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Analysis of the Spatial Distribution, and Recruitment of Native and Non-Native Tunicate Species on *Zostera marina* in New Jersey

Sarah Hoffman

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Abstract

Many tunicate species have invaded the Western North Atlantic coast in the last century. These tunicates can have negative impacts on important industries like shellfish aquaculture, but they also foul natural and anthropogenic surfaces leading to displacement of native communities. During the summer of 2017 and 2018, the spatial distribution and recruitment of tunicate species in *Zostera marina* (Eelgrass) beds in Barnegat Bay, New Jersey were assessed. Samples were collected during the summer by excavating all eelgrass from a 0.125m² area. Samples were then assessed for tunicate presence, as well as percent coverage of tunicates. In 2017, *Botrylloides violaceus* was the most commonly identified species and was found at Barnegat Inlet and Ham Island. *Botryllus schlosseri*, *Didemnum vexillum*, and *Asciidiella aspersa* were also identified, but were less abundant. Tunicate coverage increased substantially at Barnegat Inlet from June to August, but decreased at Ham Island during this period. *Didemnum vexillum* was only found at Ham Island, while *Asciidiella aspersa* was only found at Barnegat Inlet. In 2018, a broader survey of Barnegat Bay was done, which included numerous sample sites throughout the bay. *Botryllus schlosseri* was the most commonly identified species during this larger survey. A regression analysis demonstrated that there was a significant positive relationship between *Z. marina* biomass and tunicate biomass. This suggests that when there is more seagrass biomass there is more spatial area for colonization and growth of ascidians. However, there was one outlier that showed the potential negative effects of tunicate growth on seagrass. This outlier is evidence of the fact that tunicates can overgrow and kill seagrass. These results suggest that fouling invasive tunicates could have a negative impact on eelgrass communities by smothering blades and reducing plant viability. This research is the first evaluation of invasive tunicates living among the seagrass beds in New Jersey and indicates both spatial and temporal variability in presence and prevalence.

MONTCLAIR STATE UNIVERSITY

Analysis of the spatial distribution, and recruitment of native and non-native tunicate species on

Zostera marina in New Jersey

By

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A THESIS

Submitted in partial fulfillment of the requirements
For the degree of Master of Science

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2020

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By Sarah Rachel Hoffman

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Introduction

Zostera marina (eelgrass) is an ecologically important angiosperm species of seagrass on the northeastern coast of North America (Green and Short 2003). In an area that is known for low seagrass diversity, *Z. marina* is the dominant species in the Western North Atlantic (Kennish et al. 2008). It grows in lower intertidal and shallow subtidal areas and is generally found in lagoons and estuaries. *Zostera marina* is important ecologically, but it is also important to humanity. Healthy *Z. marina* beds support high biodiversity, support fisheries, filter coastal waters, dissipate wave energy, and stabilize sediment (Green and Short 2003, Short et al. 2007). The roots and rhizomes of *Z. marina* binds sediment, which stabilizes the sediment during storm events and aids in sediment accumulation as well as dissipates wave energy (Koch et al. 2006, Bos et al. 2007). The shoots of *Z. marina* are also important in maintaining water quality by providing a layer over the benthos and preventing sediment from becoming resuspended in the water column and thus decreasing turbidity (Short and Short 1984). *Zostera marina* maintains water quality by aiding in sedimentation and the uptake of nutrients, organic pollutants, and contamination that could lead to eutrophication and harmful algal blooms. These nutrients will then only be released through decomposition and consumption, which is a much slower process (Hemminga and Duarte 2000). Seagrass has a direct value to humanity as well. They provide a nursery habitat for commercially, recreationally, and ecologically important fisheries (Bertelli and Unsworth, 2014). It also provides a habitat for commercially important shellfish and serves as a food source for waterfowl species (Heck and Valentine 2007) Seagrass species such as *Z. marina* also have an important role in carbon sequestration. *Zostera marina* can be seen as a keystone species, as the amount of carbon dioxide that becomes bound into organic matter is disproportionate to their size (Duarte and Cebrian 1996, Short and Neckles 1999). Seagrass is a global primary producer for the oceans. It is

an important part of both marine and terrestrial food webs, and it is an important pathway to high secondary production (Schmidt et al. 2011). Decreases in vascular plants like seagrasses often signal the decline in ecosystem health (Bologna et al. 2007).

Globally, seagrass beds are declining at an accelerated rate due to anthropogenic stressors (Orth et al. 2006). One anthropogenic stressor is coastal development such as dredging, filling, land reclamation, dock building and jetty construction. Some fisheries and aquaculture practices also have a direct effect on seagrass beds and their ecosystems (Short et al. 2007). The potential stress from human impacts that Barnegat Bay, NJ is facing has only increased during the last few decades (Kennish et al. 2007). Stressors resulting from eutrophication like epiphytic growth, macroalgae, and phytoplankton blooms can account for seagrass loss as well. In Barnegat Bay, NJ, it has been documented that macroalgal blooms have caused rapid decline in seagrass coverage due to decreased light availability and direct smothering (Bologna et al. 2007).

The invasion of non-native ascidian species is a new, increasing source of stress that may inhibit *Z. marina* growth and cause shoot weakening or mortality, leading to the further decline of *Z. marina* populations. Along with blocking sunlight, epiphytes such as ascidians can also form large mats and weigh down the canopy (Wong and Vercaemer 2012). When ascidians invade a new habitat, they have the ability to outcompete native species and change community dynamics. It is a known fact that invasive ascidians can cause changes in the structure and function of benthic communities (Dijkstra et al. 2007), because they can overcome many abiotic challenges in new environments by having a wide range of salinity and temperature tolerances. An experiment determined that *Botryllus schlosseri* can survive in salinities from 14-38‰ and *Botrylloides violaceus* from 20-38‰ (Dijkstra et al. 2008). *B. schlosseri* can survive in temperatures between 10 and 25 °C and *B. violaceus* can survive in temperatures between 5 and 25°C. They can also

survive rapid temperature changes. *Didemnum spp.* can survive daily water temperature changes of up to 11°C (Epelbaum et al. 2009). They can also tolerate heavy metal pollution that many invertebrates cannot, such as mercury, copper, and iron (Epelbaum et al. 2009). It has been documented in San Francisco, California that invasive ascidians have reduced the number of other benthic species, which changed the community structure (Zhan et al. 2015).

Invasive ascidians can also affect benthic pelagic coupling. Dense mats of colonial ascidians, such as *Didemnum vexillum*, could prevent filter feeders from foraging in a benthic community (Zhan et al. 2015). Invasive epibiotic ascidians living on macrophytes, such as seagrass, could affect biogeochemical cycling by shading the plant and reducing its photosynthetic rate. This could affect both the dissolved oxygen and carbonic acid concentrations (Mercer et al. 2009).

Invasive ascidians also affect aquaculture. Removal of biofouling on cultured species such as oysters are expensive and impact the growth potential of bivalves by competing for food. In eastern Canada, there was a 50% loss in shellfish harvest after the population of *Styela clava* overgrew aquaculture infrastructure (Colautti et al. 2006). The cost to control biofouling organisms such as ascidians on aquaculture accounts for 5-10% of production cost. This is the equivalent of 1.5-3 billion dollars a year (Fitridge et al. 2012).

Colonial species such as *Botryllus schlosseri* have a complex life history. They reproduce both sexually and asexually, and also have two life history morphs. A single population can exhibit both characteristics of these life history morphs. The first is called semelparous colonies, where there is rapid growth before the first reproduction and high reproductive effort. They have an early age of first reproduction and die immediately after the release of the first clutch (Grosberg 1988). The second morphological type is referred to as an iteroparous colony, where a zooid will postpone

reproduction for twice as long and grow at half the rate of the semelparous colony. A zooid in an iteroparous colony will also produce a minimum of three clutches before dying (Grosberg 1988). When a larva recruits onto a substrate it quickly asexually makes a colony that is made of zooids that are genetically identical to that first larval recruit. Zooids reproduce and feed individually, but all of the zooids are connected by one large vascular system (Grosberg 1987). These zooids are covered in a gelatinous tunic, which is where their name was derived from. Every five to seven days an asexual cycle occurs, and every five to ten asexual cycles, a sexual reproductive event occurs. Sexual reproduction within the same colony doesn't occur often, because ovulation occurs several days before the testes mature (Grosberg 1987). *Botryllus schlosseri* colonies grow exponentially. They have been documented in laboratory settings to reach 1400 zooids in 69 days (Chadwick-Furman and Weissman 1995). Since the reproductive cycles of each zooid are the same, the larvae are released at the same time. The sibling larvae are then released as a temporary egg clutch to begin their short life stage as planktonic larvae (Grosberg 1988). In *B. schlosseri*, each zooid produces up to ten egg clutches with an average of one or two eggs per clutch and a maximum of five eggs per clutch. Colonial tunicates are known for their high fecundity, releasing up to eight thousand eggs per colony (Chadwick-Furman and Weissman 1995). The offspring that are produced from sexual reproduction will then settle onto a substrate often no more than one meter from where it was released (Grosberg 1987), meaning that *B. schlosseri* will usually recruit back into the parental population (Osman and Whitlatch 1998). *Botrylloides violaceus* is another colonial ascidian with a short larval stage. Their larvae are lecithotrophic, which means that their larval stage is only minutes to days long (Osman and Whitlatch 1998). One might think that tunicate larvae would be a high value prey item due to the fact that tunicates are lecithotrophic with rich yolk resources. However, larvae are also chemically defended, limiting predation and

allowing adult colonies to release larvae during the day instead of nighttime, unlike most of their invertebrate community mates (Lindquist et al. 1992).

