Hurricane events facilitate the establishment of nonnative invertebrate species in harbors

Brenna Hutchings
University of North Carolina Wilmington College of Arts and Sciences
Emma Stiles
University of North Carolina Wilmington College of Arts and Sciences
Susanna Lopez-Legentil (lopezlegentils@uncw.edu)
University of North Carolina Wilmington College of Arts and Sciences https://orcid.org/0000-0001-8737-6729

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Abstract

The coastal location and shallow depths of harbors suggest that fouling communities will be greatly affected by extreme weather events. Within fouling communities, ascidians are conspicuous animals and their sessile nature makes them ideal targets to assess community resilience. We established ascidian diversity and abundance at eighteen harbors and marinas along the coast of North Carolina (United States) a year after Hurricane Florence landfall in 2018 (post-hurricane) and compared results with those obtained in 2014 (pre-hurricane). The distribution and community structure of native and introduced ascidians were analyzed using presence-absence and relative abundance similarity matrices. Both geographic location (North vs. South) and distance between harbors had a significant effect on ascidian community composition. When compared with pre-hurricane data, a decrease in the number of native species and an increase of introduced and cryptogenic species was noted, although these trends were only statistically significant for the number of introduced species based on presence-absence data. Monthly photo transects spanning pre- and post-hurricane periods to monitor the ascidian community at the harbor located where the hurricane made landfall, revealed that all but one species disappeared from the docks after the hurricane. Recolonization occurred slowly, and one year later, only two non-native species were present. Further, we report the arrival of the globally introduced species *Styela canopus* and *Distaplia listerianum* in North Carolina. This study significantly advances our understanding of the impact of hurricanes on fouling communities inhabiting harbors and the speed of natural recovery.

Introduction

Artificial structures such as harbors and marinas with submerged pilings offer refuge for a variety of marine lifeforms, creating rich fouling communities along our coastlines. However, extreme weather events such as hurricanes have the potential to alter the composition of both fouling and natural benthic communities. The harsh environmental conditions that characterize hurricanes often result in cleared substrata available for invertebrate recolonization once the disturbance has passed (Bythell et al. 2000), and when accompanied by precipitation, decreases in seawater temperature and salinity (Jacob and Koblinsky 2007). Although studies investigating the impact of hurricanes on harbor fouling communities are rare, research indicates that some introduced marine species may take advantage of environmental disturbances to outcompete native species (Altman and Whitlatch 2006). For example, following the passage of Hurricanes Irma and María (Puerto Rico, September 2017), native seagrass populations were displaced due to the combined effects of altered physical conditions and the rapid domination of the invasive species *Halophila stipulacea* (Hernández-Delgado et al. 2020). Similarly, hurricanes in the Atlantic were suggested to have facilitated the spread of invasive lionfish from Florida to the Bahamas islands (Johnston and Purkis 2015). In other cases, however, the extreme conditions brought by hurricanes resulted in the eradication of introduced species from previously dominated areas, potentially providing a chance for native species to recolonize the space. For instance, the invasive green algae *Caulerpa brachypus* forma *parvifolia* went from 90% cover in southeastern Florida coral reefs to complete eradication after Hurricanes Frances and Jeanne (2004) and Hurricane Wilma (2005) (Lapointe et al. 2006). Because of the varying reports of non-native species response to hurricane disruptions, it is imperative to gather taxa-specific data to understand the effect of hurricane passage on established communities.

Ascidians (Chordata: Tunicata), also known as sea-squirts, are commonly observed within fouling communities. As sessile marine invertebrates with a larval life stage lasting from a few minutes to a few days (Cloney 1982, Svane and Young 1989), ascidian larvae will often settle within a few meters of their parents (Lambert 2005). However, recreational boating and the global shipping industry provide an alternative pathway for the dispersion and introduction of species to new habitats (Seebens et al. 2013, López-Legentil et al. 2015). Species introductions can have serious consequences for community dynamics, in some cases decreasing species richness (Blum et al. 2007) and lowering biodiversity due to spatial competition (Christianson and Eggleston 2021). Additionally, some species have demonstrated resiliency to variable conditions in temperature and salinity (Epelbaum et al. 2009, Li et al. 2021, Nagar and Shenkar 2016, Pineda et al. 2012b), metal pollution (Osborne et al. 2018, Pineda et al. 2012a, Tzafriri-Milo et al. 2019), pressure (Sumida et al. 2015), and overall water quality (Naranjo et al. 1996). Such characteristics often facilitate dominance of non-native species over the more sensitive native populations (Lambert 2005).

Ascidian communities in harbors and marinas along the coast of North Carolina (N.C.) are well characterized, with a total of three introduced, two cryptogenic, and eight native species at sixteen harbors and marinas (Villalobos et al. 2017). The presence of the Intracoastal Waterway, which extends from Virginia to Florida, and the abundance of both recreational and industrial shipping, offers ascidians plentiful opportunities to colonize new habitats. In addition, N.C. is periodically impacted by hurricanes, with a hurricane season that runs from June through the end of November (Collins 2020). Thus, coastal N.C. is an ideal study area for monitoring the influence of storm damage on well-established fouling communities. On September 14, 2018, Hurricane Florence made landfall in Wrightsville Beach, N.C., after weakening from a Category 4 hurricane to a Category 1 (NOAA 2018). Hurricane Florence caused extensive damage, uprooting trees and powerlines, and causing widespread power outages across the Carolinas. Furthermore, due to the slow motion of the storm, rain fell for several days, causing major flooding along the N.C. coast (from New Bern to Wilmington) and producing rainfall exceeding 100” across much of southeastern and southcentral N.C. (Steward and Berg 2019). Wilmington, in particular, was cut off entirely from the rest of the mainland and experienced 26.98” of rainfall by September 17, 2018 (NOAA 2018). Additionally, Hurricane Florence’s landfall resulted in a storm surge of 9–13 feet, which led to life threatening conditions in coastal N.C. (NOAA 2018).

In this study, we investigated how harbor ascidian communities along the whole coast of N.C. were impacted by Hurricane Florence. Sixteen harbors that were monitored in July 2014 by Villalobos et al. (2017) were revisited in July 2019. We followed the same methods described in Villalobos et al. (2017) and recorded ascidian diversity and relative abundance at each harbor. Since the hurricane made landfall in Wrightsville Beach (South N.C.), we hypothesized that southern harbors would be more severely impacted than northern sites and have a different community composition than northern sites. In addition, a monthly photo-transect survey to estimate abundance and coverage of ascidian species at Seapath Yacht Club (Wrightsville Beach) started in January 2018 (pre-hurricane) and ended in December 2019 (post-hurricane). This survey allowed us to establish the immediate impact of Hurricane Florence on an ascidian community and its recovery patterns over time.

