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Spatial Distribution and Recruitment of the Botrylloides violaceus and Botryllus schlosseri Tunicates in Barnegat Bay, New Jersey

Kaitlyn Maguire

Abstract

Botrylloides violaceus and *Botryllus schlosseri*, colonial ascidians, are known invasive species of the North American Atlantic coast. The tunicates take residence and grow on native eelgrass (*Zostera marina*) blades, potentially negatively affecting eelgrass growth rate by means of smothering which reduces the plants ability to photosynthesize. *Zostera marina* is an important habitat, food source, and an indicator of environmental health. Invasive tunicates can decrease the amount of light that eelgrass receives which in turn, decreases the rate of growth of eelgrass. In the summer months of 2021, the relationship and abundance between the invasive tunicates *B. schlosseri* and *B. violaceus* and *Z. marina* were assessed in multiple locations in Barnegat Bay, New Jersey. Two collection methods were used in June and August. In June, a 2.4 meter transect (0.5m wide) was used to assess the presence of tunicate colonies on eelgrass blades in Barnegat Inlet and Oyster Creek. In August, Ham Island, Rt. 72 Bridge and Barnegat Inlet were visited. A 90 meter transect was used and all grass present within a 25cm x 25cm quadrat at each 10 meter mark was excavated for analysis of the presence of invasive tunicates as well as plant demography. Four settling plates were also placed in Barnegat Inlet between June 30th – August 12th 2021 and August 12th – September 25th 2021 to observe the recruitment of each tunicate species on an artificial environment. A regression analysis indicated a positive relationship between the number of tunicate zooids and eelgrass blade area for both tunicate species in June, but only for the *B. violaceus* zooids at the Rt. 72 Bridge and Barnegat Inlet sites in August. There was a negative relationship between the number of zooids and *Z. marina* blade area for *B. violaceus* tunicates in Ham Island and *B. schlosseri* at all three sites in August. In June, the most abundant tunicate species in Oyster Creek was *B. violaceus* and in Barnegat Inlet the dominant species was *B. schlosseri*. In August, *B. violaceus* tunicates were the most abundant at both Ham Island and Rt. 72 Bridge while the *B. schlosseri* tunicates were the most abundant at Barnegat Inlet. The tunicate abundance results from this study are consistent with results from a study conducted in the same area in 2017-2018. The abundance of *B. violaceus* and *B. schlosseri* tunicates were similar for the two study periods in Barnegat Inlet and Ham Island. A difference was found between the sites over the abundance of the *B. violaceus* tunicates in Ham Island in 2017-2018 and in 2021 with the tunicates covering more area in Ham Island in 2021 and in Barnegat Inlet in 2017-2018. These results suggest that compared to past studies, the invasive tunicates are still present on the *Z. marina* blades and could have a potential negative impact on the plant. This research is the first evaluation of the number of zooids present on eelgrass blades in Barnegat Bay, New Jersey.

MONTCLAIR STATE UNIVERSITY

Spatial distribution and recruitment of the *Botrylloides violaceus* and *Botryllus schlosseri* tunicates in Barnegat Bay, New Jersey

by

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Introduction

An ecologically important plant that dominates the eastern coasts of Northern America from Nova Scotia to North Carolina is the common eelgrass, *Zostera marina*. *Zostera marina* is known to grow in shallow marine and estuarine areas in patches known as beds or meadows all around the world (Green and Short 2003). This angiosperm is highly productive and provides food, shelter, substrate, and nursery-like habitats for many marine species (Thormar et al. 2016). Eelgrass alters sediment dynamics by reducing turbidity and minimizing hydrodynamic energy from waves, creating a habitat with finer sediments that could be resuspended into the environment (Bos et al. 2007; Widdows et al. 2008). However, the roots and rhizomes of the plant are able to stabilize sediments by binding particulate matter which increases sediment accumulation (Bos et al. 2007; Koch et al. 2007). The shoots of *Z. marina* are an important factor in maintaining water quality. The shoots provide a layer over different types of marine organisms and prevents the sediment from resuspending which decreases turbidity (Short and Short 1984). The plant can also aid in sedimentation and the uptake of a variety of different pollutants, as well as nutrients that could cause algal blooms. The nutrients are released from the plant through consumption and decomposition (Hemminga and Duarte 2000). Healthy eelgrass beds positively affect humans by providing nurseries for fish species that support offshore fisheries and other habitats such as shellfish beds, mangrove forests, and eelgrass is often consumed by coastal populations (Short et al. 2007).

Scientists have documented a decline in the global abundance of eelgrass beds due to natural and anthropogenic stressors. The most common cause of eelgrass declines is attributed to poor water quality (Orth et al. 2010). Poor water quality can be caused by runoff containing metals, fertilizers, pesticides, and other human-derived pollutants. Since eelgrass is an excellent indicator of water quality, any reductions in its distribution and vitality could indicate poor water quality (Dennison et al. 1993). Specifically, in Chesapeake Bay, Virginia, *Zostera marina* beds have declined due to increased salinity, which caused a reduction in seed germination rates (Xu et al. 2016; Johnson et al. 2021). The decline of water quality is usually caused by nutrient loading from watersheds and from sewage systems (Nahirnick et al. 2020). This is a major problem since eelgrass meadows expand very slowly because of their clonal growth. Eelgrass goes through cell division in their apical rhizome meristem to create new branches and spread (Larkum et al. 2006). This method of growth is a slow process and for *Z. marina*, rhizomes that have a growth rate of 26 cm per year (Larkum et al. 2006), have a slower growth rate than smaller species. This makes it harder for these eelgrass meadows to grow back at a more rapid rate.

In recent years, the global spread of invasive ascidians has been shown to be a stressor to the growth and expansion of *Z. marina* beds throughout the Northwest Atlantic. These non-native ascidians will attach themselves to the eelgrass, block them from sunlight and even cause canopy collapse by weighing down the plant’s blades (Wong and Vercaemer 2012). In New England, *Didemnum vexillum* uses eelgrass as a substrate to grow on and is presumed to block the blades from sunlight, which can block the blades to release their seeds. (Carman and Grunden 2010). Non-native ascidian species are considered high profile due to their ability to consistently outcompete and displace native organisms (Costello et al. 2021). In a tide pool at Sandwich, Massachusetts, the invasive ascidian, *D. vexillum*, was shown to be responsible for smothering bivalves, sea scallops, mussels, oysters, and other marine invertebrates which negatively impacts shellfish aquaculture (Valentine et al. 2007). These invasive tunicates can also negatively affect aquaculture. In 2006, experiments suggest that there was a significant loss of about 50% in the shellfish harvest in Canada after the overgrowth of the *Styela clava* ascidian (Colautti et al. 2006). Removal methods for the fouling tunicates include manual removal, chemical treatments, and biological treatments. However, these methods can be extremely labor intensive, costly, and dangerous to the native shellfish species (Switzer et al. 2011).

Tunicates are also able to survive and reproduce across a wide range of temperatures and salinities. The *D. vexillum* were shown to be able to reattach to eelgrass at temperatures ranging from 6-10°C in Martha's Vineyard, Massachusetts (Carman et al. 2014). An experiment showed that the *Botryllus schlosseri* tunicate species can survive in environments with salinities from 10-44psu, while the *Botrylloides violaceus* tunicates can survive in salinities above 15psu (Dijkstra et al. 2008). Another experiment that tested the temperature tolerance of the ascidian species showed that *B. violaceus* can survive in temperatures ranging from 5-25°C, while *B. schlosseri* can survive in temperatures ranging from 10-25°C, and *Botrylloides leachi* can survive in temperatures ranging from 16-26°C (Epelbaum et al. 2009a).

In Barnegat Inlet, a major stressor to *Z. marina* has been associated with the invasive ascidians *Botrylloides violaceus* and *Botryllus schlosseri* (Hoffman 2020). These tunicates have been observed smothering eelgrass that blocks the plant from direct sunlight. Smothering prevents eelgrass from properly photosynthesizing, which can kill the plant. The tunicate species *B. violaceus* is a native ascidian to the Northwest Pacific Ocean of Japan (Zhan et al. 2015) and *B. schlosseri* are a native species to the Mediterranean Sea, northeastern Atlantic Ocean, and the North Sea (Carver et al. 2006). Both species are types of colonial tunicates made up of zooids, which are tiny individual organisms that asexually bud to form sheet-like colonies, as well as sexually reproducing individuals that produce motile swimming larvae (sensu Kowarsky et al. 2021). The parent colonies of the tunicates are primarily hermaphroditic and will produce tadpole larvae sexually or asexually in their blastozooids. The tadpole larvae, which are able to travel to new locations, will quickly turn into an oozooid, the first zooid of a brand new colony (Watterson 1945; Epelbaum et al. 2009b). The oozooids will then multiply asexually to form a colonial tunicate with multiple identical zooids (Epelbaum et al. 2009b). Specifically, *B. schlosseri* colonies are made up of small brown or purple zooids about 1-2mm in diameter and form a star-like or stellate pattern, giving them the name 'star tunicate' (Carver et al. 2006), while *B. violaceus* are made up of larger, orange-colored zooids that are about 2-4mm in size and form long, irregular rows that surround a common aperture (Carver et al. 2006). The zooids of the colonial tunicates form together in these intersiphonal bands that change over time to create unique patterns in the sheet-like formations (Watterson 1945). Within these bands, zooids continue to reproduce asexually, increasing the size of the colony. Although, after the new generation of zooids are produced, the parent zooids will degenerate or destroy themselves and the new zooids are responsible for reproducing and adding on to the colony (Watterson 1945). This method of reproduction allows the colonial tunicates to multiply rapidly and easily spread to other locations.

Since 1945, these invasive tunicate species have shown to be prevalent in eelgrass beds in the Northwest Atlantic waters (Carman et al. 2019). Similar to other widely dispersed invasive species, tunicates are likely transported around the world by attaching themselves to boat hulls (Ramsay et al. 2008). Anthropogenic structures along shorelines are a known contributor to the invasion process by giving the tunicate species a strong substrate use as attachment sites (e.g., floating docks, ships, and seawalls; Simkanin et al. 2012). When the tunicates attach to these artificial substrates, they transform into easily detachable lobes which can spread via bottom currents, allowing them to spread onto the sea floor and other surfaces (Tyrrell and Byers 2007).

Therefore, when tunicates are introduced to a new environment, they often initially use anthropogenic structures such as floating boat docks to establish their populations, and then later spread into eelgrass beds (Lins et al. 2018; Wagstaff 2017). Commonly, *B. schlosseri* can be found as deep as 200m, while *B. violaceus* are found in more shallow areas that are less than 50m deep (Carver et al. 2006). The spread and success of invasive tunicates throughout the Northwest Atlantic waters could be due to traits of the eelgrass growth and reproduction. Eelgrass provides a sturdy structure within sandy or muddy areas that would be unsuitable for tunicates to survive, therefore the blade structure increases potential recruitment area. Eelgrass might also serve as a dispersal mechanism for the tunicates, since eelgrass sheds dead leaves which drift away and could transport intact tunicate colonies to new areas (Carman et al. 2016).

Throughout the Northwest Atlantic, research has been conducted looking at the distribution and species contributions of invasive tunicate species present in eelgrass beds (Carman et al. 2016; Carman et al. 2019), including a 2017-2018 study in Barnegat Bay, NJ by Hoffman (2020). The research presented in this thesis assesses potential changes from previous surveys and better defines colony size and biomass of *Botrylloides violaceus* and *Botryllus schlosseri* among eelgrass beds to determine potential negative impacts of the presence of these invasive tunicate species on eelgrass beds in New Jersey.

Methods

Study Site

Barnegat Bay is a shallow back-barrier lagoon type estuary that is located on the Atlantic coast of central New Jersey. This bay is fed by two large rivers in the northern part of the bay, Toms River and Metedeconk River, as well as numerous smaller tributaries that feed into the bay. Barnegat Bay is a eutrophic body of water that contains approximately 75% of the estuarine submerged aquatic vegetation habitats in the state of New Jersey (Lathrop et al. 2001). Due to nutrient loading, eutrophication, and shading from algal blooms, eelgrass beds are declining size in Barnegat Bay (Fertig et al. 2013). Specifically, the increased nutrients and sediments from the watersheds and the loss of adjacent natural habitats like salt marshes has negatively impacted the eelgrass population. Specific sites within Barnegat Bay were chosen based on known eelgrass beds that contained *B. violaceus* and *B. schlosseri* from past studies (Carman et al. 2019; Hoffman 2020). Four sites, including Barnegat Inlet, Oyster Creek, Rt. 72 Bridge, and Ham Island, were visited between June and August 2021, with Barnegat Inlet being sampled on two occasions (Fig. 1).

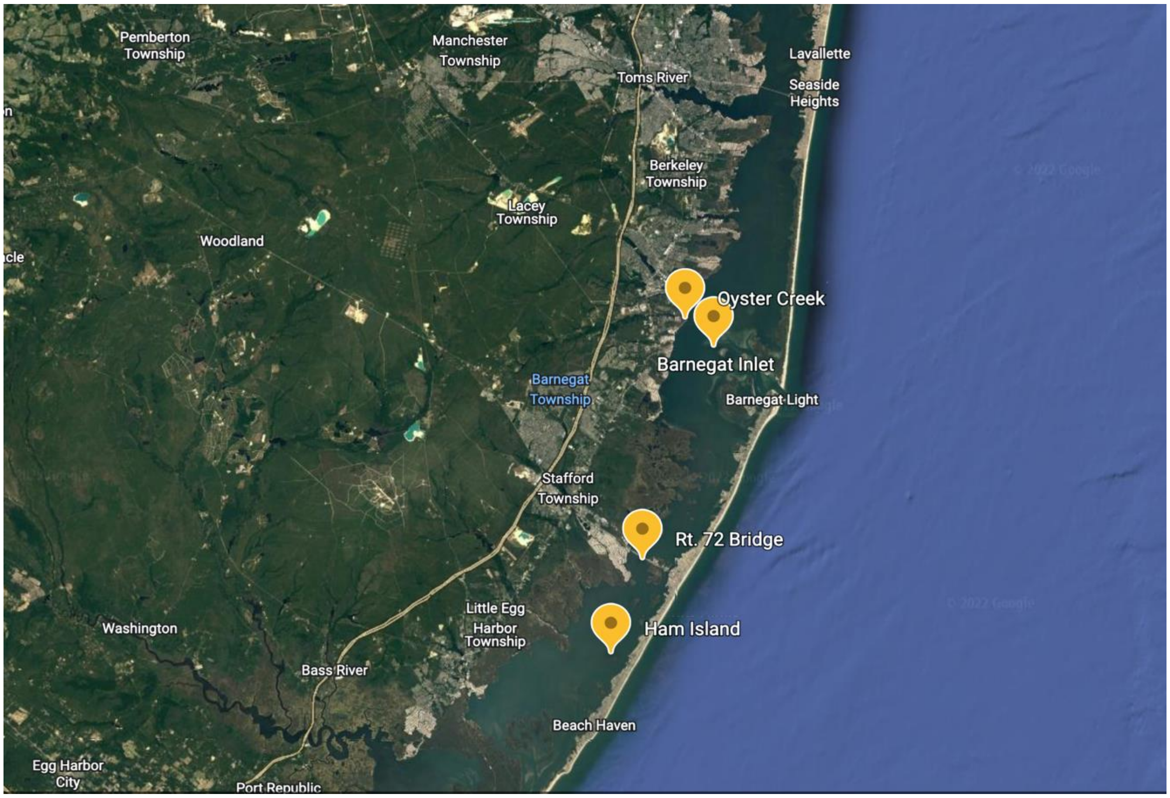

Figure 1. Specific sites in Barnegat Bay, New Jersey that were sampled in the summer of 2021 for invasive tunicate presence over the course of three months. Site identification, GPS coordinates and dates of collection can be found in Appendices A and B.

