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EXPLORATION OF MARINE LICHENIZED FUNGI AS BIOINDICATORS OF COASTAL OCEAN POLLUTION IN THE BOSTON HARBOR ISLANDS NATIONAL RECREATION AREA

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abstract. This preliminary exploration of marine lichenized fungi (lichens) as bioindicators of water pollution examined the distribution of intertidal lichen communities in the Boston Harbor Islands National Recreation Area with respect to recorded pollution throughout the harbor. We found significant negative associations between pollution measurements and the health of the lichen community based on cover and species richness. We also observed significant differences in species composition between areas of higher pollution and areas of lower pollution, though not enough data are available to establish the pollution sensitivity or tolerance of individual species. We note that difficulties in the collection and identification of marine lichens hamper efforts to use them broadly as bioindicators. This study suggests that marine lichens could prove useful as bioindicators, but more research is needed to understand the differential effects of pollution on individual species as well as to establish practical procedures both for quantifying marine lichen community health and for widespread bioindication using marine lichens. Finally, one species collected during this study, *Verrucaria ceuthocarpa*, represents a first report for the Boston Harbor Islands National Recreation Area.

Key words: environmental impact, intertidal zone, lichen diversity, marine ecology, ocean health, water pollution

Marine ecosystems are simultaneously some of the most important and diverse components of the biosphere and some of the most vulnerable (Kite-Powell et al. 2008). Increasing anthropogenic pollution and terrestrial habitat loss have led to the destruction of many marine ecosystems and have created health and safety risks for human populations. Because coastal pollution is likely to increase, coastal ecosystem health must be analyzed to appropriately evaluate environmental concerns (Sindermann 1995).

Bioindicators are organisms that can be used to judge the health of an ecosystem (Markert et al. 2003). These organisms can be effective in identifying locations that require further inspection or intervention (Brodo 1966; Markert et al. 2003). Lichens frequently have been used as indicators of air quality because of the sensitivity of some species to various pollutants (Brodo 1966; Nimis et al. 2002; Wolseley et al. 2006), particularly heavy metals and other particulates that are often deposited into the lichen thallus (Barglagi 2016; Parvianen et al. 2019; Purvis et al. 2005).

There have been numerous studies into the response of freshwater aquatic lichens to acidification (Gilbert and Giavarini 1997, 2000), eutrophication (Gilbert and Giavarini 2000; Hauck 2010), siltation (Gilbert 1996), and heavy metal pollution (Monnet et al. 2005, 2006). The use of lichens (Gilbert and Giavarini 2000; Nascimbene et al. 2013) and bryophytes (Vanderpoorten 1999) as general biomonitors of freshwater pollution has been reviewed. The examinations of lichen distribution with respect to acidification, siltation, and eutrophication suggest that freshwater lichens are sensitive to all these anthropogenic effects and that siltation and acidification in particular pose threats to freshwater lichen biota (Gilbert 1996; Gilbert and Giavarini 1997, 2000). Studies on heavy metal pollution have primarily been laboratory investigations of heavy metal accumulation in *Dermatocarpon luridum* (Dill. ex With.) J. R. Laundon, a semi-aquatic lichen that has a sensitivity to copper pollutants. These studies suggest the potential use of *D. luridum* as a biomonitor for copper pollution (Monnet et al. 2005, 2006), along with certain species of mosses or bryophytes (Bruns et al. 1997). In discussing general biomonitoring, Nascimbene et al. (2013) posed several challenges and perspectives suggesting that widespread implementation is possible, but difficult due to complications involved in the collection and identification of freshwater lichens and the accurate comparison of field data from different sources.

Considerations of marine lichens—those that inhabit the intertidal and sublittoral zones—as bioindicators have been much more limited.

Most research was conducted in Wales in the late 1960s and early 1970s at University College of North Wales, as summarized by Fletcher and Crump (2002). There was an observed spike in coastal lichen mortality associated with the use of emulsifiers to clean up the Torrey Canyon oil spill off the coast of Britain in 1967 (Ranwell 1968). The toxicity of the emulsifiers to *Lichina pygmaea* (Lightf.) C. Agardh and *Xanthoria parietina* (L.) Th. Fr. was examined by Brown (1972, 1973), and the recovery of these lichen communities in coastal Wales after the oil spill was documented by Gilbert (2001). These studies established coastal and marine lichen sensitivity to emulsifiers used to clean up oil spills. Other studies are rare and generally components of larger papers on marine algae (Stengel et al. 2004) or on the transfer of marine pollutants onto land (Scerbo et al. 1999; Wen and Carignan 2009). Stengel et al. (2004) suggested that the lichen *Ramalina siliquosa* (Huds.) A. L. Sm., which grows on rocks above the high tide line (LaGreca et al. 2020), is sensitive to heavy metal pollution—supporting the investigation of marine lichens as pollution biomonitors. The literature up to this point examines sensitivities of marine lichens to specific pollutants, but there is yet to be a study on the general use of marine lichens as bioindicators of coastal pollution. This study aims to explore the potential of marine lichens as bioindicators of oceanic pollution by examining their distribution with respect to sources and types of pollution.

