Testing ecological theories in the Anthropocene: alteration of succession by an invasive marine species

KAYLA A. CHRISTIANSON AND DAVID B. EGGLESTON†

Department of Marine, Earth, and Atmospheric Sciences, Center for Marine Sciences and Technology, North Carolina State University, Morehead City, North Carolina 28557 USA

Abstract. Ecologists employ a diverse body of theory to help explain patterns and processes in ecological systems, with the consistency of ecological theories tested against an increasingly altered world. 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. Recently, a species of colonial tunicate not previously present, *Clavelina oblonga,* has become prominent within the marine fouling community of Beaufort, North Carolina, USA. Fifty years ago, researchers tested theories of ecological succession developed in terrestrial systems and found that this marine community was characterized by a heterogeneous mixture of species that varied inter-annually (i.e., multiple community states) and increased in diversity over time. A recent follow-up study found that the fouling community is 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. The present study addressed the question: Will patterns of community development identified over 50 yr ago still operate under reduced cover of invasive *C. oblonga*? This study also quantified the impacts of two large-scale environmental disturbances (extremely cold winter and hurricane) on the abundance of *C. oblonga* and the resulting fouling community. *Clavelina oblonga* remains a dominant component of the fouling community due to its rapid growth and strong seasonal recruitment. Under conditions of reduced percent cover of *C. oblonga*, the local fouling community displayed unique community states that became even more distinct over time, consistent with the pattern of multiple community states identified by researchers over 50 yr ago, and that were dependent upon date of disturbance. Natural disturbances in this study caused by a harsh winter and a hurricane greatly reduced the presence of *C. oblonga*. This experiment advanced our understanding of marine community ecology by testing whether the concept of multiple community states identified over 50 yr ago is still operable in the absence of invasive *C. oblonga,* and highlights how natural environment disturbances can potentially moderate the spread of this invasive tunicate.

Key words: biodiversity; *Clavelina oblonga*; disturbance; experimental manipulation; fouling community; invasive species; marine community ecology; tunicate.

† **E-mail:** eggleston@ncsu.edu

INTRODUCTION

Ecological theory encompasses concepts and predictive models that help explain patterns and processes in ecological systems (Levin 1981, Palmer et al. 2004, May and McLean 2007, Jiang and DeAngelis 2013 and references therein), with the consistency of ecological theories tested against an increasingly altered world (Vitousek et al. 1997, Malhi et al. 2020). The global redistribution of species is a prominent impact of climate change and human-mediated biological invasions, 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). The 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). In marine systems, invasive 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 species invasions in marine and estuarine systems in North America, shipping was listed 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 yr 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, and in many cases favoring invasive organisms (Glasby et al. 2007, Tyrrell and Byers 2007, 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 are 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 invasive species impact the environment and communities they invade. 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.

The marine fouling community (hereafter referred to as "fouling community") has often served as a model system to experimentally test mechanisms underlying patterns of community development (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 the following: (1) The community is often space limited, (2) larval supply varies over time, and (3) 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 interannually (Sutherland and Karlson 1977, Cifuentes et al. 2007, Theuerkauf et al. 2018), and since larvae are often short-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 with 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 and Karlson (1977) manipulated the timing of disturbance by exposing larval settlement plates (terracotta tiles) suspended from a dock at different times to test whether 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 and no multiple community states. In this study, we tested whether community development patterns under reduced *C. oblonga* cover would result in multiple community states and increasing species diversity over time, as demonstrated originally by Sutherland and Karlson (1977). The present study also takes advantage of two natural disturbances (particularly cold winter and a hurricane) to test how *C. oblonga* and resulting patterns of 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 (NC), 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, indicate that *C. oblonga* remains a prominent species, and it has been observed at high abundance on docks up to ~10 km from the site of the original studies (Sutherland and Karlson 1977), suggesting its presence was not an artifact in the previous study, nor the result of a single recruitment event (Theuerkauf et al. 2018).

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 non-native species in this community. Transportation of *C. oblonga* on boat hulls has been suggested as a mechanism for introduction in Brazil (Rocha et al. 2012). It seems most likely that *C. oblonga* was introduced to the Morehead City, NC, 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 shipping and climate 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. We sought to determine whether *C. oblonga* is still present and recruiting as previously observed by Theuerkauf et al. (2018) and hypothesized that (H_1) *C. oblonga* will exhibit seasonal recruitment patterns that peak during May–July each year. If *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 (H_2) species richness will decrease with increasing percent cover of *C. oblonga*. We further hypothesized that (H_3) 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 their impacts on the fouling community were quantified. We hypothesized that (H_4) 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.

METHODS

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, <3 km from the Beaufort Inlet where water from the Atlantic Ocean enters the estuary and mixes with water from the Newport River (Fig. 1). The NC State Port of Morehead City is also located 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 m. Recent data (2017–2018) show average monthly water temperature varies from about 7°C in the winter to 29°C during the summer (Appendix S1: Fig. S1). During those two years, the minimum hourly water temperature reached was about 1°C (8 January 2018) and the maximum was 31°C (30 June 2018). Salinity often reaches seawater salinities and tends to be relatively stable (34 psu; Kirby-Smith and Costlow 1989); however, 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).

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), but with an additional *Clavelina* removal treatment. Terra cotta settlement plates (232 cm^2) were suspended horizontally approximately 0.3 m 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 applied: (1) larval recruitment (LR), (2) community development (CD), and (3) *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 *C. 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).