While the pathway of invasion is unknown for many ascidians due to a lack of systematic taxonomy knowledge throughout their history, simple dispersal is unlikely (Zhan et al. 2015). While large-scale ascidian dispersal can happen through co-traveling, like on a wreck of seagrass (Worcester 1994) or on top of animals such as rock crabs and American lobsters (Bernier et al. 2009), it is unlikely that these pathways would lead to distant invasions. Additionally, because of the short larval stage of ascidians, it is likely that large-scale invasions are the result of human activities and not natural dispersal. The three main theories of how invasive ascidians came to the Northeast region of North America is through hull fouling, ballast water, and accidental co-traveling through aquaculture (Dijkstra et al. 2007). The introduction of *B. schlosseri* and *B. violaceus* have been credited to aquaculture twice in the past. In the Gulf of Maine, ascidians were introduced with the Pacific oyster (*Crassostrea gigas*) and in Prince Edward Island, Canada, they were introduced along with mussels (Dijkstra et al. 2007, Locke et al. 2007). Increased shipping due to an increase in global trade and aquaculture expansion gave rise to new pathways of invasion for non-indigenous species and all continents have reported invasive ascidians except Antarctica (Zhan et al. 2015).

Since invasive ascidians have been documented to cause negative impacts on eelgrass growth and survival, it is important to document the presence of these species in Barnegat Bay, New Jersey. Currently there are no extensive surveys of the presence of non-native tunicate species in New Jersey and this research is the first to address the spatial and temporal distribution of invasive tunicates within seagrass habitats in New Jersey. Previously there were two small surveys done in Barnegat Bay as part of larger studies looking at tunicate distribution between New Jersey

and Newfoundland, Canada (Carman et al. 2016, Carman et al. 2019). The objective of this research was to identify non-native ascidian species living on *Z. marina* seagrass beds in Barnegat Bay, New Jersey, as well as determine where they are colonizing within the bay.

Methods

Study Site

Barnegat Bay, New Jersey is a 67.2 km lagoonal system in Ocean County New Jersey (Fig. 1). It is fed by two large rivers, including the Toms River and the Metedeconk River, as well as numerous smaller tributaries. It is connected to the Atlantic Ocean through two natural inlets in the southern and middle region of the bay and is connected in the northern region as part of the Atlantic Intercoastal Waterway. It has been classified as highly eutrophic (Kennish et al. 2007) and continues to suffer from eutrophication (Fertig et al. 2014). Over the last few decades land use in the watershed has substantially changed in Barnegat Bay through loss of natural habitats and increased urban development (Lathrop and Bogner 2001). The dominant submerged aquatic vegetation (SAV) is *Z. marina*.

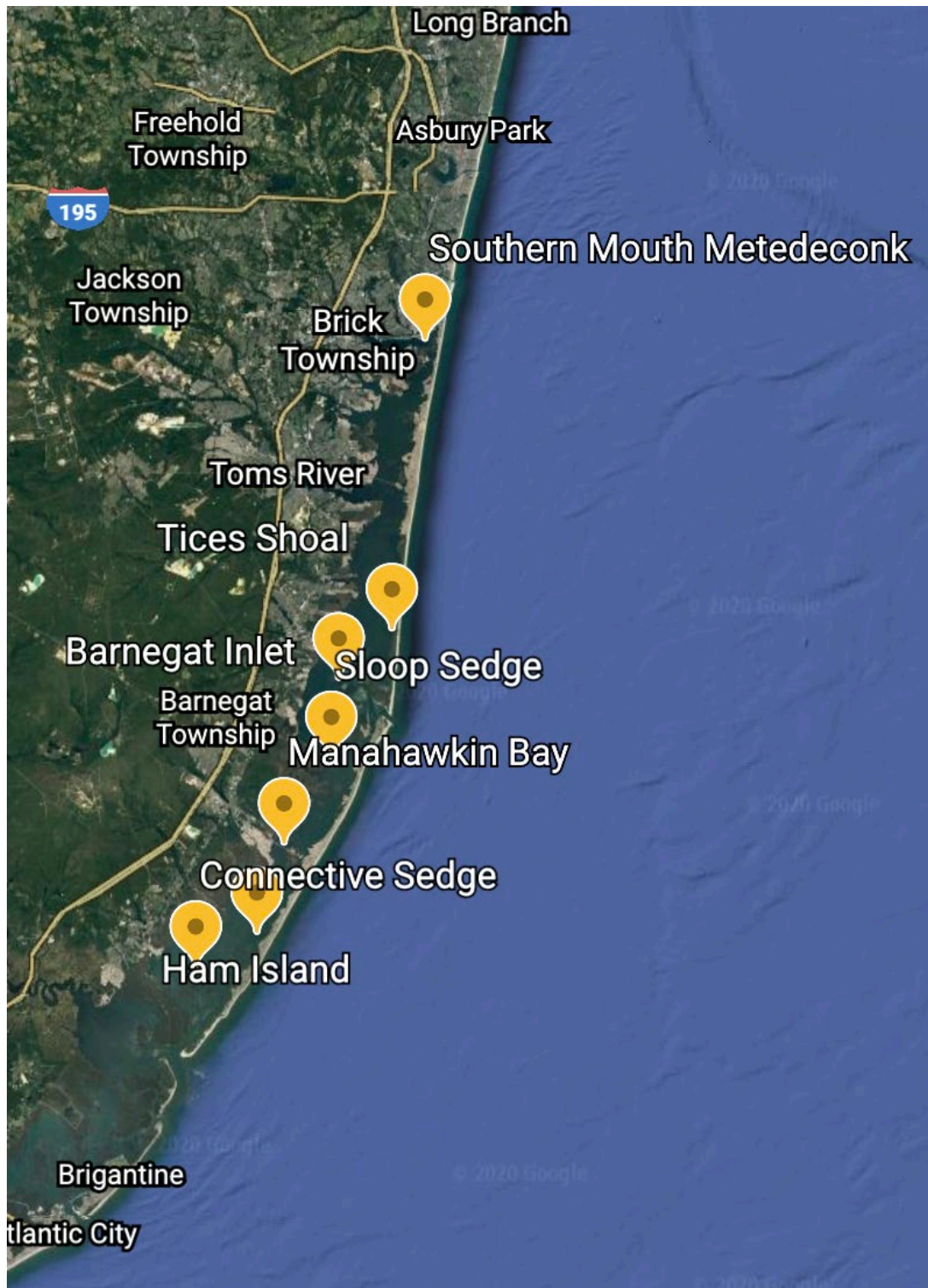


Figure 1. Field sites sampled in 2017 and 2018 for monthly tunicate coverage and the bay-wide surveys. Site identification, GPS Coordinates, and specific dates of collection are listed in Appendices A, B, and C.

Site-specific Survey Methods

In 2017, three sites in Barnegat Bay, New Jersey, were surveyed for presence, spatial distribution, and settlement patterns of tunicates during June, July, and August to assess seasonal distributions. During each sampling event, salinity, temperature, and dissolved oxygen were measured for each site using a YSI® multi-meter. The sites sampled include Ham Island, Barnegat Inlet, and Silver Bay East, New Jersey (Fig. 2). The first two sites were comprised of *Zostera marina* seagrass beds, while Silver Bay East was dominated by *Ruppia maritima*. To analyze presence and species distribution of tunicates, three 100m transect lines were set up randomly at each site within the seagrass meadows. The GPS coordinates of both endpoints were recorded (Appendix E). Samples of the seagrass were collected every 10 meters on alternating sides for each transect and consisted of removing all vegetation from a 0.125m² quadrat (25cm x 25cm). All seagrass shoots inside the quadrat were excavated at the sediment-water interface, placed into a fine mesh bag, transferred to labeled plastic Ziploc bag, and placed on ice until they were returned to Montclair State University. Samples were then processed in the laboratory by separating seagrass shoots and evaluating the presence, identity, and coverage of tunicate species on each shoot.

In 2018, Ham Island and Barnegat Inlet were again surveyed in June and August, but not July. Silver Bay was eliminated as a site, since no tunicates were present associated with *R. maritima* in 2017. The sampling protocol was changed slightly in 2018. Instead of sampling on a 100 meter transect line, 10 random samples were taken, removing all vegetation from a 0.125m² quadrat (25cm x 25cm). Five random 10x1 meter transect lines were set up at each site to count colonies in the field without excavation. At all sampling times, temperature, salinity, and dissolved oxygen were measured using a YSI® multi-meter.

