

Research Article

Copper pollution enhances the competitive advantage of invasive ascidians

Kristin L. Osborne^{1,*} and Helen C. Poynton²¹Marine Science, Safety, and Environmental Protection Department, Massachusetts Maritime Academy, Buzzards Bay, MA 02532, USA²School for the Environment, University of Massachusetts Boston, Boston, MA 02125, USAAuthor e-mails: kosborne@maritime.edu (KLO), helen.poynton@umb.edu (HCP)

*Corresponding author

Co-Editors' Note: This study was first presented at the 2018 International Invasive Sea Squirt Conference held at Woods Hole, Massachusetts, USA, May 2-4, 2018 (<https://web.whoi.edu/sea-squirt-conference/>). Since its inception in 2005, the IISCC series has provided a venue for marine biologists and people concerned with invasive ascidians, to explore the biology, ecology, impacts, management options for control, and other relevant topics.

Citation: Osborne KL, Poynton HC (2019) Copper pollution enhances the competitive advantage of invasive ascidians.

Management of Biological Invasions 10(4): 641–656, <https://doi.org/10.3391/mbi.2019.10.4.05>

Received: 3 January 2019

Accepted: 9 September 2019

Published: 28 October 2019

Handling editor: Mary Carman

Copyright: © Osborne and Poynton

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

OPEN ACCESS**Abstract**

Invasive species dominate and often outcompete native species in marine harbors. The abundance of introductions due to shipping activity combined with artificial substrates and poor water quality helps to facilitate invasion success in these areas. Colonial ascidians, in particular, have broad environmental tolerances that allow them to invade novel ecosystems including urban harbors. While the effects of invasive species and poor water quality on native species have been explored, the relative influence and how these pressures may interact to degrade biodiversity is not well characterized. The purpose of this study was therefore to understand the role of interspecific competition and pollution on the native *Aplidium glabrum* and the invasive *Botrylloides violaceus*. Colony surface area has been previously identified as a sensitive toxicity endpoint and was measured weekly to assess impacts both separately and together at 0, 1, 10, 20, and 40 µg/L copper concentrations. *A. glabrum* was significantly impacted at 1 µg/L in the presence of interspecific competition and at 10 µg/L with no competition; however, the copper EC₅₀ revealed increased sensitivity in the presence of *B. violaceus* (11.3 µg/L versus 6.9 µg/L while in competition). Conversely, significant impacts to *B. violaceus* growth did not occur until much higher copper concentrations with an EC₅₀ of 37.1 µg/L while alone and 31.7 µg/L when in competition. Interspecific competition was found to significantly impact *A. glabrum* surface area growth at 1, 10, and 40 µg/L, while there was no significant effect on *B. violaceus* at any of the concentrations tested. Finally, an interaction effect was found between copper pollution and competition status only for *A. glabrum*. The results of this study support the hypothesis that invasive species are more tolerant of pollution while also revealing the interactive effects of pollution exposure and interspecific competition.

Key words: metal toxicity, marine, benthic, invertebrates, competition, tolerance

Introduction

Invasive species pose significant threats to benthic marine communities in urban environments via competition, predation, and disease transmission (Wilcove et al. 1998; Parker et al. 1999; Lodge et al. 2006). Their presence in marine ecosystems is increasing with no sign of saturation (Seebens et al. 2017). Invasive species threaten vulnerable native ecosystem structure and function (Maxwell et al. 2016; Walsh et al. 2016; Tilman et al. 2017) The presence of invaders can lead to declining reproduction rates and

survival, ultimately impacting population growth (Lockwood et al. 2007). Further, US economic losses associated with terrestrial, freshwater, and marine invasive species management are estimated at approximately \$120 billion annually (Pimentel et al. 2005).

Frameworks have been proposed to identify critical steps in the invasion process for species to become successful in a novel environment. Blackburn et al. (2011) developed a unified framework from existing models to establish universal language describing the invasion process while including new information about invasion barriers and management strategies, all in the context of invasion stage. This model was then later applied to ascidians to identify the invasion barriers and factors which enable their success (Zhan et al. 2015). Anthropogenic impacts such as pollution and invasion have the potential to interact (Piola and Johnston 2006) and these disturbances can possibly result in decreased biodiversity (Ruiz et al. 1997). The interaction between anthropogenic stressors and the invaders themselves could significantly influence their colonization, distribution, and overall impacts in novel environments (Ruiz et al. 1999).

The Invader Tolerance Model (ITM) developed by Osborne et al. (2018) provides a framework to explain the reduction of biodiversity by invasive species and marine pollution. This framework proposes that invaders are more tolerant to pollution relative to native species, and that native species diversity will decrease following an exposure event. This decrease will further enable invasives to settle, thus decreasing overall native species diversity even more. However, within the ITM there are unanswered questions regarding the relative importance of pollution and invasive species on native species as well the combined effects.

Invasive ascidians have been identified in several anthropogenically-disturbed environments along the Northwest Atlantic coast through rapid assessment surveys (e.g. McIntyre et al. 2013; Wells et al. 2014). The colonial ascidian species *Botrylloides violaceus* (Oka, 1927) has been found in the Gulf of Maine (GOM) since the 1980s (Dijkstra et al. 2007). *B. violaceus* was likely first introduced in the Damariscotta River in Maine via oyster aquaculture imports and has since spread rapidly both north and south along the coastline, demonstrating strong competitive abilities in the GOM (Dijkstra et al. 2007; Dijkstra and Harris 2009). Conversely, *Aplidium glabrum* (formerly “*Amaroucium glabrum*”) (Verrill, 1871), is native to the North Atlantic Ocean (Van Name 1945) and is distributed along the North American coasts of the Bay of Fundy to Cape Hatteras (Van Guelpen et al. 2005). The distribution of *A. glabrum* is likely temperature-dependent, as this species tends to reside in cooler, often deeper waters (Van Name 1945). Both species are available for collection in New England (Wells et al. 2014), the larvae are easy to retrieve in the laboratory (*pers. obs.*), and rapidly reproduce. For these reasons, *B. violaceus* and *A. glabrum* serve as excellent model species to investigate the interacting impacts of competition

and pollution exposure and can concurrently provide a native-invasive tolerance comparison.