Species of marine lichens are found in the orders Collempsidiales and Verrucariales, and especially among the genera *Collemopsidium* Nyl. (Collempsidiales), *Hydropunctaria* C. Keller, Gueidan & Thüs, *Verrucaria* Schrad., and *Wahlenbergiella* Gueidan & Thüs (Verrucariales) (Pérez-Ortega et al. 2016). Species in these genera are characterized by dark, crustose thalli and small perithecia (Taylor 1982). They can withstand the extreme and variable conditions of the intertidal zone and frequently colonize rocks or rock-like substrates (Kohlmeyer and Kohlmeyer 1979; LaGreca et al. 2005; Le Devehat et al. 2014; Nascimbene et al. 2013; Satyam and Thiruchitrambalam 2018). The Boston Harbor Islands National Recreation Area (NRA) drumlin archipelago provides an ideal study site because of the many islands of varying distances from the mainland and river discharges that present a range of exposures to pollution (see, e.g., Haelewaters et al. 2018; Roman et al. 2005). Additionally, the Massachusetts Water Resources Authority (MWRA) has been monitoring pollution in the harbor for decades in response to concern about historical sewage discharge into Boston Harbor (Taylor 2018). The Boston Harbor Islands have been subjected historically to significant pollution from sewage and industrial discharge; land use has also changed over

many decades (Richburg and Patterson 2005; Roman et al. 2005; Taylor 2018). Boston began systematically discharging sewage into the harbor in the late 18th century from a waste treatment plant on Deer Island and later Nut Island (National Park Service 2015). This continued until the mid-1980s when efforts to clean up Boston Harbor intensified (Taylor 2018). The MWRA has since led attempts to reverse harbor eutrophication and improve water quality (Taylor 2018). The rivers that flow into Boston Harbor have also been significant sources of pollution, particularly the Charles River and Weir River (Urban Harbors Institute 2002).

Varied land use patterns on the examined islands (National Park Service 2015; Roman et al. 2005) also contribute to pollution in the harbor. These variable land uses on the islands over time are an important feature given the longevity of the species of lichen under study, some of which may live to be over 100 years old (Taylor 1982). Islands such as Grape, Peddocks, and Thompson were primarily agricultural until the mid-19th century, when they were converted to educational, military, and/or recreational purposes. World's End peninsula was also agricultural, and during the 20th century, it was further exposed to pollution from the nearby Weir River (Urban Harbors Institute 2002). Peddocks Island housed a group of independently owned cottages, many of which remain today (National Park Service 2015). Bumpkin Island has an extensively varied history, once home to fish-drying industries and later housing a children's hospital, only to eventually become a recreational area (National Park Service 2015). Military bases on Georges Islands, Lovells Island, and Peddocks Island fell out of use after World War II (National Park Service 2015; Taylor 2018).

This study investigated marine lichen communities in Boston Harbor with the aim of documenting patterns between measured levels of pollution and the distribution of marine lichens throughout the harbor. We hypothesized a decrease in the extent of coverage of pollution-sensitive lichens in surveyed plots near greater pollution sources and areas with higher levels of pollution measured by the MWRA. We expected pollution-tolerant lichens to dominate in plots near more heavily polluted areas.

MATERIALS AND METHODS

We tested potential for the use of marine lichens as bioindicators by examining their distribution in the Boston Harbor Islands NRA and compared those findings with land use histories and water pollution analyses in select locations throughout the park (Figure 1). Six pollution types were examined for this study, data for most of which came from the