Materials & Methods

Sample collection and identification

Ascidian diversity and relative abundance surveys were conducted at eighteen harbors and marinas along the coast of North Carolina in July 2019 (Fig. 1), with water temperature and salinity recorded at each site (Table S1). To maintain consistency in sampling procedures and perform comparative analyses between ascidian communities in 2014 and 2019, the same methods described in Villalobos et al. (2017) were used. In short, the Rapid Assessment Method (Campbell et al. 2007) was used to determine the relative abundance of each species observed at each site as follows: (1) rare, one or few specimens of a species observed, (2) common, species frequently observed but in low numbers, (3) abundant, species frequently observed in moderate numbers, or (4) very abundant, species frequently observed in high numbers. All docks at each marina were surveyed by 3 researchers and samples were collected from 0 to 2 m depth, mostly from floating docks, pilings, ropes, and boat bumpers. Surveying organism diversity in this fashion has been previously validated (Grey 2009). Photographs of ascidians were taken *in situ* whenever possible or immediately after collection.

For morphological identification and to relax the zooids, samples were placed in Ziploc® bags filled with seawater and a few menthol crystals for at least two hours. Samples were then fixed in 10% seawater formalin buffered with sodium borate. Species were identified using relevant morphological keys and species descriptions (Van Name 1945; Monniot F. 1974, 1983; Monniot C. 1983a, b; Monniot and Monniot 1984; Goodbody 1994; Rocha et al. 2012b), followed by classification as native, introduced, or cryptogenic (native or introduced status cannot be determined) as defined in Carlton (1996, 2009) and Blackburn et al. (2011). Species were assigned to each category based on Shenkar and Swalla (2011), Zhan et al. (2015), Simkanin et al. (2016), Shenkar et al. (2017), and Villalobos et al. (2017). For genetic barcoding, a piece of each colonial species was placed in a 20 mL scintillation vial with absolute ethanol. Solitary species were dissected *in situ* to remove the tunic before fixing the remaining animal in absolute ethanol. All samples were kept at -20^{o}C until further processed.

Ascidian barcoding

For DNA extraction, colonial species were dissected under a stereomicroscope to separate the zooids from the tunic, while for solitary species a piece of the branchial sac was separated. Ethanol was fully evaporated using an Eppendorf® Vacufuge® centrifuge. DNA extractions were performed with the DNeasy® Blood and Tissue Kit (QIAGEN) following manufacturer’s instructions. A fragment of the mitochondrial gene Cytochrome Oxidase I (COI) was amplified by PCR using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994), or the primer set Tun_forward and Tun_reverse2 (Stefaniak et al. 2009). Each PCR reaction consisted of 0.5µL of the forward and of the reverse primers, 11µL of PCR water, 12.5µL of MyTaq HS Mix, and 0.5µL of DNA. Samples using LCO1490 and HCO2198 primers underwent PCR first at 95° C for 1 minute, then 35 cycles at 95° C for 15 seconds, 45° C for 15 seconds, and 72° C for 10 seconds, and finished with one cycle at 72° C for 1 minute. PCR steps using the Tun_forward and Tun_reverse2 primers were identical to those used with the LCO1490 and HCO2198 primers, but with a total of 40 cycles and an annealing temperature of 42° C instead of 45° C. All PCR amplifications were conducted on an Eppendorf Mastercycler Nexus X2.

Samples were sequenced using BigDye™ terminator v.3.1 and the same primers used for PCR amplification. Sequencing was completed on an Applied Biosystems 3500 genetic analyzer available at UNCW Center for Marine Science. The resulting DNA sequences were aligned using Geneious (v. R11. Biomatters, Auckland, New Zealand) and compared with ascidian sequences available in GenBank® using BLASTn searches. Sequences obtained in this study have been deposited in GenBank® (accession numbers MW621870 to MW621909; Table S2).

Ascidian diversity and relative abundance in N.C. harbors in 2019

To compare ascidian diversity and structure across the eighteen harbors visited, two similarity matrices were constructed using presence-absence and relative abundance data, and the Bray-Curtis index. Since data was semi-quantitative, no transformation was applied. Results were visualized with non-metric multidimensional scaling (nMDS) plots. Permutational analyses of variance (PERMANOVA) to determine the effect of latitudinal position (North or South, Fig. 1) on ascidian diversity were performed with the PRIMER v6.1.10 statistical package (Clarke and Gorley 2006) and the PERMANOVA + Beta20 module (Anderson et al. 2008). Correlation between geographic distances among harbors and ascidian community dissimilarity was established with a mantel test using both the presence-absence and relative abundance matrices and the ade4 package for R (Dray and Dufour 2007). GPS coordinates were used to calculate the shortest surface distances between pairs of harbors using Bryers (1997) software.

Ascidian diversity and relative abundance in N.C. harbors pre- and post-hurricane

To compare ascidian diversity in N.C. harbors before (2014, Villalobos et al. 2017) and after (2019) Hurricane Florence (September 14, 2018), only the sixteen harbors that were visited in both 2014 and 2019 were analyzed. Similarity matrices were constructed using the Bray-Curtis index and presence-absence and relative abundance data, for all species found in both years (2014 and 2019), native species only, and introduced species only (uncategorizable species from 2019 were excluded from the last two analyses). Results were visualized with nMDS plots and PERMANOVA analyses were conducted to determine the effect of year (2014, 2019) and region (North, South) on ascidian community similarity. Plots and analyses were performed using PRIMER v6.1.10 (Clarke and Gorley 2006) and the PERMANOVA + Beta20 module (Anderson et al. 2008). The total number of ascidian species observed in 2014 and 2019 at each harbor, and the number of introduced, cryptogenic and native species were calculated for each site and visualized with a heat map created in Microsoft Excel.

To analyze changes in ascidian abundance between 2014 and 2019, we calculated the average abundance value for each species across all harbors. To calculate the average relative abundance, the summative relative abundance across all harbors for each species was divided by the number of harbors in which the species was found. Then, the 2014 average abundance value was subtracted from the corresponding 2019 value. Relative abundance values are semi-quantitative (1 to 4, see above) and non-linear, since a change from category 1 (rare) and 2 (common) is numerically smaller than between 3 (abundance) and 4 (very abundant). However, this type of comparison allows us to rapidly visualize important changes in species abundances.

Community composition pre- and post- Hurricane Florence in Wrightsville Beach

Changes in the ascidian community over time at Seapath Yacht Club (Wrightsville Beach; 34.212880˚, -77.805698˚; Site G, Fig. 1) were established using monthly photo transects from January 2018 (pre-hurricane) to December 2019 (post-hurricane). Ten pictures were randomly taken along each of the four docks (A, B, C, and D) at the marina for a total of 40 pictures per month. Hurricane Florence made landfall in Wrightsville Beach September 14, 2018, and unsafe conditions for travel in the area prevented us from acquiring the September 2018 data until October 3. Pictures were taken between 0 m and 0.5 m depth with an Olympus C-7070 camera equipped with an underwater case and fixed to an aluminum frame (inside edge: 11.716 cm x 17.526 cm, 13 cm from frame to camera lens). The aluminum frame was attached to the camera to keep a consistent focal distance when taking pictures. At the same time of image collection, seawater temperature was also measured (Table S3).

Pictures were analyzed using ImageJ v.1.48 software (Schneider et al. 2012). Every ascidian species photographed was identified and the numbers of individuals (solitary species) or colonies per m^2 was calculated. Small or newly recruited ascidians (i.e., individuals or colonies < 0.5 cm^2) could not be unambiguously identified and were not quantified. Since colonial ascidians are two-dimensional organisms in nature, surface area of each colonial species was also measured. The total area occupied for each colonial species was divided by the total area of the photograph to obtain a percent cover per species for each photo.