Copper pollution is one of the primary active ingredients in many antifouling hull paints since the widespread ban of tributyltin (TBT) in the 1980s. It is commonly found in urban environments at concentrations of approximately 1 µg/L (Hall Jr. et al. 1998; Lawes et al. 2016) but can reach upwards of 80 µg/L in some areas including heavily trafficked locations of Chesapeake Bay (Hall Jr. et al. 1998). Chronic copper toxicity of the invasive *B. violaceus* and the native *A. glabrum* was previously assessed (Osborne et al. 2018) to identify sublethal concentrations impacting growth and morphology. The results of the study indicated that *B. violaceus* is more tolerant of copper pollution than *A. glabrum* in laboratory conditions. Both the number of zooids and colony surface area were significantly higher in the invasive species relative to the native at the conclusion of the 11-week experiment. Further, heart rates indicated greater physiological stress to the natives at lower concentrations relative to invasive species.

Biotic resistance, or the hypothesis that increased native community biodiversity can stave off invader establishment, is attributed to high species diversity and thus increased competitor presence (Elton 1958). Therefore, to understand how these sensitivity differences between invasive and native ascidians will play out within the biofouling community, interspecific competition must be directly explored. The purpose of this study was to investigate if and how competition and pollution exposure factors interact, focusing on the model ascidian species *B. violaceus* and *A. glabrum*. This approach tests important predictions of the ITM that the dual impacts of pollution exposure and competition will have a negative synergistic effect on the ability of *A. glabrum* to persist. Survival, growth, and reproduction of the invasive and native species were assessed both separately and together, and while exposed or not to elucidate if an interaction effect exists between these two factors.

Materials and methods

Experimental Design

We conducted a 10-week exposure to 0, 1, 10, 20, and 40 µg/L Cu to test the combined and separate effects of copper pollution and interspecific competition on the native *A. glabrum* and the invasive *B. violaceus*. We found surface area to be a sensitive endpoint for negative impacts on colonial ascidians (Osborne et al. 2018), and therefore it was used as a primary endpoint in the present study. Table 1 describes the experimental treatment design used to test factors separately and together. Five replicates per treatment condition were included. Although it would have been worthwhile to also include a treatment of two larvae from the same species

Table 1. Organization of experimental conditions to show how factors were tested either separately or together. Treatments are named by the following scheme: “A” = *A. glabrum* (native), “B” = *B. violaceus* (invasive), “CTRL” = control, “CU” = copper treatment; either 1, 10, 20, or 40 µg/L concentration.

Condition	Native	Invasive	Copper
A CTRL	+	–	–
B CTRL	–	+	–
AB CTRL	+	+	–
A CU	+	–	+
B CU	–	+	+
AB CU	+	+	+

to test for possible intraspecific competition, this would have almost doubled the number of treatments and controls and was not possible due to space and resource constraints. However, because space was not a limiting factor in these studies (percent cover of the disks never exceeded 80%) we believe that intraspecific competition would not be a major factor influencing colony growth.

To account for the more rapid growth and colony area of *B. violaceus* compared with *A. glabrum* and allow for direct comparison of interactive effects due to competition and copper exposure, the percent growth was calculated by dividing the colony growth in all treatments with their controls. Particular interest was given to the impacts on colony growth at concentrations within the range of previously identified EC₅₀ values for each species, 10 µg/L for *A. glabrum* and 40 µg/L for *B. violaceus*. P-values indicate differences in actual growth between competition and no competition treatments at these concentrations.

Larvae Collection and Initial Settlement

We collected larvae using a modified version of the Watanabe and Lambert (1973) larvae collector design (as described in Osborne et al. 2018). Briefly, adult colonies were retrieved from the field, immediately added to the larvae collector tanks, and allowed to passively release their larvae. Colonies were collected from the floating dock at Massachusetts Maritime Academy (MMA) in Buzzards Bay, Massachusetts (41°44'19.58"N; 70°37'36.44"W) and transported to the MMA aquaculture laboratory. Colonies were held in larvae collector tanks with a continuous low-flow of 100 µm filtered seawater until larvae release was observed; no additional food was added. Larvae release occurred within 1–2 hours of collection. All adult colonies were obtained simultaneously and therefore larvae were released at approximately the same time. Larvae were directed to a submerged Nitex mesh filter by a current generated at the gravity-fed carboy water input, where they were then collected. Only larvae which were free-swimming were used for settlement and unmoving larvae were discarded. We gently pipetted larvae by hand to ensure no damage was imposed once collected in the Nitex filter.

Larvae were settled on 20 mm diameter white plastic disks according to the methods in Osborne et al. (2018). Since treatments were designed to determine both individual effects and interactions of pollution exposure and interspecific competition, disks contained either 2 larvae (1 larvae of each species) settled 1 mm apart, or 1 larvae of a single species. Once larvae metamorphosed, disks were transferred to compartmentalized plastic containers and grown for five days until the exposure period began. Prior to exposure, all juveniles were visually inspected with a dissecting microscope to ensure a healthy appearance (strong heartbeat, plump and organized ampullae, siphons open and feeding, and a well-formed zooid) and probed to monitor stimuli reaction. Juveniles were fed 20 μ L of Shellfish Diet 1800[®] (Reed Mariculture Inc., CA, USA) every alternating day for 5 days prior to exposures.

Experimental Units

Each disk with settled juveniles was suspended inside a 1 L plastic container with 750 ml of water. Copper concentrations of 0, 1, 10, 20, and 40 μ g/L were used in this study and have been previously identified as sub-lethal concentrations for both species in chronic toxicity assays (Osborne et al. 2018). Stock solutions were prepared in deionized water using cupric chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; MP Biomedicals, Santa Ana, CA). Experimental concentrations were prepared by adding the appropriate stock to 5 μ m filtered seawater at 32–34 psu salinity and ambient laboratory temperature (18–21 °C). We used a static renewal system for these exposures, changing water 3 times per week. Each container was fed 40 μ L of Shellfish Diet 1800[®] (Reed Mariculture Inc., CA, USA) following water changes.

Toxicity Endpoints

Surface area was recorded once weekly from photographs taken with a USB microscope (USB-2-Micro-200X, Plugable Technologies) on both front and back surfaces of each disk. Individual zooids were counted during this time. Large-scale morphological observations were recorded on all water change days. Surface area was analyzed on photographs using ImageJ photo processing software (Schneider et al. 2012) and was calculated for all treatments. The number of zooids were counted using a dissecting microscope (EZ4, Leica) under low power (4x magnification) for each colony. Measurements for each pair of competition treatments at each pollution concentration were then compared with one another to determine at which copper concentrations competition significantly impacted surface area for each species separately. For example, the competition and no competition surface areas of *A. glabrum* were compared at 1 μ g/L and were separately analyzed from those of *B. violaceus*.

Statistical Analyses

Statistical analyses were performed using R version 3.3.0. For all statistical tests, p-values < 0.05 were considered statistically significant and indicates those treatments where competition, pollution, or an interaction between the two factors had a significant impact on surface area growth.

