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FINAL REPORTS
OF THE

Tibor T. Polgar Fellowship Program 2020

Sarah H. Fernald,
David J. Yozzo, and
Helena Andreyko
Editors

A JOINT PROGRAM OF:

Hudson River Foundation
for Science and
Environmental Research

NYS Department
of Environmental
Conservation

Hudson River
National Estuarine
Research Reserve

REPORTS OF THE TIBOR T. POLGAR

FELLOWSHIP PROGRAM, 2020

Sarah H. Fernald, David J. Yozzo,
and Helena Andreyko

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A Joint Program of
The Hudson River Foundation
and The New York State
Department of Environmental Conservation

December 2021

ABSTRACT

Four studies completed within the Hudson River Estuary under the auspices of the Tibor T. Polgar Fellowship Program during 2020 have been included in the current volume. Major objectives of these studies included: (1) documenting skin lesion prevalence in bottlenose dolphins in the New York-New Jersey Harbor Estuary as a proxy for evaluating environmental stress, (2) evaluating the impact of urban pollution on fish microbiomes, (3) assessing the presence of *Legionella pneumophila* in saline and fresh urban and suburban water bodies and street runoff at multiple locations along the Hudson River Estuary, (4) utilizing microsatellite DNA markers to determine the contribution of Hudson River striped bass to the Montauk mixed stock recreational fishery.

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PREFACE

The Hudson River estuary stretches from its tidal limit at the Federal Dam at Troy, New York, to its merger with the New York Bight, south of New York City. Within that reach, the estuary displays a broad transition from tidal freshwater to marine conditions that are reflected in its physical composition and the biota its supports. As such, it presents a major opportunity and challenge to researchers to describe the makeup and workings of a complex and dynamic ecosystem. The Tibor T. Polgar Fellowship Program provides funds for students to study selected aspects of the physical, chemical, biological, and public policy realms of the estuary.

The Polgar Fellowship Program was established in 1985 in memory of Dr. Tibor T. Polgar, former Chairman of the Hudson River Foundation Science Panel. The 2020 program was jointly conducted by the Hudson River Foundation for Science and Environmental Research and the New York State Department of Environmental Conservation and underwritten by the Hudson River Foundation. The fellowship program provides stipends and research funds for research projects within the Hudson drainage basin and is open to graduate and undergraduate students.

Prior to 1988, Polgar studies were conducted only within the four sites that comprise the Hudson River National Estuarine Research Reserve, a part of the National Estuarine Research Reserve System. The four Hudson River sites, Piermont Marsh, Iona Island, Tivoli Bays, and Stockport Flats exceed 4,000 acres and include a wide variety of habitats spaced over 100 miles of the Hudson estuary. Since 1988, the Polgar Program has supported research carried out at any location within the Hudson estuary.

The work reported in this volume represents four research projects conducted by Polgar Fellows during 2020. These studies meet the goals of the Tibor T. Polgar Fellowship Program to generate new information on the nature of the Hudson estuary and to train students in estuarine science.

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PHOTO IDENTIFICATION AND SKIN LESION PREVALENCE OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS)* IN THE NEW YORK-NEW JERSEY HARBOR ESTUARY

A Final Report of the Tibor T. Polgar Fellowship Program

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Trabue, S.G., M.L. Rekdahl, and H.C. Rosenbaum. 2021. Photo-identification and Skin Lesion Prevalence of Bottlenose Dolphins (*Tursiops truncatus)* in the New York-New Jersey Harbor Estuary. Section I: 1-30 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2020. Hudson River Foundation.

ABSTRACT

Investigating species health helps scientists monitor their well-being within an environmental context. The health of sentinel species can reflect environmental stressors and be used to infer ecosystem health. Due to their life history traits, cetaceans can serve as sentinel species in marine environments. In cetaceans, one method for determining individual and population health is by examining skin conditions. For two decades, skin lesions have been increasingly documented in coastal populations of bottlenose dolphins (*Tursiops truncatus*) worldwide. Lesion presence indicates underlying disease or diminished health and can reflect environmental stressors. Here, lesion prevalence of bottlenose dolphins in the New York-New Jersey Harbor Estuary was documented during their seasonal presence from spring to fall. Photographs of distinct individuals sighted from May to October 2017-2020 were compiled into a catalog and skin lesions were categorized and counted. Annually, the lowest lesion prevalence was in 2018 (P = 0.28) and highest in 2017 (P = 0.48). By month, prevalence decreased from spring to fall. Overall lesion prevalence in this population was lower than reported estimates for North Carolina, South Carolina, and Georgia, and roughly equivalent to Florida. The most common lesion types observed have been associated with viral infections and may be exacerbated by environmental stressors. This research establishes an important baseline for further studies into bottlenose dolphin population health in the New York-New Jersey Harbor Estuary and surrounding waters. Understanding the health of the bottlenose dolphin population in this heavily human dominated area is particularly important in the face of continued expansion of anthropogenic activities, including those related to forthcoming offshore wind development.

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INTRODUCTION

There is currently worldwide concern about the health of the oceans (Borja et al. 2020) as the effects of climate change and environmental degradations become apparent across the globe (Bossart 2010). In order to better understand marine ecosystem health and the potential impacts of environmental stressors, a range of species have been selected based on their life history traits to serve as sentinels (Fossi and Panti 2017). These sentinel species can signal when there is cause for concern for wildlife and humans alike, which can subsequently inform management efforts (Fossi and Panti 2017). In marine systems, bottlenose dolphins (*Tursiops truncatus*) can be considered sentinel species for coastal ecosystems, as they are long-lived, easily-observed apex predators that are accessible for monitoring, and they are known to concentrate contaminants from the environment with potential health consequences (Wells et al. 2004; Bossart 2010; Fossi and Panti 2017). By understanding the health of this species within the context of their environment, inferences can be made regarding the health of the habitat they are utilizing and conservation measures can be established (Wells et al. 2004; Fossi and Panti 2017). While there are many logistical challenges to assessing dolphin health in the wild, the presence of skin conditions or lesions provides a visible morphological indicator of potential underlying disease (Duignan et al. 2020).

Skin lesions have been documented in bottlenose dolphin populations worldwide for the past two decades (Wilson et al. 1999), yet little is known about the pattern of development and the distribution of lesions on individuals and across populations. Although typically nonlethal, skin lesions are considered to be an indicator of disease or diminished health, and have been found in association with unusual mortality events for inshore dolphins (Bearzi et al. 2009; Toms et al. 2020). Lesions may be viral, fungal, or bacterial in origin and their prevalence and severity

can be influenced by environmental factors, such as sea surface temperature and salinity, and anthropogenic influences, including chemical pollutants (Wilson et al. 1999; Bearzi et al. 2009; Hart et al. 2012). Therefore, skin lesion prevalence may be an indicator of environmental or anthropogenic stressors in the ecosystem that may lead to individual and/or population level health concerns (Taylor et al. 2020; Toms et al. 2020). Furthermore, lesion types have varying etiologies (Toms et al. 2020). When considering bottlenose dolphins as sentinel species, it is important to consider the critical differences in development and spread across skin lesion types. Some lesion types appear to be more strongly correlated with environmental conditions than others; thus, inferences about population or ecosystem health may vary based on lesion types observed.

Due to the macroscopic qualities of skin lesions, photo-identification ("photo-ID") methods have been co-opted for analyzing lesions on bottlenose dolphin individuals and calculating prevalence of lesions across populations (Taylor et al. 2020). Photo-ID has been increasingly used to monitor marine mammal populations as it offers a cost-effective, non-invasive approach and can additionally be used as a baseline for comparing lesion prevalence across space and time (Urian et al. 2014; Toms et al. 2020). While photo-ID has limitations, including inaccessibility to certain parts of the dolphin's body and lack of a conclusive etiology, this method nonetheless provides a useful, straightforward approach for characterizing visible skin lesion types and calculating a minimum prevalence estimate (Taylor et al. 2020; Hart et al. 2012).

Bottlenose dolphins are present in the New York-New Jersey Harbor Estuary ("Harbor Estuary" throughout) from spring to fall, migrating south to North Carolina during the cold weather months where they overlap with other Atlantic stocks (Hayes et al. 2018). These

bottlenose dolphins belong to the Western North Atlantic Northern Migratory Coastal Stock, which is estimated to have over 6,500 individuals (Hayes et al. 2018). Anecdotally, bottlenose dolphins have been observed both more frequently and for an extended period in and around the Harbor Estuary in recent years (Stinnette et al. 2018), potentially due to efforts to restore the habitat (Taillie et al. 2020); however, little is known about the population in the Harbor Estuary and how they interact with this habitat.

The area in which bottlenose dolphins are typically found in and around the Harbor Estuary also encompasses the largest port on the Eastern seaboard and is exposed to intense vessel traffic (Port Technology 2020). Other maritime industries found in the Harbor Estuary and surrounding waters include fishing, tourism, and planned offshore renewable energy (Pirani et al. 2018; BOEM 2020); therefore, there is considerable overlap between habitat use by bottlenose dolphins and human use of these waters. Though this ecosystem is exposed to high levels of anthropogenic disturbance, no previous analyses of skin lesion prevalence have been conducted on the bottlenose dolphins in the Harbor Estuary. The Harbor Estuary also experiences seasonal fluctuations in sea surface temperature (Balcom et al. 2008) and variations in salinity due to the oceanic flushing during tides and freshwater contributions from its many tributaries, including the mainstem Hudson River (Pirani et al. 2018). Both of these environmental characteristics have been previously linked to fluctuations in skin lesion prevalence in dolphins (Wilson et al. 1999).

Given the extent of bottlenose dolphin presence in the Harbor Estuary and the level of anthropogenic disturbance, the aim of this project was to gain a better understanding of population health in the bottlenose dolphins in the Harbor Estuary by characterizing the types of skin lesions present and variations in skin lesion prevalence by season. Given that the estuarine

environment in this study experiences fluctuations in salinity and sea surface temperature, both of which are considered natural stressors that contribute to lesion development, their influence on lesion prevalence was also investigated (Wilson et al. 1999; Hart et al. 2012). Lastly, comparing skin lesion prevalence during and before the global COVID-19 pandemic provided a unique opportunity to investigate whether lesion prevalence may be correlated with a period of reduced marine traffic (March et al. 2020).

METHODS

Data Collection and Building a Photo-Identification Catalog of Individuals

Non-systematic, vessel-based surveys were conducted in the Harbor Estuary from May to October in 2017-2020. Vessels ranged in size from 9-15 m, and maintained survey speeds of 13-17 knots during daylight hours and Beaufort sea states of $\delta 3$. Trained observers were stationed around the vessel such that a clear 360° view was maintained throughout the survey. Once bottlenose dolphins were sighted, they were approached to collect photographs (Figure 1). Photographs were collected using a Nikon D2700 digital camera with a Nikon AF-S Nikkor 70-200mm lens.

All photographs containing bottlenose dolphins were analyzed using Adobe Bridge and categorized based on photo quality and the distinctiveness of the individual. Photographs of insufficient quality (e.g. blurry or not suitable for identification) were excluded from the analysis and the remaining photographs were subsequently sorted by individual distinctiveness using natural markings (Urian et al. 2014). Because this population migrates across large distances, the probability of resighting an individual is reduced compared to smaller, resident populations (Urian et al. 2014). To ensure that resights were true positives, a high threshold for individual

distinctiveness was used; only individuals with natural markings that were distinct enough to eliminate the potential for "twin" individuals were included (Urian et al. 2014). Each photo containing identifiable individuals was cropped to include just the body of the individual and the individual was given a unique ID. A catalog of unique individuals was built by comparing all the distinctive fins to one another; each distinctive fin was either matched to an individual in the catalog and noted as a resight or added to the catalog as a new individual.

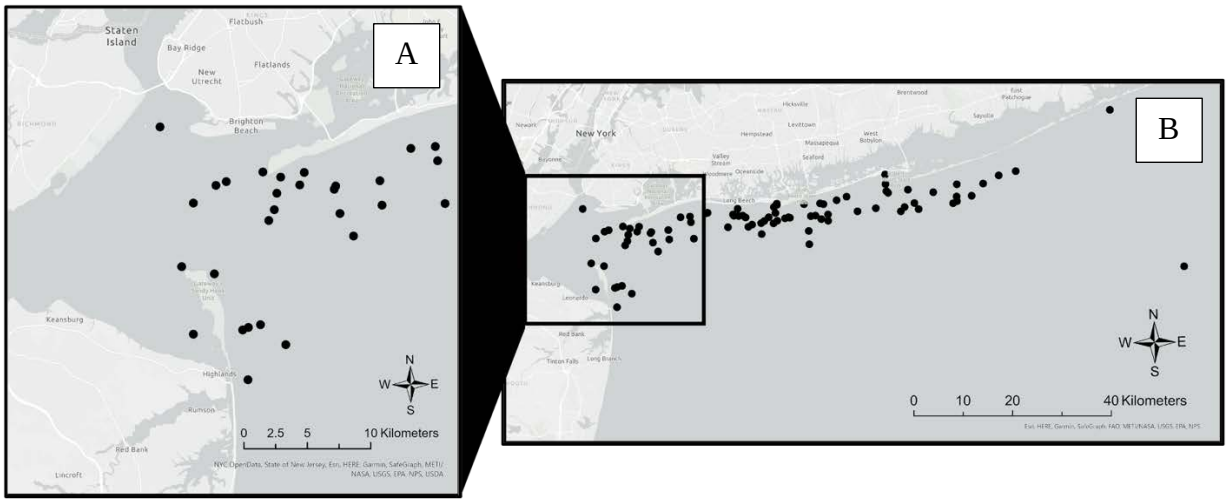

Figure 1. Bottlenose dolphin sightings in the New York-New Jersey Harbor Estuary (A) and surrounding waters (B) from 2017-2020.

Skin Lesion Categorization

Only catalogued individuals were analyzed for skin lesion prevalence in order to reduce the potential for repeat sampling of the same individual. If a catalogued individual was resighted within the same year, only the first high quality photograph of the individual from the year was included in the analysis. Catalog photos were also excluded from the lesion analysis if the surface of the skin was not visible due to poor lighting conditions or if only the dorsal fin was visible above the water line. Each of the remaining photographs were visually screened and

coded for the presence (1) or absence (0) of visible lesions. If present, lesion types were categorized according to Toms et al. 2020, and an additional "other" category. The categories included black amorphous, dark spots, lunar, white amorphous, cloudy white spots, white freckles, dark fringe, white fringe, tattoo, spotted, vesicular, mottled, orange hue, orange patches, discolored head, pathogenic rake mark-associated lesions, non-pathogenic rake mark-associated lesions, and other (Figure 2). Rake marks are caused by aggressive contact between one dolphin's teeth and another's body, usually resulting in long, thin parallel lines on the surface of the skin (Scott et al. 2005). All rake mark-associated lesions were excluded from the study, as previous lesion analyses do not include them. The number of lesion types of for each individual dolphin was calculated and the most common skin lesion type was determined for each year and for the full study period.

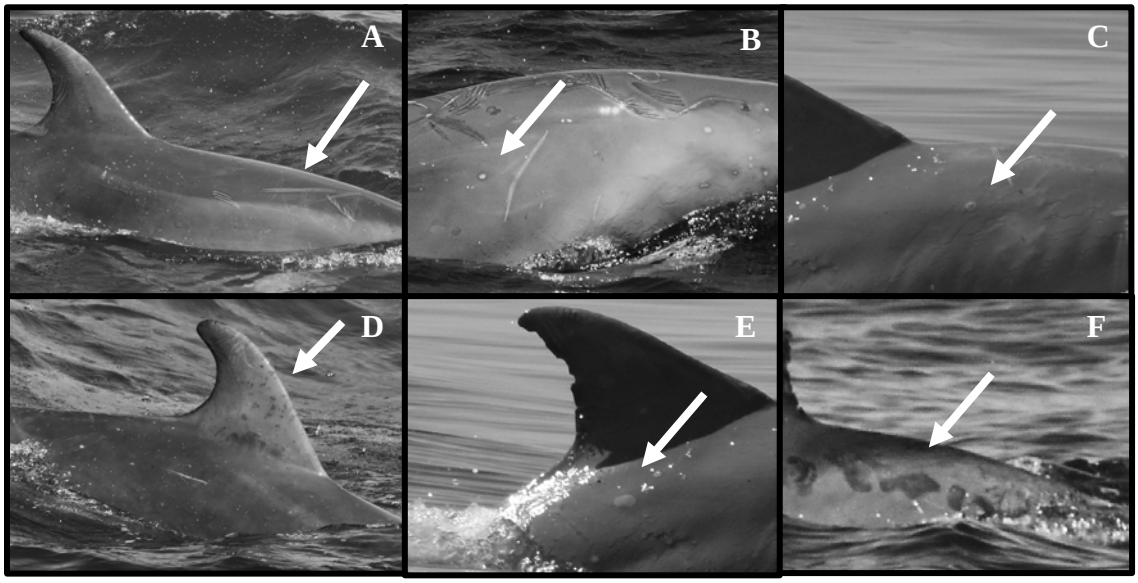

Figure 2. Bottlenose dolphins exhibiting various lesion types, including a) non-pathogenic rake marks, b) dark-fringe lesions, c) white-fringe lesions, d) dark spot lesions, e) cloudy white spots, and f) black amorphous lesions.

Spatial and Temporal Variations in Skin Lesion Prevalence

Overall prevalence for skin lesions in the Harbor Estuary was calculated and compared to reported overall prevalence estimates for other sites along the Atlantic Coast, including populations in North Carolina, South Carolina, Florida, and Georgia (Hart et al. 2012; Taylor et al. 2020; Toms et al. 2020; Table 1, Equation 1). To investigate temporal variations, annual and monthly prevalence were calculated (Taylor et al. 2020; Toms et al. 2020; Table 1, Equations 2 and 3). Because there was only one sighting in May, monthly prevalence was calculated from June to October.

Table 1. Equations for calculating overall, annual, and monthly prevalence based on Taylor et al. 2020.