2018 Bay-wide Survey

In August 2018, a bay-wide survey was conducted to assess the regional tunicate populations in Barnegat Bay, New Jersey. Seven sites were sampled throughout Barnegat Bay (Fig. 1). Each of these sites comprised benthic habitats dominated by eelgrass, *Z. marina*. Ten (10) 0.125m² quadrat (25cm x 25cm) samples were collected haphazardly from each site in the same manner as described above. Samples were collected, transported, and analyzed in the same manner as well. Additionally, five 10m long by 1m wide transect lines were set up to visually assess colonies in the field to increase total sampling area evaluated (50m² vs. 1.25m² quadrats). Tunicate colonies were counted and identified along the transect to estimate field spatial coverage of all species. At all sampling times, temperature, salinity, and dissolved oxygen were measured using a YSI® multi-meter.

In-lab sample processing

Once in the lab, seagrass samples were defrosted in cool water. In each sample, every seagrass shoot was counted, and tunicates were identified using a marine fouling organisms guide from the Massachusetts Invasive Species Program as well as a key given by Mary Carman as part of the northeast regional study (Carman pers. comm.). Shoots with *B. schlosseri*, *B. violaceus*, *A. aspersa*, and *Didemnum spp.* were isolated and counted to determine prevalence and percent cover within each sample. Tunicates were then removed from the seagrass using a single-sided safety razor. The shoots were placed into an aluminum weighing dish and the tunicates were placed into a separate aluminum weighing dish. The weighing dishes were labeled and weighed before samples were placed inside and then placed into a 80°C drying oven. Once the samples were dry to constant weight (72h - 120h), they were weighed, covered with aluminum foil, and placed into

a muffle furnace for 8 hours at 500°C. After they were removed from the furnace, they were uncovered and weighed again. This process generated ash free dry weights (AFDW) for eelgrass plant samples and tunicate biomass.

Statistical Analyses

Using The SAS® System, the presence of invasive ascidians at Ham Island and Barnegat Inlet were compared during the months of June, July, and August for 2017 using a 2-way ANOVA with month and site as independent variables and ascidian cover (individual species and the summation) as the dependent variable in the model. Additionally, a 2-way ANOVA was conducted on the number of blades per quadrat for each site and month. In 2018, a 2-way ANOVA was conducted on the ascidian cover and shoot abundance between months and sites for Ham Island and Barnegat Inlet again. The August 2018 bay-wide survey was analyzed with a 1-way ANOVA comparing the blades per quadrat and the cover of *B. violaceus* and *B. schlosseri* at each site as well as the total percent coverage of invasive ascidians at each site. A least squares means test ($\alpha = 0.05$) was done to determine significant differences between individual sites.

Results

Non-Native Tunicate Occurrence

During surveys in 2017 and 2018, two sites, Silver Bay East and Seaside Heights, were found to contain the seagrass *Ruppia maritima* only. Each of these sites showed no presence of any tunicates and were subsequently excluded from the analyses. Surveys of Ham Island and Barnegat Inlet demonstrated that there were four ascidian species identified including: *Botryllus schlosseri*, *Botrylloides violaceus*, *Didemnum spp.*, and *Asciidiella aspersa*. These four species are considered non-native in New Jersey, although they have been recorded in the region for a

considerable amount of time. The most common species found were *Botryllus schlosseri* and *Botrylloides violaceus*.

2017 Results

In 2017, there was a difference in eelgrass coverage between Ham Island and Barnegat Inlet. The average number of blades/m² was found to be 152.2 in Ham Island and 216.7 in Barnegat Inlet (Table 1). There were significantly more total ascidians found in Barnegat Inlet than Ham Island ($F_{1,80}=27.92$, $P < 0.0001$), but individual species varied between sites. There was a significant difference in the percent of *Z. marina* blades with *B. violaceus* from Ham Island to Barnegat Inlet in 2017 ($F_{1,80}=26.43$, $p<0.0001$; Fig. 2). Overall, there was greater cover of *B. violaceus* at Barnegat Inlet than Ham Island. While the amount of *B. violaceus* increased from June to August in Barnegat Inlet, it decreased at Ham Island. There was also a significant difference between Ham Island and Barnegat Inlet with regards to *B. schlosseri* percent cover ($F_{1,80}=5.02$, $P<0.0278$; Fig. 3). Both sites decreased in *B. schlosseri* coverage. However, Barnegat Inlet had a much larger percent coverage at each sampling month. The sites also differed in respect to the rare invasive tunicates, with *A. aspersa* being found only at Barnegat Inlet in July of 2017 and *D. vexillum* being only found at Ham Island in August of 2017. There is a significant regression between seagrass biomass and tunicate biomass ($F_{1,80}=27.92$, $P < 0.0001$; Fig. 4), indicating that as seagrass biomass and leaf surface area increases, so does the presence of invasive tunicates.

Table 1. Monthly characterization of the average *Zostera marina* blade density and biomass (g AFDW), tunicate biomass (g AFDW), and water quality parameters for each site in 2017.

Site	Month	<i>Z. marina</i> blade density	Temperature	Salinity	Oxygen	Tunicate biomass	Seagrass biomass
			°C	ppt	mg/l	g	g
Barnegat Inlet	June	290.85	26.1	25.3	9.42	1.35	28.36
Barnegat Inlet	July	202.2	28.4	28.7	6.3	0.50	36.77
Barnegat Inlet	August	166.8	28	28.4	12.76	0.83	25.95
Ham Island	June	229.47	24.9	23.56	5.12	0.18	11.72
Ham Island	July	131.15	28.1	30.1	5.13	0.35	15.04
Ham Island	August	93.27	26.8	27.8	7.16	0.05	8.88

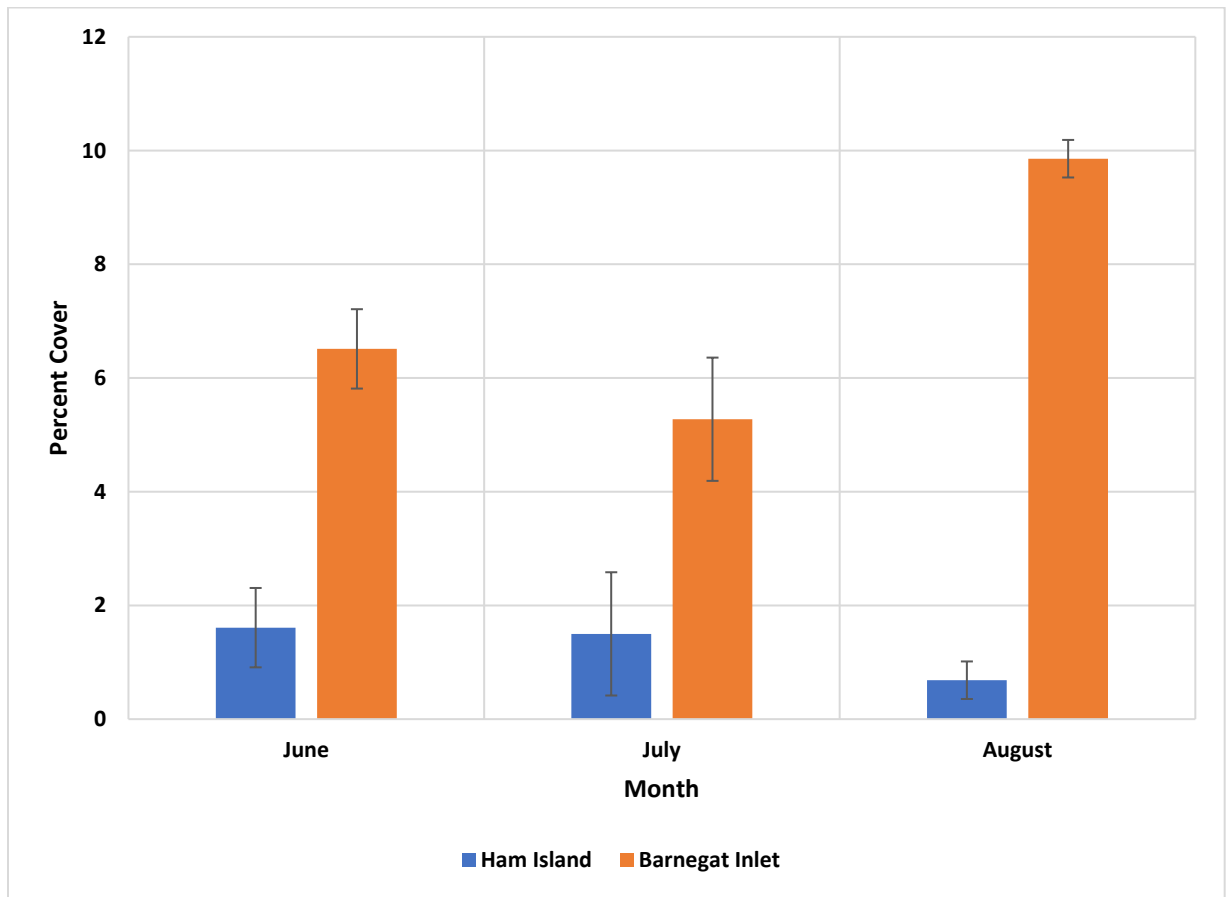


Figure 2. *B. violaceus* percent cover (\pm SD) on *Z. marina* between the two sites in 2017