The effect of copper between treatment concentrations was determined using ANOVA (normally distributed, homogeneous data) followed by Tukey's HSD or a Kruskal-Wallis (non-normally distributed, homogeneous data) followed by Dunn's test. Separate t-tests were used to determine differences between competition/no competition treatments at each concentration.

A factorial ANOVA was used to determine if an interaction effect exists between competition status and copper treatment. Competition status (competition, no competition) and copper pollution (0, 1, 10, 20, and 40 µg/L) were the independent variables and surface area was the dependent variable. Both variables will affect the outcome and the effect of changes in one variable will depend on the level of the other variable if an interaction exists; if an interaction does not exist, the effects of changes in one variable are the same at every fixed level of the other variable (Seltman 2015).

The Lowest Observable Effect Concentration (LOEC; lowest copper concentration which elicits a significant effect on surface area) was calculated using ANOVA. EC₅₀ concentrations (the Effective Concentration which negatively impacts 50% of the animals tested) were derived from dose-response curves constructed using a log-logistic regression model (Hasenbein et al. 2017).

Results

Species Differences in Growth Rate

Surface area was assessed as a measure for colony growth throughout the 10-week exposure period (see Figure 1). In the absence of any copper treatment or competition, *B. violaceus* showed an accelerated growth rate compared with *A. glabrum*. Surface area growth generally followed a linear trend for both species, although final control surface area measurements for *B. violaceus* were an order of magnitude higher than those of *A. glabrum*. As shown in Figure 1, this trend began as early as four weeks post-settlement when the average colony size of *B. violaceus* was over 100 mm², while *A. glabrum* average colony size was approximately 10 mm². Zooid counts followed a similar trend, as observed in previous studies (Osborne et al. 2018). These results indicate that in the absence of any stressors, the invasive species has a significant growth advantage over the native species.

Pollution and Competition Effects

In all treatment types, copper exposure had negative impacts on colony growth of both native and invasive ascidians. Copper treatment significantly

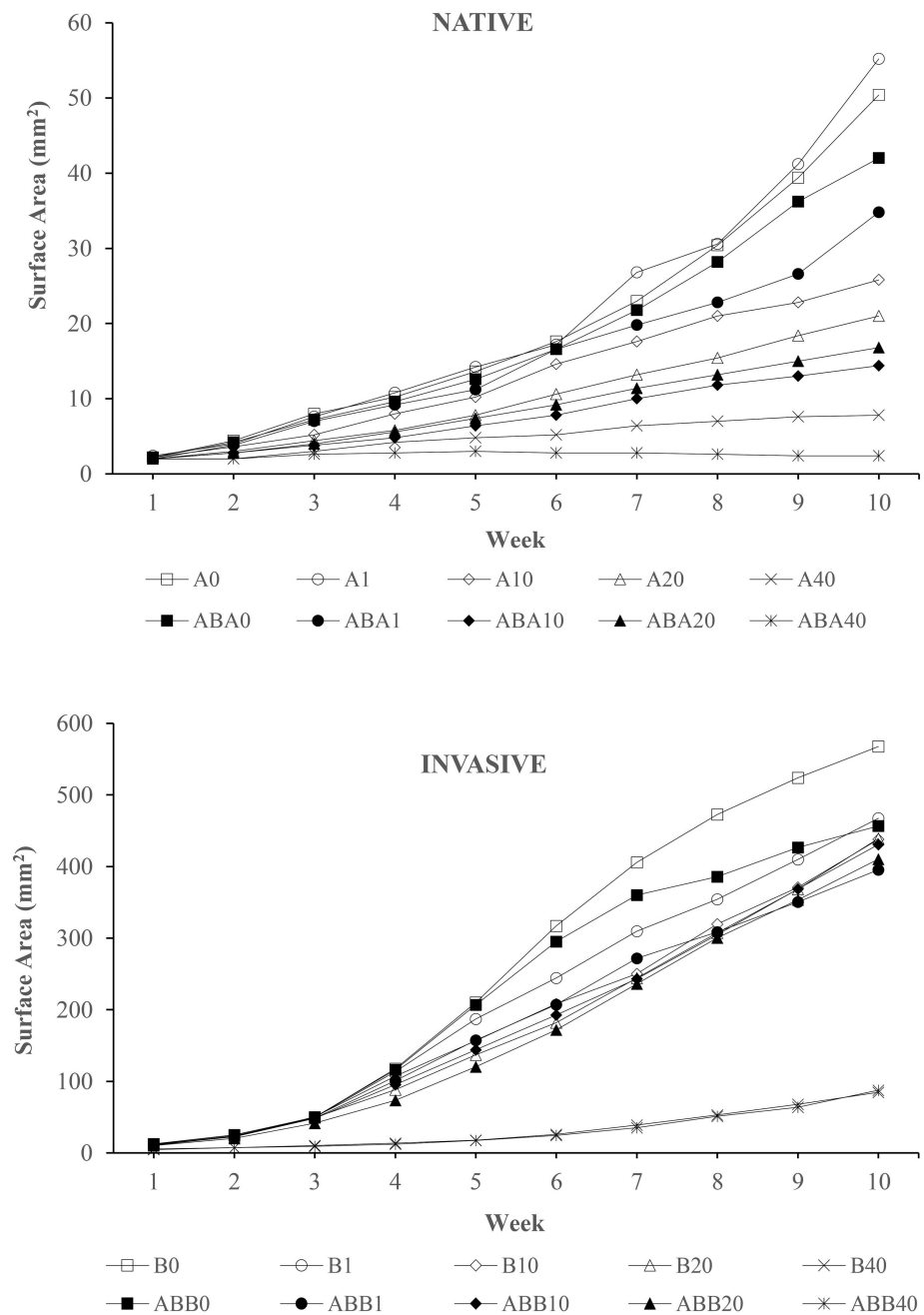


Figure 1. Weekly growth measurements for each species by treatment. “A” and “B” denotes *A. glabrum* and *B. violaceus* only treatments, respectively. “ABA” and “ABB” denotes *A. glabrum* and *B. violaceus* treatments while in competition, respectively.

impacted *Aplidium glabrum* colony growth ($p = < 0.00001$) and post-hoc analysis indicated significant effects at 10 $\mu\text{g/L}$ ($p = 0.0001$) and all other higher concentrations ($p = < 0.00001$). *Botrylloides violaceus* was also impacted by increasing copper concentrations, but only the 40 $\mu\text{g/L}$ exposure resulted in a significant effect on colony growth ($p = 0.0007$). As shown in Figure 2, there was a dramatic decrease in *A. glabrum* growth between the 1 and 10 $\mu\text{g/L}$ exposures, while *B. violaceus* did not appear to be similarly affected until 40 $\mu\text{g/L}$. Dose-response curves were generated to determine the EC_{50} values (Table 2) for colony growth, or the concentration

