ABSTRACT

CHRISTIANSON, KAYLA ANN. An Unfolding Invasion: Impacts of a Recently Introduced Exotic Marine Species on Patterns of Community Development. (Under the direction of Dr. David Eggleston).

The global redistribution of species is a prominent impact of climate change and human-mediated biological invasions, and often results in negative impacts to ecosystems. Invasive species generally exert adverse effects on communities by displacing native species, leading to losses of biodiversity with concomitant changes in trophic cascades and negative economic consequences. This research builds on previous experimental results to characterize the community level impacts of an exotic marine species, and thereby inform predictions of how future invasions might cause shifts in community composition. In the last five years, a species of colonial tunicate not previously present, *Clavelina oblonga*, has become prominent within the fouling community of Beaufort, North Carolina. Fifty years ago Sutherland and Karlson (1977) found that this community was characterized by a heterogeneous mixture of species that varied inter-annually and increased in diversity over time. Recently, Theuerkauf et al. (2018) found that the community is now dominated by *C. oblonga* with patterns of community development and structure that led to the loss of alternative community states, domination by *C.* oblonga, and reduced species diversity. This study characterized the impact of *Clavelina oblonga* on the fouling community by experimentally reducing the percent cover of *C. oblonga* to test if patterns of community development and structure resembled patterns described 50 years ago in the absence of this invasive tunicate. The impacts of two large-scale, environmental disturbances (extremely cold winter &hurricane) on the abundance of *C. oblonga* and the fouling community were also quantified.

We found that *Clavelina oblonga* is still recruiting seasonally and is present at high abundances in the community. Species richness decreases as abundance of *C. oblonga* increases, and the community exhibits greater seasonal variability and a more heterogeneous mixture of species when the presence of *C. oblonga* is reduced. *C. oblonga* is a successful invader in this community due to its rapid growth and strong seasonal recruitment. Disturbance caused by a harsh winter and a hurricane appeared to limit the presence of *C. oblonga.* This experiment advanced our understanding of marine community ecology by testing if the concept of multiple community states identified over 50 years ago by Sutherland and Karlson (1977) is still operable in the absence of invasive *C. oblonga,* and highlights how the environment could moderate the spread of this invasive tunicate.

An Unfolding Invasion: Impacts of a Recently Introduced Exotic Marine Species on Patterns of
Community Development.

by
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DEDICATION

This thesis is dedicated to my best friend and husband, Blake. Thank you for standing by my side through all the long weekends, late nights, and utter chaos that this project has brought. Without your constant love and support this thesis would not have happened.

BIOGRAPHY

Kayla spent her early childhood in Grayslake, Illinois before moving to Charlotte, North Carolina during middle school. As she grew up so did her interest in science, leading her to attend the North Carolina School of Science and Mathematics for her junior and senior years of high school. While there, she was able to take elective science classes and discovered her interests in Ecology and Aquatic Ecology.

For her undergraduate career, Kayla attended NC State University where she majored in Marine Science with a Concentration in Biological Oceanography and minors in both Biology and Zoology. After initially having a strong interest in chemistry and briefly working in Dr. Christopher Osburn's Lab, she shifted her focus to biological oceanography. The summer after her sophomore year she spent a month studying abroad in New Zealand with the Department of Parks, Recreation and Tourism Management, where she explored principles of conservation and invasive species. She also worked in the organic chemistry teaching labs stockroom as a stockroom assistant for two years, where she contributed to a paper looking at the effectiveness of supplemental videos in chemistry education.

During her last summer as an undergraduate, Kayla was able to participate in a NSF Research Experience for Undergraduates at the Central Caribbean Marine Institute. There she conducted a research project on the relationship between coral reef sponges and reef resilience under the advisement of Dr. Steve Whalan. Then, with the assistance of Dr. Astrid Schnetzer she was able to use this project as a capstone for both the MEAS Departmental and University Honors Programs, graduating with honors in December 2016.

While she was a graduate student at NC State University she focused a portion of her coursework on geospatial information courses, earning a Graduate Certificate in Geospatial

Information Science. In addition to running an 18 month settlement plate field experiment, she

mentored an undergraduate Doris Duke Conservation Scholar in the completion of a summer

research project. She also presented her preliminary results at the 2019 Benthic Ecology meeting,

in St. Johns, Newfoundland, for which she received partial funding from the Southern

Association of Marine Laboratories Student Support Program. Kayla hopes to start a career in

GIS analysis.

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1. INTRODUCTION

The introduction and establishment of non-indigenous, exotic species is increasing globally in accord with climate change, resulting in alterations to food webs, development and structure of ecological communities, and ecosystem structure and function (Vitousek et al. 1997, Dukes and Mooney 1999, Sakai et al. 2001, Levine et al. 2002, Simberloff et al. 2013, Chan and Briski 2017 and references therein). This increase in the introduction and establishment of exotic species is due to a range of processes, including species range extensions with changing abiotic conditions, such as temperature and rainfall patterns, accidental and intentional introductions of species, and global transportation of humans and products (Ruiz et al. 2000, Kelly and Goulden 2008, Sorte et al. 2010, Rindone and Eggleston 2011, Sardain et al. 2019 and references therein). In marine systems, exotic species are becoming established at exponential rates due to human activities such as shipping, opening and closing of navigational canals, aquaculture, and habitat modification, such as artificial reefs that can serve as "stepping stones" (Ruiz et al. 2000, Williams et al. 2013, Bishop et al. 2017, Sardain et al. 2019).

In a review of exotic species invasions in marine and estuarine systems in North America, shipping was attributed as the mechanism of introduction for over half of all invasions (Ruiz et al. 2000). With increasing globalization and growth, shipping is predicted to increase the probability of marine invasions dramatically over the next 30 years by facilitating the connectivity of different ecosystems and the transport of organisms between them (Sardain et al. 2019). Artificial substrates may also increase the interconnectivity of these environments by increasing settlement space for marine organisms, both native and invasive-in many cases favoring invasive organisms (Glasby et al. 2007, Tyrell and Byers 2007, Airoldi et al 2015, Firth et al. 2016, Bishop et al. 2017). Both the addition of artificial substrates to coastal ecosystems

and the increasing global shipping network facilitate the movement of species across

biogeographical barriers that would otherwise prevent their spread. The combined impacts of

human-mediated changes to ecosystems and their interconnectivity is giving rise to an increased

likelihood of invasions by non-native species. As the risk of invasion increases, it becomes even

more important to understand how exotic species impact the environment and communities they

invade. The marine fouling community has long been considered a model community for

ecological study, and a recent invasion by a marine invertebrate in coastal North Carolina, USA

provides a unique opportunity to determine how this organism is altering patterns of species

diversity and community development.

1.1 The Marine Fouling Community as a Model for Community Ecology

The marine fouling community (hereafter referred to as 'fouling community') has often served as

a model system to experimentally test the mechanisms underlying patterns of community

development (e.g., Sutherland and Karlson 1977, Dean 1981, Cifuentes et al. 2007, Nydam and

Stachowicz 2007, Theuerkauf et al. 2018). The fouling community is composed of species that

colonize hard, subtidal structures (e.g., docks, ship hulls), such as ascidians, bryozoans, hydroids,

and sponges (Nydam and Stachowicz 2007). Community development and structure of the

fouling community is shaped by numerous factors, including: (i) the community is often space

limited, (ii) larval supply varies over time, and (iii) predation often controls survival. Species that

can best take advantage of available space via high growth rates or competitive overgrowth can

be dominant competitors due to the limited space (Nandakumar et al. 1993, Claar et al. 2011,

Lord and Whitlatch 2015). Larval recruitment varies seasonally and inter-annually (Sutherland

and Karlson 1977, Cifuentes et al. 2007, Theuerkauf et al. 2018), and since larvae are often short

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lived and sometimes non-feeding there is no "seed bank" as in many terrestrial communities, such that community structure is often shaped by disturbance date (Sutherland and Karlson 1977, Cifuentes et al. 2007). Predation can shape community structure either by impacting survival of recruits (Osman and Whitlatch 2004, Nydam and Stachowicz 2007), or through preferential consumption of dominant species (Osman et al. 1992, Nydam and Stachowicz 2007).

Disturbances, defined as events that alter interactions between organisms, are a major contributor to patterns of community development. Disturbances can result in a change in available resources such as food, mates, or habitat (White and Pickett 1985). Both biological and physical disturbances are important factors in shaping both terrestrial and marine community structure (Dayton 1971, Grime 1977). In marine communities, predation and competition are sources of biological disturbance, and wave exposure and tropical storms are possible physical disturbances (Connell 1972, Dollar and Tribble 1993). Increasingly, anthropogenic disturbances can impact community structure, especially through the establishment of exotic species (Lambert and Lambert 2003, Bulleri and Chapman 2010). Anthropogenic changes to disturbance regimes may also make ecosystems more susceptible to invasion (Hobbs and Huenneke 1992).

Fifty years ago, Sutherland and Karlson (1977) tested the development and stability of the fouling community in Beaufort, North Carolina, USA to compare and contrast to patterns expected under the terrestrial paradigm of succession (Odum 1969). At the time, succession was thought of as an (1) orderly and directional process in community development that was facilitated by (2) modification of the physical environment, resulting in a (3) climax community (Odum 1969). Sutherland Karlson (1977) manipulated the timing of disturbance by exposing larval settlement plates (terra-cotta tiles) suspended from a dock at different times to test if they ultimately resulted in similar climax communities. They found that succession in the classical

sense does not occur in this system because initial development was variable, residents impeded subsequent development instead of enhancing it, and there was no stable climax community (Sutherland and Karlson 1977). Recently, Theuerkauf et al. (2018) replicated Sutherland and Karlson's study and found that initial community development was still dependent on the date of disturbance that opened-up space for new larval settlers, however long-term development was dominated by a single species, the tunicate *Clavelina oblonga*, which had not been observed historically within the community and resulted in decreased species diversity over time. In this study, we manipulated the abundance of this exotic tunicate to test how patterns of community development compare and contrast with those identified over 50 years ago in the absence of *C. oblonga* (Sutherland and Karlson 1977), and in the presence of *C. oblonga* (Theuerkauf et al. 2018). The present study also takes advantage of two natural disturbances (particularly cold winter and a hurricane) to test how *C. oblonga* and resulting community development responded to these disturbance events.