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
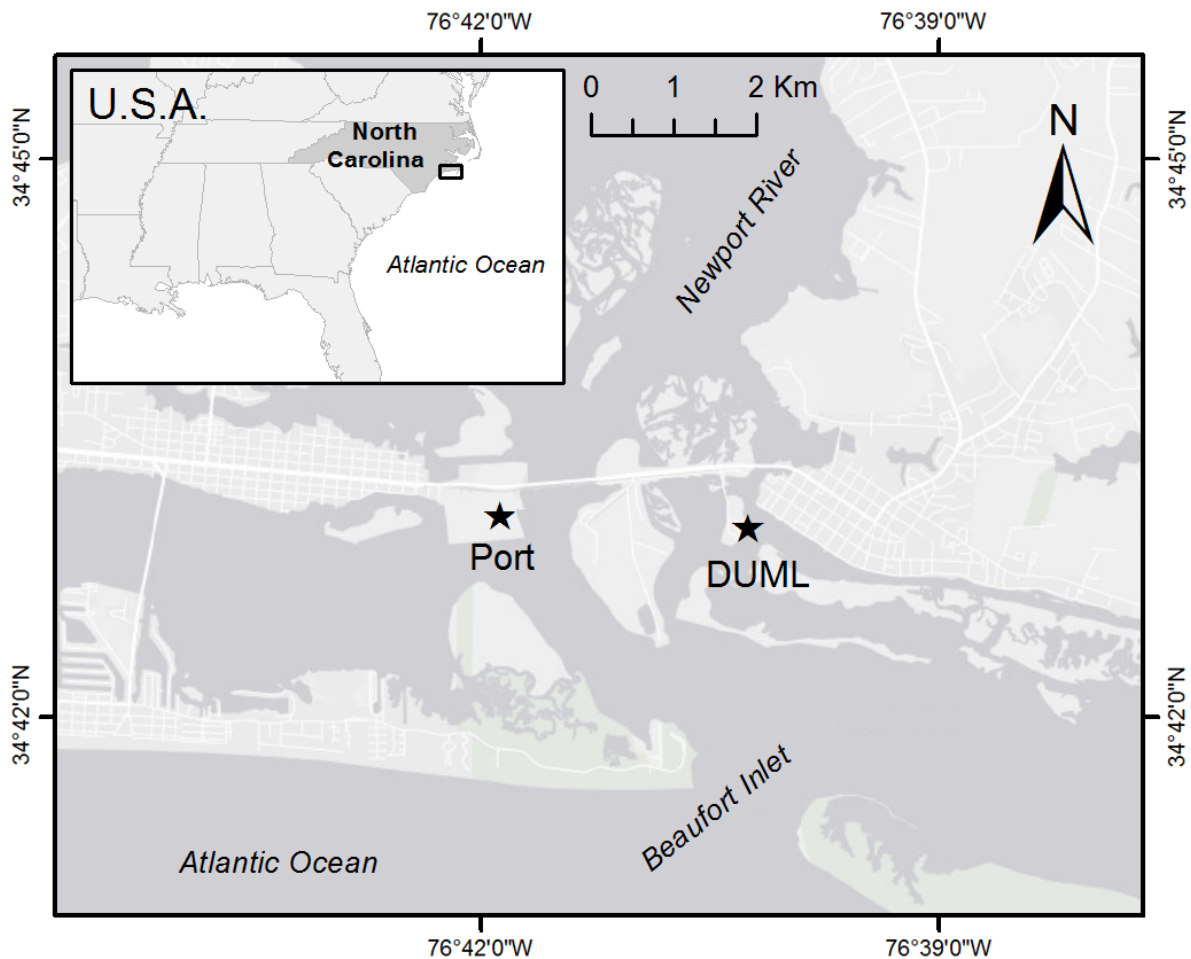
Fig. 1. Location of the study site at the Duke University Marine Lab (DUML) dock in Beaufort, NC, USA, with the Port of Morehead City and neighboring water bodies for reference.

on the underside of the plate using a custom frame (16.5 cm length × 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 photograph. 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 (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, that is, the tubes of *Corophium* sp. Where

living species grew on top of one another, both species were noted, sometimes resulting in a percent cover >100%. 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).

Larval recruitment

To test H_1, 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 to September 2018 and sampled every four weeks from June 2017 to October 2018. Four replicates were recorded at all sample dates except September 2018, where one plate was lost.

Community development

To test H_2 and H_3, species diversity and community development were quantified on plates with varying percent cover of *C. oblonga*. Two batches of community development plates were deployed at different times: (1) May 2017–October 2018 (referred to as CD1), and (2) 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.

Clavelina *removal*

To test the hypothesis that species diversity would increase in the absence of this invasive tunicate (H_2), *C. 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 as described above: (1) May 2017–October 2018, referred to as CR1 plates in which *C. oblonga* was removed once every 4 weeks, and (2) April 2018–October 2018, referred to as CD2 plates in which *C. 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 the 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 h of work or greater than approximately 95% of material removed, which ever was reached first. Percent cover of *C. oblonga* was generally reduced to <10%. Due to the ability of *C. oblonga* to regrow from small portions of tunics (Berrill 1951), it was necessary to repeat this procedure every time the plates were sampled.

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 (1) species richness, (2) Margalef's index, (3) Pielou's evenness index, (4) Shannon's diversity index, and (5) Simpson's diversity.

The various species diversity indices all showed the same patterns—when percent cover of *C. oblonga* increased, species diversity declined (see Results: Community development patterns 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 (H_2) 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 (H_3) using nonmetric multidimensional scaling (NMDS) ordination, similarity profile (SIMPROF), and similarity percentage (SIMPER) analyses using PRIMER v7 (Clarke and Gorley 2015; see Results: Community development patterns below).

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 were 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,* and *C. oblonga*) was 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 *C. oblonga* recruitment would occur during early to mid-summer, as observed during a recent two-year study (Theuerkauf et al. 2018). 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*.

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 whether species richness was a negative function of increasing percent cover of *C. oblonga* using linear least-squares regression models (for CD and CR treatments separately). Next, to test whether community development patterns varied with the relative percent cover of *C. oblonga*, we first visualized the CD and CR treatments using NMDS ordination (50 random starts) based on the 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 (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 (Sutherland and Karlson 1977).

To compare the community development (CD1 and CD2) and *Clavelina* removal (CR1 and CR2) treatments, the 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 months) 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; $\alpha = 0.05$) 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.

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). During this study, the fouling community experienced two environmental disturbances that appeared to impact the community and percent free space: (1) a severe winter during 2017–2018, and (2) Hurricane Florence

during September 2018. To assess the impact of these disturbances, we examined 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 whether there were distinct communities BEFORE the disturbance event vs. 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 on 8 and 9 September 2018, and AFTER was sampled on 20 and 21 October 2018.

RESULTS

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 (*B. neritina* and *Schizoporella* sp.), four species of tunicates (*Ascidia interrupta*, *C. oblonga*, *Didemnum* sp., and *S. plicata*), three species of hydroid (*Ectopleura crocea*, *Eudendrium carneum*, and *Hydractinia* sp.), oysters (*Crassostrea virginica*), and one unidentified encrusting species. Note: Recruitment of *A. 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., *Aplidium 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.

Recruitment patterns

Patterns of larval recruitment for the fouling community varied both seasonally and annually (Fig. 2a), which is characteristic of this study

Table 1. Total species presence over the course of the study as a function of three experimental treatments: (1) larval recruitment, (2) community development, and (3) *Clavelina* removal.

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			
Aplidium 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 fornicate		x	x

Note: Two additional species were unable to be classified.