To determine whether Hurricane Florence yielded significant changes in species' abundances and to take into account natural abundance variation due to ascidian seasonality, all species and all colonial species abundances observed from January to August 2018 were compared to their corresponding abundances the same month but in 2019 using a two-tailed paired t-test in Microsoft Excel. The same analyses were repeated but comparing colonial ascidian coverage instead of abundance.

Results

Ascidian diversity in 2019

Two of the marinas, Southport Marina and Harbor Pointe Marina (Table 1), within the southern region did not have any ascidians, while the remaining sixteen marinas contained a total of thirteen species (Table 2). Five of these species were considered native [*Aplidium stellatum* (Verrill 1871); *Ascidia interrupta* Heller 1878; *Clavelina oblonga* Herdman 1880; *Molgula manhattensis* (De Kay 1843); *Perophora viridis* Verrill 1871], two were cryptogenic [*Distaplia bermudensis* Van Name 1902; *Eusynstyela tincta* (Van Name 1902)], four were considered introduced [*Polyandrocarpa anguinea* (Sluiter 1898); *Polyandrocarpa zorritensis* (Van Name 1931); *Styela canopus* (Savigny 1816); *Styela plicata* (Lesueur 1823)], and two species were only identified to the genus level (*Aplidium* sp.; *Eudistoma* sp.: Figure S1). Of these recorded species, the introduced ascidian *Styela plicata* was the most abundant and common, occurring at all but two (South Harbor Village Marina, Joyner Marina) of our sixteen sampled sites where ascidians were present (Table 2). Barcode sequences were obtained for seven of the species: *Aplidium stellatum*, *Eudistoma* sp., *Molgula manhattensis*, *Perophora viridis*, *Polyandrocarpa zorritensis*, *Styela canopus*, and *Styela plicata* (Table S2).

Table 1

Sampling sites in coastal North Carolina and number of ascidian species observed at each site in 2019 for each of the introduction categories (native, cryptogenic, introduced). The two species classified only to the genus level, *Aplidium* sp. *and Eudistoma* sp., were found in Bridge Tender Marina and Portside Marina and are not included in this table

Code	**Harbor/Marina**	**Latitude**	**Number of Ascidian Species**		
			Native	Cryptogenic	Introduced
A	South Harbor Village Marina	South	1	0	0
B	Southport Marina	South	0	0	0
C	Harbour Point Marina	South	0	0	0
D	Joyner Marina	South	1	0	0
E	Inlet Watch Yacht Club	South	1	0	1
F	Masonboro Yacht Club & Marina	South	2	0	1
G	Seapath Yacht Club	South	2	1	2
H	Bridge Tender Marina	South	3	0	1
I	Wrightsville Beach Marina	South	0	0	2
J	Harbour Village Marina	South	1	0	2
K	Crow’s Nest Yacht Club	North	3	0	2
L	Portside Marina	North	4	1	3
M	Olde Towne Yacht Club	North	3	0	2
N	Harker’s Island	North	2	0	1
O	Ocracoke Ferry Landing	North	1	0	2
P	Hatteras Harbor Marina	North	1	0	2
Q	Hatteras Landing Marina	North	1	0	2
R	Cape Pointe Marina	North	1	0	1

Table 2

Observed ascidian species in 2019 and their relative abundance at each site. *A.* sp = *Aplidium* species, *A. st* = *Aplidium stellatum*, *A. in* = *Ascidia interrupta*, *C. ob* = *Clavelina oblonga*, *D. be* = *Distaplia bermudensis*, *E.* sp = *Eudistoma* species, *E. ti* = *Eusynstyela tincta*, *M. ma* = *Molgula manhattensis*, *P. vi* = *Perophora viridis*, *P. an* = *Polyandrocarpa anguinea*, *P. zo* = *Polyandrocarpa zorritensis*, *S. ca* = *Styela canopus*, *S. pl* = *Styela plicata*. 1 = present but rare (one or a few specimens of a species observed), 2 = common (species frequently observed but not overly abundant), 3 = abundant (species occurring frequently), 4 = very abundant (species occurring frequently and in great numbers or clusters). Codes correspond to map shown in Fig. 1. Southport Marina and Harbour Point Marina are not listed, because no ascidians were observed at these sites

		Ascidian Species & Relative Abundance												
Code	Harbor/Marina	*A.* sp	*A. st*	*A. in*	*C. ob*	*D. be*	*E.* sp	*E. ti*	*M. ma*	*P. vi*	*P. an*	*P. zo*	*S. ca*	*S. pl*
A	South Harbor Village Marina								4					
D	Joyner Marina								1					
E	Inlet Watch Yacht Club								1					1
F	Masonboro Yacht Club & Marina			1					2					3
G	Seapath Yacht Club			1				1		2		1		4
H	Bridge Tender Marina	1		2			1		1	1				4
I	Wrightsville Beach Marina											1		4
J	Harbor Village Marina								1				2	3
K	Crow’s Nest Yacht Club			2					2	1			3	4
L	Portside Marina	3	1	2	4	2				1	1	2		4
M	Olde Towne Yacht Club			2	4					1		2		3
N	Harker’s Island			1						2				3
O	Ocracoke Ferry Landing									3			2	1
P	Hatteras Harbor Marina									2			2	2
Q	Hatteras Landing Marina									2			2	3
R	Cape Pointe Marina									2				3

Along the coast of North Carolina, the number of species observed at our sampled sites ranged from 1 to 9 (Table 1), with the greatest species diversity observed at Portside Marina (northern site, Table 1, Fig. 1). While most of the species observed along the coast were common in both latitudes (*Aplidium* sp., *Ascidia interrupta*, *Molgula manhattensis*, *Perophora viridis*, *Polyandrocarpa zorritensis*, *Styela canopus*, *Styela plicata*), there were some species only present in southern locations (*Eudistoma* sp., *Eusynstyela tincta*) and some that were unique to northern locations (*Aplidium stellatum*, *Clavelina oblonga*, *Distaplia bermudensis*, *Polyandrocarpa anguinea*, Table 2). The influence of latitude on ascidian community structure was also evident in the nMDS plots based on presence-absence (Fig. 2A) and relative abundance data (Fig. 2B). Accordingly, PERMANOVA analyses showed significant differences in ascidian communities between the two regions for both presence-absence ($p = 0.009$) and relative abundance ($p = 0.020$) data. Furthermore, mantel tests correlating geographic distance between sites and community dissimilarity were significant for presence-absence ($p = 0.001$) and relative abundance ($p = 0.001$) of species. Thus, ascidian communities that were geographically closer together were more similar in ascidian composition than those further apart.