Clavelina oblonga (Herdman 1880) is a species of tunicate with transparent tunics that forms large, approximately spherical, colonies. It was first described in Bermuda and has been observed in Florida and the West Indies (van Name 1945), and has been documented as an introduced species in Brazil, the eastern Atlantic, and the Mediterranean Sea (Rocha et al. 2012, Ordóñez et al. 2016). It can negatively impact shellfish aquaculture in the Mediterranean through competitive overgrowth of equipment and species of interest (Ordóñez et al. 2016). Historically, *C. oblonga* had not been identified in Beaufort, North Carolina during extensive field surveys of the fouling community (McDougall 1943, Sutherland and Karlson 1977, Karlson and Osman 2012). Recent observations of the fouling community in high salinity areas surrounding Beaufort, NC indicates that *C. oblonga* remains a prominent species, and it has been observed at

high abundance on docks up to ~10km from the DUML, suggesting its presence was not an artifact in the previous study, nor the result of a single recruitment event (Theuerkauf et al. 2018, and see below).

The presence of the Port of Morehead City within this estuarine system may make this community particularly susceptible to invasions due to the high volume of shipping traffic that reaches the state port (Bishop et al 2006). Much of this traffic is large shipping vessels containing ballast water, a potential source of propagule pressure that could lead to the establishment of nonnative species in this community. Transportation of *Clavelina oblonga* on boat hulls has also been suggested as a mechanism for introduction in Brazil (Rocha et al 2012). It seems most likely that *C. oblonga* was introduced to this area rather than established via northward expansion given the short larval distances that are characteristic of many ascidians (Olson 1985) and the successful introduction of *C. oblonga* into other areas (Rocha et al. 2012, Ordóñez et al. 2016).

With the increasing potential of invasion due to global change, there is a growing need to understand the impact of exotic species on the communities they invade. The goals of this study were to 1) examine whether *C. oblonga* is well established within the community and 2) characterize the impacts of *C. oblonga* on development and structure of the fouling community in Beaufort, NC. We sought to determine whether *Clavelina oblonga* is still present and recruiting as previously observed by Theuerkauf et al. (2018) and hypothesized that (H1) *Clavelina oblonga* will exhibit seasonal recruitment patterns that peak during May-July each year. If we determined *C. oblonga* was still present and recruiting, we wanted to know whether the presence of *C. oblonga* would reduce species diversity and whether it would interrupt patterns of succession established by Sutherland and Karlson (1977). We hypothesized that (H2)

species richness will decrease with increasing percent cover of *C. oblonga.* We further

hypothesized that (H3) the presence of *C. oblonga* will result in a distinct fouling community

dominated by this invasive tunicate, whereas multiple distinct communities will be present under

reduced percent cover of *C. oblonga* and these distinct communities will vary over time.

Additionally, over the course of the study two environmental disturbances occurred and we

wanted to evaluate the impact of these disturbances on the fouling community. We hypothesized

that (H4) large-scale environmental disturbances, such as an extremely cold winter or hurricane,

will reduce the cover of *C. oblonga*, resulting in increased species richness and community

states.

2. MATERIALS AND METHODS

2.1 Study System

This study was conducted at the Duke University Marine Lab (DUML) dock in Beaufort, NC.

This dock is located in the Newport River estuary system, less than 3 km from the Beaufort Inlet

where water from the Atlantic Ocean enters the estuary and mixes with water from the Newport

River (Figure 1). The NC State Port of Morehead City is also within 5 km of the DUML dock,

which regularly brings large shipping vessels into the estuary as described above. This area

experiences diurnal tides of approximately 1 meter. Recent data (2017-2018) shows average

monthly water temperature varies from about 7 °C in the winter to 29 °C during the summer

(Figure A.1). During those two years the minimum hourly water temperature reached was about

1 °C (January 8th, 2018) and the maximum was 31°C (June 30th, 2018). Salinity often reaches

seawater salinities and tends to be relatively stable (34 psu; Kirby-Smith and Costlow 1989), but

pH varies significantly (Johnson et al. 2013). The North Carolina coast is often considered the

northern limit of many southern-associated species and the southern limit of many northern

associated species (Karlson and Osman 2012).

2.2 Experimental Design

This experiment replicated the methods outlined in two previous fouling community studies

carried out at the DUML dock (Sutherland and Karlson 1977, Theuerkauf et al. 2018). Terra

cotta settlement plates (232 cm^2) were suspended horizontally approximately 0.3 meters below

the lowest tide level via a center hole using vinyl-wrapped steel cable. The plates were weighted

down to minimize movement within the water column and remained continuously submerged.

The community on the underside of the settlements plates was observed (see sampling

procedures below).

Three experimental treatments were used: (i) Larval Recruitment (LR), (ii) Community

Development (CD), and (iii) Clavelina Removal (CR). Each plate was sampled every 4 weeks,

approximately at the new moon. Four replicates were used in each treatment. All plates were

initially deployed in late spring to ensure *Clavelina oblonga* would be present in the community,

as it had previously been observed to recruit to plates primarily during May – June in this system

(Theuerkauf et al. 2018).

2.3 Characterizing Community Composition

Settlement plates were carefully retrieved from the dock and immediately submerged in

seawater. Plates were then placed in 2 cm of filtered seawater in a plastic tray and a standardized

digital photograph was taken of the community on the underside of the plate using a custom

frame (16.5 cm length x 16.5 cm width; 25 mm focal length). Additional photographs, videos,

and notes were taken to aid in accurate identification of basal species on each plate. Standardized photographs were read into qGIS 2.18.15 software and a grid of points (729) was applied to the photo. A set of 50 random points were then selected out of the grid and the basal species under each point was identified. Different random points were used for each plate and each sample date. The percent cover of each species was quantified by converting point count, the number of times each species was found under one of the 50 points, to percent cover (Sutherland and Karlson 1977). Photographic point count methods are commonly used to reliably assess fouling community composition (e.g., Osman and Whitlatch 2007, Sorte and Stachowicz 2011, Lord et al. 2015). Additional video and photographs of each plate were spatially referenced with the standardized photographs to ensure accurate identification of the species under each point.

Following the methods used by both Sutherland and Karlson (1977) and Theuerkauf et al. (2018), only basal fouling species that exceeded 10% cover at some point in the study were quantified. This excluded extremely rare species and mobile species, except where sessile components of mobile species were present on the settlement plate, i.e. the tubes of *Corophium* sp. Where living species grew on top of one another, both species were noted, sometimes resulting in a percent cover greater than 100 percent. Free space was also quantified as a measure of mortality events for Community Development and Clavelina Removal treatments. Species were identified to the species level where possible using available guides and taxonomic keys (Ruppert and Fox 1988, Pollock 1998).

2.4 Larval Recruitment

To test H1, monthly patterns of larval recruitment were observed by deploying clean settlement plates each sampling period to understand what species were settling on the plates in the absence

of other fouling community members that could compete for space. These plates were deployed every four weeks from May 2017 – September 2018 and sampled every four weeks from June 2017 – October 2018. Four replicates were recorded at all sample dates except September 2018, where one plate was lost.

2.5 Community Development

To test H2 and H3, species diversity and community development were quantified on plates with varying percent cover of *Clavelina oblonga*. Two batches of Community Development plates were deployed at different times: (i) May 2017 – October 2018 (referred to as CD1), and (ii) April 2018 – October 2018 (referred to as CD2). The CD1 batch had three replicates as one was lost shortly after the initial deployment date. Community development plates were sampled every four weeks. CD plates were carefully removed from the dock and photographed as described above for the LR plates, and then carefully returned to the dock with as little disturbance as possible.

2.6 Clavelina Removal

To test the hypothesis that species diversity would increase in the absence of this invasive tunicate (H2), *Clavelina oblonga* was removed from a second set of plates. Two batches of Clavelina Removal plates were deployed and monitored at the same time as the CD1 And CD2 plates described above: (i) May 2017 – October 2018, referred to as CR1 plates in which *Clavelina oblonga* was removed once every 4 weeks, and (ii) April 2018 – October 2018, referred to as CD2 plates in which *Clavelina oblonga* was removed every 2 weeks. The

frequency of *C. oblonga* removal was increased from every 4 to every 2 weeks in an effort to reduce presence of *C. oblonga* to ~0%.

As described above for the LR and CD treatments, plates were removed from the dock and photographed. *Clavelina oblonga* was carefully removed from the community using forceps after photographs and notes were taken. As removing all tissue was nearly impossible, removal was limited to about 1 hour of work or greater than approximately 95% of material removed, which ever was reached first. Percent cover of *C. oblonga* was generally reduced to less than 10%. Due to the ability of *C. oblonga* to regrow from small portions of tunics (see Berrill 1951), it was necessary to repeat this procedure every time the plates were sampled.

3. STATISTICAL ANALYSES

Patterns of larval recruitment and species diversity were visualized using heat maps, as well as time-series of recruitment patterns and percent empty space. A suite of community-level response variables were generated using the DIVERSE module within the software program PRIMER v7 (Clarke and Gorley 2015), including: (i) species richness, (ii) Margalef's index, (iii) Pielou's evenness index, (iv) Shannon diversity index, and (v) Simpson Diversity.

The various species diversity indices all showed the same patterns – when percent cover of *Clavelina oblonga* increased, species diversity declined (see below). Species richness was selected for simplicity in further analyses below. The relationship between increasing percent cover of *C. oblonga* and corresponding changes in species richness (H2) was tested with linear least-squares regression models for the CD and CR treatments separately. Changes in the fouling community as a function of experimental treatment (CD vs CR) and over time were also tested statistically (H3) using non-metric multidimensional scaling (NMDS) ordination, Similarity

profile (SIMPROF), and Similarity percentage (SIMPER) analyses using PRIMER v7 (Clarke and Gorley 2015) (see below).

3.1 Recruitment Patterns

Heat maps were generated to visualize temporal trends in percent cover of fouling species for each of three treatments (LR, CD, and CR). Percent cover data was averaged for each treatment for each sample date, and then graphically represented with a color gradient, where white indicates the absence of a species and black indicates 100 percent cover.

Mean percent cover for each of the three dominant fouling species (*Bugula neritina, Styela plicata, Clavelina oblonga*) were then graphed over time and as a function of the three treatments (LR, CD, and CR) to compare and contrast with patterns observed in Sutherland and Karlson (1977) and Theuerkauf et al. (2018). The hypothesis in this case is that *Clavelina oblonga* recruitment would occur during early- to mid-summer, as observed during a recent two-year study (Theuerkauf et al. 2018). (See below for how patterns were compared with Sutherland and Karlson 1977). Mean percent cover of invasive *C. oblonga*, as well as mean percent free space, was then graphed over time and as a function of treatment (CD, CR) to assess the effectiveness of the CR treatment in reducing *C. oblonga* and making space available for other species to recruit to the plates. We hypothesized that once *C. oblonga* recruited to the plates, the percent cover of the other dominant species would be reduced. Conversely, percent cover of the other dominant species would increase on plates with reduced *C. oblonga*.