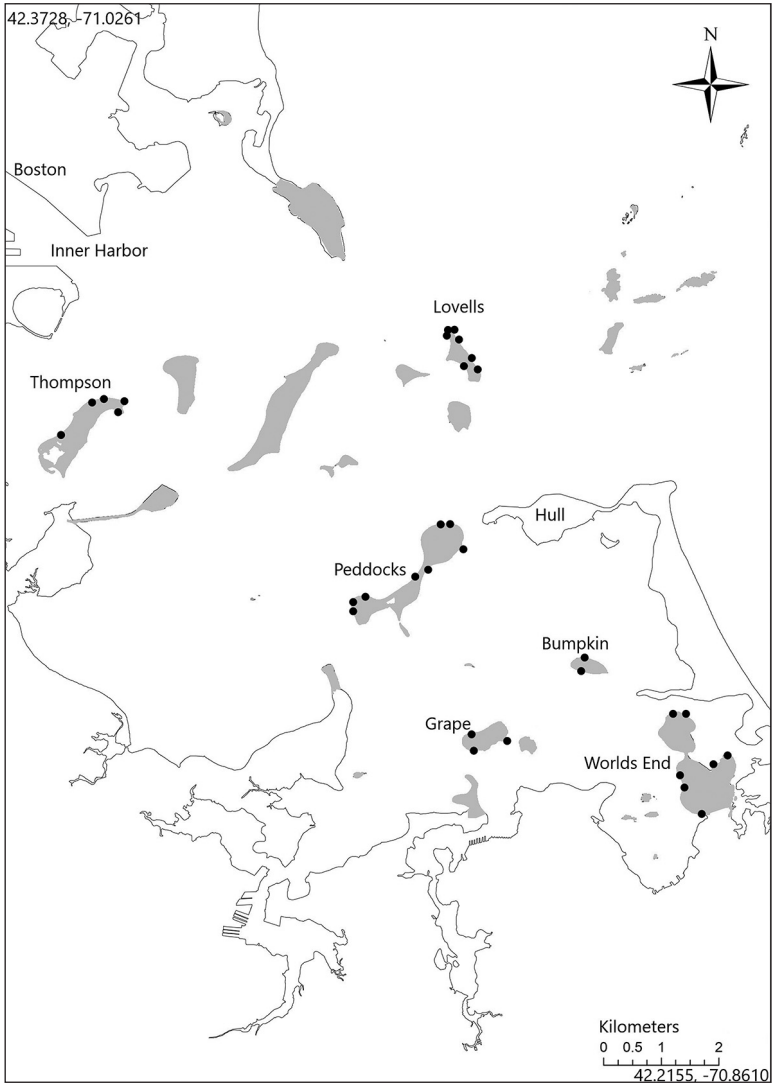

Figure 1. Map of the Boston Harbor Islands National Recreation Area (edited from Haelewaters et al. 2015), plot locations marked by black dots (excluding plot IHP1, taken near the visitor center on Long Wharf). Islands and peninsulas in gray are part of the Boston Harbor Islands National Recreation Area.

recent MWRA *Boston Harbor Water Quality Update* (Taylor 2018). The data we used included measurements of nitrogen concentration, chlorophyll-a concentration, total suspended solids (TSS), turbidity, qualitative classifications of depositional, erosional, and intermediate sediment types, and the nature of pollution of the Weir River Area of Critical Environmental Concern (Pahlevan et al. 2018; Taylor 2018; Urban Harbors Institute 2002).

Collecting procedures were approved by the National Park Service under permit #BOHA-2019-SCI-0005. Six areas were sampled based on accessibility and distance from historical or current point sources of pollution in the Boston Harbor: Bumpkin Island, Grape Island, Lovells Island, Peddocks Island, Thompson Island, and World's End peninsula. The coastline was also examined at Rowes Wharf in Boston. All sampling occurred throughout the month of August in 2019. We did not take neap and spring tides into account due to difficulties in scheduling travel to the islands; however, the range of the height of the tide on sampling days was approximately two feet (US Harbors 2019). We sampled along the entire length of transects of 1 m width that stretched from the low tide mark to the high tide mark based on mean lower-low and mean higher-high water data (National Oceanic and Atmospheric Administration 2011). In total, 33 transect sites were surveyed. Different islands had different numbers of transects based on the estimated length of suitable rocky habitat along the shore, with each estimated 100 m corresponding to one sampling site. Satellite images of the Boston Harbor were used to estimate shoreline lengths and to observe possible habitats (Terrametrics 2020). Sites were chosen ahead of time by randomly selecting an integer between 1 and 5000 and estimating the site location by moving that many meters counterclockwise from the landing dock on each island and from the entrance at World's End. When selected locations did not have suitable habitat for marine lichens, new points were chosen using the same method.

Specimens were identified through microscopy following Taylor (1982). For five specimens that proved difficult to verify morphologically, identifications were confirmed using deoxyribonucleic acid (DNA) extractions and polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) ribosomal DNA region. We used the DNeasy PowerPlant Pro Kit (Qiagen, Valencia, CA) for DNA isolation following the manufacturer's protocols. Amplification of the ITS was done in 25 µL reactions with 12.5 µL of Promega 2× PCR Master Mix (Promega Co., Madison, WI), 1.25 µL of forward and reverse 10 µM primer, 9.0 µL of H_2O, and 1.0 µL of template DNA. Different primer combinations were