system (Sutherland and Karlson 1977, Theuerkauf et al. 2018). A total of 15 different species were observed recruiting to the LR plates (Table 1, Fig. 2a). *Clavelina oblonga* settled exclusively during summer months May–August each year, with relatively high recruitment in 2018 compared to 2017 (Figs. 2a, 3). A few genera, such as *Balanus* sp. and *Corophium* sp., displayed relatively high recruitment through much of the summer and fall months, dropping to almost no recruitment during winter and early spring (Fig. 2a). Some species, such as *B. neritina*, *Crassostrea* sp., and *E. carneum*, had relatively short settlement periods whose timing varied slightly between years. *Bugula 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
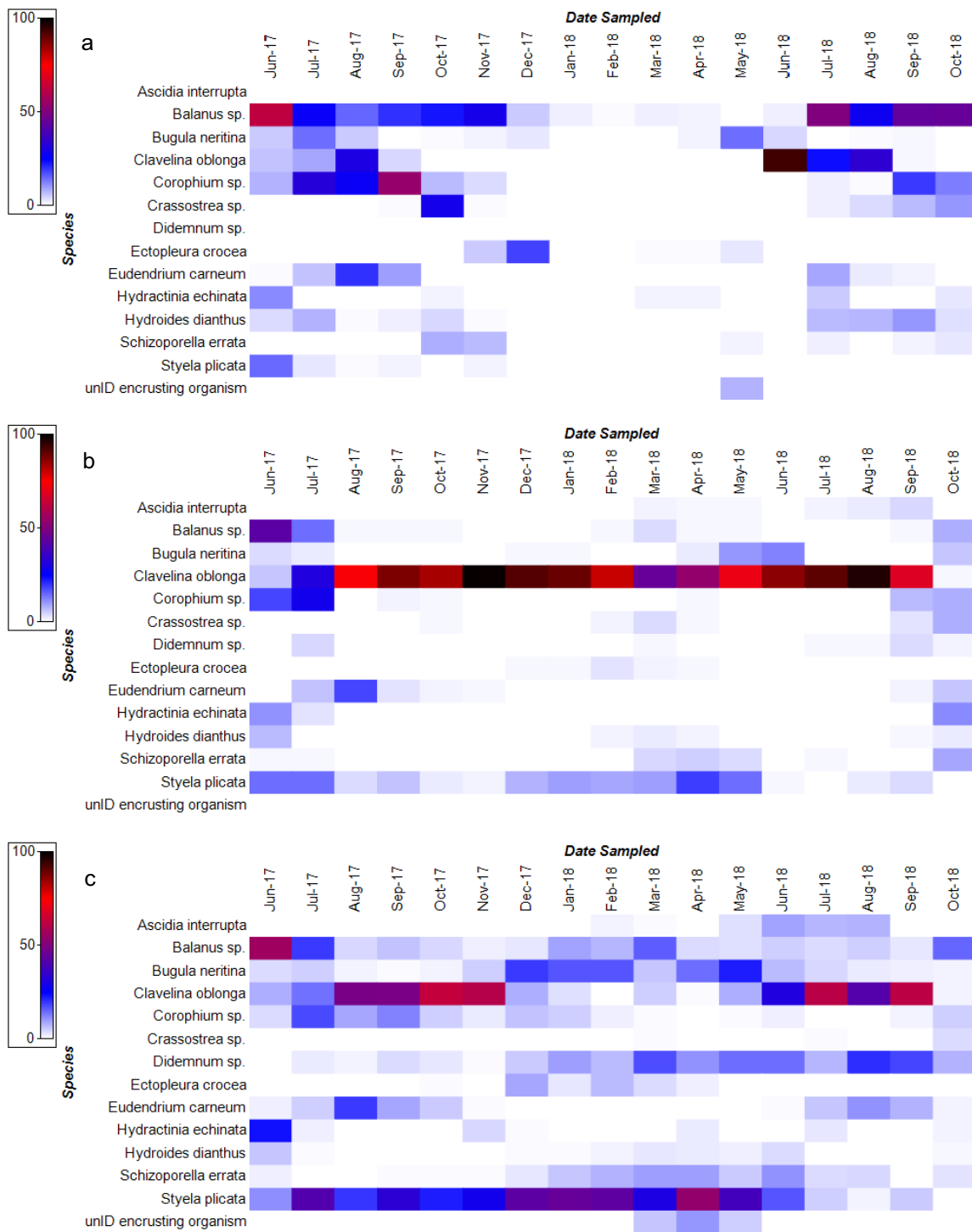
Fig. 2. Heat map of average percent cover of species present on (a) Larval recruitment, (b) community development, and (c) Clavelina removal plates sampled June 2017 through October 2018. Black color indicates maximum abundances (100% average cover), and white indicates the absence of a species (0% average cover).
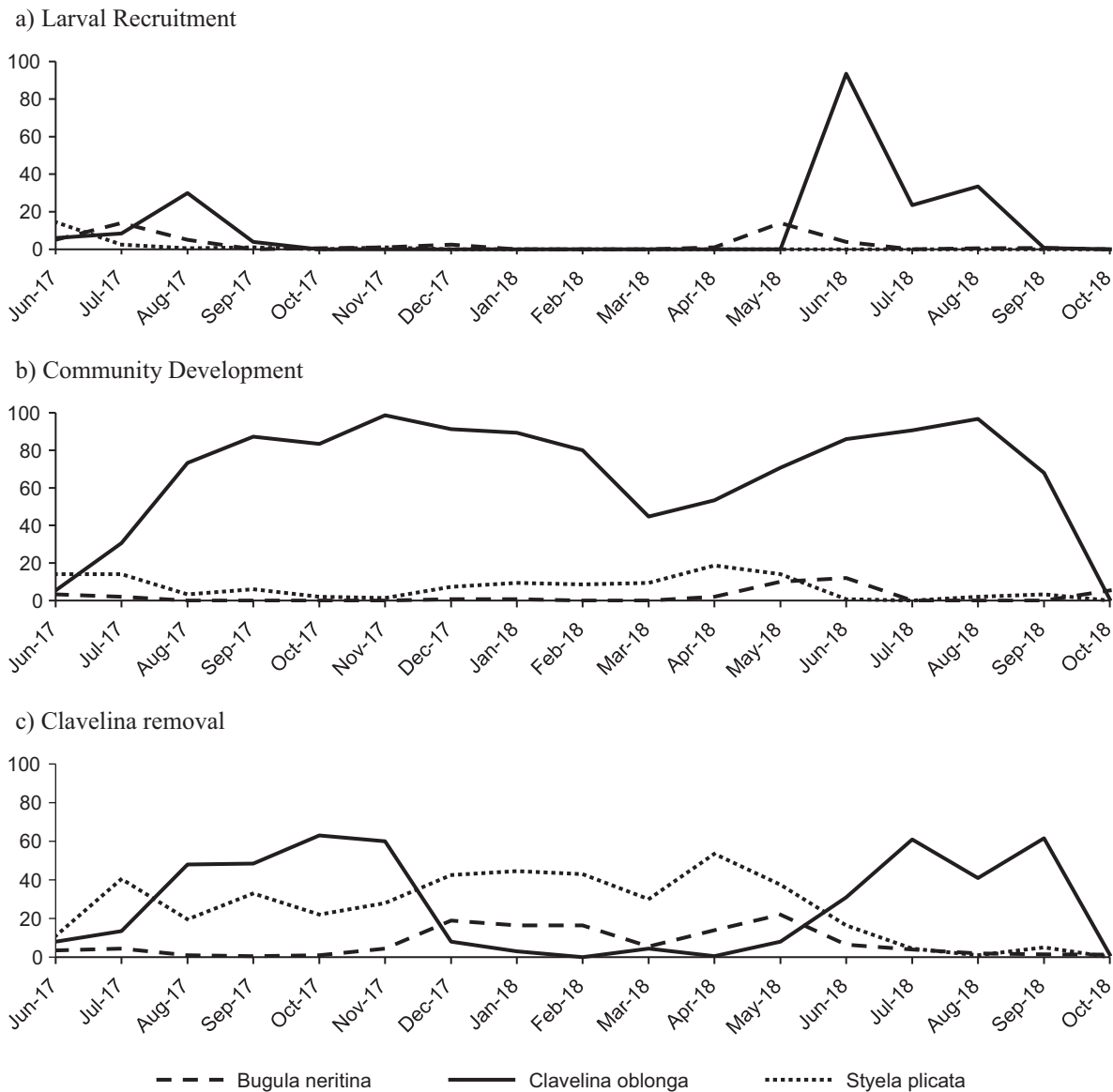
a) Larval Recruitment