Site-specific survey methods

On June 30th, 2021, the Barnegat Inlet and Oyster Creek sites were sampled. Water quality data were collected including dissolved oxygen (mg/L and %), temperature (°C), and salinity (ppt) using a YSI® multimeter. The GPS coordinates were recorded at each site (Appendix A) where the boat was anchored. The seagrass beds at both of these sites consisted of *Zostera marina*, although *Ruppia maritima* is known to also occur in these regions. To measure the spatial distribution of both *B. violaceus* and *B. schlosseri* at Barnegat Inlet, a 2.4m long transect was placed on top of haphazardly selected eelgrass regions within the larger bed. Nine transects were completed by evaluating all of the tunicate colonies present within a 0.5m wide survey (0.25m on either side of the rope) along the length of the transect (area = 1.2m^2). For each transect, all of the eelgrass that had tunicate colonies present were collected in mesh bags and transferred to labeled Ziplock bags (n = 9). The bags were placed on ice until they were transported back to Montclair State University where they were frozen prior to laboratory evaluations. The same procedure was used at Oyster Creek, but only in six randomly selected areas over eelgrass beds were sampled due to deteriorating weather (n=6). Three plastic (5cm x 5cm) and one ceramic (4.9cm x 4.9cm x 1cm) settling plates were strung together with rope and then tied to a marked buoy at Barnegat Inlet on June 30 and then retrieved on August 12, 2021 to assess tunicate larval recruitment in this environment. On August 12, 2021, these settling plates were placed in a Ziplock bag full of water from the site and kept in an ice cooler while being transferred back to Montclair State University. The plates were placed in saltwater aquaria with air diffusers to ensure organisms remained alive until the plates could be examined for the presence of recruiting tunicates.

On August 12th, 2021, three sites were sampled including Ham Island, Rt. 72 Bridge, and Barnegat Inlet. A new method of collection was used for these sites which included using a 90m transect rope with marked labeling flags at every 10m and larger plastic PVC pipes (~3 m) that were placed across a seagrass bed at each site. The GPS coordinates (Appendix B) were recorded at regular intervals along the transect (0m, 30m, 60m, 90m) and the dissolved oxygen (mg/L and %), temperature (°C), and salinity (ppt) readings were taken using a YSI® multimeter. At 10m intervals along the transect line (0-90m), a 25cm x 25cm (0.125m^2) quadrat was placed on the benthos and all existing seagrass shoots were excavated from the sediment and collected in a mesh bag. The samples from each station (n=10) were then placed into plastic Ziplock bags and stored in an ice cooler until transported back to Montclair University where they were then placed in a freezer. This mode of collection was used at all three sites with the exception of Barnegat Inlet where extra samples were collected (n = 12) due to sampling error. A second set of settling plates were placed at Barnegat Inlet from August 12 - September 25, 2021 to assess the tunicate recruitment during the end of the summer. After collecting the settling plates, they were placed in a Ziplock bag full of water from the site and kept in an ice cooler to be transferred back to Montclair State University. The plates were placed in saltwater aquaria with air diffusers to ensure organisms remained alive until the plates could be examined for the presence of recruiting tunicates.

Laboratory Evaluation of Samples

For the June samples, each sample bag with the eelgrass and tunicate colonies was removed from the freezer and placed in a glass container in cool water to defrost. The water from the container was drained after each sample was defrosted and the contents were placed into the same glass container to be counted and measured. Before counting started, 57 mm disposable aluminum foil dishes for both *B. violaceus* or *B. schlosseri* colonies and a 12 cm x 6.3 cm x 5.2 cm aluminum loaf pan for the eelgrass were labeled with the site, transect number and weighed (g). Using a counter and ruler, all of the individual blades of eelgrass that had tunicate colonies present were counted and measure by their lengths and widths (cm) and then placed into the corresponding eelgrass tin. The tunicate colonies that were on the blades of eelgrass were peeled off using tweezers and placed into a plastic petri dish with tap water and observed under a dissecting microscope. Each zooid of each tunicate colony was counted and summed for each sample and then placed in the corresponding dish. The tins were then weighed for their wet weight (g) and then placed in an 80°C drying oven until all of the water had evaporated. After a few days, each tin was taken out of the drying oven and weighed to get the dry weight (g). Using aluminum foil, each tin was covered and placed in a 500°C muffle furnace for 12 hours to burn the eelgrass and tunicate samples. The tins were taken out of the muffle furnace and placed in the drying oven until the tins were cool enough to pick up and were then uncovered and weighed for the ash weight (g). The difference between the dry weight of the sample (Dry weight of sample – Pan weight) and the ashed weight of the sample (Ashed Weight – Pan weight) was used to calculate the Ash Free Dry Weight (AFDW) of each sample. The average surface area of *Z. marina* per m^2 (cm^2/m^2) was calculated by taking the total surface areas from each sample and dividing that by the plot area ($1.2m^2$) and then taking the average of all the surface areas per sample.

For the August samples, each bag was removed from the freezer, placed into a glass container and defrosted using cool tap water. When the samples were defrosted, the excess tap water was poured down the sink and the contents of the bag were placed into the same glass container to be counted and measured. Before counting started, 57 mm disposable aluminum foil dishes for both *B. violaceus* or *B. schlosseri* colonies and a 12 cm x 6.3 cm x 5.2 cm aluminum loaf pan for the eelgrass were labeled with the site, transect number and weighed (g). Using a counter and ruler, individual blades that did not have the *B. violaceus* or *B. schlosseri* tunicates were counted and measured by length and width (cm) and placed into the corresponding eelgrass tin. The eelgrass blades that had the tunicates present were separated to be observed under a dissecting microscope. All of the tunicates on the blades of eelgrass were removed, isolated and counted. The eelgrass blades were then individually counted and measured by their lengths and widths (cm) and then placed in the same tin as the eelgrass without tunicates. Looking under the microscope, each zooid of a tunicate colony was counted and summed for each sample and then placed in the corresponding tin. Samples were then weighed, ashed, and re-weighed as described above to generate the AFDW of tunicate species and eelgrass biomass. The average surface area of *Z. marina* per m^2 (cm^2/m^2) was calculated by taking the total surface areas from each sample and dividing that by the plot area ($0.125m^2$) and then taking the average of all the surface areas per sample.

To assess tunicate presence on each of the 4 settling plates, the settling plates were removed from the salt water aquaria and into a big glass bowl that was filled with the same salt water from the aquaria. Each settling plate was placed into their corresponding glass bowl facing upwards and lined up in order to be evaluated under a dissecting microscope. Looking under the microscope, the percent coverage of each tunicate type along with other organisms (bacterial mats, algae, cnidaria, eggs, barnacles, etc.) on each side of the plates were recorded on a settling plate data sheet. For each settling plate, pictures were taken with an iPhone while the samples were under the microscope.

Statistical Analysis

Using the SAS© statistical system, a one-way ANOVA was used to assess the differences in the number of zooids per *Z. marina* blade surface area (cm^2), the number of zooids per m^2, and AFDW of *Z. marina*, *B. violaceus* and *B. schlosseri* among sites. Using the Microsoft© Excel data analysis, a regression analysis was also used to compare the number of zooids per *Z. marina* blade surface area. Sites were used as the independent variable against the measured dependent variables, with two independent analyses conducted for samples collected on June 30th, 2021 from Oyster Creek and Barnegat Inlet and samples collected on August 12th, 2021 from Ham Island, Rt. 72 Bridge, and Barnegat Inlet.

Results

Oyster Creek and Barnegat Inlet June Results

On June 30th, 2021, both *Botrylloides violaceus* and *Botryllus schlosseri* tunicates were found on *Zostera marina* blades at both Barnegat Inlet and Oyster Creek (Table 1, see Appendices C and D). Between the two sites, there was no significant difference in the density of *B. violaceus* zooids ($F_{1,13}$ = 1.56; P = 0.23) or in the number of *B. schlosseri* zooids ($F_{1,13}$ = 0.66; P = 0.43). The average surface area per m^2 of the *Z. marina* blades with both tunicate species at each site/sample was calculated and found to be 244.65 cm^2/m^2 for *B. violaceus* and 234.77 cm^2/m^2 for *B. schlosseri* in Oyster Creek and 226.91cm^2/m^2 for *B. violaceus* and 572.65 cm^2/m^2 for *B. schlosseri* in Barnegat Inlet (Table 1, see Appendix E). The mean AFDW for *Z. marina* was significantly greater at Barnegat Inlet compared to Oyster Creek ($F_{1,13}$ = 5.56; P = 0.0333), but the AFDW for *B. violaceus* and *B. schlosseri* were not significant between the two sites ($F_{1,13}$ = 0.11; P = 0.786 BVWT; $F_{1,13}$ = 1.21; P = 0.2916 BSWT). At both sites, there was a positive relationship between the number of *B. violaceus* zooids and eelgrass blade area (cm^2), showing that as the area of the blade increased, the number of zooids increased ($F_{1,71}$ = 320.1; $P < 0.0001$ OCBV; $F_{1,98}$ = 80.2; $P < 0.0001$ BIBV, Fig. 2). Oyster Creek had the highest number of *B. violaceus* zooids counted on a single colony with 2019 zooids. For the *B. schlosseri* tunicates, there was also a significant difference between the number of zooids and *Z. marina* blade area ($F_{1,95}$ = 121.1; $P < 0.0001$ OCBS; $F_{1,233}$ = 245.4; $P < 0.0001$ BIBS, Fig. 3), showing that as the area of the eelgrass blades increased the number of zooids present increased. Just like the *B. violaceus* tunicates, Oyster Creek had the highest number of *B. schlosseri* zooids counted on a single colony with 3204 zooids. There were no significance differences between the average density of zooids for both tunicate species at each site ($F_{1,13}$ = 1.56; $P = 0.23$ BV, $F_{1,13}$ = 0.66; $P = 0.43$ BS). However, at Oyster Creek there was a higher average density of *B. violaceus* zooids than at Barnegat Inlet (Fig. 4), while the opposite was found with *B. schlosseri* with Barnegat Inlet having the higher average of 5478.4 zooids/m^2 and Oyster Creek having a lower average of 4111.1 zooids/m^2 (Fig. 5).

Table 1. Characterization of the average *Z. marina* blade biomass (g AFDW) and total area with tunicate species present for all samples (cm^2/m^2), average tunicate biomass for all samples (g AFDW) and water quality parameters for the sites visited on June 30th, 2021.

Site	**Oyster Creek**	**Barnegat Inlet**
Date	6/30/2021	6/30/2021
Temperature (°C)	27.5	25.5
Dissolved Oxygen (mg/L)	9.11	6.36
Salinity (ppt)	27.5	26.4
Average area of all *Z. marina* blades with *B. violaceus* (cm^2/m^2)	244.65	226.91
Average area of all *Z. marina* blades with *B. schlosseri* (cm^2/m^2)	234.77	572.65
Average *Z. marina* biomass (g)	3.36	6.68
Average *B. violaceus* biomass (g)	0.3691	0.2916
Average *B. schlosseri* biomass (g)	0.3098	0.5283

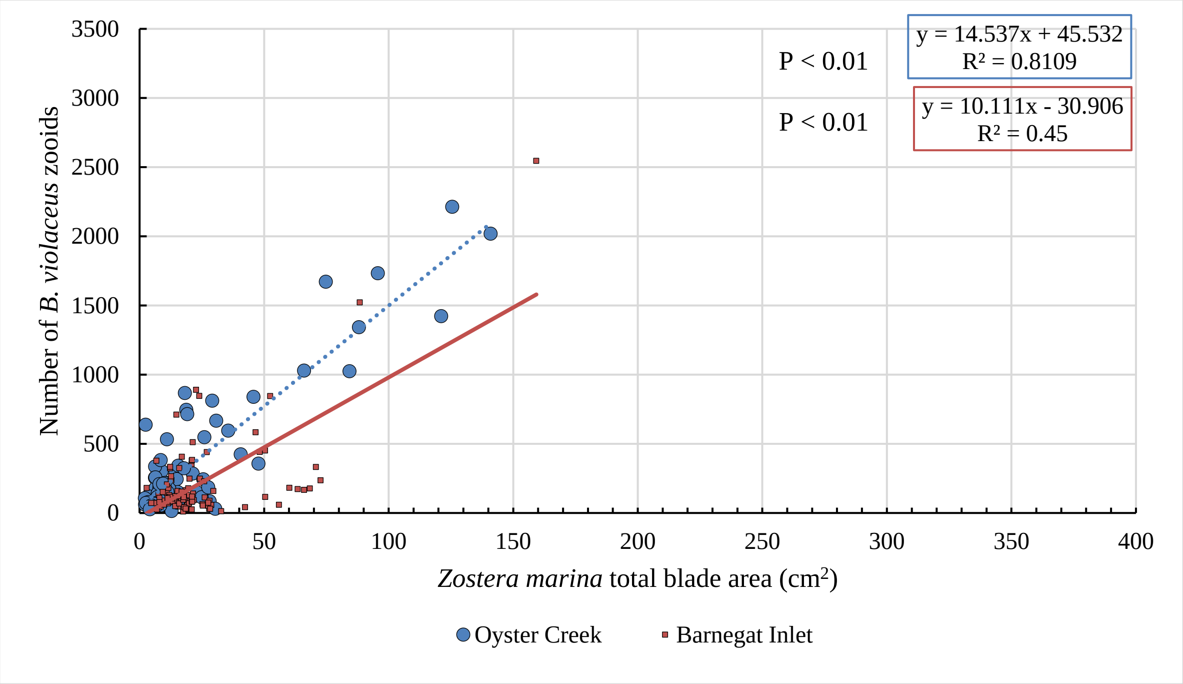

Figure 2. Regression analysis showing the positive relationship between the total blade area (cm^2) of *Z. marina* and the number of *B. violaceus* zooids at Oyster Creek and Barnegat Inlet on June 30th, 2021.