Ascidian diversity pre- and post-hurricane

Three native species: *Didemnum lutarium*, *Distaplia corolla*, and *Eudistoma capsulatum*, and one cryptogenic species *Distaplia stylifera* found in July 2014 (pre-hurricane) were not observed in 2019 (post-hurricane). Additionally, one cryptogenic species *Eusynstyela tincta* (Figure S1A), one introduced species *Styela canopus* (Figure S1B), and two uncategorizable species *Aplidium* sp. (Figure S1C), and *Eudistoma* sp. (Figure S1D) not found in 2014 were observed in 2019 (Table 3). Other species such as the cryptogenic *D. bermudensis*, the native *A. stellatum*, and the introduced species *P. anguinea* that were commonly observed in southern harbors before the hurricane, were conspicuously absent after the hurricane, with these same species prevailing in northern harbors.

Table 3

Comparison of total number of species (Tot Sp.) observed in harbors visited in 2014 and 2019; total number of introduced species (Int Sp.) observed in 2014 and 2019; total number of cryptogenic species (Cry Sp.) observed in 2014 and 2019; total number of native species (Nat Sp.) observed in 2014 and 2019. Harbors only visited in 2019 were excluded from this comparison

Code	**Harbor/Marina**	**2014**				**2019**			
		Tot Sp.	Int Sp.	Cry Sp.	Nat Sp.	Tot Sp.	Int Sp.	Cry Sp.	Nat Sp.
A	South Harbor Village Marina	2	1	0	1	1	0	0	1
B	Southport Marina	0	0	0	0	0	0	0	0
C	Harbour Point Marina	0	0	0	0	0	0	0	0
D	Joyner Marina	0	0	0	0	1	0	0	1
E	Inlet Watch Yacht Club	0	0	0	0	2	1	0	1
F	Masonboro Yacht Club & Marina	2	1	0	1	3	1	0	2
G	Seapath Yacht Club	9	3	2	4	5	2	1	2
H	Bridge Tender Marina	6	3	1	2	4	1	0	3
I	Wrightsville Beach Marina	6	3	0	3	2	2	0	0
J	Harbour Village Marina	3	2	0	1	3	2	0	1
K	Crow’s Nest Yacht Club	3	1	0	2	5	2	0	3
L	Portside Marina	5	2	0	3	8	3	1	4
M	Olde Towne Yacht Club	7	2	0	5	5	2	0	3
N	Harker’s Island	2	1	0	1	3	1	0	2
O	Ocracoke Ferry Landing	2	1	0	1	3	2	0	1
P	Hatteras Harbor Marina	2	1	0	1	3	2	0	1

There were two instances where the relative abundance of ascidian species changed remarkably between 2014 and 2019. The native but globally distributed species *Clavelina oblonga* went from having a relative abundance of 1 (rarely seen) at the Olde Towne Yacht Club (North) in 2014 to having a relative abundance of 4 (very abundant) in 2019. In addition, the species was also found with a relative abundance of 4 at the Portside Marina (North) in 2019, where it was absent in 2014 (Table 4). The introduced species *Polyandrocarpa zorritensis* dropped from a relative abundance of 4 (very abundant) at the Bridge Tender Marina and Wrightsville Beach Marina (both in the South) in 2014 to a relative abundance of 1 (rarely seen) in 2019 (Table 4).

Table 4

Comparison of relative abundance of ascidian species that were observed both in the 2014 and 2019 surveys of N.C. harbors and marinas. *A. st* = *Aplidium stellatum*, *A. in* = *Ascidia interrupta*, *C. ob* = *Clavelina oblonga*, *D. be* = *Distaplia bermudensis*, *M. ma* = *Molgula manhattensis*, *P. vi* = *Perophora viridis*, *P. an* = *Polyandrocarpa anguinea*, *P. zo* = *Polyandrocarpa zorritensis*, *S. pl* = *Styela plicata*. 1 = present but rare (one or a few specimens of a species observed), 2 = common (species frequently observed but not overly abundant), 3 = abundant (species occurring frequently), 4 = very abundant (species occurring frequently and in great numbers or clusters). Codes correspond to map shown in Fig. 1. Southport Marina and Harbour Point Marina are not listed, since no ascidians were observed at these sites in either 2014 or 2019

		Ascidian species and relative abundance																	
Code	Harbor/Marina	2014									2019								
		A. st	*A. in*	*C. ob*	*D. be*	*M. ma*	*P. vi*	*P. an*	*P. zo*	*S. pl*	*A. st*	*A. in*	*C. ob*	*D. be*	*M. ma*	*P. vi*	*P. an*	*P. zo*	*S. pl*
A	South Harbor Village Marina					2				1					4				
D	Joyner Marina														1				
E	Inlet Watch Yacht Club														1				1
F	Masonboro Yacht Club & Marina					1				3		1			2				3
G	Seapath Yacht Club	1	1		3			1	1	4		1				2		1	4
H	Bridge Tender Marina				1		1	1	4	4		2			1	1			4
I	Wrightsville Beach Marina		3			1	1	1	4	4								1	4
J	Harbour Village Marina		1					1		4					1				3
K	Crow’s Nest Yacht Club		3				1			4		2			2	1			4
L	Portside Marina	1	2					1		4	1	2	4	2		1	1	2	4
M	Olde Towne Yacht Club	1	1	1				1		4		2	4			1		2	3
N	Harker’s Island						1			1		1				2			3
O	Ocracoke Ferry Landing						1			1						3			1
P	Hatteras Harbor Marina						1			1						2			2

Despite these observations, the nMDS plots of community similarity based on presence-absence (Fig. 3A) and relative abundance (Fig. 3B) of species observed in 2014 or 2019 showed no clear groupings of harbors based on year. Accordingly, PERMANOVA analyses displayed no significant difference in presence-absence (p = 0.127) or relative abundance (p = 0.168) of species observed in 2014 and 2019. For native species (Figure S2), PERMANOVA analyses displayed no significant difference in presence-absence (p = 0.799) or relative abundance (p = 0.593) between harbors sampled in 2014 and 2019. However, there were significantly (p = 0.038) more introduced species based on presence-absence observed in 2019 than in 2014 (Fig. 4A) but not in terms of relative abundance (p = 0.111; Fig. 4B).

Seapath and Wrightsville Beach marinas (both in the South) were the two marinas with the greatest loss of species after Hurricane Florence, and these were mostly native species (Table S4). On the other hand, Portside marina (in the North) gained the most species, one introduced, one cryptogenic and one native (Table S4). All other marinas either gained, lost or remained the same in terms of number of species over time (Table S4). When the average relative abundance of ascidian species observed across all harbors in 2014 was compared to 2019, only three species displayed a change in average relative abundance greater than 0.5. *Clavelina oblonga* had by far the largest increase in average abundance, followed by *Perophora viridis.* Of all species, only *Polyandrocarpa zorritensis* relative abundance decreased over time (Figure S3).