Equations for Calculating Prevalence	
Equation 1: Overall Prevalence	$P_{overall} = \frac{\#\ of\ individuals\ with\ at\ least\ one\ lesion\ (2017-2020)}{total\ \#\ of\ individuals\ screened\ (2017-2020)}$
Equation 2: Annual Prevalence	$P_{annual} = \frac{\#\ of\ individuals\ with\ at\ least\ one\ lesion\ during\ a\ particular\ year}{total\ \#\ of\ individuals\ screened\ during\ a\ particular\ year}$
Equation 3: Monthly Prevalence	$P_{monthly} = \frac{\#\ of\ individuals\ with\ at\ least\ one\ lesion\ during\ a\ particular\ month}{total\ \#\ of\ individuals\ screened\ during\ a\ particular\ month}$

Skin Lesion Prevalence and Environmental Variables

Each bottlenose dolphin sighting in the catalog was matched with the Julian day in which it was observed. The time period during which bottlenose dolphins were sighted in the Harbor Estuary was partitioned into 22 weeks from May 31 to October 31. For each bottlenose dolphin sighting, sea surface temperature (°C) was found using the OSTIA L4 SST analysis dataset from the UK Met Office and sea surface salinity (PSU) was determined using the SMOS/MIRAS L3 daily sea surface salinity dataset from NOAA. For each week, the proportion of individuals with

lesions, mean sea surface temperature, and mean salinity were calculated. Generalized linear models were built in R (v. 3.4.3, R Core Team 2020) to explore how the weekly proportion of lesions correlated with temporal and environmental characteristics. Akaike Information Criterion (AIC) scores were used to determine the optimal model fit. The models used a log-link function and weeks were weighted by the number of days included. Akaike Information Criterion (AIC) scores were used to determine the optimal model fit (Wagenmakers and Farrell 2004). The model with the lowest AIC score was used (Bursac et al. 2008), and for models with comparative AICs, the one with the fewest terms was selected (Harrison et al. 2018). Figures were produced in R using the package ggplot2 (Wickham 2016).

RESULTS

Characteristics of Identified Individuals

Photographs of bottlenose dolphins were collected during the months of May to October in 2017-2020. In the final photo-ID catalog, there were 221 unique individuals. Out of these, 7% (n = 16) were resighted and 3% (n = 7) were resighted within the same year (Figure 3). Once within-year resights and catalog photos with poor lighting or only the dorsal visible were excluded, the 203 remaining photographs were analyzed for lesions.

Figure 3. Photographs of individual U071, sighted on September 20, 2018 (left) and again on May 31, 2019 (right) with a new lesion to the left of the dorsal fin.

Skin Lesion Categorization

The number of lesion types per individual ranged from 0-4, with a mean of 0.57 ± 0.03 types. In this population, 59% of individuals lacked visible lesions, while 30% had one lesion type, and 11% had two or more types of lesions (Figure 4). The five most common lesion types overall were cloudy white spots (n = 24, 12% of individuals), white amorphous lesions (20, 10%), mottled lesions (15, 7%), tattoo lesions (11, 5%), and dark-fringe lesions (9, 4%). Cloudy white spots were the most common lesion type in 2017 (5, 16%), 2019 (13, 13%), and 2020 (5, 16%), while in 2018 mottled lesions were most common (3, 8%); therefore, it appears that certain lesion types remain prevalent across years.

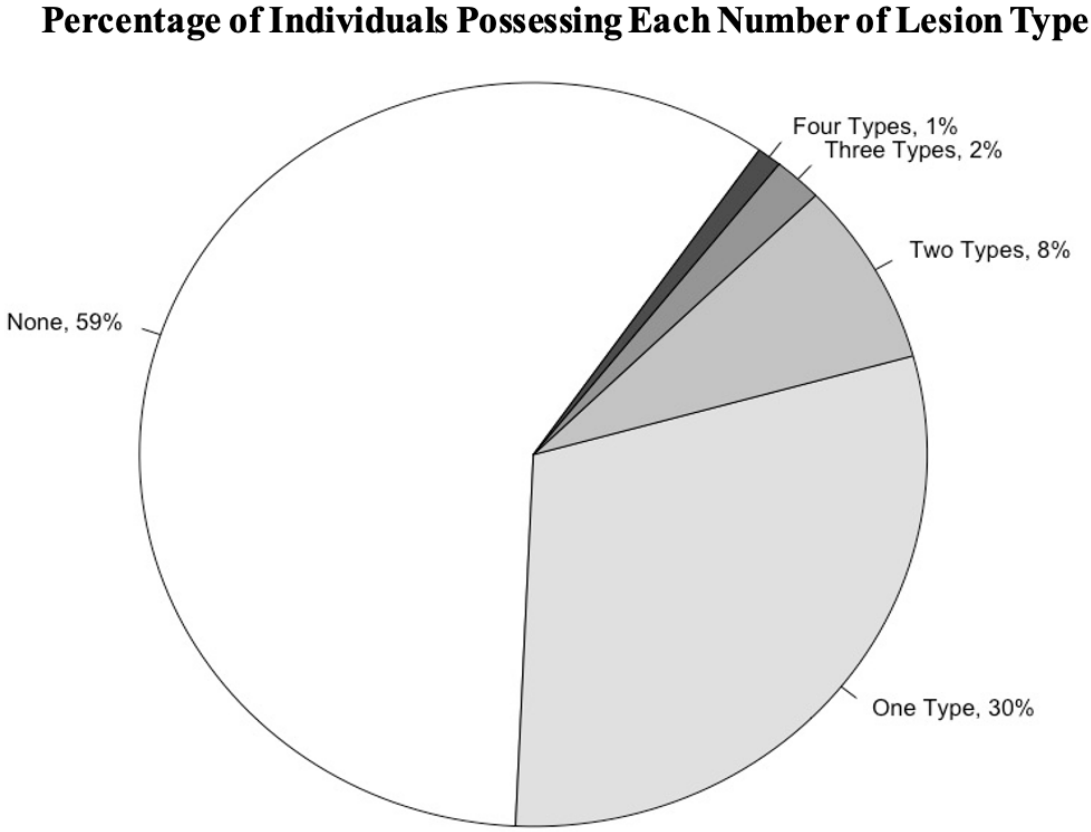

Figure 4. Percentage of individuals with none, one, two, three or four types of lesions present.

Spatial and Temporal Variations in Skin Lesion Prevalence

Skin lesion prevalence in the Harbor Estuary varied annually, with the highest prevalence observed in 2017 and the lowest in 2018 (Table 2). Although 2019 had a larger sample size compared to other years, skin lesion prevalence was moderate. 2020 had a similar sample size to 2017 and 2018, and had the second lowest lesion prevalence estimate. Skin lesion prevalence in the Harbor Estuary also varied by month. The highest prevalence was found in June ($P = 0.68 \pm 0.10$), with lower prevalence during July ($P = 0.36 \pm 0.069$), August ($P = 0.30 \pm 0.081$), and October ($P = 0.37 \pm 0.070$), and a spike in September ($P = 0.46 \pm 0.073$; Figure 5). From June to July, prevalence decreased by 32%. Overall skin lesion prevalence in the Harbor Estuary (n =

203, P = 0.41, 95% CI = 0.35-0.48) was low relative to populations in North Carolina (n = 169, P = 0.49, 95% CI = 0.42-0.57), South Carolina (n = 351, P = 0.49, 95% CI = 0.43-0.54), and Georgia (n = 322, P = 0.59, 95% CI = 0.53-0.64), and roughly equivalent with populations in Florida (n = 266, P = 0.38, 95% CI = 0.32-0.44; Hart et al. 2012; Taylor et al. 2020).

Table 2. Overall and annual minimum prevalence estimates for skin lesions.

Annual and Overall Skin Lesion Prevalence					
	2017	**2018**	**2019**	**2020**	**Overall**
n	31	36	104	32	203
P	0.48	0.28	0.45	0.38	0.41
95% CI	0.31-0.66	0.13-0.43	0.36-0.55	0.21-0.55	0.35-0.48

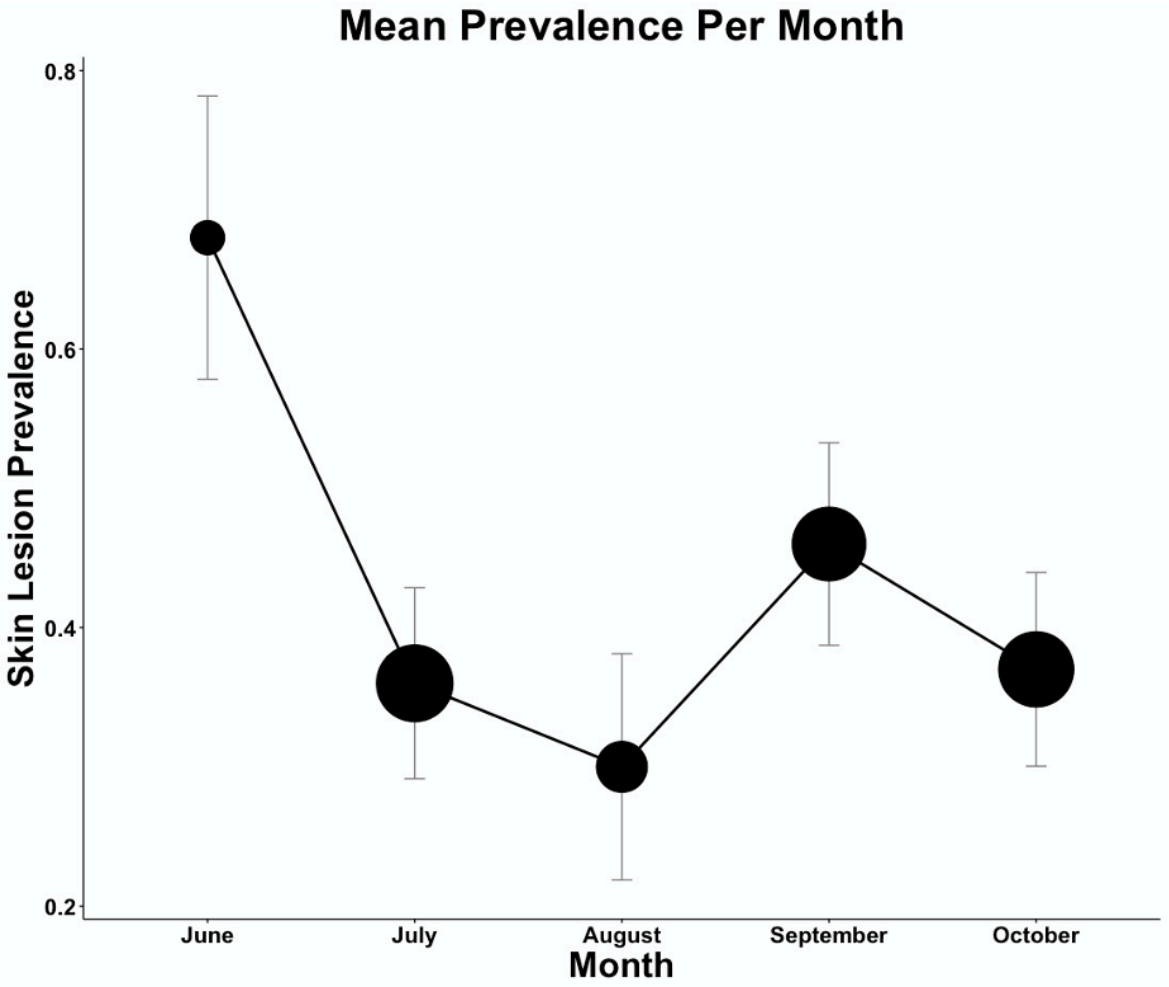

Figure 5. **Decrease in mean monthly prevalence of skin lesions from June to October. In September, a small spike in prevalence was observed. The size of the points reflects the number of individuals screened for each month across all years.**

Skin Lesion Prevalence and Environmental Variables

The optimal model included salinity and sea surface temperature as explanatory variables (Table 3). In this optimal model, both sea surface temperature (coefficient: -0.04°C, 95% CI: -0.08, -0.01, p = 0.02) and salinity (coefficient: -0.14 PSU, 95% CI: -0.23, -0.05, p = 0.01) significantly correlated with weekly skin lesion prevalence. Skin lesion prevalence was higher in colder and less saline waters (Figure 6).

Table 3. Table showing the five models with the lowest AIC scores. The optimal model is bolded.

Number	Model	df	AIC	AIC weight
1	**sea surface temperature + salinity**	**4**	**1.1**	**0.538**
2	sea surface temperature + salinity + week	5	2.3	0.287
3	salinity	3	5.1	0.071
4	salinity + week	4	6.6	0.034
5	sea surface temperature	3	7.4	0.022

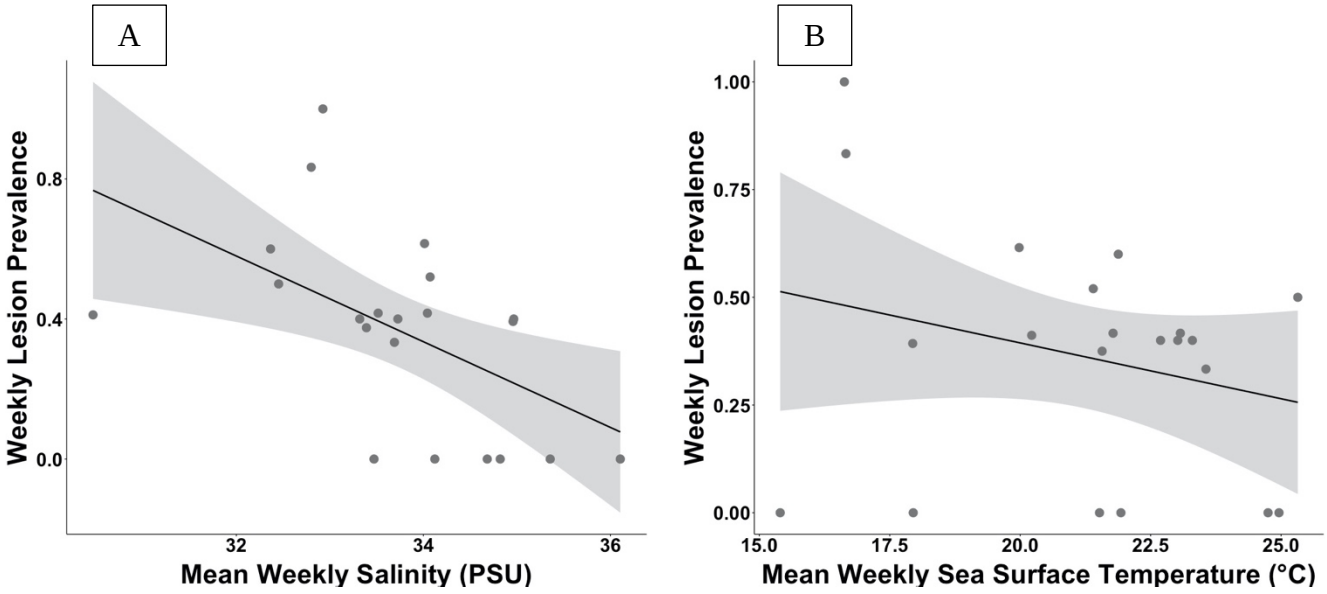

Figure 6. Line graphs showing how salinity (A) and sea surface temperature (B) correlate with weekly lesion prevalence. Observed data are indicated by dark grey points and 95% confidence intervals indicated by the light grey shaded region.

DISCUSSION

Overall, skin lesion prevalence was not stable temporally within the New York-New Jersey Harbor Estuary, or spatially across the Atlantic coast. Approximately four out of every ten bottlenose dolphins in this study had at least one lesion and there were annual and monthly fluctuations observed that may reflect environmental stressors (Hart et al. 2012; Taylor et al. 2020).

Spatial and Temporal Variations in Skin Lesion Prevalence

Skin lesion prevalence fluctuated temporally at multiple scales. Annually, skin lesion prevalence varied by as much as 20% between years. Other West Atlantic populations have more stable prevalence estimates across years, with the largest annual difference being 11% (Taylor et al. 2020). Interestingly, 2020 was not the year with the lowest lesion prevalence, despite the reduction in maritime traffic during the COVID-19 global pandemic (March et al. 2020). The effects of this reduction may be delayed, masked by other factors, or vessel activity may not be a significant contributing factor to lesion development in this area, but further research is needed.

By month, there was an apparent negative trend from June to October. Other bottlenose dolphin populations show a similar trend, with prevalence highest in spring before decreasing over the summer months into the fall (Taylor et al. 2020; Hart et al. 2012). Although this study started at the onset of summer, the highest prevalence was observed in the earliest month, with lesion prevalence in June nearly double that of July. Prevalence subsequently decreased throughout the summer and fall months, with a small peak in September. This trend may reflect seasonal changes in environmental conditions that influence lesion development. For instance, the negative relationship between prevalence and salinity resembles the decrease in prevalence by month, potentially because the Harbor Estuary may have lower salinity during the spring as increased rainfall and melting snow may produce an influx of freshwater into the estuarine ecosystem. Alternatively, prevalence may be highest in spring when water temperatures are colder, and decrease as the water temperature increases over the summer and into the fall.

In addition to fluctuations in prevalence over time, there were spatial variations. Skin lesion prevalence around the Harbor Estuary was within the range of prevalence estimates for

populations inhabiting different regions across the Atlantic Coast of the United States, but was relatively lower than most of the comparison sites (Taylor et al. 2020; Hart et al. 2012). Moreover, skin lesion prevalence estimates for Atlantic coast populations (P = 0.38-0.59) are much lower than those reported for other parts of the globe (P = 0.63-1.0; Hart et al. 2012; Wilson et al. 1999). Differences in prevalence across sites may be attributed to varying methodology, environmental conditions, and population demographics (Fury and Reif 2012).

Skin Lesion Prevalence and Environmental Variables

In the Harbor Estuary, salinity was significantly and negatively correlated with lesion prevalence. A similar trend has been found in other populations, with more lesions observed in fresher waters (Fury and Reif 2012; Wilson et al. 1999). Skin lesion prevalence was also significantly higher during colder water temperatures in the Harbor Estuary, similarly to other locations (Hart et al. 2012; Wilson et al. 1999). Given that the majority of photographs were collected from June to October, the strength of the relationship between sea surface temperature and lesion prevalence might be influenced by data collection bias during warmer months. Once more data has been collected across a broader temporal range, the relationship between prevalence and environmental variables can be further explored.