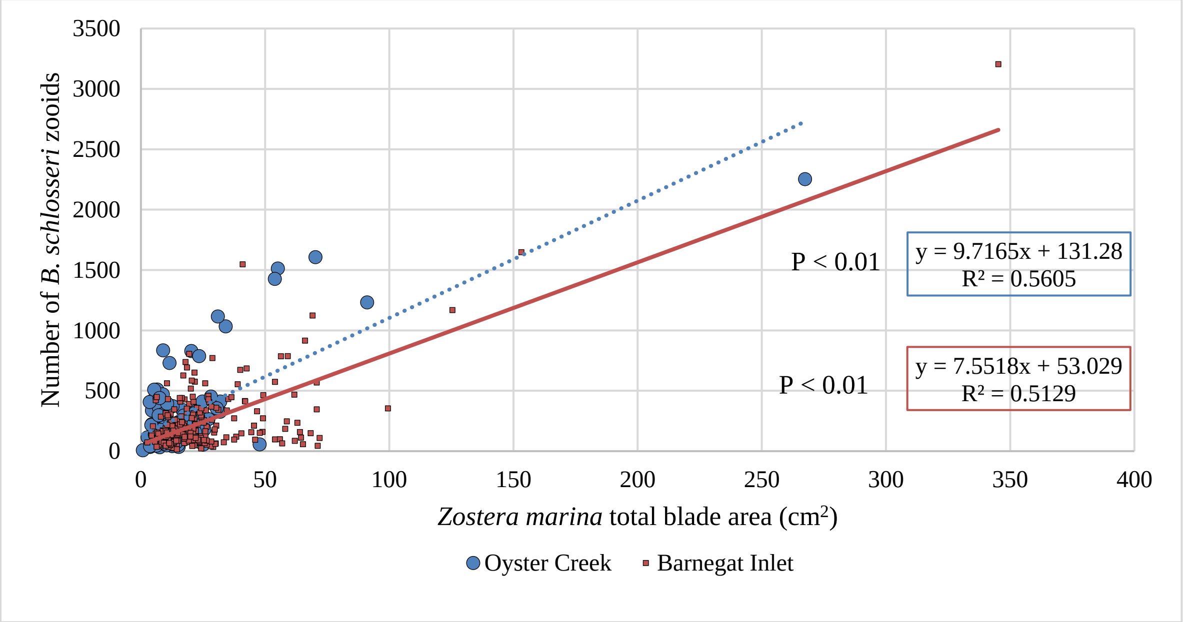

Figure 3. Regression analysis showing the positive relationship between the total blade area (cm^2) of *Z. marina* and the number of *B. schlosseri* zooids at Oyster Creek and Barnegat Inlet on June 30th, 2021.

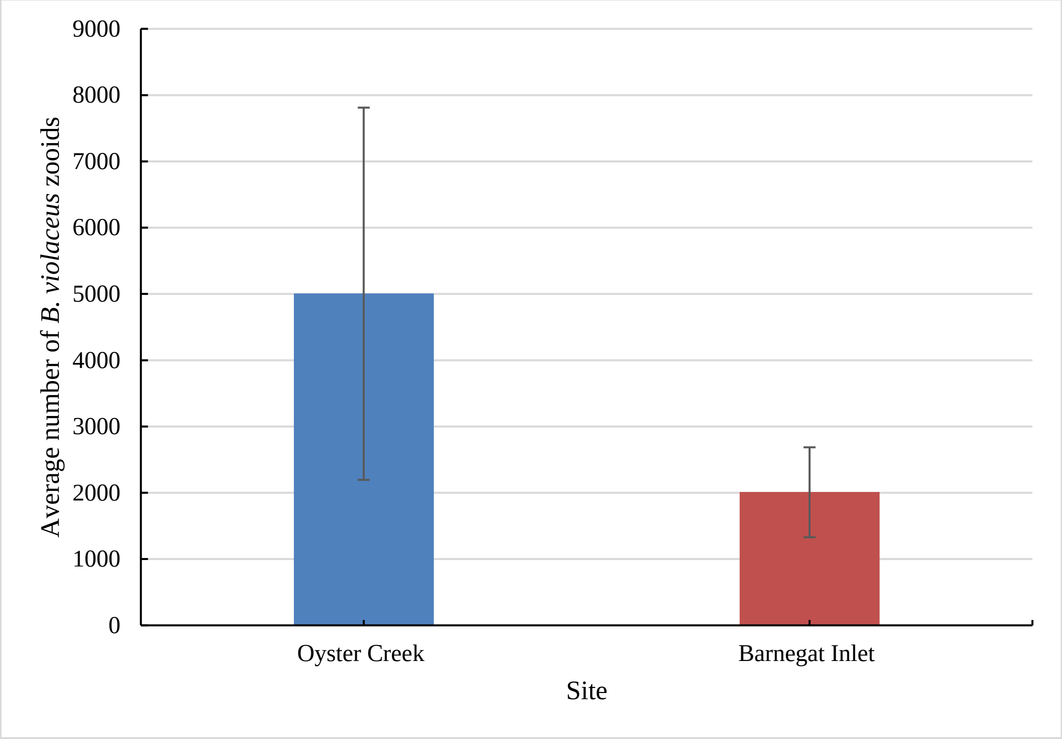

Figure 4. Average density of *B. violaceus* zooids (±SE) per m^2 at Oyster Creek and Barnegat Inlet in June 2021.

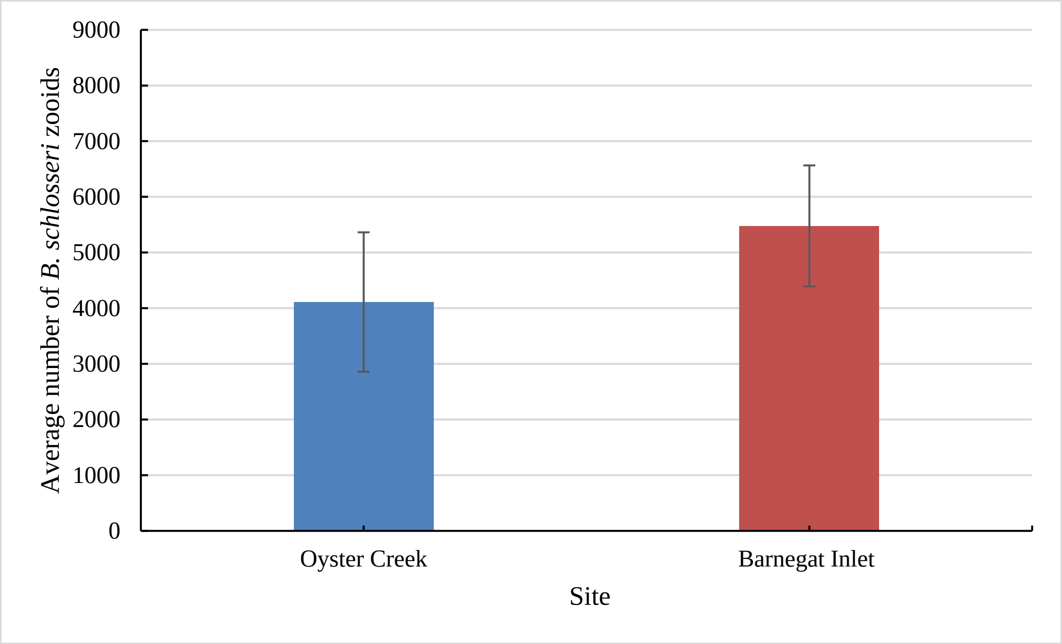

Figure 5. Average density of *B. schlosseri* zooids (± SE) per m^2 at Oyster Creek and Barnegat Inlet in June 2021.

Ham Island, Rt. 72 Bridge and Barnegat Inlet August Results

All three sites on August 12th, 2021 showed the presence of both *B. violaceus* and *B. schlosseri* tunicate species (see Appendices C and D). Among the three sites, there was no significant difference in the number of *B. violaceus* zooids ($F_{2,27} = 0.05$; P = 0.95) or in the number of *B. schlosseri* zooids ($F_{2,27} = 0.73$; P = 0.49). The total surface area of the *Z. marina* blades with both tunicate species at each site/sample was calculated. Ham Island had a blade area of 184.52 cm^2 with *B. violaceus* and 62.96 cm^2 with *B. schlosseri*, Rt. 72 had a blade area of 149.9 cm^2 with *B. violaceus* and 57.24 cm^2 with *B. schlosseri* and Barnegat Inlet had a blade area of 282.28 cm^2 with *B. violaceus* and 352.62 cm^2 with *B. schlosseri* (Table 2, see Appendix F). There was a significant positive linear regression between *B. violaceus* zooids and *Z. marina* blade area in the Rt. 72 Bridge and Barnegat Inlet samples ($F_{1,10} = 16.9$; P < 0.01 RTBV; $F_{1,13} = 14.9$; P = 0.00l BIBV, Fig. 6). However, in the Ham Island samples, there was a negative non-significant relationship between *B. violaceus* zooids and *Z. marina* blade area ($F_{1,14} = 0.001$; P = 0.99, Fig. 6). For all sites and samples, there was a negative linear regression between *B. schlosseri* zooids and *Z. marina* blades; as the area of the eelgrass blade increased, the number of zooids decreased ($F_{1,3} = 20.5$; P = 0.02 HIBS; $F_{1,4} = 1.3$; P = 0.31 RTBS; $F_{1,15} = 0.06$; P = 0.82, Fig. 7). There was not a significant difference between the average density of *B. violaceus* zooids per m^2 among the three sites ($F_{2,27} = 0.05$; P = 0.95), but Ham Island had the highest average density of *B.* violaceus zooids with 2587.2 zooids/m^2, Rt. 72 Bridge had the next highest average of 2207.2 zooids/m^2, and Barnegat Inlet had the lowest average of 1887.2 zooids/m^2 (Fig. 8). There was also not a significant difference in the average density of *B. schlosseri* zooids among the three sites ($F_{2,27} = 0.73$; P = 0.49), but the pattern was reversed. Barnegat Inlet had the highest average density of *B. schlosseri* zooids, 3004 zooids/m^2, then Rt. 72 Bridge with a narrowly higher average than Ham Island with 1249.6 zooids/m^2 and Ham Island with the lowest average of 1211.2 zooids/m^2 (Fig. 9). The average AFDW for *Z. marina* among the three sites was not statistically different ($F_{2,27} = 2.00$; $P = 0.15$), as well as the AFDW for *B. violaceus* and *B. schlosseri* ($F_{2,27} = 0.08$; $P = 0.9$; $F_{2,27} = 0.35$; $P = 0.7$, respectively).

Table 2. Characterization of the average total *Z. marina* blade area (cm^2/m^2) with tunicate species present for all samples, *Z. marina* blade biomass (g AFDW) and average tunicate biomass (g AFDW) for all samples and water quality parameters for the sites visited on August 12th, 2021.

Site	**Ham Island**	**Rt. 72 Bridge**	**Barnegat Inlet**
Date	8/12/2021	8/12/21	8/12/21
Temperature (°C)	27.7	28.2	27.1
Dissolved Oxygen (mg/L)	6.86	7.10	10.44
Salinity (ppt)	28.0	25.0	26.9
Average area of all *Z. marina* blades with *B. violaceus* (cm^2/m^2)	147.62	119.92	225.82
Average area of all *Z. marina* blades with *B. schlosser*i (cm^2/m^2)	50.37	45.79	282.10
Average *Z. marina* biomass (g)	8.22	5.66	9.01
Average *B. violaceus* biomass (g)	0.1229	0.1290	0.0809
Average *B. schlosseri* biomass (g)	0.0329	0.1078	0.0853

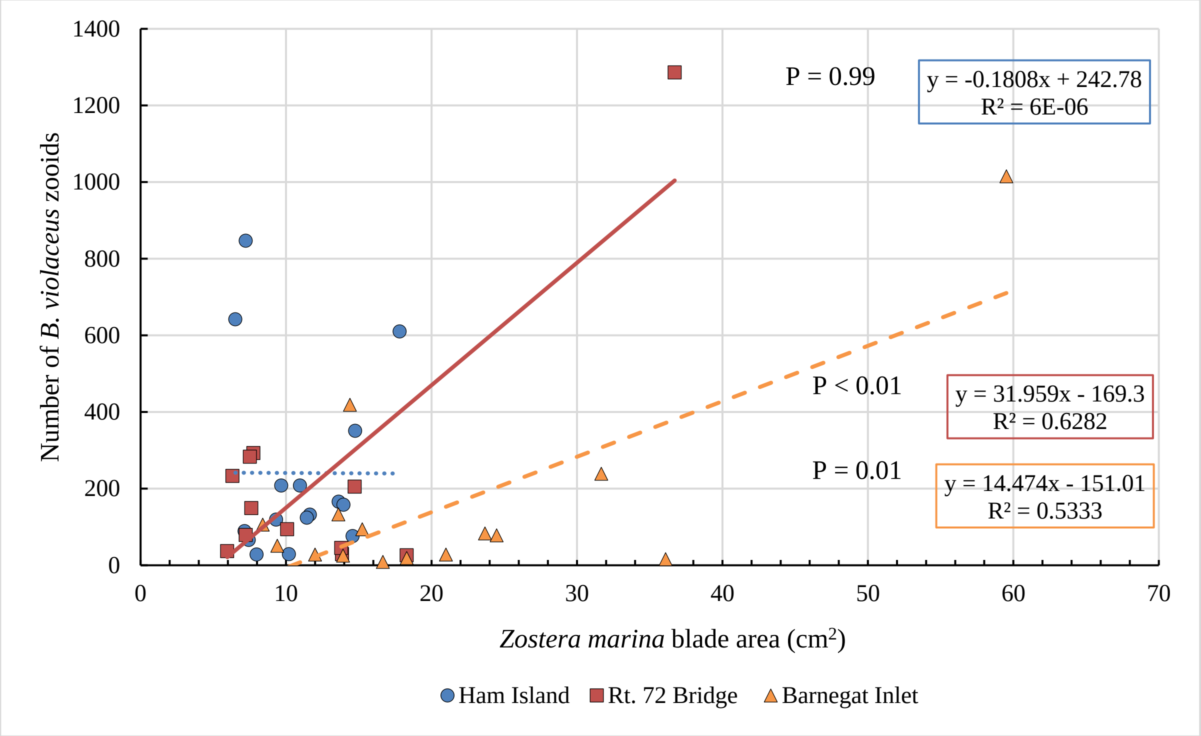

Figure 6. Regression analysis showing the relationships between the total blade area (cm^2) of *Z. marina* and the number of *B. violaceus* zooids at Ham Island, Rt. 72 Bridge and Barnegat Inlet on August 12th, 2021.

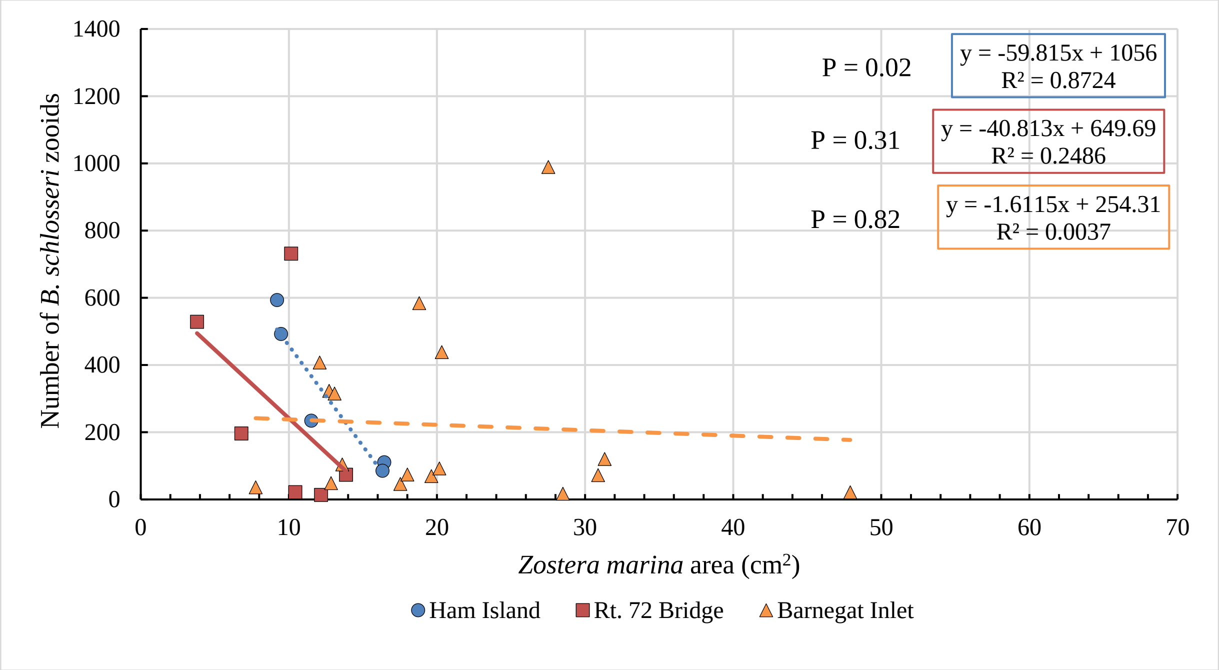

Figure 7. Regression analysis showing the negative relationships between the total blade area (cm^2) of *Z. marina* and the number of *B. schlosseri* zooids at Ham Island, Rt. 72 Bridge and Barnegat Inlet on August 12th, 2021.