Community composition pre- and post-hurricane in Wrightsville Beach

A total of eight ascidian species were observed at the Seapath Yacht Club in 2018: the solitary ascidian *Styela plicata* (Table S3), and the colonial ascidians *Diplosoma listerianum* (Milne Edwards 1841), *Distaplia bermudensis*, *Eudistoma capsulatum* (Van Name 1902), *Polyandrocarpa anguinea*, *Aplidium stellatum*, *Didemnum lutarium* Van Name 1910, and *Clavelina oblonga* (Fig. 5, Table S3 and S5). During the winter months of 2018, *S. plicata* was the most abundant species with average abundances of > 180 individuals per m^2 (Fig. 5A). Second in terms of abundance was *D. listerianum*, often observed growing over *S. plicata.* In winter and early spring of 2018, *D. listerianum* was particularly abundant, with a maximum abundance (68 individuals per m^2) observed in March 2018 (Fig. 5A) and extensive coverage (Fig. 5B). *D. bermudensis* was abundant in the late winter and early spring months, with a maximum abundance (9 colonies per m^2) recorded in April 2018 (Fig. 5A) but less coverage than *D. listeranium, E. capsulatum* and *D. lutarium* (Fig. 5B). *E. capsulatum* was abundant from January 2018 to September 2018 and peaked in abundance (16 colonies per m^2) during April 2018 (Fig. 5A), becoming the second species with the largest coverage in winter (Fig. 5B). *P. anguinea* was recorded from January 2018 until March 2018, with its highest abundance values (4 colonies per m^2) recorded in January 2018 (Fig. 5A). *D. lutarium* was only observed in March and June 2018, while *A. stellatum* only appeared in March 2018 (Fig. 5A). The species *C. oblonga* did not appear until May of 2018 with an abundance of 3 colonies per m^2 (Fig. 5A) and by summer it was the second-most widespread species in terms of coverage (Fig. 5B).

Following Hurricane Florence in September 2018, all species except for *S. plicata* disappeared from the docks (Fig. 5A). The population of *S. plicata* was also drastically reduced after the hurricane but recovered relatively fast. In August 2018 (pre-hurricane), the abundance of *S. plicata* was 96 individuals per m^2, in October 2018 (6 weeks after landfall), only 1 individual was observed, but by March 2019 (6 months after the hurricane), 92 individuals per m^2 were recorded, which was just under the pre-Florence abundance levels (Fig. 5A). By June 2019 (less than a year after Hurricane Florence), there were 712 individuals per m^2 (Fig. 5A), well above any value recorded thus far and forming massive agglomerations that covered all available space (Figure S4).

The first species to reappear in our surveys after Hurricane Florence was *D. listerianum* with an abundance of 1 individual per m^2 in March 2019 (Fig. 5A), similar to pre-hurricane values (2 individuals per m^2). The species also recovered quickly in terms of coverage, rapidly becoming the dominant species in winter of 2019 (Fig. 5B). In July 2019, the native species *E. capsulatum* reappeared in our transects and reached an abundance of 3 colonies per m^2 in November 2019, still considerably lower than the 16 colonies per m^2 noted pre-hurricane (Fig. 5A), and with no substantial recovery in terms of coverage (Fig. 5B). *C. oblonga* did not reappear until November 2019, but when it did, it reached the same abundance as when it first appeared in our transects, 3 colonies per m^2 (Fig. 5A). A *Botrylloides* species (Figure S5) not observed in 2018 appeared in September 2019, reaching values of > 20 colonies per m^2 during the following 3 months, and a maximum of 82 colonies per m^2 in October 2019 (Fig. 5A), when it also reached maximum coverage (Fig. 5B). After this sudden appearance and rapid growth in terms of number of colonies and coverage, the species disappeared from our transects in December 2019.

When comparing ascidian abundances for each species and month from January to August 2018 and for the same months in 2019, *Eudistoma capsulatum* was the only species to display a significant decrease in abundance ($p = 0.000$) after the hurricane (Table S3). When considering the sum of all colonial species in our transects, there was also a significant change ($p = 0.012$) of species abundances following Hurricane Florence (Table S3). However, when considering all species together (all colonials plus *S. plicata*) there were no significant differences ($p = 0.786$) detected before and after the hurricane. The lack of significance in this case is clearly driven by the constant presence of *S. plicata* in our transects, and the species' fast recovery and rapid increase in number of individuals per m^2 during the months following Hurricane Florence (Fig. 5B, Table S3). Similarly, *E. capsulatum* was the only colonial species to display significantly ($p = 0.05$) lower percent coverage after the hurricane, with the colonial species' community as a whole also displaying a significant ($p = 0.008$) loss in percent coverage following the hurricane (Fig. 5B, Table S5).

Discussion

We found a significant impact of hurricanes on fouling communities inhabiting harbors, where non-native species were more resilient and able to recover and recolonize open spaces faster than native ones. Accordingly, when compared with pre-hurricane data, a decrease in the number of native species and an increase of introduced species along the whole North Carolinian coast was observed. Two globally introduced species never reported in N.C. were also observed after Hurricane Florence: the solitary ascidian *Styela canopus* and the colonial species *Distaplia listerianum*. In July 2019 (one year after Hurricane Florence), we found thirteen ascidian species in sixteen harbors and marinas along North Carolina's coast, with five of these species identified as native, two as cryptogenic, four as introduced, and two that were classified only to the genus level. Similarly, thirteen distinct ascidian species were observed by Villalobos et al. (2017) in July 2014. However, not all species observed in 2014 were documented in our study and vice-versa. Three native species *Didemnum lutarium* (observed in two southern marinas), *Distaplia corolla* (observed in one northern marina), and *Eudistoma capsulatum* (occurring in one southern and two northern marinas), and one cryptogenic species *Distaplia stylifera* (observed in one southern marina) were not retrieved in 2019. On the other hand, four previously unreported species were observed in 2019: the cryptogenic species *Eusynstyela tincta*, the introduced species *Styela canopus*, and two uncategorizable species: an *Eudistoma* and an *Aplidium*. In both 2014 and 2019, the most abundant and widespread species was the solitary *Styela plicata*, which has a global distribution and an unknown origin (Barros et al. 2009; Pineda et al. 2011).

Based on presence-absence data, there were significantly more introduced ascidian species in 2019 than 2014, but there was no significant difference in the presence-absence or relative abundance of native species. The greater presence of introduced species in 2019 is likely driven by an increase in the number of harbors containing non-native species, and the appearance of a new introduced solitary species, *Styela canopus*, in several of the northern harbors. The barcoding sequences obtained here for *S. canopus* were identical or nearly identical to those reported by Barros and Rocha (2021) for Florida and Georgia (U.S.A.) specimens. Since the species was not observed in the southern-most harbors of N.C., our data suggested that *S. canopus* was likely introduced by fouling the hull of ships navigating North along the Intracoastal Waterway, rather than by natural range expansion. Independent of its introduction pathway, *S. canopus* is a species widely introduced across the globe (Barros and Rocha 2021) and thus likely to spread rapidly in N.C.