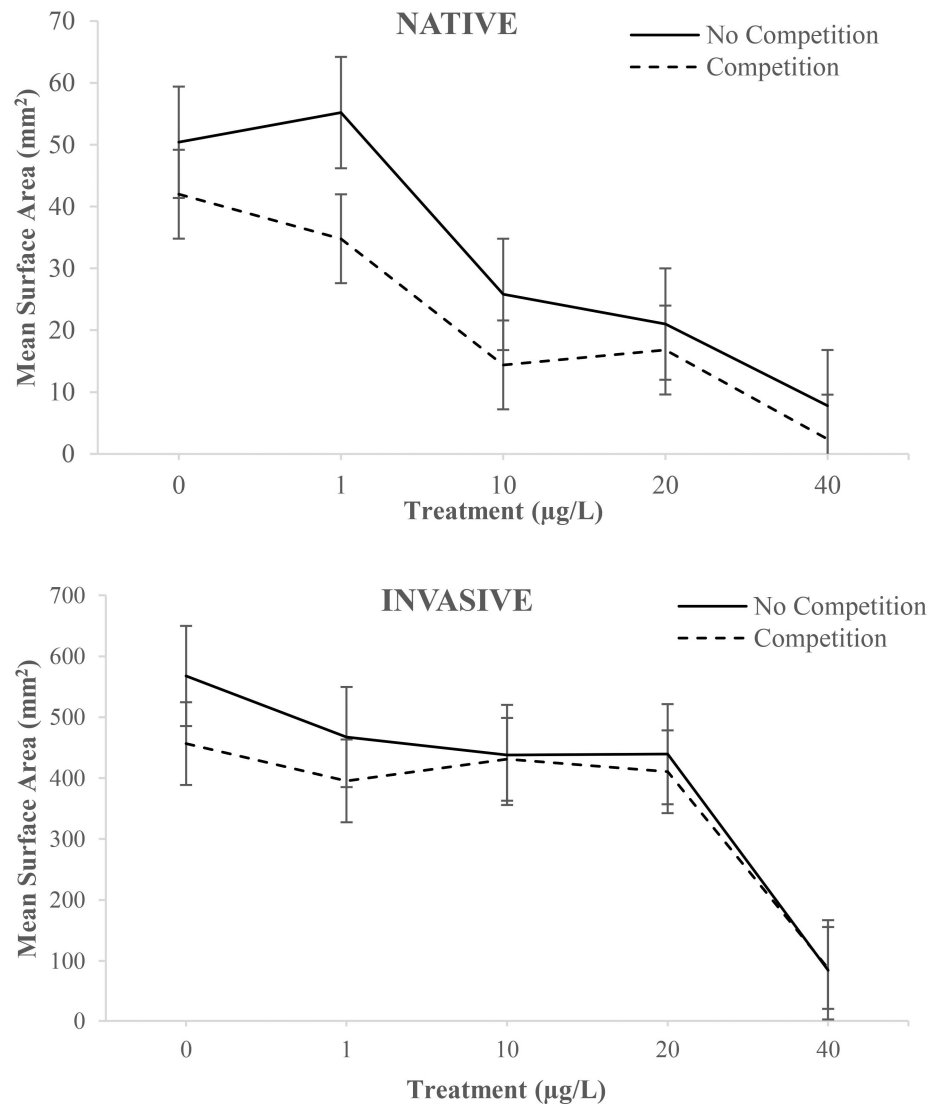


Figure 2. Effect of copper exposure and interspecific competition on ascidian growth. Growth is represented by the mean surface area after 10 weeks post-settlement in the presence or absence of copper or competition. (A) the native species, *A. glabrum*. (B) the invasive species, *B. violaceus*.

Table 2. EC₅₀ and LOEC concentrations (µg/L) impacting surface area of native (*A. glabrum*) and invasive (*B. violaceus*) ascidians in both competition treatment types. n = 5 for each treatment tested.

Species	Toxicity Threshold	SURFACE AREA		NUMBER OF ZOOIDS	
		No Competition	Competition	No Competition	Competition
NATIVE	EC ₅₀	11.3 ± 2.1	6.9 ± 4.0	13.2 ± 1.9	8.4 ± 1.7
	LOEC	10	1	10	1
INVASIVE	EC ₅₀	37.1 ± 8.6	31.7 ± 4.7	52.3 ± 1.4	41.5 ± 3.0
	LOEC	40	40	40	40

resulting in a 50% reduction in surface area. Again, the native species shows increased sensitivity with an EC₅₀ value of less than a third of the value for *B. violaceus*.

In the presence of low concentrations of copper (1 µg/L), competition played a role in further suppressing native ($p = 0.009$), but not invasive species growth (Figure 2). Competition also had a significant effect on *A. glabrum* in the 10 and 40 µg/L copper exposures (Figure 2). The surface

Table 3. Final surface area measurements with standard deviations of native (*A. glabrum*) and invasive (*B. violaceus*) ascidians in both competition treatment types at the conclusion of the 10-week exposure period.

Species	Treatment ($\mu\text{g/L}$)	No Competition	Competition	P-Value (* < 0.05)
NATIVE	0	50.4 \pm 9.3	42.0 \pm 5.6	0.13
	1	55.2 \pm 7.8	34.8 \pm 7.5	0.003*
	10	25.8 \pm 3.4	14.4 \pm 3.9	0.001*
	20	21.0 \pm 6.7	16.8 \pm 4.5	0.28
	40	7.8 \pm 4.5	2.4 \pm 2.5	0.05*
INVASIVE	0	567.6 \pm 117.5	456.6 \pm 49.4	0.12
	1	467.4 \pm 53.0	395.4 \pm 50.8	0.06
	10	438.0 \pm 71.6	430.8 \pm 45.4	0.85
	20	439.2 \pm 55.2	410.4 \pm 39.1	0.37
	40	84.6 \pm 32.1	87.6 \pm 26.4	0.88

Table 4. Factorial ANOVA results indicating a significant interaction between copper concentration (0, 1, 10, 20, and 40 $\mu\text{g/L}$) and competition status (competition/no competition) on final surface area measurements.

		df	Sum Squares	Mean Squares	F Value	Pr (>F)
<i>A. glabrum</i> (native)	Competition Status	1	137.8	137.8	34.79	6.564e-07 *
	Treatment Concentration	4	1428	357.1	90.17	< 2.2e-16 *
	Competition x Pollution	4	46.52	11.63	2.937	0.03 *
	Residuals	40	158.4	3.96		
<i>B. violaceus</i> (invasive)	Competition Status	1	2592	2592	6.639	0.07
	Treatment Concentration	4	123790	30948	79.26	< 2e-16 *
	Competition x Pollution	4	2518	629.4	1.612	0.19
	Residuals	40	15618	390.4		

area of *A. glabrum* was 50% less compared to the control without competition at 10 $\mu\text{g/L}$, and nearly 65% less while in competition ($p = 0.001$). At 40 $\mu\text{g/L}$, surface area was 85% less without competition and 95% less in the presence of competition ($p = 0.05$).