tested for optimal results, including ITS1F (Gardes and Bruns 1993) and ITS9mun (Egger 1995) as forward primers, and ITS4 (White et al. 1990) and ITS4A (Larena et al. 1999) as reverse primers. Thermocycler conditions were as follows: initial denaturation at 94 °C for 3:00 min; 35 cycles of denaturation at 94 °C for 1:00 min, annealing at 50 °C for 0:45 min, and extension at 72 °C for 1:30 min; and final extension at 72 °C for 10:00 min (Haelewaters et al. 2018). Purification and Sanger sequencing were outsourced to Genewiz (South Plainfield, NJ). Forward and reverse reads were assembled and edited in Sequencher 5.2.3 software (Gene Codes Co., Ann Arbor, MI), and final sequences were submitted to the National Center for Biotechnology Information GenBank sequence database (accession numbers MT809480–MT809484). Collections verified with sequences are specified in Table 1. Finally, we compared our samples with voucher specimens from LaGreca et al. (2005). Nomenclature follows Index Fungorum (2020). Specimens are preserved at FH (Farlow Herbarium, Harvard University, Cambridge, MA). Note that field identifications on Bumpkin Island were made using Taylor (1982) and comparison with herbarium specimens from other islands. No voucher specimens were collected from this island owing to practical limitations.

We measured species richness (i.e., the number of species) in each transect and total coverage, size, number of thalli, and reproductive potential (density of perithecia) for each species. Size, cover, and count of lichen thalli within each sampling site were calculated with a combination of measurement in the field (i.e., counts, measurements, and field identifications) and analysis of photographs taken at each site. Size was measured by the longest diameter of each thallus, and cover was estimated by placing a grid on the photograph of each transect and using field notes and pictures to estimate percent cover of the transect. We measured perithecial density with collected specimens by finding the average number of perithecia within three grid templates of 1 cm^2 placed on each collection using a dissecting microscope.

Because our study design and data violated conditions for most parametric statistical analyses (according to the Anderson-Darling test and D'Agostino K^2 tests for normality), we used nonparametric models and significance tests to analyze our data. For all significance tests, $\alpha = 0.05$ unless otherwise specified or Bonferroni corrected. We used Spearman's Rho and associated two-tailed significance tests to assess nonparametric correlation (MacFarland and Yates 2016), with the following hypotheses: H_0: $\rho = 0$, H_a: $\rho \neq 0$. To compare species richness among different areas of differing levels of pollution, we used the Mann-Whitney U test (pairwise) (MacFarland and Yates 2016) and Bonferroni-corrected

Table 1. List of specimens collected by date and island. "*" denotes verification of identification with DNA sequencing, "^" denotes a specimen collected outside of the intertidal zone. All specimens are deposited at FH.

Species	Location	Date (2019)	Accession
Aspicilia cinerea (L.) Körb.^*	World's End	August 22	BHI-F1158
Collemopsidium halodytes (Nyl.) Grube & B. D. Ryan*	Thompson Island	August 10	BHI-F1146
C. halodytes	Thompson Island	August 10	BHI-F1147
C. halodytes	Thompson Island	August 10	BHI-F1145
C. halodytes	Lovells Island	August 15	BHI-F1151
C. halodytes	World's End	August 22	BHI-F1159
C. halodytes	World's End	August 22	BHI-F1160
C. halodytes	Peddocks Island	August 24	BHI-F1166
C. halodytes	Peddocks Island	August 24	BHI-F1165
C. halodytes	Peddocks Island	August 24	BHI-F1167
C. halodytes	Grape Island	August 31	BHI-F1168
Verrucaria ceuthocarpa Wahlenb.*	World's End	August 22	BHI-F1155
Verrucaria ditmarsica Erichsen	Thompson Island	August 10	BHI-F1144
V. ditmarsica	World's End	August 22	BHI-F1154
V. ditmarsica	Peddocks Island	August 24	BHI-F1164
V. ditmarsica	Grape Island	August 31	BHI-F1169
Verrucaria erichsenii Zschacke	Lovells Island	August 15	BHI-F1148
Verrucaria halizoa Leight.	Lovells Island	August 15	BHI-F1149
V. halizoa	Lovells Island	August 15	BHI-F1150
V. halizoa	World's End	August 22	BHI-F1152
V. halizoa	World's End	August 22	BHI-F1156
V. halizoa	Peddocks Island	August 24	BHI-F1162
Wahlenbergiella mucosa (Wahlenb.) Gueidan & Thüs	World's End	August 22	BHI-F1153
*W. mucosa**	Peddocks Island	August 24	BHI-F1161
Wahlenbergiella striatula (Wahlenb.) Gueidan & Thüs*	Peddocks Island	August 24	BHI-F1163

significance tests with hypotheses H_0: $\eta_1 = \eta_2$, H_a: $\eta_1 \neq \eta_2$. To show differences in species composition among locations of different levels of pollution, we used nonmetric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity index (McCune et al. 2002). We used analysis of similarities to test for significant differences between species composition of different groups, again using Bray-Curtis dissimilarity and with the following hypotheses: H_0: there is no difference between the means of two or more groups of (ranked) dissimilarities, and H_a: there is a difference between the means of two or more groups of (ranked) dissimilarities. Finally, to analyze the main contributors to differences in species composition, we used the similarity percentage analysis (SIMPER).