In this study, the latitudinal location of harbors and the distance among them had a significant effect on community similarity for both presence-absence and relative abundance of ascidian species. This observation contrasted with results reported by Villalobos et al. (2017) for 2014, where only distance between harbors had a significant impact on the relative abundance of ascidians. The most parsimonious explanation for these differences is the varying severity of environmental impacts sustained by each area after Hurricane Florence. Ascidians, as sessile invertebrates unable to avoid local abiotic stressors (e.g. temperature and salinity changes), will be affected more severely than mobile organisms (Patrick et al. 2020) and will likely display varied levels of resilience based on their proximity to a disturbance. Hurricane Florence made landfall in Wrightsville Beach (South) and serious flooding was reported to occur between Wilmington (a mile away from Wrightsville Beach) and New Bern (90 miles North), but sparing the northernmost locations in N.C. Accordingly, we expected ascidian communities in southern harbors to have been impacted differently than those in the North. Support for this expectation included, when examining the southern sites as a whole, a loss of four species: the native species *Aplidium stellatum* and *Didemnum lutarium*, and the cryptogenic species *Distaplia bermudensis* and *Distaplia stylifera*. Additionally, we observed the disappearance of all species except for *S. plicata* following Hurricane Florence at Seapath Yacht Club, our southern long-term monitoring site, with colonial species displaying significantly decreased abundance per m^2 and percent coverage.

Following Hurricane Florence, the first species to recover its original numbers (and even increase these) at Seapath Yacht Club was the only solitary species in our photo-transects, *Styela plicata*. This species has a global distribution (Pineda et al. 2011) and is the most common and abundant species in N.C. harbors and marinas (Villalobos et al. 2017, here). In addition, both adults and larvae are known to tolerate a wide range of salinity and temperature conditions (Thiyagarajan and Qian 2003, Pineda et al. 2012a,b) and in other temperate locations, the species is able to reproduce all year long (Pineda et al. 2013). Thus, *S. plicata* has all the makings of an opportunistic species, and here it was able to rapidly colonize all available substrate left by species that perished as a result of the hurricane.

Similarly, the globally introduced ascidian *Diplosoma listerianum* (here reported in N.C. for the first time) was the second species recovering from Hurricane Florence at Seapath Yacht Club. *D. listerianum* was previously documented in the states above (Virginia) and below (South Carolina) N.C. (Pérez-Portela et al. 2013), and was observed here in 2018 and 2019. Thus, although it is possible that the species is a new arrival to N.C., the most parsimonious explanation for the lack of previous observations in N.C. is the species' life cycle. As shown here, *D. listerianum* numbers and species coverage were particularly high in fall and winter, with numbers and coverage decreasing in spring and few to no individuals observed in summer. The seasonal life pattern of *D. listerianum*; however, did not prevent the species from recovering from the drastic environmental changes caused by Hurricane Florence after a few months. In fact, the success of *D. listerianum* as a wide-spread introduced species is attributed to its ability to produce cross-fertilized zygotes as late as one month after sperm has been stored (Bishop and Ryland 1991), foster diverse genotypes within fused colonies (Sommerfeldt and Bishop 2002), and tolerate a wide range of salinities (Gröner et al. 2011). Thus, the first two species to recolonize available substrate after Hurricane Florence were globally introduced species, which is in agreement with previous studies showing that non-native species are typically opportunistic and more resilient than their native counterparts (Lambert 2005).

All colonial species disappeared during several months after Hurricane Florence and all experienced a significant decrease in abundance and percent cover. Among the colonial ascidians present at Seapath Yacht Club in 2018 and 2019, the native *Eudistoma capsulatum* was the most severely impacted by Hurricane Florence in terms of number of individuals and coverage. Other species within the *Eudistoma* genus have displayed decreased settlement success, increased juvenile mortality, and failed metamorphosis to the adult stage at low salinities (Vázquez and Young 2000). The sensitivity of *E. capsulatum* and other ascidians to changes in abiotic conditions (in particular decreased salinity) is well described in the literature (Vázquez and Young 1996, 2000, Epelbaum et al. 2009, Pineda et al. 2012a, Nagar and Shenkar 2016); thus, further work should focus on establishing why these changes affect native and introduced species differently and the mechanisms behind increased resilience, not only to hurricanes but also to anthropogenic disturbances.

Declarations

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Data availability

The PRIMER7 input files and raw survey data are available from the corresponding author. All sequences have been deposited in GenBank® (accession numbers MW621870 to MW621909). All other data generated and analyzed during this study are included in this published article and its supplementary materials.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Study conception and design were done by Susanna López-Legentil. Material preparation, data collection and analyses for the 2019 surveys were performed by Brenna Hutchings and Susanna López-Legentil. Material preparation, data collection and analyses for the monthly photo transects were done by Emma Stiles and Susanna López-Legentil. The first draft of the manuscript was written by Brenna Hutchings and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

