

Research Article

Invasive tunicate (Ascidiacea) metabolic and filtration rates in comparison to native tunicate and bivalve speciesYingqi Zhang^{1,2,*}, Linda Deegan³ and Mary R. Carman⁴¹University of Southern California, Los Angeles, California 90089, USA²Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA³Woods Hole Research Center, Falmouth, Massachusetts 02540, USA⁴Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USAE-mail: yingqizh@usc.edu (YZ), lddeegan@whrc.org (LD), mcarman@whoi.edu (MC)

*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 IISSC 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: Zhang Y, Deegan L, Carman MR (2019) Invasive tunicate (Ascidiacea) metabolic and filtration rates in comparison to native tunicate and bivalve species. *Management of Biological Invasions* 10(4): 617–625, <https://doi.org/10.3391/mbi.2019.10.4.03>

Received: 24 December 2018**Accepted:** 9 September 2019**Published:** 30 October 2019**Handling editor:** Stephan Bullard**Copyright:** © Zhang et al.

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**

Several invasive species of tunicates (Ascidiacea) have become cosmopolitan and widely distributed in coastal areas worldwide over the past few decades. These non-indigenous tunicates have consequently caused fouling problems in aquaculture and marine harbors. The goal of our project was to enrich the understanding of how invasive tunicates interact with other organisms in the ecosystem. Two species of invasive tunicates (*Didemnum vexillum* and *Botrylloides violaceus*) and one species of native tunicate (*Aplidium glabrum*) were evaluated for their metabolic rates. The filtration rates for native blue mussels (*Mytilus edulis*) and invasive tunicates (*Diplosoma listerianum*) were determined. *D. vexillum* regenerated NH_4^+ at a faster rate than *A. glabrum* and *B. violaceus*. Both tunicates and blue mussels were feeding on phytoplankton as their major food source, although the size of particles utilized by different organisms was not examined in this study. Invasive tunicates were strongly competing with mussels to filter feed, but were not inhibiting mussel's filtration rate.

Key words: colonial tunicates, metabolic rate, blue mussels, filtration rate**Introduction**

Facilitated by global trade and shipping, invasive species have become a growing worldwide problem (Lambert 2007; Keller and Perrings 2011). Shallow coastal ecosystems are especially susceptible to invasion by exotic species, as they are often influenced by human activities, including ballast water transfer and aquarium releases, shoreline development, and aquaculture (Carlton and Geller 1993). Biodiversity and the physical environment of global ecosystems are shaped by anthropogenic influences. Oceans are expected to continue to become warmer and more acidic with the increase of atmospheric CO_2 concentration (Solomon et al. 2007). Multiple studies suggest that climate change might provide a competitive advantage for invasive species and enable them to colonize new habitats and replace endemic species (Anthony et al. 2009; Rahel and Olden 2008; Hellmann et al. 2008).

Tunicates (Ascidiacea), commonly known as sea squirts, are marine biofouling organisms that commonly distribute by attaching to the hulls of recreational boats and commercial ships (McKenzie et al. 2016). Once transported to new locations, non-endemic species may be able to quickly colonize local natural or artificial substrates, reproduce, and establish populations (Lambert 2007). Repeated introductions often occur over time so that different haplotypes of the same species can co-occur in the same area, such as with the colonial tunicates *Botryllus schlosseri* (Pallas, 1766) (Yund et al. 2015) and *Didemnum vexillum* (Kott, 2002) (Stefaniak et al. 2009). Several species of non-native tunicates from East Asia and Europe were introduced to New England in the 1970s and 1980s (Valigra 2005). Non-native, introduced tunicates compete with other species for food and space (Dijkstra et al. 2007; Colarusso et al. 2016) and can be found on a variety of artificial and natural substrates, ranging from rocks and moorings to eelgrass and shellfish (Carman et al. 2010, 2016a). The hard surface of cultured mussels, oysters, and other shellfish and aquaculture gear provides an ideal platform for tunicate fouling and their negative impact on bivalve shellfish aquaculture community, including increased maintenance cost and reduced shellfish growth, is the cause for concern to the aquaculture industry (Carman et al. 2010, 2016b; Adams et al. 2011; Auken et al. 2014). Bryozoans often co-occur with tunicates in the wild on shellfish. More research is needed to understand how interactions of native and non-native fouling species will impact economically important coastal resources, namely native shellfish.

The focus of our study was to better understand the influence of tunicates, including *Aplidium glabrum* (Verrill, 1871), a native species; and *Didemnum vexillum*, *Botrylloides violaceus* (Oka, 1927), and *Diplosoma listerianum* (Milne-Edwards, 1841), invasive species, in coastal ecosystems. Tunicates are likely utilizing similar food sources as blue mussels (Buschbaum and Saier 2001; Bone et al. 2003; Bullard and Carman 2009; Colarusso et al. 2016). Tunicates efficiently consume 1–2 micron size particles while mussels efficiently consume particles that are > 4 microns (Petersen 2007). Therefore we used a food source that includes a range of particle sizes. The epibiotic presence of tunicates may inhibit the ability of shellfish to filter-feed (Lutz-Collins et al. 2009).