All tests and models were calculated with the 'vegan' package in the R language and environment for statistical computing (Oksanen et al. 2019; R Core Team, Vienna, Austria), with the exception of the pairwise analysis of similarities, for which we used PAST software (Oslo, Norway), and SIMPER, for which we used PRIMER-7 (Quest Research Limited, Auckland, New Zealand).

RESULTS

In total, 26 lichen collections were made at Grape Island, Lovells Island, Peddocks Island, Thompson Island, and World's End peninsula; details of all collections are given in Table 1. *Aspicilia cinerea* (L.) Körb., though present in Table 1, was not included in the analyses because it was collected outside of the intertidal zone. We identified eight species of intertidal lichens in the six sampled areas of the Boston Harbor Islands NRA (Table 2). Synopses of pollution statistics for each island are also included in Table 2. Groups assigned for analyses are based on levels of turbidity: low (7.5–10.0), medium–low (10.1–12.5), medium–high (12.6–15.0), and high (15.1–17.5) (measured in spectral remote sensing reflectance, S_{rs}).

A summary of a Mann-Whitney pairwise U-test for differences in medians for species richness in plots in each pollution group is included in Table 3, including Bonferroni-corrected *p*-values ($\alpha = 0.05$). We failed to reject the null hypothesis—i.e., no significant differences were observed in species richness—in any pairwise comparison.

Figure 2 displays a NMDS plot based on the percent cover of each species in each plot. The stress of the NMDS plot was approximately 0.17, indicating a fair conformity of the multivariate distance to a two-dimensional plot. Points in the NMDS plots are labeled by their plot, consisting of the first letter of the island where the plot was taken and a number to differentiate it from other plots studied on said island (i.e., "L3" represents plot 3 on Lovells Island). The low pollution and medium–low pollution groups formed separate concentrations of points, but the high and medium-high pollution groups were scattered throughout the plot. The low pollution group was, however, entirely in the positive half of the axis NMDS1, and the vast majority of the other plots fell on the negative half (with the exception of L3, W2, W3, and T3). Plots in which no species were found had to be removed so they did not form their own group and increase the stress of the NMDS.

Table 2. List of plots by island with information on pollution and species found in each plot. Turbidity is measured in spectral remote sensing reflectance (Srs); chlorophyll-a (Chlor) and total suspended solids (TSS) are measured in µg/L and mg/L, respectively; all values are averages. Species are abbreviated as follows: *Collemopsidium halodytes* (*C. hal*), *Hydropunctaria amphibia* (*H. amp*), *Verrucaria ceuthocarpa* (*V. ceu*), *Verrucaria ditmarsica* (*V. dit*), *Verrucaria erichsenii* (*V. eri*), V*errucaria halizoa* (*V. hal*), *Wahlenbergiella mucosa* (*W. muc*), *Wahlebergiella striatula* (*W. str*). An "x" denotes presence in a plot whereas a "–" denotes absence in a plot.

Island	Plot ID	Turbidity	Chlor	TSS	*C. hal*	*H. amp*	*V. ceu*	*V. dit*	*V. eri*	*V. hal*	*W. muc*	*W. str*
Thompson	TP1	14	6	8	–	–	–	–	–	–	–	–
	TP2				x	–	x	x	–	–	–	–
	TP3				x	–	–	–	–	–	–	–
	TP4				x	–	–	x	–	–	x	–
	TP5				x	–	–	x	–	–	x	–
Inner Harbor	IHP1	13	5	3	–	–	–	–	–	–	–	–
Lovells	LP1	11	3	5	–	–	–	–	–	–	–	–
	LP2				–	–	–	–	–	–	–	–
	LP3				x	–	–	x	x	x	–	–
	LP4				x	–	–	x	x	–	–	–
	LP5				–	–	–	x	x	x	–	–
	LP6				x	–	–	x	x	–	x	–
	LP7				–	–	–	–	–	–	–	–
World's End	WEP1	16	3	3	–	–	–	–	–	–	–	–
	WEP2				x	–	–	–	x	x	x	–
	WEP3				x	–	x	x	–	x	–	–
	WEP4				–	–	–	x	–	x	x	–
	WEP5				–	–	–	–	x	–	x	–
	WEP6				–	–	–	–	x	–	x	–
	WEP7				–	–	–	–	–	–	–	–