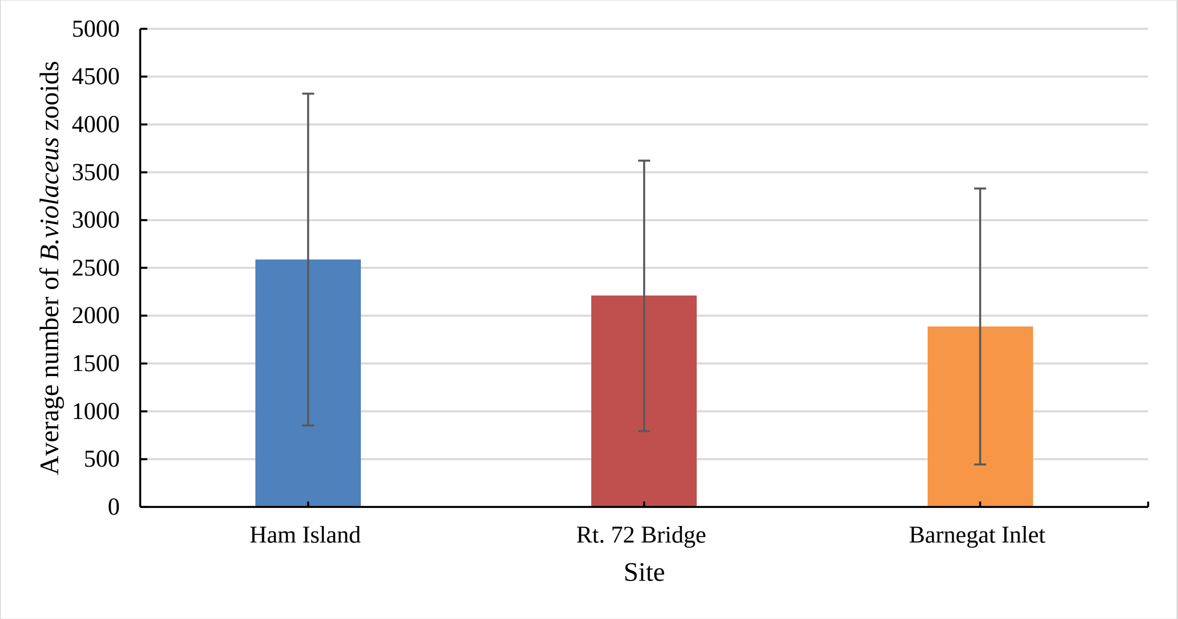

Figure 8. Average density of *B. violaceus* zooids (± SE) per m^2 at Ham Island, Rt.72 Bridge and Barnegat Inlet in August 2021.

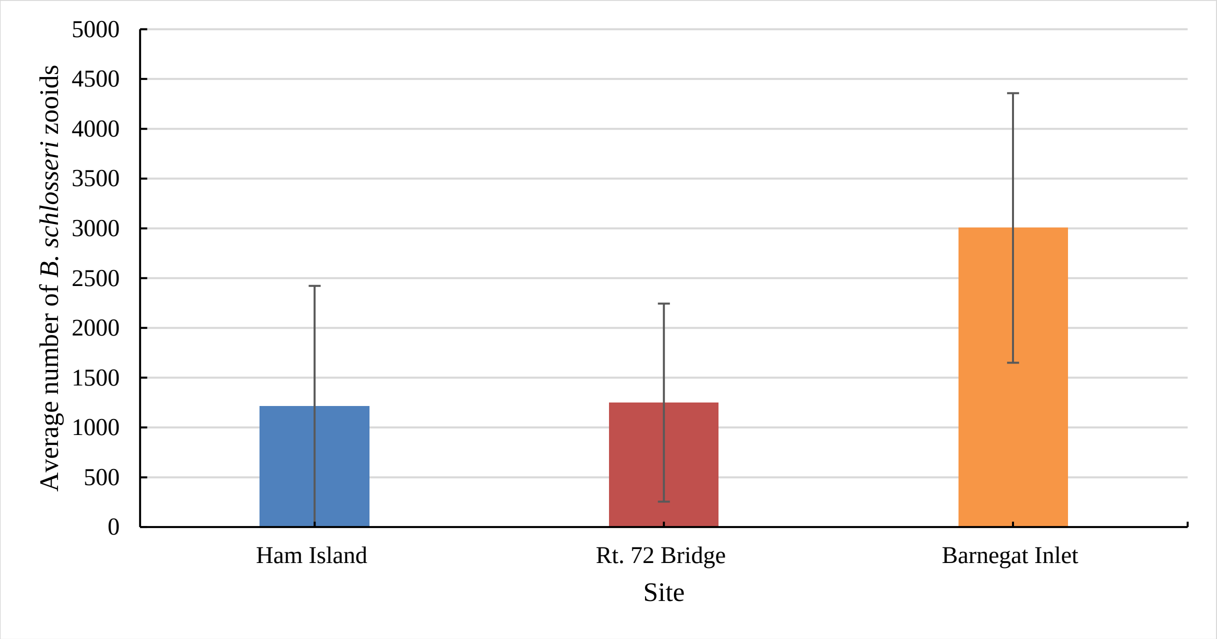

Figure 9. Average density of *B. schlosseri* zooids (±SE) per m^2 at Ham Island, Rt.72 Bridge and Barnegat Inlet in August 2021.

Barnegat Inlet June-August

Since there were two collection methods between June and August at Barnegat Inlet, the data were standardized per m^2 to allow for comparison. Between June and August, there was a noticeable decline in the total surface area of *Z. marina* blades with the *B. schlosseri* colonies present at Barnegat Inlet, but not a big difference for *B. violaceus*. A positive linear regression is still observed between *Z. marina* blade area and *B. violaceus* zooids between June and August (Fig. 10). For the *B. schlosseri* tunicates, a positive linear regression between the number of zooids and blade surface area was observed at Barnegat Inlet in June, but a negative linear regression was observed in August (Fig. 11). There was a higher density of *B. violaceus* and *B. schlosseri* zooids/m^2 at Barnegat Inlet in June than there were in August (Fig. 12 and 13). In June, the average density was 2008.1 zooids/m^2 for *B. violaceus* and 5478.4 zooids/m^2 for *B. schlosseri*. In August, the average density of *B. violaceus* zooids was 1887.2 zooids/m^2 and for *B. schlosseri* tunicates the average was 3004 zooids/m^2.

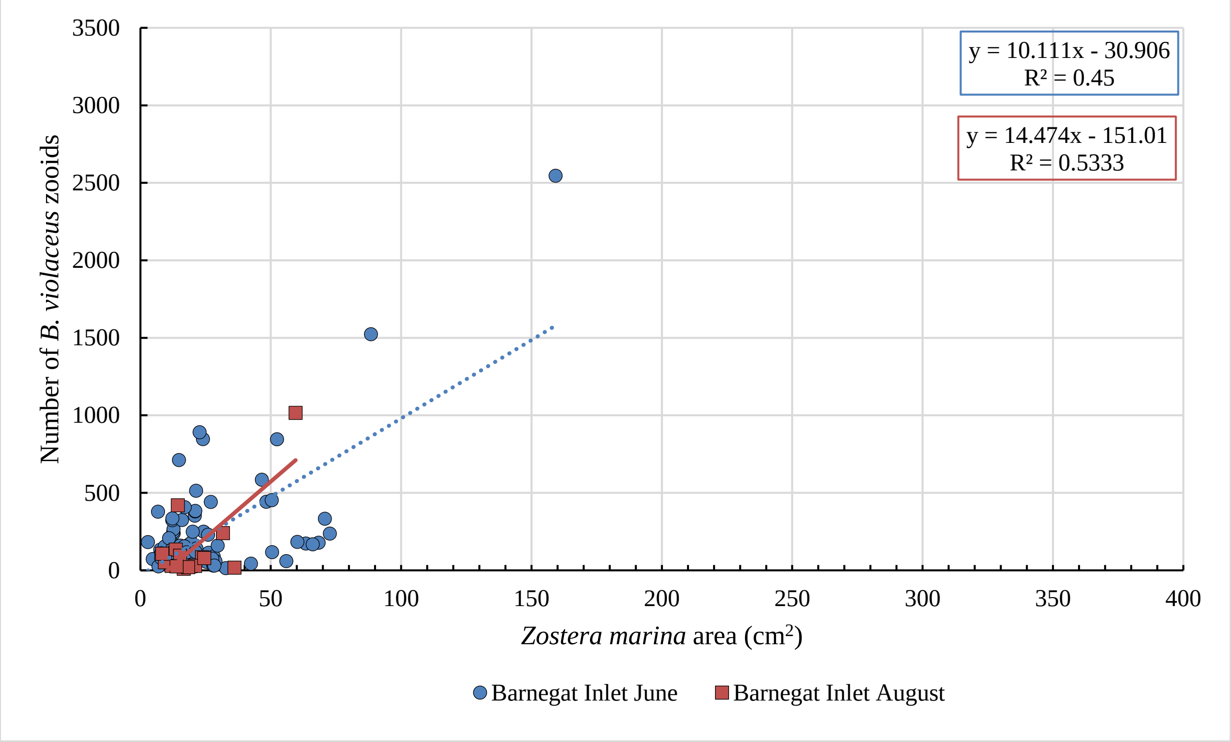

Figure 10. Regression analysis showing the relationships between the total blade area (cm^2) of *Z. marina* and the number of *B. violaceus* zooids at Barnegat Inlet between June and August 2021.

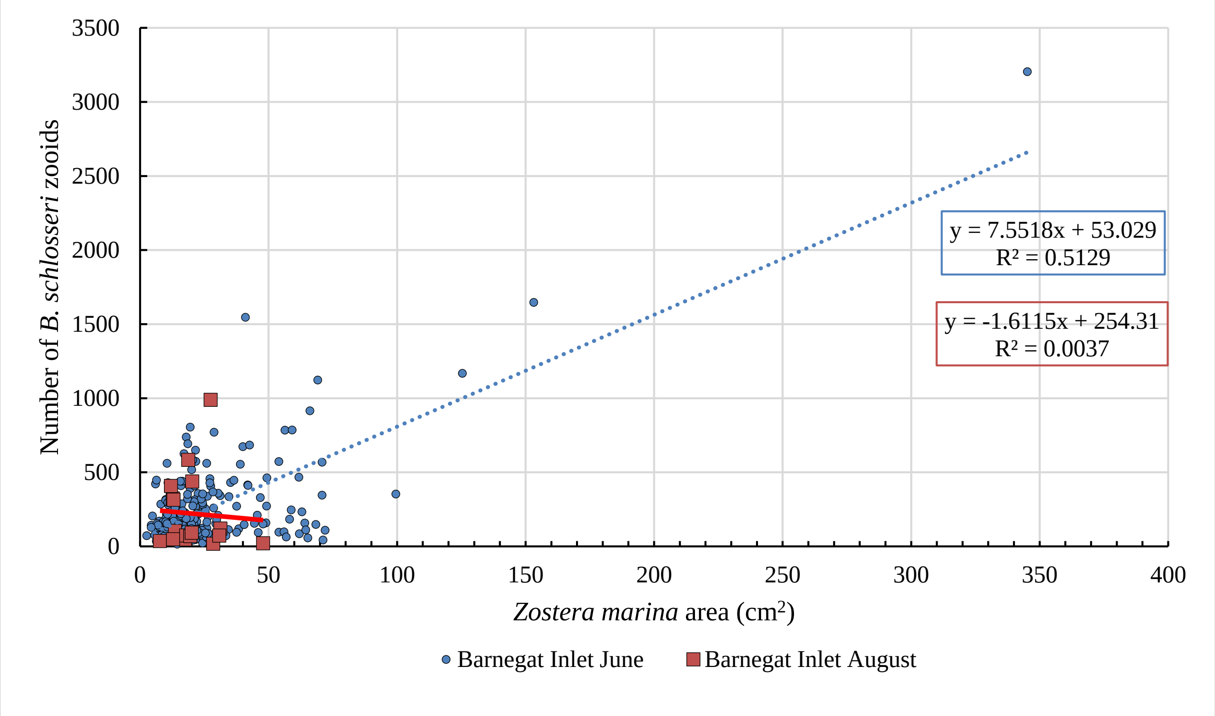

Figure 11. Regression analysis showing the relationships between the total blade area (cm^2) of *Z. marina* and the number of *B. schlosseri* zooids at Barnegat Inlet between June and August 2021.

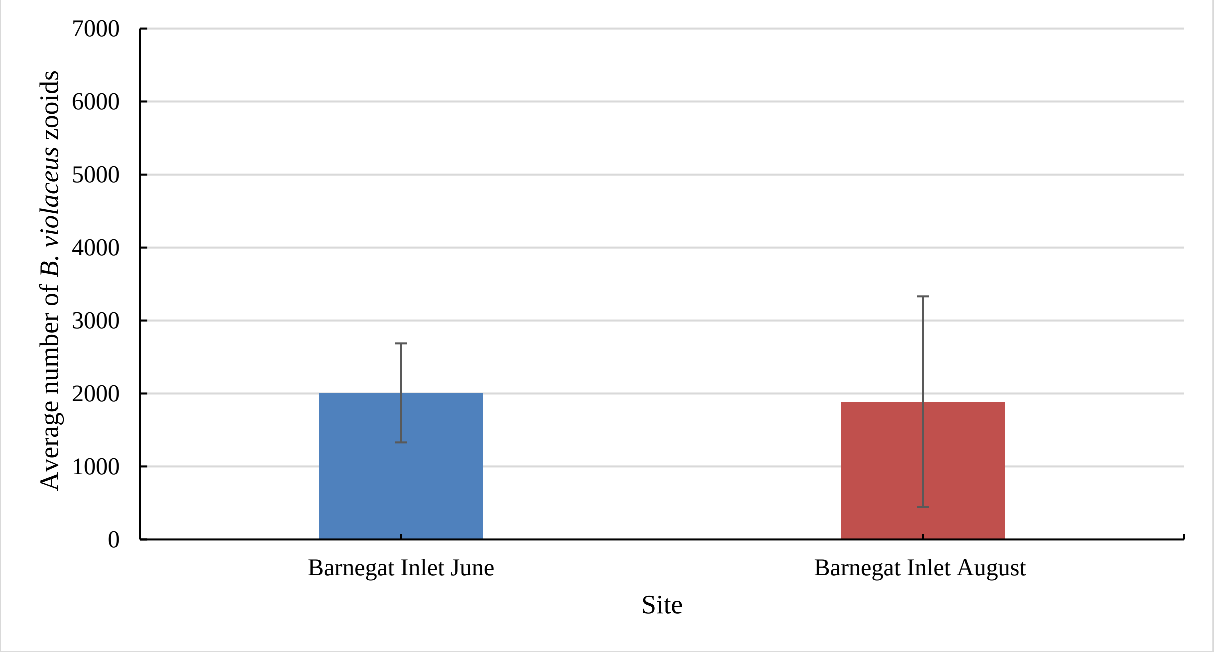

Figure 12. Density of *B. violaceus* zooids (±SE) per m^2 at Barnegat Inlet between June and August 2021.