b) Community Development

c) Clavelina removal

– – – Bugula neritina   —— Clavelina oblonga   ········ Styela plicata

Fig. 3. Mean percent cover (N = 3–4 for a given sample date) of three of the most abundant species on (a) larval recruitment, (b) community development, and (c) *Clavelina* removal plates across sampling periods. Species include *Bugula neritina*, *Clavelina oblonga*, and *Styela plicata*. *Bugula neritina* and *S. plicata* were chosen as a means to compare against patterns shown in Sutherland and Karlson (1977).

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, *C. 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 (Fig. 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, *B. neritina* and *S. plicata*, remained low (<20%) throughout the course of the experiment on the CD treatment (Fig. 3b). In the CR treatment, *C. oblonga* remained a significant part of the community but no longer dominated the community entirely (Figs. 2c, 3c). As shown in Fig. 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 toward the very end of the experiment in which the high recruitment of *C. oblonga* during June–August 2018 reduced the distinctions between treatments (Fig. 4a). 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 Results: Environmental disturbances below).

Low percent cover of *C. oblonga* corresponds to increases in percent free space (Fig. 4b). 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 peaked in October 2018 (44%). These patterns of free space also correspond to two environmental disturbances (see Results: Environmental disturbances below). With the exception of periods of high *Clavelina* recruitment (May 2017 and 2018), mean species richness was 2–3 times higher on CR than on CD plates (Fig. 4c).

Community development patterns

Patterns of community development were dominated by *C. 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 (Fig. 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 (Fig. 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 (Fig. 2c) shows less dominance by a single species, but instead several prominent species. More seasonal 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 as described by Sutherland and Karlson (1977).

There was a significant negative relationship between increasing percent cover of *C. oblonga* and diversity of fouling species—the strength of the negative relationship was reduced when the percent cover of *C. oblonga* was experimentally reduced (Fig. 5). 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 illustrates that dominance by *C. oblonga* depresses 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* (Table 2, Fig. 6a). 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 vs. CR plates had increased (compare Fig. 6a vs. b). Nine months after plates were deployed, the distinction between fouling communities on CD vs. CR plates was even more apparent (compare Fig. 6a, 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*
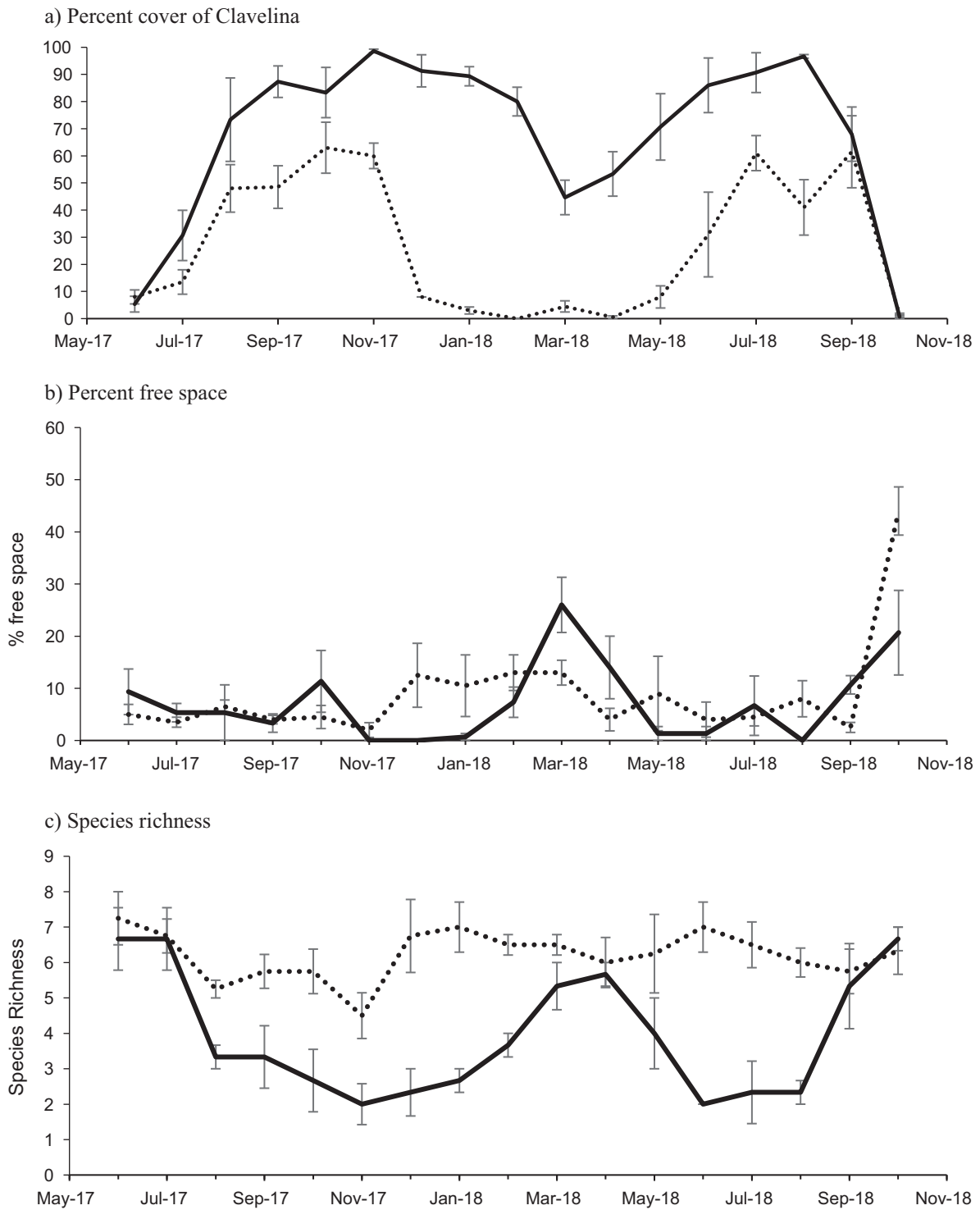
a) Percent cover of Clavelina