These findings suggest that as copper concentration increased, the effect of competition became stronger on *A. glabrum*. The presence of competition affected the growth of *A. glabrum* at all concentrations, further decreasing overall growth beyond the impacts of copper alone. For example, in the 10 $\mu\text{g/L}$ exposure with the presence of competition, colony growth of *A. glabrum* is lower than the growth at 20 $\mu\text{g/L}$ in the absence of competition (Table 3, Figure 1). Dose-response curves revealed that the EC_{50} values were lower in the presence of competition. In addition, EC_{50} values were nearly five times greater for *B. violaceus* than *A. glabrum* in treatments with competition, indicating a heightened sensitivity of the native species relative to the invader, especially when in competition with *B. violaceus* for resources.

A factorial ANOVA was used to compare the main effects of competition status and copper exposure (as reported in the sections above) and determine if there was an interaction effect on ascidian growth. The results reported in Table 4 indicated that a competition status-copper exposure interaction was significant for *A. glabrum* ($p = 0.03$) but not for *B. violaceus* at environmentally relevant concentrations.

Discussion

Understanding the mechanisms responsible for the success of marine invasive species is critical for their management. Under this context, the influences of copper pollution and interspecific competition were compared on a prolific marine invader, *Botrylloides violaceus* and a native species, *Aplidium glabrum*. Both species are abundant along the Northwest Atlantic coast where they compete for resources within the marine biofouling community. We argue that colony surface growth is a strong predictor of invasion success because space is a primary resource and under constant competition from the benthic community (e.g. Stachowicz et al. 2002). The major findings of this study indicate that 1) competition does not play a significant role in limiting *A. glabrum* surface area growth until copper is introduced and 2) *B. violaceus* is not significantly impacted by competition at any copper concentration tested. Additionally, similar EC₅₀ values to those previously established (Osborne et al. 2018) confirm relatively higher copper tolerance in invaders.

B. violaceus continues to be globally transported and field studies have confirmed the dominance of *B. violaceus* in the overlapping range of *A. glabrum* (Dijkstra and Harris 2009; Sorte and Stachowicz 2011; McIntyre et al. 2013; Wells et al. 2014; Dijkstra et al. 2017). Here, *A. glabrum* demonstrated a much slower proliferation overall than *B. violaceus*. Since this disparity in growth rate has been observed in the field (*pers. obs.*), this study provides a potential mechanism for this occurrence.

It was previously established that *B. violaceus* is more tolerant to copper pollution compared with native species, *A. glabrum*. The current study confirmed these findings where significant impairment of growth was observed in *A. glabrum* at 10 µg/L, while *B. violaceus* showed no sign of reduced growth until concentrations nearly four times higher. The physiological “tipping point” is therefore measurably lower in the native versus the invasive species. Copper toxicity to colonial ascidians has been previously investigated on a large-scale using community experiments (Piola and Johnston 2008, 2009; Crooks et al. 2011) and also specific species (Bellas et al. 2004; Osborne et al. 2018). In general, native species displayed lower tolerance to copper. In the community experiments, the lower native tolerance resulted in decreased native biodiversity, which in turn, further enabled the growth and spread of invasive species. To conclude, Piola and Johnston (2008) emphasized the need to consider pollution effects not on diversity as a whole, but to distinguish native and non-native species as they were found to respond to exposure differently.

In addition to copper, the increased tolerance of invasive species to other environmental stressors may facilitate their spread. Studies have shown that *B. violaceus* can tolerate broad temperature fluctuations ranging 5–25 °C (McCarthy et al. 2007; Sorte et al. 2010) and wide salinity changes of

10–38 psu (Dijkstra et al. 2008; Epelbaum et al. 2009a). These observations are in direct contrast to native species sensitivity to environmental factors. In a temperature tolerance study by Sorte et al. (2010), the recovery period following a simulated heat wave was significantly longer for native than invasive ascidians, with natives demonstrating an overall lower recovery in general. Further, juvenile invasive ascidians were not significantly impacted by the heatwave, yet the abundance of the native colonial species decreased by 40% (Sorte et al. 2010). Lagos et al. (2017) investigated the relative tolerance of several native ascidians (i.e. *Pyura dalbyi* Rius and Teske, 2011, *Pyura doppelgangera* Rius and Teske, 2013, *Herdmania grandis* (Heller, 1878), and *Botrylloides magnicoecum* (Hartmeyer, 1912)) and invasive ascidians (i.e. *Styela plicata* (Lesueur, 1823), *Styela clava* Herdman, 1881, *Didemnum* spp., and *Diplosoma* spp.) to hypoxia. Invasive species were found to be nearly twice as tolerant as natives to low oxygen conditions. Further, it was determined that manmade structures, including those found within marinas, lead to reductions in local oxygen levels, which likely impacts native species survival and growth (Lagos et al. 2017). The increased tolerance of invasive species to low oxygen, may help explain why invasive species occur in great abundance on artificial structures in urban harbors and marinas (e.g. Simkanin et al. 2012; Cordell et al. 2013). Taken together, these studies provide further evidence that environmental stressors can impact species biodiversity, by inhibiting the growth of native species, reducing biotic resistance, and providing habitat for invasive species to settle.

However, the relative importance of competition versus environmental stress and whether these stressors interact to further inhibit native species growth has not been directly explored. Therefore, in the current study we went beyond measuring the difference in tolerances between *A. glabrum* and *B. violaceus*, to investigate if competition impacts ascidian growth both in the presence and absence of copper pollution. *A. glabrum* was not inhibited by invasive competitive pressure alone, but the combined effects of competition and pollution, even at low levels, were extremely detrimental to native growth. Concentrations of 1 µg/L caused a difference in surface area growth of *A. glabrum* in the presence of *B. violaceus* despite no observable impacts of competition in controls. Further, 1 µg/L is an environmentally concentration in urban marine settings (Stauber et al. 2000; Schiff et al. 2007), supporting the possibility that copper is negatively influencing native species. Previous studies have found that copper concentrations in urban environments can range from 0–80 µg/L (Manisseri and Menon 1995; Hall Jr. et al. 1998; Negri and Heyward 2001; Katranitsas et al. 2003; Bellas et al. 2004). According to these estimates, areas with low levels of copper pollution are at risk for invasion once their ability to compete is compromised. Although copper inhibited *A. glabrum*

at relatively lower concentrations than *B. violaceus*, the crucial finding is that the pressure of competition overwhelmed the native species only once copper was introduced.