Potential Health Risks Associated with Lesions

Four of the five most common lesion types identified on bottlenose dolphins in this study have been previously associated with viral infections. Cloudy white spots have been associated with herpesvirus, while white amorphous lesions have been associated with herpesvirus in addition to a number of other potential causes, including healing after trauma, ectoparasite attachment, previous viral infection, and inflammation (Hart et al. 2012; Toms et al. 2020). Mottled lesions have an unknown origin, and both tattoo lesions and dark-fringe lesions have

been associated with poxvirus (Toms et al. 2020; Hart et al. 2012). Neither herpesvirus nor poxvirus appear to be fatal in bottlenose dolphins, but may have sublethal effects that ultimately contribute to reduced population health (Bossart and Duignan 2018; van Elk et al. 2009). The causes and effects of these viruses are important to understand, particularly given the level of anthropogenic disturbance that bottlenose dolphins are exposed to in the Harbor Estuary which may directly contribute to population health decline or exacerbate the effect of natural stressors.

Herpesvirus is hypothesized to spread through sexual contact of free-ranging bottlenose dolphins (van Elk et al. 2009). Though typically nonlethal, dolphin herpesviruses are associated with minor mucosal or epidermal lesions and have occasionally been found in relation to fatal infections of the nervous system (Bossart and Duignan 2018). Because the distribution and development are hypothesized to be socially driven and the genetic make-up of the herpesvirus can reflect the geographic origin of the dolphin, there may be implications for determining the directionality of the spread within and across populations (van Elk et al. 2009). This is particularly relevant for this population considering that during cold water months the bottlenose dolphin population in this study overlaps spatially and temporally with other populations that have higher lesion prevalence, such as the North Carolina population, suggesting that herpesvirus may be dispersing into the study population during the winter season.

Whereas herpesvirus spread is socially driven, development of poxvirus lesions has been correlated with environmental conditions (Flom and Houk 1979; Powell et al. 2018). Typically, poxvirus lesion prevalence increases as salinity and sea surface temperature decrease (Hart et al. 2012; Fury and Reif 2012). In addition to these natural stressors, differential prevalence of poxvirus expression may be associated with chemical pollutants in the water, which may have immunosuppressive effects and contribute to lesion development (Bossart and Duignan 2018).

Lastly, depressed immune systems can be caused by stress, which could ultimately lead to an increased prevalence of poxvirus lesions (Fury and Reif 2012). Poxvirus lesions have been found with varying prevalence in the Atlantic and Pacific Oceans, as well as the North, Mediterranean and Tasman Seas, suggesting that there are either common environmental stressors in these different areas or that many different stressors can contribute to poxvirus lesion development (Maldini et al. 2010; Fury and Reif 2012).

Given that bottlenose dolphins exhibit lesions when there are stressors in the environment, it has been suggested that lesion prevalence can be used as an indication of population health and ecosystem degradation (Powell et al. 2018; Bossart and Duignan 2018). When considering bottlenose dolphins as sentinel species, it is important to consider the critical differences in development and spread across lesion types, because some types appear to be more strongly correlated with environmental conditions than others; inferences about population or ecosystem health may vary based on lesion types observed. For instance, poxvirus lesions, including dark-fringe and tattoo lesions, have been highly correlated with environmental conditions whereas the development and distribution of herpesvirus-related lesions, including cloudy white spots and white amorphous lesions, are more likely socially driven (Fury and Reif 2012; Flom and Houk 1979; Powell et al. 2018; van Elk et al. 2009). Therefore, the presence of dark-fringe and tattoo lesions are more reliable indicators of environmental stressors than cloudy white spots or white amorphous lesions. As further monitoring occurs for the Harbor Estuary population of bottlenose dolphins, tracking fluctuations in poxvirus lesions would be beneficial to assess potential underlying environmental stressors to provide insight on ecosystem health.

In the Harbor Estuary, bottlenose dolphins may be developing poxvirus lesions for a variety of reasons. In addition to natural stressors, there are multiple contaminants of concern

found in the Harbor Estuary, including polychlorinated biphenyls (PCBs), mercury, dioxins and furans, pesticides and polyaromatic hydrocarbons (Lodge et al. 2015). Contaminants have been found in high concentrations in fish tissues in the Harbor Estuary (Lodge et al. 2015), and as bottlenose dolphins consume these fish, they may bioaccumulate contaminants. In turn, these contaminants may reduce immune functioning and contribute to lesion development (Bossart and Duignan 2018). It is possible that lesion prevalence is highest in spring because storm water runoff moves contaminants from tributaries into and throughout the Harbor Estuary system, where dolphins are found (Lodge et al. 2015). Furthermore, bottlenose dolphins in the Harbor Estuary may experience high levels of stress due to the intense anthropogenic disturbance in this region that may ultimately contribute to depressed immune systems and increased susceptibility to diseases (Fury and Reif 2012). While poxvirus, like herpesvirus, has a low mortality rate in odontocetes it may still indicate poor health and can factor into calf mortality (Powell et al. 2018; Bossart and Duignan 2018); therefore, the presence of poxvirus lesions in bottlenose dolphins in the Harbor Estuary is cause for concern and further research into the causes and effects of these lesions is needed.

Limitations of the Data

Photographic identification of individual animals offers a cost-effective and noninvasive way to explore the potential variation in skin lesion prevalence which can then be supplemented by other methods such as capture and release (Taylor et al. 2020; Bossart et al. 2019; Hart et al. 2012). Photographic identification is limited by photo quality, visible parts of the dolphin's body, and variation in methods of photographic assessment across studies (Fury and Reif 2012). Photographic analysis and visual screening of lesions are subject to some degree of observer bias (Toms et al. 2020; Urian et al. 2014). Additionally, standards for lesion categorization have not

been solidified, and there is variation in both the number and type of categories included in analyses of lesion prevalence (Toms et al. 2020); however, despite these limitations, this method provides valuable data for inferring population health and making comparisons across time and space, as well as providing insight into the health of the underlying ecosystem (Toms et al. 2020; Fossi and Panti 2017).

Future Directions and Conclusions

Continuing to use photo-ID to expand the catalog and monitor the population of bottlenose dolphins in the Harbor Estuary will increase the sample size for resighted individuals, which can then be analyzed for longitudinal patterns in skin lesions for individuals in addition to the population as a whole. This would allow for demographic analyses of skin lesion prevalence. At other locations, immature bottlenose dolphins demonstrate significantly higher prevalence of poxvirus-associated lesions than mature individuals, suggesting that this lesion type is not distributed evenly throughout the population (Van Bressem et al. 2003; Powell et al. 2018). Demographic information of the catalogued individuals from the Harbor Estuary is not yet readily available due to the relatively recent undertaking of photo-ID analysis in this region; however, as the catalog expands and the understanding of this population improves, it will be possible to analyze variation in lesion prevalence based on individual characteristics, such as age. This will provide more nuanced insights on how bottlenose dolphins may be affected by stressors in this environment, which could be taken into consideration when drawing inferences about the underlying ecosystem health.

Future studies should also incorporate other methods of assessing disease prevalence in this bottlenose dolphin population, such as tissue analyses. Integrating multiple approaches for assessing health contributes to a comprehensive understanding of the development and spread of

lesions in Atlantic populations of bottlenose dolphins. For instance, information on the origin and persistence of lesions on the skin surface could indicate whether lesion presence reflects environmental stressors in the current habitat or previous habitats throughout the migration route. Additionally, by understanding the etiology of the lesions, it might be possible to consolidate lesion types based on the causal agent. Currently, if the macroscopic qualities of a lesion change drastically throughout the development of an infection, it could be categorized in multiple ways at different stages. By linking observable characteristics of an infection to the causal agent, those categories could be grouped by etiology and stage of development, which would aid in the standardization of skin lesion categories and allow for more accurate comparisons across study sites.

While this study included two environmental variables commonly associated with skin lesions, sea surface temperature and salinity, future studies on this population should include other anthropogenic stressors in the environment, such as chemical pollutants. Moreover, if there are multiple stressors in an area, the population may be more susceptible to the development of lesions, with consequences to fitness or survival (Powell et al. 2018). For instance, if there are chemical pollutants with immunosuppressive effects in an environment with low salinity, lesion development may be more likely, potentially contributing to higher rates of calf mortality. Therefore, possible cumulative and interactive effects between multiple stressors and the potential consequences of those effects should be further investigated, especially given that the study population migrates across large distances and is potentially exposed to many stressors throughout their migration.

In summary, 41% of the bottlenose dolphins in this study had at least one visible skin lesion and 11% had multiple lesion types, indicating that diseased individuals are common in this

population. Temporal fluctuations of lesion prevalence in the Harbor Estuary demonstrate that prevalence is dynamic in this population, potentially reflecting variations in environmental stressors. Further research is needed to supplement and build upon this data to better understand the interactions between bottlenose dolphins and the natural and anthropogenic features of the Harbor Estuary. This study provides a straightforward framework for the continued monitoring of this bottlenose dolphin population during their seasonal presence, which is particularly important given forthcoming developments planned in this region. As sentinel species, understanding the health of these bottlenose dolphins can provide insight on the health of the underlying ecosystem with potential implications for coastal management and conservation efforts. This is particularly important given the continued expansion of anthropogenic activity in this heavily human dominated region. Monitoring environmental stressors and their impacts on bottlenose dolphin health as these developments occur can signal when there may be cause for concern for humans and wildlife alike.

ACKNOWLEDGMENTS

Thank you to the Hudson River Foundation and the Tibor T. Polgar Fellowship Program for providing support for this research. In particular, the authors would like to express their gratitude to Helena Andreyko, David Yozzo, and Sarah Fernald for their guidance throughout the process. Finally, thank you to Carissa King, Emily Chou, and Sara Kross for all of the friendly encouragement and valuable feedback.

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INFLUENCE OF URBAN SEWAGE POLLUTION ON FISH MICROBIOMES

A Final Report of the Tibor T. Polgar Fellowship Program

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Garrison, E.G. and G.D. O'Mullan. 2021. Influence of Urban Sewage Pollution on Fish Microbiomes. Section II: 1-27 pp. *In* S.H. Fernald, D.J. Yozzo, and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2020. Hudson River Foundation.

Abstract

The impact of urban pollution on fish microbiomes in a coastal megacity was evaluated. Fecal indicator bacteria (FIB) concentrations in water were paired to antibiotic resistant microorganism (ARM) concentrations in both fish guts and associated environmental water along a gradient of urbanization. A gradient of urban sewage impact across study sites was established based on historical data. This gradient was not significant based on sampling within the scope of this study; however, this was likely due to under-sampling. ARMs were detected in 95% of the individual fish tested. A positive correlation was found between FIB and ARMs at sites as well as between ARMs in water and in guts. Proteobacteria was the most abundant phyla detected in fish gut microbiomes; moreover, genus *Ralstonia* made up 63% of all ARM isolates. While environmental impacts on fish microbiomes are explored widely in the literature, this study is novel in its attempt to understand these impacts in the context of urban sewage pollution.

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INTRODUCTION

The majority of the world's metropolises have been built on coasts and estuaries because they have historically provided safe locations for ports with inland connectivity (Ross 1995). These environments are also typically rich in local biodiversity. As cities have grown, these environments have been subject to similarly rich and diverse contamination. Despite the substantial environmental degradation that has resulted from urban development, these environments still host diverse wildlife including species on high priority lists for conservation (Ives et al. 2015). While many resources are dedicated to the monitoring, management, and conservation of urban wildlife, a complete understanding of how urban pollution impacts resident species is still lacking.

Species that live in cities, particularly those that live in aquatic environments, are subject to both persistent historical and modern pollution sources. Urban waterways receive a variety of contaminants from stormwater runoff and wastewater, both treated and untreated. Of particular concern are the results of combined sewage systems, which confer untreated sewage into local waterways via combined sewage outfalls (CSOs) to avoid overwhelming sewage infrastructure during wet weather events. These sources discharge a variety of contaminants including heavy metals, PAHs, pesticides, pharmaceuticals, hormones, solvents, petroleum products, debris, and microorganisms (Rodenburg et al. 2010; Hendricks and Pool 2012; Eaton et al. 2013; Cantwell et al. 2018; Walter et al. 2019). Antibiotic resistant microorganisms (ARMs), in particular, have been found to enter waterways via CSOs, drastically increasing in concentration with wet weather events, closely correlated with fecal indicator bacteria (Young et al. 2013), suggesting that ARMs may be a helpful metric of urban sewage impact.

Furthermore, chemical contaminants can alter microbial environments through coselection (Nguyen et al. 2019). These conditions exhibit spatial heterogeneity, with highly localized conditions (Rouff et al. 2013). Furthermore, sediments can act as a reservoir for contaminants, including microorganisms both with and without antibiotic resistance (O'Mullan et al. 2019; Rodgers et al. 2018).

Recent developments recognizing the role that environment plays in shaping an organism's microbiome and the resultant impact this has on organismal health underscores a critical gap in the understanding of how urban pollution affects local aquatic species. Microbiomes made up of microbiota, the assemblage of microorganisms that reside within a given environment, their genes, and their habitat (Marchesi and Ravel 2015), are critical to organismal health. Gut microbiomes, in particular, impact fish development, immunity, nutrition, fecundity, survival, hormonal regulation, and energy homeostasis (Mehdinejad et al. 2019; Nayak 2010). Fish microbiomes are exposed to urban contamination through a variety of pathways. Early environment shapes the development of fish gut microbiomes (Giatsis et al. 2015); therefore, exposure to contaminants and microbiota originating from urban sewage sources can significantly impact fish in early life stages. Diet plays an integral role in shaping fish gut microbiomes (Tarnecki et al. 2017). Thus, benthivorous foraging behavior may expose fish microbiomes to contaminants and microbiota present in sediment reservoirs. Furthermore, while poorly documented, instances of fish feeding at outfalls of both treated and untreated wastewater have been recorded in both press (Tompkins 2015; Van Velzer 2017) and the literature (Moore 1932; Campbell 1939; Allen et al. 1976; Russo

1989). This suggests that fish microbiomes may reflect the highly localized spatial heterogeneity of urban pollution.

Urban contaminants can disrupt gut microbiome diversity, assemblages, and distribution. In fact, gradients of urbanization alone have been recently revealed to influence the microbiomes of resident species (Gaday et al. 2019; Murray et al. 2020). The Hudson River Estuary is home to New York City, its pollution, and a variety of fish species. Estuaries are unique in the critical roles they play in the life cycles of many fish species: providing nursery habitats for juvenile fish and passage for anadromous and catadromous species; therefore, New York City's waterways host both permanent and transient residents, some of which are at their most vulnerable life stages. Despite significant evidence that microbiomes influence fish health, and that urban contamination may alter these microbiomes, the influence of urban sewage pollution on the microbiomes of fish has not yet been directly studied.

In order to address this gap, the effect of urban pollution on fish microbiomes was explored along a gradient of sewage influence using antibiotic resistant bacteria as a metric of impact. The goals of this study were: (1) to establish a gradient of urban sewage impact using historical water quality data; (2) to confirm whether ARMs could be found in fish gut microbiomes; (3) to determine if there is a connection between ARM concentrations in the fish guts and contamination in their local environment; and (4) to characterize the taxonomic identity of the ARMs present in gut microbiomes. It was hypothesized that ARMs are present in fish gut microbiomes and vary along gradients of sewage impact in urban environments.

METHODS

Site Selection

Three sites with similar salinities and expected to have decreasing degrees of urban sewage pollution were chosen for sample collection: Flushing Creek; Little Neck Bay; and Hobart Beach (Figure 1; Table 1). Flushing Creek is located in a densely populated part of Queens, New York, and is adjacent to several high volume combined sewage outfalls which release upwards of 500 million gallons of untreated sewage into the waterway per year (Table 1; NYC DEP 2019). By comparison, Little Neck Bay, which lies on the boundary between Queens and Nassau County, receives wastewater/stormwater by separated stormwater pipes with only one major CSO approximately one mile from the study site, and has a lower population density (NYC DEP 2015). The Little Neck Bay study site itself is adjacent to a stormwater sewer designated solely for street water runoff (NYC DEP 2015). Finally, Hobart Beach, Eatons Neck, represents the least impacted of the study sites. This location exists on a recreational beach located along a spit, somewhat removed from direct street runoff, in an area of relatively low population density. In order to confirm a gradient of urban sewage pollution, historical datasets retrieved from the National Water Quality Monitoring Council (2020) for Hobart Beach (site ID 21NYBCH-NY477769-01) and the New York Water Trail Association (2020) for Little Neck Bay and Flushing Creek, were analyzed for overall fecal indicator bacteria trends.

Figure 1: **Map of study sites, FC=Flushing Creek, LNB=Little Neck Bay, HB=Hobart Beach. Base map imagery retrieved from Esri (2021).**

Table 1: **Description of study sites. Population density data based on 2010 census data (United States Census Bureau 2010) of adjacent community districts Queens CD7, Queens CD11, and Eatons Neck representing Flushing Creek, Little Neck Bay, and Hobart Beach, respectively.**

Site	Coordinates	Population Density	Site Description
Flushing Creek (FC)	40.761198, -73.835539	20,927 people/mile^2	This sample site is adjacent to Combined Sewer Overflow (CSO) outfalls TI-010 and TI-022, which release 463 million and 65 million gallons/year, respectively.
Little Neck Bay (LNB)	40.779155, -73.768005	12,386 people/mile^2	This sample site is adjacent to a storm sewer which is designated for street runoff only.
Hobart Beach (HB)	40.922675, -73.404104	1,396 people/mile^2	This sample site is adjacent to a beach designated for recreation which is located on a spit a distance from direct runoff from the street.

Sample Collection

During fall 2020, four sampling events occurred at FC and LNB and three sampling events occurred at HB. With each sampling event, three fish guts were sampled along with water from their environment. Mummichogs (*Fundulus heteroclitus*) were used as a model organism as they are found throughout the Harbor, including sites of extreme pollution, and have a small home range of <200 m (Skinner et al. 2012).

Mummichogs were collected using baited minnow traps, which were set to receive the incoming tide and left for no more than 2 hours. Once fish were collected, they were euthanized using a 0.4% solution of MS-222 followed by submersion in an ice slurry, consistent with methods outlined in the literature (e.g. Givens et al. 2015; Lloyd et al. 2016) in accordance with institutional guidelines (IACUC approved protocol #192). Euthanized fish were then wiped with 95% ethanol, dissected, and whole GI tracts were removed. Gut contents were extracted using sterile tweezers and placed in 1mL sterilized environmental water along with the whole gut. Samples were placed on ice and subsamples were processed for cultivation-based enumeration within six hours of extraction, and the remainder was frozen for future molecular analysis.