b) Percent free space

c) Species richness

Fig. 4. Mean percent (a) cover of *Clavelina oblonga*, (b) free space, and (c) species richness (± standard error; N = 3–4 for a given sample date) for community development (CD1, solid) and Clavelina removal (CR1, dashed) treatments during June 2017 to October 2018.
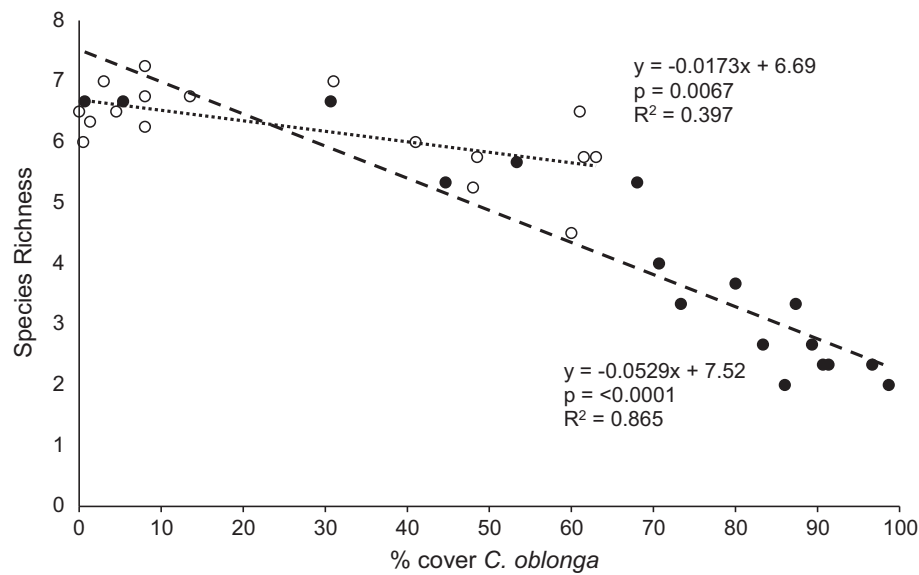
Fig. 5. Regression of average percent cover of *Clavelina oblonga* and species richness for community development (CD1, solid circles, dashed line) and Clavelina removal (CR1, empty circles, dotted line) treatments.

(Fig. 6d). This seems to be the result of *C. oblonga* recovering after the low winter temperatures and regaining space within the community.

Environmental disturbances

Two large-scale environmental disturbances, in which free space exceeded 20% for at least one treatment (Fig. 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 2017–2018 where water temperatures reached as low as 1°C. In the last 25 yr, 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 yr. This seems to indicate that the winter of 2017–2018 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 (Fig. 2a). In the community dominated by *C. oblonga* (CD, Fig. 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, Fig. 2c), percent cover of *C. oblonga* was low before free space peaked. Other prominent species in the community declined in March 2018, including *S. plicata* and

B. 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 (Table 3, Fig. 7). 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 *C. 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.; Fig. 2a–c, compare September vs. 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

Table 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 NMDS plots of 3, 6, 9, and 12 months of development of settlement plates, shown in Fig. 6.

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
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

Notes: NMDS, nonmetric multidimensional scaling; SIMPER, similarity percentage. Clusters composed of a single plate are excluded.

(Fig. 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 *C. oblonga* during June–August 2018 as described above, whereas after the hurricane, the community displayed four distinct states (Table 4, Fig. 8).

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, *C. oblonga*, is established in high salinity (>25 ppt) estuarine waters in the Beaufort/Morehead City area of North Carolina, USA (and other parts of the world as described
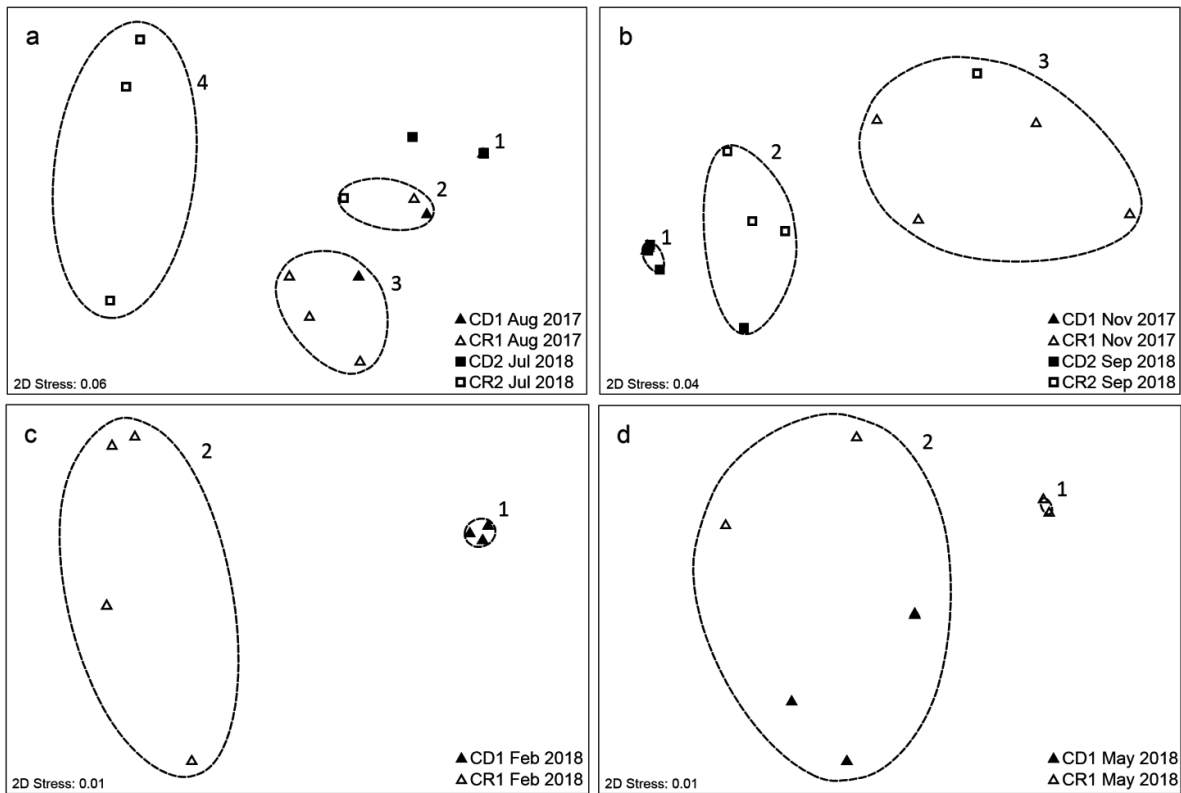
Fig. 6. Nonmetric, multidimensional scaling plots depicting community development and Clavelina removal treatments at (a) 3 months, (b) 6 months, (c) 9 months, and (d) 12 months after deployment of plates. Ellipses show results of SIMPROF analyses, indicating significant differences in community structure (alpha = 0.05). Ellipsis numbers correlate with clusters in SIMPER results (Table 2).

above), with significant impacts on the development and structure of the local fouling community. For example, similar to other invasive 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).

The ability to replicate exactly the community ecology experiments conducted over 50 yr ago by Sutherland and Karlson (1977), and recently by Theuerkauf et al. (2018), yet with the addition of a *Clavelina* removal treatment, allows us to advance our understanding of marine community ecology by testing whether the concept of

multiple community states identified over 50 yr ago by Sutherland and Karlson (1977) is still operable in the absence of invasive *C. oblonga*. Moreover, the presence of two large-scale environmental disturbances (severe winter and hurricane) during the course of this experiment highlights how environmental disturbances 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 is their ability to swamp local communities with

Table 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 NMDS plot of settlement plate communities before and after the impacts of winter, shown in Fig. 7.