3.2 Community Development Patterns

Patterns of community development were evaluated by first comparing species richness between

the CD and CR treatments and over time, and then testing if species richness was a negative function of increasing percent cover of *Clavelina oblonga* using linear least-squares regression models (for CD and CR treatments separately). Next, to test if community development patterns varied with the relative percent cover of *C. oblonga*, we first visualized the CD and CR treatments using non-metric multidimensional scaling (NMDS) ordination (50 random starts) based on Bray-Curtis similarity matrices of the untransformed percent cover data. Untransformed data can effectively evaluate the role of dominant species in a community without overestimating the importance of rare species, as described by Clarke et al. (2014). We hypothesized that in the presence of *C. oblonga*, the fouling communities would coalesce into those dominated by this invasive tunicate (e.g., Theuerkauf et al. 2018), whereas under reduced *C. oblonga* and with increasing time, the fouling communities would reflect the date at which the plates were exposed to potential recruits (e.g., Sutherland and Karlson 1977).

To compare the Community Development (CD1 and CD2) and Clavelina Removal (CR1 and CR2) treatments, Bray-Curtis similarity matrices and NMDS plots were generated for sample periods 3, 6, 9, and 12 months after initial deployment dates. Each NMDS plot has an associated two-dimensional stress value that indicates the amount of mismatch between the two-dimensional NMDS plot and the predicted values from the regression of the similarity matrix. This value represents the accuracy of the two-dimensional plot in capturing the similarity between all points under consideration. Stress values <0.2 indicate the NMDS ordination is generally interpretable, with even smaller stress values indicating a lower risk of misinterpretation.

Hierarchical cluster analysis was then performed to group the plates in each age group (3, 6, 9, or 12 month) into clusters based on similarity of community structure. A cluster analysis

was carried out for each Bray-Curtis similarity matrix using a group average linking method (Clarke et al. 2014). Similarity profile (SIMPROF) analyses were then performed (999 permutations; α= 0.05) to test the null hypothesis that there were no differences in community structure within groups of plates identified in the cluster analyses. The results of the SIMPROF analyses were overlain on the NMDS plots to visualize significant similarities in community structure as a function of treatment (CD vs CR) and time (6, 9, 12 months). Similarity percentage (SIMPER) analyses were used to determine the similarity of plates within a cluster and which species contributed most to that similarity.

3.3 Environmental Disturbances

As described above, there is a rich ecological history of examining the role of environmental disturbances and the resulting changes to the diversity and structure of biological communities (Dayton 1971, Grime 1977, White and Pickett 1985, Dollar and Tribble 1993, Mumby 1999, Bertness et al. 2001 and references therein). During this study, the fouling community experienced two environmental disturbances that appeared to impact the community and percent free space: a severe winter during 2017-18, and Hurricane Florence during September 2018. To assess the impact of these disturbances, we examined the changes in percent cover of species from before to after these events via the recruitment heat maps, and then applied NMDS, cluster analysis, SIMPROF, and SIMPER as described above to test if there were distinct communities BEFORE the disturbance event versus AFTER. For the severe winter analysis, BEFORE was established to be January 2018 as this was before the marked increase in free space on CD plates, and AFTER was established as March 2018 which was the peak in free space on CD plates. For

the hurricane analysis, BEFORE the hurricane was sampled September 8th and 9th, 2018, and AFTER was sampled October 20th and 21st, 2018.

4. RESULTS

4.1 Fouling Community

Over the course of the study, a total of 22 different fouling species/genera were observed (Table 1). Fourteen of these taxa were observed with ≥10% cover. These include a species of tube building polychaete (*Hydroides dianthus*), barnacles (*Balanus spp.*), amphipods (*Corophium* spp.), two bryozoans (*Bugula neritina* and *Schizoporella* sp.), four species of tunicates (*Ascidia interrupta, Clavelina oblonga, Didemnum* sp., and *Styela plicata*), three species of hydroid (*Ectopleura crocea, Eudendrium carneum,* and *Hydratinia* sp.), oysters (*Crassostrea virginica*), and one unidentified encrusting species. Note: recruitment of *Ascidia interrupta* and *Didemnum* sp. was not observed during the course of this experiment but these species were observed as adults. An additional eight species/genera were observed over the course of the study but never reached 10% cover on any plate: *Spirorbus* spp., *Aplidum stellatum*, *Botryllus* sp., *Distaplia bermudensis*, *Perphora veridis*, *Anomia simplex*, *Crepidula fornicata*, and one unidentified red encrusting organism. Of the taxa occurring at ≥10% cover, all were found on both the CD and CR treatments at some point during the study, but CD plates tended to have a lower species richness than the CR plates.

4.2 Recruitment Patterns

Patterns of larval recruitment for the fouling community varied both seasonally and annually (Figure 2a), which is characteristic of this study system (Sutherland and Karlson 1977,

Theuerkauf et al. 2018). A total of 15 different species were observed recruiting to the LR plates (Table 1; Figure 2a). *Clavelina oblonga* settled exclusively during summer months May-August each year, with relatively high recruitment in 2018 compared to 2017 (Figures 2a &3). A few genera, such as *Balanus* sp. and *Corophium* sp., displayed relatively high recruitment through much of the summer and fall months and dropping to almost no recruitment during winter and early spring (Figure 2a). Some species, such as *Bugula neritina, Crassostrea* sp., and *Eudendrium carneum*, had relatively short settlement periods whose timing varied slightly between years. *B. neritina* recruited at highest density in July 2017 but peaked earlier the next year in May 2018. *Crassostrea* sp. recruited almost exclusively in October 2017 and displayed increasing recruitment starting in July 2018 and continuing through October 2018, but at lower densities than in 2017. *E. carneum* had highest recruitment in August 2017 but peaked in recruitment in July at lower density. *Styela plicata* was only observed recruiting in 2017.

In the Community Development treatment, *Clavelina oblonga* rapidly increased in percent cover, reaching almost 75% cover by August 2017 and remained at high percent cover for several months, before declining in March of 2018 to 45% cover (Figure 3b). Percent cover of *C. oblonga* then increased again, reaching 86% cover by June 2018 and remaining relatively high until October, when the impacts of Hurricane Florence reduced cover of *C. oblonga* to almost zero. Percent cover of other prominent species, *Bugula neritina* and *Styela plicata*, remained low (<20%) throughout the course of the experiment on the CD treatment (Figure 3b). In the CR treatment, *Clavelina oblonga* remained a significant part of the community but no longer dominated the community entirely (Figure 2c & 3c). As shown in Figure 3c, percent cover of *C. oblonga* was high seasonally, and when its percent cover was low other species were able to attain higher percent cover in its place.

After the first sampling date, percent cover of *C. oblonga* was consistently higher on the CD treatment than the CR treatment, except towards the very end of the experiment in which the high recruitment of *C. oblonga* during June-August 2018 reduced the distinctions between treatments (Figure 4). Both treatments showed a similar pattern over time, in which percent cover of *C. oblonga* was highest in late summer and fall and declined in winter, and with C. oblonga almost entirely excluded from the CR treatment from December 2017 to May 2018. Percent cover *C. oblonga* was also nearly zero for both treatments in October 2018 after the impact from Hurricane Florence (see below).

Low percent cover of *C. oblonga* correspond to increases in percent free space (Figure 5). Free space was highest for the CD treatment in March 2018 (26%) and October 2018 (21%). Free space was moderately high throughout winter (~13%) on the CR treatment, and also peaked in October 2018 (44%). These patterns of free space also correspond with two environmental disturbances (see below).

4.3 Community Development Patterns

Patterns of community development were dominated by *Clavelina oblonga* over the majority of the study, indicating that it is both still present and present at high abundances as was observed in a previous study (Theuerkauf et al. 2018). The heat maps of CD and CR treatments (Figure 2b,c) qualitatively show the sharp contrast between a community dominated by *C. oblonga* and a community with reduced presence of *C. oblonga*. The CD community (Figure 2b) is dominated by *C. oblonga,* with few other species achieving significant percent cover and only notable seasonal variation in a few species. In contrast, the heat map of the CR treatment (Figure 2c) shows less dominance by a single species, but instead several prominent species. More seasonal

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variability is also present in the CR treatment than the CD. The lack of a single dominant species and the presence of seasonal variation are both historical characteristics of this community described by Sutherland and Karlson (1977).

There was a significant negative relationship between increasing percent cover of *Clavelina oblonga* and diversity of fouling species – the strength of the negative relationship was reduced when the percent cover of *C. oblonga* was experimentally reduced (Figure 7). The percent cover of *C. oblonga* strongly (R^2=0.865) explains variation in species richness for the undisturbed CD treatment, and more weakly (R^2=0.397) explains variation in species richness for the manipulated CR treatment. The difference between treatments is likely a result of the experimental manipulation and may also be influenced by the lack of high percent cover data points for the CR treatment. The strong negative relationship shown by the CD treatment clearly illustrates that dominance of *C. oblonga* depresses the species diversity of this community.

Community development varied as a function of experimental treatment and time. For example, three months after plates were deployed, there were three distinct fouling communities when percent cover of *C. oblonga* was experimentally reduced, and a fourth distinct community in the presence of high percent cover of *C. oblonga* (Figure 8a). This pattern of distinct community states depending upon the date the plates were deployed, and the reduced percent cover of *C. oblonga*, is similar to patterns of alternative community states identified by Sutherland and Karlson (1977) in the mid-1970s. Six months after plates were deployed, the number of distinct communities had reduced to three and the distinction between CD versus CR plates had increased (compare Figures 8a vs. 8b). Nine months after plates were deployed, the distinction between fouling communities on CD versus CR plates was even more apparent (compare figures 8a, b, and c). Conversely, 12 months after the plates were deployed, the plates

were shuffled between the two distinct community states, with the more common community dominated by *C. oblonga* (Figure 8d). This seems to be the result of *C. oblonga* recovering after the pressure of low winter temperatures was released and regaining space within the community.

