped rims fringing deeper central basins. However, this analysis of foraging habitat showed inconsistent relationships in terms

of depth, chlorophyll *a*, and sea surface temperature between Georges Bank/the southern Gulf of Maine and the Bay of Fundy (Powers *et al.* 2017). The breeding biology of two closely related shearwaters, Short-tailed Shearwater *A. tenuirostris* and Cory's Shearwater *Calonectris borealis* (Bradley *et al.* 1999, Campioni *et al.* 2020), indicates that age-specific demographics may also play an important role in Great Shearwater movement ecology in the Gulf of Maine, which is postulated as a wintering area for mostly non-breeding birds (Huetmann & Diamond 2000, Powers *et al.* 2017, Ronconi *et al.* 2018).

Platform terminal transmitters (PTTs) provide geographical information that is tracked by Argos, an international satellite system. Using this information, researchers can describe foraging and wintering ranges, migration corridors, and seasonal movements of seabirds (e.g., Pinet *et al.* 2011, Jodice *et al.* 2015). Such information allows mapping of the broad-scale distribution of a population, which is often related to oceanographic variables. Spatial data can also provide insight into fine-scale distribution, which is mostly influenced by prey distribution (Schneider & Piatt 1986, Fauchald *et al.* 2000, Davoren *et al.* 2003, Suryan *et al.* 2016). However, exploration into the role of prey in seabird distributions requires further examination of seabird diet in tandem with tracking data.

Here, we report results of Argos data from PTTs placed on 58 Great Shearwaters that were captured in the southwestern Gulf of Maine over six years (2013–2018). We used these results to characterize the spatial and temporal extent of a defined portion of their wintering range in the Northwest Atlantic over a timeframe that can accommodate cycles of seasonal and inter-annual variability. We explored differences in utilization distributions (UDs), estimating range sizes and interactions with other individuals on an annual basis. We derived behavioral traits from the aggregated spatial data to assess revisitation and flight patterns between areas of differing foraging intensity, in order to corroborate if foraging behaviors align with changes in bird density. Temporal shifts in overall distribution over the Northwest Atlantic were examined on a monthly basis to determine the timing of bird movement through the region and the extent of movement between and within areas in the study region. Characteristics of key foraging areas and the distributions of preferred prey were compared with information on prey selection, which was determined by fecal DNA analysis. Age demographics were determined from primary molt scores and the timing of departure, in comparison with a gonad and bursa analysis of fishery-related bycaught birds. We suggest that primarily young non-breeding Great Shearwaters utilize the southern Gulf of Maine and Georges Bank during their wintering period. This pattern of habitat utilization is associated with the distribution of a key prey

Fig. 1. Map of study area in the northwestern Atlantic Ocean. The shearwater capture area is noted by the green polygon in the southwestern Gulf of Maine. Bycatch specimens were collected in NOAA/NEFOP Statistical Area 521, which is noted by white dashed line east of Cape Cod, Massachusetts, USA.

species that has distinct biological, physical, and geographical habitat preferences.

STUDY AREA AND METHODS

Data collection

We tagged Great Shearwaters from 2013 to 2018 off the coast of Massachusetts in the southwestern Gulf of Maine, from the Stellwagen Bank National Marine Sanctuary to the coastal waters east of Cape Cod (41.3° to 43.0° latitude, -70.5° to -69.5° longitude; Fig. 1). We used small vessels to access birds at known foraging areas and attracted birds to the vessel using cut fish and squid. Once they were close enough to the vessel, birds were caught with handnet landing nets.

Over six consecutive years, we placed PTTs on 58 shearwaters (*ca.* 10 yr⁻¹; Table 1). Solar PTT-100 tags (15 g, Microwave Telemetry, 8835 Columbia 100 Parkway, Suites K & L, Columbia, MD 21045, USA) were used with duty cycles of 24 hours on (i.e., continuous). Tags were attached dorsally, central to the body mass of the bird below the nape and between the wings, using four subcutaneous sutures (Prolene suture 4.0 Ethicon) as per MacLeod *et al.* (2008). All tags eventually stopped transmitting, and we expected that they fell off when the Prolene sutures degraded. Tags represented less than 3% of body mass for all tagged birds to minimize effects on flight efficiency (see Phillips *et al.* 2003). Tags were typically deployed in July, except for 2017, when poor weather conditions delayed tagging until early August, and a single bird in September 2016. Body weight and primary molt were scored using the BTO method (Ginn & Melville 1983) for each tagged bird. Sex was identified genetically from blood or feather samples provided (see Acknowledgements).

Data analysis

Range estimation using kernel density

We used the Time Local Convex Hull (T-LoCoH, <http://tlocoh.r-forge.r-project.org>) method (Lyons *et al.* 2013) in R version 3.4.0 (R Development Core Team 2018) to analyze our Argos location dataset. T-LoCoH generalizes the non-parametric UD construction method LoCoH (Getz *et al.* 2007) and integrates time with space in the construction of local hulls through a scaling that relates distance and time to the individual's characteristic velocity (Lyons *et al.* 2013). A hull is defined as a minimum convex polygon constructed around each Argos location point using a set of 'nearest neighbors' (i.e., other Argos location points). Hulls represent space-use; if constructed with time, they are localized in space and time (Lyons *et al.* 2013). The resulting hulls also enable estimation of behavioral metrics describing both 1) movement phase and 2) multiple dimensions of time use, including revisitation and duration (Lyons *et al.* 2013). Space-state models (Jonsen *et al.* 2005, Jonsen *et al.* 2007) provide temporal normality and flight behavior to asynchronous satellite telemetry data, but the incorporation of time into range construction using the T-LoCoH approach expands the concept of UDs beyond a traditional density gradient facilitated via state-space models to spatial models of movement and time (Lyons *et al.* 2013). Location error is problematic with Argos locations using either method with respect to partitions of space or edges of utilization. But, compared to location error introduced via either modeling approach, gaps in temporal resolution have

larger consequences on space and time use as well as behavioral characterizations (Powers *et al.* 2017 supplement). PTTs in this study transmitted continuously, and temporal resolution was limited only by satellite availability.

Argos locations were converted to Universal Transverse Mercator (UTM) projections using Easting (X) and Northing (Y) coordinates based on UTM Zone 19 for this analysis. This Mercator projection preserves angles and direction but distorts distance over multiple zones. So, we confined the analysis to an area bounded by 38° to 50° latitude and -50° to -73° longitude (Fig. 1). Argos location classes in the set {3, 2, 1, 0, A, B} were used for the observed data. T-LoCoH's 's' parameter controls the degree to which the time difference between two locations is factored into the calculation of their physical 'distance', which is used to identify 'nearest neighbors' (Lyons 2014). Nearest-neighbor selection is based on a distance metric (time-scaled distance, TSD) (Lyons *et al.* 2013), and we selected 's' to scale hull creation into 24-hour intervals per Argos location. The effect of time-distance is to separate points that are far away in time even though they may be close in two-dimensional space (Lyons *et al.* 2013). Hulls were created using the adaptive *a*-method, which selects all points whose cumulative distance to a parent point, ordered smallest to largest, is less than or equal to *a*. The *a*-method (vs. *r*-fixed radius method and *k*-nearest neighbor method) produces more robust UDs because this method is the least sensitive to suboptimal choices for its kernel parameter *a* (Getz *et al.* 2007); thus, it provides good results in both dense and sparse areas of use. In this study, UDs were created from density isopleths of hull parent points (i.e., centroid locations) for 95%, 50%, and 25% kernel densities, with the 25% kernel UD representing areas of greatest bird concentration and 95% kernel UD areas of the largest spatial extent. We then determined the amount of overlap or individual interactions within these UDs, both among individuals in the same year and among all individuals during the entire study. Physical covariates (depth and substrate properties) were assigned according to the parent point of each hull (centroid) using available data layers. Substrate properties included sediment sand content (%) and mean grain size (mm) for the United States northeast continental shelf (NOAA/NCCOS unpubl. data).

Correlation of individual range size and PTT duration was determined statistically using Pearson's product moment test. If a correlation was found, we then examined whether assumptions of homogeneity of variance differed in individual tag duration by year and number of tag days by year using Bartlett's test before proceeding to ANOVA. ANOVA (*F*-statistic) was used to determine whether UD groups (by year) or behavioral groups were different, using Tukey's test in post-hoc analyses. We also used the Kruskal-Wallis test to determine whether characteristics (e.g., tag days, range size, body weight, molt scores, sex ratio) of population distributions were identical when we could not assume a normal distribution.

Time-use and behavioral characteristics

Time-use metrics were used as another line of evidence to examine spatial use (Lyons *et al.* 2013). We used an inter-visit gap (IVG) period of 24 h to understand daily movements. IVG is a unit of time that must pass before another occurrence in a hull is considered a separate visit. So, for daily foraging behavior, we did not want two locations that were a day apart to be considered nearest neighbors, even if they were close in space. To explore the relationships among the distribution of hulls in time-use space, we examined duration

(i.e., the number of locations in a hull within the IVG) and equated that to an amount of time a bird spent in a hull on each visit. We expected that birds should spend more of their time each day in areas of higher foraging intensity. We also expected that revisitation, or the number of times a bird revisited the same hull outside of the IVG, would reflect longer periods of daily usage of an area.

Estimation of directional movement of shearwaters was based on a behavioral metric: eccentricity (Lyons *et al.* 2013). Eccentricity is measured on the bounding ellipsoid of a hull. The eccentricity of an ellipse varies from 0 for a perfect circle to 1 for a line. Hulls with low eccentricity represent areas of non-directional movement, such as area-restricted searching or foraging, whereas higher values indicate areas where an animal is moving directionally, such as migrating or traversing habitat of low resource value (Lyons *et al.* 2013). The *a*-method reduces the number of neighbors per hull in outlying areas, where hulls become more eccentric (more directional travel), compared to hulls constructed in areas of more equidistant neighbors, which are less eccentric (more area-restricted searching). We also examined temporal patterns of spatial use (latitude and longitude, by month) within the 95%–50%, 50%–25%, and < 25% bands of kernel density by using the aggregate dataset.

Fecal DNA analysis

For food habits analysis, birds were captured in 2017–2018 and temporarily placed into individual pet carriers on mesh netting suspended over an aluminum foil covered floor. Fecal material captured on the foil was collected for each individual and frozen either dry or in ethanol. Fecal samples were transported to a genetics lab and stored at –20°C until processing. Frozen samples were scraped into 2.0-ml. tubes using flame-sterilized forceps and frequent glove changes. Genomic DNA was extracted from each sample using a Qiagen (Valencia, California, USA) stool mini-kit following manufacturer protocols, with the following modifications: samples were incubated at 60°C overnight in the first buffer and we added 1 µL of carrier RNA after the second incubation. We employed polymerase chain reaction (PCR) to target a fragment of the mitochondrial 16S gene that is about 110–130 bp long. We used the Chord_16S_F_TagA (forward) and Chord_16S_R_Short (reverse) primers from Deagle *et al.* (2009), standard PCR conditions, and a blocking primer (GrSh-block1: 5'-TGTGGAACTTAAATCAACGA-3') that we designed for this study to terminate in a C3 spacer, which would limit predator reads. Previous work demonstrated that this primer set (forward, reverse, and blocking) could reconstruct vertebrate prey items from seal fecal samples collected from the Gulf of Maine, with species-level taxonomic resolution for most items (K. Flanders pers. comm.). Pre-PCR and PCR workflows took place in dedicated labs in separate buildings to minimize the potential for contamination.

Amplicons were cleaned and sent to a genomics facility, where they were indexed and sequenced on 1/8th of a flow cell on an Illumina (San Diego, California, USA) MiSeq using 2×300 bp chemistry (*ca.* two million reads). Forward and reverse reads were assembled in PANDAseq (Masella *et al.* 2012) using the default primer-match threshold. After trimming adapters and primers, *de novo* operational taxonomic units (OTUs) were picked using a threshold of 97% sequence similarity using QIIME2 (Caporaso *et al.* 2010).

OTUs with < 2 reads were trimmed using the “phyloseq” package (McMurdie & Holmes 2013) in R version 3.2.5 (R Development

Core Team 2018). Remaining OTUs above the 1% threshold of Deagle *et al.* (2009) were identified to the lowest possible taxonomic resolution with NCBI Blast+ in the Cloud software accessed through Amazon Web Services Marketplace (Seattle, Washington, USA), which queried OTUs against a server copy of the nucleotide database. Taxonomy was assigned to each OTU based on the lowest expect value (*e*-value), with a maximum threshold of 1e–20 (see Thomas *et al.* 2014). If multiple database matches shared the same lowest *e*-value, we selected the match whose species is known to occur in the study area. If the OTU matched best (i.e., lowest *e*-value) to more than one geographically appropriate species, the OTU was assigned to the lowest taxonomy shared between the matches (e.g., family or genus instead of species). OTUs with identical taxonomy were combined using the “phyloseq” package, and those identified as obviously not prey (e.g., human, shearwater) were removed as contaminants.

Distributions of potential prey

We examined the National Oceanic and Atmospheric Administration's (NOAA) bottomfish population assessment database for comparisons of potential prey distribution to Great Shearwater distribution (Politis *et al.* 2014). NOAA trawl logs included location (latitude and longitude), depth, and number of fish caught, but we independently assigned substrate properties (sand content and grain size) based on location using our substrate data layers (NOAA/NCCOS unpubl. data). Based on insight gleaned from the fecal analysis, we investigated distributions of Northern sand lance *Ammodytes dubius*, Atlantic mackerel *Scomber scombrus*, and Atlantic herring *Clupea harengus*. Mackerel feed throughout the water column, so bottomfish surveys may be inconclusive in mackerel stock assessments. Atlantic menhaden *Brevoortia tyrannus* is a mid-water fish, so it was not represented in these bottom trawl surveys. While sand lance is not specifically targeted in trawl surveys, it is caught as bycatch and is included in trawl survey datasets. We examined spring (April–May) and fall (October–November) logs of trawls from 2013 to 2017 covering the northeastern US continental shelf (east of –73°) including Georges Bank. These surveys did not include the Scotian Shelf in Canada. We identified all trawl stations as spatial points to define the scope of bottom trawl sampling, and we characterized bathymetry, substrate, surface temperatures, and bottom temperatures at locations where targeted fish were caught. Sampling of fish was uniform across the northeastern US continental shelf for each spring and fall survey (Politis *et al.* 2014), so we summed the locations of fish caught over the entire period.

Age analysis using bycaught birds

Data from bycaught individuals was used to examine age and sex demographics in the southwestern Gulf of Maine. Bycaught shearwaters were obtained from the US NOAA Northeast Fisheries Observer Program (NEFOP) in the Gulf of Maine (Northeast Statistical Area 521 in Appendix A of NEFSC FSB 2016) and from scientific longline surveys around Gough Island in the Tristan da Cunha archipelago in the South Atlantic. Individuals were frozen upon collection and necropsied as part of a larger study following the methods of van Franeker (2004). Morphometric data were collected on live birds captured in the southwestern Gulf of Maine (see Fig. 1) in July 2019 for comparison to bycaught specimens.

Within this study, we relied upon gonad development, molt score, and the presence/size of the bursa of Fabricius to gauge sex and likely age. Based on visual assessment of gonad color and quantitative measurement of gonad size, gonad development was scored as one of four classes: hatch year, immature, sub-adult/non-breeder, or breeding bird. As noted in previous studies incorporating necropsy data, the sub-adult category (Class 3) includes both immature birds close to maturity and non-breeding adults, since gonadal development of these two stages is very similar (Cortes *et al.* 2018, Hector *et al.* 1986). Molt score was examined following BTO methods, and each specimen was assigned a score from 0 to 50. Bursa presence and size was also noted when possible because these can be used as non-plumage-based evidence to estimate the age of procellariiform seabirds (Broughton 1994) instead of the nape plumage characteristics noted by Pyle (2008). The bursa begins to enlarge shortly after hatching and reaches its maximum size in four to six months (Broughton 1994). Involution is typically complete by the end of a bird's first year of life, and the bursa is absent in adult birds (Glick 1983, Mase & Oishi 1991). Vestigial bursas have internal walls that cannot be visibly differentiated from the cloacal wall (Broughton 1994).

Gonad development, molt score, and bursa presence/size were assessed together for each bird to assign a likely age class: young or mature. Collected measurements were compared between Cape Cod (Northwest Atlantic as assumed non-breeders) and Gough Island (South Atlantic as suspected or confirmed breeders). The Wilcoxon test was used to compare parameter means as we could not assume that the measurements were normally distributed.

RESULTS

PTTs provided an average of 19.1 Argos locations per day per tag. These were used to create TSD hulls for individual Great Shearwaters with an IVG of 24 hours. A total of 73 302 convex hulls were determined for 4 477.5 days (Table 1).

Annual ranges, expressed in TSD units, were calculated per bird (Table 1). The 50% kernel UD in any year typically represented 21.8% of an individual's 95% kernel UD. The number of range intersections among individual birds in any year increased proportionately with range size: 32.3% in the 25% kernel UD and 62.3% in the 50% kernel UD, relative to total intersections within the 95% kernel UD. But, the area of intersection was disproportionately larger in the 95% kernel UD, and 83.6% of the overlap occurred outside of the 50% kernel UD (Table 1).

Comparing range size by year, we found a positive correlation between tag days and the estimated 95% kernel UD ($P = 3.578 \times 10^{-6}$, $R^2 = 0.552$). As a result we examined, but found no difference in, both the length of individual tag duration (variance) by year (Bartlett's $K' = 2.1842$, $df = 5$, $P = 0.8231$) and the number of tag days (sampling effort) by year ($F = 1.0712$, $df = 5$ and 52, $P = 0.3871$). However, the 95% kernel UD size by year was significantly different ($F = 3.162$, $df = 5$ and 52, $P = 0.0144$) between 2013 and 2015 (Tukey's test, $P = 0.028$) and between 2015 and 2018 (Tukey's test, $P = 0.035$; Table 1). Plotting 95% kernel UDs by year (Fig. 2) showed that most individuals in 2013 and 2018 stayed within the Gulf of Maine, as opposed to 2015, when

TABLE 1
Annual breakdown of platform terminal transmitter (PTT) metrics related to sampling effort, kernel density range estimates as scaled by time-scaled distance (TSD), individual range intersections, and morphological measurements for Great Shearwaters ($n = 58$) tagged in 2013–2018

Statistic	Density	2013	2014	2015	2016	2017	2018
Total tags (PTT)		10	11	10	10	9	8
Mean tag duration (day)		59.2	85.1	96.2	69.3	79.7	72.1
Total tag days		591.8	936.4	962.0	693.3	717.6	576.4
Total convex hulls		10 878	14 037	12 981	13 079	11 889	10 438
Mean range by density (TSD*1e-6)	0.25	2 319	6 160	8 655	2 806	1 251	2 212
	0.50	9 144	11 256	21 399	13 222	11 777	6 879
	0.95	33 457	74 914	98 053	48 388	49 160	31 434
Mean tag intersections by density	0.25	13	25	17	23	19	7
	0.50	31	35	37	32	27	15
	0.95	45	55	45	44	36	28
Mean intersection area by density (TSD*1e-6)	0.25	325	982	1 275	950	774	617
	0.50	594	2 990	2 845	1 842	1 545	886
	0.95	3 742	17 685	21 935	8 417	8 863	4 939
Mean body weight per bird (g)		754	940	874	859	716	734
Mean primary molt score (0–50)			45.6	35.8	48.9	48.9	47.6
Range primary molt score			41–50	6–50	45–50	44–50	41–50
Gender (M/F)				5/4	4/6	2/6	5/3
Tagging date (dd-mm)		12-Jul	20-Jul	09-Jul	12-Jul	04-Aug	9-Jul

the UD of half of the individuals extended east to the Scotian Shelf and north to the Grand Banks. The breadth of the 95% kernel UD in 2018 was also underrepresented, as two individuals (ID numbers: 112710 and 112717), within three weeks of capture, moved east of the study area ($< -50^\circ$ longitude): one to the Flemish Cap (47° , -44°) and the other to the mid-Atlantic Ridge (41° , -30.5°). Thus, the overall wintering range of Great Shearwaters extends east of Atlantic Canada (-50° longitude) and beyond the longitudinal boundary used in this study.

In the context of UD size, we rearranged metrics by individual to derive a table showing birds that primarily used the Gulf of Maine compared to those that also extended to the Scotian Shelf or the Grand Banks (Table 2). In this configuration, there was a significant difference in tag days per range group (Kruskal-Wallis $\chi^2 = 7.3174$, $df = 2$, $P = 0.02577$), so we could not statistically compare UD sizes. Birds restricted to the Gulf of Maine had the smallest 95% kernel UD compared to those that also used the Scotian Shelf (median

20250 vs. 87000 TSD* $1e-6$) or the Grand Banks (median 115500 TSD* $1e-6$). Body weight was significantly different among these range groups (Kruskal-Wallis $\chi^2 = 8.671$, $df = 2$, $P = 0.0131$) and increased with larger UD sizes (Table 2). Primary molt score was not statistically different between groups (Kruskal-Wallis $\chi^2 = 3.7689$, $df = 2$, $P = 0.1519$). However, all birds in the Grand Banks group were in active molt, whereas most of the Gulf of Maine group were not in molt when tagged. There was no significant difference in sex between these regional groups (Kruskal-Wallis $\chi^2 = 1.1681$, $df = 2$, $P = 0.5576$).

In a spatial context, individual 50% kernel UD of Great Shearwaters intersected each year along the 100-m isobath east of Cape Cod and south to the Great South Channel. In several years, this area extended north to include the Stellwagen Bank National Marine Sanctuary (2014–2017) or east along the same isobath across northern Georges Bank (2013–2016). Multiple individuals had 50% kernel UD in the Bay of Fundy in 2014 and 2018, and on

Fig. 2. Plots of Great Shearwater 95% kernel utilization distributions (UDs) derived from convex hulls of individual birds tagged per year (2013–2018). Individual UD are filled in green. Red borders denote intersections of individuals within that year. In 2018, two UD extended east of -45° longitude outside of the study area.

the southern New England shelf west of Nantucket Shoals in 2017. Thus, the majority of 50% kernel UD were in tangential areas across the southwestern Gulf of Maine, but the 25% kernel UD shifted from year to year.

The UD of all individuals tagged in 2013–2018 were collectively plotted to show individual boundaries with aggregated 95%, 50%, and 25% kernel density isopleths (Fig. 3). Isolated from an annual perspective, 25% kernel UD became contiguous within the southwestern Gulf of Maine, along with separate isolated pockets in the northern Gulf of Maine (including Bay of Fundy and the northeastern corner of the Scotian Shelf (Banquereau)). The centralized 25% kernel UD within the Gulf of Maine were connected by the 50% kernel UD, which also encapsulated those 25% kernel UD on the Scotian Shelf (Fig. 3).

In the aggregate view, more hulls from between the annual 25%–50% kernel density isopleths collapsed into a single 25% kernel UD, and the overall number of hulls between the aggregate 50%–95% isopleths were disproportionately reduced. A comparison of behavioral characteristics by density groups (Table 3) showed

significant differences in revisitation ($F = 3.347$, $df = 2$ and 71.623 , $P < 2.2e-16$), duration ($F = 622$, $df = 2$ and 71.623 , $P < 2.2e-16$), and eccentricity ($F = 497$, $df = 2$ and 71.623 , $P < 2.2e-16$) (Table 3).

Fig. 3. Aggregate plot of all individual utilization distributions of Great Shearwaters ($n = 58$) from 2013 to 2018 with kernel density groupings based on entire convex hull dataset: < 25% (red), 25%–50% (yellow), and 50%–95% (green). Shading is caused by overlapping ranges of differing density.