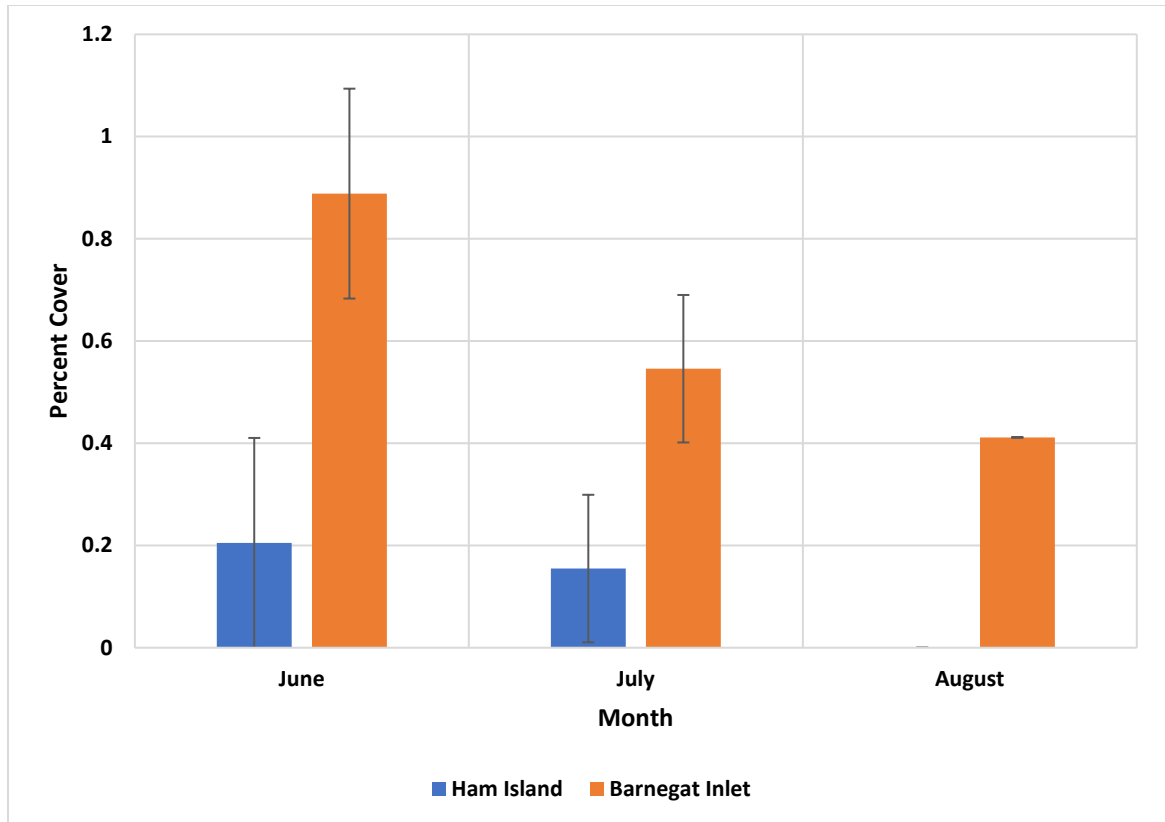


Figure 3. *B. schlosseri* percent cover (\pm SD) on *Z. marina* between the two sites in 2017

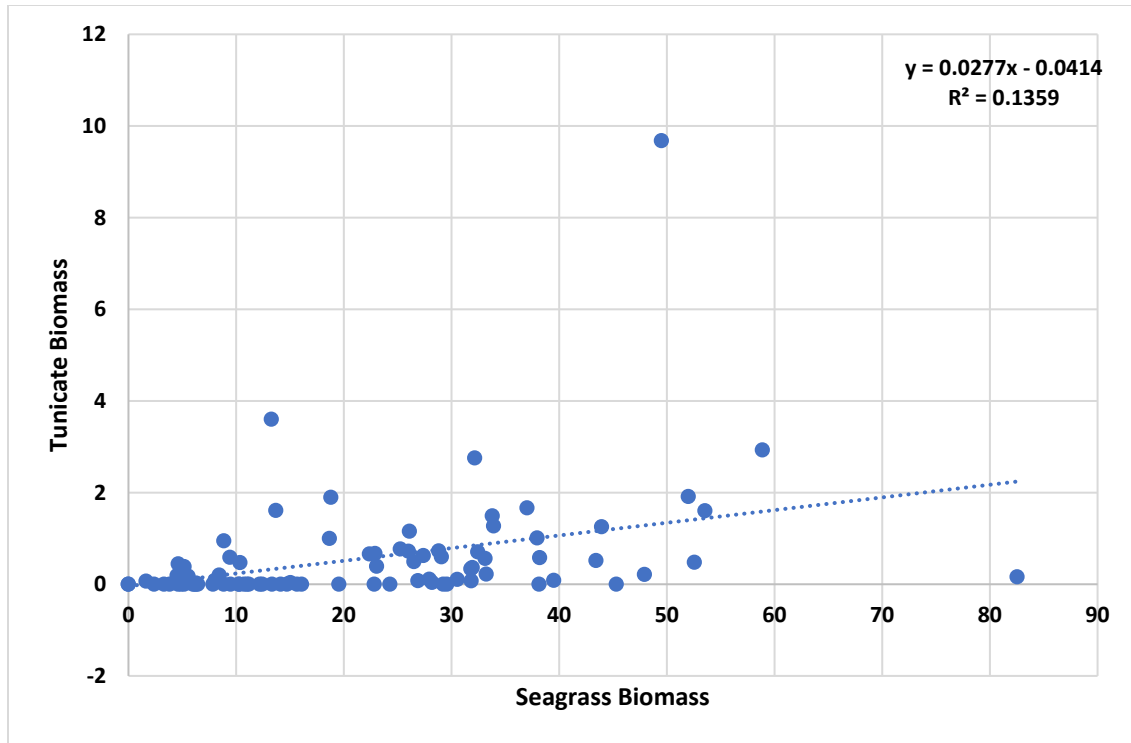


Figure 4. Regression analysis showing a significant positive relationship between seagrass biomass and tunicate biomass

2018 Barnegat Inlet and Ham Island Results

In 2018, these two sites were sampled in June and August and they differed significantly in respect to coverage among species. Specifically, no *B. violaceus* was found at Ham Island, but they were present at Barnegat Inlet demonstrating a significant site difference ($F_{1,36}=11.51$, $P<0.0001$; Fig. 5). Additionally, there was significantly more *B. violaceus* in June than in August ($F_{1,36}=11.51$, $P<0.0001$; Fig. 5). There was a significant decline in *B. schlosseri* percent coverage from June to August at Barnegat Inlet ($F_{1,36}=10.10$, $P<0.0030$; Fig. 6), while at Ham Island the percent cover increased from 0% in June to 0.47% in August (Fig. 6). The eelgrass blade density decreased significantly from June to August at both Ham Island and Barnegat Inlet ($F_{1,36}=5.57$, $P<0.0238$; $F_{1,36}=5.57$ $p<0.0238$; Table 2).