4.4 Environmental Disturbances

Two large-scale environmental disturbances, in which free space exceeded 20% for at least one treatment (Figure 3), were observed during this experiment. The first occurred in March 2018, which seemed to be the result of organisms sloughing off after the harsh winter of 2017 -18 where water temperatures reached as low as 1 °C. In the last 25 years, minimum winter temperatures have ranged from 0.9 °C to 8.1 °C, but were only below 2°C in three of the last 25 years. This seems to indicate that the winter of 2017-18 reached lower temperatures than most winters but is not atypical. The harsh winter reduced overall recruitment on the LR treatment to almost zero from January 2018 through April 2018 (Figure 2a). In the community dominated by *Clavelina oblonga* (CD, Figure 2b), percent cover of *C. oblonga* was notably depressed, with slight increases in percent cover of many other species. In the experimentally manipulated treatment (CR, Figure 2c), percent cover of *C. oblonga* was low before free space peaked. Other prominent species in the community declined in March 2018, including *Styela plicata* and *Bugula neritina*. Two species seemed to take advantage of the available free space: *Balanus* sp. and *Didemnum* sp. increased in percent cover. Community development varied BEFORE and AFTER the extreme winter temperatures (Figure 9). Prior to the winter disturbance, the CD treatment had a single, distinct community. After the disturbance, this community became two distinct communities that were also distinct from their prior state. The CR treatment was composed of two distinct communities prior to the winter disturbance and three communities

after the disturbance, but the BEFORE and AFTER communities had significant overlap. This may indicate that the *Clavelina oblonga* dominated community is less resilient to environmental disturbance than the community with reduced presence of *C. oblonga.*

The second disturbance occurred in October 2018, in which percent free space increased sharply as a result of the passage of hurricane Florence. The passage of hurricane Florence appeared to reduce the cover of all species except barnacles (*Balanus* sp.) (Figure 2a, b, c, compare September versus October 2018). Disturbance from the passage of hurricane Florence increased species diversity. For example, species with relatively high percent cover in September were greatly reduced, and in some cases new species were able to successfully recruit (Figure 2, compare September vs. October 2018). Community development also varied from BEFORE to AFTER the hurricane. For example, prior to the hurricane the community had a single, distinct state dominated by the high and consistent recruitment of *Clavelina oblonga* during June – August 2018 as described above, whereas after the hurricane the community displayed four distinct states (Figure 10).

5. DISCUSSION

Exotic species are continually introduced in most regions of the world, yet all do not become established. The results from this study indicate that the exotic tunicate, *Clavelina oblonga* is established in high salinity (> 25 ppt) estuarine waters in the Beaufort/Morehead City area of North Carolina, USA with significant impacts on the development and structure of the local fouling community. For example, similar to other exotic species that compete for space in aquatic systems, such as zebra mussel in the Great Lakes (Johnson and Carlton 1996) and Asian clam in San Francisco Bay (Carlton et al. 1990), *C. oblonga* displays a remarkable ability to

completely dominate available space in the fouling community, resulting in decreased species diversity and a distinct community dominated by this invasive tunicate (Theuerkauf et al. 2018, this study).

The ability to replicate exactly the community ecology experiments conducted over 50 years ago by Sutherland and Karlson (1977), and recently by Theuerkauf et al. (2018), yet with the addition of a Clavelina Removal treatment, allow us to advance our understanding of marine community ecology by testing if the concept of multiple community states identified over 50-years ago by Sutherland and Karlson (1977) are still operable in the absence of invasive *C. oblonga*. Moreover, the presence of two, large-scale environmental disturbances (severe winter; hurricane) during the course of this experiment highlight how the environment could moderate the spread of this invasive tunicate. In this study, under conditions of reduced percent cover of *C. oblonga*, the local fouling community displayed unique community states that became even more distinct over time (3 vs. 6 vs. 9 months), consistent with the pattern of multiple community states identified by Sutherland and Karlson (1977) that were dependent upon date of disturbance (i.e., date when plates were exposed to the available larval pool). A feature of successful invaders in aquatic systems in their ability to swamp local communities with consistently high larval supply (Chan and Briski 2017). That appeared the case in this study, in which incredibly high recruitment of *C. oblonga* in 2018 appeared to swamp even the Clavelina removal treatments, such that community development after 12 months coalesced into a single community state dominated by C. oblonga. Several large-scale environmental disturbances may reduce the spread of *C. oblonga*: (i) extremely cold winters, and (ii) hurricanes and tropical cyclones (see below).

5.1 Mechanisms of Invasion by *Clavelina oblonga*

Clavelina oblonga likely established itself within this system due to high propagule pressure

from the nearby Port of Morehead City, which handles a high volume of shipping traffic (Bishop

et al. 2006). *C. oblonga* originates from tropical waters and was likely transported here from

Brazil or other parts of the western Atlantic that it has previously colonized. Whether propagule

pressure was due to ballast water, as is commonly reported of invasive species in the literature

(Carlton and Geller 1993, Chan and Briski 2017 and references therein), or via transport on boat

hulls, as suggested by Rocha et al (2012), remains to be determined.

Two characteristics of *Clavelina oblonga* likely contributed to its success in this

community after its introduction: its ability to rapidly grow and overtake space, and its intense

yearly recruitment. Other exotic tunicates, such as *Ciona intestinalis*, also display strong

seasonal recruitment and rapid grow rates as methods of invasion (Ramsay et al 2009). *C.

oblonga*'s rapid growth, and its remarkable ability to regrow after being almost entirely removed

from the community, seem to imply its dominance in this community is achieved via competitive

overgrowth rather than predation release.

Clavelina oblonga's marked seasonal recruitment may also have facilitated its invasion

of this community. Seasonality in recruitment and high density of *C. oblonga* has been observed

in other areas (Casso et al 2018, Ordóñez et al 2016), which supports the idea that this is a

typical characteristic of the species and not an artifact of the present study. The intense summer

recruitment coupled with the seasonal variability of the environment likely allowed the

establishment of *C. oblonga* in this community, as has been seen with other ascidians (Bullard et

al. 2013, Astudillo et al 2016, Chan and Briski 2017). Many invasive fouling species exhibit

increased recruitment and or growth at higher temperatures (Djikstra et al. 2011, Lord 2017), so

the early summer recruitment of *C. oblonga* allows it to take advantage of highest seasonal growth potential before less than ideal winter conditions limit its growth and survival.

5.2 Role of Invasive Species in Altering Patterns of Community Development

Invasive species alter patterns of community development by interrupting the normal interactions of species within the community and with the environment. In this community, *Clavelina oblonga* quickly monopolizes resources after settlement, altering recruitment patterns of other species and ultimately community development. This pattern is consistent with other studies that have found fouling organisms which are able to monopolize resources and persist through time alter patterns of recruitment and community assembly (Vieira et al. 2018). This is also seen in other communities, such as the deserts of North America where invasive grasses change patterns of succession by increasing fire frequency and subsequently out-competing native species for resources. (Brooks and Pyke 2002). The resulting decrease in species diversity disrupts the patterns of community development that were driven by the diverse assemblage of species in the community (Brooks and Pyke 2002). When the presence of *C. oblonga* in the fouling community is experimentally reduced, the community seems to return to previously observed patterns of succession: the long-term (3-9 month) community is composed of a heterogeneous mixture of species that varies over time, with increasing diversity over time (Sutherland and Karlson 1977). In other communities, such as the native ant community in northern California, the addition of an invader not only reduced species diversity, but also broke down patterns of community assembly (Sanders et al. 2003). This is further evidence that the breakdown of patterns of community development and organization is a common result of invasive species.

Clavelina oblonga alters patterns of community development by disrupting the normal disturbance regime of the community, in which large adults frequently senesce from the community opening up new space for settlement. Many terrestrial cases of invaders altering disturbance regimes and the concomitant changes to community development exist (Mack and D'Antonio 1998, and references therein). Some plant invaders, for example, alter fire regimes by increasing or decreasing the volume of fuel in the environment, leading to modified communities due to altered disturbance regimes (Mack and D'Antonio 1998, Brooks and Pyke 2002). One of the most frequent disturbances in the fouling community shapes patterns of community development directly: the regular loss of adult organisms as they senesce, which opens free space for other organisms to settle and grow. This normally results in a diverse assemblage of species that varies over time, but in the presence of *C. oblonga* this process is halted and the tunicate remains dominant. Since the frequent, regular disturbances are now prevented from occurring, the community is tending towards lower species diversity, as would be predicted by the intermediate disturbance hypothesis (Connell 1978). Infrequent, large-scale disturbance may have a greater importance in maintaining this ecosystem in the presence of *C. oblonga*, but these disturbances may also increase the risk of other invasions (Hobbs and Huenneke 1992).

5.3 Role of Abiotic and Biotic Processes in Controlling the Spread of Exotic Species

A number of abiotic and biotic factors impact the spread of invasive species. In the case of *Clavelina oblonga,* it appears that abiotic factors are more successful in limiting its dominance within the community. The thermal range of our study system may be a limiting factor to the spread of *C. oblonga*: during the winter months, percent cover of *C. oblonga* rapidly declined and did not recover until temperatures increased. Many invasive species in the fouling

community, especially tunicates, exhibit increased growth with increased temperature, and therefore decreased growth at low temperatures (Djikstra et al. 2011, Lord 2017). The strong effect of winter on the naturally *C. oblonga* dominated community follows this pattern and may indicate that it is at or near the northern limit of its potential range. This idea aligns with the common understanding that coastal North Carolina is the upper limit of many tropical species: even though *C. oblonga* is not native to this area, this environment may represent the upper limit of its biological tolerances (Karlson and Osman 2012). The effects of winter also likely limit the dominance of *C. oblonga* in this community as other species have the opportunity to regain space within the community during the coldest parts of the year. Similarly, minimum winter temperature has been used to map potential ranges of invasive plants (Gassó et al. 2012), and has been found to limit the spread of the invasive hemlock woolly adelgid (Paradis et al. 2008).

Timing of substrate availability is likely another limiting factor in the spread of *Clavelina oblonga* and may prevent this system from becoming a monoculture in the long term. For example, in plant communities, success of an invader depends on the mechanisms by which it impacts the community and the successional stage of the invaded plant community: if these two factors are not aligned correctly it is less likely that the invader will succeed (Catford et al. 2012). While the priority effect (see Munguia et al. 2010) may not limit the presence of *C. oblonga* in the community, large disturbances may do so instead. Large-scale disturbance events, such as severe winter temperatures or tropical storms, may reduce the abundance of *C. oblonga* while simultaneously making substrate available for other species to recruit, allowing other species to persist in the community. These effects are likely strengthened by the seasonal recruitment pattern of *C. oblonga*: since it primarily recruits during a short window, any large disturbance outside of that window may favor other species in the community.

Two common biotic factors that limit the spread of invasive species are competition and predation (Weis 2011, Bando 2006). For example, the range of the introduced European green crab in eastern North America is limited by predation by the native blue crab (deRivera et al. 2005). Some species of non-native tunicates, such as *Ciona intestinalis* and *Didemnum vexillum,* are limited by predation while others, such as *Botrylloides violaceus*, are not (Cahill et al 2010, Dumont et al. 2011, Forrest et al. 2013). *Clavelina oblonga* does not appear to be predation limited in this system; even if predators are present, its high growth likely allows it to compensate and remain dominant. For example, although predation on colonial ascidians can reduce their overall colony size, their colonial life style actually limits the role of predation in restricting their occurrence (Hiebert et al. 2019). The impacts of biotic control processes may change over time if *C. oblonga* remains abundant in the system. Since this is a relatively recent introduction, there is the potential that predators in the area will eventually begin to show a pattern of preferential predation of *C. oblonga* as the dominant species and therefore the most plentiful prey item (Osman et al. 1992, Nydam and Stachowicz 2007, Pratt and Grason 2007).