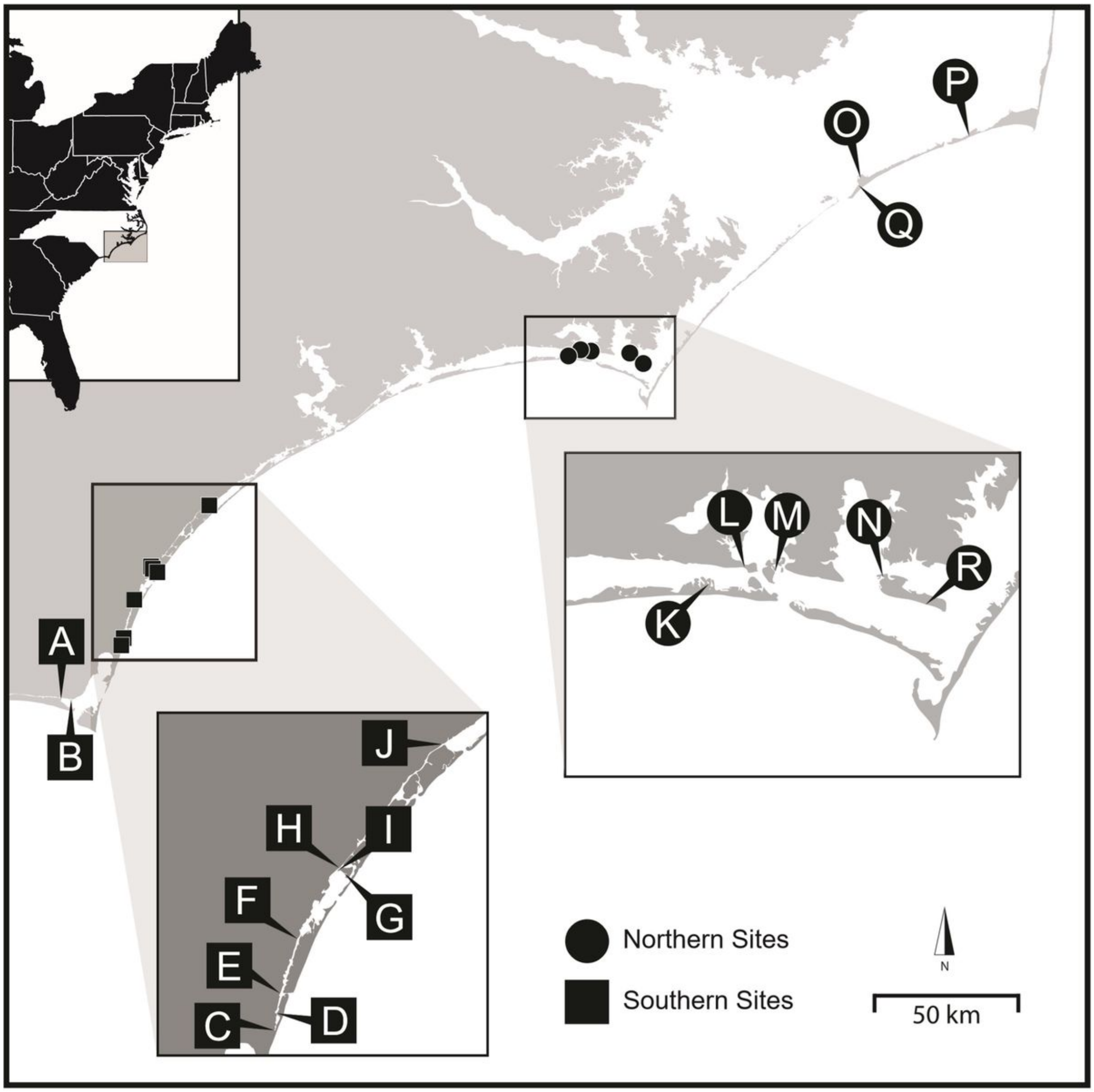

Figure 1

Sampling locations across the coast of North Carolina, distinguished by latitude: northern sites (circles) and southern sites (squares). Site identities are: South Harbor Village Marina (A); Southport Marina (B); Harbour Point Marina (C); Joyner Marina (D); Inlet Watch

Yacht Club (E); Masonboro Yacht Club & Marina (F); Seapath Yacht Club (G); Bridge Tender Marina (H); Wrightsville Beach Marina (I); Harbour Village Marina (J); Crow's Nest Yacht Club (K); Portside Marina (L); Olde Towne Yacht Club (M); Harker's Island (N); Ocracoke Ferry Landing (O); Hatteras Harbor Marina (P); Hatteras Landing Marina (Q); Cape Pointe Marina (R)

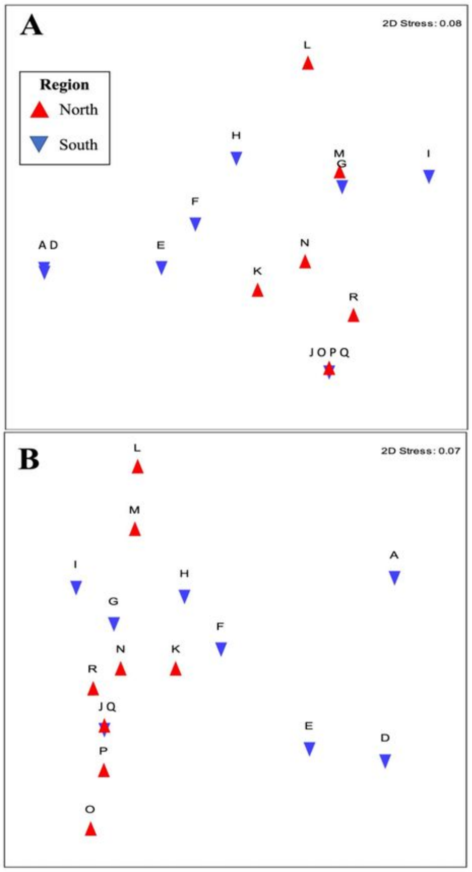

Figure 2

Non-metric multidimensional scaling (nMDS) plots of 2019 ascidian community similarity based on presence-absence (A) and relative abundance (B) data. Latitudinal position of each harbor is indicated by red (North = up-arrow) and blue (South = down-arrow) symbols. Letters correspond to the harbors listed in Table 1

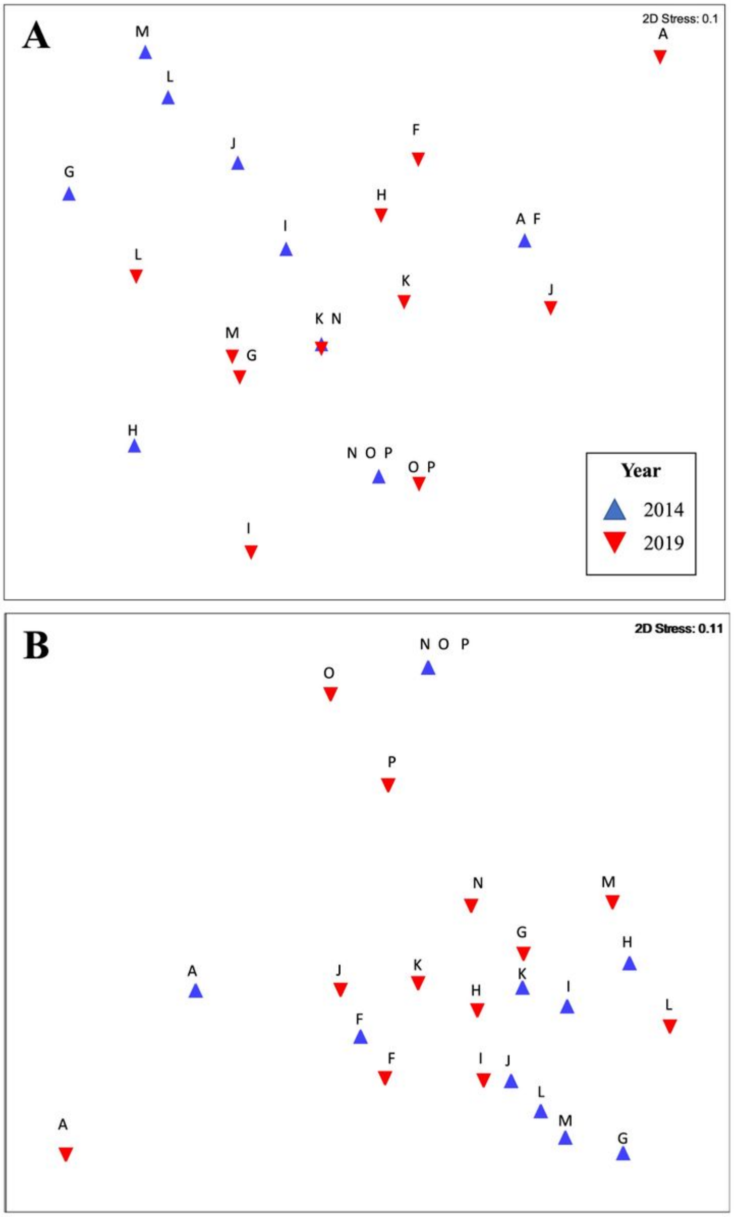

Figure 3

Non-metric multidimensional scaling (nMDS) plots comparing community similarity based on presence-absence (A) and relative abundance (B) of ascidian species observed in 2014 and 2019. Year that each community (individual harbors) was sampled is indicated by blue (2014 = up-arrow) and red (2019 = down-arrow) symbols. Harbor identity corresponds to those listed in Table 1