TABLE 2
Platform terminal transmitter metrics related to sampling effort, area of 95% kernel utilization distribution (UD) as scaled by time-scaled distance (TSD), and morphological measurements by inclusive regions of the continental shelf in the Northwest Atlantic for Great Shearwaters ($n = 58$) tagged in 2013–2018 (median, 1st–3rd quartiles)

Measurement	Gulf of Maine	Scotian Shelf	Grand Banks
Total individuals	32	14	12
Tag days	59.2 (29.9–97.2)	85.1 (98.0–119.7)	96.2 (46.8–188.3)
Area of 95% kernel UD (TSD*1e–6)	20 250 (6 395–38 150)	87 000 (60 525–112 250)	115 500 (61 075–138 500)
Body weight (g)	758 (699–853)	858 (793–885)	898 (826–943)
Primary molt score	50 (45–50)	49 (46–50)	46 (43–48)
Total male	10	4	2
Total female	9	6	4

TABLE 3
Physical properties and behavioral characteristics of convex hull locations for Great Shearwaters ($n = 58$) tracked in 2013–2018, as summarized by three density groupings with median, 1st–3rd quartiles of each group. Substrate properties represent locations within only the Gulf of Maine and on the western Scotian Shelf.

Measurement	< 25%	25%–50%	50%–95%
Convex hulls ¹	41 637	17 007	12 982
Depth (m) ²	64 (43–103)	108 (57–171)	102 (65–174)
Eccentricity (0–1) ³	0.82 (0.71–0.90)	0.85 (0.74–0.93)	0.87 (0.76–0.94)
Revisitation ⁴	5 (2–8)	3 (2–5)	2 (1–4)
Duration ⁵	16.8 (10.9–27.0)	14.0 (9.7–22.0)	15.0 (10.0–22.0)
Sand content of sediment (%) ⁶	61.9 (45.7–77.4)	37.1 (13.8–75.0)	44.5 (28.9–82.2)
Mean grain size of sediment (mm) ⁷	0.577 (0.206–0.961)	0.202 (0.044–0.531)	0.287 (0.084–0.457)

¹ Hulls are minimum convex polygons constructed around each location point (Argos) from a set of nearest neighbors (other locations).

² Water depth determined at the centroid of each convex hull.

³ Eccentricity is the degree of elongation of each individual hull and indicates directionality of movement.

⁴ Revisitation is the number of daily visits to the same hull.

⁵ Duration is the number of locations counted at each hull when it was visited.

⁶ Sand content is percent amount of sand in the sediment per hull.

⁷ Mean grain size is the predicted diameter of sediment grain structure.

Revisitation significantly increased from 2 to 5 visits per hull as kernel density decreased (i.e., 95%–25% density), indicating that birds went back to the same hulls on more different days in the < 25% kernel band. Regarding revisitation, each of the density bands was statistically different (Tukey's test, $P = 0.00$). As expected, duration was significantly higher in the collective < 25% kernel density band, indicating that birds stayed in those hulls for longer periods during each daily visit: 16.8 locations hull⁻¹ vs. 14–15 locations hull⁻¹ when kernel density was 25%–95% (Table 3). Outside of the < 25% kernel density band, there was no significant difference in duration between the 25%–50% or the 50%–95% kernel density groups (Tukey's test, $P = 0.775$). Once outside of the primary foraging areas, birds did not linger but instead moved to other areas. Regarding eccentricity, the < 25%

density group was significantly different from both the 25%–50% group (Tukey's test, $P = 0.004$) and the 50%–95% group (Tukey's test, $P = 0.0002$). Hull shapes were rounder, indicating substantially more “area-restricted search” behavior within the 25% kernel density isopleth, where eccentricity was 0.82 vs. 0.85–0.87 outside of it (Table 3). Thus, calculated behavioral traits that were measured corroborated more intense foraging activity within the aggregated 25% kernel UD.

Physical characteristics of the aggregate kernel density groups also showed significant differences in depth ($F = 3.024$, $df = 2$ and 71 623, $P < 2.2e-16$), sand content in the substrate ($F = 1.550$, $df = 2$ and 54 653, $P < 2.2e-16$), and mean grain size of sediment ($F = 1.597$, $df = 2$ and 54 653, $P < 2.2e-16$). None of the density groups overlapped (Tukey's test, $P = 0.00$). The < 25% kernel density band had the shallowest depths (median 64 m), highest sand content (median 62%), and coarsest grain size (median 0.578 mm). Hulls in the 50% and 95% kernel density groups were in deeper water (> 100 m), where the substrate had lower sand content (< 45%) and finer grain sizes (< 0.287 mm) (Table 3). There was no single surficial sediment data layer for the entire study area, so we used a single sediment layer for only the Gulf of Maine, Georges Bank, and the western Scotian Shelf including the Bay of Fundy (NOAA/NCCOS unpubl. data; Table 3). A separate sediment layer (Fader *et al.* 1982) was used for hulls located on the eastern Scotian Shelf (Browns Bank and Lurcher Shoals north to Laurentian Channel). Almost half of the Scotian Shelf hulls (44% or 3 892 of 8 927) were located over gravel sediment with > 50% sand and an average depth of 67 m. Seventy percent of these locations with sandier substrates occurred around Sable Island, Banquereau, Western Bank, and Browns Bank (see Fig. 1).

Since birds were tagged near Cape Cod, all individual movements across all years emanated from the same general area starting in July, except for 2017 when birds were tagged in early August (Fig. 4). Birds in the < 25% density group consistently stayed in the same general area centered at 42°, –70° (southwest Gulf of

Fig. 4. Histograms of monthly (Jul–Nov) average latitude (A) and longitude (B) by kernel density groups (< 25%, 25%–50%, 50%–95%) from 2013 to 2018. All birds were tagged off Cape Cod in July, except for 2017 when one was tagged in early August. Most birds (32 of 58) remained the Gulf of Maine during their tag lifespan.

TABLE 4
Number of samples (count) and frequency of occurrence (FOO) of prey groups representing food habits of Great Shearwaters. Data were derived from metagenomic analysis of 35 successfully sequenced fecal samples collected in waters off Massachusetts, USA.

Prey group	Common name	Count	FOO
<i>Ammodytes</i> spp.	Sand lance	25	71.4%
<i>Brevoortia tyrannus</i>	Atlantic menhaden	17	48.6%
<i>Scomber scombrus</i>	Atlantic mackerel	11	31.4%
<i>Leucoraja erinacea</i>	Little skate	7	20.0%
<i>Peprilus triacanthus</i>	Atlantic butterfish	3	8.6%
<i>Clupea harengus</i>	Atlantic herring	2	5.7%
<i>Squalus acanthias</i>	Spiny dogfish	2	5.7%
<i>Morone saxatilis</i>	Striped bass	1	2.9%
<i>Merluccius bilinearis</i>	White hake	1	2.9%
Pleuronectiformes	Flatfishes (flounders)	1	2.9%
<i>Tautoglabrus adspersus</i>	Cunner	1	2.9%

Maine) from July through November. Birds in the 25%–50% and 50%–95% density groups showed northward progressions from July through September, a pattern that then shifted southward from October through December (Fig. 4A). Their monthly longitudinal progression was increasingly eastward while in the study area (Fig. 4B). The majority of the 25%–50% group was contained within the Gulf of Maine, with some reaching the Bay of Fundy to the north in September while staying west of Nova Scotia ($< -65^\circ$ longitude) through October. In November there was considerable movement east of Nova Scotia ($> -65^\circ$ longitude) as tagged birds left the study area (Fig. 4B). The 50%–95% group showed the largest latitudinal and longitudinal expansions over time. The northernmost points reached 48° in August to September, after which the birds progressively moved southward to 41° by December (Fig. 4A, also Fig. 2 for years 2014 and 2015). By September most of this group was east of Nova Scotia ($> -65^\circ$), extending to the Grand Banks ($< -55^\circ$) (Fig. 4B). In 2018, individuals (ID number: 112710 and 112717) moved east of the Grand Banks to -30° in August. The eastward trend continued into December while average latitude was decreasing southward. Thus, eastward movement out of the study area occurred over a wide latitudinal band (40° to 47°) and was most pronounced in the outer parts of their collective UD (50%–95% kernel density) after September. Of the 58 birds in this study, 18 were tracked south of the Equator, 3 by the end of September and the remaining 15 in November and December. Only two of the birds tracked south of the Equator visited the Tristan da Cunha archipelago and neither stayed more than a single day before returning to the Patagonian Shelf.

Fecal DNA analysis

Prey sequences were successfully obtained for 35 of the 47 fecal samples. Samples were collected in August and September 2017 and in July and November 2018. Average prey diversity was 2.11 ± 0.28 (mean ± 1 SE, min = 1, max = 7). Most samples (71.4%) contained sand lance; the next most frequent prey item was menhaden (48.6%, Table 4). Only 14.3% (5 of 35) of samples lacked sand lance or menhaden. Mackerel, Atlantic butterfish *Peprilus triacanthus*, and herring were also identified in Great Shearwater diets at lower frequencies. Sand lance, menhaden, and mackerel were consumed in both years sampled (2017 and 2018).

In the remaining six prey groups, it is unclear whether shearwaters were taking juvenile fish directly (which might explain the occurrence of striped bass *Morone saxatilis*, white hake *Merluccius bilinearis*, cunner *Tautoglabrus adspersus*, and a pleuronectiform flatfish) or whether birds were scavenging chum or bait from fishing vessels (which might explain the occurrence of little skate *Leucoraja erinacea* and spiny dogfish *Squalus acanthias*). Alternatively, primary prey may have contained bait or chum that we detected indirectly (Sheppard *et al.* 2005).

Depth, substrate, and prey distribution

Foraging in 25% kernel UD was linked to shallow depths and substrates with high sand content of coarser grain sizes (Table 3). During the 2013–2017 period, 88 of 2319 trawls conducted by NOAA's National Marine Fisheries Service (NMFS) captured sand lance, while 67% of trawls with sand lance were over the aggregate 25% kernel UD of Great Shearwaters (Fig. 5). The physical properties of these sand lance locations were similar to those hulls in the shearwater 25% kernel UD having a mean depth of 61.6 m

(± 48.5 m) with 71.1% ($\pm 22.7\%$) sand content of relatively coarse grain size (median 0.685 mm). In addition, 89% (78 of 88) of the NMFS trawls with sand lance also occurred within the 100-m isobath. Of these 88 sand lance locations for which we could extract sand content and grain size information, we found that 64% (56 of 88) were found over substrates with $> 50\%$ sand and grain sizes in the range of 0.35–2.00 mm. This range of grain sizes encompasses coarse to very coarse sand (Krumbein & Sloss 1963; Fig. 5). Of the Great Shearwater locations in the 25% kernel UD, 63% (26 355 of 41 637) were within the 100-m isobath with $> 50\%$ sand content; 49% (20 562) were also coincident within this inclusive range of sediment grain sizes.

Mackerel were caught at 688 locations on the northeastern US continental shelf, with greatest abundance near Race Point off Cape Cod, eastward along the 100-m isobath to northern Georges Bank. Among trawls with mackerel, 38% (259 of 688) occurred within the shearwater 25% kernel UD, and the fish were caught at an average depth of 111.1 ± 68.2 m over substrate with $51.4\% \pm 30.1\%$ sand content and a median grain size of 0.238 mm. Herring were caught in 1 225 locations, with highest abundance over western Nantucket Shoals, northern Georges Bank, and in Cape Cod Bay north to Jeffreys Ledge. Among trawls with herring, 36% (447 of 1 225) occurred within the shearwater 25% kernel UD, and the fish were caught at an average depth of 125.7 ± 72.1 m over substrate with $44.6\% \pm 29.0\%$ sand content and a median grain size of 0.174 mm.

Age analysis using bycatch

Necropsies were performed on Great Shearwaters caught as bycatch near Gough Island in the Tristan da Cunha archipelago and near Cape Cod. South Atlantic specimens were landed in April 2018 ($n = 23$, 16 male and 7 female), December 2012 ($n = 1$, male), and October 2018 ($n = 1$, female). Northwest Atlantic specimens were landed in August 2017 ($n = 71$, 27 male and 44 female).

The median gonad area in males was 8 mm^2 for Northwest Atlantic birds and 32 mm^2 for South Atlantic birds. In females, median gonad area was 33 mm^2 for Northwest Atlantic birds and 100 mm^2 for South Atlantic birds. Gonad size was significantly different in both sexes between regions (males: $W = 429$, $P = 1.248\text{e-}06$; females: $W = 327$, $P = 0.000136$). A truth table was developed to sort age categories and determine maturity (Table 5). All birds from the South Atlantic were mature, but only two males and six females from the Northwest Atlantic region were mature. We then compared gonad size of young vs. mature birds for both sexes between the Northwest Atlantic and the South Atlantic groups. In terms of gonad size, young birds from the Northwest Atlantic were significantly different (smaller) from the South Atlantic birds (males: $W = 424$, $P = 4.41\text{e-}06$; females: $W = 301$, $P = 1.648\text{e-}05$), whereas there was no significant difference for mature birds (males: $W = 5$, $P = 0.1404$; females: $W = 26$, $P = 0.8461$). Thus, 11% (8 of 71) of the Northwest Atlantic bycatch specimens were sub-adults or adults who may have been possible breeders, while 89% (63 of 71) were juvenile or immature individuals considered to be pre-breeders.

We analyzed 53 of the 71 Northwest Atlantic birds and all 25 South Atlantic individuals for bursas. For the Northwest Atlantic specimens, bursas were identified in 13 males and 24 females and deemed absent (involved) in 7 males and 9 females. Bursas were not found in any South Atlantic individuals, as they were all mature. Thus, of those

birds examined for bursa, 70% of the pre-breeders from the Northwest Atlantic specimens were juvenile or hatch-year individuals.

We also compared primary molt score and head-bill length (mm) between the regional necropsy samples (Northwest vs. South

Atlantic) and 18 live shearwaters caught in July 2019 within the tagging area. The median primary molt scores among these areas (NwAtl-necropsy, NwAtl-live, SAtl-necropsy) were 50, 46.5, and 0, respectively. The average head-bill lengths for the same areas were 101.4 ± 3.0 mm, 103.5 ± 4.5 mm, and

Fig. 5. Plot of aggregate 25% kernel utilization distributions (UDs) of Great Shearwaters, sand lance trawl captures, and substrate properties common to both species in the southwestern Gulf of Maine and Georges Bank. Most sand lance (at least 64%) caught in US NOAA/NMFS bottomfish trawl samples (2013–2017) occurred within the 100 m isobath over sediment with > 50% sand content and grain sizes ranging from 0.35–2.00 mm in diameter. Within the 25% kernel UD, 67% of sand lance captures occurred and 49% of shearwater hull locations intersected with these sediment properties.

TABLE 5
Parameters used to designate “mature” and “young” age categories of necropsied Great Shearwaters

Parameter	Distinguishing features between ages	Notes
Molt score	HY ¹ = 50, non-HY = < 50	HY birds typically complete primary molt before arriving in the southwestern Gulf of Maine; immature and sub-adult individuals complete molt following migration
Gonad class	Classes 1 & 2 = young Classes 3 & 4 = mature	Visual assessment of color and gonad size were used to define gonad class
Gonad size comparison	Within min-max range of suspected post-breeding birds	Birds considered “mature” if gonad area overlapped the range of gonad area observed in suspected post-breeding adult/sub-adult individuals from Gough Island
Bursa presence/size	Present, bulbous = HY	Bursa present in only HY individuals

¹ HY = Hatch year

105.3 $\pm$ 2.5 mm, respectively. The necropsied birds from the Northwest Atlantic had the smallest head-bill lengths and had predominantly completed molt, suggesting young individuals. The live birds from the Northwest Atlantic showed a mixture of younger and older birds, with most in active molt and larger head-bill lengths. The necropsied birds from the South Atlantic (April 2018 samples) were all older with the largest head-bill lengths and old primary feathers (molt score = 0).

DISCUSSION

We used individual tracks from 58 Great Shearwaters over a six-year period (2013–2018) to characterize the spatiotemporal utilization of Great Shearwaters over a large portion of their wintering range in the Northwest Atlantic. This study improves our understanding of Great Shearwater use of the Gulf of Maine and Atlantic Canada by providing temporal and behavioral dynamics across the entire region, along with a more comprehensive examination than available previously of how age is integrated into spatial use. In general, Great Shearwaters begin to arrive on Georges Bank in late May (Powers 1983) and are abundant across the southern Gulf of Maine in June and July (Powers *et al.* 2017). They undergo a rapid molt upon arrival, which does not render them flightless (Brown 1986, Brown 1988). Molt scores of birds in this study showed that primary molt was > 90% complete (average score 45.4 of possible 50) in July (Table 1). Molt should have been completed in August, as has been noted in the Bay of Fundy (Powers *et al.* 2017), yet we found a preponderance of completed primary molt in July (20 of 48 scored). This indicated that many of our captures were hatch-year or, less likely, immature pre-breeders that molted in the South Atlantic prior to coming north (Watson 1971, Bugoni *et al.* 2015).

Our molt data were supported by our necropsy data on gonad size, which showed that only 11% had gonad sizes comparable to that of individuals from the Gough Island breeding colony and that 89% were non-adults. The presence of visually prominent bursa indicates that 70% of non-adult birds in the study area may be hatch-year birds. The non-breeding status of the birds was further supported by our tracking data. No birds that we tracked into the South Atlantic for this study spent any significant time near the breeding islands of Tristan da Cunha. As characterized by nape plumage, 89% of Great Shearwaters in the Gulf of Maine were thought to be less than three years of age (Powers *et al.* 2017). However, that methodology was derived from Pyle (2008) and has not been thoroughly vetted in the literature. In addition, birds tagged in the Bay of Fundy by Powers *et al.* (2017) were confirmed as non-breeders (Ronconi *et al.* 2018). An evaluation of mass strandings of Great Shearwaters along the eastern coast of the United States noted that birds stranded in the southeastern US were primarily juveniles while those in the northeast were a mix of adults and juveniles (Haman *et al.* 2013). The breeding population of Great Shearwaters has been estimated at 5–6 million pairs (10–12 million birds) (Ryan 2007) and the global population (inclusive of pre-breeding birds) is thought to be around 16.5 million (Fishpool & Evans 2001), as noted by Ronconi *et al.* (2018). These population estimates indicate that 27%–40% of the world population may be non-breeding. So, if our Gulf of Maine sample consists of 89% non-breeders, it may be an important area used primarily by younger non-breeding birds. Most adult birds may be wintering in areas further north on the Grand Banks, as suggested by Brown (1986).

The 95% kernel UD of birds in this study indicated that Great Shearwaters utilized much of the continental shelf from southern New England across the Scotian Shelf to the Grand Banks after their initial arrival to the southwestern Gulf of Maine and Georges Bank. Nearly half (45%) of the birds moved to Atlantic Canada by August, while the remainder (55%) stayed within the Gulf of Maine until November. Wong *et al.* (2018) noted the highest densities of Great Shearwaters in the Bay of Fundy in early September, which is coincident with the start of southern migration for 'early' migrants (Powers *et al.* 2017). The spatial spread over time was generally northward until October and eastward into November. The frequency of birds staying in the Gulf of Maine was consistent from year to year, but the number of birds with ranges that extended to Atlantic Canada varied annually.

It's not entirely clear why some birds stayed in the Gulf of Maine and others undertook more extensive travelling, but it may be related to age and experience. With increasing maturity, individuals identify new resources across their wintering range. Cory's Shearwaters do not become familiar with main migratory routes until they reach four years of age, when they broadly use the same routes as older immatures and adults (Campioni *et al.* 2020). Beyond this age their migratory behavior becomes progressively adult-like until time of first breeding (Campioni *et al.* 2020). This observation of older Great Shearwaters expanding their UD on the wintering range is supported by our data showing heavier birds tending to move out of the Gulf of Maine before lighter birds. While age at first breeding is unknown for Great Shearwaters, the related Short-tailed Shearwater breeds at six or seven years old (Bradley *et al.* 1999) and Cory's Shearwater at nine years (Campioni *et al.* 2020); Great Shearwaters might be similar. The long duration of immaturity in Wandering Albatross *Diomedea exulans* relates to a long period of experiential learning about spatial, behavioral, and resource constraints (Riotte-Lambert & Weimerskirch 2013). The ontogeny of long-distance migratory strategies and related patterns of spatial distribution in young Cory's Shearwaters progressively changes with age (Campioni *et al.* 2020).

Thus, most Great Shearwaters (55%) stayed within the Gulf of Maine from July to November instead of moving to Atlantic Canada. Individual 50% kernel UD sizes typically represented 22% of a bird's 95% kernel UD, and 84% of individual range interactions occurred outside of the 50% kernel UD, indicating that foraging occurs over wide areas each year. When we aggregated all individuals across all years into a single dataset, the contrast of utilization reversed, and 82% of the hulls were within the 50% kernel UD. The merge of all tangential 50% kernel UDs from each year created a singular 25% kernel UD, which included 58% of all hulls. Thus, birds were foraging in similar areas each year with significant overlap among years. Behavioral traits in the high-density region supported more intense daily foraging activity as birds remained in hulls for longer periods of time (duration), revisited the same hulls on different days more often (revisitation), and showed more area-restricted search behavior (eccentricity). The aggregate 25% kernel UD also had commonality in its physical characteristics, as it was shallow (median 64 m) with high sand content (median 62%) of coarser grains (median 0.58 mm) than the more peripheral bands of the 50% or 95% kernel UDs.

The physical characteristics of shallow habitat with coarsely grained sand selected by Great Shearwaters was also spatially aligned with that of sand lance, which our fecal samples identified as their main

prey (71% Frequency of Occurrence). The habitat preferences (deeper, with less sand and smaller grains) of mackerel and herring were less aligned with that of shearwaters, and these species were identified as lesser prey in our fecal samples (mackerel 31%, herring 6%). Research trawl data showed that 67% of locations containing sand lance fell within the Great Shearwater 25% kernel UD, while far fewer locations containing mackerel and herring fell within this UD (38% and 36%, respectively). Further, at least 64% of trawls with sand lance occurred in waters where depth was < 100 m, sand content was > 50%, and sediment grains were 0.35–2.00 mm in diameter. This combination of factors was also coincident with 49% of shearwater locations in their 25% kernel UD. This indicates that sand lance and the habitats that support them are key factors in shearwater habitat use. Depth (< 100 m) was a significant predictor for the presence/absence of Great Shearwaters in the Gulf of Maine, and this may be related to their reliance on sand lance as forage (Powers *et al.* 2017).

Shearwater utilization of the eastern Scotian Shelf was also associated with banks having shallow waters and high sand content. Sand lance on the Scotian Shelf occur on most of the major banks, where the bottom consists of sand or fine gravel (Scott 1982), and in the shallower areas of the Grand Banks, particularly in the northeastern and southeastern areas (Winters 1983). Previous assessments of Great Shearwater diets in the southwestern Gulf of Maine noted sand lance as a common food item (Powers & Backus 1987), while euphausiids *Meganyctiphanes norvegica*, herring, mackerel, and short-finned squid *Illex illecebrosus* dominate shearwater diets in the Bay of Fundy (Brown *et al.* 1981, Ronconi *et al.* 2010). Around Newfoundland, spawning aggregations of capelin *Mallotus villosus* and immature short-finned squid attract shearwaters (Brown *et al.* 1981, Davoren *et al.* 2012).