Competition also does not seem to play a significant role in controlling the spread of *C. oblonga* since it dominates the community so thoroughly. As discussed above, *C. oblonga* is likely a strong competitor in this community due to its intense seasonal recruitment, rapid growth, and potential to recover from substantial damage. The combination of these characteristics likely makes it difficult for other species in the community to successfully compete with and limit the growth of *C. oblonga*. This may be a result of confounding factors: in a study comparing the success of a non-native and native seagrasses it was found that the non-native species was successfully limited by competition from the native species, but only in the absence of disturbance (Bando 2006). It could be that in the case of *C. oblonga,* unknown

factors, such as altered disturbance regimes, sub-ideal environmental conditions, or increased predation, prevent other species in the community from limiting its dominance via competition.

5.4 Conclusions

The recent invader *Clavelina oblonga* remains a dominant species in the fouling community. It likely reached this community via shipping activities and was able to become established due to its high seasonal recruitment and rapid growth. Its presence and proliferation within this community has altered patterns of community development by monopolizing space for settlement of other organisms, thereby reducing species richness. Large-scale environmental disturbances appear to be the only factor controlling the dominance of *C. oblonga*. Further study is needed to determine how *C. oblonga* will impact the community in the long term, whether it will cause local extirpation of native species or simply reduce abundances, or whether the large scale disturbances that seem to control the spread of *C. oblonga* may make the community vulnerable to other invasions.

Table 1: Total species presence over the course of the study as a function of three experimental treatments: (i) Larval Recruitment, (ii) Community Development, and (iii) Clavelina Removal. Two additional species were unable to be classified.

| Species	Larval Recruitment	Community Development	Clavelina Removal
Annelida			
Hydroides dianthus	x	x	x
Spirorbus spp.	x		
Arthropoda			
Balanus spp.	x	x	x
Corophium sp.	x	x	x
Bryozoa			
Bugula neritina	x	x	x
Schizoporella errata	x	x	x
Chordata			
Aplidum stellatum			x
Ascidia interrupta		x	x
Botryllus sp.	x		
Clavelina oblonga	x	x	x
Didemnum sp.		x	x
Distaplia bermudensis			x
Perphora veridis	x	x	x
Styela plicata	x	x	x
Cnidaria			
Ectopleura crocea	x	x	x
Eudendrium Carneum	x	x	x
Hydractinia sp.	x	x	x
Mollusca			
Anomia simplex	x		
Crassostrea sp.	x	x	x
Crepidula fornicata		x	x
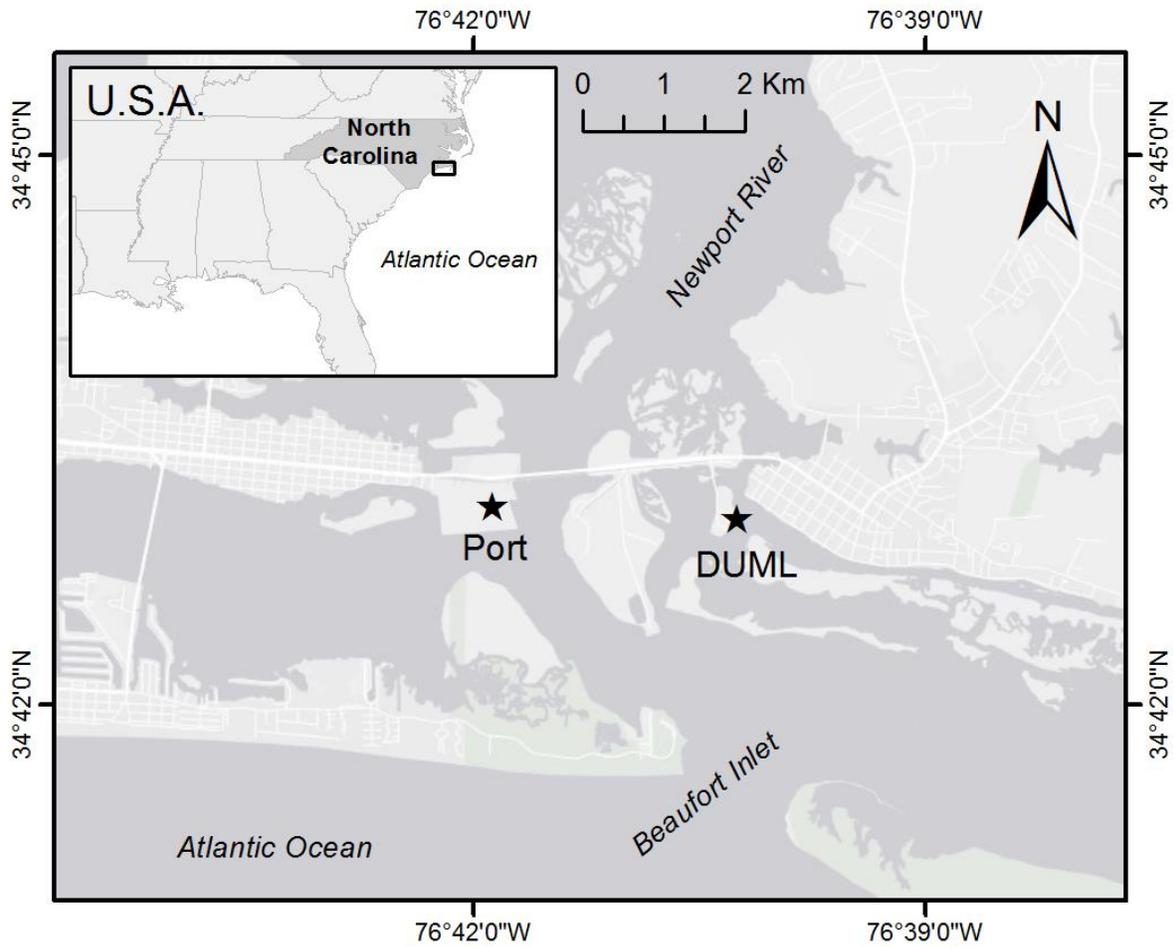
Figure 1: Location of the study site at the Duke University Marine Lab (DUML) dock in Beaufort, NC, USA, with the Port of Morehead City and neighboring bodies of water for reference.
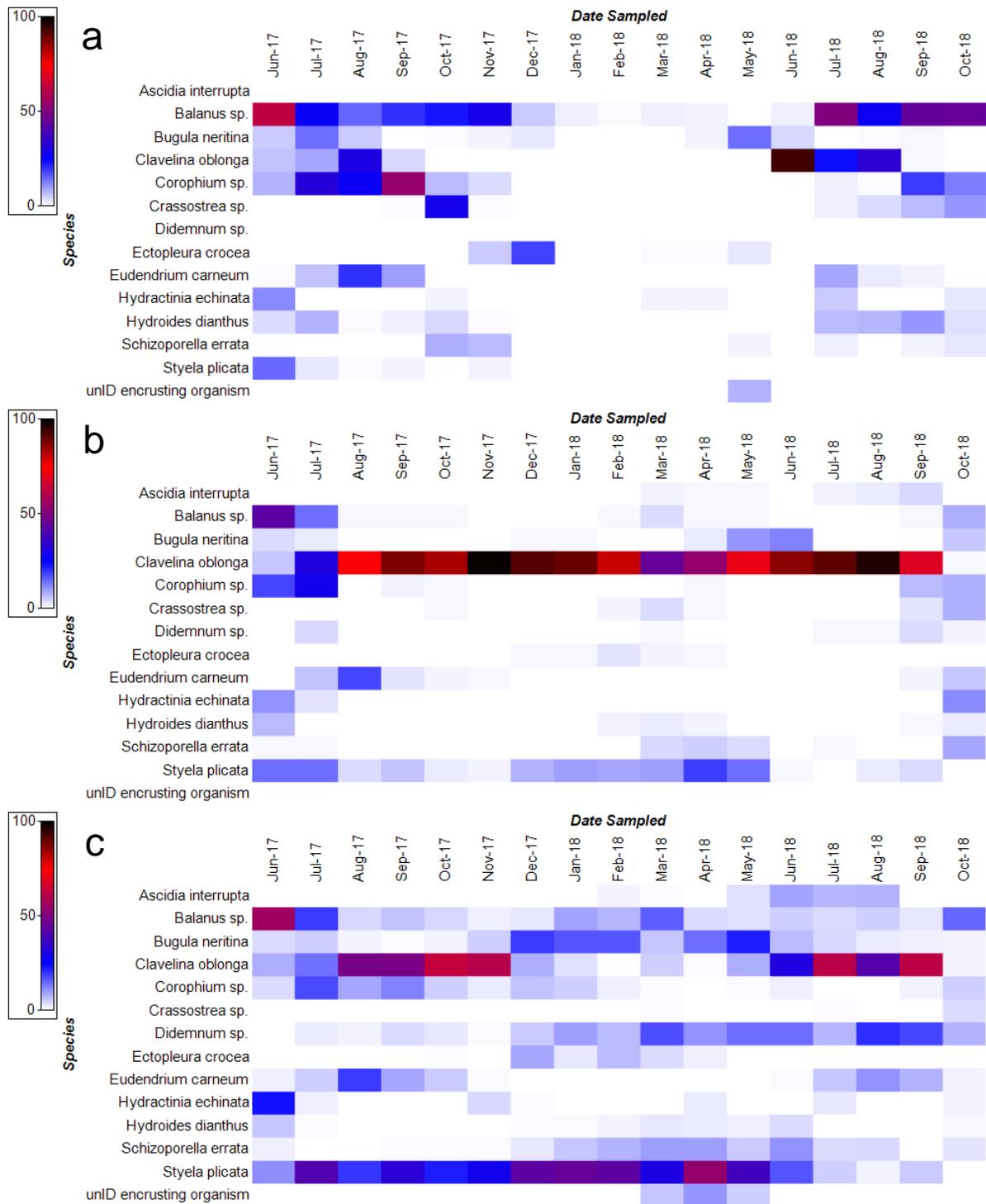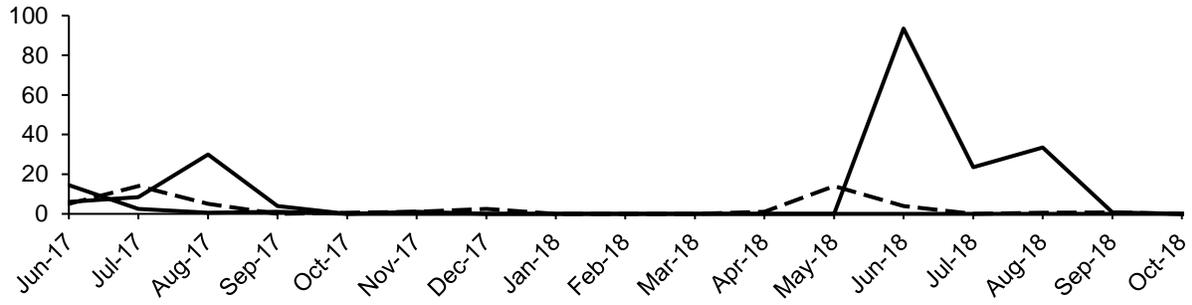
Figure 2: Heat map of average percent cover of species present on (a) Larval Recruitment, (b) Community Development, and (c) Clavelina Removal plates sampled June 2017 through October 2018. Black indicates maximum abundances (100% average cover) and white indicates absence of a species (0% average cover).