Previous studies have demonstrated that invasive species success significantly increases with the availability of free space (Stachowicz et al. 2002). Although we did not observe total resource exploitation during the 10-week duration of this experiment, percent cover has also been utilized as a proxy of competitive pressure in other studies (Stachowicz et al. 1999; Paetzold et al. 2012). The release of inhibitory chemical compounds by invaders (allelopathy) has been proposed as a potential important non-resource interaction with native counterparts (Hierro and Callaway 2003). Paetzold et al. (2012) determined that *B. violaceus* only inhibited its competitor *Ciona intestinalis* (Linnaeus, 1767) on pre-settled plates and not on bare plates which were available for settlement to both concurrently. The authors therefore suggest that allelopathic impacts may be derived from established *B. violaceus* colonies in pre-settled treatments only. Although possible, it is unlikely that competition for food played a role because effects of food type and concentration have been previously explored (Epelbaum et al. 2009b). Further, concentrations were doubled from recently published values (Osborne et al. 2018) to ensure abundant food availability.

It has been proposed that rapid environmental disturbances may limit native species with prior advantageous adaptations while in competition with non-native species (Byers 2002). Our findings provide key evidence for the Invader Tolerance Model (ITM) that native species are relatively more impacted than invaders by the dual pressures of copper exposure and competition, as described in Osborne et al. (2018). The ITM provides a framework to test the hypothesis that invasive species are more tolerant of marine pollution than natives, and high native species diversity is a crucial component of the community to resist invasion. Our study has shown that in the absence of an additional stressor (i.e. copper), *A. glabrum* growth is not hindered by the presence of invasive species. This implies that under pristine conditions, *A. glabrum* and likely other native ascidians, have the capacity to defend against invasive species through biotic resistance. Further studies with additional native ascidians exposed to other environmental stressors are necessary to confirm the generality of this finding. In addition, future studies should account for propagule pressure of invasive species, which was not possible with our current experimental set-up. However, this is the first known attempt to begin to untangle the effects of pollution, competition, and native/invasive status with a widespread marine invader. This important interaction effect sheds new light on community biotic resistance; tolerances to external pressures including marine pollution may play a much more significant role than originally proposed in forming a biotic defense against invaders.

The management of invasive colonial ascidians poses many challenges, including their ability to be transported on ship hulls, tolerate broad physical conditions, and utilize multiple reproductive strategies to proliferate rapidly (Carlton 1989; Carlton and Geller 1993; Collin et al. 2013). Biological (Carman et al. 2009; Switzer et al. 2011), chemical (Switzer et al. 2011, Carman et al. 2016), and physical (Switzer et al. 2011) removal treatments have been employed, but there has been little success in large-scale field applications. Therefore, management practices should continue to strive for avoiding initial introduction by identifying areas at risk, or hot spots of invasion. As seen here, invasive ascidians are stronger competitors for resources than native ascidians and copper pollution compounds the stress impacting native ascidians. Understanding that native species are threatened by both stressors, both independently and together, highlights the importance of multipronged approaches to invasive species management that includes reducing marine pollution.

We found that low and environmentally relevant concentrations of copper significantly impaired the native ascidians species in this study. This finding offers support for using copper regulation as a new management tool for combating biological invasions. Currently, the U.S. EPA marine water quality criteria (WQC) values are 2.0 µg/L for acute and 1.3 µg/L for chronic copper protection (U.S. EPA 2016). Considering the concentration yielding an interactive effect with competition in this study and measured field copper concentrations together highlights the importance of tightening pollution control to be a more effective prophylactic regulatory strategy. The U.S. National Invasive Species Council's (2016) current invasive species management strategies include introduction prevention, eradication, containment, and ecosystem restoration. This approach focuses on the invasive species themselves and does not consider the management of other potentially additive or synergistic stressors. A trans-disciplinary approach combining invasive species management with stricter copper and perhaps other heavy metal pollution regulations will produce more effective defense strategies for the communities both separately seek to protect.

Acknowledgements

This work was partially supported by the Woods Hole Sea Grant (grant number C120343) and the Coastal America Foundation's general Marines Sciences Research Program grant. We would like to thank Massachusetts Maritime Academy, especially Fran Veale, Tom Pham, and Bill Hubbard for their continued support and access to laboratory and floating dock space. Thank you to Dr. William Robinson and Dr. Jarrett Byrnes from the University of Massachusetts Boston, as well as Dr. Judith Pederson of MIT Sea Grant for their insightful contributions and suggestions throughout the development of this project. Finally, we would like to thank the reviewers advising on the revision process for their improvements to this manuscript.