Sand lance distributions are patchy with considerable annual fluctuations in abundance, and larval forms are prey to both herring and mackerel (Staudinger *et al.* 2020). Populations increased 5- to 10-fold in the Gulf of Maine from 1976 to 1981, but abundance subsequently dropped between 1982 and 1987 (Nelson & Ross 1991). 2016 to 2018 were poor years for herring (and likely mackerel), which may have contributed to the high frequency of occurrence of sand lance found in shearwater fecal samples. Age-1 recruitment of herring in the northeastern US has been below average since 2013, and four of the six lowest annual recruitment estimates have occurred since that year (NEFSC 2018b). Since 2005, diets of Atlantic Puffins *Fratercula arctica* and Razorbills *Alca torda* in the Gulf of Maine switched from herring to sand lance due to the decline in herring (Scopel *et al.* 2019). Based on fisheries model results and sensitivity, the mackerel stock in the northeastern US was estimated to be at an all-time low in 2012, having experienced increasing exploitation (overfishing) through the early 2000s to a high in 2010 (NEFSC 2018a). We note that concurrent with obtaining some fecal samples, there was an unusual event of mackerel driving yearling menhaden into the shoreline at Race Point on Cape Cod, Massachusetts in August 2017. Menhaden were taken by shearwaters in both 2017 and 2018, but in 2017 we observed hundreds of Great Shearwaters feeding on beached menhaden along the shoreline. Menhaden nursery grounds in New England have become significant contributors of age-1 fish (Anstead *et al.* 2016). Since 2015, abundance of both adult and juvenile menhaden has increased in the Gulf of Maine (ASMFC 2017). Menhaden and mackerel were previously unknown in diets of Great Shearwaters in the northeastern US, so their importance as prey may be increasing

in this region. The availability of any suitable prey is important, but Great Shearwaters in the southwestern Gulf of Maine in 2013–2018 foraged most intensively over the same habitat used by sand lance.

Latitudinal differences in trophic levels of Great Shearwaters, as measured by stable isotopes (feathers and blood) between populations in Newfoundland, the Bay of Fundy, and southwestern Gulf of Maine, could be attributable to preferences in primary prey (Ronconi *et al.* 2010, Gulka *et al.* 2017, Hong *et al.* 2019). The dietary niche of shearwaters in coastal Newfoundland was found to narrow with increased availability of capelin, and shearwaters were uniquely qualified to forage over broader scales to continuously maintain contact with capelin patches (Gulka *et al.* 2017). Similarly, Great Shearwaters in the southwestern Gulf of Maine and northern Georges Bank showed selectivity for habitats with higher availability of preferred prey (sand lance).

In summary, a combination of PTT tracking and necropsy data indicate that the Gulf of Maine is a key wintering area for young (0–2 years) Great Shearwaters. The characteristics of identified foraging areas and DNA from fecal samples from birds indicate that shallow, sandy-bottomed marine habitat and sand lance are important aspects of their habitat use and survival. Since juveniles represent an important demographic in the viability of any species (Hazen *et al.* 2012), we suggest that management actions in the Gulf of Maine that would impact their preferred habitat and prey should consider Great Shearwaters in their decision-making.

ACKNOWLEDGEMENTS

We thank Peter Hong, Tammy Silva, Kent Hatch, Sara Williams, students from the Boston University Marine Program (especially Megan Feddern, Lindsay Smith, Montana Airey, and Lucy McGinnis), and the crew of NOAA R/V *Auk* for assistance in collecting shearwater data. Laboratory work was contributed by J. Biagini of the College of Arts and Sciences at the University of New England. We are grateful to Peter Ryan and Veronica Perold at the Fitzpatrick Institute of African Ornithology and Maëlle Connan from Nelson Mandela University for sample collection in the South Atlantic, collaboration, and necropsy assistance. We thank Justin Suca for his critical review of an earlier version of the manuscript. NOAA's Northeast Fisheries Science Center provided bottom fish trawl information. NOAA's National Centers for Coastal Ocean Science (NCCOS) provided surficial sediment datasets for the Northeast and Mid-Atlantic areas of the US continental shelf. Genetic analyses of blood and feather samples were done by Animal Genetics, Inc., 1336 Timberlane Rd., Tallahassee, FL 32312-1766, USA. All applicable international, national, and institutional guidelines for the care and use of animals were followed. Permits to capture, handle, and tag birds were obtained from the US Department of the Interior (permit #21963). Permits to handle birds were obtained from the Massachusetts Division of Fisheries and Wildlife (permit #202-17SCB).

Funding was provided by the Volgenau Foundation, Bureau of Ocean Energy Management, Stellwagen Bank National Marine Sanctuary, National Marine Sanctuary Foundation, Mudge Foundation, Boston University Marine Program, and Pew Charitable Trusts. The NOAA Nancy Foster Scholarship provided support to A. Robuck. Laboratory work was supported by a Summer Undergraduate Research Experience fellowship through the College of Arts and Sciences at the University of New

England. The comments of two anonymous reviewers greatly helped us to improve the clarity of this paper.

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The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the US Fish and Wildlife Service, NOAA, or the US Department of Commerce. This publication does not constitute an endorsement of any commercial product or intend to be an opinion beyond scientific or other results obtained by these agencies.

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Sensitivity of sand lance to shifting prey and hydrography indicates forthcoming change to the Northeast US shelf forage fish complex

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Abstract

Northern sand lance (*Ammodytes dubius*) and Atlantic herring (*Clupea harengus*) represent the dominant lipid-rich forage fish species throughout the Northeast US shelf and are critical prey for numerous top predators. However, unlike Atlantic herring, there is little research on sand lance or information about drivers of their abundance. We use intra-annual measurements of sand lance diet, growth, and condition to explain annual variability in sand lance abundance on the Northeast US Shelf. Our observations indicate that northern sand lance feed, grow, and accumulate lipids in the late winter through summer, predominantly consuming the copepod *Calanus finmarchicus*. Sand lance then cease feeding, utilize lipids, and begin gonad development in the fall. We show that the abundance of *Calanus finmarchicus* strongly influences sand lance parental condition and recruitment. Atlantic herring can mute this effect through intra-guild predation. Hydrography further impacts sand lance abundance as increases in warm slope water decrease overwinter survival of reproductive adults. The predicted changes to these drivers indicate that sand lance will no longer be able to fill the role of lipid-rich forage during times of low Atlantic herring abundance— changing the Northeast US shelf forage fish complex by the end of the century.

Keywords: forage fish, trophic ecology, recruitment, climate change, *Calanus*, changepoint

Introduction

1 Small pelagic fishes, or forage fishes, are critical components of both neritic and oceanic
2 food webs. They represent an important link between planktonic production and a diversity of
3 larger predators, including numerous endangered species. However, forage fish abundance and
4 recruitment are often sensitive to temperature and secondary production, leading to large
5 fluctuations in abundance, making this trophic link highly variable in space and time (Chavez *et*
6 *al.*, 2003; Clausen *et al.*, 2018; Thompson *et al.*, 2019; Sydeman *et al.*, 2020). Further, numerous
7 studies show that fluctuations in forage fish abundance can lead to marked effects on predator
8 condition and breeding success (Barrett *et al.*, 2012; Kadin *et al.*, 2012; Engelhard *et al.*, 2013;
9 Church *et al.*, 2019). Owing to the sensitivity of forage fish to climate regimes, and, in turn, the
10 breeding and condition of their predators, there is great interest in understanding climate–forage
11 fish relationships and using them to project forage fish abundances into the future (Fréon *et al.*,
12 2005; Alder *et al.*, 2008; Checkley *et al.*, 2017) .

13 Sand lances (family Ammodytidae) are one such group of quintessential forage fishes
14 throughout temperate and sub-polar shelf ecosystems of the Northern Hemisphere. This is
15 particularly true for northern sand lance (*Ammodytes dubius*) in the Northeast US Shelf
16 ecosystem (Figure 1a). Of 72 regional predators consuming sand lance, populations of humpback
17 whales (*Megaptera novaeangliae*) and numerous seabirds are particularly reliant on this resource
18 (Staudinger *et al.*, 2020). However, the lack of a directed sand lance fishery and, therefore,
19 regular assessments, has hampered our understanding of basic sand lance ecology that is needed
20 to predict the species' characteristically large interannual fluctuations in abundance (Figure 1b,c;
21 (Nelson and Ross, 1991; Richardson *et al.*, 2014; Staudinger *et al.*, 2020).

22 Evidence for other sand lances of the genus *Ammodytes* indicate they are particularly
23 sensitive to prey availability and temperature regimes at multiple life stages throughout their
24 range (Winslade, 1974; Tomiyama and Yanagibashi, 2004; van Deurs *et al.*, 2011, 2014, 2015;
25 Régnier *et al.*, 2017, 2018; Clausen *et al.*, 2018; Wright *et al.*, 2017; Murray *et al.*, 2019; Von
26 Biela *et al.*, 2019). Larval prey availability and adult exposure to elevated overwintering
27 temperatures are hypothesized to drive variability in catch of the species *A. marinus* (T. Régnier
28 *et al.*, 2017; Wright *et al.*, 2017). Further, significant reductions in size-at-age were observed for
29 *A. personatus* off Alaska during the warm and zooplankton-poor “blob” event, and reductions in
30 catch of *A. japonicus* were correlated with decreasing zooplankton abundance off Japan (Von
31 Biela *et al.*, 2019; Nishikawa *et al.*, 2020). These relationships indicate that recruitment and
32 condition of sand lances are linked to prey availability, although existing studies have not
33 explicitly connected adult prey availability and condition to recruitment. Despite numerous
34 studies on congeners, little to no information currently exists on the drivers of northern sand
35 lance abundance throughout its range.

36 In addition to most forage fish species individually exhibiting population fluctuations,
37 they often oscillate out-of-phase with one another, leading to one species replacing the role of the
38 other when environmental conditions change (Bakun and Broad, 2003; Möllmann *et al.*, 2008;
39 Alheit *et al.*, 2014). The Northeast US Shelf forage fish complex has historically exhibited an
40 oscillation between dominance of northern sand lance and Atlantic herring (*Clupea harengus*;
41 Sherman *et al.*, 1981; Richardson *et al.*, 2014). The causes of this oscillation remain unknown,
42 especially regarding sand lance. Intra-guild predation, zooplankton prey availability, and habitat
43 suitability may be contributing factors (Sherman *et al.*, 1981; Fogarty *et al.*, 1991; Richardson *et*
44 *al.*, 2014). Intra-guild predation, when a species not only competes with (e.g. for food) but also

45 consumes another species, can lead to an overwhelming competitive advantage in food webs,
46 driving oscillatory patterns of competitor abundance (Polis *et al.*, 1989). Zooplankton regimes in
47 the region also fluctuate on seasonal to decadal scales, resulting in temporally inconsistent prey
48 availability for small pelagic fishes (Kane, 2007; Morse *et al.*, 2017; Perretti *et al.*, 2017).

49 Beyond natural variability in zooplankton regimes, the Northeast US shelf has
50 experienced dramatic warming of both surface and bottom waters throughout the late 20th and
51 early 21st century. Warming has been linked to changes in heat flux from atmospheric forcing
52 and changes in Gulf Stream dynamics that alter advective processes in the region (Greene and
53 Pershing, 2007; Pershing *et al.*, 2015; Kavanaugh *et al.*, 2017; Gangopadhyay *et al.*, 2019; Chen
54 *et al.*, 2020). This recent warming has negatively impacted temperate fish species occupying the
55 shelf, driving both implied direct mortality and range shifts of numerous fishes (Pershing *et al.*,
56 2015; Henderson *et al.*, 2017; Kleisner *et al.*, 2017).

57 Here, we combine stomach content, lipid content, and growth analyses of sand lance to
58 better understand their ecology and phenology, and then use these results to inform a mechanistic
59 approach to understanding the drivers of sand lance population fluctuations across the Northeast
60 US shelf. We hypothesize that northern sand lance abundance on the Northeast US shelf is
61 controlled by a combination of adult prey availability, intra-guild predation on larvae, and winter
62 hydrographic conditions. We then predict sand lance abundance throughout the 21st century on
63 the Northeast US shelf using projections of the primary drivers of sand lance abundance that we
64 identified: *Calanus finmarchicus* abundance, Atlantic herring abundance, and warm slope water
65 proportions.

66 **Materials and Methods**

67 *Seasonal feeding and reproductive ecology of sand lance*

68 *Sand lance collections*

69 Adult sand lance (age 1+) were collected from Stellwagen Bank with a 1.0×0.5 m,
70 small-mesh (0.63 cm) beam trawl (Figure 1a). Stellwagen Bank, a sandy bank in Massachusetts
71 Bay within the Gulf of Maine, is a top predator hotspot and representative of typical sand lance
72 habitat throughout the Northeast US shelf (Hazen *et al.*, 2009; Richardson *et al.*, 2014). Thus,
73 our insights from Stellwagen Bank collections were used to better understand the ecology and
74 predict environmental drivers of this species across their range.

75 Collections occurred monthly in 2019 (except January, July, and December) and
76 opportunistically on cruises from 2016 through 2018 (Table S1). Trawl duration was 10 minutes
77 and distances ranged from 0.5–1 km. Collected sand lance in 2019 were flash frozen in foil in
78 liquid nitrogen upon retrieval. Sand lance collected prior to 2019 were placed in a -20°C freezer
79 and then stored in ice for transportation. All specimens were collected in accordance with the
80 guidelines and approval of Woods Hole Oceanographic Institution’s Institutional Animal Care
81 and Use Committee (approval #23453).

82 Stellwagen Bank can be split into three larger “regions”, south, central, and north (Figure
83 1a). Collections aimed to cover both the northern and southern regions of Stellwagen Bank
84 where sand lance density is historically high, except for February when collections solely
85 occurred in the southern and central regions of the bank due to limited ship time.

86 *Zooplankton collections*

87 Simultaneous CTD and zooplankton tows were performed at each station where sand
88 lance were captured. A SeaBird 19plus was lowered to 1 m above the bottom, coupled with a 30-
89 cm diameter, 150- μ m mesh zooplankton net that sampled vertically on the upcast. This resulted

90 in a cast from 3 m above the bottom to the surface, allowing for the calculation of water volume
91 sampled. Zooplankton samples were immediately preserved in 95% ethanol upon retrieval.

92 Prior to processing, zooplankton samples were reduced to approximately 500 organisms
93 with a modified box splitter. Zooplankton were sorted, counted, and identified to the lowest
94 possible taxa at the Polish Plankton Sorting and Identification Centre and in the lab at Woods
95 Hole Oceanographic Institution following Kane (2007).

96 *Sand lance dissections*

97 Adult sand lance were thawed to near room temperature in the laboratory for dissections.
98 Standard length, fork length, whole weight, eviscerated weight, liver weight, stomach content
99 weight, and gonad weight were recorded for each fish. Stomach contents were preserved in 95%
100 ethanol after weighing. Heads of each fish were removed for otolith extraction and ageing by
101 making a vertical cut immediately posterior to the opercula. The remaining portion of each fish
102 (stomach contents and head removed) was weighed and freeze dried for subsequent lipid
103 extraction (see lipid extractions). Gonadosomatic index was calculated as the proportion of
104 gonad weight compared to somatic weight.

105 *Stomach content identification*

106 Stomach contents of each fish that contained prey were examined to identify the specific
107 zooplankton prey of sand lance and how consumption varies seasonally. Details of stomach
108 content identification methods can be found in the supplementary information. Feeding incidence
109 was defined as the proportion of stomachs inspected on each sampling date that contained prey
110 items. An overall proportion of prey taxa by number and biomass was also calculated for each
111 month of collection.

112 For pre-2019 samples, feeding incidence was assessed as the presence or absence of prey
 113 in the stomach under a microscope after dissection. Samples collected before 2019 were not flash
 114 frozen, and thus prey items were often degraded and thus may have biased results toward items
 115 that were more robust to digestion. We therefore did not calculate diet by biomass or number for
 116 pre-2019 collected sand lance.

117 *Age and growth*

118 A subset of fish from March, April, June, August and November of 2019 were
 119 haphazardly selected for otolith-based ageing. Full details of ageing analysis can be found in the
 120 supplementary information. For abundant age classes, a von-Bertalanffy growth curve was fit to
 121 standard length (SL) by month (m) for each age class with sufficient number of individuals (n
 122 >30) following the equation:

$$123 \quad SL_m = SL_{\infty}(1 - e^{-k(m-m_0)})$$

124 where k is the growth coefficient. Von-Bertalanffy growth curves were fit primarily to estimate
 125 the parameter SL_{∞} in order to determine which month standard length of individuals in an age
 126 class approached their annual maximum when somatic growth ceases. The equation thus follows
 127 the standard von-Bertalanffy growth equation except for using month as the time scale. Von-
 128 Bertalanffy curve fitting was performed using the vB function in the *FSA* package in R (Derek
 129 and Wheeler, 2020).

130 *Lipid extraction and content analysis*

131 Lipid extractions were performed for opportunistically collected sand lance prior to 2019
 132 following the exact modified Folch method presented in Post & Parkinson (2001). For 2019
 133 collected sand lance, up to 25 fish per month of collection were processed for lipid extraction

134 following a further modified method to minimize lipid oxidation. Details of this method are
135 described in the supplementary material.

136 Percent lipid values were then fit with a cyclic penalized cubic regression spline with day
137 of collection as the predictor and region of collection on Stellwagen Bank (north, central, south)
138 as a factor via the *mgcv* package in R to determine the effect of day of year on sand lance lipid
139 content while accounting for regional variability (Wood, 2017). Region of collection within
140 Stellwagen Bank (north, central, south) was included due to observed differences in zooplankton
141 and hydrography by region (Figure S1, S2). The model was written as follows:

$$142 \quad \text{Percent Lipid} = \beta_0 + s(DOY) + \text{Region}$$

143 Where percent lipid represents percent lipid of whole sand lance, $s(DOY)$ represents the fitted
144 smoothing function for day of year (DOY), and region is fit as a categorical predictor. We report
145 change in deviance explained (ΔDE) from the full model for each variable as an estimate of their
146 relative importance.

147 Eighteen individuals were selected for duplicates, encompassing two individuals from
148 each month of collection, to determine intra-fish variability in lipid content estimates. Duplicates
149 indicated $< 3\%$ intra-fish difference.

150 *Shelf-wide estimates and drivers of sand lance abundance*

151 *Northeast US shelf sand lance abundance*

152 Estimations of Northeast US shelf sand lance abundance came from the spring Northeast
153 Fisheries Science Center (NEFSC) bottom trawl survey, spanning from 1968 to present. During
154 each survey, 300-400 stations are sampled in a random stratified design, ranging from the
155 Western Scotian Shelf to Cape Hatteras (Figure 1b,c). At each station, all sand lance are weighed
156 and individuals are measured, with sub-sampling occurring when catches were large. Further

157 details of the survey and sampling gear are found within Politis *et al.* (2014). The log area
158 weighted stratified mean abundance in catch of each survey was calculated to estimate
159 interannual variability in sand lance abundance (Richardson *et al.*, 2014). The spring survey
160 typically occurs in March and April, well within the feeding window of sand lance (see Results),
161 and has largely been consistent in timing, gear, and vessel—allowing the survey to provide a
162 consistent index of northern sand lance abundance (Politis *et al.* 2014). This is opposed to the fall
163 survey which occurs at a transitional window in sand lance life history from feeding to preparing
164 for fall reproduction, leading to possible conflation of abundance trends with inter-annual
165 variability in behavior (see Results; Murray *et al.* 2019). The survey is also not explicitly
166 designed to sample sand lance habitat and thus there are rarely multiple trawls within a stratum
167 that represent equivalent sand lance habitat quality. We therefore focused on the log area
168 weighted stratified mean abundance for the entire survey region, assuming the broad spatial
169 range and large sample size mitigated the likelihood of spurious or small-scale behavior related
170 biases in abundances estimates. Sand lance abundance exhibits major fluctuations, ranging
171 orders of magnitude, allowing the survey to represent sand lance abundance trends despite being
172 imprecise.

173 In 2009, the survey changed vessels and gear, notably switching the net liner from 1.27 to
174 2.54 cm, leading to a noticeable decrease in sand lance catchability during the calibration study
175 (Miller *et al.*, 2010). The time series of sand lance abundance thus ends in 2008 to avoid
176 conflation of gear selectivity with abundance trends. We do not have age estimates for each year
177 of the survey, and thus all age classes of sand lance are included in the log area-weighted
178 stratified mean abundance. The most comprehensive ageing of sand lance from the NEFSC
179 spring trawl survey suggests >90% of individuals collected in the survey are age-2 or age-3

10

180 (Nelson and Ross, 1991). Due to this finding, we assumed that abundance of age-2 and age-3
181 individuals were responsible for the changes in overall sand lance abundance in the survey.
182 While the survey provides a robust estimate of the abundance of age-2 and age-3 sand lance
183 throughout the Northeast US Shelf, overall abundance of sand lance is likely underestimated due
184 to low selectivity of age-1 individuals (Nelson and Ross, 1991).

185 *Historical EcoMon zooplankton data*

186 *Calanus* abundance anomalies were calculated following Kane (2007) and Perretti *et al.*,
187 (2017) from collections of the NEFSC EcoMon sampling program spanning 1977 to present.
188 Briefly, a mean abundance by date is computed by area for *Calanus finmarchicus*. This is
189 accomplished by binning all samples for a given species to bi-monthly collection dates based on
190 median cruise date, taking the mean abundance, then fitting a spline interpolation between mean
191 bi-monthly abundance to give expected abundance on any given day of the year. Data were
192 spatially constrained to the Gulf of Maine ecological production unit (Lucey and Fogarty, 2013).
193 All years (1977–2008) were used because annual sampling effort included at least 35% spatial
194 coverage of the Gulf of Maine ecological production unit (Morse *et al.*, 2017). The Gulf of
195 Maine was chosen as opposed to the full Northeast US shelf as this region serves as the source of
196 *Calanus finmarchicus* for much of the Northeast US shelf. The Gulf of Maine has deep basins
197 that provide habitat for diapausing *Calanus* and the general southward flow of the region advects
198 *Calanus* to the other regions of the Northeast US shelf (Kane, 2005, 2007; Runge *et al.*, 2015; Ji
199 *et al.*, 2017).

200 Zooplankton data were constrained to cruises centering around bi-monthly dates from
201 January to July to correspond with our observed feeding patterns for sand lance. Winter-summer
202 *Calanus* abundance anomalies were then lagged to approximate an average Gulf of Maine

203 *Calanus* abundance at 3–4 years prior to the spring bottom trawl survey estimate of sand lance
204 abundance. A 3–4 year lag was selected as this represents the years in which the parents of fish
205 collected in the survey (age-2s and 3s) would be feeding on *Calanus* prior to spawning in the
206 fall. The specific selection of this time lag was based upon our observations of sand lance
207 feeding, growth, and lipid content being tightly coupled with their feeding on *Calanus*. The time
208 lag used therefore represents a test of the parental condition hypothesis, whereby higher
209 abundances of *Calanus* available to parents would result in either more or higher quality eggs,
210 and thus higher abundances of sand lance in the survey three and four years later when they
211 become catchable in the survey as age-2 and age-3 individuals.

212 *Atlantic herring index*

213 Atlantic herring are intra-guild predators of northern sand lance, meaning that adults of
214 each species are competitors for prey but Atlantic herring also consume the larvae and juveniles
215 of northern sand lance (Suca *et al.*, 2018). Hence, Atlantic herring may exert a strong top-down
216 influence on northern sand lance abundance by consuming the early life stages of sand lance
217 (Polis *et al.*, 1989).

218 Similar to sand lance, Atlantic herring catchability in the NEFSC bottom trawl survey
219 changed during the time series. Catchability of Atlantic herring substantially increased on the
220 survey in the mid-1980s, possibly due to a change in trawl doors (which has been consistently
221 noted; NEFSC, 2018; Miller *et al.*, 2010). Richardson *et al.* (2014) thus developed a composite
222 index of Atlantic herring abundance, from 1968 through 2010, combining numerous state and
223 federal fisheries independent surveys of Atlantic herring using a non-linear least-square
224 optimization procedure. This composite index represents an estimation of the overall Atlantic
225 herring abundance on the Northeast US Shelf. It is worth noting that Atlantic mackerel likely

226 also exert a top-down effect on sand lance via intra-guild predation but their transitory use of the
227 region and tendency to avoid gear used in standardized surveys precluded effective use of
228 Atlantic mackerel abundance as a predictor (Mbaye *et al.*, 2020).