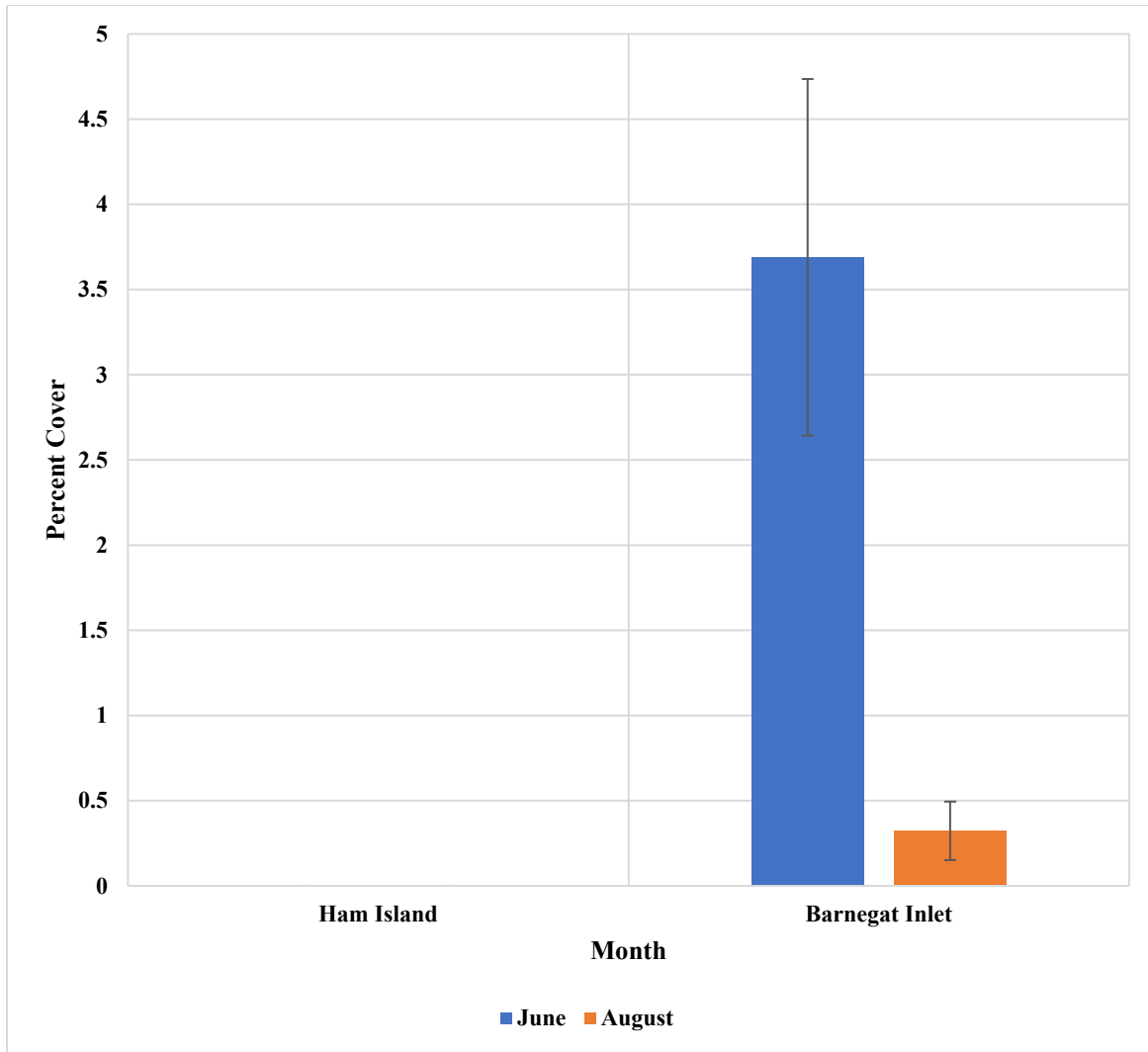


Figure 5. Percent cover of *B. violaceus* (\pm SD) growing on eelgrass beds from June to August in both Barnegat Inlet and Ham Island in 2018. Cover was significantly greater at Barnegat Inlet, but significantly declined from June to August.

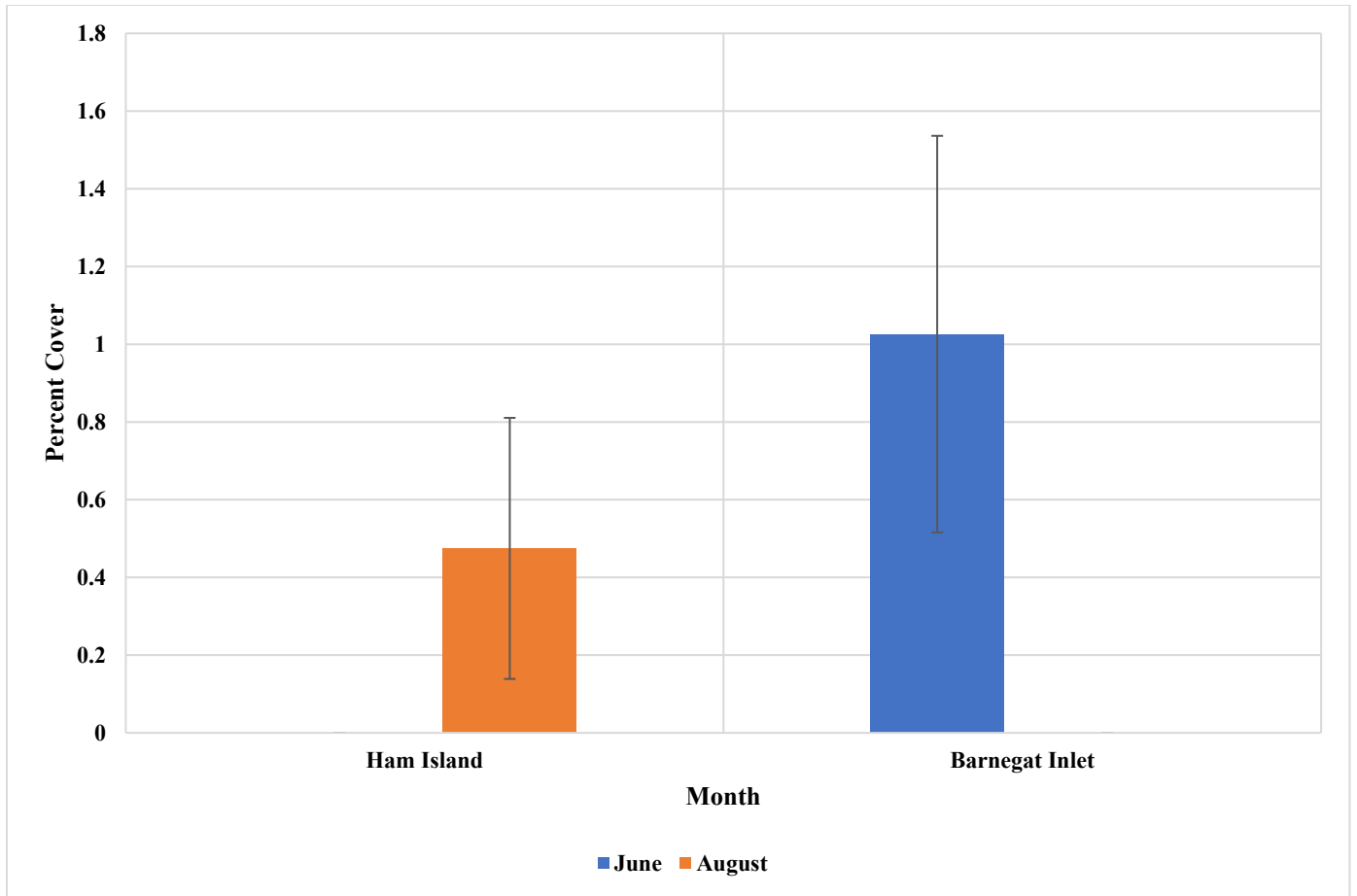


Figure 6. Percent cover of *B. schlosseri* (\pm SD) from June and August 2018 from Ham Island and Barnegat Inlet. Complete opposite patterns were observed between sites and months.

Table 2. Monthly characterization of the average *Zostera marina* blade density and biomass (g AFDW), tunicate biomass (g AFDW), and water quality parameters for each site in 2018.

Site	Month	<i>Z. marina</i> blade density	Temperature	Salinity	Oxygen	Tunicate biomass	Seagrass biomass
			°C	ppt	mg/l	g	g
Barnegat Inlet	June	136.1	21.1	27.6	6.83	0.15	14.8
Barnegat Inlet	August	97.2	26.6	28	7.01	0.07	8.87
Ham Island	June	110.2	24.6	26.6	5.48	0	4.53
Ham Island	August	110.2	27.9	29.6	4.41	0.01	3.73

2018 Bay-wide Survey

Tunicate coverage was not uniform throughout the seven eelgrass sites sampled. In August of 2018, five sites were found to have invasive tunicates, while two sites (Manahawkin Bay and Connective Sedge) did not have invasive tunicates present (Fig. 7). Sites also varied in species present and relative abundance ($F_{6,62}=7.04$, $P<0.0001$). Sloop Sedge was found to have the highest percent cover of invasive tunicates, followed by Tices Shoal and Southern Mouth Meteteconk. Eelgrass blade density/quadrat was also not uniform among sites ($F_{6,62}=7.14$, $P<0.0001$). Connective Sedge had the lowest blade density and Tices Shoal had the greatest at 216.3 (Fig. 8). Complete raw data for this sampling survey are presented in Appendices D (Quadrat Data) and E (Transect Data). When seagrass biomass was regressed against tunicate biomass, a weak, but significant regression occurred ($F_{1,107}=8.0$, $P<0.005$), suggesting that greater blade density has a positive effect on tunicate presence (Fig. 9).