Microbial Enumeration

A spread plate technique was utilized to provide a relative enumeration of antibiotic resistant microorganisms (ARMs) across samples and to allow initial isolation of resistant colonies; therefore, both heterotrophic and antibiotic resistant microorganisms were quantified to enable a comparison. Four tenfold dilutions of water and gut samples were prepared, using sterilized (autoclaved) estuarine water. 0.1 mL sub samples of gut extractions and environmental water were added to plates containing solid R2A Agar

media with or without antibiotics to quantify heterotrophic and antibiotic resistant microorganisms, respectively. Following methodology outlined in Young et al. (2013), concentrations of 50mg/L and 10 mg/L of Ampicillin or Tetracycline, respectively, were used for antibiotic containing plates. Plates were subsequently incubated at 28°C for three days and colonies were visually enumerated heterotrophic (het), ampicillin resistant (ARB) and tetracycline resistant (TRB) bacteria.

Water samples were also processed for enterococci within six hours of collection, as specified in EPA method 1600 (US EPA 2006), using the IDEXX Enterolert methodology, consistent with prior literature (e.g., Eaton et al. 2013; Young et al. 2013; O'Mullan et al. 2019). Enterolert media was dissolved into 90mL of sterile deionized water, 10mL of sample water was added creating a ten-fold dilution, as recommended for brackish water samples (IDEXX 2019). The solution was sealed in a Quanti-Tray2000 container and incubated at 41°C for 24 hours in accordance with Enterolert procedures (IDEXX 2019). Post incubation enterococci concentration was quantified through visual enumeration of fluorescent quanti-tray wells under UV light and subsequently compared with the associated MPN table and multiplied by the dilution factor, consistent with IDEXX protocols (2019).

Molecular Techniques

Isolated bacterial colonies were picked from heterotrophic, ampicillin, and tetracycline plates using a micropipette tip and placed in 40 µL sterile hyclone water, and frozen until shipping. Single pass colony DNA sequences of the 16S rRNA gene were obtained through Sanger Sequencing performed by Eton Bioscience (Union, NJ). The DNA sequences were then aligned to a reference database from the Ribosomal Database

Project (Cole et al. 2014) and taxonomic identity determined at the genus level using the RDP Classifier tool (Wang et al. 2007).

Statistical Analysis

Non-parametric tests including Kruskal-Wallis and Dunn's adjusted multiple comparison were performed using GraphPad's Prism software (version 6.0; San Diego, CA) in order to compare enterococci concentrations and ARM concentrations between and across sites. These non-parametric tests were selected for comparison because of the non-normal distribution of microbial counts. Spearman's coefficient was used to conduct pairwise comparisons between concentrations of enterococci and ARMs in water as well as concentrations of ARMs in water and fish guts.

RESULTS

Urban Sewage Impact at Sites

A gradient of urban sewage impact was confirmed using historical enterococci data retrieved from the National Water Quality Monitoring Council (2020) and the New York Water Trail Association (2020) (Figure 2). Enterococci concentrations were found to be significantly different across all three sites (Kruskal-Wallis, $p<0.001$; Dunn's adjusted multiple comparison, $p<0.001$ for all pairwise comparisons). Flushing Creek experienced the highest median enterococci concentration, followed by Little Neck Bay and finally Hobart Beach (Figure 2). Enterococci samples collected through the course of this study demonstrated a similar trend to the historical data among sites (Figure 2; Kruskal-Wallis, $p=0.025$), but only the pairwise comparison of Flushing Creek and

Hobart Beach were significantly different (Dunn's adjusted multiple comparison, p=0.037).

The abundance of enterococci had a positive correlation with both ARB (Figure 3; Spearman r=0.813, p=0.004) and TRB (Spearman r = 0.7576, p = 0.009, data not shown) in paired water samples.

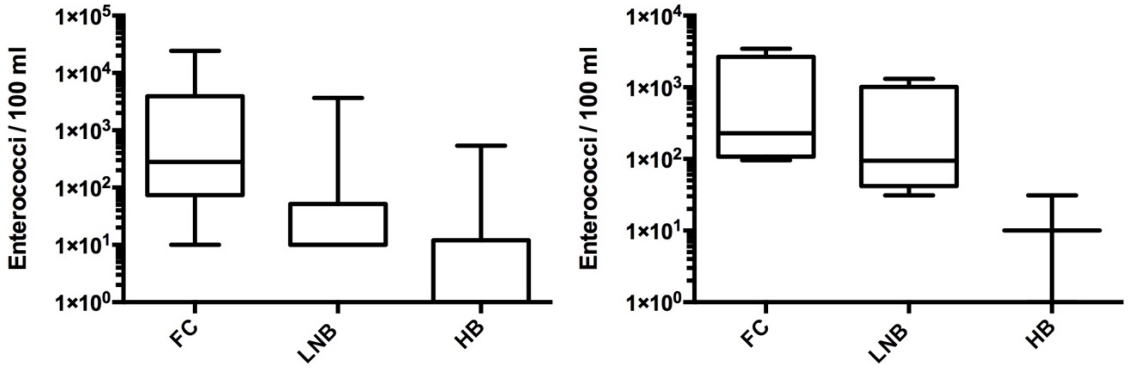

Figure 2: **Box plots of enterococci concentrations based on historical data (left) and samples collected through the study (right) across sites Flushing Creek (FC), Little Neck Bay (LNB), and Hobart Beach (HB). Historical data: FC: n=7, LNB: n=88, HB: n=132. This study: FC: n=4, LNB: n=4, HB: n=3.**

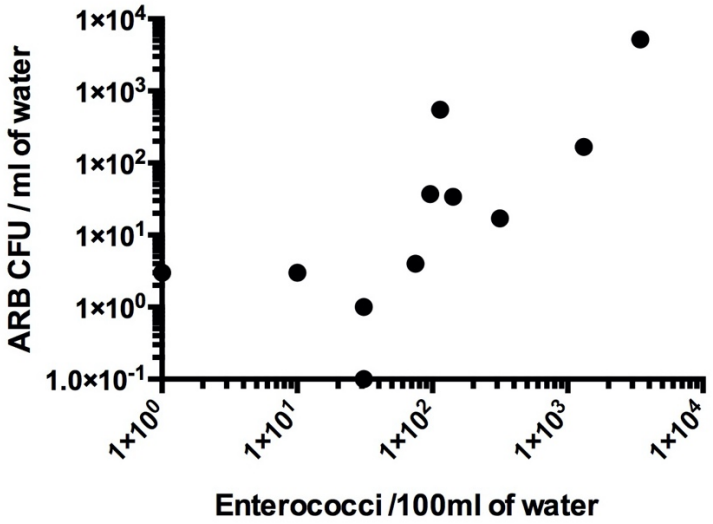

Figure 3: **Pairwise comparison of Ampicillin Resistant Bacteria and enterococci in water samples.**

Antibiotic Resistant Microorganisms in Fish Microbiomes

ARMs were detected in 95% of fish gut samples (n=41) collected, with concentrations of ARMs varying by up to five orders of magnitude. Cross site comparisons of ARM concentrations yielded no significant difference among sites (Kruskal-Wallis p=0.0628 and p=0.186 for ARB and TRB, respectively). The median and range of values for both ARB and TRB, however, were several orders of magnitude greater at Flushing Creek compared to Little Neck Bay and Hobart Beach (Figure 4). Little Neck Bay ARB samples also varied by several orders of magnitude compared to Hobart Beach, but this was not reflected in TRB samples (Figure 4). Despite differences in the range of values, TRB samples reflected similar medians across sites (Figure 4b). Analysis of samples across sites indicated a positive correlation between ARMs in gut microbiomes and ARMs in water samples for both ARB (Figure 5; Spearman r = 0.7527, $p<0.001$) and TRB (Spearman r = 0.6178, p=0.008, data not shown).

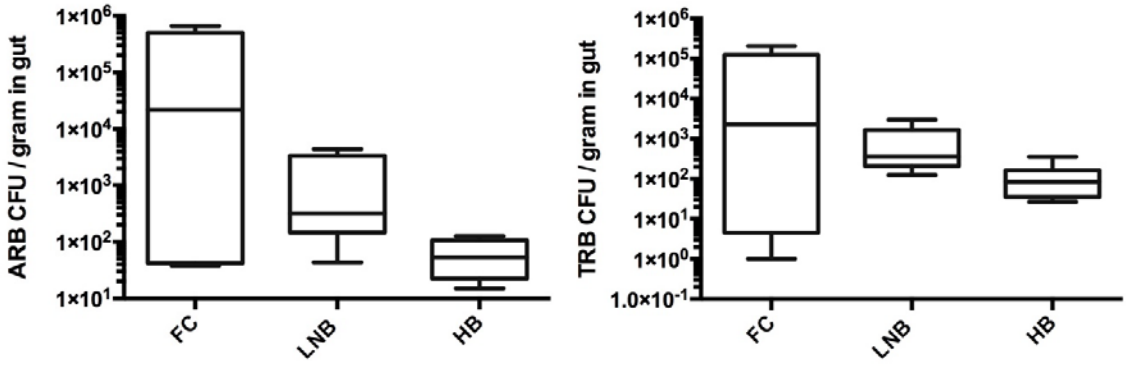

Figure 4: **Box plots for ARM concentrations across sites. Both Ampicillin resistant bacteria (ARB) concentrations (left) and Tetracycline resistant bacteria (TRB) concentrations (right) are shown. Flushing Creek (FC; n=5), Little Neck Bay (LNB; n=6), and Hobart Beach (HB, n=6).**

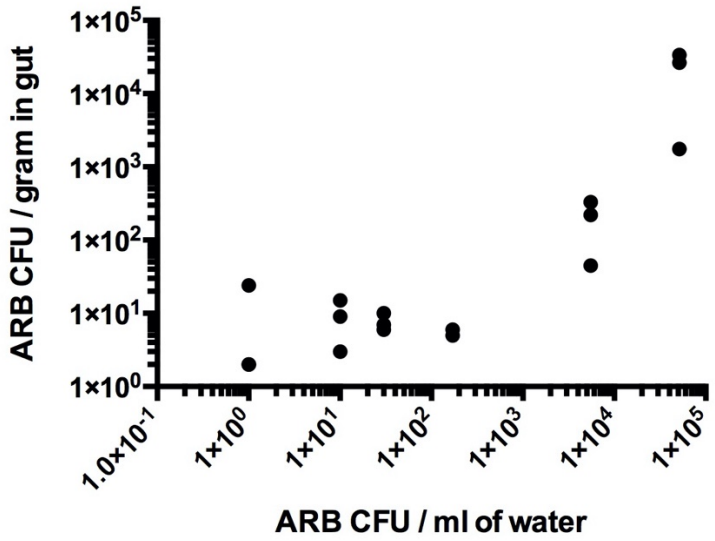

Figure 5: Pairwise comparison of Ampicillin Resistant Bacteria in fish guts and water samples.

Identity of Detected Microorganisms

Despite a relatively small number (n=53) of gut microbiome isolates being characterized, ARMs from fish microbiomes consist of a phylogenetically diverse assemblage, spanning the phyla Actinobacteria, Firmicutes, and Proteobacteria based on 16S rRNA gene sequences of isolates from the fish gut. Proteobacteria was the most abundant phyla detected in fish microbiomes (Table 2). The genus *Ralstonia* (phylum Proteobacteria) made up 63% of all ARM isolates and was dominant in both ARB and TRB isolates across all sites but was not detected among heterotrophic isolates. Genera *Aeromonas* and *Pseudomonas* were also common in both heterotrophic and ARB isolates, although not detected in TRB. Although very few isolates from water were sequenced (n= 16), two of the three most abundant genera in fish microbiomes (*Pseudomonas* and *Ralstonia*) were also detected in isolates from water (data not shown).

Table 2: Classification of 16S rRNA gene sequences from Het, ARB, and TRB isolates of fish gut microbiomes at Flushing Creek (Het: n=3; ARB: n=7; TRB n=10), Little Neck Bay (Het: n=11; ARB: n=9; TRB n=10), and Hobart Beach (Het: n=0; ARB: n=1; TRB n=2) based on the classifier tool of the Ribosomal Database Project with genera reported at 95% confidence unless otherwise indicated. [a] Indicates that all samples were between 57 and 85% confidence, [b] indicates 2 samples above 95%, remaining between 37 and 50% confidence in genus level assignment.

Microorganism Type	Phylum	**Genus**	Percent of identified colonies		
			FC (%)	**LNB (%)**	**HB (%)**
Het	Actinobacteria	*Microbacterium*		27	
	Firmicutes	*Lactococcus*		9	
		Staphylococcus		9	
		Paenibacillus	33[a]		
	Proteobacteria	*Aeromonas*	33		
		Citrobacter		9	
		Pseudomonas		9	
		Serratia		36[b]	
		Shewanella	33		
ARB	Actinobacteria	*Streptomyces*		11	
	Proteobacteria	*Aeromonas*	43		
		Pseudomonas	14	22	
		Ralstonia	29	56	100
		Raoultella	14	11[a]	
TRB	Firmicutes	*Enterococcus*	10		
		Lactococcus		10	
	Proteobacteria	*Klebsiella*	10		
		Ralstonia	50	70	100
		Serratia	30[b]	20[a]	

DISCUSSION

Establishing a Gradient of Impact

The gradient of impact established using sewage input is consistent with patterns of increasing urban characteristics such as population density (Table 1), impervious surface cover, and overall development supporting this study's hypothesis. Flushing Creek, the site with the greatest annual input of combined sewage overflow, containing both sanitary wastewater and stormwater runoff had the highest median and greatest range in enterococci concentrations (Figure 2). Little Neck Bay, being adjacent to a separated stormwater pipe is a site of intermediate sewage impact, which is reflected in the intermediate median enterococci concentrations. Finally, Hobart Beach, a recreational beach site, with no combined or separate stormwater pipes in the immediate vicinity, and relatively little direct runoff due to its location on a spit, had relatively low enterococci concentrations with a smaller range. The lack of significance of this gradient in the samples taken through the course of this study likely reflects the limited statistical power due to the small number of samples taken rather than a lack of gradient. An increase in sampling effort would likely result in a significant FIB gradient.

Previous studies have demonstrated the close association of ARMs and FIB, which were positively correlated in water samples from New York Harbor increasing in abundance during wet weather events when combined sewage and stormwater runoff are actively flowing (Young et al. 2013). The findings of this study of a correlation between enterococci and ARMs (Figure 3) confirm this phenomenon, demonstrating that ARM concentration is a reasonable metric of sewage impact. Considering the historical pattern of enterococci differences when larger sample sizes are analyzed combined with the

correlated abundance of FIB and ARMs, these patterns suggest that with more sampling the site gradient would likely be reflected in significantly differing ARM concentrations as well, despite historical data of ARMs at sites not being available for verification and the inability to demonstrate this pattern with the current sampling effort. Expanded sampling could test this prediction.

ARMs in Fish Microbiomes

Mummichogs have a small home range, spending the majority of their lives in an environment of <200 m (Skinner et al. 2012). It is reasonable to predict that their microbiomes are likely to reflect this highly localized environment and the heterogeneous water quality conditions typical of New York Harbor; therefore, ARMs detected should be representative of the conditions associated with the immediate surroundings of the sample site. Furthermore, ampicillin and tetracycline attack microorganisms via two different pathways (by inhibiting cell wall growth and translation, respectively) commonly used by two major classes of antibiotics (NCBI 2021; Chopra and Roberts 2001) and, therefore, likely representing a range of organisms that utilize diverse pathways of resistance.

ARMs were detected in the gut microbiomes in the vast majority of fish sampled, including the lowest impact site, Hobart Beach. Hobart Beach fish did, however, exhibit a smaller range in microbiome ARMs compared to the highest impact site, Flushing Creek, which was consistent with trends in enterococci concentrations along the site gradient. Differences in salinity have been shown to impact fish microbiomes (Schmidt et al. 2015), but sites were intentionally chosen based on the similarity of their salinity profiles, to control for this factor. Overall, findings suggest that, similar to enterococci trends,

expanded sampling may result in a significant difference among sites that vary in sewage impact.

The combined positive correlations between ARMs in water and guts (Figure 5), and between ARMs and enterococci (Figure 3) suggest that sewage pollution may be impacting fish microbiomes, even if this current sampling effort did not resolve a significant difference along the site gradient. The implications of these correlations are twofold. First, that sewage pollution may impact fish microbiomes which, based on prior literature, would then be expected to impact fish health on several levels including development, nutrition, reproductive success, and survival (Mehdinejad et al. 2019; Nayak 2010). Second, the other contaminants present in discharge of both combined sewage and stormwater runoff, in particular heavy metals, PAHs, and pharmaceuticals, all which have been found to impact fish microbiomes in other contexts (Walter et al. 2019; Zhai et al. 2017; Navarrete et al. 2008) are likely impacting fish health through similar pathways in urban environments as well. ARMs have been previously detected in New York Harbor mummichog microbiomes, consistent with locations of high heavy metal contamination, which was attributed to co-selection of antibiotic and heavy metals resistance in microorganisms (Lloyd et al. 2016). CSO pipes are known contributors of heavy metals and ARMs to surrounding waterways (Eaton et al. 2013; Young et al. 2013), which affects the immediate environment around CSO discharge, but also may persist in sediments which may additionally influence benthivores. Co-selection in both environment and microbiomes may, therefore, play a role in the levels of ARMs detected in fish.

Identity of Detected Microorganisms

The most dominant genus identified, *Ralstonia*, was not detected in heterotrophic isolates, indicating an ARM dominant genus. With larger sampling of heterotrophic isolates it is expected that *Ralstonia* would be detected, but they are of increasing importance when antibiotic selection decreases overall diversity of isolates. *Ralstonia* was also included within the ARB isolates detected in water by Young et al. (2013) in other New York Harbor locations. *Ralstonia* has also been previously identified in association with the guts of other fish species, making up one of the three dominant genera identified in the guts of marine fishes with diverse feeding habits and associated with several digestion pathways (Huang et al. 2020). *Ralstonia* may be common in a broader range of local fish species and may play a role in fish health through metabolism. Some species of *Ralstonia* are human pathogens (e.g., *R. picketti*); therefore, a species level identity may be useful in further understanding the implications of these microorganisms.