References

- Bellas J, Beiras R, Vázquez E (2004) Sublethal effects of trace metals (Cd, Cr, Cu, Hg) on embryogenesis and larval settlement of the ascidian *Ciona intestinalis*. *Archives of Environmental Contamination and Toxicology* 46: 61–66, <https://doi.org/10.1007/s00244-003-0238-7>
- Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JR, Richardson DM (2011) A proposed unified framework for biological invasions. *Trends in Ecology & Evolution* 26: 333–339, <https://doi.org/10.1016/j.tree.2011.03.023>
- Byers JE (2002) Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos* 97: 449–458, <https://doi.org/10.1034/j.1600-0706.2002.970316.x>
- Carlton JT (1989) Man's role in changing the face of the ocean: biological invasions and implications for conservation of near-shore environments. *Conservation Biology* 3: 265–273, <https://doi.org/10.1111/j.1523-1739.1989.tb00086.x>
- Carlton JT, Geller JB (1993) Ecological roulette: the global transport of nonindigenous marine organisms. *Science* 261: 78–82, <https://doi.org/10.1126/science.261.5117.78>
- Carman MR, Allen HM, Tyrrell MC (2009) Limited value of the common periwinkle snail *Littorina littorea* as a biological control for the invasive tunicate *Didemnum vexillum*. *Aquatic Invasions* 4: 291–294, <https://doi.org/10.3391/ai.2009.4.1.30>
- Carman MR, Lindell S, Green-Beach E, Starczak VR (2016) Treatments to eradicate invasive tunicate fouling from blue mussel seed and aquaculture socks. *Management of Biological Invasions* 7: 101–110, <https://doi.org/10.3391/mbi.2016.7.1.12>
- Collin SB, Edwards PK, Leung B, Johnson LE (2013) Optimizing early detection of non-indigenous species: estimating the scale of dispersal of a nascent population of the invasive tunicate *Ciona intestinalis* (L.). *Marine Pollution Bulletin* 73: 64–69, <https://doi.org/10.1016/j.marpolbul.2013.05.040>
- Cordell JR, Levy C, Toft JD (2013) Ecological implications of invasive tunicates associated with artificial structures in Puget Sound, Washington, USA. *Biological Invasions* 15: 1303–1318, <https://doi.org/10.1007/s10530-012-0366-y>
- Crooks JA, Chang AL, Ruiz GM (2011) Aquatic pollution increases the relative success of invasive species. *Biological Invasions* 13: 165–176, <https://doi.org/10.1007/s10530-010-9799-3>
- Dijkstra JA, Harris LG (2009) Maintenance of diversity altered by a shift in dominant species: implications for species coexistence. *Marine Ecology Progress Series* 387: 71–80, <https://doi.org/10.3354/meps08117>
- Dijkstra J, Harris LG, Westerman E (2007) Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. *Journal of Experimental Marine Biology and Ecology* 342: 61–68, <https://doi.org/10.1016/j.jembe.2006.10.015>
- Dijkstra J, Dutton A, Westerman E, Harris L (2008) Heart rate reflects osmotic stress levels in two introduced colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus*. *Marine Biology* 154: 805–811, <https://doi.org/10.1007/s00227-008-0973-4>
- Dijkstra JA, Westerman EL, Harris LG (2017) Elevated seasonal temperatures eliminate thermal barriers of reproduction of a dominant invasive species: A community state change for northern communities? *Diversity and Distributions* 23: 1182–1192, <https://doi.org/10.1111/ddi.12604>
- Elton CS (1958) *The Ecology of Invasion by Plants and Animals*. Methuen, London, England, 181 pp, <https://doi.org/10.1007/978-1-4899-7214-9>
- Epelbaum A, Herborg LM, Therriault TW, Pearce CM (2009a) Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. *Journal of Experimental Marine Biology and Ecology* 369: 43–52, <https://doi.org/10.1016/j.jembe.2008.10.028>
- Epelbaum A, Therriault TW, Paulson A, Pearce CM (2009b) Botryllid tunicates: culture techniques and experimental procedures. *Aquatic Invasions* 4: 111–120, <https://doi.org/10.3391/ai.2009.4.1.12>
- Hall Jr. LW, Scott MC, Killen WD (1998) Ecological risk assessment of copper and cadmium in surface waters of Chesapeake Bay watershed. *Environmental Toxicology and Chemistry* 17: 1172–1189, <https://doi.org/10.1002/etc.5620170626>
- Hasenbein S, Peralta J, Lawler SP, Connon RE (2017) Environmentally relevant concentrations of herbicides impact non-target species at multiple sublethal endpoints. *Science of the Total Environment* 607: 733–743, <https://doi.org/10.1016/j.scitotenv.2017.06.270>
- Hierro JL, Callaway RM (2003) Allelopathy and exotic plant invasion. *Plant and Soil* 256: 29–39, <https://doi.org/10.1023/A:1026208327014>
- Katranitsas A, Castritsi-Catharios J, Persoone G (2003) The effects of a copper-based antifouling paint on mortality and enzymatic activity of a non-target marine organism. *Marine Pollution Bulletin* 46: 1491–1494, [https://doi.org/10.1016/S0025-326X\(03\)00253-4](https://doi.org/10.1016/S0025-326X(03)00253-4)
- Lagos ME, Barneche DR, White CR, Marshall DJ (2017) Do low oxygen environments facilitate marine invasions? Relative tolerance of native and invasive species to low oxygen conditions. *Global Change Biology* 23: 2321–2330, <https://doi.org/10.1111/gcb.13668>

- Lawes JC, Clark GF, Johnston EL (2016) Contaminant cocktails: Interactive effects of fertiliser and copper paint on marine invertebrate recruitment and mortality. *Marine Pollution Bulletin* 102: 148–159, <https://doi.org/10.1016/j.marpolbul.2015.11.040>
- Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Malden, Massachusetts, USA, 312 pp
- Lodge DM, Williams S, MacIsaac HJ, Hayes KR, Leung B, Reichard S, Mack RN, Moyle PB, Smith M, Andow DA, Carlton JT (2006) Biological invasions: recommendations for US policy and management. *Ecological Applications* 16: 2035–2054, [https://doi.org/10.1890/1051-0761\(2006\)016\[2035:BIRFUP\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2)
- Manisseri MK, Menon NR (1995) Copper-induced damage to the hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni* - an ultrastructural study. *Diseases of Aquatic Organisms* 22: 51–57, <https://doi.org/10.3354/dao022051>
- Maxwell SL, Fuller RA, Brooks TM, Watson JEM (2016) The ravages of guns, nets, and bulldozers. *Nature News* 536: 143–145, <https://doi.org/10.1038/536143a>
- McCarthy A, Osman RW, Whitlatch RB (2007) Effects of temperature on growth rates of colonial ascidians: a comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. *Journal of Experimental Marine Biology and Ecology* 342: 172–174, <https://doi.org/10.1016/j.jembe.2006.10.036>
- McIntyre CM, Pappal AL, Bryant J, Carlton JT, Cute K, Dijkstra J, Erickson R, Garner Y, Gittenberger A, Grady S, Haram L, Harris L, Hobbs N, Lambert C, Lambert G, Lambert W, Marques A, Mathieson A, McCuller M, Mickiewicz M, Pappal A, Pederson J, Rock-Blake R, Smith J, Sorte C, Stefaniak L, Wagstaff M (2013) Report on the 2010 rapid assessment survey of marine species at New England floating docks and rocky shores. Massachusetts Office of Coastal Zone Management, 37 pp
- National Invasive Species Council (2016) Management Plan: 2016–2018. Washington, DC, 50 pp
- Negri AP, Heyward AJ (2001) Inhibition of coral fertilisation and larval metamorphosis by tributyltin and copper. *Marine Environmental Research* 51: 17–27, [https://doi.org/10.1016/S0141-1136\(00\)00029-5](https://doi.org/10.1016/S0141-1136(00)00029-5)
- Osborne KL, Hannigan RE, Poynton HC (2018) Differential copper toxicity in invasive and native ascidians of New England provides support for enhanced invader tolerance. *Marine Ecology Progress Series* 595: 135–47, <https://doi.org/10.3354/meps12555>
- Paetzold SC, Giberson DJ, Hill J, Davidson JD, Davidson J (2012) Effect of colonial tunicate presence on *Ciona intestinalis* recruitment within a mussel farming environment. *Management of Biological Invasions* 3: 15–23, <https://doi.org/10.3391/mbi.2012.3.1.02>
- Parker IM, Simberloff D, Lonsdale WM, Goodell K, Wonham M, Kareiva PM, Williamson MH, Von Holle B, Moyle PB, Byers JE, Goldwasser L (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1: 3–19, <https://doi.org/10.1023/A:1010034312781>
- Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273–288, <https://doi.org/10.1016/j.ecolecon.2004.10.002>
- Piola RF, Johnston EL (2006) Differential tolerance to metals among populations of the introduced bryozoan *Bugula neritina*. *Marine Biology* 148: 997–1010, <https://doi.org/10.1007/s00227-005-0156-5>
- Piola RF, Johnston EL (2008) Pollution reduces native diversity and increases invader dominance in marine hard-substrate communities. *Diversity and Distributions* 14: 329–342, <https://doi.org/10.1111/j.1472-4642.2007.00430.x>
- Piola RF, Johnston EL (2009) Comparing differential tolerance of native and non-indigenous marine species to metal pollution using novel assay techniques. *Environmental Pollution* 157: 2853–2864, <https://doi.org/10.1016/j.envpol.2009.04.007>
- Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist* 37: 621–632, <https://doi.org/10.1093/icb/37.6.621>
- Ruiz GM, Fofonoff P, Hines AH, Grosholz ED (1999) Non-indigenous species as stressors in estuarine and marine communities: assessing invasion impacts and interactions. *Limnology and Oceanography* 44: 950–972, https://doi.org/10.4319/lo.1999.44.3_part_2.0950
- Schiff K, Brown J, Diehl D, Greenstein D (2007) Extent and Magnitude of Copper Contamination in Marinas of the San Diego Region, California, USA. Technical Report #483. Southern California Coastal Water Research Project 19 pp, <https://doi.org/10.1016/j.marpolbul.2006.10.013>
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675, <https://doi.org/10.1038/nmeth.2089>
- Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pysek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jager H, Kartesz J, Kenis W, Kreft H, Kuhn A, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rossinelli S, Roy H, Scalera R, Schindler S, Stajero K, Tokarska-Guzik B, van Kleunen M, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications* 8: 14435, <https://doi.org/10.1038/ncomms14435>