Table 2. *continued*

Island	Plot ID	Turbidity	Chlor	TSS	*C. hal*	*H. amp*	*V. ceu*	*V. dit*	*V. eri*	*V. hal*	*W. muc*	*W. str*
Peddocks	PP1	10	2	3	x	–	–	x	–	x	x	–
	PP2				x	x	–	x	–	x	x	x
	PP3				x	x	–	x	x	x	x	x
	PP4				x	–	–	–	–	x	x	–
	PP5				x	–	–	x	–	–	x	–
	PP6				x	–	–	x	x	x	x	x
	PP7				x	–	–	x	x	x	x	x
	PP8				x	–	–	x	x	x	x	x
Bumpkin	BP1	15	3	5	x	–	–	–	x	–	x	x
	BP2				–	–	–	–	–	–	–	–
Grape	GP1	14	3	5	–	–	–	–	–	–	–	–
	GP2				x	–	–	x	x	–	x	–
	GP3				–	–	–	–	–	–	–	–

Table 3. Table listing pairwise Mann-Whitney U test Bonferroni-corrected *p*-values, comparing species richness between each pollution group. Pollution groups are based on levels of turbidity: low (7.5–10.0), medium–low (10.1–12.5), medium–high (12.6–15.0), and high (15.1–17.5) (measured in spectral remote sensing reflectance, S_{rs}).

Group	High	Medium–high	Medium–low	Low
High	–	1.0000	1.0000	0.2586
Medium–high	–	–	0.9984	0.0802
Medium–low	–	–	–	0.6618
Low	–	–	–	–

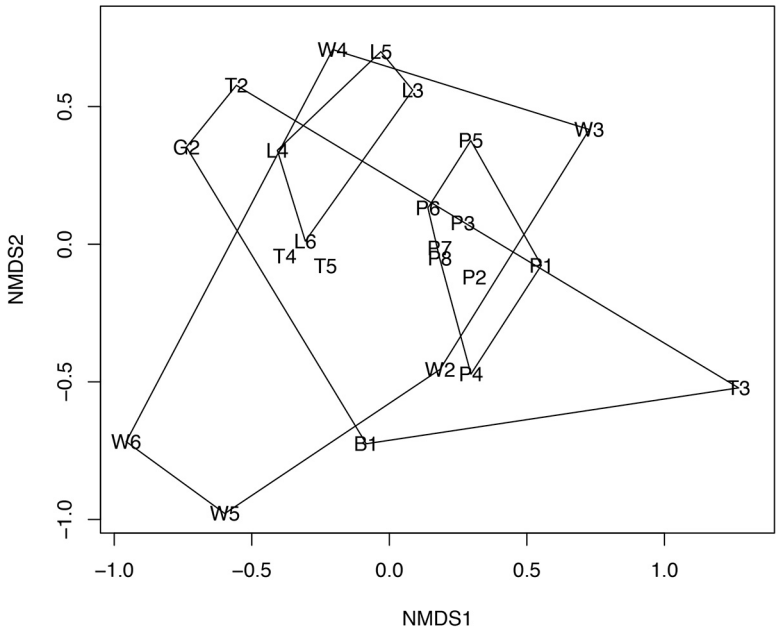

Figure 2. Nonmetric multidimensional scaling plot depicting the relationship between species composition at different pollution levels. Points are labeled by the first letter of the island of the plot they describe followed by their plot number on that island. Convex hulls encapsulate the plots from each pollution group. The pollution groups break down as follows: low pollution contains P1–P8, medium–low pollution contains L3–L6, medium–high pollution contains B1, G2, and T2–T5, and high pollution contains W2–W6.

Table 4. Analysis of similarities, pairwise Bonferroni-corrected *p*-values for significance of the difference between species composition of each pollution group. Pollution groups are based on levels of turbidity: low (7.5–10.0), medium–low (10.1–12.5), medium–high (12.6–15.0), and high (15.1–17.5) (measured in spectral remote sensing reflectance, S_{rs}).

Group	High	Medium–high	Medium–low	Low
High	–	0.4602	1.0000	0.0150
Medium–high	–	–	1.0000	0.0096
Medium–low	–	–	–	0.0132
Low	–	–	–	–

Table 5. SIMPER analysis depicting the species contributing most to the between-plot similarity in each group. Species are no longer listed after 75% cumulative contribution.

Species	Average	Contribution % similarity
Low pollution	87.23	
Verrucaria ditmarsica	19.56	22.42
Collemopsidium halodytes	18.54	21.25
Verrucaria halizoa	15.44	17.70
Wahlenbergiella mucosa	13.94	15.98
Medium–low pollution	67.61	
Verrucaria ditmarsica	29.73	43.97
Verrucaria erichsenii	26.19	38.74
Medium–high pollution	40.67	
Collemopsidium halodytes	19.35	47.57
Verrucaria ditmarsica	14.40	35.41
High pollution	40.08	
Verrucaria erichsenii	16.65	41.55
Wahlenbergiella mucosa	13.51	33.71

The results of the analysis of similarities test are shown in Table 4. We rejected the null hypothesis in any comparison between the low pollution group and the others, suggesting significant differences in the species composition of the low composition group. We failed to reject the null hypothesis in all other cases, indicating only fair discriminatory power of species richness for estimating pollution level. The SIMPER analysis in Table 5 presents the species that typify each group through ranking their contribution to between-plot similarities within each group. Low pollution and medium–low pollution groups were characterized by the dominance of *Verrucaria ditmarsica* Erichsen. In contrast, the high pollution group was dominated by *V. erichsenii* Zschacke and

Wahlenbergiella mucosa (Wahlenb.) Gueidan & Thüs. The low pollution group also displayed greater diversity in terms of common species (four rather than two in each other case). The small size of the data set and lower coverage in many plots may explain low diversity of common species among contributors to similarities within groups.