Additionally, ARM genera *Aeromonas* and *Pseudomonas*, which were common in fish gut samples, have been previously identified as among the most common ARMs detected in New York Harbor water samples (Young et al. 2013). This further supports a connection between microorganisms found in the aquatic environment and those found in fish guts. While *Aeromonas* was not detected in water samples from this study, this is likely due to the limited number of water isolates sequenced. It should also be noted that the cultivation-based methodologies used in this study do not provide a comprehensive insight into all microorganisms present in both water and fish guts, selecting only for those microorganisms that proliferate in the cultivation conditions used. Further

exploration using whole gut sequencing tools and known antibiotic resistant genes to better characterize these microbiomes would be beneficial.

Significance and Future Work

Sewage impacts such as FIB and ARMs are typically considered sanitary issues, of concern to the protection of human health but not necessarily ecological health. The relationship between urban sewage sources and fish health has wide implications for fisheries management and conservation. Current approaches focus on management planning and reporting, biodiversity and monitoring surveys, and creation of habitat structures. There is no current consideration of how urban sewage pollution impacts fish populations through their microbiomes. The local connection between sewage and organisms demonstrated in this study further illustrates the urgency with which urban centers like New York City should address sewage contamination. Solutions to FIB and ARM influx are often focused on limiting combined sewage through gray infrastructure which can increase conveyance and detention, but often disrupts urban landscapes and can be expensive (Hakimdavar et al. 2014). These impacts will be compounded by future increases in high intensity rainfall events and resulting increases in stormwater runoff and CSO discharge due to climate change (Horton et al. 2014; Fortier and Mailhot 2015). Green infrastructure captures stormwater before it enters sewage systems, providing a less invasive, lower footprint alternative with additional benefits, including the capture of direct runoff not fully addressed by grey infrastructure.

Management decisions related to upgrading urban infrastructure are often determined by cost-benefit considerations. This study highlights a negative consequence of sewage pollution on fish populations, an interaction generally overlooked when

considering possible benefits that would occur from sewage pollution reductions. This is similar to the mostly overlooked interaction of sewage pollution and greenhouse gas production (Brigham et al. 2019) or the connections between sewage pollution in water and microbial air quality (Dueker and O'Mullan 2014; Dueker et al. 2018). If the full range of impacts from sewage pollution would be considered, there would be added incentive to accept the cost of urban infrastructure upgrades.

The impact of urban sewage on fish microbiomes remains understudied, providing many pathways for future work. This study would benefit from an overall increase in sampling effort which may further support findings and produce significant insights. Future studies should consider exploring additional sites, fish species, and contaminants to provide a more comprehensive understanding of the relationship between urban sewage and fish microbiomes. Additionally, sampling that focused on comparing fish microbiomes in wet and dry weather and monitoring the shifts in microbiomes that occur after wet weather events would help to determine the timescale of microbiome responses that occur from CSO and stormwater influx and their role in shaping fish microbiomes.

ACKNOWLEDGEMENTS

We would like to thank the Hudson River Foundation and the members of the Polgar Committee for supporting and guiding this project, especially in a year when many student projects were not realized. Additional thanks are due to Azlan Maqbool, Jenny Liu, Danny Fabricant, and Ann Dougherty, for their assistance and support in lab and field work.

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INVESTIGATING THE DISTRIBUTION OF *LEGIONELLA PNEUMOPHILA* IN URBAN AND SUBURBAN HUDSON RIVER WATERSHEDS

A Final Report of the Tibor T. Polgar Fellowship Program

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Maqbool, A., G. O'Mullan, and M.E. Dueker. 2021. Investigating the Distribution of *Legionella* in Urban and Suburban Hudson River Watersheds. Section III-1-23 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2020. Hudson River Foundation.

ABSTRACT

The presence of *Legionella pneumophila* was assessed using a cultivation-based approach in New York City waterways, a freshwater portion of the lower Hudson River Estuary near Kingston NY, and in urban and suburban street water. *Legionella pneumophila* was detected in 51% of brackish New York City Estuary samples, most with concentrations near minimum detection (10 organisms/ mL). In contrast, the bacterium was detected in 22% of suburban freshwater Hudson River Estuary samples. Levels of the bacterium were found to be higher during wet weather compared to dry weather in the highly dense urban setting, but not in the less dense suburban/rural settings. *Legionella pneumophila* was also detected in 95% of New York City street water samples and in 88% of suburban street water samples. These results presented a strong initial indication of wet weather contamination from street water discharge into the estuarine environment. This is the first study to document the widespread occurrence of *Legionella pneumophila* in street water and to establish a clear pattern of increased concentrations of *Legionella pneumophila* during wet weather in an urban estuarine environment.

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INTRODUCTION

Legionella pneumophila, the causative agent in Legionnaires Disease (LD), is a bacterium with widespread distribution and is of increasing concern especially in densely populated environments where components of the built environment influence its distribution and exposure patterns. The recognition of *Legionella* came about after a large outbreak at an American Legion conference hotel in Philadelphia, PA, in 1976. Following the convention, 82 attendees became ill with serious forms of atypical pneumonia, and 29 died (McDade et al. 1977). The bacterium was isolated a few months later and named after the event that caused the outbreak (McDade et al. 1977). It wasn't until over a year later when the cooling tower atop the hotel was identified as the culprit. Since then, there have been several outbreaks of LD across the United States involving man-made water systems. As *Legionella* has been increasingly tied to sources such as cooling towers and HVAC systems, there has been limited research into other potential reservoirs in built environments that may also harbor the bacteria.

Although primarily studied in the built environment, *Legionella* has been reported in literature to have a widespread naturally occurring presence in lakes, river systems, and soils (van Heijnsbergen et al. 2015; Declerck et al. 2010; Steele et al. 1990; Fliermans et al. 1981). A 2010 study detected *Legionella* in 42% (185 out of 388) of Mt. Hope Bay, Massachusetts estuarine samples, indicating the *Legionella* can be found in widely different saline environments and grown in saline conditions (Gast et al. 2011). A 2014 study documented the growth of *L. pneumophila*, and associated amoeba biofilm, in subsurface water layers in three separate Poland lakes (Żbikowska et al. 2014). In soils, the presence of *Legionella* was detected in six garden

soils that were mixed with composted materials (Hughes and Steele 1994). Furthermore, there is evidence that natural soil is a reservoir and source of *Legionella* (Wallis and Robinson 2005). There are currently more than 58 species that have been described in published articles (Prussin et al. 2017). Of these, approximately 25 are linked to disease, including *Legionella pneumophila* species serogroup 1, 3, 4, and 6. *Legionella pneumophila* serogroup 1 is the most virulent strain causing the majority of infections (Walser et al. 2014). Urban environments are perhaps the key centers where exposures of *Legionella pneumophila* occur. One relevant source in the context of urban waterways is wastewater.

Wastewater treatment plants (WWTPs) have been confirmed to contain *Legionella* (Buse et al. 2012; Caicedo et al. 2018; Vantarakis et al. 2016) which can play an important role in community cases and outbreaks of LD. Studying *Legionella* in WWTPs is noteworthy as the quantity of municipal wastewater produced worldwide is drastically increasing as a result of growing population numbers. This, coupled with the discharge of inefficiently treated wastewater, particularly during rain events, into surrounding surface water sources serves as a direct threat to water quality, marine life, and humans. The persistence of *Legionella* in aeration tanks and wastewater treatment plants, is complicated by the bacterium's ability to interact with a variety of protozoan species. Once infiltrated, *Legionella* can hide, repair, and replicate within its host organism. The host cell protects *L. pneumophila* from harsh environmental conditions while providing a nutrient rich replicative niche (Abdel-Nour et al. 2013; Boamah et al. 2017). This ability is likely what causes *L. pneumophila* to survive despite water disinfection procedures.

In highly dense urban centers like New York City, coastal water quality has been clearly linked to wet weather-related discharge and bacterial contamination. For example, previous

research in the Hudson River Estuary (HRE) has shown an increased concentration of antibiotic resistant bacteria following rainfall (Young et al. 2013), increased estuarine greenhouse gas emissions following nutrient addition (Montero et al. 2015), and high levels of fecal indicator bacteria (FIB) delivered from urban street water to coastal waterways (Montero and O'Mullan 2018). While there has been plenty of evidence linking bacteria of concern to degradation of coastal water quality, there is less known about the distribution of *Legionella* in relation to water quality and sewage pollution.

The prior literature has not established an expected distribution in urban stormwater sources, and there is no clear expectation for how the distribution of *Legionella* will change along estuarine gradients and between urban and suburban environments. The goals of this study were to: 1) determine if *Legionella pneumophila* can be detected in urban and suburban Hudson River watershed environments; 2) determine if the concentration of *Legionella pneumophila* in coastal water is influenced by wet weather events; and 3) examine the distribution of *Legionella pneumophila* in urban and suburban street water. The hypothesis tested was that *Legionella* would be detected in estuarine and freshwater Hudson River environments and that concentrations would increase following wet weather due to precipitation linked pollution discharge from urban and suburban environments.

METHODS

In the summer of 2019, a total of 22 New York City sites were sampled during a 4-month period from June 2019 to October 2019 (Figure 1a). Of these 22 sites, fifteen were estuarine sites in western Long Island Sound and East River tributaries of New York City. Two sites were combined sewer overflow sites (BB08 and BB06) located in Flushing Bay (40.761858 N,

73.845919 W; 40.760250 N, -73.854587 W). Five street water sites (Figure 1a) were also sampled proximal to the Queens College campus. The chosen estuarine sites were a subset of sampling locations sampled at the time through the Riverkeeper water quality monitoring program conducted by the O'Mullan laboratory at Queens College. Urban estuarine sites shown in Figure 1a were accessed by boat. Once on site, 50 mL centrifuge tubes were triple rinsed with sample water before collection and then immediately stored on ice in a cooler to protect from sunlight until processing (Young et al. 2013). Samples were then returned to the lab shortly after where Legiolert, a cultivation method (IDEXX Laboratories, Westbrook, ME) based on a most probable number approach, was utilized to enumerate total *Legionella pneumophila* in water samples. The 1.0 mL protocol for non-potable water was used before transferring sample into a 96-well Legiolert Quanti-tray (IDEXX Laboratories, Westbrook, ME) and placed into an incubator at 37° C. After a 7-day incubation period, samples were taken out and analyzed for positive wells, which is indicated by a change in color as compared to the negative control tray. Results are given in most probable number (MPN) of *Legionella pneumophila* cells per 100 ml. The detection limit for the 1.0 mL assay is ≥100 organisms in 100 mL or ≥1 organism per mL. In parallel, Enterolert, an assay also developed by IDEXX Laboratories, was used to assess enterococci concentrations in all water samples (Young et al. 2013). Samples were transferred into Quanti-tray/2000 and incubated at 41° C. After 24 hours, trays were taken out and analyzed under a UV light. Any samples that presented a blue fluorescence were counted as positive.

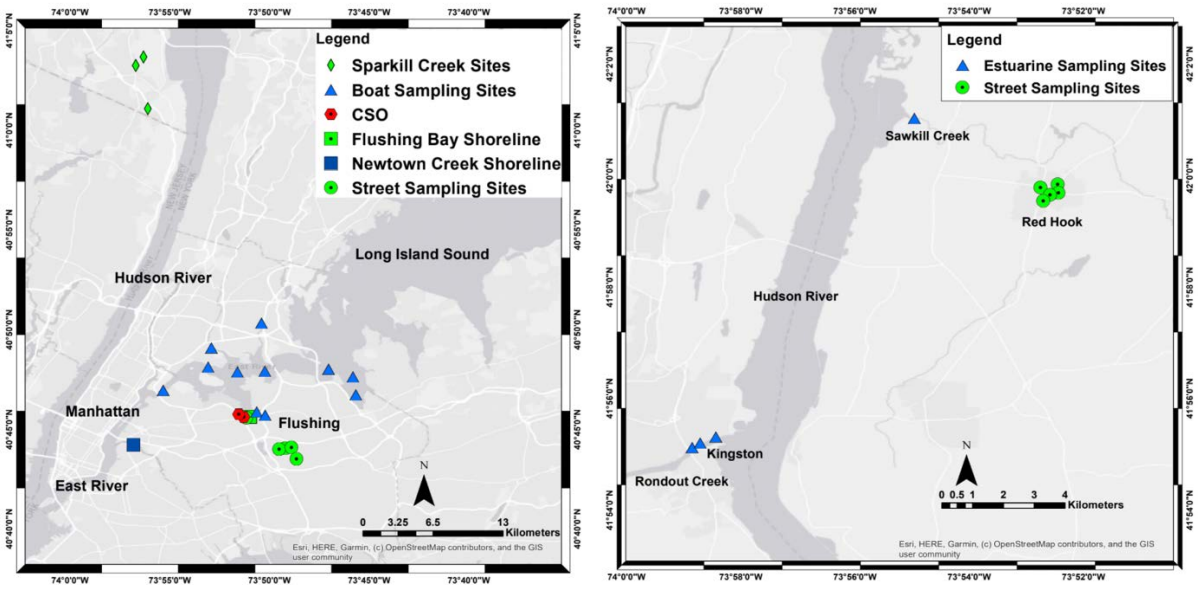


Figure 1. **Map of Study Sites: a) 22 sites were regularly sampled in the New York City area. b) 9 sites sampled regularly in mid-Hudson Valley tributaries (Kingston, NY) and street water in Red Hook, NY.**

During the fall of 2020 (September 2020 to November 2020), a total of nine sites and 44 samples were collected in the mid-Hudson River estuary watershed (Figure 1b) near Kingston, and Red Hook, NY. Four estuarine sites were sampled; three were located in the tidal reach of Rondout Creek, one was located at the tidally influenced mouth of the Sawkill Creek. Five street water sites located in the small suburban community of Red Hook, NY, were also sampled during wet weather events. Three additional samples were taken at Sparkill Creek sites, near Piermont, NY, above the dam and, therefore, not influenced by tide or salinity. Samples were sent to the O'Mullan laboratory at Queens College and were tested for *Legionella pneumophila*, with parallel samples analyzed in the Dueker Laboratory at Bard College for fecal indicating bacteria (FIB): enterococci, coliform, and *E. coli*.

Statistical analyses were run using Prism statistical analysis software (Version 6). Non-parametric tests were performed on the *Legionella pneumophila* and enterococci data to evaluate differences between the abundance of wet and dry weather bacteria because microbial data were non-normally distributed. Specifically, the Mann–Whitney and Kruskal–Wallis tests were used on microbial counts. Spearman's coefficient was used to evaluate the correlation between the fecal indicator, *Enterococcus,* and *L. pneumophila*.

RESULTS

During the summer 2019 sampling period, *Legionella pneumophila* was detected in 53% of estuarine samples. Many of the positive detects were mid-level detections (<=500 organisms/100mL) relative to the maximum detection level except for one sample, FB5 (110,970 organisms/100mL), which was taken during a wet weather event and is located near BBO8, one of New York City's largest combined sewage overflow (CSO) outfalls. In suburban estuarine samples, taken during fall 2020, *Legionella pneumophila* was detected in 26% of samples, mostly at low levels (<=110 organisms/ 100mL) near the assay's minimum detection limit apart from one Sparkill Creek sample (<=740 organisms/ 100mL). Urban estuarine samples had significantly higher concentrations (*Mann Whitney p=0.0086***)** of *L. pneumophila* than suburban estuarine samples (Figure 2).

To investigate the association of *Legionella* abundance with fecal indicating bacteria, enterococci concentrations were compared to *Legionella* concentrations in paired samples across all estuarine sites (Figure 3) and were found to be correlated (Spearman $r = 0.4571$, $p<0.001$). Although a similar relation was found when examining only the urban estuarine samples

(Spearman r =0.5380, p<0.001), the suburban estuarine samples on their own were not significantly correlated (Spearman r = 0.04728, p = 0.8304).

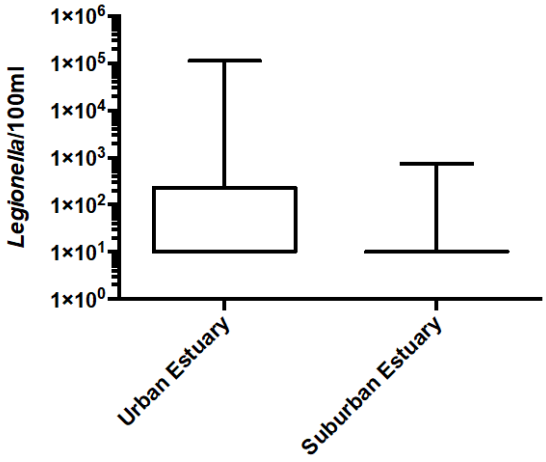


Figure 2. *Legionella* MPN in estuary box plot- Urban (boat + shore) vs. Suburban.

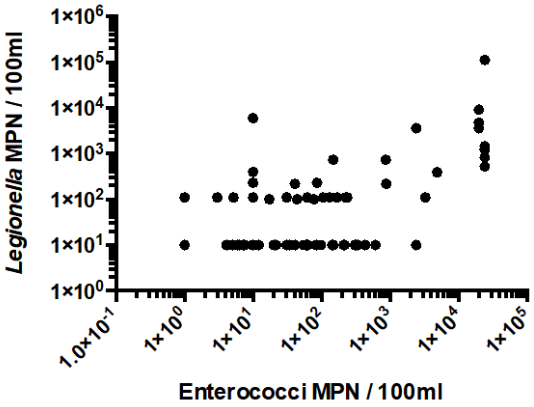


Figure 3. Enterococci-*Legionella* correlation for all estuary samples urban (boat and shore) and suburban (includes Sparkill Creek and mid-Hudson River estuary).

Wet vs dry weather results

The influence of rainfall on *Legionella* concentration was assessed by comparing samples collected in both wet and dry weather conditions. 102 samples were taken in wet weather events, while 38 samples were taken in dry weather events across all sampling sites: urban and suburban.

Enterococci concentrations during wet weather events were significantly higher than the dry weather counts in the urban environments, but not the suburban environment (Figure 4; Mann Whitney, p = 0.0136, p = 0.3171, respectively) when compared across sites. Conversely, *Legionella* concentrations were observed to be significantly higher during wet weather sampling in the urban environment (Figure 5; Mann Whitney, p = 0.003) but not in the suburban environment.