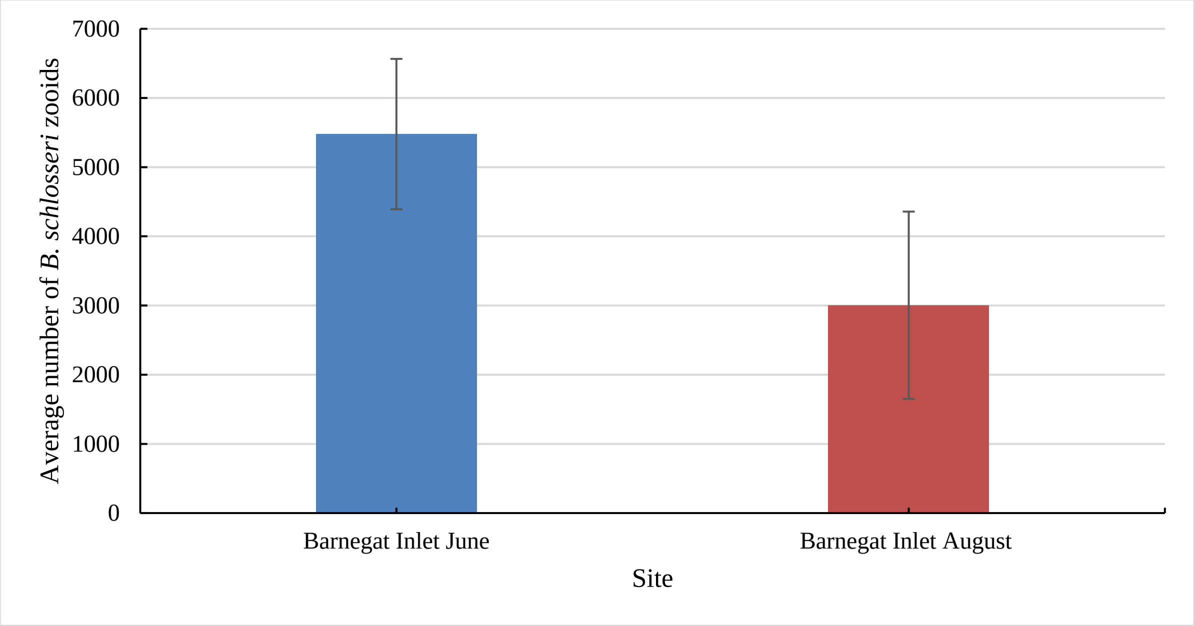

Figure 13. Density of *B. schlosseri* zooids (± SE) per m^2 at Barnegat Inlet between June and August 2021.

Settling plates June-September 2021

Between June 30th – August 12th 2021, both tunicate species had colonized the settling plates indicating sexual reproduction and larval release. On the first settling plate, there were no *B. violaceus* tunicates present, but the *B. schlosseri* tunicates had covered ~5% of the bottom of the plate (Fig. 14a). On the second settling plate, *B. schlosseri* covered ~1% of the bottom of plate 2 and *B. violaceus* covered ~10% (Fig. 14b). Both tunicate species were present on the third plate with *B. violaceus* covering <1% of the top of plate 3 and 40% of the bottom of plate 3, while *B. schlosseri* only covered 20% of the bottom (Fig. 14c). On the fourth ceramic settling plate, only *B. schlosseri* (red arrow) were present, but only covered <1% of the bottom of plate 4 (Fig. 14d). There were a variety of other organisms and plants that covered each settling plate. These species include, *Enteromorpha* algae, red filamentous algae, *Obelia spp.*, *Bugula turrita*, spirorbids, barnacles, and bacterial mats that covered anywhere between 1-100% of the plates (see Appendix G).

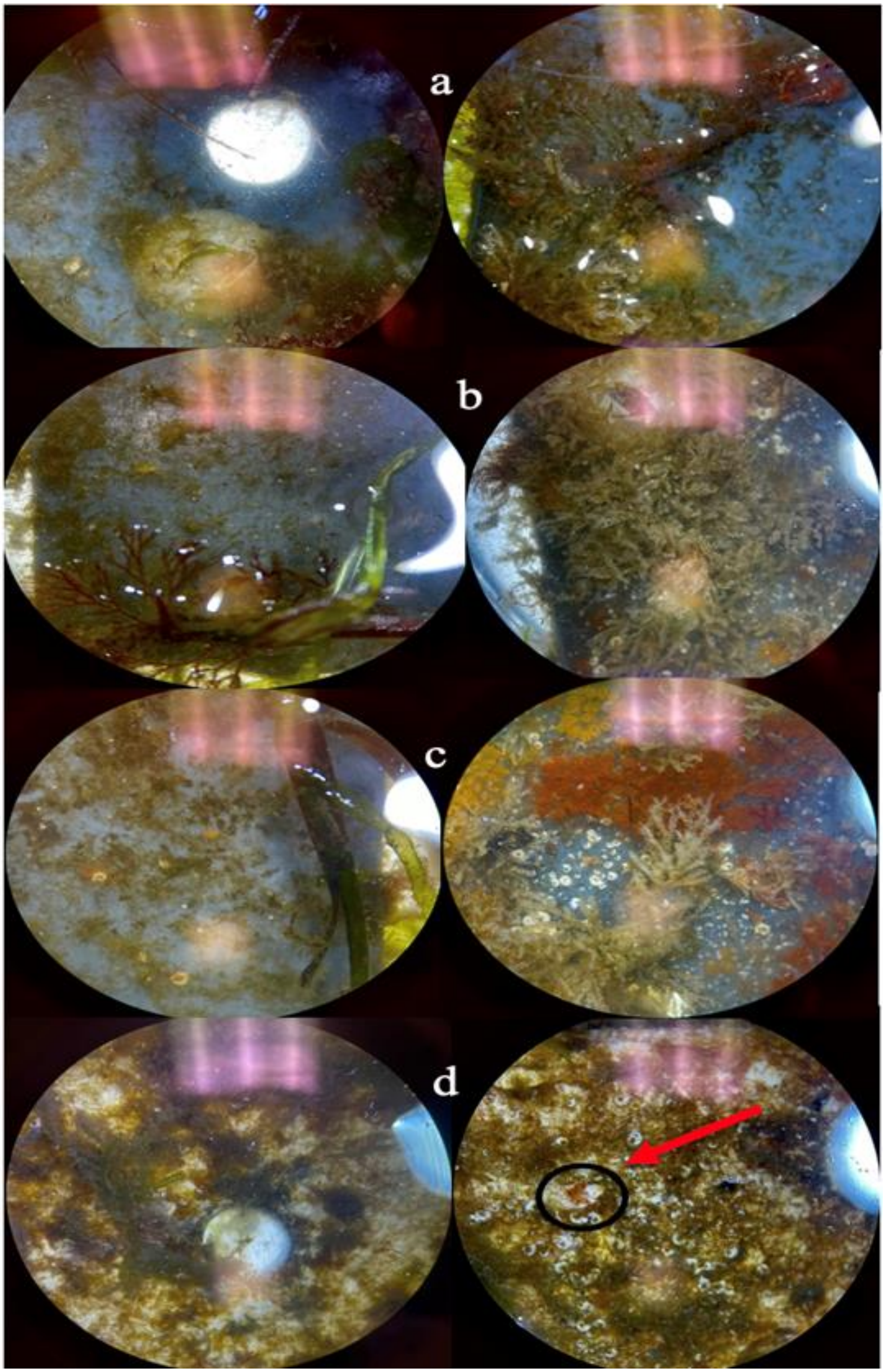

Figure 14. Photographs of *B. violaceus* and *B. schlosseri* tunicate settlement on four settling plates; plate 1 top/bottom (a), plate 2 top/bottom (b), plate 3 top/bottom (c), and plate 4 top/bottom (d) at Barnegat Inlet from June 30th – August 12th, 2021.

The second deployment from August 12th – September 25th 2021, there was a higher percent coverage of both species on the settling plates. There were no tunicates present on either side of plate 1 (Fig.15a). Only the *B. schlosseri* tunicates were present on plate 2 and covered ~75% of the bottom (red arrow) (Fig. 15b). Both tunicate species were present on plate 3 with *B. violaceus* (yellow arrow) covering ~15% of the top and ~85% of side 2 and *B. schlosseri* (red arrow) covered <1% of the bottom (Fig. 15c). On the fourth ceramic settling plate, both species were present, but only covered the bottom. *B. violaceus* (yellow arrow) covered ~50% and *B. schlosseri* (red arrow) covered ~35% of the bottom (Fig. 15d). There were a variety of other organisms and algae that covered each settling plate including *Enteromorpha spp.*, red filamentous algae, *Obelia spp.*, *Bugula turrita*, spirorbids, barnacles, egg cases/eggs and bacterial mats that covered anywhere between 1-100% of the plates (see Appendix H).

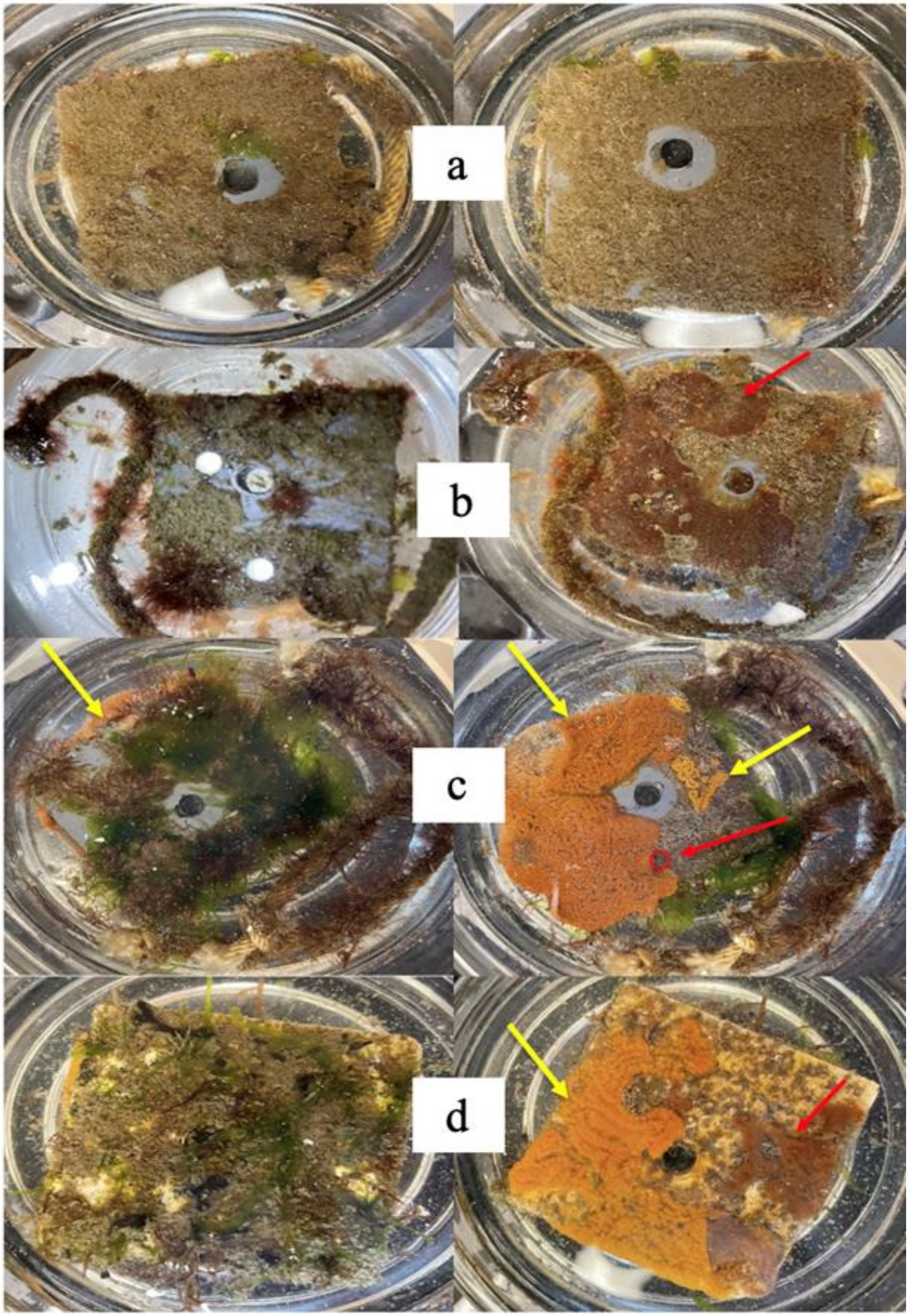

Figure 15. Photographs of *B. violaceus* and *B. schlosseri* tunicate settlement on four settling plates; plate 1 top/bottom (a), plate 2 top/bottom (b), plate 3 sides top/bottom (c), and plate 4 top/bottom (d) at Barnegat Inlet from August 12th – September 25th, 2021.

Discussion

This study focused on assessing any potential changes from previous surveys and to better define colony size and biomass of *Botrylloides violaceus* and *Botryllus schlosseri* among eelgrass beds and to determine their potential negative impacts on the eelgrass beds in Barnegat Bay, New Jersey. Though Barnegat Inlet had a higher total *Z. marina* blade surface area (cm^2) than Oyster Creek, there was a higher density of *B. violaceus* zooids on eelgrass blades at Oyster Creek (Fig. 4). This could be due to the differences in water temperatures and salinities between the two sites. On June 30th, 2021, the water temperatures and salinities measured were 27.5°C and 27.5 ppt for Oyster Creek and 25.5°C and 26.4 ppt for Barnegat Inlet (Table 1). Both the *B. violaceus* and *B. schlosseri* tunicates favor warmer temperatures for optimal growth as shown in an experiment by McCarthy et al. (2006). When compared to the aggressive *Didemnum spp.* tunicate in different temperature conditions, both the *B. violaceus* and *B. schlosseri* tunicates showed an increase in growth in bins filled with water that was either 2°C above or 4-5°C above ambient temperatures. Another study showed that the *B. violaceus* tunicates tend to grow faster in warmer temperatures ranging from 19-23°C, than in colder temperatures ranging from 14.5-19°C (Dijkstra et al. 2008). This study also found that lower salinities can delay the reproduction of this tunicate species and they found the best reproductive success at salinities above 20ppt (Dijkstra et al. 2008) and the salinity at all sites were higher than 20 ppt, which made it optimal for the *B. violaceus* to grow. Additionally, an experiment conducted by Epelbaum et al. (2009a) found that the optimal temperature and salinity for the growth of *B. violaceus* was 20-25°C and 26-38 ppt. However, it was found that with rising water temperatures throughout the world, the *B. violaceus* tunicates are able to acclimate and grow in these rising temperatures. Looking at the sexual and asexual cycles of the *B. violaceus* tunicates in the Gulf of Maine, there was an increase in the brooding duration as the temperatures increased (10°C - 32°C) (Dijkstra et al. 2017). They predict that a 3°C increase will add more sexual generations of the *B. violaceus* tunicate.