229 The composite Atlantic herring index was lagged to represent Atlantic herring abundance
230 2–3 years prior to the bottom trawl survey estimate of sand lance. This lag indicates the
231 abundance of Atlantic herring on the Northeast US shelf when survey-collected sand lance were
232 larvae and early juveniles and thus susceptible to predation by Atlantic herring.

233 *Slope water proportions*

234 We then further explored hydrographic drivers of variability in sand lance abundance on
235 the Northeast US Shelf in conjunction with trophic (prey availability and predation) effects. We
236 tested the hypothesis that increasing warm slope water proportion would have a direct
237 detrimental effect on adult northern sand lance abundance on the Northeast US shelf. Slope
238 waters enter the Gulf of Maine through the Northeast Channel (Figure 1) and are composed
239 largely of two sources, colder and fresher Arctic-influenced Labrador slope water (LSW) and
240 warmer, saltier Slope Sea and Gulf Stream-influenced “warm” slope waters (WSW) of which the
241 relative proportions of each source vary interannually (Mountain, 2012). These slope waters then
242 mix and circulate around the Gulf of Maine, becoming a substantial portion of the shelf water of
243 the Northeast US shelf (Mountain, 2012), the primary habitat of northern sand lance on the
244 Northeast US shelf. These warm slope waters have been shown to have deleterious effects to
245 wintertime populations of *Calanus*, the primary prey of sand lance (Record *et al.*, 2019). This
246 deleterious effect of warm slope water on *Calanus* does not appear to carry through the spring,
247 with local production decoupling warm slope water proportions and integrated annual *Calanus*
248 abundance (Record *et al.*, 2019). Thus the mechanism behind warm slope water’s effect on sand

249 lance abundance could manifest in two ways: indirectly through decreasing prey availability to
250 spawners—thus lowering parental condition and spawning output—or through a direct negative
251 effect on over-winter survival of adult sand lance via elevated bottom temperatures. Our
252 observations suggest that the latter scenario is more likely as biological sampling showed that
253 reproductive sand lance have their lowest energy reserves in winter, making mature individuals
254 (age-2+) susceptible to mortality from increased metabolism and lack of prey availability from
255 elevated warm slope water proportions. Thus, we hypothesized that increasing warm slope water
256 proportions would have a deleterious effect at a 0-year lag, given the slope water conditions that
257 either favor age-2+ survival or mortality would occur in the winter prior to the spring trawl
258 survey (i.e. in the same year as the survey). We also note that warm slope water may influence
259 the catchability of sand lance in the survey due to its effect on bottom temperature. However,
260 schooling behavior of congeners have been shown to be primarily linked to feeding and food
261 availability (van Deurs *et al.* 2011), thus we believe this effect would be small relative the
262 changes in true abundance given the survey overlaps with peak feeding for sand lance (see
263 Results).

264 Slope water proportions were extracted from the *ecodata* package in R (Fratantoni, 2019;
265 Mountain, 2012). Slope water proportions were derived from bathythermograph data, CTD data,
266 and water samples collected from Niskin bottles from Northeast Fisheries Science Center
267 surveys from the 1977 to present between 150–200 m in the Northeast Channel (42.2–42.6° N,
268 66–66.8°W, Figure 1). Data collected from 150–200 m are assumed to be waters sourced from
269 three sources, Scotian Shelf water (2°C, 32 salinity) , Labrador slope water (6°C, 34.7 salinity),
270 and warm slope water from off the Northeast US Shelf (10°C, 35 salinity). The relative
271 proportion of each source was calculated via a 3-point mixing algorithm following Mountain,

14

(2012). These temperature and salinity end-member values of each water mass are assumed to be constant over time, though local heat fluxes and mixing likely alter these values. Despite these fluctuations, warm slope water proportion provides a reliable estimate of warmer waters entering the Gulf of Maine and provides an estimate of winter thermal conditions for sand lance over this period. While buoy data exist in the Northeast Channel from 2004–2018 (NERACOOS buoy N), annual estimates of slope water proportions were selected in order to extend the time series back to 1977, thus matching the length of available zooplankton data.

Sand lance abundance regression

Log sand lance abundance from NEFSC bottom trawl surveys were related to *Calanus* abundance, the composite Atlantic herring index, and warm slope water proportion using a multiple linear regression written as follows:

$$\log(SL_t) = \beta_0 + \beta_1 * Calanus_{t-3,4} + \beta_2 * Atlantic_herring_{t-2,3} + \beta_3 * WSW_t$$

Where SL_t represents stratified mean spring trawl survey abundance of sand lance in year t , *Calanus* represents winter-summer *Calanus* abundance anomaly at a 3–4 year lag, *Atlantic herring* represents the Atlantic herring index at a 2–3 year lag, and *WSW* represents warm slope water proportion in year t . Predictor variables were not strongly correlated ($r < 0.5$). We inspected model residuals for temporal autocorrelation (see Figure S11) and used generalized additive models to confirm linear relationships and use of linear regression. We also considered a model including a population dynamics term either in the form of a transformed response variable $\log(SL_t/S_{t-3})$ and an autoregressive term (SL_{t-3}) to allow the model to represent the current abundance relative to the parent population.

We performed a leave-one-out cross-validation to test the predictive capability of our regression within our dataset. This method iteratively trains the model using $n-1$ observations

295 and uses the model fitted to the training set to predict the left-out observation. The R^2 and root-
 296 mean-square-error for the predicted vs. observed values were calculated to determine model
 297 predictive performance.

298 *Change in slope water proportions with time*

299 A changepoint beta regression was fit to the time series of warm slope water to determine
 300 if a changepoint occurred in warm slope water proportion after 2000, concurrent with other
 301 changes in regional hydrography near this time (Andres, 2016; McCarthy *et al.*, 2018;
 302 Gangopadhyay *et al.*, 2019; Friedland *et al.*, 2020a). Beta distributions were used because warm
 303 slope water values are represented as proportions. The stepwise beta regression was written as
 304 follows:

$$\begin{aligned}
 305 \quad \log \left(\frac{WSW}{(1 - WSW)} \right) &= \beta_o + \beta_1 t + \beta_2 (t - \tau) I_\tau(t) \\
 306 \quad I_\tau(t) &= 0 \text{ if } t \leq \tau \\
 307 \quad &= 1 \text{ if } t > \tau
 \end{aligned}$$

308 where WSW represents the proportion of warm slope water, t is time in years, and τ is the
 309 changepoint. The parameters β_o , β_1 , β_2 , τ , and θ (the dispersion parameter) were fit via maximum
 310 log likelihood using the `fmincon` function in Matlab. Significance ($\alpha = 0.05$) was determined
 311 through parametric bootstrap (1000 simulations). The p-value was defined as the proportion of
 312 times the simulated difference in maximized log likelihood of the changepoint beta regression
 313 and the beta regression without a changepoint exceeded the difference between these regressions
 314 using our observed values, as described in the following equation:

$$\begin{aligned}
 315 \quad p &= \frac{\sum_{i=1}^{1000} X(i)}{1000} \\
 316 \quad X(i) &= 1 \text{ if } (LL(i)_{CP_{sim}} - LL(i)_{\beta_{sim}}) > (LL_{CP_{obs}} - LL_{\beta_{obs}})
 \end{aligned}$$

$$= 0 \text{ if } (LL(i)_{CP_{stim}} - LL(i)_{\beta_{stim}}) < (LL_{CP_{obs}} - LL_{\beta_{obs}})$$

Where LL represents the maximum log likelihood, CP represents the changepoint regression, and β represents the standard beta regression.

Projections of sand lance abundance

Log sand lance abundance was projected from 2020–2100 using multiple combinations of time-series predictor variables to represent several future scenarios. Eight scenarios were simulated, encompassed by four groupings (“current trajectory,” “optimistic,” “average herring,” “reduced warm slope water”; Table 1; Figure S3). Owing to observed declines in Atlantic herring biomass and recruitment (NEFSC, 2018), predicted declines in *Calanus* abundance (Grieve *et al.*, 2017), and our observation of increases in warm slope water proportion, we termed the predictors following this pattern as the “current trajectory” scenario. Long-term projections indicate suitable *Calanus* habitat throughout the Northeast US Shelf will decrease in the coming century, although there is regional variability in observations and projections of this trend (Reygondeau and Beaugrand, 2011; Grieve *et al.*, 2017; Ji *et al.*, 2017). Atlantic herring recruitment has been below average since 2013, with six of the eight worst recorded recruitment years occurring since 2013 (NEFSC, 2020). Herring spawning stock biomass is also below average and declining, despite stable to decreasing fishing mortality rates (NEFSC, 2020). Such declines, which are not easily attributable to fishing mortality, led to speculation that aspects of the rapidly changing Northeast US shelf ecosystem may be the cause of poor herring recruitment and result in continued declines in Atlantic herring (though egg predation by haddock may also be a cause; Richardson *et al.*, 2011). While it is impossible to know if Atlantic herring populations will eventually recover, it will likely at least take multiple years for the stock to recover from the historically poor recruitment levels seen since 2013.

340 Importantly, in each of the four groupings of projection scenarios, *Calanus* abundance
 341 was represented by a normal random variable with a linearly decreasing abundance of *Calanus*
 342 from the mean of the existing time series to 68% percent of its mean to represent the projected
 343 decrease in *Calanus* under Representative Concentration Pathway (RCP) 4.5 or to 50% of its
 344 mean, representing the projected decrease in *Calanus* abundance under RCP 8.5 (variance was
 345 assumed to remain constant in time; rates of decline follow Grieve *et al.*, 2017). Each predictor
 346 was simulated independently owing to the lack of strong correlation among predictor variables.
 347 Sand lance abundance was then estimated using our fitted multiple linear regression and these
 348 simulated regressors. This was then repeated 1000 times to get a mean simulated log sand lance
 349 abundance, along with interquartile range, for each year from 2020–2100.

350 *Projection metrics*

351 For each scenario, we calculated the percent decline in sand lance abundance from the
 352 beginning of the simulation through the following equation:

$$353 \quad \text{Percent Decline} = \left(\frac{\overline{SL}_x - \overline{SL}_{2020}}{\overline{SL}_{2020}} \right) * 100$$

354 Where $\overline{SL}_x$ represents the average projected sand lance abundance in year x .

355 We also calculated the proportion of years in each simulation of each scenario that had
 356 below average sand lance abundance and proportion of years that had below average abundances
 357 of both sand lance and Atlantic herring in the same year. This latter metric is perhaps most
 358 informative as this represents an ecosystem with below average abundance of both dominant
 359 lipid-rich forage fishes on the Northeast US shelf.

360 **Results**

361 *Sand lance feeding, lipid accumulation, and growth*

362 Feeding incidence (the percentage of stomachs with prey) varied monthly for sand lance
363 collected in 2019, with highest values occurring in the winter and spring (52–84%; $n = 212$;
364 Figure 2a). Feeding incidence decreased to zero in August and remained at zero through October,
365 followed by a reappearance of prey in the guts in November—consistent with patterns of
366 monthly feeding incidence from previous years (Figure 2a; Figure S4). During months of feeding
367 (other than November), the copepod *Calanus finmarchicus* (*Calanus* hereafter) dominated
368 stomach contents, ranging from 30.4% of diet by biomass in February to 78% of the diet by
369 biomass in April (Figure 2b).

370 Whole body lipid content of sand lance varied significantly by day of year and by region
371 within Stellwagen Bank, peaking throughout the bank in August ($n = 197$; deviance explained =
372 35.2 %; Figure 2c; Figure S5). Notably, lipid content decreased rapidly between October and
373 November in connection with spawning during this time window as evidenced by the sharp
374 increase and subsequent decrease in gonadosomatic index (day of year $\Delta DE = 32.5\%$, Figure 2e).
375 Additionally, lipid content varied spatially within the bank, with highest lipid content values
376 occurring on the northern portion of the bank in each month (region $\Delta DE = 3.0\%$).

377 Intra-annual size-at-age for both age-2 ($n = 54$) and age-3 ($n = 45$) fish approached their
378 annual maximum in the summer, concurrent with the cessation of feeding and lipid accumulation
379 (Figure 2d). Age-2 fish reached their annual maximum length in June (mean SL = 168.3 mm,
380 median SL = 171.0 mm, $L_{\infty} = 170.8$ mm) and age-3 fish reached their annual maximum length by
381 August (mean SL = 184.3 mm, median SL = 182.5 mm, $L_{\infty} = 184.0$ mm).

382 *Sand lance abundance regression*

383 Sand lance abundance and lagged winter-summer *Calanus* abundance anomalies were
384 significantly correlated throughout the time series ($R^2 = 0.21$, $p = 0.015$), and particularly well in
385 the 1980s and 1990s ($R^2 = 0.46$, $p < 0.01$; Figure 3a). However, the relationship decouples later
386 in the time series, concurrent with an increase in Atlantic herring in the region (Figure 3a).
387 Lagged Atlantic herring index and sand lance abundance were negatively correlated ($R^2 = 0.28$, p
388 < 0.01) throughout the time series, with Atlantic herring in low abundance during the 1980s
389 “boom” of sand lance and in high abundance when the sand lance time series began to decouple
390 from lagged *Calanus* abundances in the late 1990s and early 2000s (Figure 3a).

391 Warm slope water proportion was negatively correlated to log sand lance abundance (R^2
392 $= 0.52$, $p < 0.01$; Figure 3b). A cross-correlation analysis indicated that only a 0-year lag
393 between warm slope water proportion and sand lance abundance was significant (adjusted for
394 autocorrelation; Figure S6). Incorporating 3–4 year lagged *Calanus* abundance, 2–3 year lagged
395 Atlantic herring index, and unlagged warm slope water proportion in a multiple linear regression
396 explains a majority of the interannual variance in the northern sand lance spring trawl survey,
397 indicating these three mechanisms are likely the dominant drivers of northern sand lance
398 throughout the Northeast US shelf ($R^2 = 0.76$; Figure 3; Table 2). Inclusion of population
399 dynamics terms either as a transformed response variable or as an autoregressive term did not
400 improve model fit and thus were not included.

401 Leave-one-out cross-validation indicated high predictive performance within the dataset
402 ($R^2=0.68$, RMSE=1.07) and showed no notable outliers between the predicted and observed
403 values (Figure S7).

404 *Warm slope water changepoint*

405 The fitted changepoint beta regression indicated a significant changepoint in 2009
406 ($p < 0.001$), with the rate of warm slope water proportions increasing with time rising by over ten-
407 fold after 2009 ($\beta_1 = 0.01$; $\beta_2 = 0.26$; Figure 3b; Table 3). Slope water proportions showed no
408 significant mean change with time prior to this observed change point (i.e. from 1977–2008;
409 Figure 3b; Table 3).

410 *Projections of sand lance abundance*

411 Rates of sand lance decline were similar for each RCP projection in the four scenarios
412 (current trajectory, reduced warm slope water, average Atlantic herring, and optimistic). The
413 average decline in sand lance abundance was 45.6% and 74.6% by 2100 for RCP 4.5 and 8.5,
414 respectively (Figure 4). The minimum rate of decline for both RCP 4.5 was in the “reduced
415 warm slope water” scenario, with percent declines of 38.5% and in the “optimistic” scenarios for
416 RCP 8.5 at 71.4% . The maximum percent decline for both RCP 4.5 was in the “average Atlantic
417 herring” scenario at 52.0% and in the “current trajectory” scenario at 76.9%.

418 Scenarios varied drastically in both the proportion of years with below average sand
419 lance abundance and proportion of years with below average sand lance and Atlantic herring
420 abundance (Figure 4, Table 4). The “reduced warm slope water” scenario had the lowest average
421 proportion of years with below average sand lance abundance, with 34% and 47% of years
422 having below average sand lance abundance for RCP 4.5 and 8.5. The “average Atlantic herring”
423 scenario resulted in the highest number of years with below average sand lance abundance,
424 averaging 97% and 98% of years for RCP 4.5 and 8.5, respectively.

425 When Atlantic herring are included in the calculation of below average years of
426 abundance, the “current trajectory” scenario represents the maximum amount of below average
427 years, while the “optimistic scenario” results in the lowest number of these years (Figure 5). An

428 average of 20% of years in RCP 4.5 and 26% of years in RCP 8.5 of the “optimistic” scenario
429 had both negative sand lance and negative Atlantic herring anomalies. However, an average of
430 94% of years in RCP 4.5 and 96% of years in RCP 8.5 of the “current trajectory” scenario had
431 both negative sand lance and negative Atlantic herring anomalies indicating nearly all years
432 having below average sand lance and Atlantic herring in the future.

433 **Discussion**

434 Our work indicates that the future of adult northern sand lance in the Northeast US shelf
435 ecosystem is tenuous, owing to the observed and predicted changes in hydrography and prey
436 availability across the region. The sensitivity of sand lance to these changing factors are directly
437 linked to their capital breeding strategy, whereby the species has a defined feeding and growth
438 period, and individual lipid reserves undergo a two-fold change within one breeding season to
439 allocate energy for reproduction. Further, if ongoing declines in Atlantic herring recruitment and
440 biomass persist for decades to come (NEFSC, 2018), the Northeast US shelf will likely be in a
441 state of low adult abundances of its two most dominant lipid-rich forage fishes for much of the
442 21st century.

443 Projections of long-term declines in sand lance abundance indicate marked change in the
444 forage fish complex of one of the world’s most economically valuable shelf ecosystems
445 (Hoagland *et al.*, 2005) as concurrent negative anomalies of northern sand lance and Atlantic
446 herring have only occurred in six years from 1968-2008. Such a shift may open niche space for
447 more warm, temperate small pelagic species such as Atlantic butterfish (*Peprilus triacanthus*)
448 and Atlantic menhaden (*Brevoortia tyrannus*), diversifying the forage fish complex (Friedland *et*
449 *al.*, 2020b). This will likely alter the food web of the Northeast US shelf and represent a
450 northward contraction of the region’s “lipidscape” (Record *et al.*, 2018). The timing and

451 implications of a shift in forage fish community warrants continued, targeted estimates of forage
452 fish abundances and concurrent measurements of the condition of top predator taxa throughout
453 the Northeast US shelf. Tropicalization and shifts in forage community are likely to be
454 detrimental to lipid-rich piscivores, such as humpback whales and bluefin tuna (*Thunnus*
455 *thynnus*), while simultaneously ushering in piscivores commonly seen at lower latitudes such as
456 black sea bass (*Centropristis striata*) and summer flounder (*Paralichthys dentatus*) (Henderson
457 *et al.*, 2017; Kleisner *et al.*, 2017). This shift in community composition, from forage fish to top
458 predators, would change much of the food web and fisheries of this region.

459 Declines in *Ammodytes* condition and catch have been documented in the Pacific,
460 suggesting this key type of forage fish may be in global jeopardy due to shifting climate
461 (Nishikawa *et al.*, 2020; Von Biela *et al.*, 2019). Suggestions of sensitivity of European
462 congeners to temperature further this concern (Lindegren *et al.*, 2018). Many protected species
463 on the Northeast US shelf, such as humpback whales and seabirds, consistently target sand lance
464 as their primary forage and may be in jeopardy from declining sand lance regardless of Atlantic
465 herring's abundance (Staudinger *et al.*, 2020). Populations of seabirds in the Northeast Atlantic
466 suffer high chick mortality when local sand eel (*A. marinus*) abundances decline (Harris and
467 Wanless, 1991; Rindorf *et al.*, 2000). Recent reports indicate that declines in local sand lance
468 populations even decrease adult survival of Arctic terns (*Sterna paradisaea*) in Iceland (Peterson
469 *et al.*, 2020). We may thus expect similar levels of repercussions to sand lance-reliant top
470 predators on the Northeast US shelf within this century with recent data suggesting that declines
471 in productivity of the endangered roseate tern (*Sterna dougallii*) in the Northeast US due to
472 decreasing sand lance are already underway (J. Walsh, pers. comm.).

473 The lines of concurrent evidence of a capital breeding strategy for northern sand lance are
474 consistent with observations of congeners around both the Pacific and Northeast Atlantic
475 (Robards *et al.*, 1999; Wright *et al.*, 2017; Von Biela *et al.*, 2019). However, one major
476 difference is timing of the dormancy period. *Ammodytes* in the Northeast Atlantic are largely
477 considered to be dormant in the winter, along with *Ammodytes dubius* populations in Greenland
478 (van Deurs *et al.*, 2011; Danielsen *et al.*, 2016; Wright *et al.*, 2017). Our observations of late
479 summer dormancy indicate aestivation—a pattern observed in the warmer water *Ammodytes*
480 *japonicus*—as opposed to hibernation observed in Northeast Atlantic congeners (Kuzuhara *et al.*,
481 2019). Robards *et al.* (1999) also suggest that *Ammodytes personatus* in the Pacific undergo
482 winter dormancy, though they did not analyze stomach contents and their observations of
483 seasonal lipid accumulation match the pattern we observe. It is therefore possible *Ammodytes*
484 *personatus* also undergoes aestivation and feeds in the winter, contrary to current theory.

485 Our work further supports the importance of prey availability, namely lipid-rich
486 zooplankton, to the life history of *Ammodytes*. Studies throughout the Pacific have indicated sand
487 lance growth is linked to food availability (Kuzuhara *et al.*, 2019; Von Biela *et al.*, 2019) and,
488 further, that recruitment follows changes in zooplankton abundance on decadal scales
489 (Nishikawa *et al.*, 2020). We observed that the seasonal feeding pattern of *Ammodytes dubius*
490 was consistent with times of historically high *Calanus* abundance in the Northeast US Shelf
491 ecosystem where peaks in abundance typically occur from April through June, after which
492 *Calanus* enter diapause in the late summer (Kane, 2007; Figure S8,9). This suggests the timing
493 of sand lance feeding and subsequent reproduction may be linked to the phenology of *Calanus*.
494 Additionally, the overlap of lipid accumulation and somatic growth with feeding, and the
495 subsequent cessation of feeding, growth, and lipid accumulation during gonad development,

496 indicate that northern sand lance condition and reproduction may be sensitive to bottom-up
497 forcing of prey availability. Indeed, even spatial differences in *Calanus* availability appear to
498 drive differences in lipid accumulation in northern sand lance (Figure 2c). Elevated lipid content
499 on the northern portion of Stellwagen bank corresponds with localized increases in seasonal
500 *Calanus* abundance brought by the western Gulf of Maine Coastal Current that intercepts this
501 region (Figure S1, S2). Such a link between sand lance condition and *Calanus* abundance
502 indicates that projected decreases in *Calanus* on the Northeast US shelf will likely lead to a
503 decrease in availability of high-quality prey for sand lance, and thus may decrease parental
504 condition and spawning output (Grieve *et al.*, 2017).