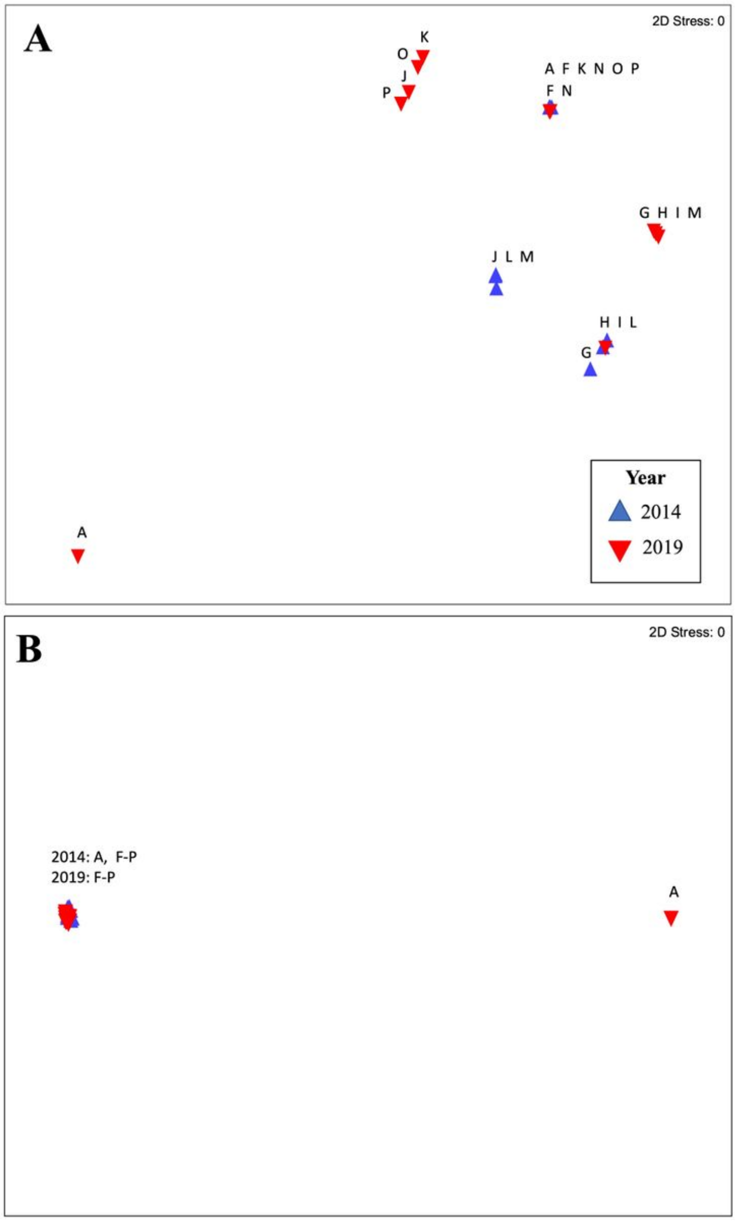

Figure 4

Non-metric multidimensional scaling (nMDS) plot of community similarity based on presence-absence (A) and relative abundance (B) of introduced species observed in harbors sampled in 2014 and 2019. Year that each harbor was sampled is indicated by blue (2014 = up-arrow) and red (2019 = down-arrow) symbols. Harbor identity corresponds to Table 1

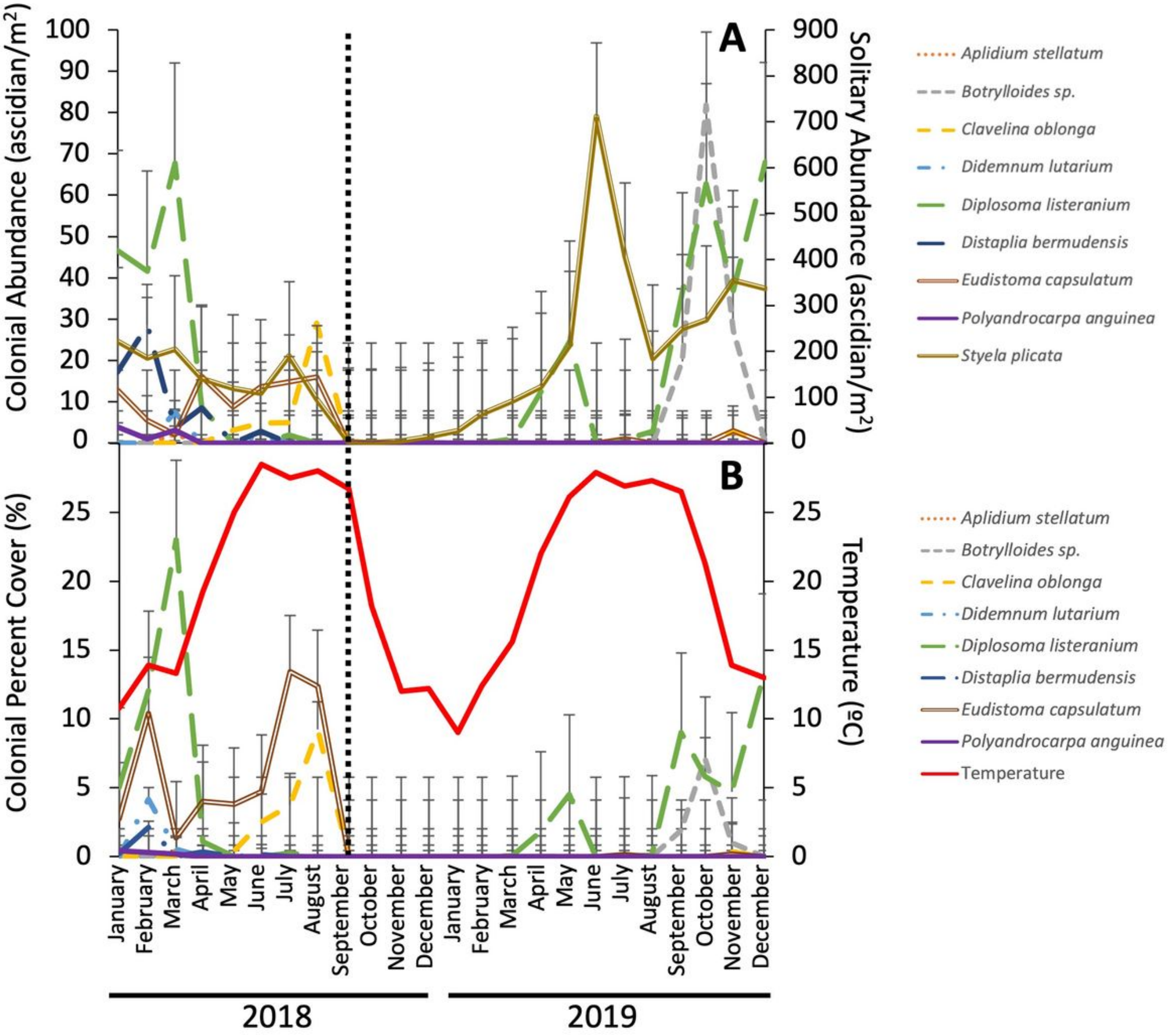

Figure 5

Abundances per m^2 (+SD) (A) for the ascidian species identified at the Seapath Yacht Club, Wilmington, N.C., from January 2018 to December 2019 (24 months). Percent cover (+SD) (B) of ascidian species at Seapath Yacht Club. Monthly temperatures are represented by the solid red line for both graphs. Vertical dashed black line represents landfall of Hurricane Florence on September 14, 2018