a) Larval Recruitment

b) Community Development
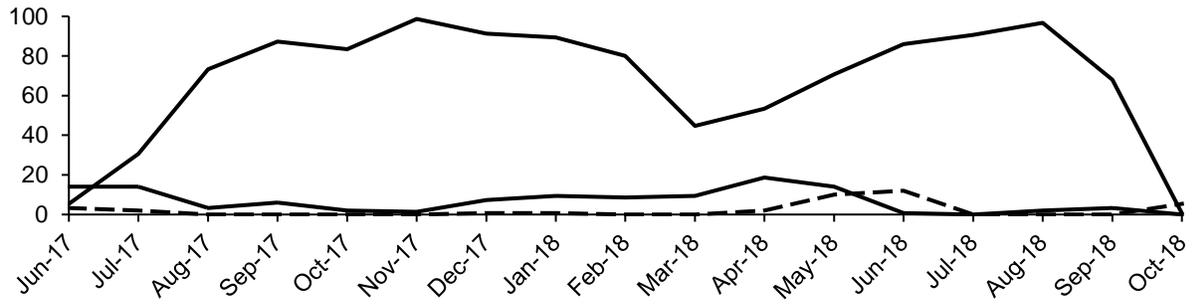
c) Clavelina removal
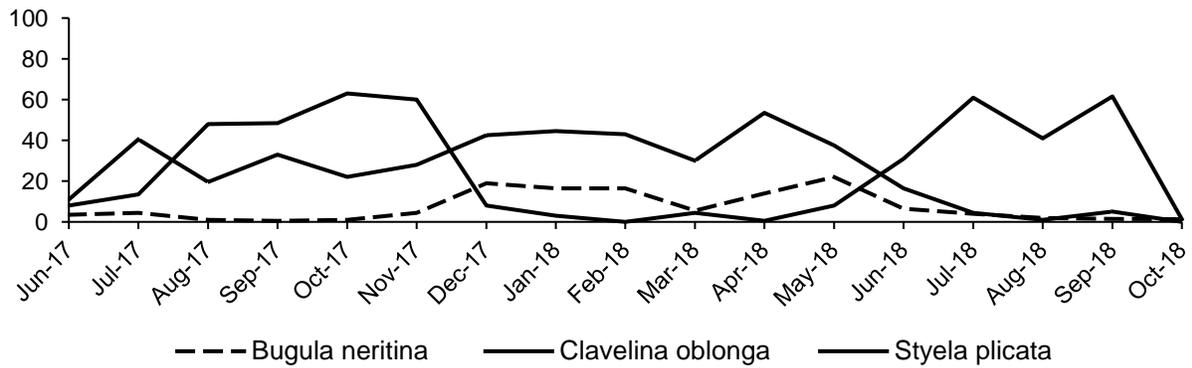
- - - Bugula neritina ——— Clavelina oblonga ——— Styela plicata