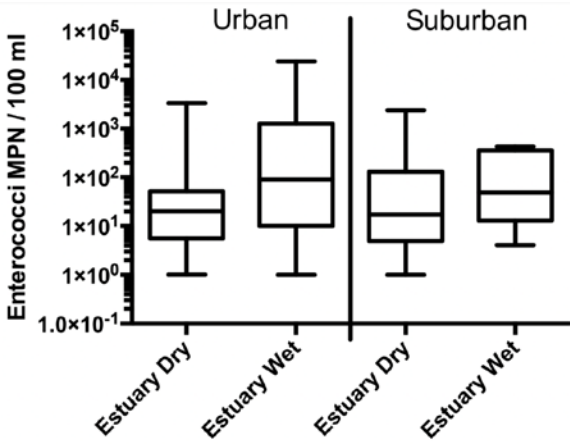


Figure 4. *Enterococcus* wet vs. dry urban estuary and suburban estuary (urban wet, n= 46; urban dry, n=18; suburban wet n= 15; suburban dry, n=15)

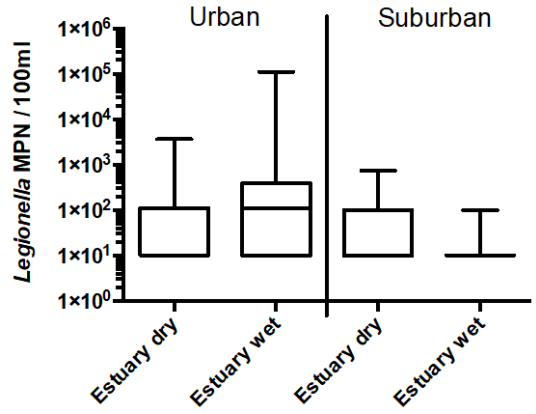


Figure 5. *Legionella* wet vs. dry urban estuary and suburban estuary (urban wet, n= 46; urban dry, n=18; suburban wet n= 15; suburban dry, n=15).

Street water results and comparisons

Although the concentration of enterococci was significantly higher in urban than the suburban street water (Mann Whitney, p <0.001, Figure 6), the concentration of *L. pneumophila* did not differ across urban and suburban street environments (Kruskal Wallis p = 0.691, Figure 7). Concentrations of *L. pneumophila* at CSO sites also did not significantly differ from urban and suburban concentrations of the bacteria in street water. A paired comparison of *Legionella* and enterococci across all sites was found to be weakly correlated (Spearman r = 0.4171, p= 0.01); however, a strong positive correlation was found between *L. pneumophila* and enterococci concentrations in the urban estuarine and street water environment (Figure 8b; Spearman r =0.8613, p<0.001).

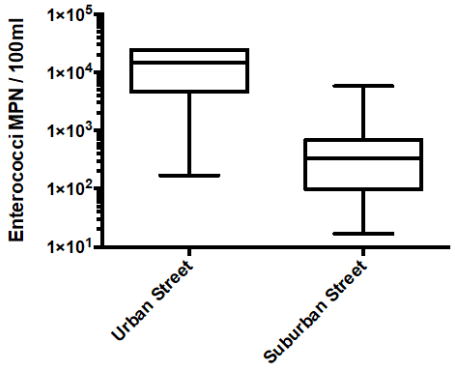


Figure 6. *Enterococcus* Street Urban vs Suburban.

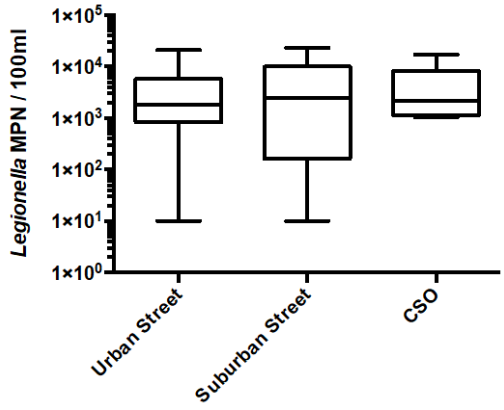
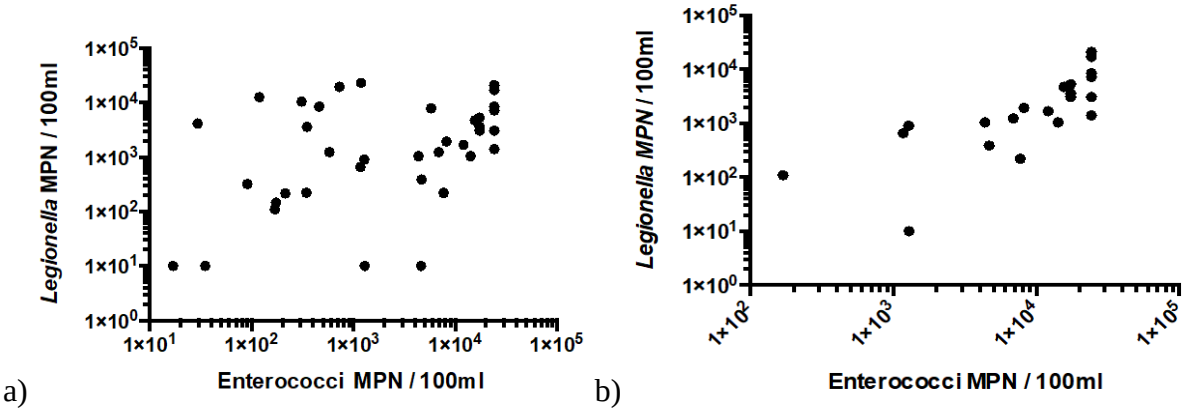


Figure 7. *Legionella* Street Urban vs. Suburban vs. CSO.



a) b)

Figure 8. (a) Enterococci-*Legionella* correlation for all samples urban and suburban (b) Enterococci-*Legionella* correlation for urban samples only.

DISCUSSION

Urban vs Suburban Estuarine Results

The abundance of *L. pneumophila* in the estuarine environment was found to be significantly higher in urban proximal waterways than the less-saline suburban estuarine environment (Figure 2). Of the 15 suburban estuarine samples, only 4 samples were positive for *L. pneumophila* (26% of samples). This result is consistent with findings of the bacterium in similar aquatic environments (Walczak et al. 2013; Dutka and Ewen 1983; Fliermans et al. 1981). Walczak et al. 2013 reportedly detected *L. pneumophila* species in 12.42% of surface waters in five lakes in Poland. The lower detection of *L. pneumophila* in wet weather conditions in suburban estuarine was an unexpected result. This could relate to the lower number of samples collected/sampling locations, or it could do with a variety of physio-chemical parameters not directly assessed in this study. This result requires further investigation. As far as the detection of *L. pneumophila* in the urban estuarine environment, prior studies did not contain results from an urban waterway such as the lower Hudson River estuary. The results in this study detected *L.*

pneumophila in 51% of urban estuarine samples. This higher detection at the urban level is most likely due to wet-weather related discharges from the high density of outfalls in New York City. Tracking wet-weather discharges often involve FIB such as enterococci, which has been linked to both sanitary sewage and stormwater inputs (Suter et al. 2011; Montero and O'Mullan 2018).

Enterococci connection

Levels of *L. pneumophila* followed a similar distribution pattern to enterococci in the urban estuarine environment ($p > 0.001$) but did not follow that pattern in suburban estuarine waters ($p > 0.8304$). This pattern was also evident between wet and dry weather events as there was a significantly higher concentration of *L. pneumophila* observed in urban estuary wet weather samples versus urban dry weather samples. The suburban estuary wet versus dry weather events did not show a difference which most likely indicates a lower influence of wet-weather related discharge in less densely populated environments. The positive correlation between *L. pneumophila* and *enterococci* in the urban environment does not necessarily indicate a connection to sanitary sewage. Although the waterways in the urban environment had higher concentrations than suburban waterways, the levels of *L. pneumophila* in the suburban street samples were not significantly different than in urban streets (Figure 2 and 4); therefore, the difference between urban and suburban waterways may be more related to relative abundance and proximity to urban high-density outfalls. Anthropogenic sources then are likely contributors to higher levels of both FIB and *L. pneumophila.*

Highest abundance of Legionella pnuemophila found in stormwater

Prior literature has reported a clear connection between stormwater and elevated levels of enterococci (Montero and O'Mullan 2018). Similarly, this study found a clear connection

between street water and elevated levels of *L. pneumophila*. The surfaces of the built environment, whether that is an urban or suburban environment, are primarily impervious where little infiltration occurs. Contaminants such as metals, chemicals, and pathogens can all accumulate on street surfaces. In this study, the highest levels of *L. pneumophila* were observed in street water. Coupled with the uniformly high levels of *L. pneumophila* in urban, suburban and CSO samples (Figure 7) this suggests that stormwater is likely the major source to both CSO discharge and to waterways. Moreover, it is likely the quantity of stormwater runoff, relative to waterway volume, is a determinant of *L. pneumophila* concentration in the coastal environment.

Ecology and Possible Sources

There is not much literature on whether the persistence of *L. pneumophila* differs in freshwater versus saline waters. Carvalho et al. (2007) did link a higher diversity of *Legionella* species with downstream, sewage-impacted waters when compared to lesser detection of *Legionella* in an upstream freshwater aquatic environment. The study was conducted along the Itanham River system in the Atlantic Forest of Brazil and did not definitively pinpoint salinity as the parameter affecting elevated detections. Rather, Carvalho et al. 2007 pointed to several factors related to anthropogenic sources such as the high level of organics and the presence of amoeba which allow for intracellular reproduction of *Legionella.* The host cell environment is known to protect *L. pneumophila* from harsh environmental conditions (Abdel-Nour et al. 2013). In the context of the urban environment and man-made water systems, this relationship between *Legionella* and protozoa does contribute to its overall persistence. Moreover, this relationship speaks to the importance of further investigation into the distribution of *Legionella* in the built environment and on street surfaces.

Management Relevance

Wet-weather contamination is closely connected to coastal water quality in the Hudson River Estuary. In New York City, the primary mechanism of sewage contamination is attributed to combined sewer overflow (CSOs) delivering a mixture of stormwater and untreated sanitary sewage to waterways. New York City has 426 CSO outfall pipes lining the city's coast which release approximately 20 to 25 billion gallons of untreated sewage and stormwater every year (NYC 2016). There are both national and regional monitoring programs that have been implemented to curtail this contamination. In 2012, NYC implemented a Long-Term Control Plan to deal with CSO contamination and related violations of the Clean Water Act (NYC 2012). This initially included a $1.7 billion-dollar investment in engineering measures and another $187 million toward green infrastructure to capture stormwater (NYSDEC 2012). Another management connection to bacterial pollution to coastal waterways, as pointed out by Montero and O'Mullan 2018, was the Municipal Separate Storm Sewer System (MS4) regulations (NYC 2016). MS4 is a system that transports stormwater in pipes separated from the sanitary wastewater system. Wastewater is delivered to a WWTP where it is treated, while untreated stormwater from separated sewers is discharged into a waterbody without treatment (NYC 2020). NYC's MS4 plan emphasizes preventing illicit discharges to stormwater pipes, controlling pollution in stormwater, and green or grey infrastructure initiatives. Additional management of stormwater under the MS4 permitting process may provide mechanisms to reduce both FIB and other microbes of concern, including *Legionella*. Currently, New York City does have legislations requiring action limits of *Legionella* concentrations in non-potable water systems. The New York state action limit is ≥ 20 per mL and the New York city action limit is ≥ 10 per mL (IDEXX 2019). The management of *Legionella* is especially important given the spike in associated outbreaks. During 2013-2014, drinking water reports showed a widespread

distribution of *Legionella* contamination (Benedict et al 2017). While *Legionella* is not considered a major groundwater contaminant, it does account for many CDC-reported drinking water illness outbreaks. *Legionella* was responsible for 57% of water–associated outbreaks and 13% of illnesses (Benedict et al 2017).

Aside from stormwater management, aerosolization management is also vital in preventing the spread of *Legionella*. New York City, thus far, has been one of the leading municipalities with legislation requiring regular screenings of large HVAC systems atop buildings across the city. A potential area of concern that remains is aerosolization sources in waterways such as Newtown Creek. Increased culturable bio-aerosols were reported in the near-shore environment of Newtown Creek (Dueker and O'Mullan 2014) when the aeration process was occurring. Bioaerosol contaminants are of increasing importance to society. The SARS-CoV-2 pandemic will undoubtedly have an impact on the biocontrol management solutions used in built environments. It is important to better understand aerosolization sources and the consequences of airborne transmission of microbial contaminants, especially in densely populated areas.

CONCLUSION

Higher levels of *L. pneumophila* were detected in urban estuarine waterways compared to suburban waterways. The lower-level detection of *L. pneumophila* in wet weather conditions in the suburban environment was an unexpected outcome, and further analysis of this result is required. Although there was very little signal of *L. pneumophila* in the suburban estuarine samples, the suburban street environment contained high levels of *L. pneumophila*, comparable to the urban street environment and urban CSO samples. Thus, the difference between urban and

suburban waterways is most likely related to the quantity of stormwater input from the built environment into an associated waterway. In highly dense urban centers like New York City, controlling the quantity of stormwater input into waterways is of management relevance given the public health consequences of aquatic pollution. While efforts have been made to manage *L. pneumophila* occurrence in cooling towers, there is not yet adequate information to minimize the occurrence and transmission of *Legionella* from other aerosolization sources such as WWTPs and aerated waterways. There is evidence to suggest that bio-aerosols can transmit pathogens a considerable distance. Thus, to minimize risk from Legionnaires' disease outbreaks, it is important to understand and manage environmental sources. Additional study is needed to evaluate the public health consequences of the widespread distribution of *Legionella* in urban and suburban water systems.

ACKNOWLEDGEMENTS

We would like to thank the Hudson River Foundation for supporting this project and the assistance of the Dueker Laboratory at Bard College. A special thanks to Grace Carter for all her efforts in helping to collect and process suburban water samples. Lastly, I would like to acknowledge the COVID-19 pandemic as a major event during this thesis and associated sample collection, lab analysis, and write-up. As a result, I would like to thank friends and family for all their encouragement and support throughout this difficult period.

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CONTRIBUTION OF THE HUDSON RIVER STRIPED BASS POPULATION TO THE MIXED COASTAL RECREATIONAL HARVEST AT MONTAUK POINT, NY

A Final Report of the Tibor T. Polgar Fellowship Program

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Hasegawa, E.H., I. Wirgin, L. Maceda, and N. Roy. 2021. Contribution of the Hudson River Striped Bass Population to the Mixed Coastal Recreational Harvest at Montauk Point, NY. Section IV: 1-32 pp. *In* S.H. Fernald, D.J. Yozzo, and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2020. Hudson River Foundation.

ABSTRACT

Striped bass, *Morone saxatilis,* support one of the most lucrative recreational and commercial fisheries along the Atlantic Coast of the U.S. Yet, today, striped bass numbers are down significantly along the Atlantic Coast and new management initiatives have been implemented in 2020 to reduce the catch coastwide. In the middle of its range along the Atlantic coast of the U.S., striped bass are seasonally migratory, ranging from the Bay of Fundy, Canada, to the Outer Banks of North Carolina. This coastal migratory stock of striped bass is primarily supported by spawning populations in the Hudson River and Chesapeake Bay, with smaller contributions from the Delaware River and Roanoke River populations. Spawning success and recruitment within these populations vary temporally and is often asynchronous suggesting that they likely require differing levels of protection over time. Therefore, an important prerequisite to effective management of these populations is a quantitative estimate of their relative contributions to coastal landings. Montauk Point is probably the site within New York State that hosts the largest striped bass recreational fishery and is geographically sited to host migrant striped bass from all possible populations that contribute to the coastal migratory stock. Therefore, the stability of the Hudson River contributions to the recreational harvest of striped bass at Montauk Point in 2019 were evaluated. Mixed Stock Analysis (MSA) and Individual Based Assignment (IBA) were used to test microsatellite DNA data. In the Montauk mixed-stock fishery, results showed that Chesapeake fish are the largest contributors (88%), followed by Hudson River fish (8.2%), and, lastly, Roanoke River fish (3.4%). Data from this study will be useful for NOAA managers in their stock assessment modeling for striped bass since variations in recruitment of contributing stocks vary over time, which reflects their differing population abundances.

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INTRODUCTION

The Hudson River is home to three glamour species of fishes that are of important commercial and recreational interests. Striped bass, *Morone saxatilis*, is one of these species (the others being American shad, *Alosa sapidissima*, and Atlantic sturgeon, *Acipenser oxyrinchus*, that at one time supported lucrative commercial fisheries within the Hudson River (Limburg et al. 2006). While the Hudson River still supports a robust seasonal recreational fishery and lucrative charter fishing operations, its commercial fishery for striped bass was closed river-wide in 1976, and remains closed today because of elevated levels of PCBs in its population, exceeding the U.S. Food and Drug Administration limit of 2.0 ppm for human consumption (Ashley et al. 2000). Restoring this fishery to its full potential requires monitoring the abundance of striped bass within the river itself, but also its quantitative vulnerability to mixed stock harvests along the Atlantic Coast. In 2016, the Atlantic States Marine Fisheries Commission (ASMFC) highlighted stock composition analysis as a high priority for striped bass fishery management (ASMFC 2016). Determining the population origin of striped bass in mixed coastal fisheries such as at Montauk Point is critical in evaluating the impact of mortality in mixed coastal fisheries on individual populations, such as the Hudson River, comprising the coastal migratory stock. These results are important to the New York State Department of Environmental Conservation (NYSDEC), managers of the Hudson River component of the resource, as well as the ASMFC charged with managing the resource coastwide. This is especially important today given the precarious state of the

fishery and the stated need by the ASMFC in 2019 to significantly reduce landings and catch and release mortality in 2020 (ASMFC 2019).

Striped bass in marine waters of the northeastern U.S. are termed the coastal migratory stock and are supported by spawning in the Hudson River, eight tributaries of the Chesapeake Bay, and to a lesser extent the Delaware (Waldman and Wirgin 1994) and Roanoke rivers (Callihan et al. 2015; Wirgin et al. 2020). Striped bass spawned in these rivers are anadromous and adults seasonally migrate coastally from the Outer Banks of North Carolina to the Bay of Fundy in Atlantic Canada (Wirgin et al. 2020). The proportion of specimens from the different spawning populations at any single coastal site probably varies significantly over time depending on the relative recruitment success in the individual populations which contribute to the mixed coastal stock. Relative reproductive and recruitment success among the contributing populations may be asynchronous: one population may experience much higher success than another population in the same year. For instance, the reproductive success in the Hudson River striped bass population was noticeably higher than that of Virginia populations in 2017, with approximate geometric mean values of nine and 17, respectively (Figures 1 and 2, NYSDEC 2020; Gallagher et al. 2018). Similarly, 2011 was an outstanding year for Virginia populations (mean=27) and just average in the Hudson (mean=11). These results highlight the different dynamics experienced by these populations and suggest that these populations be managed separately rather than pooling them into one unit.