For *B. schlosseri*, Barnegat Inlet had a higher density of zooids than Oyster Creek, but the biomass was the complete opposite. In an experiment conducted by Westerman et al. (2009) on the brooding patterns of the invasive tunicates, they found that the recruitment for *B. schlosseri* occurred at much cooler temperatures than *B. violaceus*. In one of their native environments, specifically in a lagoon in Venice, it was found that the adult tunicates are able to grow and survive optimally from temperatures of 11-26°C and salinities from 25-40 psu (Cima et al. 2015). In their non-native environment in British Columbia, it was found that the optimal growth temperature and salinity was found to be between 15-20°C and 20-30 ppt and the optimal reproductive temperature and salinity was 25°C and 26 ppt (Epelbaum et al. 2009a). Although the optimal growing temperatures for *B. schlosseri* are between 11-26°C, they can be found globally in temperatures ranging from 2.8-30.6°C and are predicted to increase in abundance as global water temperatures increase (Cockrell and Sorte 2013).

For the three sites in August, there was a higher density of *B. violaceus* zooids at Ham Island than there was at both Barnegat Inlet and Rt. 72 Bridge (Fig. 8). In Hoffman's study however, she found that in August 2017, Ham Island had a lower precent coverage of *B. violaceus* than at Barnegat Inlet and found no *B. violaceus* at Ham Island in 2018 (Hoffman 2020). In my study, a different pattern was observed with *B. schlosseri*, with Barnegat Inlet having the highest density, Rt. 72 Bridge having the second highest and Ham Island having the lowest density (Fig. 9). The same was found in Hoffman's (2020) study where the percent cover of *B. schlosseri* was higher at Barnegat Inlet than at Ham Island in August of 2017 and 2018.

There was no huge difference in temperatures between Ham Island and Barnegat Inlet between this study and Hoffman's 2017-2018 study. The temperatures in Ham Island and Barnegat Inlet for 2017-2018 were 26.8°C and 27.9°C in Ham Island and 28°C and 26.6°C in Barnegat Inlet respectively (Hoffman 2020). During my 2021 study, the temperature was 27.7°C at Ham Island and 27.1°C at Barnegat Inlet. With the differing dominance with the *B. violaceus* tunicates between Ham Island and Barnegat Inlet between the two studies, this could be due to the aggressive behavior of the tunicates. This was shown in one study in Woods Hole, Massachusetts on the colonization and recruitment of different non-native ascidians (Valentine 2016). In this study, he found that both the *B. violaceus* and *B. schlosseri* tunicates had growth interactions with other colonial tunicates which resulted in competitive standoffs. The *B. violaceus* tunicates were able to outcompete and overgrow other tunicates such as *Schizoporella unicornis*, *D. vexillum*, and *B. schlosseri*; while the *B. schlosseri* tunicates were observed to compete with the *B. violaceus* and *Aplidium glabrum*, but *D. vexillum* were able to outcompete and overgrow *B. schlosseri* (Valentine 2016). Other than temperature differences, the aggressive growth behavior of both the *B. violaceus* and *B. schlosseri* tunicates could contribute to the differences in dominance in Barnegat Inlet and Ham Island between the 2017-2018 and 2021 studies.

In August, Ham Island, Rt. 72 Bridge, and Barnegat Inlet had fewer blades of *Z. marina* with tunicates present than sites in June. There was still a positive linear regression for *B. violaceus* zooids and *Z. marina* blade surface area for Barnegat Inlet and Rt. 72 Bridge, but there was a negative relationship observed in Ham Island (Fig. 6). There was also a negative relationship between the *B. schlosseri* zooids and *Z. marina* blade surface area for all three sites (Fig. 7). This change in results for the August samples could be because of blade shedding due to temperature tolerance, and reduced light exposure. The survival of *Z. marina* blades in high temperatures was studied by Hammer et al. (2018) at Goodwin Island, Virginia and it was found that there were negative effects on blade growth, survival and rhizome growth at 30°C with a heat stress threshold of 26-30°C. Since New Jersey waters heat up relatively fast throughout the summer months, heat stress becomes a huge factor for eelgrass and causes them to release their leaves. Another study conducted in Chesapeake Bay, Virginia showed that during the hotter months of the summer between June-August, there is a decreased presence of *Z. marina* in the environment (Shields et al. 2019). They found that there was a net decline when the marine temperatures were above the average of 26°C. More specifically, at temperatures >28°C there was more than a 50% decline in eelgrass in Chesapeake Bay (Shields et al. 2019). The water temperatures at all three sites did increase in August with temperatures ranging from 27.1°C at Barnegat Inlet to 28.2°C at Rt. 72 Bridge (Table 2). With these higher temperatures, there were fewer eelgrass blades with tunicates present in August than in June.

Another factor that causes eelgrass to shed their blades is reduced light exposure. Eelgrass growth is highly dependent on water temperature and light conditions. As water temperatures decrease, eelgrass only requires a low amount of light for photosynthesis, but as the temperatures increase, so does the light requirement for photosynthesis to balance respiratory demands of the plant (Kim et al. 2015). With events such as Brown-tides, microalgal blooms, inflows of turbidity plumes, sediment loading, and resuspension of organic matter from watershed development that reduce the water clarity, there has been a rapid decline in eelgrass abundance (Bologna et al. 2007; Kim et al. 2015; Lefcheck et al. 2017). Due to the ability of both *B. violaceus* and *B. schlosseri* tunicates to rapidly grow and cover the eelgrass blade surface, the light availability to the eelgrass decreases, which blocks the plant from going through photosynthesis. This can ultimately kill the shoots, so the plant sheds the dead blades with the tunicate colonies to minimize the stress. With less eelgrass present in a specific area, there will be fewer tunicates present on the blades (Shields et al. 2019). But as the eelgrass shed their blades, this can aid in tunicate dispersal. In their study, Carman et al. (2016) talk about how as the number of tunicates living on eelgrass blades increases, the eelgrass could be providing that dispersal mechanism for the tunicates by means of released plant debris.

At Barnegat Inlet in June and August, only the *B. violaceus* and *B. schlosseri* tunicates were observed. In a similar study by Hoffman (2020) in Barnegat Inlet, they found the presence of the invasive tunicates *B. violaceus*, *B. schlosseri*, *D. vexillum*, and *A. aspersa*. Looking at the number of *B. violaceus* zooids per *Z. marina* blade surface area in Barnegat Inlet in June and August, there was a positive linear regression, but there were more total zooids and more blades that had tunicates present on them (Fig. 10). For the *B. schlosseri* zooids per *Z. marina* blade surface area, a positive linear regression was observed in June, but a negative linear regression was observed in August (Fig. 11). This could be due to the shedding of eelgrass blades because of heat shock or the smothering of the tunicates blocking the blades from sunlight and killing them. The density of *B. violaceus* zooids was higher in June than in August as well as the density of *B. schlosseri* zooids. (Figs. 12 and 13). Similarly, Hoffman (2020) observed a larger percent cover of *B. violaceus* than *B. schlosseri* in June 2017, as well as in June 2018. Since both *B. violaceus* and *B. schlosseri* tunicates are known to be aggressive colonizers, one could be the dominant species one month and then the next month the other species could dominate more space (Carman et al. 2016; Dijkstra et al. 2017).

The tunicates show a similar pattern on the settling plates with their ability to cover the plates during the summer months. From June 30th – August 12th, 2021, there was very minimal coverage of both the *B. violaceus* and *B. schlosseri* tunicates with all but the third plate having a minimal 1-5% coverage (Fig. 14). From August 12th – September 25th, 2021, there was a massive increase in the percent cover of both tunicate species on the plates with the highest percent cover of ~85% on plate 3 (Fig. 15). It is likely that active recruitment during this time lead to the increase in cover. This trend was also seen in a study comparing the spatial variation in the population dynamics of the *B. schlosseri* tunicates in Maine. It was found that population sizes varied based on the summer months with August-September reaching peak densities of the tunicates (Yund and Stires 2002). In another study testing the growth rate of invasive ascidians, it was found that with elevated temperatures, both *B. violaceus* and *B. schlosseri* had an increased growth rate with elevated temperatures, but *B. violaceus* was able to double its growth rate and density in the warmer water conditions (Lord and Whitlatch 2015).

Future studies that look at the growth of *B. violaceus* and *B. schlosseri* tunicates in Barnegat Bay, New Jersey can further assess how rapidly these invasive ascidians can grow during the summer months. Because these tunicates are known to be extremely aggressive colonizers (Carman et al. 2016), it is extremely important to monitor these species in order to find new ways to protect the eelgrass beds and other native species that these tunicates manage to outcompete.

Conclusion

There was a difference in the number of zooids per blade area (cm^2) and average density of zooids at the sites in June and August 2021. The sites in June had a higher density and number of zooids on blades of eelgrass for both tunicate species than the three sites in August. Compared to Hoffman’s study, there was a difference in which tunicate species dominated Ham Island in 2017-2018 and 2021, with *B. violaceus* being the dominant species at Ham Island in

2021, rather than in Barnegat Inlet. This research is the first evaluation of the number of zooids present on eelgrass blades in Barnegat Bay, New Jersey. Due to the different sampling methods and a three month sampling window, more data needs to be collected in future years to be able to accurately assess the growth of *B. violaceus* and *B. schlosseri* zooids in *Z. marina* beds.

Although *B. violaceus* and *B. schlosseri* are ideal invasive organisms to study because of their large temperature and salinity tolerances, they still pose a serious threat to marine communities.

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Appendix A. GPS coordinates for all sites visited in June 2021.

Site	GPS °N	GPS °W	Date
Barnegat Inlet	39.78758	-74.14996	6/30/21
Oyster Creek	39.80476	-74.17197	6/30/21

Appendix B. GPS coordinates of the transect markers (0, 30, 60, 90 meters) for the sites visited in August 2021

Site	GPS °N	GPS °W	Date
Ham Island	39.60089	-74.22909	8/12/21
Ham Island	39.60110	-74.22934	8/12/21
Ham Island	39.60131	-74.22986	8/12/21
Ham Island	39.60153	-74.23008	8/12/21
Rt. 72 Bridge	39.65820	-74.20490	8/12/21
Rt. 72 Bridge	39.65842	-74.20480	8/12/21
Rt. 72 Bridge	39.65864	-74.20472	8/12/21
Rt. 72 Bridge	39.65886	-74.20462	8/12/21
Barnegat Inlet	39.78730	-74.14847	8/12/21
Barnegat Inlet	39.78752	-74.14869	8/12/21
Barnegat Inlet	39.78773	-74.14893	8/12/21
Barnegat Inlet	39.78794	-74.14915	8/12/21

Appendix C. Raw data of *B. violaceus* zooids and *Z. marina* blade area collected from each site in the summer of 2021.

Site	**Date**	***Z. marina* area (cm^2)**	***B. violaceus* zooids**
Oyster Creek	6/30/21	140.98	2019
Oyster Creek	6/30/21	88.08	1343
Oyster Creek	6/30/21	18.24	867
Oyster Creek	6/30/21	40.64	424
Oyster Creek	6/30/21	47.8	356
Oyster Creek	6/30/21	11.12	217
Oyster Creek	6/30/21	14.5	295
Oyster Creek	6/30/21	21.36	285
Oyster Creek	6/30/21	11.92	127
Oyster Creek	6/30/21	12.08	162
Oyster Creek	6/30/21	16.4	88
Oyster Creek	6/30/21	18.78	746
Oyster Creek	6/30/21	14.96	244
Oyster Creek	6/30/21	8.88	302
Oyster Creek	6/30/21	6.28	336
Oyster Creek	6/30/21	8.7	158
Oyster Creek	6/30/21	84.34	1024
Oyster Creek	6/30/21	29.2	812
Oyster Creek	6/30/21	19.2	714
Oyster Creek	6/30/21	125.58	2213
Oyster Creek	6/30/21	6.24	255
Oyster Creek	6/30/21	6.48	180
Oyster Creek	6/30/21	121.12	1423
Oyster Creek	6/30/21	66.08	1029
Oyster Creek	6/30/21	26.08	548
Oyster Creek	6/30/21	2.52	638
Oyster Creek	6/30/21	12.6	83
Oyster Creek	6/30/21	2.24	60
Oyster Creek	6/30/21	30.8	667
Oyster Creek	6/30/21	35.6	595
Oyster Creek	6/30/21	11.04	533
Oyster Creek	6/30/21	95.68	1733
Oyster Creek	6/30/21	15.42	150
Oyster Creek	6/30/21	10.08	167
Oyster Creek	6/30/21	25.6	243
Oyster Creek	6/30/21	12.88	14
Oyster Creek	6/30/21	23.2	148
Oyster Creek	6/30/21	4.5	111
Oyster Creek	6/30/21	6.4	257
Oyster Creek	6/30/21	3.54	120
Oyster Creek	6/30/21	12.6	107
Oyster Creek	6/30/21	28.2	84
Oyster Creek	6/30/21	8	87

Oyster Creek	6/30/21	8	182
Oyster Creek	6/30/21	8.52	382
Oyster Creek	6/30/21	2.16	107
Oyster Creek	6/30/21	7.32	141
Oyster Creek	6/30/21	3.48	78
Oyster Creek	6/30/21	2.6	72
Oyster Creek	6/30/21	17.94	103
Oyster Creek	6/30/21	30.4	30
Oyster Creek	6/30/21	12	174
Oyster Creek	6/30/21	15.6	342
Oyster Creek	6/30/21	24.96	114
Oyster Creek	6/30/21	27.6	185
Oyster Creek	6/30/21	11.22	152
Oyster Creek	6/30/21	5.82	49
Oyster Creek	6/30/21	7.2	115
Oyster Creek	6/30/21	7.68	70
Oyster Creek	6/30/21	6.3	69
Oyster Creek	6/30/21	8.4	54
Oyster Creek	6/30/21	6.6	49
Oyster Creek	6/30/21	6.3	65
Oyster Creek	6/30/21	45.78	839
Oyster Creek	6/30/21	17.82	323
Oyster Creek	6/30/21	10.2	217
Oyster Creek	6/30/21	4.8	55
Oyster Creek	6/30/21	7.98	208
Oyster Creek	6/30/21	9.9	101
Oyster Creek	6/30/21	4.14	27
Oyster Creek	6/30/21	9.48	211
Oyster Creek	6/30/21	74.82	1672
Oyster Creek	6/30/21	86.08	1785
Barnegat Inlet	6/30/21	20.9	352
Barnegat Inlet	6/30/21	14.56	92
Barnegat Inlet	6/30/21	12.8	237
Barnegat Inlet	6/30/21	24.24	248
Barnegat Inlet	6/30/21	13.9	120
Barnegat Inlet	6/30/21	19	83
Barnegat Inlet	6/30/21	25.3	64
Barnegat Inlet	6/30/21	18.2	44
Barnegat Inlet	6/30/21	11.5	69
Barnegat Inlet	6/30/21	14.8	50
Barnegat Inlet	6/30/21	10.9	144
Barnegat Inlet	6/30/21	25.4	53
Barnegat Inlet	6/30/21	16	76
Barnegat Inlet	6/30/21	32.82	13
Barnegat Inlet	6/30/21	42.4	42
Barnegat Inlet	6/30/21	18.24	106
Barnegat Inlet	6/30/21	15.5	46
Barnegat Inlet	6/30/21	11.6	181