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

Notes: NMDS, nonmetric multidimensional scaling; SIMPER, similarity percentage. Clusters composed of a single plate are excluded.
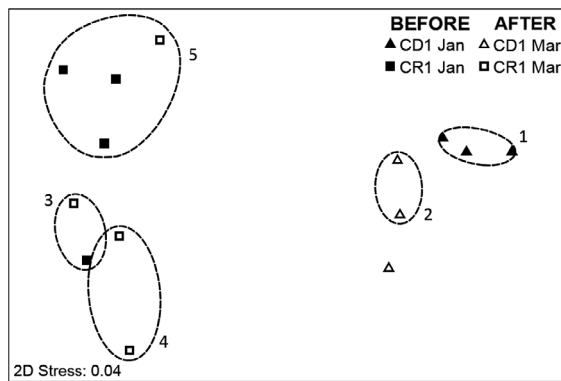

Fig. 7. Nonmetric multidimensional scaling plot depicting all plates sampled BEFORE (January 2018) and AFTER (March 2018) winter-associated decreases in percent cover decrease occurred. Ellipses show result of SIMPROF analyses, indicating significant differences in community structure (alpha = 0.05). Ellipsis numbers correlate with clusters in SIMPER results (Table 3).

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*: (1) extremely cold winters, and (2) hurricanes and tropical cyclones.

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). *Clavelina 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 *C. oblonga* likely contributed to its success in this community after its introduction: (1) ability to rapidly grow and overtake space, and (2) intense yearly recruitment. Other invasive tunicates, such as *Ciona intestinalis*, also display strong seasonal recruitment and rapid growth rates (Ramsay et al. 2009). *Clavelina oblonga*'s rapid growth, and its

Table 4. 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 NMDS plot of settlement plate communities before and after the impacts of Hurricane Florence, shown in Fig. 8.

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

Notes: NMDS, nonmetric multidimensional scaling; SIMPER, similarity percentage. Clusters composed of a single plate are excluded.
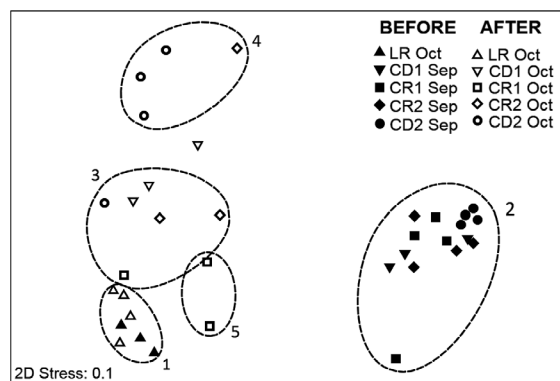

Fig. 8. Nonmetric, multidimensional scaling plot depicting all plates sampled BEFORE (September 2018) and AFTER (October 2018) Hurricane Florence. Sampling dates were approximately 1 week prior and 5 weeks post-Hurricane Florence. Ellipses show results of SIMPROF analyses, indicating significant differences in community structure (alpha = 0.05) Ellipsis numbers correlate with clusters in SIMPER results (Table 4).

remarkable ability to regrow after being almost entirely removed from the community, seems 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* have been observed in other areas (Ordóñez et al. 2016, Casso et al. 2018), 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 (Dijkstra 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.

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 their environment. In this community, *C. 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 that are able to monopolize resources and persist through time alter patterns of recruitment and community assembly (Vieira et al. 2018). This pattern 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 was experimentally reduced, the community returned to previously observed patterns of succession—the long-term (3–9 months) 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 disturbance 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 frequent, regular disturbances may be prevented in the presence of *Clavelina*, the community is tending toward lower species diversity, as would be predicted by the intermediate disturbance hypothesis (Connell 1978). Infrequent, large-scale disturbances 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).

Role of abiotic and biotic processes in controlling the spread of an invasive species

A number of abiotic and biotic factors impact the spread of invasive species. In the case of *C. oblonga*, it appears that abiotic factors are more successful in limiting its dominance within the community than biotic processes. The thermal range of our study system may be a limiting factor to the spread of *C. oblonga*—during the winter months, the 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 (Dijkstra et al. 2011, Lord 2017). The strong effect of winter on the *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 *C. 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 (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 disturbance effects are likely strengthened by the seasonal recruitment pattern of *C. oblonga* since it recruits primarily 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 (Bando 2006, Weis 2011). 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 *C. 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*. In a study comparing the success of a non-native and native seagrasses, 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.

Predictions of ecological patterns from theory can be strengthened through replication of foundational studies under different environmental conditions (Theuerkauf et al. 2018). The present study demonstrates that the invasive tunicate, *C. oblonga*, is still a dominant component of the fouling community, causing a decline in species diversity of the community over time. Conversely, under reduced cover of *Clavelina*, community development patterns display multiple community states over time, similar to the patterns (not necessarily same species) identified by Sutherland and Karlson (1977). 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.

ACKNOWLEDGMENTS

Literature Cited

Astudillo, J. C., K. M. Y. Leung, and T. C. Bonebrake. 2016. Seasonal heterogeneity provides a niche opportunity for ascidian invasion in subtropical marine communities. Marine Environmental Research 122:1–10.

Bando, K. J. 2006. The roles of competition and disturbance in a marine invasion. Biological Invasions 8:755–763.

Berrill, N. J. 1951. Regeneration and budding in tunicates. Biological Reviews 26:456–475.

Bertness, M. D., S. D. Gaines, and M. E. Hay, editors. 2001. Marine community ecology. Sinauer Associates, Sunderland, Massachusetts, USA.

Bishop, M. J., et al. 2017. Effects of ocean sprawl on ecological connectivity: impacts and solutions. Journal of Experimental Marine Biology and Ecology 492:7–30.

Bishop, M. J., R. B. Carnegie, N. A. Stokes, C. H. Peterson, and E. M. Burreson. 2006. Complications of a non-native oyster introduction: facilitation of a local parasite. Marine Ecology Progress Series 325:145–152.

Brooks, M. L., and D. A. Pyke. 2002. Invasive plants and fire in the deserts of North America. Pages 1–14 in K. E. M. Galley and T. P. Wilson, editors. Proceedings of the Invasive Species Workshop: The Role of Fire in the Control and Spread of Invasive Species. Fire Conference 2000: The First National Congress on Fire Ecology, Prevention, and Management. Miscellaneous Publication No. 11. Tall Timbers Research Station, Tallahassee, Florida, USA.

Bullard, S. G., C. V. Davis, and S. E. Shumway. 2013. Seasonal patterns of ascidian settlement at an aquaculture facility in the Damariscotta River, Maine. Journal of Shellfish Research 32:255–264.

Bulleri, F., and M. G. Chapman. 2010. The introduction of coastal infrastructure as a driver of change in marine environments. Journal of Applied Ecology 47:26–35.