Table 6 presents the value and significance of Spearman's Rho (r_s), a nonparametric measure of correlation. The test suggested that there was a significant, moderately strong, negative correlation between percent cover and turbidity ($p = 0.0017$) as well as between percent cover and chlorophyll concentration ($p = 0.0002$), with r_s values of −0.53 and −0.60, respectively. The relationship between TSS and percent cover was considerably weaker ($R = -0.34$), though still significant ($p = 0.0499$).

We observed significant differences in species composition between plots in the low pollution group compared with each of the other pollution levels. This was likely not entirely due to differences in species richness between groups as evidenced by the results of the Mann-Whitney test. Nonparametric correlation suggested that as measures of pollution increased in the vicinity of any given plot, there was a relatively strong decrease in the marine lichen cover. Overall, results indicated changes in species composition and measures of lichen community health (such as cover) associated with higher pollution.

DISCUSSION

Some of the specimens collected during this study represent interesting records from a distributional point of view. Of the eight reported species of lichen, one was found for the first time at the Boston Harbor Islands NRA (LaGreca et al. 2005): *Verrucaria ceuthocarpa* Wahlenb., a relatively common seashore species in North America (Flenniken and Gibson 2003). It was collected once at World's End peninsula. Our collections of *V. ditmarsica* from Grape Island, Peddocks Island, Thompson Island, and World's End; *V. erichsenii* from Lovells Island; and *V. halizoa* Leight. from Lovells Island and World's End are the first ones from these localities (LaGreca et al. 2005). We also identified *Hydropunctaria amphibia* (Clemente) Cl. Roux on Peddocks Island. This species has not yet been reported from the Boston Harbor Islands NRA (LaGreca et al. 2005). However, no voucher material has been collected and so we cannot formally report it as new.

The results of this study suggest that there is a significant negative association between measurements of pollution (surface level turbidity, chlorophyll-a concentration, and TSS) and the percent cover of intertidal lichens in any surveyed plot. This trend corresponds to previous findings

Table 6. Table presenting Spearman's rho (r_s) and the two-tailed *p*-value for r_s for the correlation between cover and turbidity, chlorophyll-a, and total suspended solids (TSS).

Measure	Statistic	Turbidity	Chlorophyll	TSS
Cover	r_s	−0.53	−0.60	−0.34
	p (two-tailed)	0.0017	0.0002	0.0499

regarding terrestrial lichens, where diversity, health, and cover deteriorate in proximity with sources of air pollution, such as city centers and sources of atmospheric heavy metals (e.g., Brodo 1966; Gutiérrez-Larruga et al. 2020; Paoli et al. 2015). Toxic heavy metals may be taken up by the thalli of lichens, making them particularly useful as bioindicators of heavy metal pollution (Barglagi 2016; Lange et al. 2004; Purvis et al. 2005; Riget et al. 2000). Lichens can also be affected by changes in climate and weather patterns, which are a secondary manifestation of human pollution and influences in many areas (Ellis and Coppins 2010; Ellis et al. 2007). The majority of previous research on lichen response to marine pollution covers marine lichen mortality and later recovery as a result of the emulsifiers used to clean up the Torrey Canyon oil spill (Brown 1972, 1973; Gilbert 2001; Ranwell 1968). Other studies have investigated the reactions of lichens growing above the intertidal zone (Stengel et al. 2004). The trends that we observed seem to indicate that marine lichens are sensitive to water pollution and, pending further research, may be employed as bioindicators.

We observed an intertidal lichen desert near the Weir River Area of Critical Environmental Concern on World's End, which is an area with known heavy metal pollution and sediment runoff from nearby communities, including from sewage and landfills, and numerous storm drain outfalls into Hull Bay (Urban Harbors Institute 2002). We noticed a layer of sediment deposition on surfaces in this area as well. We saw a similar intertidal lichen desert at Rowes Wharf, which is part of Boston's urban seafront.