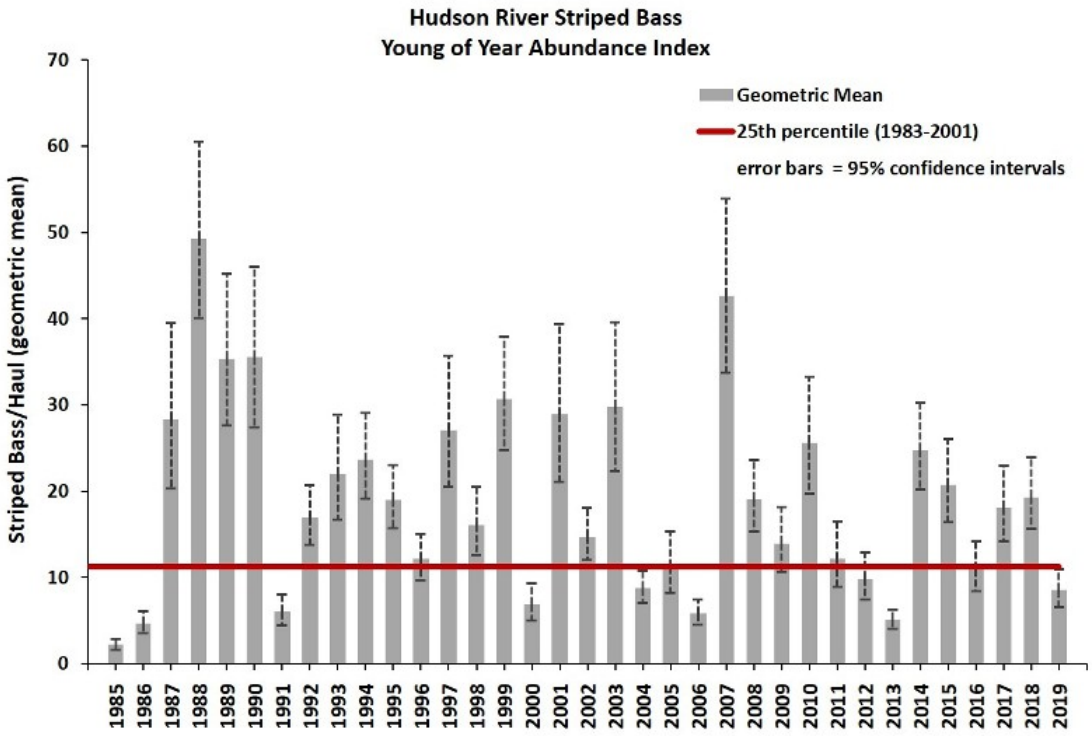

Figure 1. Reproductive success of striped bass in the Hudson River (Young of Year Striped Bass Abundance, NYSDEC 2020).

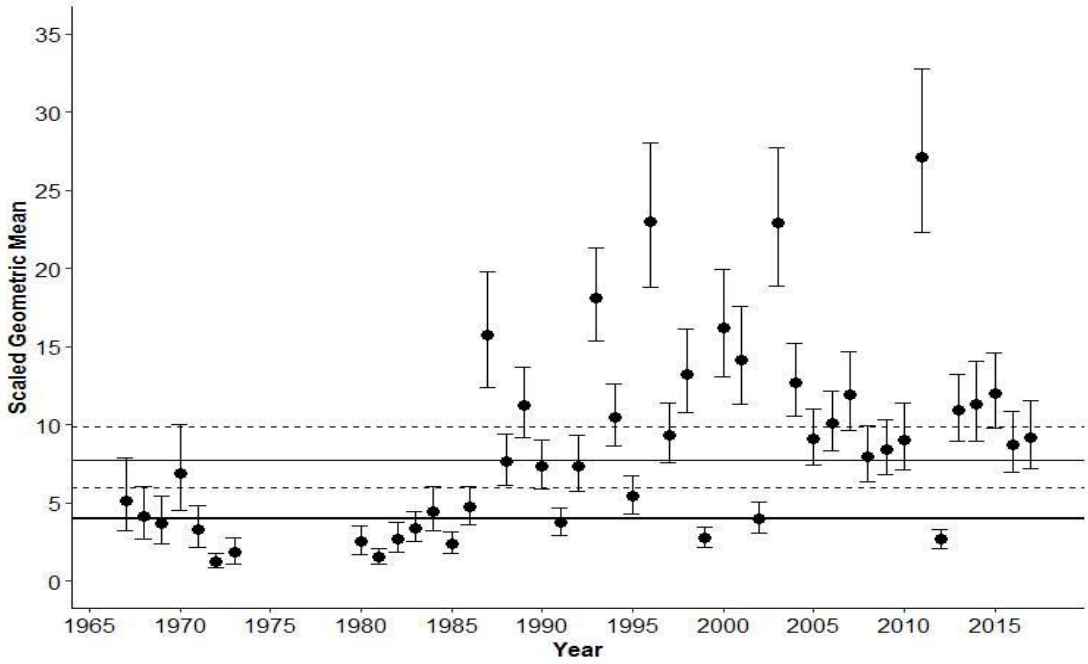

Figure 2. Reproductive success of striped bass in Virginia (Gallagher et al. 2018).

Their popularity as a sport fish and profitable commercial fishery along the Atlantic coast is a testament to how valued striped bass are as an ecosystem resource. In New York alone, $1.165 billion was added to the state's gross GDP by the recreational fishery in 2016 (Southwick Associates 2019). Striped bass spawned in the Hudson are subject to significant mortality from commercial and sport fisheries in coastal waters from North Carolina to Atlantic Canada. Based on past data, the coastal migratory stock is composed primarily of Chesapeake and Hudson River fish, with smaller contributions from other populations (Waldman et al. 1997). The abundance of striped bass populations is known to fluctuate significantly depending on spawning success, recruitment, and vulnerability of populations to within-river and coastal harvest. Reproductive success of a population is believed to be subject to a variety of physical environmental parameters and density dependent biological factors. These factors resulted in a coastwide crash of abundances of striped bass populations in the early 1980s followed by recovery in the early to mid-1990s. This was followed by two decades of recruitment success and what was considered a totally rebuilt fishery; however, the past several years witnessed alarming signs of population declines as evidenced by a significant reduction in female spawning biomass and a decrease in recruitment success (Limburg et al. 2006). As a result, severe cuts in harvest were being enacted for the year 2020 by the ASMFC, including several measures that would ensure an overall 18% decrease in fishing mortality for the coastal migratory stock (ASMFC 2019). For example, Virginia has imposed a complete moratorium on the landings of adults during the 2020 trophy spawning season and a 50% reduction of harvest during the fall season by reducing the possession limit from two to one fish (Commonwealth of

Virginia Marine Resources Commission 2020). Closures on harvests have also been declared for select populations in North Carolina (Ingram 2021).

Understanding genetic population structure is important in fisheries management because it informs fishery managers and researchers on the presence of individual reproductively isolated units within a species' distribution that may differ in: 1) reproductive and recruitment success, 2) growth, maturity, and natural mortality rates, 3) migratory behavior, and 4) exploitation rates. As a result, managers need to know the origins of fish in mixed stock fisheries to set appropriate and effective regulations to best protect the most vulnerable populations (Waldman et al. 1997); however, striped bass is still managed as a single stock coastwide despite the advantages of management on a stock specific basis and evidence that discrete stocks exist.

A sensitive method to genetically distinguish stocks from each other is needed. In this study, informative striped bass microsatellite DNA markers are used (Wirgin et al. 2020) to accurately determine the Hudson River's contribution to the Montauk mixed stock recreational fishery. Microsatellites for striped bass were isolated by Rexroad et al. (2006) and Roy et al. (2000) and were recently shown to be effective in distinguishing striped bass populations that contribute to the coastal migratory stock (Wirgin et al. 2020). Individual Based Assignment (IBA) was used to assign each individual Montauk specimen to its most likely spawning population and Mixed Stock Analysis (MSA) was used to quantify the percentage contribution of each spawning population to the Montauk harvest. This study was the first to evaluate the population composition of the Montauk fishery over an entire fishing season rather than a brief snapshot. It provides a stock composition estimate for the 2019 fishing season.

Techniques used in the past to distinguish striped bass populations and to assign individuals to specific population origins include meristics, morphometrics, scale shape, trace element composition of scales, fatty acids, and parasite assemblages (reviewed in Waldman et al. 1988), along with molecular techniques such as protein electrophoresis (Morgan et al. 1973), restriction fragment polymorphism analysis (RFLP) of mitochondrial DNA (mtDNA) (Wirgin et al. 1990), DNA fingerprinting (Wirgin et al. 1991), single nucleotide polymorphisms (SNPs) (Wirgin et al. 2005), microsatellite DNA (Robinson et al. 2004; Brown et al. 2005; Gauthier et al. 2013; Anderson et al. 2014; Wirgin et al. 2020), and next generation single nucleotide polymorphism analysis (Leblanc et al. 2018). However, some of these methods have met some challenges; for instance, allozymes did not vary substantially (Morgan et al. 1973) and mtDNA variation was very low in striped bass (Wirgin et al. 1990).

Microsatellite DNA analysis has provided a valuable tool in the identification of the population structure of many economically and ecologically important fishes. In many instances, microsatellites provided informative data in a management context for species and populations that heretofore proved refractory to other less sensitive methods of stock identification. This is because of the high levels of allelic variability often exhibited at microsatellite loci (Kumar et al. 2019). As reviewed by Abdul-Muneer (2014), microsatellite markers have been used to determine whether hatchery-raised fish are genetically differentiated from wild populations, to distinguish natural populations, analysis of pedigree, genomic variation, and studies of evolution. These highly polymorphic loci are ideal as genetic tags in aquaculture, stock discrimination, and population genetics.

Several studies have attempted to determine the relative contribution of spawning populations of striped bass to mixed coastal collections. In the most cited study, and most referred to by management, Berggren and Lieberman (1978) used a suite of five morphometric measurements to find an overwhelmingly large Chesapeake (90.2%) and small Hudson (6.6%) contribution to 1975 coastwide collections from multiple locales ranging from Cape Hatteras to Maine. In a genetic study, Wirgin et al. (1993) used RFLP analysis of mtDNA to estimate that the Hudson River contribution to a fall collection in 1989 from the eastern Long Island haul seine fishery was much larger, at 73%. Both studies suffered from shortcomings. Berggren and Lieberman's (1978) effort relied on markers that are known to be subject to environmental influence and therefore temporal instability, and Wirgin et al.'s (1993) investigation used DNA that exhibited limited levels of variation. Furthermore, given the dynamic nature of reproductive success in these populations and likely fluctuating contributions to coastal migrations, it is time to revisit this issue with a more sensitive and stable genetic technique.

An added benefit from a contemporary quantification of the relative contributions of these populations to the Montauk sport fishery contribution is more informed human health risk from the consumption of PCB-contaminated striped bass from the Hudson River. Despite six years of remediation, recent evidence suggests that striped bass from the lower Hudson River still exhibit significantly higher levels of lipid normalized total PCBs than striped bass from other systems (Klawinski and Greenberg 2017).

It is hypothesized that smaller contribution of the Hudson River population to the Montauk mixed stock recreational striped bass fishery in 2019 than in 1990 will be found. According to young-of-year striped bass surveys done by the New York State

Department of Environmental Conservation (NYSDEC), there were fewer juvenile striped bass per haul in 2019 compared to 1990 (Figure 1, NYSDEC 2020). Furthermore, it is likely that the proportional contributions to the coastal migratory stock have changed over time based on differing relative recruitment success in the contributing populations. Lastly, there is likely no significant difference in stock composition by total length or collection date.

METHODS

Montauk Specimen Collections

410 adult striped bass were collected by angling weekly from June 23 through Oct 31, 2019, and were provided by one of the captains of the Montauk charter boat fleet. Date of capture and total length were recorded for each specimen. Montauk supports a significant recreational fishery, is central to the migratory route of the coastal stock of striped bass, and may be the coastal locale where the population composition is most representative of mixed aggregations of specimens from the Hudson, Chesapeake, Delaware, and Roanoke populations.

Figure 3. Sampling locations of striped bass; Montauk Point fish were collected in 2019 and all other specimens were collected in past years. US Census 2019 Cartographic Boundary Files, Rivers of the US (National Operational Hydrologic Remote Sensing Center); Map made with ArcMap 10.8.1.

Reference Specimen Genotyping

To compare Montauk specimen genotypes to those of reference population specimens, genotypes from reference population specimens were analyzed from the Hudson River, Roanoke River, Delaware River, and the tributaries of the Chesapeake

Bay from past years. Specimens were collected from the Upper Bay, Choptank, Patuxent, Pocomoke, Nanticoke, Rappahannock, and Potomac rivers. These reference samples allowed for evaluation of temporal stability of diagnostic microsatellite genotypes in these three populations that are the major contributors to the coastal migratory stock.

DNA isolations

Due to four specimens getting lost or yielding very little DNA, a total of 406 Montauk specimens were used for analysis. 765 reference specimens were used to compare with Montauk DNA. Genomic DNA was isolated from fin clips by their incubation in hexadecyltrimethylammonium bromide buffer (CTAB) buffer and digestion with proteinase K, followed by standard phenol-chloroform extractions and alcohol precipitations. Briefly, fin clips were 1) placed in phosphate-buffered saline (PBS) for 10 min, 2) placed in water for 10 min, 3) placed in vials of CTAB and proteinase K, and 4) placed in a water bath at 37°C overnight. DNA was extracted by 1) centrifuging specimens in 180 µl of chloroform:isoamyl alcohol at a 24:1 ratio, 2) taking out the aqueous layer and adding 90 µl of phenol and centrifuging, 3) placing the aqueous layer into 90 µl of phenol, adding 90 µl of chloroform:isoamyl alcohol, then centrifuging, 4) adding 180 µl of chloroform:isoamyl alcohol and centrifuging, 5) placing the supernatant to 200 µl of isopropanol and 1.8 µl of glycogen then mixing very well, and finally 6) placing the vials in a -80°C freezer overnight. Each centrifugation was done at 13,000 rpm for 5 min.

To isolate and purify the DNA pellet, 1) vials were centrifuged 2) isopropanol was dumped out, then 200 µl of 70% ethanol was added, 3) vials were centrifuged, 4)

ethanol was removed, and vials were overturned to dry for 1 hour, and 5) 110 µl of sterile water was added, and vials were incubated at 37°C for 1 hour. Centrifugations were done at 13,500 rpm for 30 min. DNA concentrations of each sample were quantified using a Nanodrop ND-100 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware) and adjusted to 25 ng/µl for standardization of subsequent procedures. Each sample had a final volume of 125 µl.

DNA analysis

Eight microsatellite loci were used in total. Four loci, MSM1357, MSM1598, MSM1584, and SB108, were selected from the battery developed by Rexroad et al. (2006). Wirgin et al. (2020) previously used these four loci to successfully distinguish spawning populations of striped bass coastwide and used MSA and IBA in determining the status and population origin of specimens in two rivers in Atlantic Canada, including the Saint John and Annapolis. Four newly developed loci, MSM1626, MSM1557, MSM1556, and MSM1592, were used since they showed efficacy in distinguishing striped bass populations. Polymerase chain reactions (PCR) were performed as described in Robinson et al. (2004). Polymerase chain reactions for MSM primers were conducted in 12.5 µl total reaction volumes in 96 well plates, where each well contained 3.5 µl of DNA, 6.6 µl of sterile water, 1.25 µl of KlenTaq1 reaction buffer (AB Peptides, Inc., St. Louis, Missouri), 0.5 µl of forward and reverse primers (1 µM stock primer) (Integrated DNA Technologies, Coralville, IA), 0.1 µl of dNTPs (250 µM stock of each) (GE Healthcare, Chicago, IL.), and 0.03 µl of KlenTaq1 enzyme (AB Peptides, Inc., St. Louis, Missouri). Polymerase chain reactions volumes for SB108 contained 3.5 µl of DNA, 5.6 µl of sterile water, 1.25 µl of Taq polymerase reaction buffer (Roche Diagnostics

Corporation, Indianapolis, IN), 0.1 µl of dNTPs, 1.0 µl of forward and reverse primers, and 0.05 µl of Taq polymerase (Roche Diagnostics Corporation, Indianapolis, IN). All forward primers were labeled with Well-Red dyes on their 5' end (Sigma Aldrich, St. Louis, MO). Cycling parameters were as follows: initial denaturation at 95°C for 5 min, followed by 69 cycles of denaturation at 95°C for 15 s, annealing at 56°C, 58°C, 62°C for 15 s, extension at 72°C for 30 s, and final extension at 72°C for 7 min.

Characterization of microsatellite genotypes were performed using a Beckman Coulter CEQ8000 capillary-based DNA sequencer (Beckman Coulter, Inc., Fullerton, CA). To make economical use of the sequencer, 0.5-4.5 µl of product from each of up to four PCR reactions were multi-pooled and loaded onto 96-well plates along with 0.5 µl of Beckman Coulter CEQ DNA Size Standard-400 and 36 µl of Sample Loading Solution (Beckman Coulter). All analyses were performed using the FRAG 1.

Statistical Analysis

Composite microsatellite genotypes at the eight loci were compiled for all Montauk and reference population specimens. Departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed with GENEPOP version 4.1.0 (Rousset 2008) using default parameters. The program STRUCTURE version 2.3.4 (Pritchard et al. 2000) was used to evaluate population clusters with K = 1 – 11, where 11 was the total number of sampling locations. The web-based program StructureSelector (Li and Liu 2017) was used to visualize results. IBA tests implemented in GeneClass2 (Piry et al. 2004) were used to identify the population origin of each specimen collected from Montauk. IBA tests using multi-locus likelihood functions were used to determine the likelihood of each individual's genotype being found in the

Hudson, Delaware, Chesapeake, or Roanoke reference collections. MSA implemented in the ONCOR program (Kalinowski et al. 2008) was used to estimate the proportion of each reference population in the mixed sample of Montauk fish and to determine the probability of each individual's belonging to each of the reference collections. In contrast to IBA, MSA applies mixture modelling, taking into consideration the genotypes of individual fish across multiple loci, multilocus genotype distributions of the reference samples, and the multilocus genotype distribution in the mixture samples. Mixture proportions and their 95% confidence limits were determined based on 10,000 bootstraps. Tests to determine whether there were genetic differences by stock, Pearson's chi-square tests were performed in RStudio 1.4.1106.