505 While consistent with other studies in indicating the importance of *Calanus* to
506 *Ammodytes*, the mechanism suggested from our data differs from larval match-mismatch
507 hypotheses proposed to drive *Ammodytes* recruitment in the Northeast Atlantic (T. Régnier *et al.*,
508 2017) in that our data suggest *Calanus* abundance is linked to adult condition and growth. While
509 the three year lag of sand lance behind *Calanus* abundance may represent both an effect of
510 *Calanus* on parental condition of age-2 fish and larval feeding for age-3 fish, the strongest time-
511 lag correlations between sand lance abundance and *Calanus* anomalies occur at a 3–4 year lag as
512 opposed to a 2–3 year lag, indicating the effect of parental condition is, at the very least, more
513 pronounced than an effect of *Calanus* abundance on larval feeding (Figure S10). However, it is
514 important to note that this does not exclude the possibility that *Calanus* abundance plays a role in
515 the early life history of sand lance and further research is needed regarding this question. An
516 effect of parental condition on offspring abundance and/or survival has been both theorized and
517 observed in multiple systems (Green, 2008; Hare, 2014; Hixon *et al.*, 2014; Saenz-Agudelo *et*
518 *al.*, 2015), including for haddock on Georges Bank (Friedland *et al.*, 2008), and has even been

519 incorporated into population models for herring in Iceland (Oskarsson and Taggart, 2010). It is
520 noteworthy that this effect of *Calanus* on sand lance recruitment appears to be largely
521 independent of the sand lance spawning stock as population dynamics terms did not improve
522 model fit. This may be due to parental condition overwhelming an effect of abundance and/or the
523 per capita benefit of increased *Calanus* on parental condition being reduced in years and areas
524 with high densities of sand lance. Both scenarios could lead to present populations of sand lance
525 being largely independent of the size of their parents' population, as observed here. We are also
526 presently unsure if increased *Calanus* abundance only plays a role in lipid accumulation, or a
527 role in both lipid accumulation and growth. Future studies are needed to assess changes in
528 length-at-age and lipid accumulation for sand lance with changing zooplankton abundance and
529 regimes.

530 In addition to the capital breeding strategy driving recruitment through parental
531 condition, the large intra-annual change in lipid content for sand lance makes them susceptible to
532 overwinter conditions. Individuals are at their lowest energy reserves after spawning and are thus
533 sensitive to increased temperatures and shortages of prey. Such sensitivity to winter conditions
534 has been shown in *Ammodytes marinus*, indicating this scenario is not unique to northern sand
535 lance (van Deurs *et al.*, 2011). This sensitivity becomes problematic for sand lance populations
536 with recent shifts in hydrography, particularly the changepoint in warm slope water proportion
537 entering the Northeast Channel (Figure 3). This changepoint in hydrography will likely lead to
538 northern sand lance enduring winter regimes with consistently high temperatures and low prey
539 (Record *et al.*, 2019).

540 The changepoint in warm slope water proportion is noteworthy for the Northeast US
541 shelf ecosystem. Other changepoints have been identified in advective processes in the Northeast

542 US shelf region since 2000, notably a near doubling in the number of warm core rings shed from
543 the Gulf Stream, a westward shift of the Gulf Stream destabilization point, and a rapid shift to an
544 earlier spring transition date throughout the Northeast US shelf after 2008 (Friedland *et al.*, 2015;
545 Andres, 2016; Gangopadhyay *et al.*, 2019). The cause of this changepoint is still undetermined,
546 but hypotheses include a slowing of the Gulf Stream and warming of Slope Sea waters on the
547 coastal side of the Gulf Stream (Andres, 2016; Gangopadhyay *et al.*, 2019; McCarthy *et al.*,
548 2018). While a change in survey gear precludes consistent estimates of northern sand lance
549 abundance after 2008, this rapid increase in warm slope water proportion following 2009 is
550 likely to be detrimental to sand lance populations throughout the Northeast US shelf via
551 increasing overwinter mortality of this species.

552 Our work also indicates that sand lance populations may be heavily regulated by intra-
553 guild predation from Atlantic herring. This mechanism has been proposed before (Fogarty *et al.*,
554 1991; Polis *et al.*, 1989), and subsequent diet studies indicate larval sand lance are important
555 prey for Atlantic herring and Atlantic mackerel (though the overall abundance of the former is
556 much greater; Suca *et al.*, 2018). These dynamics largely allow either Atlantic herring or
557 northern sand lance to be the dominant forage fish throughout the Northeast US shelf in any
558 given year. However, when other drivers prevent a species from rebounding when their intra-
559 guild predator is low, concurrent low abundances of both species can occur. While this has not
560 happened often in the case of the Northeast US shelf, our projections indicate this situation of
561 low northern sand lance and Atlantic herring abundances will happen frequently in the coming
562 decades, and thus the ecosystem level implications of this change require monitoring.

563 Perhaps the most alarming result of our projections is that the “current trajectory”
564 scenario—based on current trends and knowledge regarding the future of *Calanus*, herring, and

565 warm slope water— indicates the worst outcome for the future of forage fish complex on the
566 Northeast US shelf. These projections were also robust to the rate of decline of *Calanus*, with
567 both RCP 4.5 and 8.5 rates of decline resulting in greater than 90% of years between 2020-2100
568 having below average abundance of both sand lance and Atlantic herring. Given concurrent
569 changepoints in warming rates and advection throughout the region (Andres, 2016;
570 Gangopadhyay *et al.*, 2019; Friedland *et al.*, 2020a), and *Calanus*' sensitivity to changing
571 temperature and salinity (Grieve *et al.*, 2017), the least certain aspect of these projections is the
572 future of Atlantic herring. However, even if Atlantic herring are to return to oscillating around
573 their historic mean, it would take a drastic decline in warm slope water proportions to allow sand
574 lance and Atlantic herring abundances on the Northeast US shelf to return to similar levels
575 observed prior to 2008. Even this “optimistic” scenario results in a higher proportion of years
576 with negative anomalies of sand lance and Atlantic herring than observed from 1968-2008,
577 showing the projections of change to the forage fish complex on the Northeast US shelf—most
578 notably a clear decline in sand lance—are consistent among all projections. However, the true
579 disappearance of northern sand lance may take longer than the NEFSC trawl survey indicates.
580 The deleterious effect of warm slope water occurs for post-spawning adults (age-2+) and thus it
581 is likely age-1 sand lance will be able to withstand this change in hydrography. These younger
582 fish may not be sufficient for many predators though, as the ongoing decline in roseate tern
583 productivity indicates (J. Walsh, pers. comm.). Further, if warm slope water affects catchability
584 of sand lance in the NEFSC spring survey, even if the effect is small relative to changes in
585 abundance, this would lead to sand lance disappearing at a slower rate than many of our
586 projections suggest. Such an effect would result in a future more closely following either the
587 “reduced warm slope water” or “optimistic” scenario. Sand lance focused surveys could better

28

588 resolve this question and refine projections of their abundance. While the future of the forage
589 fish on the Northeast US shelf will certainly have a great degree of spatiotemporal complexity
590 not incorporated or assessed in our projections, large scale changes to sand lance abundance are
591 likely to have ecosystem-wide effects and need to be considered in management and protection
592 of top predator populations throughout the region.

593 **Supplementary Materials:** Supplementary methods, figures, and tables are available
594 at *ICESJMS* online.

595 **Acknowledgements**

596 We would like to thank Page Valentine and Dann Blackwood of USGS for assistance in
597 collecting data. We also thank the captain and crew of the *R/V Auk*, particularly Amy Meloski
598 and David Slocum. Research was funded by the Bureau of Ocean Energy Management (IA
599 agreement M17PG0019; D.N.W, L.K., H.B., and J.K.L.), including a subaward via the National
600 Marine Sanctuary Foundation (18-11-B-203). Additional support came from the National
601 Oceanic and Atmospheric Administration Woods Hole Sea Grant Program (NA18OAR4170104,
602 Project No. R/O-57; J.K.L., H.B., D.N.W.) and a National Science Foundation Long-term
603 Ecological Research grant for the Northeast US Shelf Ecosystem (OCE 1655686; J.K.L.). J.J.S
604 was funded by the National Science Foundation Graduate Research Fellowship program. A.R.R
605 was funded by a NOAA Nancy Foster Scholarship. Manuscript drafts were improved from
606 suggestions by Jessica Perelman, Carin Ashjian, and two anonymous reviewers. No authors have
607 any conflict of interest to declare.

608 **Data Availability Statement:** Data are available to those with inquires upon request to the
609 corresponding author.

610 **Author Contribution Statement:** J.J.S, D.N.W, T.L.S., H.B., L.K., and J.K.L designed
 611 research, J.J.S, T.L.S, A.R.R., S.G., E.C., T.G., M.A.T., and P.H. performed the research; J.J.S.,
 612 D.E.R., A.R.S., and J.K.L. contributed to data analysis and interpretation, and J.J.S wrote the
 613 paper.

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Figure Legends

Figure 1. Hydrography of the Northeast US Shelf and sand lance abundance. (A) Overview of the Northeast US Shelf with blue arrows indicating prevailing surface currents of the region, red arrow indicating path of warm slope water entering the Northeast Channel (green box), and the purple arrow indicating flow of Labrador slope water entering the Northeast Channel. Inset displaying the 40 m isobath of Stellwagen Bank, and the three regions of the bank (North, Central, and South) including trawl locations referenced throughout the text. (B) Mean interpolated cube-root catch of sand lance over the Northeast US shelf as part of the NEFSC spring bottom trawl survey from 1978–1982. (C) Same as (B), except for the period of 1991–1995.

Figure 2. Ecology and phenology of adult sand lance collected on Stellwagen Bank in 2019. (A) Feeding incidence by day of year for fish collected in 2019 ($n = 202$). (B) Sand lance diet by proportion of biomass for each month ($n = 70$ fish containing prey, $n = 15,279$ prey). (C) Percent lipid of whole fish for sand lance separated by region and day of year on Stellwagen Bank ($n = 197$). Solid lines represent fitted values from a generalized additive model including month and region with shading representing 95% confidence intervals (deviance explained = 38.5%). (D) Standard length by day of year for age-2 ($n = 54$) and age-3 ($n = 45$) and dashed lines represent fit von Bertalanffy curves for each age class. (E) Gonadosomatic index (GSI) by day of years for males ($n = 105$) and females ($n = 107$) along with mean $\pm$ SEM GSI for each sex by monthly collection. Horizontal axis ticks in each plot represent first of the respective month.

Figure 3. Time series of the environmental drivers of sand lance abundance. (A) NEFSC spring bottom trawl sand lance abundance (log scale; blue), 3–4 year lagged winter-summer *Calanus* abundance anomaly in the Gulf of Maine (red), and 2–3 year lagged Atlantic herring index (green). (B) NEFSC spring bottom trawl sand lance abundance (log scale; blue) and warm slope water proportion entering the Northeast Channel (gold). Dashed line indicates fitted changepoint beta regression (changepoint = 2009, $p < 0.001$).

Figure 4. Projections of sand lance abundance under various scenarios. Predicted sand lance abundance (log) under “reduced warm slope water” scenario (dotted lines), “optimistic” scenario (thin solid lines), “current trajectory” scenario (thick solid lines), and “average Atlantic herring” scenario (dashed lines). Color represents the projected decline of *Calanus* under RCP 4.5 (blue) or the projected decline of *Calanus* under RCP 8.5 (red). Shaded regions represent bounds for the upper (75%) and lower (25%) quantiles. Black dashed line represents mean sand lance abundance from 1968–2008.

Figure 5. Histograms representing proportion of years between 2020 and 2100 that each simulation had negative sand lance abundance anomalies and negative Atlantic herring anomalies for (A) the “current trajectory” scenario, (B) “optimistic” scenario, (C) “average herring” scenario, and (D) “reduced warm slope water” scenario. Color represents RCP 4.5 (blue) and 8.5 (red) rates of *Calanus* decline. Thick dashed line represents proportion of years from 1968–2008 with both negative sand lance and Atlantic herring anomalies.

Table 1. Description of scenarios used in predictions of sand lance abundance through the end of the 21st century.

Scenario	Description	<i>Calanus</i>	Atlantic herring	Warm slope water
Current Trajectory	○ Declining <i>Calanus</i>	Normal random variable with linearly decreasing mean (32% decrease in RCP 4.5, 50% decrease in 8.5). Constant variance with time corresponding to variance of used time series (1977-2008)	Normal random variable with mean and variance corresponding to previous decline (1973-1988)	Beta random variable with mean and dispersion parameter following fitted changepoint regression
	○ Low Atlantic herring			
	○ Increasing warm slope water			
Optimistic	○ Declining <i>Calanus</i>	Normal random variable with linearly decreasing mean (32% decrease in RCP 4.5, 50% decrease in 8.5). Constant variance with time corresponding to variance of used time series (1977-2008)	Normal random variable where $\mu=0$, $\sigma=1$	Beta random variable with mean and dispersion parameter corresponding to pre-2009 time series (1977-2008)
	○ Average Atlantic herring			
	○ Pre-2009 warm slope water			
Average Herring	○ Declining <i>Calanus</i>	Normal random variable with linearly decreasing mean (32% decrease in RCP 4.5, 50% decrease in 8.5). Constant variance with time corresponding to variance of used time series (1977-2008)	Normal random variable where $\mu=0$, $\sigma=1$	Beta random variable with mean and dispersion parameter following fitted changepoint regression
	○ Average Atlantic herring			
	○ Increasing warm slope water			
Reduced Warm Slope Water	○ Declining <i>Calanus</i>	Normal random variable with linearly decreasing mean (32% decrease in RCP 4.5, 50% decrease in 8.5). Constant variance with time corresponding to variance of used time series (1977-2008)	Normal random variable with mean and variance corresponding to previous decline (1973-1988)	Beta random variable with mean and dispersion parameter corresponding to pre-2009 time series (1977-2008)
	○ Low Atlantic herring			
	○ Pre-2009 warm slope water			

Table 2. Fitted parameter estimates for the multiple linear regression of sand lance abundance anomaly (n=25 yrs).

Regressor	Slope Estimate	p-value
Gulf of Maine <i>Calanus</i> Anomaly	2.48	0.009
Atlantic herring index	-1.27	<0.001
Warm slope water proportion	-5.89	<0.001

Table 3. Fitted parameter estimates for the changepoint beta regression of warm slope water proportion with time (n=39 yrs). Model p-value <0.001.

Parameter	Estimate
β_0	-18.8
β_1	0.01
β_2	0.26
τ	2009

Table 4. Percent sand lance decline under four scenarios and each RCP projection of *Calamus* decline and proportion of years from 2020–2100 with negative sand lance anomalies and both negative sand lance anomalies and negative Atlantic herring anomalies.

Scenario	RCP 4.5 Decline (%)	Proportion of Negative Sand Lance Anomalies	Proportion of Negative Sand lance & Herring Anomalies	RCP 8.5 Decline (%)	Proportion of Negative Sand Lance Anomalies	Proportion of Negative Sand lance & Herring Anomalies
Current trajectory	-51.6	0.96	0.95	-76.9	0.98	0.97
Reduced warm slope water	-38.5	0.34	0.33	-74.8	0.47	0.46
Average	-52.0	0.97	0.47	-75.1	0.98	0.48
Atlantic herring Optimistic	-40.4	0.64	0.20	-71.4	0.72	0.26

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20-00597

Critical Review

A.O. De Silva et al.

PFAS Exposure Pathways

PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/etc.4935.

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(Submitted 3 August 2020; Returned for Revision 17 September 2020; Accepted 5 November 2020)

Abstract: Here we synthesize current understanding of the magnitudes and methods for assessing human and wildlife exposures to poly- and perfluoroalkyl substances (PFAS). Most human exposure assessments have focused on two to five legacy PFAS and wildlife assessments are typically limited to targeted PFAS (up to ~30 substances). However, This article is protected by copyright. All rights reserved.

shifts in chemical production are occurring rapidly and targeted methods for detecting PFAS have not kept pace with these changes. Total fluorine (TF) measurements complemented by suspect screening using high resolution mass spectrometry are thus emerging as essential tools for PFAS exposure assessment. Such methods enable researchers to better understand contributions from precursor compounds that degrade into terminal perfluoroalkyl acids (PFAA). Available data suggest that diet is the major human exposure pathway for some PFAS but there is large variability across populations and PFAS compounds. Additional data on TF in exposure media and the fraction of unidentified organofluorine are needed. Drinking water has been established as the major exposure source in contaminated communities. As water supplies are remediated, and for the general population, exposures from dust, personal care products, indoor environments and other sources may be more important. A major challenge for exposure assessments is the lack of statistically representative population surveys. For wildlife, bioaccumulation processes differ substantially between PFAS and neutral lipophilic organic compounds, prompting a revaluation of traditional bioaccumulation metrics. There is evidence that both phospholipids and proteins are important for the tissue partitioning and accumulation of PFAS. New mechanistic models for PFAS bioaccumulation are being developed that will assist in wildlife risk evaluations.

Keywords: organofluorine, exposure assessment, bioaccumulation, drinking water, toxicants, wildlife

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1. INTRODUCTION

Poly- and perfluoroalkyl substances (PFAS) are a class of thousands of anthropogenic substances containing an aliphatic fluorinated carbon chain (Buck 2011; OECD 2018). The PFAS family includes: (a) perfluoroalkyl substances, mainly perfluoroalkyl acids (PFAA) such as perfluoroalkyl carboxylates (PFCA) and perfluoroalkyl sulfonates (PFSA), as well as perfluoroalkane sulfonamide substances, and (b) polyfluoroalkyl substances such as fluorotelomer monomers, including fluorotelomer alcohols (FTOH), fluorotelomer olefins (FTO) and fluorotelomer iodides (FTI), and polyfluoroalkyl ether acids. Both perfluoroalkyl substances and polyfluoroalkyl substances can be polymers or non-polymers. The extraordinary strength of the C-F bond in the perfluoroalkyl moiety imparts unique properties (Smart 2001; Biffinger 2004) and has led to widespread industrial and commercial uses of PFAS. With over 7800 PFAS chemical structures identified to date (US EPA 2020a) and thousands registered for regulatory purposes (e.g., chemical inventories) (OECD 2018), they play a prominent role in modern society.

PFAS present a societal challenge. On one hand, PFAS represent some of the most innovative developments in materials chemistry and provide innumerable societal benefits (Johns 2000). However, following decades of widespread global use, and because many PFAS are highly persistent and mobile, concerns have been raised about the ecological and human health impacts of PFAS exposures. PFAS use categories include personal care products (PCP), cosmetics, ski wax, aqueous film forming foams

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(AFFF), textile treatments for stain and water repellency, food contact materials, medical devices, membranes in fuel cells, and membranes in chlor-alkali processes. This list of PFAS applications, though not encompassing of the full scale of PFAS use, captures their diversity. Prior work established the concept of “essential uses” of PFAS by first reviewing where they are abundantly used and when such uses can be replaced by safer alternatives (Cousins 2019).

Multiple strategies have been implemented to reduce emissions, production and use of specific PFAS. Manufacturers have phased out production of certain PFAS and in some cases replaced them with new PFAS or chemical substitutes. For example in textile treatments, many polymers containing long perfluoroalkyl side chains (more than seven perfluorinated carbons) were replaced by analogs containing short perfluoroalkyl side chains (six or four perfluorinated carbons) or fluorine-free moieties (e.g., siloxanes and hydrocarbon polymers) (Schellenberger 2019a). Further efforts are underway to constrain leachable content in fluoropolymers and side-chain fluorinated polymers. This leachable content consists of unbound monomers (such as fluorotelomer alcohols), oligomers, and other non-polymeric PFAS used during the polymer manufacturing process (such as surfactants and chain transfer reagents). Governments have implemented plans to restrict the usage, manufacture and import of certain PFAS, typically on a chemical-by-chemical basis and with certain exemptions. However, based on the recalcitrance of PFAS terminal products, their ubiquitous presence, and continued usage, PFAS exposure to humans and wildlife continues (Scheringer 2014).

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The volume of research publications over the past decade identifying PFAS in environmental media, humans and wildlife is staggering with several comprehensive review publications (Houde 2011b; Jian 2018; Sunderland 2019; Wang 2019). However, our ability to quantify how production and environmental releases of PFAS translate into PFAS tissue burdens in humans and wildlife is still limited. PFAS exposure science was a key area explored in the SETAC Focused Topic Meeting on Environmental Risk Assessment of PFAS held in Durham, NC, USA August 12-15, 2019.

Here we synthesize current understanding of PFAS exposure sources for humans and wildlife and identify key knowledge gaps that inhibit the development of informed regulatory decisions based on sound science. Specifically, we review: (a) PFAS sources and environmental transport pathways; (b) analytical methods used to assess PFAS exposures and their strengths and limitations; (c) methods for assessing human exposures to PFAS; (d) current understanding of the relative importance of different human exposure pathways; and (e) current understanding of PFAS bioaccumulation in wildlife.

2. OVERVIEW OF PFAS SOURCES AND ENVIRONMENTAL PATHWAYS

Figure 1 provides a schematic of the sources and spatial scales of PFAS transport and accumulation. Releases to the environment occur during the production, use, and disposal of materials containing PFAS. For example, legacy emissions of perfluorooctanoic acid (PFOA) were dominated by its manufacture and use to manufacture fluoropolymer products (Prevedouros 2006), whereas emissions of PFOS were dominated by its release during use of consumer and industrial products (e.g., surface treatments, AFFF, insecticides) (Armitage 2009b; Paul 2009; Wang 2017a).

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Existing chemical production and release inventories for PFAS have largely focused on PFCA, PFSA and their precursor compounds (e.g., FTOH and perfluorooctane- sulfonamides and -sulfonamidoethanols: FASA) (Armitage 2006; Prevedouros 2006; Yarwood 2007; Armitage 2009a; Armitage 2009b; Paul 2009; Wang 2014a; Wang 2014b; Kotthoff 2015; Shi 2015; Wang 2017b; Boucher 2019). A major shift in chemical production occurred between 2000-2002 with the voluntary phase-out of the base chemical POSF (perfluorooctane sulfonyl fluoride) to PFOS and perfluorooctane sulfonamide-based chemistry by 3M, the major global manufacturer at the time (3M Company 1999). Stewardship programs for PFOA in the United States and Europe have similarly been very successful at phasing out releases of this compound. We now know PFOS, PFOA and other long-chain legacy PFAS compounds represent only a small proportion of the total number of PFAS (US EPA 2020a). Understanding of the global environmental production and distribution of the legacy PFAS has been greatly facilitated by academic partnerships with industry that allowed the development of global emissions inventories for some compounds (Prevedouros 2006; Armitage 2009a; Armitage 2009b; Paul 2009; Wang 2014a; Wang 2017a, 2017b). We thus recommend greater transparency and collaboration between academia, industry and government to prioritize and improve understanding of global environmental releases of the thousands of PFAS structures on US EPA Master List (US EPA 2020a).

The effects of the phase out in chemical production of PFOS and its precursors as well as the PFOA stewardship program are well documented and illustrate the potential benefits of coordinated action curbing chemical releases. For example, ocean modeling studies forced by changes in riverine discharges to the North Atlantic show a large

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decline in surface (0-10 m depth) seawater PFOS concentrations at their peak (median > 60 pg L⁻¹) around the year 2000 to <40 pg/L in 2020 (Zhang 2017). In juvenile North Atlantic pilot whales, perfluorooctane sulfonamide: FOSA (a precursor to PFOS) accounted for 84% of the 15 targeted PFAS measured in the year 2000 but declined to 34% by 2013 (Dassuncao 2017). Human cohort studies in Denmark, Australia, Japan, Germany, and the Faroe Islands all indicate large declines in PFAS exposure for the general population outside of contaminated areas over this same time period (Olsen 2012; Okada 2013; Yeung 2013a; Toms 2014; Bjerregaard-Olesen 2016; Dassuncao 2018).

Sources of local scale contamination include fluorochemical manufacturing facilities, other manufacturing facilities where PFAS are used, PFAS-containing AFFF, wastewater treatment plants, and landfills. High environmental concentrations of PFAS result in exposure through consumption of contaminated drinking water, agricultural products, or fish and game. For more diffuse PFAS exposures driven predominantly by use of consumer products, population has been established as a good proxy for contamination of surface waters and coastal ecosystems (Paul 2012; Xie 2013; Li 2015; Zhang 2017).