Barnegat Inlet	6/30/21	13.2	84
Barnegat Inlet	6/30/21	18.88	27
Barnegat Inlet	6/30/21	16.8	108
Barnegat Inlet	6/30/21	18.4	141
Barnegat Inlet	6/30/21	27	440
Barnegat Inlet	6/30/21	12.6	247
Barnegat Inlet	6/30/21	12.72	265
Barnegat Inlet	6/30/21	6.36	72
Barnegat Inlet	6/30/21	7.62	132
Barnegat Inlet	6/30/21	26.18	112
Barnegat Inlet	6/30/21	18.5	164
Barnegat Inlet	6/30/21	14.4	50
Barnegat Inlet	6/30/21	17.52	10
Barnegat Inlet	6/30/21	9.4	89
Barnegat Inlet	6/30/21	12.24	323
Barnegat Inlet	6/30/21	19.6	178
Barnegat Inlet	6/30/21	21	379
Barnegat Inlet	6/30/21	52.44	845
Barnegat Inlet	6/30/21	15.3	159
Barnegat Inlet	6/30/21	21.1	383
Barnegat Inlet	6/30/21	48.3	441
Barnegat Inlet	6/30/21	4.72	72
Barnegat Inlet	6/30/21	88.44	1522
Barnegat Inlet	6/30/21	24	846
Barnegat Inlet	6/30/21	46.6	584
Barnegat Inlet	6/30/21	16	324
Barnegat Inlet	6/30/21	21.4	512
Barnegat Inlet	6/30/21	22.72	890
Barnegat Inlet	6/30/21	13.08	104
Barnegat Inlet	6/30/21	16	122
Barnegat Inlet	6/30/21	9.36	152
Barnegat Inlet	6/30/21	20.04	129
Barnegat Inlet	6/30/21	11.84	113
Barnegat Inlet	6/30/21	19.28	57
Barnegat Inlet	6/30/21	50.5	116
Barnegat Inlet	6/30/21	63.5	172
Barnegat Inlet	6/30/21	8.5	46
Barnegat Inlet	6/30/21	16	68
Barnegat Inlet	6/30/21	19.9	69
Barnegat Inlet	6/30/21	21	25
Barnegat Inlet	6/30/21	21	81
Barnegat Inlet	6/30/21	6.96	25
Barnegat Inlet	6/30/21	28	30
Barnegat Inlet	6/30/21	28.1	88
Barnegat Inlet	6/30/21	27.68	51
Barnegat Inlet	6/30/21	14.8	711
Barnegat Inlet	6/30/21	26	229
Barnegat Inlet	6/30/21	12.32	334

Barnegat Inlet	6/30/21	68.4	177
Barnegat Inlet	6/30/21	66.1	167
Barnegat Inlet	6/30/21	70.8	332
Barnegat Inlet	6/30/21	8	109
Barnegat Inlet	6/30/21	21.5	140
Barnegat Inlet	6/30/21	2.9	181
Barnegat Inlet	6/30/21	28.8	58
Barnegat Inlet	6/30/21	27.6	74
Barnegat Inlet	6/30/21	50.4	452
Barnegat Inlet	6/30/21	11	206
Barnegat Inlet	6/30/21	29.68	159
Barnegat Inlet	6/30/21	60.2	182
Barnegat Inlet	6/30/21	72.7	236
Barnegat Inlet	6/30/21	16.92	154
Barnegat Inlet	6/30/21	56	59
Barnegat Inlet	6/30/21	11.2	81
Barnegat Inlet	6/30/21	18	42
Barnegat Inlet	6/30/21	21.84	93
Barnegat Inlet	6/30/21	21.2	82
Barnegat Inlet	6/30/21	17.6	96
Barnegat Inlet	6/30/21	9.8	60
Barnegat Inlet	6/30/21	28.4	30
Barnegat Inlet	6/30/21	159.3	2545
Barnegat Inlet	6/30/21	17.8	115
Barnegat Inlet	6/30/21	12	130
Barnegat Inlet	6/30/21	6.76	377
Barnegat Inlet	6/30/21	20.08	248
Barnegat Inlet	6/30/21	17	406
Barnegat Inlet	6/30/21	21.12	118
Barnegat Inlet	6/30/21	12	105
Barnegat Inlet	6/30/21	12.3	116
Barnegat Inlet	6/30/21	11.3	107
Barnegat Inlet	6/30/21	8	78
Barnegat Inlet	6/30/21	18.56	31
Ham Island	8/12/21	6.52	642
Ham Island	8/12/21	7.16	89
Ham Island	8/12/21	14.58	76
Ham Island	8/12/21	17.82	610
Ham Island	8/12/21	9.68	208
Ham Island	8/12/21	13.62	166
Ham Island	8/12/21	7.24	847
Ham Island	8/12/21	7.44	66
Ham Island	8/12/21	7.98	28
Ham Island	8/12/21	10.2	29
Ham Island	8/12/21	14.76	351
Ham Island	8/12/21	10.96	208
Ham Island	8/12/21	13.96	158
Ham Island	8/12/21	11.64	132

Ham Island	8/12/21	11.44	124
Ham Island	8/12/21	9.32	119
Rt.72 Bridge	8/12/21	7.76	293
Rt.72 Bridge	8/12/21	7.52	283
Rt.72 Bridge	8/12/21	36.72	1286
Rt.72 Bridge	8/12/21	7.24	79
Rt.72 Bridge	8/12/21	6.32	233
Rt.72 Bridge	8/12/21	7.62	149
Rt.72 Bridge	8/12/21	14.72	205
Rt.72 Bridge	8/12/21	13.86	29
Rt.72 Bridge	8/12/21	10.08	94
Rt.72 Bridge	8/12/21	13.8	45
Rt.72 Bridge	8/12/21	18.3	26
Rt.72 Bridge	8/12/21	5.96	37
Barnegat Inlet	8/12/21	13.6	133
Barnegat Inlet	8/12/21	12	28
Barnegat Inlet	8/12/21	23.68	83
Barnegat Inlet	8/12/21	31.68	239
Barnegat Inlet	8/12/21	16.66	9
Barnegat Inlet	8/12/21	36.1	16
Barnegat Inlet	8/12/21	21	28
Barnegat Inlet	8/12/21	9.4	51
Barnegat Inlet	8/12/21	14.4	419
Barnegat Inlet	8/12/21	59.52	1015
Barnegat Inlet	8/12/21	8.4	106
Barnegat Inlet	8/12/21	15.24	94
Barnegat Inlet	8/12/21	24.48	78
Barnegat Inlet	8/12/21	18.3	19
Barnegat Inlet	8/12/21	13.92	25

Appendix D. Raw data of *B. schlosseri* zooids and *Z. marina* blade area collected from each site in the summer of 2021.

Site	Date	*Z. marina* area (cm^2)	*B. schlosseri* zooids
Oyster Creek	6/30/21	47.8	55
Oyster Creek	6/30/21	14.5	190
Oyster Creek	6/30/21	20.4	132
Oyster Creek	6/30/21	4.64	417
Oyster Creek	6/30/21	6	116
Oyster Creek	6/30/21	10.08	245
Oyster Creek	6/30/21	91.12	1231
Oyster Creek	6/30/21	8.28	142
Oyster Creek	6/30/21	6.6	509
Oyster Creek	6/30/21	25.12	54
Oyster Creek	6/30/21	31.92	326
Oyster Creek	6/30/21	32	411
Oyster Creek	6/30/21	24.32	124
Oyster Creek	6/30/21	16.68	167
Oyster Creek	6/30/21	14.5	108
Oyster Creek	6/30/21	7.3	116
Oyster Creek	6/30/21	27.2	265
Oyster Creek	6/30/21	11.6	80
Oyster Creek	6/30/21	20.34	829
Oyster Creek	6/30/21	267.44	2252
Oyster Creek	6/30/21	55.18	1512
Oyster Creek	6/30/21	6.6	147
Oyster Creek	6/30/21	4.5	336
Oyster Creek	6/30/21	25.6	180
Oyster Creek	6/30/21	7.28	359
Oyster Creek	6/30/21	10.8	342
Oyster Creek	6/30/21	34.14	1032
Oyster Creek	6/30/21	6.36	220
Oyster Creek	6/30/21	11.2	147
Oyster Creek	6/30/21	15.04	238
Oyster Creek	6/30/21	16.8	340
Oyster Creek	6/30/21	9	834
Oyster Creek	6/30/21	3.6	406
Oyster Creek	6/30/21	30.56	355
Oyster Creek	6/30/21	21.84	158
Oyster Creek	6/30/21	22	328
Oyster Creek	6/30/21	8.28	71
Oyster Creek	6/30/21	11.2	53
Oyster Creek	6/30/21	24.8	411
Oyster Creek	6/30/21	23.5	786
Oyster Creek	6/30/21	12.4	45
Oyster Creek	6/30/21	17.4	116
Oyster Creek	6/30/21	8.4	84

Oyster Creek	6/30/21	18.8	146
Oyster Creek	6/30/21	7.56	32
Oyster Creek	6/30/21	10.2	243
Oyster Creek	6/30/21	23.12	280
Oyster Creek	6/30/21	15.2	35
Oyster Creek	6/30/21	6.96	109
Oyster Creek	6/30/21	12.64	41
Oyster Creek	6/30/21	28.24	451
Oyster Creek	6/30/21	10.56	150
Oyster Creek	6/30/21	4.68	51
Oyster Creek	6/30/21	12.8	372
Oyster Creek	6/30/21	6.96	147
Oyster Creek	6/30/21	5.04	50
Oyster Creek	6/30/21	4.96	45
Oyster Creek	6/30/21	4.5	89
Oyster Creek	6/30/21	15.06	52
Oyster Creek	6/30/21	9.6	116
Oyster Creek	6/30/21	3.72	34
Oyster Creek	6/30/21	9.12	138
Oyster Creek	6/30/21	8.7	305
Oyster Creek	6/30/21	10.56	393
Oyster Creek	6/30/21	8.88	470
Oyster Creek	6/30/21	6.9	180
Oyster Creek	6/30/21	3.76	55
Oyster Creek	6/30/21	4.4	223
Oyster Creek	6/30/21	6.96	116
Oyster Creek	6/30/21	0.8	7
Oyster Creek	6/30/21	10.56	125
Oyster Creek	6/30/21	13.14	226
Oyster Creek	6/30/21	8.8	90
Oyster Creek	6/30/21	6.36	94
Oyster Creek	6/30/21	6.3	45
Oyster Creek	6/30/21	7.74	176
Oyster Creek	6/30/21	2.7	114
Oyster Creek	6/30/21	5.6	138
Oyster Creek	6/30/21	4.2	216
Oyster Creek	6/30/21	53.92	1426
Oyster Creek	6/30/21	11.58	730
Oyster Creek	6/30/21	7.2	296
Oyster Creek	6/30/21	5.36	508
Oyster Creek	6/30/21	21.68	314
Oyster Creek	6/30/21	18	233
Oyster Creek	6/30/21	16.96	152
Oyster Creek	6/30/21	16.32	189
Oyster Creek	6/30/21	17.28	294
Oyster Creek	6/30/21	10.4	47
Oyster Creek	6/30/21	13.02	146
Oyster Creek	6/30/21	3.68	41

Oyster Creek	6/30/21	7.32	105
Oyster Creek	6/30/21	14.34	74
Oyster Creek	6/30/21	9.52	62
Oyster Creek	6/30/21	70.28	1606
Oyster Creek	6/30/21	31	1115
Oyster Creek	6/30/21	7.56	439
Barnegat Inlet	6/30/21	7.92	41
Barnegat Inlet	6/30/21	6.88	79
Barnegat Inlet	6/30/21	34.6	336
Barnegat Inlet	6/30/21	17.64	427
Barnegat Inlet	6/30/21	49.32	463
Barnegat Inlet	6/30/21	9.28	160
Barnegat Inlet	6/30/21	7.2	56
Barnegat Inlet	6/30/21	23.52	339
Barnegat Inlet	6/30/21	22	66
Barnegat Inlet	6/30/21	49	159
Barnegat Inlet	6/30/21	12.4	105
Barnegat Inlet	6/30/21	17.5	143
Barnegat Inlet	6/30/21	34.4	114
Barnegat Inlet	6/30/21	22.1	168
Barnegat Inlet	6/30/21	14	56
Barnegat Inlet	6/30/21	6.96	42
Barnegat Inlet	6/30/21	21	113
Barnegat Inlet	6/30/21	24.9	66
Barnegat Inlet	6/30/21	20.7	118
Barnegat Inlet	6/30/21	18.7	74
Barnegat Inlet	6/30/21	24.3	23
Barnegat Inlet	6/30/21	10.32	45
Barnegat Inlet	6/30/21	11.52	78
Barnegat Inlet	6/30/21	8	55
Barnegat Inlet	6/30/21	23.76	74
Barnegat Inlet	6/30/21	13.32	23
Barnegat Inlet	6/30/21	29.16	34
Barnegat Inlet	6/30/21	33.4	73
Barnegat Inlet	6/30/21	24.6	242
Barnegat Inlet	6/30/21	10	317
Barnegat Inlet	6/30/21	25.4	255
Barnegat Inlet	6/30/21	20.1	518
Barnegat Inlet	6/30/21	17.6	226
Barnegat Inlet	6/30/21	41.9	415
Barnegat Inlet	6/30/21	9.8	145
Barnegat Inlet	6/30/21	2.58	72
Barnegat Inlet	6/30/21	10.6	166
Barnegat Inlet	6/30/21	17.6	67
Barnegat Inlet	6/30/21	14.48	137
Barnegat Inlet	6/30/21	21.6	103
Barnegat Inlet	6/30/21	9	122
Barnegat Inlet	6/30/21	4.83	205

Barnegat Inlet	6/30/21	12	310
Barnegat Inlet	6/30/21	8.1	285
Barnegat Inlet	6/30/21	19.5	74
Barnegat Inlet	6/30/21	13.8	231
Barnegat Inlet	6/30/21	64.08	158
Barnegat Inlet	6/30/21	27.52	406
Barnegat Inlet	6/30/21	15.2	157
Barnegat Inlet	6/30/21	6.72	128
Barnegat Inlet	6/30/21	42	412
Barnegat Inlet	6/30/21	26.5	201
Barnegat Inlet	6/30/21	12.9	211
Barnegat Inlet	6/30/21	26.18	338
Barnegat Inlet	6/30/21	15.9	143
Barnegat Inlet	6/30/21	10.9	430
Barnegat Inlet	6/30/21	46.8	330
Barnegat Inlet	6/30/21	19.5	391
Barnegat Inlet	6/30/21	17.1	626
Barnegat Inlet	6/30/21	15	262
Barnegat Inlet	6/30/21	10.25	219
Barnegat Inlet	6/30/21	62	85
Barnegat Inlet	6/30/21	4.32	143
Barnegat Inlet	6/30/21	24.36	282
Barnegat Inlet	6/30/21	22.6	357
Barnegat Inlet	6/30/21	10.24	92
Barnegat Inlet	6/30/21	14.4	185
Barnegat Inlet	6/30/21	63	234
Barnegat Inlet	6/30/21	30.36	210
Barnegat Inlet	6/30/21	10	312
Barnegat Inlet	6/30/21	7.5	171
Barnegat Inlet	6/30/21	9.5	53
Barnegat Inlet	6/30/21	19.5	805
Barnegat Inlet	6/30/21	345.24	3204
Barnegat Inlet	6/30/21	6.6	147
Barnegat Inlet	6/30/21	12.64	26
Barnegat Inlet	6/30/21	27.2	457
Barnegat Inlet	6/30/21	11.22	102
Barnegat Inlet	6/30/21	22.8	219
Barnegat Inlet	6/30/21	6	421
Barnegat Inlet	6/30/21	18	738
Barnegat Inlet	6/30/21	24.3	295
Barnegat Inlet	6/30/21	10.5	561
Barnegat Inlet	6/30/21	10	189
Barnegat Inlet	6/30/21	25.92	561
Barnegat Inlet	6/30/21	6.4	447
Barnegat Inlet	6/30/21	41	1546
Barnegat Inlet	6/30/21	11.12	35
Barnegat Inlet	6/30/21	61.8	467
Barnegat Inlet	6/30/21	49.2	272