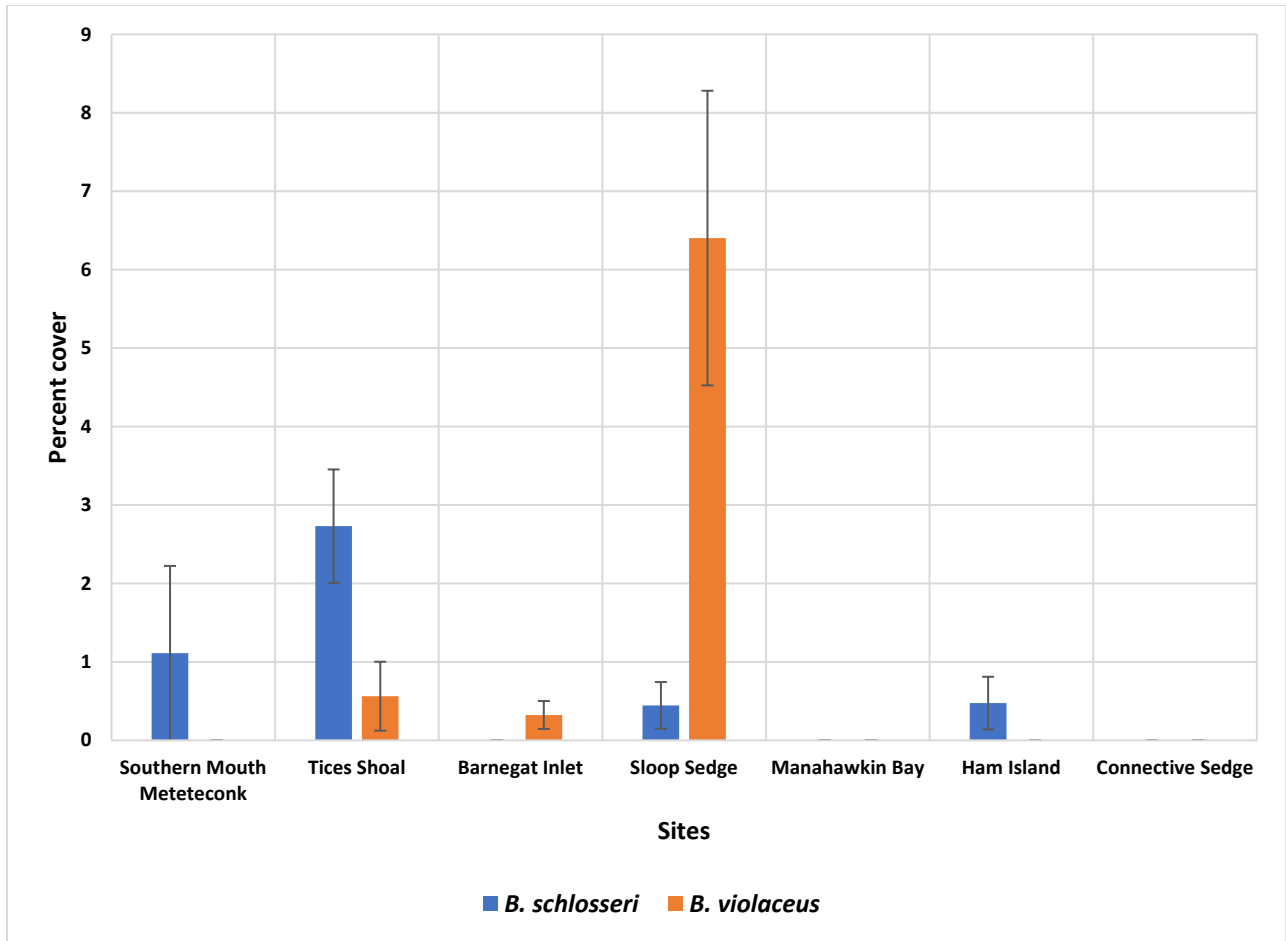


Figure 7. *B. violaceus* and *B. schlosseri* percent cover for sites sampled in 2018.

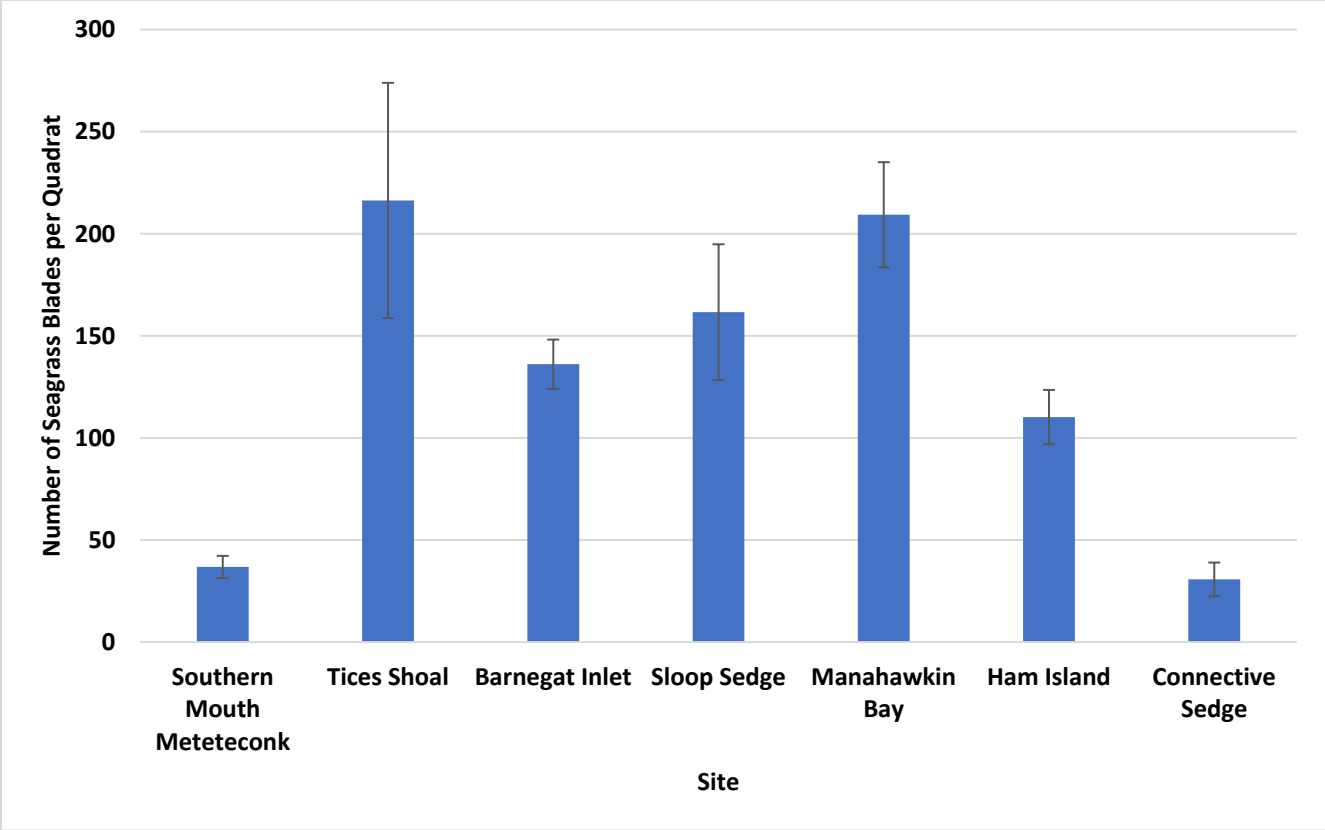


Figure 8. *Z. marina* blade density varied from site to site. The sites on the graph are organized from north to south. Southern Mouth Meteteconk being most northern and Connective Sedge being most southern.

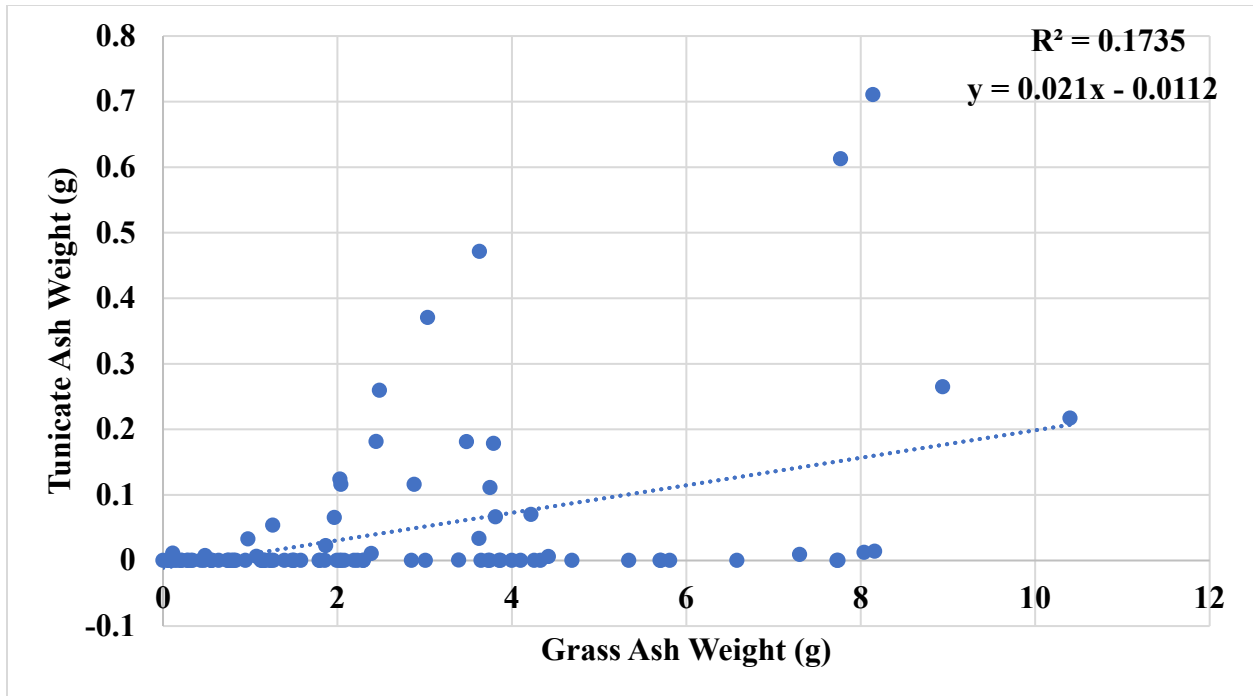


Figure 9. Tunicate biomass vs. seagrass biomass for the August 2018 survey.