Environmental concentrations and human and wildlife exposures to PFAS are typically highest at contaminated sites (Paustenbach 2007; Pistocchi 2009; Hoffman 2011; Shin 2011a; Shi 2015). Fluorochemical manufacturing sites are responsible for a large proportion of global emissions of certain types of PFAS such as PFCA, but there are relatively few of these sites. One study reported the presence of 16 fluorochemical manufacturing plants in the U.S. (Hu 2016) and another estimated that there were 33

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fluoropolymer production plants worldwide as of 2002 (Prevedouros 2006). High volume emissions from such facilities can impact large geographic areas and correspondingly large populations. For example, releases into the Ohio River from a fluoropolymer production plant in the U.S. state of West Virginia resulted in elevated level of PFAS in drinking water in communities hundreds of miles downstream (Herrick 2017).

Use of AFFF containing PFAS for fire suppression or training activities at military bases, commercial airports, and fire-training areas around the globe has contaminated many aquatic environments (Moody 1999; Barzen-Hanson 2017). Landscapes and water systems adjacent to areas of AFFF use often have high levels of PFAS in soil, sediment, groundwater, surface water, or drinking water (Karrman 2011; Houtz 2013; Anderson 2016; US DOD 2017). PFAS concentrations measured in different environmental media at ten active United States Air Force installations were highest for PFOS and included 4,300 µg/L in groundwater, 8,970 µg/L in surface water, 190,000 µg/kg in sediments, 9,700 µg/kg in surface soil and 1,700 µg/kg in subsurface soil (Anderson 2016).

Incidents of localized contamination have been linked to facilities that employ PFAS to produce goods such as plastic and textile coating facilities (VTDEC 2016; NHDES 2020; NYDEC 2020), and leather tanneries (US EPA 2020b). Numerous other industries are users of PFAS, and all of these have the potential to cause localized contamination. For example, major sources of PFAS contamination in surface waters in New York State and Rhode Island, USA included mixed industrial sources that predominately release PFOS and PFOA, metal plating industry sites, and landfills (Zhang

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2016). PFAS enter landfills as components of residual materials and can be released to the environment in leachate, and may also contribute to elevated concentrations in wastewater (Huset 2011; Lang 2017; Masoner 2020). Concentrations of PFAS in municipal solid waste vary substantially depending on the waste source (Solo-Gabriele 2020).

Wastewater treatment facilities receive PFAS in influent and discharge PFAS in treated effluent and biosolids (Sinclair 2006; Coggan 2019). Treated effluent can be a source of PFAS exposure if it is discharged to a water body that is used as a drinking water source. For example, the probability of detecting PFAS in US public drinking supplies was significantly associated with higher numbers of wastewater treatment plants within a watershed (Hu 2016). Land application of biosolids or irrigation using reclaimed water can result in accumulation of PFAS in soils and underlying groundwater, and uptake into food or fodder crops (Choi 2019; Coggan 2019; Lazcano 2019; Letcher 2020). Concentrations of PFAS are highest in effluent and biosolids for treatment plants that receive wastewater from industrial plants that use PFAS or facilities that use AFFF (3M 2001; Houtz 2016).

Following decades of releases (ca. 1958 to present), PFAS are now ubiquitous in the global environment and the ocean is thought to be the final sink for the terminal products (PFAS) associated with most global production (Armitage 2009a; Armitage 2009b; Zhang 2017). The spatial distributions of PFAS in the environment following releases reflect their physical-chemical properties (propensity for sorption vs. transport in air and water) and types of releases (air, water, soil) during manufacturing, use, and

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disposal. The relative importance of the atmospheric transport/precursor degradation pathway versus the oceanic transport/terminal end product pathway has been assessed for some PFAA. Generally, aquatic discharges and oceanic transport are more relevant next to source regions in the United States, Europe, and Japan (Prevedouros 2006; Armitage 2009a; Zhang 2017) while atmospheric transport is important in remote regions such as the Arctic and Southern Ocean for many compounds (Wang 2015; Dassuncao 2017; MacInnis 2017; Yeung 2017; Pickard 2018). Accumulation of PFAS in the oceans reflects both contemporary and historic PFAS production because terminal PFAA are not known to appreciably degrade under environmental conditions and the timescales associated with PFAS removal through burial in coastal and deep-sea sediment are long (Yamashita 2008).

A major focus of PFAS research is better understanding the releases and environmental degradation pathways of precursor compounds that degrade into terminal PFAA. The majority of precursor compounds studied to date are neutral organics with appreciable vapor pressures. This means that they have a greater propensity for atmospheric transport, whereas most PFAA have low pKa values and exist as stable ions in solution (Cheng 2009). Significant effort is being dedicated to better understanding point source releases of atmospheric PFAS (both PFAA and precursor compounds), but stack testing methods and inventories are still limited. Ionizable precursors also exist but data are scarce. Surface deposition of atmospheric PFAA emissions followed by leaching of PFAS to groundwater has been demonstrated at multiple industrial sites (Guelfo 2018; NHDES 2020; NYDEC 2020). Integration of PFAS measurements into routine atmospheric monitoring for pollutants by programs like the North American Deposition

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Program (www.nadp.org) would thus be valuable for measuring changes in atmospheric PFAS concentrations, assisting atmospheric PFAS modeling efforts (Thackray 2020), and identifying regions vulnerable to atmospheric PFAS contamination.

3. ANALYTICAL TECHNIQUES FOR MEASURING PFAS EXPOSURES

The ability of scientists and regulators to identify and rank the importance of different PFAS exposure sources is directly contingent on analytical methods available for measuring PFAS in a variety of environmental matrices and biological tissues. Analytical techniques for PFAS have advanced rapidly from an early focus on PFOA and PFOS to routine measurements of a suite of approximately 40 PFAS with commercially available analytical standards for detection using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Recent methods have been evolving for detecting volatile species, new compounds, and total fluorine in environmental and human samples. These advances have been critical for keeping pace with the rapidly changing chemical landscape of PFAS exposure sources.

3.1 Targeted PFAS analysis

Most studies report concentrations of non-volatile PFAS measured using LC-MS/MS. This is commonly referred to as “targeted” analysis because it is based on setting up the instrumental analysis to collect data on specific substances, while confirmation of these substances relies on specific mass-to-charge (m/z) ratios and retention times based on parameters determined using commercially available chemical standards. Nonetheless, this approach enables quantitative PFAS determination with high precision and high sensitivity. Targeted PFAS analysis is used for water (drinking water,

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surface water, groundwater), air/ airborne particulate, food, solids (soil, sediment, house dust), and consumer products. The same techniques are also used to determine PFAS in diverse biological tissues including plasma, sera, whole blood, urine, breast milk, muscle, and other tissues. Over the past few decades, targeted PFAS measurements has improved substantially with improved instrument sensitivity and lower detection limits, numerous laboratory intercomparisons and standard operating procedures, and the addition of more PFAS that can be detected (Guelfo 2020). A major analytical challenge is that synthesis of analytical standards for newer PFAS has lagged their production and release and a lack of reference materials for the major PFAS in routine analysis (Xiao 2017; Land 2018).

3.2 Suspect screening and non-targeted analysis

While targeted analysis is limited to a finite number of PFAS analytes, a comprehensive understanding of PFAS exposure calls for innovative techniques. Specifically, techniques that reveal the presence of emerging PFAS produced intentionally and unintentionally in various industrial processes, as well as PFAS transformation products formed in natural and engineered systems. Suspect screening and nontargeted analysis using high-resolution mass spectrometry (HRMS) allow discovery and characterization of unidentified PFAS in the environment. HRMS provides highly accurate mass to charge ratio of the analytes ($< \pm 0.001$ m/z), its fragments, and their isotopic patterns. Comparing such information with chemical databases that contain thousands of PFAS compounds enables identification of the molecular structure of analytes without analytical standards. Suspect screening and nontargeted analyses have led to the identification of emerging anionic, zwitterionic, cationic, and neutral PFAS in

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water (Strynar 2015; Barzen-Hanson 2017; Gebbink 2017; Newton 2017), sediment (Newton 2017), soil (Baduel 2017; Lin 2017), airborne particulate matter (Yu 2018), and biological samples (Rotander 2015b; Liu 2018).

A drawback of HRMS methods is that such analyses are typically qualitative, due to the absence of analytical standards. In addition, the PFAS congener can only be determined if the sample preparation has adequately recovered the analyte. Analyte spike and recovery validation is not possible without analytical standards. In addition, HRMS data analysis is labor intensive and require specialized analysts. However, it is increasingly recognized for its data-banking value, in which archived HRMS spectra for routine analyses can be re-analyzed when emerging analytes become a priority.

3.3 New methods for closing the fluorine mass balance in exposure studies

A major challenge in PFAS exposure assessment is that most studies employ targeted LC-MS/MS analyses and thus cannot assess the total burden of PFAS in environmental and biological samples. Even HRMS studies are limited by the sample preparation technique which can be discriminatory towards certain classes of PFAS. Also, because HRMS analyses are qualitative or semi-quantitative in nature, they cannot be used to develop mass budgets for total PFAS in the environment or the total burden of PFAS exposures. This has led to the development and use of several total fluorine (TF) measurements to better characterize the total burden of known and unknown PFAS in a sample.

TF methods rely on determinations of the concentration of atomic fluorine. Several strategies have been developed for the determination of the total organofluorine. This article is protected by copyright. All rights reserved.

(TOF), extractable organofluorine (EOF), or adsorbable organofluorine (AOF) content in order to assess the total PFAS content, as reviewed in prior work (Koch 2020). Known PFAS are typically determined using targeted LC-MS/MS techniques on the same sample extracts analyzed for EOF or AOF analyzed using combustion ion chromatography (CIC). The presence of organofluorine is indicative of anthropogenic substances, since organofluorines are very rare in nature as a consequence of the high energy bond between carbon and fluorine. By contrast, inorganic fluoride is the most abundant halogen on Earth and must be quantified to assess the organofluorine content. The portion of unidentified organofluorine in a sample is calculated by subtracting the concentration of target PFAS, converted to moles of fluorine, from the total organofluorine content in the same extract (Figure 2). Because the quantity of unidentified organofluorine is dependent on the targeted analysis, it is challenging to compare unidentified organofluorine among different studies. In Figure 2b, the unidentified organofluorine is shown relative to the targeted analysis of 50 PFAS congeners in human blood. In general, studies on TOF and EOF/AOF indicate a significant portion of organofluorine in biota and the environment is not captured by monitoring of the typical suite of PFAA congeners.

Different methods have been developed for total fluorine or organofluorine measurements in environmental samples and consumer products, including defluorination with sodium biphenyl (Musijowski 2007), ^{19}F Nuclear Magnetic Resonance (NMR) (Moody 2000), continuum source molecular absorption spectrometry (CS-MAS) (Qin 2012), inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) (Jamari 2018), instrumental neutron activation analysis (INAA) (Schultes 2019), particle-induced gamma-ray emission spectrometry (PIGE) (Ritter 2017), CIC (Miyake 2007a), and X-ray

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photoelectron spectroscopy (XPS) (Tokranov 2019). While some methods can distinguish between organofluorine and inorganic fluoride (NMR, XPS), others give the total concentration of fluorine in a sample (for example PIGE and CIC), therefore a pre-extraction of inorganic fluorine is needed.

Assessing PFAS in consumer products presents an important challenge for fully understanding human exposure. Some studies have measured the fluorine concentration at the material surface or sub-surface (PIGE, XPS, and INAA), while others consider the average concentrations throughout the whole sample analyzed (CIC). The unknown organofluorine content is useful for understanding TF analysis in human tissues. Concentrations of organofluorine in human serum have been measured using the CIC method, both total fluorine before extraction (Miyake 2007b) and EOF after extraction (Yeung 2016). As an example of this application, one study targeted 52 PFAS congeners in human blood from Münster, Germany including PFSA, PFCA, FASA, fluorotelomer acids (FTUCA & FTCA), and polyfluorinated phosphate esters (Yeung 2016). Through TF and EOF analysis, these PFAS accounted for approximately 80% of EOF from 1982-2006 and the unknown EOF increased to 50% from 2007 to 2009, further emphasizing that humans are being increasingly exposed to new organofluorine substances. Caution must be applied in ascribing the unknown EOF to PFAS because many pharmaceuticals contain fluorine. It may be possible to adapt methods to avoid coextraction of non-PFAS organofluorine. For example, a recent study (Figure 2b) used acetonitrile extraction followed by a dispersive graphitized carbon clean-up for human plasma (Miaz 2020), which is likely to sorb the fluorine in pharmaceuticals that is typically in the form of a $-F$ or $-CF_3$ moiety on an aromatic ring.

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The Total Oxidizable Precursor Assay (TOP) assay provides another method for estimating unknown precursors. The method has been mainly applied to aqueous samples which are subjected to oxidation via hydroxyl radicals formed in a persulfate thermolysis, to transform PFAA precursors to their terminal products (Houtz 2013; Houtz 2016). The oxidized extracts and un-oxidized extracts are analyzed by LC-MS/MS for PFAS in order to quantify the concentrations of oxidizable precursors. The TOP assay highlights the relevance of PFAA precursors to PFAA concentrations in the environment. For example, one study determined that precursors in AFFF had significantly contributed to PFCA and PFSA in groundwater from a firefighting training area (Houtz 2013).

3.4 Strengths and limitations of analytical techniques for assessing exposures

Table 1 compares the strengths and limitations of different analytical methods used to assess PFAS exposures. Comprehensive human exposure assessments need to consider a wider range of PFAS than available from targeted LC-MS/MS measurements, including precursors that transform to terminal PFAA. Non-targeted screening for a variety of matrices and the TOP assay in aqueous samples have been proven useful for detecting additional PFAS. The utility of non-targeted analysis depends on identification strategies and available suspect lists. The TOP assay provides quantitative data on the contribution of oxidizable precursors and can provide some insight on the precursor structure (e.g. functional group and chain lengths). However, the TOP assay has only reliably been performed on aqueous samples due to matrix interference in soil and biota. Further, it is not able to oxidize certain emerging PFAS like per- and polyfluoroalkyl ether acids (PFEA) such as F-53B (6:2 Cl-PFESA) and GenX (hexafluoropropylene oxide dimer

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acid). Non-specific TF methods add information on the magnitude of unknown PFAS that are not quantifiable using targeted analysis. However, some of the detected total fluorine or organofluorine may include compounds outside of those PFAS according to the current definition by the Organization for Economic Cooperation and Development (OECD).

Pharmaceuticals and pesticides with a low atomic fraction of fluorine could result in moderate or high organofluorine content in water if present in high concentrations. Other PFAS classes could be excluded from the analysis during conventional sample extraction. For example, the non-polar PFAS like perfluorobutyl side-chain fluorinated co-polymers surfactant with molecular weight >1600 g/mol was only extracted from soil, wastewater sludge and sediment using 1:1 hexane/acetone, whereas much lower recoveries were obtained using methanol or acetonitrile (Chu 2017; Letcher 2020). The removal of inorganic fluoride also needs to be validated, especially for natural waters and drinking water where fluoride concentrations can be orders of magnitude higher than PFAS concentrations. Non-specific TF methods that target atomic fluorine thus risk overestimating the PFAS content of samples but nonetheless provide a useful screening metric for identifying the magnitude of unidentified organofluorine in an environmental sample.

4. METHODS FOR ASSESSING HUMAN EXPOSURE TO PFAS

Exposure pathways for PFAS can be examined as a chain of events shown in Figure 3, linking sources to media (via fate and transport) to external exposure (via behavioral factors) to concentrations in blood, the body's central compartment (via

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toxicokinetics). Exposure routes that are typically examined for PFAS include: dietary ingestion, water ingestion (particularly in contaminated communities), and inhalation of air and dust particles. Hand-to-mouth contact and dermal absorption can also be relevant pathways.

4.1. Two approaches to PFAS exposure assessment

Exposures are typically estimated using two complementary approaches. The exposure factor (“bottom up”) approach relies on measured concentrations of certain PFAS in exposure media (e.g., food, water, air, dust) and uses estimates of exposure frequency and duration to estimate external exposure (mass/kg body weight/time). PFAS levels in media (dietary items, drinking water, and the indoor environment) can also be estimated using multi-media modeling assessments of global or local sources, transport and accumulation. An alternate method is the epidemiologic (“top down”) approach to exposure assessment, which typically involves regressing serum/blood levels against measured concentrations in different media (e.g., water, air, dust), and/or behavioral data (e.g., food consumption) to estimate the strength of association with one or more sources. With sufficient data regarding multiple pathways, either method can estimate the relative importance of different exposure routes.

Several studies have used the exposure factor approach to quantify the contribution of PFAS in the indoor environment, seafood, drinking water, and food packaging to total exposures in humans (Trudel 2008; Vestergren 2008; Harrad 2010; Gebbink 2015; Dassuncao 2018). Exposure to precursors has been linked to increased bioaccumulation in food webs (Kelly 2009; Dassuncao 2017; Boisvert 2019; Zhang 2019). Associations between serum PFAS concentrations and exposure behaviors such as

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water district of residence, consumption of tap water, and fish consumption have also been characterized in prior work (Christensen 2017; Herrick 2017; Dassuncao 2018; Barton 2020).

The two exposure assessment methods each have strengths and weaknesses and are complementary. In the exposure factor approach, one or both elements may be uncertain. For example, while measuring PFAS in food can be analytically challenging with many non-detects, average food consumption rates are better known. In contrast, measuring PFAS in house dust is easier, but dust ingestion rates are uncertain, particularly for adults. Carrying out statistically representative (and therefore extrapolatable) sampling and characterizing the fraction of exposure originating from precursors is challenging for many exposure routes. One strength of the exposure factor approach is the potential for a direct link to chemical production and environmental concentrations that drive exposures (Armitage 2009a). This information is critical for designing interventions that mitigate human and wildlife exposures.

The epidemiologic approach integrates both exposure and toxicokinetics, but often must take into account potential confounding between exposure routes as well as exposure measurement error. For example, the latter can arise when regressing serum concentrations of persistent PFAS with media measured at one point in time. The long half-life of many PFAS in humans means that serum concentrations reflect cumulative exposures over a relatively long time period, while external exposure measurements may often reflect a short exposure window. Hybrid models can be used to integrate and compare the two approaches. For example, one can use toxicokinetic models to estimate serum levels from exposure estimates (or vice versa), comparing the estimated and

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4.5. Indoor exposure via inhalation and dust ingestion

Most North Americans spend approximately 90% of their time in indoor environments. PFAS are used extensively in products designed for indoor use such as stain resistant coatings for carpet and furniture. They are found in indoor air and dust, although so far the connection to specific indoor sources has received little attention (Beesoon 2012). Thus, there is the potential for indoor exposures to PFAS via inhalation, ingestion of dust and dermally.

Investigation of indoor exposure to PFAS is more complicated than for many groups of compounds (e.g., polybrominated diphenyl ethers: PBDEs) due to the vast variety of physical-chemical properties for PFAS and the existence of precursors and polymers. Semivolatile organic compounds (SVOCs) will tend to partition between the vapor phase, suspended particulate, dust, and indoor surfaces (including skin and clothing), depending in part on their octanol/air partition coefficients (Weschler 2008). Some PFAS such as FTOH and FOSE are relatively volatile and are found in the vapor phase indoors. Other PFAS such as PFOA and PFOS are found at high concentrations in dust. There is little information available about the indoor presence and fate of fluorinated polymers (e.g., side-chain fluoropolymers used in some stain resistance formulations). In part this is due to analytical difficulties (Rankin 2015; Letcher 2020). They may be released from products to dust via physical abrasion—as has been shown for other low volatility compounds (Webster 2009)—or potentially gradually breakdown over time, releasing the fluorinated side chains as may occur in outdoor environments (Washington 2015; Letcher 2020). An important question is the amount of unidentified

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organic fluorine in air and dust. For example, TF analysis in conjunction with HRMS may be a useful first step in examining polymers or other unmeasured compounds in dust.

PFAS concentrations in indoor environments are usually measured through filtration or adsorption to a solid phase (filters or sorbents) using either active air pumping or passive samples, followed by extraction of the solid phase to recover PFAS for quantification (Martin 2002; Shoeib 2008; Padilla-Sánchez 2017; Guo 2018; Rauert 2018; Wong 2018; Yao 2018). In indoor air, concentrations can be an order of magnitude higher (ng/m^3 levels) than outdoor environments (Shoeib 2004; Shoeib 2005; Yao 2018). Indoor air sampling has tended to focus on the more volatile compounds such as the FTOHs and FASA.

PFAS concentrations in dust can be very high and have been reported at the $\mu\text{g}/\text{g}$ level (Moriwaki 2003; Kubwabo 2005; Shoeib 2005; Strynar 2008; Eriksson 2015a; Eriksson 2015b; Lankova 2015; Winkens 2018). Dust sampling has found PFAA and other PFAS, including relatively large amounts of polyfluorinated phosphate esters (diPAP) (De Silva 2012; Eriksson 2015a; Makey 2017). Methods for dust sampling and processing—e.g., where and how to sample dust in homes, sieving size—are less standardized than for air. Bigger issues are the variety of indoor environments—home, workplace, childcare facilities, vehicles, etc. (with homes being a main focus)—and the difficulty of doing representative sampling (Goosey 2012; Fraser 2013; Zheng 2020). Unlike the extraordinary efforts that have been made for representative biomonitoring (e.g., NHANES in the USA), most indoor sampling is convenience sampling that may not

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be representative of exposures across the general population. Exposure factors for inhalation are well known, but exposure factors for dust ingestion are quite uncertain (US EPA 2011). As a result, inhalation estimates are likely more reliable than those for dust ingestion. An additional source of uncertainty is the amount of conversion of precursors (e.g., FTOH, FASA) into terminal end products (e.g., PFCA, PFSA) found in serum/blood (Poonthong 2020). This issue is particularly important when trying to compare inhalation with dust ingestion, or indoor routes of exposure to diet and other sources.

Two North American studies have found associations between serum levels of some PFAA and the precursors FTOH and FASA, respectively, in indoor air (Fraser 2012; Fraser 2013; Makey 2017), but little association with PFAS concentrations in dust. A Norwegian study found associations between certain PFAS in whole blood and indoor air and/or dust (Poonthong 2020). The latter study also estimated that diet was more important than indoor exposure on average but that inhalation and dust ingestion dominated for some study participants, particularly the people with the highest blood concentrations. Some studies have found that serum PFAS concentrations increase with socioeconomic status indicating that indoor and dietary exposure may be partly correlated due to purchasing decisions (Nelson 2012). Relatively few studies have used epidemiologic techniques to examine air and dust or other pathways simultaneously (Haug 2011; Fraser 2013; Makey 2017; Poonthong 2020).

In summary, some epidemiologic evidence suggests indoor exposure is important enough to be empirically associated with serum/blood levels, and may be the dominant exposure route for some people (Fraser 2013; Makey 2017; Poonthong 2020). More

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research is needed on the differences in indoor exposure patterns between people as well as differences between countries and time trends. Relatively little research has been conducted on the connection between indoor levels and putative sources, total organic fluorine, the contribution of fluorine-containing polymers, and exposure of children.

4.6 Outdoor air exposures

Reported PFAS concentrations in outdoor air range from non-detectable or sub pg/m^3 levels to hundreds of pg/m^3 (Martin 2002; Stock 2004; Barber 2007; Jahnke 2007; Fromme 2009; Rauert 2018; Wong 2018). PFAS concentrations in urban areas are typically higher than in rural areas (Martin 2002; Stock 2004; Barber 2007; Jahnke 2007). A few studies of general populations report that outdoor air PFAS concentrations are one to two orders of magnitude lower than indoors (Shoeib 2011), presumably due to indoor sources. For such populations, we expect inhalation exposure indoors to exceed outdoor exposures.