Figure 3: Mean percent cover (N = 3-4 for a given sample date) of three of the most abundant species on a) LR, b) CD and c) CR plates across sampling period. Species include *Bugula neritina*, *Clavelina oblonga*, and *Styela plicata*. *B. neritina* and *S. plicata* were chosen as a means to compare against patterns shown in Sutherland and Karlson (1977).
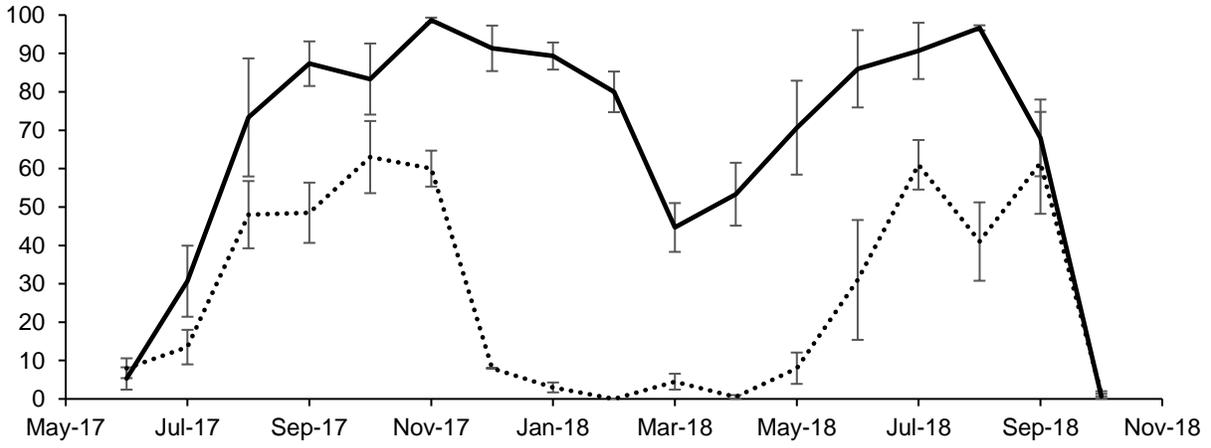
Figure 4: Mean percent cover of *Clavelina oblonga* (+/- SE; N = 3-4 for a given sample date) for Community development (CD1, solid) and Clavelina Removal (CR1, dashed) treatments for June 2017 to October 2018.
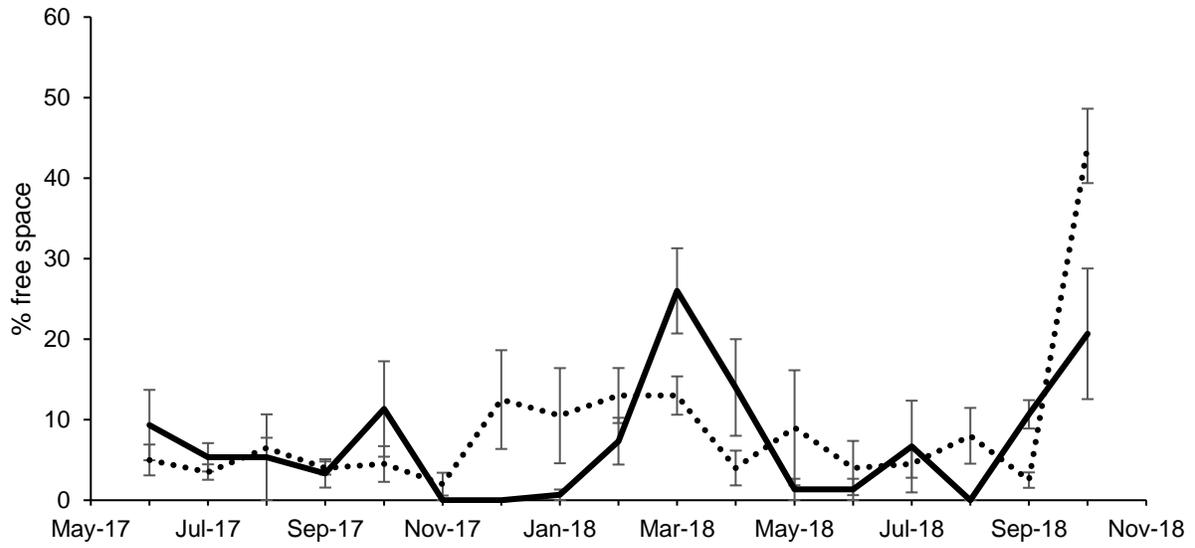
Figure 5: Mean percent free space (+/- SE; N = 3-4 for a given sample date) for Community development (CD1, solid) and Clavelina Removal (CR1, dashed) treatments for June 2017 to October 2018.
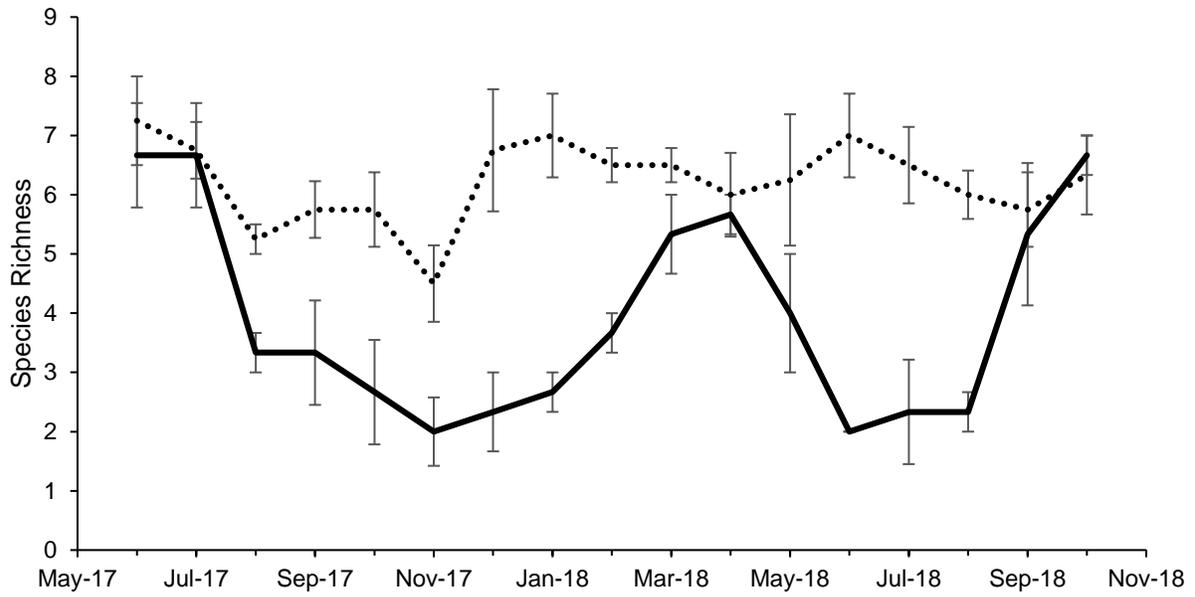
Figure 6: Mean species richness (+/- SE) calculated from average percent cover (N = 3-4 for a given sample date) for Community Development (CD1, solid) and Clavelina Removal (CR1, dashed) treatments.
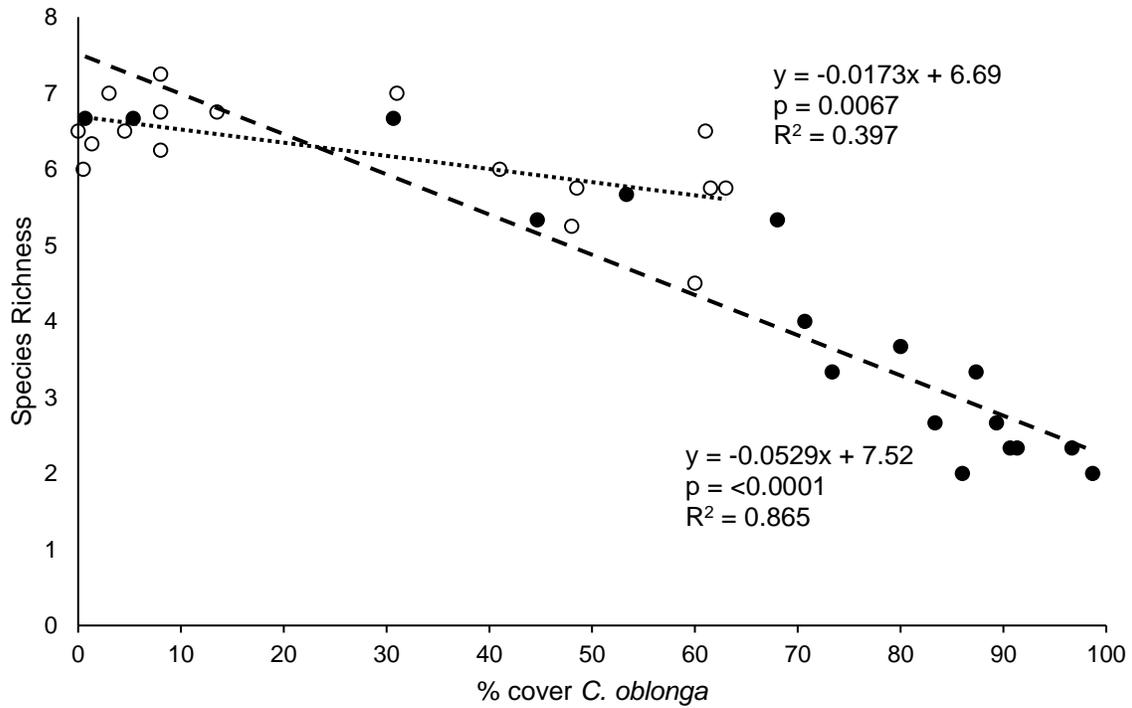
Figure 7: Regression of average percent cover of *C. oblonga* and species richness for Community Development (CD1, solid circles, dashed line) and Clavelina Removal (CR1, empty circles, dotted line) treatments. CD1: y = -0.0166x = 1.82, p = <0.0001, R^2 = 0.9477. CR1: y = -0.00578x + 1.49, p = 0.0003, R^2 = 0.5885.
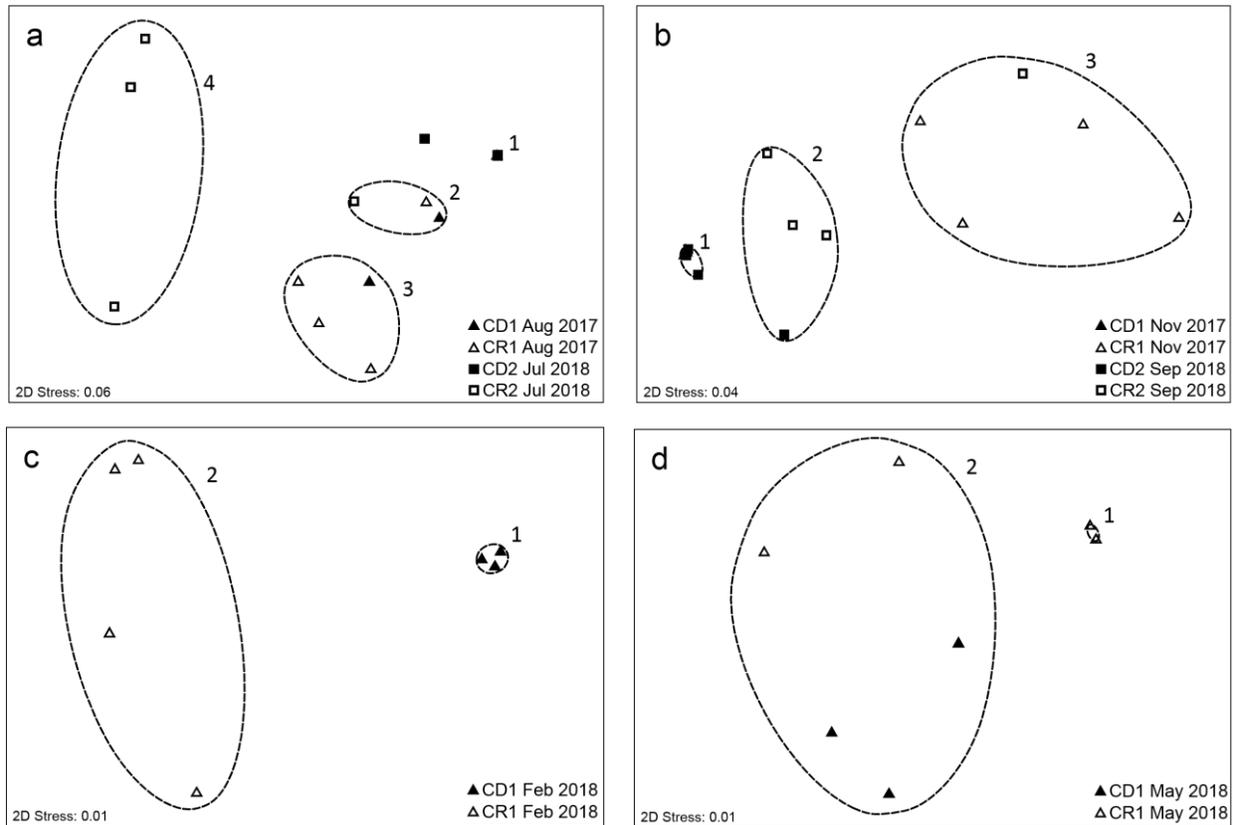
Figure 8: Nonmetric, multidimensional scaling plots depicting Community Development and Clavelina Removal treatments at a) 3 months, b) 6 months, c) 9 months, and d) 12 months after deployment of plates. Ellipses show result of SIMPROF analyses, indicating significant differences in community structure (alpha=0.05). Ellipsis numbers correlate to clusters in SIMPER results (Table A.1).
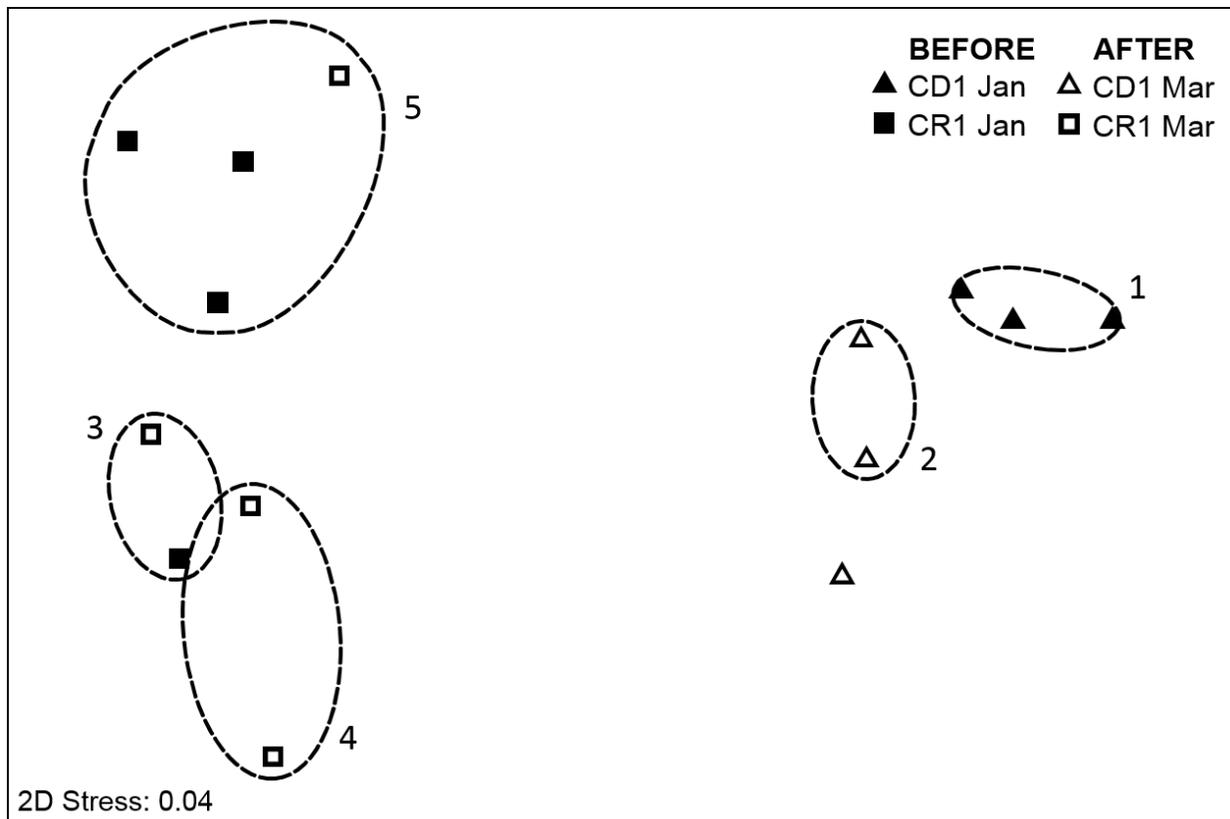
Figure 9: Nonmetric, multidimensional scaling plot depicting all plates sampled BEFORE (January 2018) and AFTER (March 2018) winter-associated percent cover decrease occurred. Ellipses show result of SIMPROF analyses, indicating significant differences in community structure (alpha=0.05). Ellipsis numbers correlate to clusters in SIMPER results (Table A.2).
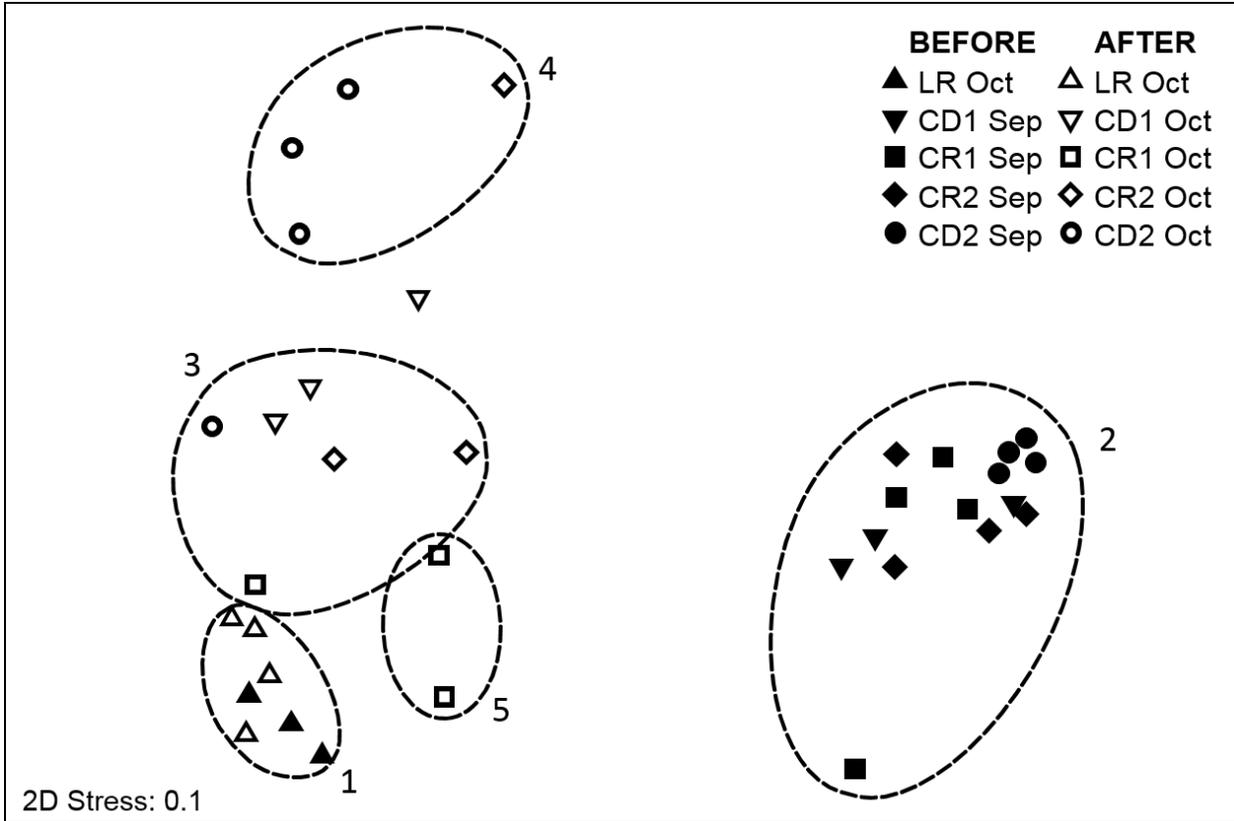
Figure 10: Nonmetric, multidimensional scaling plot depicting all plates sampled BEFORE (September 2018) and AFTER (October 2018) Hurricane Florence. Sampling dates were approximately 1 week prior and 5 weeks post Hurricane Florence. Ellipses show result of SIMPROF analyses, indicating significant differences in community structure (alpha=0.05). Ellipsis numbers correlate to clusters in SIMPER results (Table A.3).