Discussion

Ham Island and Barnegat Bay 2017 vs 2018

In 2017, Barnegat Inlet had significantly greater *Z. marina* blade density than Ham Island (Table 1). However, in 2018, the blade densities at these sites did not differ (Table 2). Barnegat Inlet also had a significantly higher amount of tunicates growing on eelgrass (Figs. 2 and 3) and the results of this study demonstrated that tunicate biomass was significantly related to *Z. marina* biomass (Fig. 4). This suggests that tunicates are more likely to colonize where there is a greater eelgrass surface area.

In 2018, the percent coverage of tunicates on eelgrass increased from June to August for Ham Island, while it decreased at Barnegat Inlet (Figs. 5 and 6). This could be due to the fact that at Ham Island the temperature only increased by 3 degrees Celsius from June to August, while in Barnegat Inlet the temperature increased by over 5 degrees (Table 2). These results are similar to Dijkstra et al. (2007), where they determined that the temporal patterns of invasive tunicate colonization was not uniform. Colonial tunicates are constantly competing with each other for space and while one species may be dominant one month, the next month another species could be dominant. This was seen in Barnegat Inlet in 2018 where *B. violaceus* was the dominant species on *Z. marina* in June, and then by August the dominant species was *B. schlosseri* (Figs. 5 and 6). There could have been new recruits in Ham Island in August due to floating eelgrass and macroalgae wrack. Tunicates continue to reproduce while floating along the surface on macroalgae and other submerged aquatic vegetation, and it is possible that new larvae from this wrack settled at Ham Island (sensu Worcester 1994). Eelgrass acts as a non-permanent home for tunicate colonization and may act to spread invasive tunicates when the shoots are released and become floating wrack (Worcester 1994). As the shoots drift, seagrass rafts can act as a possible dispersal mechanism for ascidians (Carman et al. 2016). Many species reproduce and recruit in the summer and fall. These species also experience high growth rates in the summer (Stachowicz et al. 1999). The high abundance of the two dominant species in early summer in Barnegat Bay may be deterring settlement of other species, but my survey demonstrates the first time that *A. aspersa* has been documented in New Jersey. In a future study, it would be beneficial to do a DNA sequence on samples to verify identity and relatedness of regional populations to infer invasion pathways for this species.

2018 Bay-wide Survey

The purpose of this study was to determine, for the first time, the presence, distribution, and identity of invasive ascidians living on *Zostera marina* seagrass beds in Barnegat Bay, New Jersey. In general, tunicate distribution in Barnegat Bay was found to be patchy within eelgrass beds. The two sites with the highest percent cover of invasive tunicates living on eelgrass were Tices Shoal and Sloop Sedge, which are quite close to Barnegat Inlet, where Barnegat Bay meets the Atlantic Ocean (Fig. 1). This elevated tunicate coverage could be due to the large amount of recreational boating that happens in this area. Tices Shoal, which had the second highest percent coverage, is known for being a popular spot for boaters to park and spend the day lounging and clamming. It is possible that these boats are transporting fouling organisms such as tunicates (Lambert 2001). It is also possible that these sites had the highest percent coverage because the abiotic conditions could be more stable than the sites farther away from the inlet. In a similar study, it was stated that tunicates were less likely to colonize an area that changes in salinity (Carman et al. 2010), and this fits with the lack of tunicates observed in the *R. maritima* beds surveyed where salinity is lower and fluctuates. The three most southern sites (Manahawkin Bay, Ham Island, and Connective Sedge) had fewer to no tunicates living on eelgrass, suggesting some within-bay differences in tunicate distributions exist.

Non-native ascidians were found living on eelgrass throughout Barnegat Bay. The non-native species that were present were *B. violaceus*, *B. schlosseri*, *D. vexillum*, and *A. aspersa*. These results differ slightly from a previous survey done in Barnegat Bay where the only species found were *B. violaceus* and *B. schlosseri* (Carman et al. 2016). This survey had two study sites in Barnegat Bay, the first site in Carman's study was slightly north of Tices Shoal, a site where I also documented *B. schlosseri* and *B. violaceus*. The second site surveyed by Carman et al. (2016) was

only 289 meters from the Barnegat Inlet site where *B. violaceus* was found. The difference may be due to the fact that the study done by Carman et al. (2016) was done in October of 2013. However, other factors may play a role in the colonization of habitats by invasive tunicates.

Barnegat Bay is home to several oyster and hard clam grow out facilities. One area that is known for shellfish aquaculture is in High Bar Harbor in Long Beach Township, New Jersey. In the August 2018 survey it was determined that there were two sites (Sloop Sedge and Barnegat Inlet) that had invasive ascidians living on eelgrass. Mantoloking, New Jersey is also known for shellfish grow out operations. Unfortunately, it is only about 1.60 km from a survey site that was found to have invasive tunicates growing on eelgrass. These grow out facilities may have a future economic issue if they have to start dealing with ascidians on their grow out mesh bags and screens. Another area known for shellfish aquaculture is Rose Cove in Little Egg Harbor, New Jersey. It appears that Rose Cove is in a slightly better position than High Bar Harbor. There were three sites surveyed in August that were close to Rose Cove (Connective Sedge, Ham Island, and Manahawkin Bay) and only one of those sites had invasive ascidians. Consequently, this region may be at lower risk, but since *B. violaceus*, *B. schlosseri*, and *D. vexillum* are known to be very aggressive colonizers (Carman et al. 2016). Consequently, it is important to continue monitoring the invasive tunicate population in order to protect these aquaculture grow out facilities as aquaculture is the third most valuable fishery for economic revenue (NOAA Fisheries New England/Mid-Atlantic). *Styela clava*, a major nuisance species to aquaculture, has been reported on shellfish cages in Atlantic City, New Jersey (Barnegat Oyster Collective, pers. comm.), which is south of Barnegat Bay. This study did not find any *S. clava* living on eelgrass beds in Barnegat Bay, but clearly it could be a species of concern in the future.

A future study that looks at tunicate colonization on eelgrass in New Jersey would benefit from sampling earlier than June. While similar studies based out of Woods Hole, Massachusetts, began sampling in July (Carman et al. 2016), it would be beneficial to start sampling a few months earlier in New Jersey because of the differences in water temperature. It has been documented that heat is a main source of stress for *Z. marina* in Barnegat Bay, New Jersey (Bologna and Sinnema 2011). New Jersey's water begins to warm up sooner than the water in Massachusetts, stressing out the eelgrass and causing the seagrass to release their shoots due to heat stress. Future studies should also look for the spread of *D. vexillum* in Barnegat Bay, as it was only found at one site, but is said to be an aggressive colonizer (Osman and Whitlatch 2007, Carman and Grunden 2010). Sampling in cooler months would be beneficial to determine the pattern of colonization for different tunicate species. *B. violaceus* is known to grow faster than *B. schlosseri* in warmer water, while *Didemnum spp.* is known to grow faster in cooler temperatures. (Stachowicz et al. 2002, McCarthy et al. 2007). It would also be beneficial to sample hard and anthropogenic substrates in warmer months. As the heat increases, *Z. marina* cover decreases which will lead to a decrease in occurrence of tunicate species on seagrass (Shields et al. 2019). However, it is possible that the invasive ascidians species are still thriving on hard substrates. While heat stress can lead to a decrease in *Z. marina* shoots, it has been shown to increase populations of both *B. violaceus* and *B. schlosseri* (Cockrell and Sorte 2013).

Changing the methods in 2018 (i.e., 1x10m transect counting colonies in the field) was not beneficial when it comes to documenting more species. However, it was quite beneficial in saving time. A future study may determine this to be a more effective way to determine presence and absence of invasive tunicates over a larger area.

Conclusion

There is a significant relationship between seagrass and tunicate biomass, which means that where there is more seagrass, there are more tunicates. More seagrass biomass means that there is more surface area for tunicates to recruit. This research is the first evaluation of invasive tunicates living among the seagrass beds in New Jersey. Future research in this area needs to be done to assess the extent of the impacts of invasive ascidians on *Z. marina* beds. Non-native ascidians can have negative impacts on important industries like shellfish aquaculture, but also foul numerous natural and anthropogenic surfaces leading to displacement of native communities. They are a good organism for studying invasive organisms in a lab setting because of their life history traits and resilience to abiotic conditions. However, as an invasive species they pose a significant and growing threat to native eelgrass communities, as well as potential negative consequences for a growing aquaculture industry. All invasive species pose risks, but invasive tunicates can have a disproportionately large impact in communities they have invaded.