The strongest relationships among the variables were those between percent cover and chlorophyll-a and percent cover and turbidity (Table 6), with rho values of −0.60 and −0.53, respectively. Many factors influence turbidity, giving rise to the strong relationship of cover with it, different types of pollution, and high populations of marine microbiota. The turbidity measurements were also collected much closer to any given plot than any of the MWRA data were (Pahlevan et al. 2018). This does not explain the strength of the relationship between chlorophyll-a and cover, although it is possible that the sediment deposition includes marine microalgae

(Grossi et al. 2003). A higher chlorophyll-a concentration is also associated with eutrophication (Souchu et al. 2010), to which freshwater lichens are known to be sensitive (Gilbert and Giavarini 1997; Hauck 2010). Therefore, a strong negative association between chlorophyll-a concentration and marine lichen cover might suggest marine lichen sensitivity to eutrophication.

These data may be confounded by the frequent exposure to air pollution that marine lichens face due to changing tidal levels, which exposes lichens in the upper littoral zone to air for most of any given day (Nascimbene et al. 2013; West et al. 2018). We did, however, observe high terrestrial lichen coverage on World's End peninsula near outflow points of the Weir River Area of Critical Environmental Concern. The high terrestrial lichen coverage immediately adjacent to marine lichen deserts may suggest that even in areas of good air quality, marine lichens remain affected by poor water quality. Further comparative research regarding the differential responses of lichens in different littoral zones is required to understand the degree to which lichens at varying heights in the intertidal zone are affected by water pollution vs. air pollution.

Because different lichens have displayed different levels of tolerance with respect to pollution in air pollution biomonitoring studies (Brodo 1966; Parvianen et al. 2019; Scerbo et al. 1999), it is important to also observe specific distributions of marine lichens to better investigate their potential for aquatic biomonitoring. We observed significant differences in the species composition of plots associated with different levels of pollution. However, because we did not sample physiological differences among the lichens or collect water pollution data for each plot, we cannot make firm conclusions about the response of individual species to marine pollution.

Figure 2 presents the different groups of plots with significantly different species compositions. The plots from higher pollution groups are oriented in the negative half of the NMDS1 axis, which suggests that pollution explains some of the variation in species compositions along that axis. The explanation of the variation along the NMDS2 axis is less clear because there is a lot of overlap in points from each group along that axis.

The species that characterize the low pollution and medium–low pollution groups are primarily *Collemopsidium halodytes* (Nyl.) Grube & B. D. Ryan and *Verrucaria ditmarsica*, according to the SIMPER analysis. The low pollution group has two other species that represent a substantial portion of the within-group similarity: *V. halizoa* and *Wahlenbergiella mucosa*. There is little difference among these species and the ones that apparently characterize the higher pollution groups, except for

V. erichsenii, which contributes the most to the average similarity between the high pollution plots. According to Taylor (1982), *C. halodytes*, *V. ditmarsica*, and *W. mucosa* are distributed slightly lower in the littoral zone compared to *V. erichsenii*. Certain species found only in low pollution plots (*Hydropunctaria amphibia* (Clemente) Cl. Roux and *W. striatula* (Wahlenb.) Gueidan & Thüs) also occur lower in the intertidal zone (Fletcher 1973; Taylor 1982), which may suggest that length of water immersion explains different reactions to water pollution. This does not explain the prevalence of many of the same species (*V. ditmarsica*, *C. halodytes*, and *W. mucosa*) in the higher pollution environments, even though they are lower in cover than in low pollution plots. Further research is necessary to better elucidate the nuances and causes of differential marine lichen responses to pollution, as well as the application of specific marine lichen species as bioindicators.

Marine lichens are challenging to use as bioindicators. Lichens in general are not easily identified; in particular, species of marine lichens in the Collemopsidiales and Verrucariales can be extremely difficult to distinguish morphologically (Nascimbene et al. 2013; Taylor 1982). They are also still undergoing taxonomic revision, including higher-level classification changes, and cryptic species and species complexes are common (Nascimbene et al. 2013; Orange 2012; Pérez-Ortega et al. 2016; Thüs et al. 2015). In addition, Brunialti et al. (2012) raised concerns that the assessment of lichen diversity data is highly variable depending on the skill level and experience of the assessors. They suggested that owing to high variability between lichen diversity assessments taken by different groups, despite consistent standards and instructions, comparison of lichen diversity data should be viewed cautiously until better standard techniques are developed.

The results of this study are preliminary but provide compelling evidence that marine lichens could serve as bioindicators of coastal water pollution. We observed several significant negative associations between measurements of water pollution and measurements of marine lichen cover in Boston Harbor. We also found significant differences in species composition among areas of low pollution and high pollution, and we observed differences in the species that characterized different levels of pollution. We recommend further research on the effects of water vs. air pollution on intertidal lichens, species-specific pollution responses, and appropriate standard measurements of intertidal lichen community health—in line with the Index of Atmospheric Purity or Index of Human Impact for terrestrial lichens (Gombert et al. 2004)—to use these challenging organisms as bioindicators.

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