Cahill, A., S. Crickenberger, R. Crim, and R. Selden. 2010. Dispersal limitation and post-settlement survival of an introduced ascidian (*Botrylloides violaceus*) in San Jan Islands, WA. Integrative and Comparative Biology 50:E22.

Carlton, J. T., and J. B. Geller. 1993. Ecological Roulette: the global transport of nonindigenous marine organisms. Science 261:78–82.

Carlton, J. T., J. K. Thompson, L. E. Schemel, and F. H. Nichols. 1990. Remarkable invasion of San Francisco bay (California, USA) by the Asian clam *Potamocorbula anurensis*. I. Introduction and dispersal. Marine Ecology Progress Series 66:81–94.

Casso, M., M. Navarro, V. Ordóñez, M. Fernández-Tejedor, M. Pascual, and X. Turon. 2018. Seasonal patterns of settlement and growth of introduced and native ascidians in bivalve cultures in the Ebro Delta (NE Iberian Peninsula). Regional Studies in Marine Science 23:12–22.

Catford, J. A., et al. 2012. The intermediate disturbance hypothesis and plant invasions: implications for species richness and management. Perspectives in Plant Ecology, Evolution and Systematics 14:231–241.

Chan, F. T., and E. Briski. 2017. An overview of recent research in marine biological invasions. Marine Biology 164:121.

Cifuentes, M., C. Kamlah, M. Thiel, M. Lenz, and M. Wahl. 2007. Effects of temporal variability of disturbance on the succession in marine fouling communities in northern-central Chile. Journal of Experimental Marine Biology and Ecology 352:280–294.

Claar, D. C., K. F. Edwards, and J. J. Stachowicz. 2011. Positive and negative effects of a dominant competitor on the settlement, growth, and survival of competing species in an epibenthic community. Journal of Experimental Marine Biology and Ecology 399:130–134.

Clarke, K. R., and R. N. Gorley. 2015. PRIMER v7: user manual/tutorial. PRIMER-E, Plymouth, UK.

Clarke, K. R., R. N. Gorley, P. J. Somerfield, and R. M. Warwick. 2014. Change in marine communities: an approach to statistical analysis and interpretation. Third edition. PRIMER-E, Plymouth, UK.

Connell, J. H. 1972. Community interactions on marine rocky intertidal shores. Annual Review of Ecology and Systematics 3:169–192.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science 199:1302–1310.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs 41:351–389.

Dean, T. A. 1981. Structural aspects of sessile invertebrates as organizing forces in an estuarine fouling community. Journal of Experimental Marine Biology and Ecology 53:163–180.

DeRivera, C. E., G. M. Ruiz, A. H. Hines, and P. Jivoff. 2005. Biotic resistance to invasion: Native predator limits abundance and distribution of an introduced crab. Ecology 86:3364–3376.

Dijkstra, J. A., E. L. Westerman, and L. G. Harris. 2011. The effects of climate change on species composition, succession and phenology: a case study. Global Change Biology 17:2360–2369.

Dollar, S. J., and G. W. Tribble. 1993. Recurrent storm disturbance and recovery: a long-term study of

coral communities in Hawaii. Coral Reefs 12:223–233.

Dukes, J. S., and H. A. Mooney. 1999. Does global change increase the success of biological invaders? Trends in Ecology and Evolution 14:135–139.

Dumont, C. P., C. F. Gaymer, and M. Thiel. 2011. Predation contributes to invasion resistance of benthic communities against the non-indigenous tunicate *Ciona intestinalis*. Biological Invasions 13:2023–2034.

Firth, L. B., A. M. Knights, D. Bridger, A. J. Evans, N. Mieszkowska, P. J. Moore, N. E. O'Connor, E. V. Sheehan, R. C. Thompson, and S. J. Hawkins. 2016. Ocean sprawl: challenges and opportunities for biodiversity management in a changing world. Oceanography and Marine Biology: An Annual Review 54:193–269.

Forrest, B. M., L. M. Fletcher, J. Atalah, R. F. Piola, and G. A. Hopkins. 2013. Predation limits spread of *Didemnum vexillum* into natural habitats from refuges on anthropogenic structures. PLOS ONE 8: e82229.

Gassó, N., W. Thuiller, J. Pino, and M. Vilà. 2012. Potential distribution range of invasive plant species in Spain. NeoBiota 12:25–40.

Glasby, T. M., S. D. Connell, M. G. Holloway, and C. L. Hewitt. 2007. Nonindigenous biota on artificial structures: Could habitat creation facilitate biological invasions? Journal of Marine Biology 151:887–895.

Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111:1169–1194.

Herdman, W. A. 1880. Preliminary report on the Tunicata of the challenger expedition. Part 2. Proceedings of the Royal Society of Edinburgh 10:714–726.

Hiebert, L. S., E. A. Vieira, G. M. Dias, S. Tiozzo, and F. D. Brown. 2019. Colonial ascidians strongly preyed upon, yet dominate the substrate in a subtropical fouling community. Proceedings of the Royal Society B: Biological Sciences 286:20190396.

Hobbs, R. J., and L. F. Huenneke. 1992. Disturbance, diversity, and invasion: implications for conservation. Conservation Biology 6:324–337.

Jiang, J., and D. L. DeAngelis. 2013. Strong species-environment feedback shapes plant community assembly along environmental gradients. Ecology and Evolution 3:4119–4128.

Johnson, L. E., and J. T. Carlton. 1996. Post-establishment spread in large-scale invasions: dispersal mechanisms and the zebra mussel *Dreissena polymorpha*. Ecology 77:1686–1690.

Johnson, Z. I., B. J. Wheeler, S. K. Blinebry, C. M. Carlson, C. S. Ward, and D. E. Hunt. 2013. Dramatic variability of the carbonate system at a temperate coastal ocean site (Beaufort, North Carolina, USA) is regulated by physical and biogeochemical processes on multiple timescales. PLOS ONE 8:e85117.

Karlson, R. H., and R. W. Osman. 2012. Species composition and geographic distribution of invertebrates in fouling communities along the east coast of the USA: a regional perspective. Marine Ecology Progress Series 458:255–268.

Kelly, A. E., and M. L. Goulden. 2008. Rapid shifts in plant distribution with recent climate change. Proceedings of the National Academy of Sciences USA 105:11823–11826.

Kirby-Smith, W. W., and J. D. Costlow. 1989. The Newport River estuarine system. Publication UNC-SG-89-04. UNC Sea Grant College, Raleigh, North Carolina, USA.

Lambert, C. C., and G. Lambert. 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Marine Ecology Progress Series 259:145–161.

Levin, S. A. 1981. The role of theoretical ecology in the description and understanding of populations in heterogenous environments. American Zoologist 21:865–875.

Levine, J. M., M. Vila, C. M. D'Antonio, J. S. Dukes, K. Grigulis, and S. Lavorel. 2002. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society B: Biological Sciences 270:775–781.

Lord, J. P. 2017. Impact of seawater temperature on growth and recruitment of invasive fouling species at the global scale. Marine Ecology 38:e12404.