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Appendix A: Site location, GPS coordinates and sampling dates for 2017

Site	GPS °N	GPS °W	Date
Barnegat Inlet	39.78749	-74.14827	Jun. 21, 2017
Barnegat Inlet	39.78749	-74.14827	Jul. 19, 2017
Barnegat Inlet	39.78749	-74.14827	Aug. 17, 2017
Ham Island	39.59045	-74.23027	Jun. 21, 2017
Ham Island	39.59045	-74.23027	Jul. 19, 2017
Ham Island	39.59045	-74.23027	Aug. 17, 2017

Appendix B: Site location and sampling dates for 2018

Site	GPS °N	GPS °W	Date
Barnegat Inlet	39.78749	-74.14827	Jun. 14, 2018
Barnegat Inlet	39.78749	-74.14827	Aug. 10, 2018
Ham Island	39.59045	-74.23027	Jun. 22, 2018
Ham Island	39.59045	-74.23027	Aug. 10, 2018

Appendix C: Site location and sample time data from August 2018 survey

Site	GPS °N	GPS °W	Date	Time
Southern Mouth Meteteconk	40.05031	-74.06119	Aug. 24, 2018	12:42PM
Tices Shoal	39.82571	-74.09460	Aug. 10, 2018	3:05PM
Barnegat Inlet	39.78749	-74.14827	Aug. 10, 2018	1:58PM
Sloop Sedge	39.72666	-74.15564	Aug. 10, 2018	1:05PM
Manahawkin Bay	39.65960	-74.20312	Aug. 10, 2018	12:35PM
Ham Island	39.59045	-74.23027	Aug. 10, 2018	10:10AM
Connective Sedge	39.56403	-74.29167	Aug. 10, 2018	9:30AM

Appendix D: Raw data from quadrats collected during the 2018 Bay-wide Survey

Site	Blades	Shoots	Canopy Height (cm)	Biomass (g) (ash-pan)	% cover <i>B. violaceus</i>	% cover <i>B. schlosseri</i>
Southern Mouth Meteteconk	53	14	27	0.32	0.00	0.00
Southern Mouth Meteteconk	38	10	29	0.13	0.00	0.00
Southern Mouth Meteteconk	19	5	23	0.07	0.00	0.00

Southern Mouth Meteteconk	11	3	19	0.07	0.00	0.00
Southern Mouth Meteteconk	66	16	20	0.29	0.00	0.00
Southern Mouth Meteteconk	36	8	14	0.11	0.00	0.00
Southern Mouth Meteteconk	19	4	22	0.17	0.00	0.00
Southern Mouth Meteteconk	51	13	22	0.21	0.00	0.00
Southern Mouth Meteteconk	33	11	23	0.22	0.00	0.00
Southern Mouth Meteteconk	42	12	19	0.28	0.00	0.00
Tices Shoal	649	168	12.5	10.40	0.00	1.39
Tices Shoal	392	128	18	8.14	1.28	1.53
Tices Shoal	204	85	18	3.62	0.00	1.47
Tices Shoal	264	71	21	8.94	0.00	1.52
Tices Shoal	170	50	16.5	3.03	0.00	4.71
Tices Shoal	69	23	22.5	1.87	0.00	2.90
Tices Shoal	86	26	25	3.48	0.00	8.14
Tices Shoal	145	53	17.2	2.88	0.00	2.76
Tices Shoal	115	37	16	3.65	0.00	0.00
Tices Shoal	69	16	19.5	1.96	4.35	2.90
Barnegat Inlet	155	39	47.6	4.69	0.00	0.00
Barnegat Inlet	140	34	52.2	3.75	1.43	0.00
Barnegat Inlet	128	30	43.7	4.42	0.78	0.00
Barnegat Inlet	98	27	41.5	3.79	1.02	0.00
Barnegat Inlet	101	25	33	2.30	0.00	0.00
Barnegat Inlet	58	17	38.8	3.73	0.00	0.00
Barnegat Inlet	68	28	43	1.50	0.00	0.00
Barnegat Inlet	31	14	11	0.82	0.00	0.00
Barnegat Inlet	87	25	24.8	1.50	0.00	0.00
Barnegat Inlet	106	29	37.2	2.85	0.00	0.00
Sloop Sedge	93	32	23	1.85	0.00	0.00
Sloop Sedge	126	34	32	2.45	5.56	0.00
Sloop Sedge	70	18	31	0.98	8.57	0.00
Sloop Sedge	177	50	30	7.77	7.34	0.00
Sloop Sedge	211	51	28	1.26	18.96	2.37
Sloop Sedge	292	83	22	3.63	10.96	0.00
Sloop Sedge	96	27	32	2.04	4.17	2.08

Sloop Sedge	188	54	15	1.80	0.00	0.00
Sloop Sedge	9	2	21	0.05	0.00	0.00
Sloop Sedge	354	76	27	0.55	8.47	0.00
Manahawkin Bay	339	102	32	13.73	0.00	0.00
Manahawkin Bay	170	47	39	12.63	0.00	0.00
Manahawkin Bay	333	82	32	16.42	0.00	0.00
Manahawkin Bay	243	61	29	14.61	0.00	0.00
Manahawkin Bay	141	32	50	13.75	0.00	0.00
Manahawkin Bay	97	21	27	7.30	0.00	0.00
Manahawkin Bay	229	51	25	13.98	0.00	0.00
Manahawkin Bay	173	41	27	9.21	0.00	0.00
Manahawkin Bay	232	57	26	13.11	0.00	0.00
Manahawkin Bay	136	42	21	9.89	0.00	0.00
Ham Island	44	14	19.6	0.47	0.00	0.00
Ham Island	64	19	14.5	0.48	0.00	3.13
Ham Island	185	54	23.2	2.39	0.00	1.62
Ham Island	75	21	15.2	1.17	0.00	0.00
Ham Island	86	27	17.5	1.39	0.00	0.00
Ham Island	125	37	21	2.29	0.00	0.00
Ham Island	41	7	11	0.56	0.00	0.00
Ham Island	80	18	24	2.03	0.00	0.00
Ham Island	75	17	18.2	1.15	0.00	0.00
Ham Island	92	18	27	0.74	0.00	0.00
Connective Sedge	40	10	42	1.12	0.00	0.00
Connective Sedge	40	10	45	0.95	0.00	0.00
Connective Sedge	56	19	41	0.78	0.00	0.00
Connective Sedge	35	8	55	1.17	0.00	0.00
Connective Sedge	0	0	0	0.00	0.00	0.00

Connective Sedge	29	8	40	1.14	0.00	0.00
Connective Sedge	14	6	26	0.34	0.00	0.00
Connective Sedge	3	1	39	0.09	0.00	0.00
Connective Sedge	83	23	26	1.27	0.00	0.00
Connective Sedge	7	3	22	0.64	0.00	0.00

Appendix E: Raw data from the 20m transect in field survey during the 2018 bay-wide survey

Site	Date	Transect #	<i>B. violaceous</i> colonies	<i>B. schlosseri</i> colonies
Southern Mouth Metedeconk	August 24 2018	1	0	1
Southern Mouth Metedeconk	August 24 2018	2	0	0
Southern Mouth Metedeconk	August 24 2018	3	0	0
Southern Mouth Metedeconk	August 24 2018	4	0	0
Southern Mouth Metedeconk	August 24 2018	5	0	0
Tices Shoal	August 10 2018	1	13	1
Tices Shoal	August 10 2018	2	0	0
Tices Shoal	August 10 2018	3	0	0
Tices Shoal	August 10 2018	4	23	0
Tices Shoal	August 10 2018	5	0	0
Barnegat Inlet	August 10 2018	1	11	0
Barnegat Inlet	August 10 2018	2	3	0
Barnegat Inlet	August 10 2018	3	2	0
Barnegat Inlet	August 10 2018	4	61	0
Barnegat Inlet	August 10 2018	5	16	0
Sloop Sedge	August 10 2018	1	56	0
Sloop Sedge	August 10 2018	2	6	0
Sloop Sedge	August 10 2018	3	5	0
Sloop Sedge	August 10 2018	4	18	0
Sloop Sedge	August 10 2018	5	1	0
Manahawkin Bay	August 10 2018	1	0	0
Manahawkin Bay	August 10 2018	2	0	0
Manahawkin Bay	August 10 2018	3	0	0
Manahawkin Bay	August 10 2018	4	0	0
Manahawkin Bay	August 10 2018	5	0	0

Ham Island	August 10 2018	1	1	0
Ham Island	August 10 2018	2	0	0
Ham Island	August 10 2018	3	0	0
Ham Island	August 10 2018	4	0	0
Ham Island	August 10 2018	5	0	0
Connective Sedge	August 10 2018	1	9	0
Connective Sedge	August 10 2018	2	0	0
Connective Sedge	August 10 2018	3	3	0
Connective Sedge	August 10 2018	4	0	0
Connective Sedge	August 10 2018	5	3	0