Facilities

All the facilities and instrumentation needed to conduct these studies were available in Dr. Wirgin's lab and shared facilities in NYU's Department of Environmental Medicine. Equipment available in Dr. Wirgin's labs included many gel electrophoresis chambers, power supplies, three MJ Research thermal cyclers, and five microcentrifuges. Equipment available in the shared Molecular Support Core (Dr. Wirgin is Co-Director of the NIEHS Center Molecular Cell and Analytic Services Facility Core) included a Nanodrop ND-1000 Spectrometer and Beckman Coulter CEQ8000 automated DNA sequencer.

RESULTS

Genetic Diversity of Loci and Reference Populations

The number of alleles per locus in the reference collections ranged from 15 to 32, with MSM1598 having 15 alleles and MSM1556 having 32. The effective number of alleles, which state the number of alleles with the same frequencies, ranged from 7 to 14, with MSM1598, 1557, and SB108 having 7 and MSM1556 having 14. The fixation index (Fst) values show little genetic differentiation among populations for all loci. None of the loci showed significant departure from Hardy-Weinberg equilibrium.

Table 1. Allelic characteristics of polymorphic loci and genetic differentiation between reference populations

Locus	Number of Alleles	Effective Number of Alleles	Fst
MSM1598	15	7.1	0.018
MSM1584	19	8.9	0.004
MSM1357	18	8.0	0.009
SB108	22	7.1	0.006
MSM1626	30	11.9	0.006
MSM1556	32	14.6	0.005
MSM1592	25	10.3	0.007
MSM1557	20	7.3	0.020

F'st analysis showed highly significant differences among Hudson, Delaware, pooled Chesapeake, and Roanoke populations. F'st values were used instead of Fst since F'st corrects for Fst estimates for heterozygosity within populations. Hudson, pooled Chesapeake, and Roanoke populations were significantly different from each other ($p<0.005$), while the Delaware specimens could not be distinguished from the pooled Chesapeake collection, which included the Upper Bay, Choptank, Patuxent, Pocomoke, Nanticoke, Rappahannock, York, and Potomac specimens.

Table 2. P-values of F'st pairwise comparisons between reference populations; Populations were considered significantly distinct if their p-values were <0.05.

	Hudson	Delaware	Upper Bay	York	Rappahannock	Choptank
Hudson	--	0.001	0.001	0.001	0.001	0.001
Delaware	0.001	--	0.07	0.064	0.079	0.016
Upper Bay	0.001	0.07	--	0.039	0.022	0.001
York	0.001	0.064	0.039	--	0.183	0.001
Rappahannock	0.001	0.079	0.022	0.183	--	0.001
Choptank	0.001	0.016	0.001	0.001	0.001	--
Nanticoke	0.001	0.054	0.001	0.005	0.001	0.068
Potomac	0.001	0.072	0.161	0.23	0.293	0.001
Pocomoke	0.001	0.884	0.625	0.519	0.229	0.115
Patuxent	0.001	0.294	0.005	0.029	0.003	0.003
Roanoke	0.001	0.001	0.001	0.002	0.001	0.001

	Nanticoke	Potomac	Pocomoke	Patuxent	Roanoke
Potomac	0.001	--	0.087	0.02	0.001
Pocomoke	0.293	0.087	--	0.994	0.005
Patuxent	0.001	0.02	0.994	--	0.001
Roanoke	0.001	0.001	0.005	0.001	--

Population Clusters

STRUCTURE analysis showed three major distinct reference populations: Hudson, Chesapeake, and Roanoke. Delaware specimens were not genetically distinct from Chesapeake specimens. Separate analysis of only Chesapeake tributary populations showed two distinct populations, where Choptank and Nanticoke clustered moderately differently from the other populations.

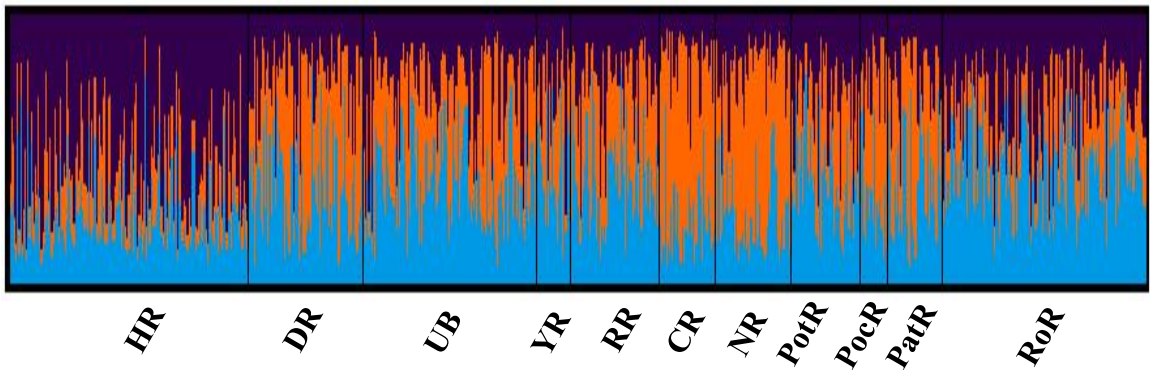

Figure 4. **Genetic clusters of reference populations based on eight microsatellite loci; HR = Hudson River, DR = Delaware River, UB = Upper Bay, YR = York River, RR = Rappahannock River, CR = Choptank River, NR = Nanticoke River, PotR = Potomac River, PocR = Pocomoke River, PatR = Patuxent River, RoR = Roanoke River**

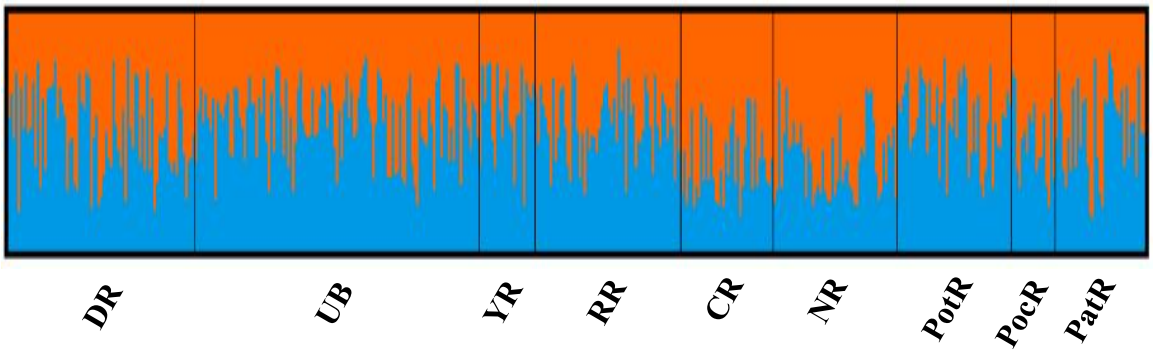

Figure 5. **Genetic clusters of reference individual Chesapeake populations based on eight microsatellite loci.**

Assignment Accuracy

Table 3. **Assignment accuracy of reference specimens with their respective 95% CIs.**

	Assignment Accuracy	95% CI
Hudson	0.9217	(0.8645, 0.9729)
Chesapeake	0.9638	(0.9118, 0.9984)
Roanoke	0.8128	(0.7251, 0.8858)

Using 100% simulations in ONCOR, the assignment accuracy of specimens to the three populations was tested. This step is required to measure how accurately specimens

are assigned back to their sampling location and indicates how useful the loci are at assigning a mixed stock fishery sample to reference populations. Assignment accuracy was highest for Chesapeake specimens with a value of 0.96, and lowest for Roanoke specimens with a value of 0.81.

Mixed Stock Analysis

MSA was performed with 10,000 bootstraps. The pooled Chesapeake population was the largest contributor to the Montauk mixed stock, followed by Hudson, then Roanoke as the smallest contributor.

Table 4. MSA performed with 10,000 bootstraps and 95% CIs.

	Population Estimate	**95% CI**
Hudson	0.0821	(0.044, 0.142)
Chesapeake	0.8839	(0.799, 0.920)
Roanoke	0.034	(0.010, 0.095)

Individual Based Assignment

IBA showed that the Montauk 2019 mixed stock was comprised of 77% Chesapeake, 14% Hudson, and 9% Roanoke specimens. Though the percentage of Chesapeake population is less in IBA than in MSA, and the Hudson and Roanoke populations are greater in IBA than in MSA, the results from both analyses are comparable since Chesapeake specimens are the predominant contributor, followed by Hudson, then Roanoke. IBA gives a confidence score of how likely an individual specimen is from a certain population. Specimens with a confidence score of 70% or greater were included.

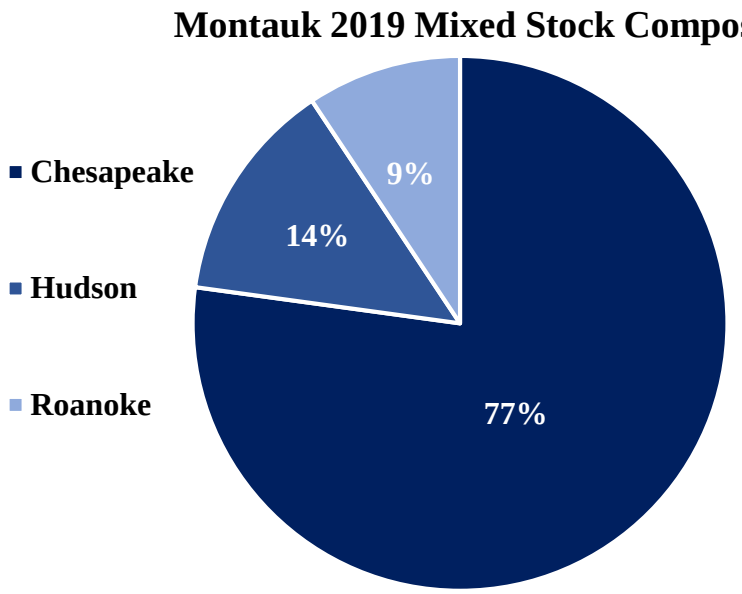

Figure 6. Percent composition of Montauk 2019 mixed stock based on IBA testing.

Genetic Differences by Collection Date and Total Length

To determine whether there were differences in stock composition by collection date or total length (cm), Montauk specimens were separated into quartiles, where the first quartile was comprised of fish caught earliest in the season or the smallest fish. IBA was done to find proportions of Chesapeake, Hudson, and Roanoke individuals per quartile. Pearson's chi-square tests were done to determine whether the stock and collection date or length were correlated. Chi-square tests did not show significant dependence between the two variables.

Table 5. Stock composition by collection date; $\chi^2 = 5.925$, p-value = 0.43

	Chesapeake (%)	Hudson (%)	Roanoke (%)
1st Quartile	76.6	13	10.4
2nd Quartile	82.8	9.4	7.8
3rd Quartile	80.0	12.0	8.0
Latest Group	69.9	19.2	11.0

Table 6. Stock composition by total length (cm); $\chi^2 = 3.715$, p-value = 0.72

	Chesapeake (%)	Hudson (%)	Roanoke (%)
1st Quartile	72.3	18.5	9.2
2nd Quartile	78.3	12.0	9.6
3rd Quartile	78.9	14.0	7.0
Largest Group	78.6	10.7	10.7

DISCUSSION

All loci used showed high numbers of alleles per locus and none significantly departed from Hardy-Weinberg equilibrium. All but one of the loci were four base-pair repeats, where SB108 was the only locus that was a two base-pair repeat. Preference for four base-pair repeats was due to how it was easier to score genotypes when there was more space between each allele. The MSA and assignment accuracy results from this study indicated that the eight loci used were reliable to differentiate populations in a mixed stock. There were an additional four loci that have been used in previous striped bass research (SB113, SB117D, SB91, and MSM1334) (Wirgin et al. 2020), though due to substantial failure in obtaining amplified PCR products, PCR specifications for these loci need to be reviewed. Although the eight loci used in this study were sufficient for achieving high assignment accuracy, more loci would increase the resolution.

Some loci were more difficult to genotype than others and there may have been errors in genotyping some alleles. Nevertheless, this issue was mostly relevant to SB108, a two base-pair repeat locus. As a result, more stutter peaks around the true allele made scoring more difficult than if the peaks were farther apart, as was true with four base-pair repeat loci. Nevertheless, most of the genotyping was done by one individual and methods were kept consistent to avoid recording false alleles.

Microsatellites have proven to be useful and reliable in distinguishing baseline populations from a mixed stock for fisheries management in commercially important species other than striped bass, including whiting (*Merlangius merlangus*), Atlantic salmon (*Salmo salar*), and Atlantic sturgeon (*Acipenser oxyrinchus*). Rico et al. (1997) found significant differences between northern and southern whiting of the North Sea using two loci. The authors found no significant differences among other sampled populations, indicating that there was high exchange of migrants. Bradbury et al. (2018) identified 26 reporting groups of Atlantic salmon in Labrador, Canada using a large panel of sequenced microsatellites. The authors achieved high levels of assignment accuracy, averaging between 88% to 91%. Wirgin et al. (2018) analyzed the mitochondrial DNA control region and microsatellite loci to determine the population of origin of subadult Atlantic sturgeon found in the Hudson River estuary north of the Battery in New York City. They found that the majority of subadult sturgeon assigned back to the Hudson River, but they also found small contributions from the Delaware River, Kennebec River, James River, and Ogeechee River with assignment accuracies above 80%. In sum, microsatellite analysis is capable of defining geographically fine-scale reporting groups from mixed stocks and resolution is improved upon addition of more loci.

This study's results are comparable to those of Berggren and Lieberman (1978), who found the Chesapeake population to be the major contributor by far to the coastal migratory stock of striped bass with Hudson and Roanoke populations being minor contributors. Berggren and Lieberman (1978) measured the number of lateral line scales, left and right pectoral rays, second dorsal rays, anal rays, upper-arm gill rakers, fork length, snout length, head length, and inter-nostril width to distinguish individuals and

used multivariate discriminant analyses to determine stock of origin. Similar population contributions were found by Kneebone et al. (2014), who determined striped bass population contribution to coastal Massachusetts waters using acoustic telemetry. The authors pooled the Roanoke and Chesapeake populations and kept the Delaware River stock separate from the Chesapeake. They also found the Chesapeake/Roanoke stock to be the largest contributor with lesser contributions from the Delaware and Hudson River stocks. Wirgin et al. (2020) recently used microsatellite DNA analysis to successfully define striped bass populations coastwide and to estimate the potential efficacy of microsatellite markers for mixed stock analysis. In their simulations, 93.2%, 96.5%, and 81.6% of the Hudson River, Chesapeake Bay-Delaware River, and Roanoke River specimens in individual collections were assigned to the correct baseline populations, respectively. Overall, the stock contributions to a mixed Atlantic stock from the present study are comparable to results reported by other studies.

Chesapeake Bay tributaries are the largest contributors to the Montauk mixed stock and the data indicates that the tributaries can be pooled into one management unit. Findings by Brown et al. (2005) also show little genetic differentiation among the Upper Bay, Choptank, Potomac, Nanticoke, Upper York, and Rappahannock tributaries when using microsatellite and mitochondrial marker estimates of population structure. Similarly, LeBlanc et al. (2018) pooled Delaware River specimens in with the Chesapeake stock and reported very low genetic differentiation among Upper Bay, Potomac River, Rappahannock River, and Delaware River specimens. The present study also showed that Choptank and Nanticoke specimens may be moderately differentiated

from collections from other Chesapeake tributaries since STRUCTURE analysis found an optimal K value of two across all sampled Chesapeake locations. LeBlanc et al. (2018) and Wirgin et al. (2020) reported similar results where Choptank and Nanticoke specimens showed heterogeneity from samples of western Chesapeake Bay tributaries. Fine-scale analyses of eastern tributary specimens are warranted.

There was a wide range of sample numbers per reference population among the Chesapeake tributaries in the present study; for instance, there were 117 specimens from the Upper Bay and only 18 from the Pocomoke River. Although previous research showed little genetic differentiation among striped bass from Chesapeake tributaries, more samples from each tributary would provide more fine-scale resolution of potential genetic dissimilarities among populations.

A recent study found that the majority of large striped bass tagged in Chesapeake Bay migrated to Massachusetts waters in the spring with a small group lingering for about 60 days in Long Island Sound, then stayed there again for about the same amount of time when they returned from Massachusetts in the fall (Secor et al. 2020). Montauk Point is a critical location in this migration corridor and any development that may interfere with movement in the area should be thoroughly evaluated.

Currently, there are plans for construction of more wind turbines at the Block Island Wind Farm off Rhode Island and connecting the cable ashore at East Hampton, NY. Construction will be in the migration route of striped bass and may impede steady movement. Interviews with recreational fishers who regularly fish around Block Island noticed seeing striped bass around the wind farm, perhaps due to wind farms creating artificial reefs that attract prey for striped bass (ten Brink and Dalton 2018); however,

construction noise from pile driving may deter striped bass, since some respondents noted seeing fewer individuals closer to turbine construction compared to other parts of the island. Threats to striped bass migration need to be considered when issuing permits for construction and more research should be done to determine how striped bass will react to wind farm expansion.

This study was the first to determine striped bass stock composition at Montauk Point, NY, over a whole fishing season from June to October, 2019. Using reference samples of baseline populations to compare with the mixed stock, the Chesapeake stock was shown to be the main contributor, with Hudson and Roanoke stocks being minor contributors. Hudson River striped bass are still recovering from overfishing and habitat loss and their reproductive success hit below the 25^{th} percentile in 2019. Recreational fishers and fisheries managers need to know whether the fish they are exploiting are sustainable; thus, knowing the main contributors to an important mixed stock with high landings is critical.

ACKNOWLEDGEMENTS

I would like to thank the Tibor T. Polgar Fellowship Committee and the Hudson River Foundation for the opportunity to complete and share this research with their continued support. I also thank my advisor Dr. Isaac Wirgin for his expert guidance and mentorship. Special thanks go out to Lorraine Maceda for her teachings and assistance, along with Dr. Nirmal Roy for his help in data analyses. This work would not have been possible without the efforts of Captain Burt Prince and the crew of the Susie E II for catching specimens. I would also like to thank Justin Prince for taking fin clips for DNA analysis.

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Special thanks to Emma Blackford for assistance in formatting manuscripts.