Little is currently known about communities with major atmospheric point sources. In Parkersburg, West Virginia, USA, local drinking water sources were contaminated primarily by air emissions of PFOA emitted by the Washington Works facility followed by deposition and groundwater transport (Davis 2007). In Fayetteville, NC, precipitation monitoring to assess the deposition of GenX via air emissions has been ongoing since 2018. Communities in Hoosick Falls, NY, Bennington, VT and Merrimack, NH have had varying extent and types of monitoring conducted in response to concerns or documentation of groundwater/drinking water contamination. An important question in these scenarios is the relative importance of exposure via inhalation

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vs. water ingestion. Using a sophisticated fate and transport model, researchers estimated PFOA concentrations in ambient air and water in the communities surrounding the Washington Works facility in West Virginia, USA over time (Shin 2011b; Shin 2011c). Their results compared well with measured water values and indoor air was assumed to be 10% of outdoor air due to partial infiltration. Transport of PFAS in air is faster than in soil and groundwater. Thus, for people living in areas with contaminated air, estimated inhalation exposure exceeded that via water ingestion in the early time period but was less than water ingestion later on (Shin 2011b; Shin 2011c). These results suggest that inhalation exposure to PFAS might exceed water ingestion in areas with continuing air emissions but mitigation of drinking water (e.g., via filtration).

4.7 Dermal exposures to PFAS

Dermal exposure to PFAS can result from contact with house dust, PCP, and other consumer products. It has received relatively little attention, with two exceptions: dust and, more recently, cosmetics/personal care products (PCP). For example, one study estimated dermal exposure of children to PFAS in dust in childcare settings using measured dust concentrations and an exposure factor for the amount of dust adhering to skin (Zheng 2020). Poothong et al (2020) used an alternative method for skin contact: measuring PFAS on hands using handwipes. Both then used the fraction dermal absorption approach, a model commonly used in risk assessment (Kissel 2011).

One study examined selected PCP with product labeling indicating PFAS ingredients such as polyfluorinated phosphate esters (PAPs) or other fluorinated compounds and reported PFCAs in the ug/g range but did not determine the levels of

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PAPs (Fujii 2013). Another study that measured 39 PFAS, as well as EOF and total fluorine, detected PAPs at up to 470 ug/g in cosmetic products (Schultes 2018). The measured PFAA accounted for only a small fraction of the EOF and TF, implying the presence of unidentified compounds, potentially including polymers or inorganic fluorine. Skin contact with PCP can depend on a number of factors including the amount of the product applied per unit area (loading), the surface area of exposed skin, the duration of use and the frequency of washing the skin. Prior work has estimated dermal absorption of PFOA in foundation cosmetics, leading to an absorbed dose through dermal exposure of <0.006-3.1 ng/kg/day, with the high end exceeding dietary exposure in Sweden (Schultes 2018). This dermal exposure estimate for PFOA does not include indirect exposure via PAP.

5. RANKING SOURCES OF PFAS EXPOSURE FOR HUMAN POPULATIONS

A major question discussed by the exposure assessment panel at the SETAC PFAS topic meeting was whether it is possible to rank the relative importance of PFAS exposure sources for different human populations at this time. Researchers and decision-makers seek to understand the relative contributions of different exposure pathways to human exposure in order to inform risk assessments and prioritize interventions. The panel concluded that there were many gaps remaining in this area, especially for general populations that have diverse exposure pathways for PFAS. A summary of present understanding is provided below.

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5.1 Occupational exposures

Occupational health effects associated with human PFAS exposures have been reported for individuals who worked in fluorochemical production plants in the US (Olsen 1999), Italy (Girardi 2019) and China (Fu 2016). Occupational exposure to PFAS occurs mainly through inhalation and dermal contact (Franko 2012). Inhalation exposure to PFAS in a workplace situation can be important due to sublimation into the gaseous phase of volatile manufacturing intermediates that are hydrolyzed to end product PFAS (Kaiser 2010). Firefighters working and training with AFFF did not show a relationship between internal exposure of PFOS and the self-reported frequency of direct skin contact with AFFF, indicating that dermal contact may not be an important pathway of exposure (Rotander 2015a). However, they had elevated blood levels of PFOS and PFHxS, which is consistent with the composition of legacy electrochemical fluorination (ECF) AFFF. Elevated levels of PFNA and other long-chain PFCAs have been connected to occupational exposure for firefighters (Laitinen 2014; Trowbridge 2020). Professional ski wax technicians showed elevated blood levels of PFCAs, which was associated with number of working years. Ski wax contains both precursor semifluorinated n-alkanes and PFCA (Plassmann 2013; Carlson 2020) and is often applied by using heat (130-220°C) which results in gaseous compounds and particles. An exposure study with internal dose measurement and personal and ambient air including airborne particles showed that inhalation of both precursor compounds and terminal end products contributed to the internal dose (Nilsson 2010).

5.2 Communities near contaminated sites

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PFAS have been detected in both surface water and groundwater sources near facilities that manufacture or process fluorochemicals. Concentrations in aqueous matrices in areas without point sources generally occur below detection limits or at the sub ng/L (parts per trillion) level but are commonly detected at the µg/L (parts per billion) level or higher in contaminated areas (Chen 2017b; Cai 2018; Pan 2018; Park 2018; Janda 2019). These include sites with historical releases of AFFF, such as military bases and airports, and wastewater treatment plants that have received industrial wastes (Herrick 2017; Worley 2017; Barton 2020). These sites have been associated with contamination of drinking water across the U.S. with at least six million people estimated to have been exposed to levels above EPA health advisories for PFOS and PFOA of 70 ng/L (Hu 2016). The total number of people exposed to PFAS in the U.S. is greater because this estimate does not include populations exposed through small drinking water systems or private wells or exposure to other PFAS.

Many biomonitoring studies have shown PFAS in drinking water near contaminated sites have led to population blood levels that are much greater than background levels (Daly 2018; Ingelido 2018; Li 2018). In such areas, serum levels are associated with concentrations in drinking water (Hoffman 2011). Drinking water has been estimated to contribute up to 75% of exposures near contaminated sites (Vestergren 2009). The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) is currently conducting biological monitoring at over 15 sites with contaminated drinking water across the country following comparable methods for collecting blood and administering exposure questionnaires. This work will generate further information about

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the contributions of drinking water exposures across a diverse set of communities near contaminated sites.

Production and use of the most common legacy PFAS (PFOS, PFOA, PFHxS) have decreased in the U.S. but an increasing number of sites contaminated with these compounds have been identified over the past decade. Data compiled by the Northeastern University's Social Science Environmental Health Research Institute (SSEHRI) show that as of May 2020 there were 393 known contaminated public drinking water systems in the U.S. (SSEHRI 2020). As background levels of legacy PFAS decrease, the relative contribution of their exposure from drinking water sources near these contaminated sites will increase (Bao 2017). Furthermore, replacement compounds, such as PFEA, including GenX, have already been detected in drinking water near manufacturing facilities (Hopkins 2018). Newer methods that measure EOF and TF are beginning to be used on drinking water with the goal of quantifying the exposure of unidentified PFAS (Hu 2019). However, the large-scale implications of changes in production have yet to be fully understood.

5.3 General population PFAS exposures

The relative importance of different PFAS exposure sources varies dramatically across general populations with diverse PFAS exposure sources. Table 2 summarizes some prior literature estimates of source contributions to overall PFAA exposures for adult populations without occupational exposure and not living in close proximity to point sources of PFAS contamination. Large variability in the relative importance of different exposure sources across studies reflects variable concentrations in

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environmental media and differing assumptions regarding exposure sources, frequencies, duration, and consideration of precursors.

Several studies have shown that the exposure to PFAS in children differs from adults due to behavioral and dietary variability. Breast feeding is known to be an important source of early-life exposure to PFAS (Mogensen 2015; Kang 2016; Papadopoulou 2016). A study from the Faroe Islands showed hand-to-mouth contact with carpeting was an important exposure source for children but not adults based on the contrasting composition of PFAS measured in serum (Hu 2018). The 2020 European Food Safety Authority (EFSA) report found that toddlers/children had a two-fold higher exposure than adults, in part due to maternal exposure (European Food Safety Authority 2020).

There is general agreement that dietary exposure is the major contributor to population exposure for PFOS and PFOA (Table 2), but more limited evidence for PFHxA, PFHpA, PFNA, PFDA, and PFHxS. In 2018, EFSA estimated that the main contributors to adult dietary exposure to PFOS were fish, meat, eggs and products with these ingredients (European Food Safety Authority 2018). The greatest sources of exposure to PFOA were eggs and dairy and products containing these ingredients. The youngest population groups were estimated to have the highest dietary exposure to PFOA and PFOS. In 2020, EFSA expanded its analysis of exposures to PFAS in foods and reported that the sources contributing to PFAS exposure to adults and children were consistent with the findings from 2018. They concluded that PFOA contributed 21%, PFNA 4%, PFHxS 10% and PFOS 66% to the sum of PFAS exposures, based on the

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Reiner 2015; Penland 2020). Several comprehensive reviews (Houde 2011a; Reiner 2015; Muir 2019) have synthesized data from available biomonitoring studies.

The highest PFAS concentrations in wildlife tend to be associated with proximity to contaminated sites. For example, one of the highest reported fish PFOS concentrations (maximum 9349 ng/g dry weight in whole fish tissue) was from an AFFF-impacted site downstream from Barksdale Air Force Base in Louisiana (Lanza 2017). Many biomonitoring studies have identified elevated exposures to legacy and emerging PFAS as the result of industrial activities (Custer 2012; Custer 2014; Liu 2017; Groffen 2019; Lopez-Antia 2019; Guillette 2020). Legacy PFAS such as PFOS are still abundant at many contaminated sites and novel PFAS are increasingly being detected. For example, one study reported PFOS was the predominant compound in fish (mean 263-348 ng/g wet weight (ww) in muscle) adjacent to a major fluorochemical production facility in Wuhan, China (Zhou 2013). Suspect and non-target screening subsequently revealed a suite of 330 novel fluorinated structures belonging to 10 different chemical classes in fish liver from the same region (Liu 2018). The profile of specific PFAS released at each contaminated site affects the accumulation of different compounds in biota and may also be relevant for determining exposure risks such as near AFFF contaminated regions (Yeung 2013c; Custer 2014; Munoz 2017; Larson 2018; Salice 2018; Langberg 2019; Munoz 2020).

Biological time series data for specific ecosystems suggest variable temporal changes in PFAS across compounds and ecosystems. In the Arctic, there is sustained or increasing (post-2010) PFAA levels in some wildlife (Muir et al. 2019). Following the

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phase out of the parent chemical to PFOS and its precursors ca. 2000-2002, several studies have noted rapid declines in perfluorooctane sulfonamide (FOSA), an atmospheric precursor to PFOS that is biotransformed by most mammals, but less consistent declines or even increases in PFOS (Smithwick 2006; Ahrens 2009a; Dassuncao 2017; Sun 2019; Schultes 2020). This likely reflects the rapid response of atmospheric concentrations to changes in chemical production but a lagged response of most aquatic ecosystems. Most time series studies have focused on targeted PFAS but recent work has shown that trends in TF indicated by EOF and other methods differ from the legacy compounds (Schultes 2020). Understanding temporal and spatial trends in emerging PFAS compounds in biota is thus an important research need (Spaan 2020).

6.2 PFAS bioaccumulation metrics

The bioaccumulation potential of persistent organic pollutants (POPs) is commonly reported based on several metrics: bioconcentration factor (BCF: the direct uptake of a chemical by an organism from water or air, i.e., $BCF = C_{fish}/C_{water}$ in a controlled laboratory experiment with no dietary intake); biomagnification factor (BMF: the concentration of an organism relative to their diet, i.e., C_{fish}/C_{prey}); bioaccumulation factor (BAF: the combined effects of all uptake pathways). BCFs are commonly measured in laboratory experiments, while BMFs and BAFs are typically field-based measurements. The trophic magnification factor (TMF) is an indicator of dietary biomagnification and is generally established empirically using slope of the relationship between trophic position in a food web based on stable nitrogen isotopes and chemical concentrations in organisms from a field-based food web. A large body of work has established that for neutral,

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hydrophobic POPs, simple partitioning between lipid and water indicated by their octanol-water partition coefficient (K_{ow}) or octanol-air partition coefficient (K_{oa}) provides a reasonable proxy for bioaccumulation propensity.

By contrast, processes governing the uptake of PFAS into organisms and partitioning across tissues are less well-understood, even for commonly studied PFAA (Figure 4). The pKa of long-chain PFAA are thought to range from less than zero to 1, indicating that they are almost completely ionized at environmentally and biologically relevant pH (Goss 2008a, 2008b). Despite being ionized, PFAS are bioavailable, and long-chain PFAA can accumulate in specific biological media to levels equivalent to lipid accumulation of neutral POPs. Given these observations, the question emerges of whether and how existing metrics for chemical accumulation in wildlife can be used to describe diverse PFAS. This formidable data gap hampers efforts to develop mechanistic models for exposure and risk assessment of PFAS, and stands as a major limitation to ecological exposure assessment of PFAS.

A synthesis of 513 laboratory-based and 931 field-based measurements indicates long-chain PFCA with 12-14 carbon-chain length generally exhibit the highest bioaccumulation potential, with whole-body BCF values ranging between 18,000-40,000 L/kg (Gobas 2020). Laboratory-based whole-body BCFs of PFCA with 8 to 11 carbon-chain lengths are generally much lower (BCF range: 4.0-4,900 L/kg). Similarly, PFOS exhibits relatively low laboratory-based whole-body BCFs, generally in the range of 100 to 1,000 L/kg. Field-based BAFs are generally in agreement with the laboratory-derived BCF values, but in some cases are somewhat higher, reflecting dietary accumulation.

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Generally, BCFs and BAFs (L/kg) of individual PFAAs in plankton, aquatic gill-ventilating invertebrates, and fish increase with increasing perfluoroalkyl chain length and hydrophobicity (Conдор 2008), though exceptions have been identified (Munoz 2017; Zhang 2019).

Avian and marine mammalian food webs exhibit the highest reported TMFs for PFAA (Kelly 2009; Tomy 2009). For example, the TMF for PFOS in these relatively long food webs containing air-breathing wildlife (e.g., marine birds and mammals) is approximately 20. TMFs in aquatic piscivorous food webs tend to be much lower. For example, TMFs of PFOS in the Lake Ontario aquatic piscivorous food webs range between 1.9 to 5.9 (Martin 2004; Houde 2008). Other studies have reported negligible biomagnification of PFOS in aquatic piscivorous food webs, with TMFs not substantially different than one (Loi 2011; Penland 2020). This behavior mirrors previous observations of food web-specific biomagnification of low K_{OW} -high K_{OA} moderately hydrophobic organic chemicals (Kelly 2007). In particular, PFOS and several other PFAS of concern, which are likewise moderately hydrophobic and poorly metabolizable substances, may not biomagnify extensively in aquatic food webs due to efficient respiratory elimination to water via gills. Conversely, these substances can biomagnify to a high degree in food webs containing air-breathing animals because elimination of these substances via lung-air exchange is negligible. More data are needed to refine these hypotheses and address variability across current data sets. Observed variability in TMFs for PFAA is likely due to selection of species, tissues, concentration normalization techniques, as well as the influence of site-specific conditions, life history stage, trophic condition of sampled individuals, and tissues and techniques used for stable isotope analyses.

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The contribution of PFAA precursors to field-based measurements of BAFs represents a major gap in understanding of PFAS bioaccumulation. For example, one study noted higher than expected accumulation of PFCA with five and six carbons in marine plankton from the Northwestern Atlantic and posited this reflects the accumulation of degraded precursor compounds (Zhang 2019). Another study that included liver tissues from marine mammals from the same region found a large fraction (30-75%) of unidentified organofluorine (Spaan 2020). Shrimp from a subtropical food-web in Hong Kong were similarly noted to have a high fraction of unknown fluorinated compounds (Loi 2011). Some precursor compounds behave more similarly to traditional POPs and may thus have enhanced bioaccumulation propensity (Dassuncao 2017). Additional data on bioaccumulation potential and health risks associated with unidentified organofluorine are thus needed, particularly as chemical manufacturing has shifted away from the legacy PFAS typically detected in biota (Section 2).

6.3. Modifications to bioaccumulation metrics for POPs needed for PFAS

Table 3 illustrates some potential modifications to key bioaccumulation metrics that better reflect the behavior of PFAS in biological systems. In deciding the appropriate metric to use and how it is to be defined (in what tissue, with what type of normalization) a key guiding question is: “For what purpose?” If a TMF is being calculated to understand the exposure of a predator organism to PFAS in its prey, it makes the most sense to use the concentration in the portion of the prey consumed by that organism to define the denominator. For example, one study calculated the BMF for PFAA in polar bears using the ratio of polar bear liver to ringed seal blubber concentrations (Boisvert 2019).

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Similarly, when considering BCFs in sport fish, PFAS concentrations in muscle tissue may be most relevant in establishing dietary guidelines for humans, but liver-specific BCFs may provide better insight for potential health consequences to the fish population itself.

Refining metrics to better capture the behavior of PFAS in biological systems requires a better understanding of how PFAS are transferred from external exposure sources to the internal environment, and how, once internalized, they distribute to different tissues and are eliminated (Figure 5). Presently there is little consensus on the tissue type (e.g., liver, kidney, muscle) that best represents PFAS bioaccumulation in wildlife and whether normalization of concentrations to protein or phospholipid is needed. For example, using tissue-specific measurements in fish, there were large variations in blood BCF and blood BAF compared to the analogous whole-body BAF and BCF whereas liver-based BCF and BAF were in the same order of magnitude for the whole body accumulation parameters (Martin 2003; Shi 2018).

PFAA are acidic compounds with low pKa, which means they are often associated with proteins (e.g., serum albumin) and phospholipids rather than storage lipids (Armitage 2012; Armitage 2013; Ng 2013, 2014; Chen 2016; Armitage 2017; Dassuncao 2019). As a result, PFAA accumulate primarily in the blood, liver, kidney, and brain of organisms rather than adipose tissue (Martin 2003; Ahrens 2009b; Borg 2010; Kowalczyk 2013; Dassuncao 2019). The affinity of different PFAA for proteins varies widely, suggesting binding-site-specific interactions and facilitated transport of some compounds. PFAA interact with protein molecules through a combination of polar, non-polar, and

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electrostatic interactions and not through covalent binding. Hence, sorption and desorption are dependent on thermodynamic gradients (i.e., chemical activity differences) in biological systems (Bischel 2010). Distribution of PFAS to structural (muscle) proteins is generally low, while binding to specific proteins (e.g. serum albumin, liver fatty acid binding protein) leads to tissue-specific accumulation patterns (Luebker 2002; Jones 2003; Martin 2003; Ahrens 2009b; Ng 2014). Phospholipids also play an important role in tissue specific partitioning (Armitage 2012). This is particularly true for the long chain PFAS and brain tissue, which is very high in phospholipid content (Dassuncao 2019). Thus, more refined approaches to account for tissue-specific uptake in bioaccumulation assessments are needed.

6.4 PFAS bioaccumulation modeling

Mechanistic bioaccumulation (i.e., toxicokinetic) models developed for neutral lipophilic contaminants such as polychlorinated biphenyls (PCBs) and organochlorine pesticides have been widely used by academics, risk assessment professionals, and regulatory authorities (Arnot 2004). Applications of these models include substance prioritization, screening-level risk assessments, development of environmental quality guidelines, total maximum daily loads, and other purposes. They use information on the physical-chemical properties of the compound of interest, as well as environmental and biological properties of the site and organisms. The success of these existing mechanistic approaches relies on good data quality on the partitioning properties of neutral compounds in biological media and tissues. Thus, a major challenge for PFAS is suitable data on their partitioning in biological compartments.

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One available approach for modeling fish bioaccumulation of ionizable organic compounds (IOCs, including PFAA) takes into account the role of thermodynamic gradients in tissue partitioning for PFAS (Armitage 2013). Specifically, the model is based on pH-dependent distribution ratios, including the chemical's octanol-water distribution ratio (D_{OW}) and membrane-water distribution ratio (D_{MW}), as well as the estimated distribution ratio between non-lipid organic matter (NLOM) and water. It provides a simple partitioning-based equation to predict steady-state concentrations of PFAA and other IOCs in aquatic organisms.

Strong relationships between empirically derived bioaccumulation metrics (BCF, BAF, TMF) and distribution ratios for protein-water (D_{PW}) and membrane-water (D_{MW}) of individual PFAA have been demonstrated for some ecosystems (Kelly 2009; Chen 2016). The relatively high degree of bioaccumulation of long-chain PFCA with 12-14 carbons in fish is likely attributable to high D_{MW} and D_{PW} (estimated values of 10^5 to $10^6 D_{MW}$ and 10^4 to $10^6 D_{PW}$). Thus, D_{PW} and D_{MW} are two key parameters that may be useful for predicting PFAS bioaccumulation potential (Table 3).

The NLOM component of the IOC model represents endogenous protein (Armitage 2013). While it performs well for many IOCs, it tends to underestimate the bioaccumulation capacity of some PFAA. This is likely due to the complex association of PFAS with proteins. Thus, a key to parameterizing this model is the difference in distribution ratios between neutral and ionized forms of the specific PFAS congener, which may require challenging experimental determinations. Further, different protein types can exhibit different sorptive capacities for IOCs (Henneberger 2016). For some

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compounds, protein-water distribution ratios (D_{pw}) in plasma protein (e.g., albumin) can be orders of magnitude greater than those in structural proteins (e.g., muscle protein). Thus, application of simple equilibrium partitioning-based models may require utilizing a series of distribution coefficients for different proteins (e.g., transporter protein-water distribution ratios (D_{TP-W}) and structural protein-water distribution ratios (D_{SP-W}). This approach was used to assess tissue-specific bioaccumulation of PFAA and other IOCs in laboratory exposed fish (Chen 2016, 2017a).

A chemical activity-based approach to ecological risk assessment bridges some gaps between traditional empirical modeling efforts and mechanistic models (Gobas 2017; Gobas 2018). This approach was used to assess bioaccumulation and exposure risks of several PFAS in wildlife at AFFF-impacted sites (Gobas 2020). The chemical activities of PFOS and other PFAA indicated that these compounds tend to primarily biomagnify in food webs composed of air-breathing wildlife (birds, mammals, terrestrial reptiles), compared to those comprising only aquatic organisms. For example, activity-based biomagnification factors in upper trophic wildlife (bird and mammals) were found to range between 10 and 20 and in aquatic organisms were close to 1, indicating negligible biomagnification. An advantage of this approach that is particularly relevant to PFAS is that it can be used effectively for both neutral and ionic substances, including anionic, cationic and zwitterionic compounds. A limitation of this approach is that solubility estimates and hence calculated activities are based on numerous assumptions regarding physicochemical properties, phase partitioning, protein-binding and toxicokinetics.

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Beyond simple partitioning-based models for substance screening, more sophisticated approaches may be required for higher resolution modeling. For example, IOC binding to intra- and extracellular protein (serum albumin, liver fatty acid binding protein), as well as membrane-associated organic anion transporters, may act to provide both enhanced sorption capacity and advective transport across biological membranes (Ng 2013). This affects uptake and elimination rates as well as tissue distribution, and helps explain the long elimination half-lives of PFAS in organisms.

Physiologically-based toxicokinetic (PBTK) models incorporating absorption, distribution, metabolism, and excretion (ADME) metrics have been developed to assess toxicokinetics of PFOS and PFOA in various animal models, including fish and mammals (Andersen 2006; Tan 2008; Consoer 2014; Fabrega 2014; Consoer 2016; Fabrega 2016; Cheng 2017; Khazaei 2018). PBTK models are particularly useful for assessing the influence of membrane transporters, and revealing important challenges in equilibrium modeling of PFAS bioaccumulation. For example, laboratory-based evaluations of bioconcentration (e.g. the OECD 305 test), a kinetic BCF can be defined as the ratio of uptake and elimination rates (k_1/k_2). However, the elimination rate for PFAS can be affected by saturable transporter-mediated renal uptake and clearance, as has been observed in rats (Han 2012) and monkeys (Andersen 2006), so that the k_2 rate becomes dose-dependent. Therefore, observations both in the laboratory and in the field (e.g. at highly contaminated sites) may be concentration dependent and thus not representable by first-order kinetics.