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APPENDIX

Table A.1: Results from SIMPER analyses that list percent similarity and rank the three most influential species that contributed to the composition of the unique assemblages identified on the NDMS plots of 3, 6, 9, and 12 months of development of settlement plates, shown in figure 8. Clusters composed of a single plate are excluded.

Cluster	Average Similarity (%)	Three Most Influential Species	Average Percent Cover in Cluster (%)	Percent Contribution to Similarity (%)	Cumulative Contribution to Similarity (%)
3 months					
1	94.25	*Clavelina oblonga*	47.25	98.56	98.56
		Ascidia interrupta	1.75	1.44	100.00
		N/A	N/A	N/A	N/A
2	83.35	*Clavelina oblonga*	35.33	81.81	81.81
		Eudendrium carneum	7.67	15.55	97.36
		Balanus spp.	1.00	0.91	98.27
3	71.83	*Clavelina oblonga*	20.25	54.26	54.26
		Eudendrium carneum	12.25	30.20	84.46
		Styela plicata	10.25	10.87	95.33
4	46.72	*Clavelina oblonga*	6.33	43.03	43.03
		Eudendrium carneum	4.00	18.01	61.04
		Schizoporella errata	4.33	10.98	72.01
6 months					
1	96.43	*Clavelina oblonga*	48.67	99.04	99.04
		Styela plicata	0.67	0.82	99.86
		Eudendrium carneum	0.33	0.14	100.00
2	86.16	*Clavelina oblonga*	40.00	94.07	94.07
		Styela plicata	3.00	2.72	96.79
		Eudendrium carneum	1.25	1.56	98.35
3	79.17	*Clavelina oblonga*	29.80	67.90	67.90
		Styela plicata	13.80	28.80	96.70
		Hydractinia echinata	1.60	1.04	97.74

Table A.1 (continued).

9 months					
1	85.90	*Clavelina oblonga*	40.00	91.00	91.00
		Styela plicata	4.33	8.19	99.19
		Ectopleura crocea	1.33	0.81	100.00
2	59.74	*Styela plicata*	21.50	67.24	67.24
		Bugula neritina	8.25	15.89	83.12
		Balanus spp.	3.50	6.06	89.19
12 months					
1	93.58	*Styela plicata*	31.00	60.78	60.78
		Bugula neritina	17.00	31.37	92.16
		Didemnum sp.	4.00	7.84	100.00
2	46.34	*Clavelina oblonga*	24.20	60.97	60.97
		Styela plicata	6.80	19.65	80.61
		Bugula neritina	5.00	11.86	92.47

Table A.2: Results from SIMPER analyses that list percent similarity and rank the three most influential species that contributed to the composition of the unique assemblages identified on the NDMS plot of settlement plate communities before and after the impacts of winter, shown in figure 9. Clusters composed of a single plate are excluded.

Cluster	Average Similarity (%)	Three Most Influential Species	Average Percent Cover in Cluster (%)	Percent Contribution to Similarity (%)	Cumulative Contribution to Similarity (%)
1	91.35	*Clavelina oblonga*	44.67	93.41	93.41
		Styela plicata	4.67	6.59	100.00
		N/A	N/A	N/A	N/A
2	74.67	*Clavelina oblonga*	25.50	89.29	89.29
		Styela plicata	5.50	10.71	100.00
		N/A	N/A	N/A	N/A
3	72.29	*Styela plicata*	13.00	33.33	33.33
		Balanus spp.	9.50	20.00	53.33
		Schizoporella errata	7.50	20.00	73.33
4	69.47	*Didemnum* sp.	15.00	39.39	39.39
		Balanus spp.	11.50	27.27	66.67
		Styela plicata	8.50	15.15	81.82
5	68.37	*Styela plicata*	26.50	69.23	69.23
		Bugula neritina	8.50	14.87	84.10
		Didemnum sp.	4.25	5.78	89.88

Table A.3: Results from SIMPER analyses that list percent similarity and rank the three most influential species that contributed to the composition of the unique assemblages identified on the NDMS plot of settlement plate communities before and after the impacts of Hurricane Florence, shown in figure 10. Clusters composed of a single plate are excluded.

| Cluster	Average Similarity (%)	Three Most Influential Species	Average Percent Cover in Cluster (%)	Percent Contribution to Similarity (%)	Cumulative Contribution to Similarity (%)
1	76.33	*Balanus* spp.	22.14	65.47	65.47
		Corophium sp.	7.71	17.51	82.98
		Crassostrea sp.	4.29	10.03	93.01
2	71.98	*Clavelina oblonga*	37.20	91.88	91.88
		Styela plicata	2.80	2.99	94.87
		Didemnum sp.	3.13	1.55	96.42
3	55.53	*Balanus* spp.	9.67	45.45	45.45
		Hydractinia echinata	4.50	16.69	62.14
		Schizoporella errata	6.67	13.43	75.57
4	53.18	*Bugula neritina*	13.75	47.53	47.53
		Hydractinia echinata	11.00	39.17	86.70
		Schizoporella errata	4.50	7.56	94.26
5	42.11	*Balanus* spp.	5.00	50.00	50.00
		Corophium sp.	2.50	12.50	62.50
		Clavelina oblonga	1.00	12.50	75.00
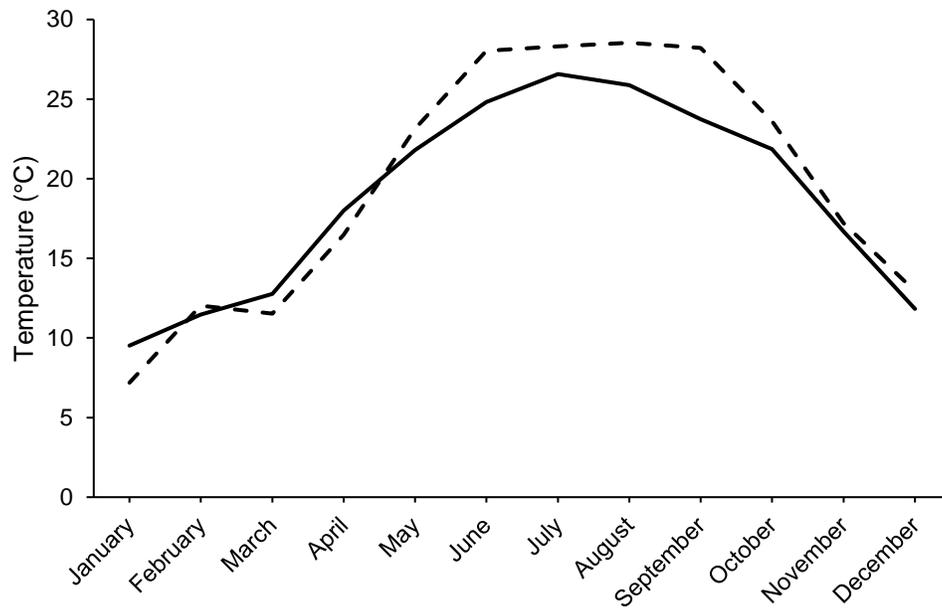
Figure A.1: Average monthly water temperature for 2017 (solid) and 2018 (dashed) at the DUML dock. Data from NOAA tide station 8656483.