Lord, J. P., J. M. Calini, and R. B. Whitlatch. 2015. Influence of seawater temperature and shipping on the spread and establishment of marine fouling species. Journal of Marine Biology 162:2481–2492.

Lord, J., and R. Whitlatch. 2015. Predicting competitive shifts and responses to climate change based on latitudinal distributions of species assemblages. Ecology 96:1264–1274.

Mack, M. C., and C. M. D'Antonio. 1998. Impacts of biological invasions on disturbance regimes. Trends in Ecology and Evolution 13:195–198.

Malhi, Y., J. Franklin, N. Seddon, M. Solan, M. G. Turner, C. B. Field, and N. Knowlton. 2020. Climate change and ecosystems: threats, opportunities and solutions. Philosophical Transactions of the Royal Society B: Biological Sciences 375:20190104.

May, R., and A. McLean, editors. 2007. Theoretical ecology: principles and Applications. Oxford University Press, Oxford, UK.

McDougall, K. D. 1943. Sessile marine invertebrates of Beaufort, North Carolina: a study of settlement, growth, and seasonal fluctuations among pile-

dwelling organisms. Ecological Monographs 13:321–374.

Mumby, P. J. 1999. Bleaching and hurricane disturbances to populations of coral recruits in Belize. Marine Ecology Progress Series 190:27–35.

Munguia, P., R. W. Osman, J. Hamilton, R. B. Whitlatch, and R. N. Zajac. 2010. Modeling the priority effects and species dominance in Long Island Sound benthic communities. Marine Ecology Progress Series 413:229–240.

Nandakumar, K., M. Tanaka, and T. Kikuchi. 1993. Interspecific competition among fouling organisms in Japan. Marine Ecology Progress Series 94:43–50.

Nydam, M., and J. J. Stachowicz. 2007. Predator effects on fouling community development. Marine Ecology Progress Series 337:93–101.

Odum, E. P. 1969. The strategy of ecosystem development. Science 164:262–270.

Olson, R. R. 1985. The consequences of short-distance larval dispersal in a sessile marine invertebrate. Ecology 66:30–39.

Ordóñez, V., M. Pascual, M. Fernández-Tejedor, and X. Turon. 2016. When invasion biology meets taxonomy: Clavelina oblonga (Ascidiacea) is an old invader in the Mediterranean Sea. Biological Invasions 18:1203–1215.

Osman, R. W., and R. B. Whitlatch. 2004. The control of the development of a marine benthic community by predation on recruits. Journal of Experimental Marine Biology and Ecology 311:117–145.

Osman, R. W., and R. B. Whitlatch. 2007. Variation in the ability of Didemnum sp. to invade established communities. Journal of Experimental Marine Biology and Ecology 342:40–53.

Osman, R. W., R. B. Whitlatch, and R. J. Malatesta. 1992. Potential role of micro-predators in determining recruitment into a marine community. Marine Ecology Progress Series 83:35–43.

Palmer, M. A., et al. 2004. Ecology for a crowded planet. Science 304:1251–1252.

Paradis, A., J. Elkinton, K. Hayhoe, and J. Buonaccorsi. 2008. Role of winter temperature and climate change on the survival and future range expansion of the hemlock woolly adelgid (Adelges tsugae) in eastern North America. Mitigation and Adaptation Strategies for Global Change 13:541–554.

Pollock, L. W. 1998. A practical guide to the marine animals of northeastern North America. Rutgers University Press, New Brunswick, New Jersey, USA.

Pratt, M. C., and E. W. Grason. 2007. Invasive species as a food source: Does a nudibranch predator prefer eating an invasive bryozoan? Biological Invasions 9:645–655.

Ramsay, A., J. Davidson, D. Bourque, and H. Stryhn. 2009. Recruitment patterns and population development of the invasive ascidian Ciona intestinalis in Prince Edward Island, Canada. Aquatic Invasions 4:169–176.

Rindone, R. R., and D. B. Eggleston. 2011. Predator-prey dynamics between recently established Stone Crabs (Menippe spp.) and oyster prey (Crassostrea virginica). Journal of Experimental Marine Biology and Ecology 407:216–225.

Rocha, R. M., L. P. Kremer, and K. H. Fehlauer-Ale. 2012. Lack of COI variation for Clavelina oblonga (Tunicata, Ascidiacea) in Brazil: Evidence for its human-mediated transportation? Aquatic Invasions 7:419–424.

Ruiz, G. M., P. W. Fofonoff, J. T. Carlton, M. J. Wonham, and A. H. Hines. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annual Review of Ecology and Systematics 31:481–531.

Ruppert, E. E., and R. S. Fox. 1988. Seashore animals of the southeast. University of South Carolina Press, Columbia, South Carolina, USA.

Sakai, A. K., et al. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics 32:305–332.

Sanders, N. J., N. J. Gotelli, N. E. Heller, and D. M. Gordon. 2003. Community disassembly by an invasive species. Proceedings of the National Academy of Sciences USA 100:2474–2477.

Sardain, A., E. Sardain, and B. Leung. 2019. Global forecasts of shipping traffic and biological invasions to 2050. Nature Sustainability 2:274–282.

Simberloff, D., et al. 2013. Impacts of biological invasions: what's what and the way forward. Trends in Ecology and Evolution 28:58–66.

Sorte, C. J. B., and J. J. Stachowicz. 2011. Patterns and processes of compositional change in a California epibenthic community. Marine Ecology Progress Series 435:63–74.

Sorte, C. J. B., S. L. Williams, and R. A. Zerebecki. 2010. Ocean warming increases threat of invasive species in a marine fouling community. Ecology 91:2198–2204.

Sutherland, J. P., and R. H. Karlson. 1977. Development and stability of the fouling community at Beaufort, North Carolina. Ecological Monographs 47:425–446.

Theuerkauf, K. W., D. B. Eggleston, and S. J. Theuerkauf. 2018. An exotic species alters patterns of marine community development. Ecological Monographs 88:92–108.

Tyrrell, M. C., and J. E. Byers. 2007. Do artificial substrates favor nonindigenous fouling species over native species? Journal of Experimental Marine Biology and Ecology 342:54–60.

Van Name, W. G. 1945. The North and South American ascidians. Bulletin of the American Museum of Natural History 84:1–476.

Vieira, E. A., A. A. V. Flores, and G. M. Dias. 2018. Persistence and space preemption explain species-specific founder effects on the organization of marine sessile communities. Ecology and Evolution 8:3430–3442.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997. Introduced species: a significant component of human-caused global change. New Zealand Journal of Ecology 21:1–16.

Weis, J. S. 2011. Invasion and predation in aquatic ecosystems. Current Zoology 57:613–624.

White, P. S., and S. T. A. Pickett. 1985. Natural disturbance and patch dynamics: an introduction. Pages 3–13 in S. T. A. Pickett and P. S. White, editors. The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, Florida, USA.

Williams, S. L., et al. 2013. Managing multiple vectors for marine invasions in an increasingly connected world. BioScience 63:952–966.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3471/full