- Seltman HJ (2015) Experimental design and analysis. Carnegie Mellon University, Pittsburgh, Pennsylvania, USA, 428 pp, <http://www.stat.cmu.edu/~hseltman/309/Book/Book.pdf>
- Simkanin C, Davidson IC, Dower JF, Jamieson G, Therriault TW (2012) Anthropogenic structures and the infiltration of natural benthos by invasive ascidians. *Marine Ecology* 33: 499–511, <https://doi.org/10.1111/j.1439-0485.2012.00516.x>
- Sorte CJ, Stachowicz JJ (2011) Patterns and processes of compositional change in a California epibenthic community. *Marine Ecology Progress Series* 435: 63–74, <https://doi.org/10.3354/meps09234>
- Sorte CJB, Fuller A, Bracken MW (2010) Impacts of a simulated heat wave on composition of a marine community. *Oikos* 119: 1909–1918, <https://doi.org/10.1111/j.1600-0706.2010.18663.x>
- Stachowicz JJ, Whitlatch RB, Osman RW (1999) Species diversity and invasion resistance in a marine ecosystem. *Science* 286: 1577–1579, <https://doi.org/10.1126/science.286.5444.1577>
- Stachowicz JJ, Fried H, Osman RW, Whitlatch RB (2002) Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. *Ecology* 83: 2575–2590, [https://doi.org/10.1890/0012-9658\(2002\)083\[2575:BIRAME\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[2575:BIRAME]2.0.CO;2)
- Stauber J, Benning RJ, Hales LT, Eriksen R, Nowak B (2000) Copper bioavailability and amelioration of toxicity in Macquarie harbour, Tasmania, Australia. *Marine and Freshwater Research* 51: 1–10, <https://doi.org/10.1071/MF99010>
- Switzer SE, Therriault TW, Dunham A, Pearce CM (2011) Assessing potential control options for the invasive tunicate *Didemnum vexillum* in shellfish aquaculture. *Aquaculture* 318: 145–153, <https://doi.org/10.1016/j.aquaculture.2011.04.044>
- Tilman D, Clark M, Williams DR, Kimmel K, Polasky S, Packer C (2017) Future threats to biodiversity and pathways to their prevention. *Nature* 546: 73–81, <https://doi.org/10.1038/nature22900>
- U.S. EPA (2016) Draft Aquatic Life Ambient Estuarine/Marine Water Quality Criteria or Copper. Office of Water, Washington DC, USA, EPA-822-P-16-001, 257 pp
- Van Guelpen L, Pohle G, Vanden Berghe E, Costello M (2005) Marine species registers for the north Atlantic Ocean, version 1, 2005. <http://www.vliz.be/vmdcdata/narms/index.php>
- Van Name WG (1945) The North and South American Ascidians. *Bulletin of the American Museum of Natural History* 84: 1–476
- Walsh JR, Carpenter SR, Vander Zanden MJ (2016) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences* 113: 4081–4085, <https://doi.org/10.1073/pnas.1600366113>
- Watanabe H, Lambert CC (1973) Larva release in response to light by the compound ascidians *Distaplia occidentalis* and *Metandrocarpa taylori*. *The Biological Bulletin* 144: 556–566, <https://doi.org/10.2307/1540308>
- Wells CD, Pappal AL, Cao Y, Carlton JT, Currimjee Z, Dijkstra JA, Edquist S, Gittenberger A, Goodnight S, Grady SP, Green LA, Harris LG, Harris LH, Hobbs N, Lambert G, Marques A, Mathieson AC, McCuller MI, Osborne K, Pederson JA, Ros M, Smith JP, Stefaniak LM, Stevens A (2014) Report on the 2013 Rapid Assessment Survey of Marine Species at New England Bays and Harbors. Massachusetts Office of Coastal Zone Management, 32 pp
- Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. *BioScience* 48: 607–615, <https://doi.org/10.2307/1313420>
- Zhan A, Briski E, Bock DG, Ghabooli S, MacIsaac HJ (2015) Ascidians as models for studying invasion success. *Marine Biology* 162: 2449–2470, <https://doi.org/10.1007/s00227-015-2734-5>