Barnegat Inlet	6/30/21	23.52	119
Barnegat Inlet	6/30/21	21	145
Barnegat Inlet	6/30/21	22	81
Barnegat Inlet	6/30/21	20.52	105
Barnegat Inlet	6/30/21	19.5	149
Barnegat Inlet	6/30/21	38.4	119
Barnegat Inlet	6/30/21	14.6	108
Barnegat Inlet	6/30/21	24.3	123
Barnegat Inlet	6/30/21	13.2	82
Barnegat Inlet	6/30/21	7.52	133
Barnegat Inlet	6/30/21	30.2	63
Barnegat Inlet	6/30/21	14	126
Barnegat Inlet	6/30/21	40.5	147
Barnegat Inlet	6/30/21	21	90
Barnegat Inlet	6/30/21	16.8	139
Barnegat Inlet	6/30/21	28.1	40
Barnegat Inlet	6/30/21	13.2	81
Barnegat Inlet	6/30/21	26.5	53
Barnegat Inlet	6/30/21	21.6	47
Barnegat Inlet	6/30/21	21.72	574
Barnegat Inlet	6/30/21	21.2	314
Barnegat Inlet	6/30/21	66.1	915
Barnegat Inlet	6/30/21	56.4	785
Barnegat Inlet	6/30/21	26	122
Barnegat Inlet	6/30/21	40	673
Barnegat Inlet	6/30/21	21.6	650
Barnegat Inlet	6/30/21	37.6	271
Barnegat Inlet	6/30/21	39	554
Barnegat Inlet	6/30/21	99.5	353
Barnegat Inlet	6/30/21	68.4	148
Barnegat Inlet	6/30/21	72	109
Barnegat Inlet	6/30/21	29.5	154
Barnegat Inlet	6/30/21	25.7	66
Barnegat Inlet	6/30/21	71.2	43
Barnegat Inlet	6/30/21	16.8	234
Barnegat Inlet	6/30/21	28.8	771
Barnegat Inlet	6/30/21	70.8	568
Barnegat Inlet	6/30/21	42.6	684
Barnegat Inlet	6/30/21	14.5	59
Barnegat Inlet	6/30/21	70.8	346
Barnegat Inlet	6/30/21	27.2	429
Barnegat Inlet	6/30/21	15.9	410
Barnegat Inlet	6/30/21	35.2	431
Barnegat Inlet	6/30/21	31.2	343
Barnegat Inlet	6/30/21	28.64	259
Barnegat Inlet	6/30/21	8.8	82
Barnegat Inlet	6/30/21	21.6	270
Barnegat Inlet	6/30/21	12.24	134

Barnegat Inlet	6/30/21	36.5	446
Barnegat Inlet	6/30/21	23.76	320
Barnegat Inlet	6/30/21	16.4	218
Barnegat Inlet	6/30/21	54	96
Barnegat Inlet	6/30/21	21	302
Barnegat Inlet	6/30/21	22	221
Barnegat Inlet	6/30/21	8.88	164
Barnegat Inlet	6/30/21	11.04	280
Barnegat Inlet	6/30/21	19.28	173
Barnegat Inlet	6/30/21	6.56	153
Barnegat Inlet	6/30/21	20.5	273
Barnegat Inlet	6/30/21	7.04	144
Barnegat Inlet	6/30/21	8.4	77
Barnegat Inlet	6/30/21	47.88	151
Barnegat Inlet	6/30/21	13.5	346
Barnegat Inlet	6/30/21	12.12	237
Barnegat Inlet	6/30/21	21.14	186
Barnegat Inlet	6/30/21	12.64	207
Barnegat Inlet	6/30/21	16.3	287
Barnegat Inlet	6/30/21	30	59
Barnegat Inlet	6/30/21	54	573
Barnegat Inlet	6/30/21	20	150
Barnegat Inlet	6/30/21	46	94
Barnegat Inlet	6/30/21	18.6	693
Barnegat Inlet	6/30/21	125.44	1168
Barnegat Inlet	6/30/21	18.5	321
Barnegat Inlet	6/30/21	16.5	440
Barnegat Inlet	6/30/21	10.8	297
Barnegat Inlet	6/30/21	10	167
Barnegat Inlet	6/30/21	30.4	359
Barnegat Inlet	6/30/21	17.3	210
Barnegat Inlet	6/30/21	28.4	80
Barnegat Inlet	6/30/21	37.6	95
Barnegat Inlet	6/30/21	22.1	117
Barnegat Inlet	6/30/21	5.68	77
Barnegat Inlet	6/30/21	59.16	786
Barnegat Inlet	6/30/21	21.24	409
Barnegat Inlet	6/30/21	10.68	217
Barnegat Inlet	6/30/21	15.68	440
Barnegat Inlet	6/30/21	20.88	450
Barnegat Inlet	6/30/21	19.5	195
Barnegat Inlet	6/30/21	18.5	351
Barnegat Inlet	6/30/21	29.8	179
Barnegat Inlet	6/30/21	23.6	84
Barnegat Inlet	6/30/21	24.4	355
Barnegat Inlet	6/30/21	44.5	156
Barnegat Inlet	6/30/21	56	98
Barnegat Inlet	6/30/21	13.1	170

Barnegat Inlet	6/30/21	9.92	55
Barnegat Inlet	6/30/21	11.76	49
Barnegat Inlet	6/30/21	56.9	64
Barnegat Inlet	6/30/21	14.72	137
Barnegat Inlet	6/30/21	20.72	42
Barnegat Inlet	6/30/21	24.2	108
Barnegat Inlet	6/30/21	4.32	129
Barnegat Inlet	6/30/21	15.92	208
Barnegat Inlet	6/30/21	18	188
Barnegat Inlet	6/30/21	17.5	67
Barnegat Inlet	6/30/21	13.3	69
Barnegat Inlet	6/30/21	9.9	71
Barnegat Inlet	6/30/21	23	94
Barnegat Inlet	6/30/21	13.4	76
Barnegat Inlet	6/30/21	9.24	68
Barnegat Inlet	6/30/21	18.6	105
Barnegat Inlet	6/30/21	10	45
Barnegat Inlet	6/30/21	153.2	1647
Barnegat Inlet	6/30/21	58.8	246
Barnegat Inlet	6/30/21	69.12	1123
Barnegat Inlet	6/30/21	20.5	584
Barnegat Inlet	6/30/21	15	149
Barnegat Inlet	6/30/21	45.6	211
Barnegat Inlet	6/30/21	26	164
Barnegat Inlet	6/30/21	28.4	368
Barnegat Inlet	6/30/21	64.5	111
Barnegat Inlet	6/30/21	15	85
Barnegat Inlet	6/30/21	65.3	57
Barnegat Inlet	6/30/21	17.6	103
Barnegat Inlet	6/30/21	6.36	36
Barnegat Inlet	6/30/21	10.2	119
Barnegat Inlet	6/30/21	58.16	184
Barnegat Inlet	6/30/21	10.7	131
Barnegat Inlet	6/30/21	26.52	88
Barnegat Inlet	6/30/21	13.6	260
Barnegat Inlet	6/30/21	22.08	108
Barnegat Inlet	6/30/21	25.3	236
Barnegat Inlet	6/30/21	19.3	89
Barnegat Inlet	6/30/21	20	120
Barnegat Inlet	6/30/21	14.24	86
Barnegat Inlet	6/30/21	12	57
Barnegat Inlet	6/30/21	11.44	66
Barnegat Inlet	6/30/21	17.5	105
Barnegat Inlet	6/30/21	17.6	118
Barnegat Inlet	6/30/21	25.44	91
Barnegat Inlet	6/30/21	15.84	221
Barnegat Inlet	6/30/21	10.5	154
Barnegat Inlet	6/30/21	16.6	240

Barnegat Inlet	6/30/21	14.5	15
Ham Island	8/12/21	11.52	234
Ham Island	8/12/21	16.44	110
Ham Island	8/12/21	9.48	492
Ham Island	8/12/21	16.32	85
Ham Island	8/12/21	9.2	593
Rt.72 Bridge	8/12/21	3.8	528
Rt.72 Bridge	8/12/21	10.16	731
Rt.72 Bridge	8/12/21	6.8	196
Rt.72 Bridge	8/12/21	13.86	73
Rt.72 Bridge	8/12/21	10.44	21
Rt.72 Bridge	8/12/21	12.18	13
Barnegat Inlet	8/12/21	12.72	323
Barnegat Inlet	8/12/21	13.6	104
Barnegat Inlet	8/12/21	31.32	120
Barnegat Inlet	8/12/21	47.9	21
Barnegat Inlet	8/12/21	27.52	989
Barnegat Inlet	8/12/21	17.52	46
Barnegat Inlet	8/12/21	28.5	17
Barnegat Inlet	8/12/21	18	74
Barnegat Inlet	8/12/21	18.8	584
Barnegat Inlet	8/12/21	13.08	315
Barnegat Inlet	8/12/21	19.62	69
Barnegat Inlet	8/12/21	20.32	438
Barnegat Inlet	8/12/21	20.16	92
Barnegat Inlet	8/12/21	7.76	36
Barnegat Inlet	8/12/21	12.84	48
Barnegat Inlet	8/12/21	12.08	407
Barnegat Inlet	8/12/21	30.88	72

Appendix E. Individual biomasses of *Z. marina*, *B. violaceus* and *B. schlosseri* from each sample from the sites in June 2021.

Site	**Date**	**Sample**	**AFDW *Z. marina* (g)**	**AFDW *B. violaceus* (g)**	**AFDW *B. schlosseri* (g)**
Oyster Creek	6/30/21	1	7.05	1.2325	0.3007
Oyster Creek	6/30/21	2	1.58	0.0413	0.1089
Oyster Creek	6/30/21	3	4.85	0.0799	0.6033
Oyster Creek	6/30/21	4	1.08	0.0104	0.0887
Oyster Creek	6/30/21	5	1.7	0.2172	-
Oyster Creek	6/30/21	6	3.92	0.6331	0.7571
Barnegat Inlet	6/30/21	1	7.35	0.1328	0.2716
Barnegat Inlet	6/30/21	2	5.73	0.0546	0.1807
Barnegat Inlet	6/30/21	3	1.51	0.015	0.2321
Barnegat Inlet	6/30/21	4	7.67	0.1795	0.2815
Barnegat Inlet	6/30/21	5	11.81	0.7883	0.643
Barnegat Inlet	6/30/21	7	8.45	0.082	0.9854
Barnegat Inlet	6/30/21	8	6.91	0.1364	0.3521
Barnegat Inlet	6/30/21	9	4.4	-	1.446
Barnegat Inlet	6/30/21	10	6.33	1.2359	0.3623

Appendix F. Individual biomasses of *Z. marina*, *B. violaceus* and *B. schlosseri* from each sample from the sites in August 2021.

Site	**Date**	**Sample**	**AFDW *Z. marina* (g)**	**AFDW *B. violaceus* (g)**	**AFDW *B. schlosseri* (g)**
Ham Island	8/12/21	0 meters	22.3	1.0116	0.3291
Ham Island	8/12/21	10 meters	5.77	0.028	-
Ham Island	8/12/21	20 meters	11.28	-	-
Ham Island	8/12/21	30 meters	5.7	-	-
Ham Island	8/12/21	40 meters	6.75	-	-
Ham Island	8/12/21	50 meters	6.21	-	-
Ham Island	8/12/21	60 meters	4.99	-	-
Ham Island	8/12/21	70 meters	6.94	0.0005	-
Ham Island	8/12/21	80 meters	5.47	0.1892	-
Ham Island	8/12/21	90 meters	6.78	-	-
Rt. 72 Bridge	8/12/21	0 meters	3.39	-	-
Rt. 72 Bridge	8/12/21	10 meters	6.35	-	-
Rt. 72 Bridge	8/12/21	20 meters	5.15	0.1213	-
Rt. 72 Bridge	8/12/21	30 meters	7.94	-	-
Rt. 72 Bridge	8/12/21	40 meters	0.82	1.0647	1.0116
Rt. 72 Bridge	8/12/21	50 meters	7.23	-	-
Rt. 72 Bridge	8/12/21	60 meters	6.91	-	0.0512
Rt. 72 Bridge	8/12/21	70 meters	8.07	-	-
Rt. 72 Bridge	8/12/21	80 meters	4.39	0.1037	0.0156
Rt. 72 Bridge	8/12/21	90 meters	6.31	-	-
Barnegat Inlet	8/12/21	0 meters	3.51	-	-
Barnegat Inlet	8/12/21	10 meters	7.59	0.093	0.135
Barnegat Inlet	8/12/21	20 meters	13.07	0.0534	-
Barnegat Inlet	8/12/21	30 meters	12.8	0.0002	-
Barnegat Inlet	8/12/21	40 meters	6.45	-	0.2353
Barnegat Inlet	8/12/21	50 meters	16.59	-	0.0179
Barnegat Inlet	8/12/21	60 meters	8.9	-	0.3723
Barnegat Inlet	8/12/21	70 meters	8.48	-	0.0057
Barnegat Inlet	8/12/21	80 meters	6.41	-	0.087
Barnegat Inlet	8/12/21	90 meters	7.19	0.6621	-

Appendix G. Raw data of the percent coverage (%) of all organisms found on the settling plates at Barnegat Inlet from June 30th – August 12th, 2021.

Organisms	**Plate 1 Up (%)**	**Plate 1 Down (%)**	**Plate 2 Up (%)**	**Plate 2 Down (%)**	**Plate 3 Up (%)**	**Plate 3 Down (%)**	**Plate 4 Up (%)**	**Plate 4 Down (%)**
Bugula turrita	-	30	15	50	40	20	25	55
Botrylloides violaceus	-	-	-	10	-	40	-	-
Botryllus schlosseri	-	5	-	1	-	1	-	<1
Enteromorpha	10	5	20	5	30	30	-	40
Filamentous red	5	-	-	-	-	5	-	-
Obelia	20	2	5	2	5	1	-	-
Barnacle	1	-	-	1	-	-	-	-
Spiorbis	>50	<100	<70	<100	20	<100	70	-
Bacterial mat	70	60	90	-	90	-	100	100

Appendix H. Raw data of the percent coverage (%) of all organisms found on the settling plates at Barnegat Inlet from August 12th – September 25th, 2021.

Organisms	**Plate 1 Up (%)**	**Plate 1 Down (%)**	**Plate 2 Up (%)**	**Plate 2 Down (%)**	**Plate 3 Up (%)**	**Plate 3 Down (%)**	**Plate 4 Up (%)**	**Plate 4 Down (%)**
Bugula turrita	20	-	-	-	-	-	15	-
Botrylloides violaceus	-	-	-	-	15	85	-	50
Botryllus schlosseri	-	-	-	75	-	<1	-	35
Enteromorpha	<5	<5	5	1	80	-	45	-
Filamentous red	15	-	40	1	30	10	20	<1
Obelia	-	15	-	40	60	50	60	50
Barnacle	-	-	-	1	-	-	-	-
Egg case/Eggs	-	50	10	-	15	40	-	15
Spiorbis	-	-	-	-	-	-	-	15
Bacterial mat	<100	<100	<100	50	50	45	<100	40