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6.5 *In vitro* and *in silico* methods to support modeling and assessment

As more focus is placed on molecular interactions between PFAS and biomolecules including proteins, transporters, and phospholipids, a variety of *in vitro* and analytical methods have been developed to support both laboratory assessments and the parameterization of mechanistic models. *In vitro* methods include cell- and vesicle-based assays to estimate and compare uptake rates into cells via passive diffusion and active transport (Luebker 2002; Katakura 2007; Nakagawa 2008; Weaver 2010; Herédi-Szabó 2012), and protein-binding assays using equilibrium dialysis (Bischel 2010; Bischel 2011), fluorescence displacement (Chen 2009; MacManus-Spencer 2010; Yang 2020), and other methods (MacManus-Spencer 2010). A key challenge of these approaches is high variability across studies, which can lead to order of magnitude differences in estimated binding affinities for the same PFAS-protein combinations (MacManus-Spencer 2010; Ng 2014). Recently, a hybrid approach used size exclusion column co-elution (SECC) with non-target mass spectrometry to identify L-FABP (liver fatty acid binding protein) ligands from complex PFAS mixtures (Yang 2020). The method identified 31 new L-FABP ligands from AFFF. Given the known importance of this protein to liver accumulation of PFAS in diverse organisms, this method illustrates the potential for combining non-target analysis with bioaccumulation potential screening.

In silico methods have been developed to predict the behavior of PFAS at the molecular level in biological systems. These methods have an advantage of high throughput testing and are not hindered by the lack of available standards and samples. Molecular docking and molecular dynamics are computational approaches originally

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developed for drug discovery, and are powerful tools for the prediction of protein-ligand interactions.

They have now been used to screen a number of legacy and emerging PFAS for binding with serum albumin (Salvalaglio 2010; Ng 2015), L-FABP (Zhang 2013; Ng 2015; Cheng 2018; Yang 2020), and peroxisome proliferator activated receptors (PPARs), which are thought to be linked to some of the toxic effects of PFAS (Li 2019). Predicted binding affinities from these kinds of studies are highly correlated with experimental observations and can be used to help parameterize mechanistic PBTK models. However, we note the predicted binding affinities are overestimated for perfluoroalkylsulfonamide and may still require anchoring to some experimental binding data.

The role of phospholipids and related application of D_{MW} in PFAS toxicokinetics and tissue has not been fully realized. *It* vitro assay protocols to assess phospholipid partitioning using laboratory-prepared liposomes (Escher 2000) are applicable to determining D_{MW} for PFAS. Solid-supported phosphatidylcholine (PC) membranes can also be applied for generating empirical data for D_{MW} for PFAS such as TRANSIL-XL membrane affinity assay kits (Droge 2019).

Finally, more empirical computational methods attempt to build predictive relationships between PFAS structure and their bioaccumulation potential or toxicity based on available data rather than mechanistic process-based approaches. These include quantitative structure activity relationships (QSARs) for predicting key physicochemical properties for PFAS, as recently reviewed (Lampic 2020), and a machine learning-based

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approach to classifying the potential bioactivity of thousands of PFAS (Cheng 2019). Hybrid in vitro/in silico approaches that generate data with which to develop a QSAR have also been used, for example to tackle the important subject of PFAS mixture toxicity (Hoover 2019).

7. SUMMARY AND KEY RESEARCH GAPS

In this manuscript, we reviewed current understanding of: (a) PFAS sources and environmental transport pathways, (b) analytical methods used to assess PFAS exposures and their strengths and limitations, (c) methods for assessing human exposures and the relative importance of different sources and pathways, and (d) PFAS bioaccumulation in wildlife. Changing geographic locations of PFAS manufacturing and the shifting chemical landscape poses a number of challenges for exposure assessments for humans and wildlife. Synthesis of analytical standards for newer PFAS has lagged their production and release and thus non-targeted methods, suspect screening and total fluorine assessments have all emerged as essential tools for understanding the total burden of PFAS in the environment, humans and wildlife. Although such data are now appearing across all media, more data are needed, particularly for food items, PCPs, dust, drinking water, and wildlife tissues that contain PFAS. For both humans and wildlife, additional research is needed to characterize the fraction of exposure originating from precursors that degrade into terminal PFAA that have been linked to adverse health effects. Existing literature on time trends in PFAS in biological tissues suggest that while some legacy PFAS have decreased over the past two decades, the percent of unidentifiable EOF has increased. There is a need for greater constraints on residual

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content (i.e. unbound fluorinated surfactants, monomers) in fluoropolymers, side-chain polymers and ether-based polymers, and for more data on their contribution to PFAS exposure. Measurement of polymers in environmental media remains an important challenge.

A major focus of past research has been contaminated sites where the highest concentrations in the environment and biota are often found. Moving forward, a better understanding of atmospheric PFAS sources and potential resulting exposures is needed, particularly as treatment systems are introduced for contaminated drinking water supplies. Integration of PFAS measurements into long-term atmospheric monitoring for pollutants would be valuable for measuring changes in atmospheric PFAS concentrations, aiding model development, and identifying vulnerable regions.

Occupational health effects associated with human PFAS exposures have been reported for individuals who worked in fluorochemical production plants. There is a reasonable consensus that near contaminated sites, drinking water is often the main pathway for human exposure to PFAS. However, data are still sparse on exposure sources for the general population and for communities where drinking water contamination has been remediated. Presently, there is a paucity of data on the indoor environment where many PFAS-containing products are found. Dermal exposures to PFAS from dust and PCP warrant additional consideration given the extremely high concentrations reported for some PCP and dust. There is general agreement that dietary exposure is the major contributor to population exposure for PFOS and PFOA, but more limited evidence for other PFAS, including that contributed by food processing and packaging. More

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information is needed about the contribution of food processing and packaging to PFAS in food.

Most prior work on human exposure pathways has not included statistically representative population surveys. Future PFAS researchers may benefit from establishing collaborations with existing representative populations. The field needs total exposure studies seeking to characterize all pathways and routes of exposure to PFAS including precursors. Empirical TK data needed to describe PFAS partitioning among tissues, facilitated transport and elimination half-lives are still limited or non-existent for many PFAS and represent an important research need. As the focus of PFAS research shifts toward issues associated with emerging compounds, additional data for parameterizing TK models represent a critical research need.

For wildlife exposures, a major challenge is adapting existing metrics for chemical accumulation to describe diverse PFAS. This data gap hampers efforts to develop mechanistic models for exposure and risk assessment of PFAS. Early PFAS bioaccumulation modeling suggested that some PFAS are efficiently eliminated through the gills of water breathing organisms but biomagnify to a greater degree in air-breathing organisms due to limited lung-air exchange (Kelly 2007). Mechanistic bioaccumulation models for PFAS are needed to understand the tissue accumulation patterns for novel PFAS, including neutral precursors and intermediates. Non-traditional approaches to probing molecular mechanisms of bioaccumulation such as protein association have important impacts on predicting tissue distribution and internal dose but also can have organismal effects if PFAS are able to perturb endogenous functions. Tissue and cellular

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partitioning of PFAS can be used to inform models; current wildlife monitoring is primarily limited to liver or muscle or blood.

Early research on PFAS releases and global transport was conducted as a partnership between industry and academia. We thus recommend greater transparency and collaboration between academia, industry and government to prioritize and then improve understanding of global environmental releases of the thousands of PFAS structures present in global commerce. One example of improved transparency could be a commitment by industry to release information on the chemical structures and analytical standards for new PFAS used in commerce. Better characterizing PFAS exposure sources for humans and wildlife is an essential first step in the design of effective risk mitigation strategies.

Acknowledgment—This manuscript was developed by participants in the Exposure Assessment Panel as part of the SETAC North America Focused Topic Meeting on Environmental Risk Assessment of PFAS held August 12-15, 2019. WH-B acknowledges funding from the NIEHS Superfund Research Program (P42ES007381). CN acknowledges support from the U.S. National Science Foundation (NSF: 1845336). AR acknowledges financial support from the U.S. National Oceanic and Atmospheric Administration (NOAA) Dr. Nancy Foster Scholarship program (NA17NOS4290028) and the NIEHS Superfund Research Program (P42ES027706). EMS acknowledges support from NIEHS (P42ES027706) and the Strategic Environmental Research and Development Program (SERDP, ER18-1280). TFW was supported in part by NIEHS (R01ES027813, R01ES028800). MS acknowledges financial support from the North

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Carolina PFAS Testing Network through the North Carolina Policy Collaboratory.

Positions or statements expressed herein do not necessarily reflect organizational views of the authors. All coauthors contributing to synthesizing information and writing this review. AOD and EMS led the synthesis team.

Data availability statement—Data, associated metadata, and calculation tools are available from the corresponding author (ems@seas.harvard.edu).

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56 pages of references omitted for brevity – available upon request

Figure 1. Conceptual representation of key emission sources and global transport pathways of perfluoroalkyl acids (PFAA) and their polyfluorinated precursors.

Figure 2. Components of the fluorine mass balance. Panel a) shows a conceptual diagram representing the relative fractions of total fluorine (TF) including fluoride, unextracted organofluorine, extractable organofluorine (EOF), and targeted PFAS. Panel b) shows actual data where unknown EOF was determined using targeted analysis of 50 PFAS congeners in blood plasma of first time mothers from Uppsala, Sweden (data from Miaz 2020).

Figure 3. Schematic of exposure assessment steps for humans that relates PFAS sources to exposure media, and internal concentrations of PFAS in blood. Not all possible exposure routes (e.g., outdoor air) or arrows are shown. ADME = Absorption, distribution, metabolism and excretion.

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Figure 4. Key bioaccumulation processes, metrics and gaps associated with PFAS in wildlife. BMR = basal metabolic rate; BAF = bioaccumulation factor; BMF = biomagnification factor; TMF = trophic magnification factor; BCF = bioconcentration factor; BSAF = biota-sediment accumulation factor.

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Figure 5. Key elements for predicting the relationship between external exposure and internal dose of wildlife and human to PFAS. The internal distribution and dose are driven by the balance of absorption, distribution, metabolism, and elimination (ADME). For PFAS, unlike for neutral organic chemicals, internal distribution is substantially influenced by protein binding and transporter uptake and efflux, leading to accumulation in the liver and blood and species and sex-specific elimination half-lives.

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Graphical Abstract: Methods for assessing human and wildlife exposures to per- and polyfluoroalkyl substances are reviewed along with current understanding of exposure sources and pathways.

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Tables

Table 1. Comparison of analytical techniques for assessing PFAS exposure.^a

Methods	Applicable matrices	Advantages	Limitations
Targeted analysis	All matrices	<ul style="list-style-type: none"> • Sensitive (0.1–1 ng/L) • High selectivity to the analysis targets 	<ul style="list-style-type: none"> • Limited inclusivity • Variable recoveries • Potential bias from sample extraction
Non-targeted analysis^b	All matrices	<ul style="list-style-type: none"> • High inclusivity • Elucidate unknown structures 	<ul style="list-style-type: none"> • Not quantitative • Potential bias from sample extraction • Need expensive instruments and highly skilled users
TOP	Mainly aqueous samples	<ul style="list-style-type: none"> • Sensitive (0.1–1 ng/L) • High selectivity to PFAS 	<ul style="list-style-type: none"> • Limited inclusivity • Variable recoveries
EOF (CIC)	All matrices	<ul style="list-style-type: none"> • High selectivity to organofluorine 	<ul style="list-style-type: none"> • Less sensitive (0.1–0.5 ppm F) than targeted analyses • Potential bias from sample extraction
AOF (CIC)	Water	<ul style="list-style-type: none"> • High selectivity to organofluorine 	<ul style="list-style-type: none"> • Not as sensitive (0.1–0.5 ppm F)
PIGE	Solids	<ul style="list-style-type: none"> • High-throughput • Non-destructive 	<ul style="list-style-type: none"> • Not as sensitive (50 nmol F/cm²)

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		<ul style="list-style-type: none"> No matrix effects Surface measurement (100-250 μm) 	<ul style="list-style-type: none"> Interference by fluoride
XPS	Solids	<ul style="list-style-type: none"> High-throughput Widely-available instrumentation Identification of perfluoroalkyl moiety Etching of surface possible to create depth profiles Surface measurement (~10 nm) 	<ul style="list-style-type: none"> High detection limits (~1% F) Small area measurement Can be affected by surface roughness and inhomogeneity

^aA more comprehensive discussion of the strengths and limitations of different analytical techniques is reviewed elsewhere (Guelfo 2020).

^bFor a more detailed discussion of the strengths and limitations of non-targeted analysis please see the following viewpoints and response (Hites 2018, 2019; Samanipour 2019).

Table 2. Literature estimates of source contributions (%) to adult exposures to PFAS^a

PFAS	Carbon length	Exposure Medium ^b				Exposure Route ^b			Study Location	Ref.
		Diet	Dust	Water	Consumer goods	Inhalation	Dermal	Indirect		
PFBA	4		4	96					NA	c
PFHxA	6	38	4	38		8		12	NA	c
PFHxA	6	87	4			2			Norway	d
PFHxS	6	57	38			5			Finland	e
PFHxS	6	94	1						Norway	d
PFHpA	7	93	1						Norway	d
PFHpS	7								Norway	d
					100					

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PFOA	8	16	11		58	14		NA & EU	f
PFOA	8	85	6	1	3		4	Germany Japan	g
PFOA	8	77	8	11		4		Norway	h
PFOA	8	66	9	24		<1	<1	US	i
PFOA	8	41		37			22	Korea	j
PFOA	8	99		<1				China	k
PFOA	8	47	8	12		6	27	NA	c
PFOA	8	95	<2.5			<2.5		Finland	e
PFOA	8	89	3			2		Norway	d
PFOA	8	91		3		5		Ireland	l
PFOS	8	66	10	7		2	16	NA	c
PFOS	8	72	6	22		<1	<1	US	m
PFOS	8	96	1	1		2		Norway	h
PFOS	8	81	15		4			NA & EU	f
PFOS	8	93		4			3	Korea	j
PFOS	8	100		<1				China	k
PFOS	8	95	<2.5			<2.5		Finland	e
PFOS	8	75				3		Norway	d
PFOS	8	100						Ireland	l
PFOPA	8							Norway	d
		100							
PFNA	9	79						Norway	d
			5			1			
PFDA	10	51						NA	c
			2	4		15	28		
PFDA	10	78						Norway	d
			1			2			
PFDS	10							Norway	d
			89		4				
PFUnDA	11	61	4			1		Norway	d
PFDoDA	12	86	2			4		NA	c
				2			5		
PFDoDA	12	48	15					Norway	d

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NA = North America; EU = Europe.

^a Adapted from (Sunderland 2019), and updated with more recent publications.

^b Where available, central tendency values are presented.

^c Data from (Gebbink 2015). Data shown here are based on the intermediate exposure scenario in Fig. 3 in their manuscript.

^d Data from (Poothong 2020)

^e Data from (Balk 2019); Values represent modeled exposures for children at 10.5 years of age.

^f Data from (Trudel 2008). Values shown here are based on the high exposure scenarios from Figs. 2 and 5.

^g Data from (Vestergren 2009). Values shown here are for the background population exposure from Figure 4a in their manuscript.

^h Data from (Haug 2011). Values shown here are based on the 50th percentile exposure scenario for women and the mid-range scenario for dust exposure.

ⁱ Data from (Lorber 2011). Data shown here are based on pathway specific intake estimates for adults.

^j Data from (Tian 2016). Data shown here are for adult exposures based on Fig. 4 in their manuscript.

^k Data from (Shan 2016). Data shown here are based on summed estimated daily intakes.

^l Data from (Harrad 2019).

^m Data from (Egeghy 2011). Values shown here represent the typical environmental exposure scenario shown in Fig. 3 in their manuscript.

Table 3. Modifications to persistent organic pollutant bioaccumulation metrics for PFAS.

Metric	Traditional Indicator	PFAS-Specific Recommendation
K_{OW} , K_{OA}	Used as surrogates for equilibrium partitioning of neutral organic chemicals to lipid tissues of aquatic and air-breathing organisms.	<p>D_{OW}: Octanol-water distribution ratio (takes degree of ionization into account);</p> <p>D_{MW}: Membrane-water partition ratio;</p> <p>Chemical activity ratios;</p> <p>K_{PW}: Protein-water partition coefficient;</p> <p>K_A, K_D: Equilibrium association and dissociation constants for specific proteins (e.g. albumin, LFABP)</p> <p><i>Key gap: relevant metric for air-breathing organisms.</i></p>
BCF: bioconcentration factor (waterborne or airborne exposure only)	Concentration in organism (whole body, lipid-normalized)/concentration in water (freely dissolved)	Concentration (chemical activity) ^l in serum/ concentration in water;
BAF: bioaccumulation factor (waterborne/airborne and/or dietary exposure)		Concentration in liver/ concentration in water;
		Concentration in organism (whole body)/concentration in water.

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<i>Key gap: (1) Selecting appropriate tissue to represent accumulation in organism. (2) Accounting for contributions of precursors to field based BAFs.</i>		
BMF: biomagnification factor	Concentration in predator (whole body, lipid-normalized)/concentration in prey (whole body, lipid-normalized)	Concentration in predator liver /concentration in prey liver;
TMF: trophic magnification factor		Concentration in predator (whole body) / concentration in prey (whole body);
<i>Key gaps: Selecting appropriate predator and prey tissues across food webs.</i>		

¹Emerging approach: activity-based metrics. See section 6.4.

APPENDIX B

TABLE OF PFAS ACRONYMS

This appendix contains the full name, abbreviations, molecular structure, CAS number, and potential uses of PFAS measured within dissertation.

Compound	Abbreviation	Chemical Formula	CAS #	PFAS Family after Buck et al. 2011	Some Potential Sources or Uses
Perfluorobutyric acid	PFBA	C4HF7O2	375-22-4	PFCA	Chromatographic reagent, manufacturing of photographic film, breakdown product of precursors or other PFCA
Perfluoropentanoic acid	PFPeA	C5HF9O2	2706-90-3	PFCA	Stain and grease proof coatings on food packaging, couches, and carpets, breakdown product of precursors and other PFCA
Perfluorobutanesulfonic acid	PFBS	C4HF9O3S	375-73-5	PFSA	Stain repellent; Replacement for PFOS
Perfluorohexanoic acid	PFHxA	C6HF11O2	307-24-4	PFCA	Breakdown product of PFHxS
1-Octanesulfonic acid, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-	4:2-FTS	C8H5F13O3S	27619-97-2	FTS	Potential impurity of 6:2 FTS creation
1-Pentanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,5-undecafluoro-	PFPeS	C5HF11O3S	2706-91-4	PFSA	Firefighting foams, stain and grease proof coatings on carpets and other textiles
Perfluoroheptanoic acid	PFHpA	C7HF13O2	375-85-9	PFCA	Surfactant
Perfluorohexanesulfonic acid	PFHxS	C6HF13O3S	355-46-4	PFSA	Firefighting foam
Perfluorooctanoic acid	PFOA	C8HF15O2	335-67-1	PFCA	Nonstick coatings, general surfactant and polymerization/processing aid
1-Octanesulfonic acid, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-	6:2-FTS	C8H5F13O3S	27619-97-2	FTS	Firefighting foam, mist suppressant
Perfluoroheptanesulfonic acid	PFHpS	C7HF15O3S	375-92-8	PFSA	Firefighting foams, stain and grease proof coatings on carpets and other textiles
Perfluorononanoic acid	PFNA	C9HF17O2	375-95-1	PFCA	Performance plastic production, general surfactant and processing aid
Perfluorooctanesulfonamide	FOSA	C8H2F17NO2S	754-91-6	FASA	Used as raw material in the electrochemical fluorination process to make substances that are used for surfactants and surface treatments. FASAs can degrade to form PFAs such as PFOS
Perfluorooctanesulfonic acid	PFOS	C8HF17O3S	1763-23-1	PFSA	Firefighting foams, stain and grease proof coatings on carpets and other textiles
Perfluorodecanoic acid	PFDA	C10HF19O2	335-76-2	PFCA	Rarely used in commercial products directly. Breakdown or process byproduct of stain and grease proof coatings, other PFCA surfactant uses
3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecanesulphonic acid	8:2-FTS	C10H5F17O3S	39108-34-4	FTS	Mist suppression, metal plating
Perfluorononanesulfonic acid	PFNS	C9HF19O3S	68259-12-1	PFSA	Impurity of production
Perfluoroundecanoic acid	PFUnDA	C11HF21O2	2058-94-8	PFCA	Rarely used in commercial products directly. Breakdown or process byproduct of stain and grease proof coatings, other PFCA surfactant uses
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	C11H6F17NO4S	2355-31-9	FASAA	Food packaging, coatings
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	C12H8F17NO4S	2991-50-6	FASAA	
Perfluorodecanesulfonic acid	PFDS	C10HF21O3S	335-77-3	PFSA	Impurity of production
Perfluorododecanoic acid	PFDoA	C12HF23O2	307-55-1	PFCA	Rarely used in commercial products directly. Breakdown or process byproduct of stain and grease proof coatings, other PFCA surfactant uses
Perfluorotridecanoic acid	PFTtDA	C13HF25O2	72629-94-8	PFCA	Replacement for PFOA. Surfactant and polymerization aid used in the production of PTFE.
Perfluorotetradecanoic acid	PFTeDA	C14HF27O2	376-06-7	PFCA	
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	HFPO-DA	C6HF11O3	13252-13-6	PFEA	Replacement for PFOA. Nonstick coatings, general surfactant and processing aid
Perfluoro-2-ethoxypropanoic acid	PEPA	C5HF9O3	267239-61-2	PFEA	
Perfluoro-2-methoxypropanoic acid	PMPA	C4HF7O3	13140-29-9	PFEA	
Perfluoro-3,5-dioxahexanoic acid	PFO2HxA	C4HF7O4	39492-88-1	PFEA	
Perfluoro(3,5,7-trioxaoctanoic) acid	PFO3OA	C5HF9O5	39492-89-2	PFEA	
Perfluoro-3,5,7,9-tetraoxadecanoic acid	PFO4DA	C6HF11O6	39492-90-5	PFEA	
Perfluoro(3,5,7,9,11-pentaoxadecanoic) acid	PFO5DoDA	C7HF13O7	39492-91-6	PFEA	Byproduct of the production of the Nafion ionomer
2-[1-[difluoro[(1,2,2-trifluoroethenyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoroethanesulfonic acid,	Nafion by-product 1 (BP1)	C7HF13O5S	29311-67-9	PFEA	
2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoroethanesulfonic acid	Nafion byproduct 2 (BP2)	C7H2F14O5S	749836-20-2	PFEA	
2,2,3,3,4,4,5,5,5,5-tetrafluoro-2-sulfoethoxy)pentanoic acid	Nafion byproduct 4 (BP4)	C7H2F12O6S	na	PFEA	
1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoro-ethoxy)ethane sulfonate	NVHOS	C4H2F8O4S	801209-99-4 (free acid)	PFEA	
1,2,2,3,3,4,4,5,5,6,6-decafluoro-4-(1,1,2,2,2-pentafluoroethyl)-cyclohexanesulfonic acid,	PFECHS	C8HF15O3S	646-83-3	cyclic PFSA	Erosion inhibitor in aircraft hydraulic fluids
4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Pentadecafluorodecanoic Acid	7:3 FTCA	C10H5F15O2	812-70-4	FTCA	Formed via oxidation of 8:2 FTOH, a fluorotelomer alcohol commonly used in textile production