Materials and methods

Four colonial species of tunicates and one bivalve species were used in our experimental trials: *Aplidium glabrum*, a native species; and *Didemnum vexillum*, *Botrylloides violaceus*, and *Diplosoma listerianum*, non-native species; and blue mussels *Mytilus edulis* (Linnaeus, 1758), a native species.

Field sampling

Aplidium glabrum, *D. vexillum*, and *B. violaceus* were collected from the Marine Biological Laboratory (MBL) docks at Eel Pond, Woods Hole,

Massachusetts. Blue mussels, with epibionts (including *D. listerianum* and bryozoans), were collected from the Martha's Vineyard Shellfish Group, Inc. dock at Lagoon Pond on Martha's Vineyard. It is unknown how long the tunicate epibionts were attached to the blue mussels. All collections were conducted during November 2016. Field samples were transported in containers of seawater to the lab and held in a running seawater facility prior to further experiments.

Preparation of experimental tunicate tiles

Colonial tunicates were divided into pieces of similar size (approximately 1 g and 4 cm²), and secured to 4.8 cm × 4.8 cm white ceramic tiles with a rough surface using rubber bands (n = 48 per species). Tiles with tunicates were held in tanks of flowing seawater for about 48 hours to allow the tunicates to attach to the tiles. Attachment was checked by gently shaking the tile underwater while lifting up the rubber band. If the organism had minimal to no change in position, then attachment was successful. Tunicates were considered viable and healthy if they attached to the tile. Tiles with attached tunicates were then used in experimental trials. Blank tiles without attached tunicates were used as controls.

Experiment #1: Metabolic rates of tunicates

Metabolism (oxygen uptake and nitrogen regeneration) was measured in a 473 mL sealed respiration chambers (n = 8 per species) over time. Each chamber contained 6 tunicate tiles of one species or 6 blanks. The chambers were held in an 18 °C incubator, gently stirred. Oxygen concentration measured with Hach HQ30D Portable Dissolved Oxygen Meters every 5 minutes until the oxygen level dropped to around 5 mg/L. The net O₂ consumption rate (mg/g/L/h) by tunicates was determined by subtracting the O₂ uptake rate of the blank-tile group from the total O₂ uptake of the tunicate-tile group and dividing by the total wet weight of the tunicates. Nitrogen regeneration (μM/g/h) was estimated from the NH₄⁺ concentration (Solorzano 1969) in the chambers at the beginning and the end of each metabolic trial, subtracting the blank-tile group, and dividing by the total wet weight of the tunicates of each tunicate-tile group to get NH₄⁺ regeneration rate per biomass.

Experiment #2: Algal filtration rates and food source dependence of mussels and tunicates

To assess the relationship between epifaunal tunicates and blue mussels, the metabolic rates of blue mussels with varying coverage of tunicates and other fouling organisms were measured. The percent coverage of epibionts (predominantly tunicates, including *Diplosoma listerianum*, with a small amount of bryozoans) on blue mussels was estimated by photographing

the blue mussels and measuring the total area of coverage by using the image-processing software *ImageJ*. Total filtration rates were determined for each live blue mussel with attached tunicates and bryozoans. Then the blue mussels' internal tissues were carefully removed and the filtration rate was determined for the same blue mussel shells with epifauna only in replaced water. Sixteen blue mussels/pairs of mussel shells were placed into sixteen jars, filled with 350 mL of seawater and 5 mL of diluted algae solution frozen algae paste (*RotiGrow+*), and then kept on slowly moving shaker tables to keep algal cells suspended and maintain oxygen levels. The amount of chlorophyll *a* in each jar was measured every two hours for eight hours (Turner Designs 10-au fluorometer). Filtration rate was determined by the change in chlorophyll *a* concentration after the first two hours. The net filtration rate ($\mu\text{g/g/L/h}$) of blue mussels was calculated as the difference between the filtration rate of whole mussel and that of its shells with just epibionts divided by the individual meat biomass. Epibiont biomass was not measured. Muscle tissue of several blue mussels and the tissues of *D. listerianum* were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the MBL stable isotope lab to assess relative importance of algae as a food source. *D. listerianum* was chosen as it was thought to be representative of colonial tunicate feeding types and was present in sufficient mass to obtain a sufficient sample. Typical isotopic values for wild local phytoplankton were retrieved from Deegan and Garritt 1997, Nelson et al. 2015, and Colarusso et al. 2016.

Statistics

One-way ANOVA was performed in R 3.5.2 to determine whether metabolic rates of different tunicate species were significantly different. Regression was used to evaluate the correlation between mussel filtration rate and epibiont coverage as well as epibiont filtration rate. A *p* value smaller than 0.05 is considered to be statistically significant.

Results

Experiment #1: Metabolic rates of tunicates

There was a strong trend for the two invasive tunicates species, *Didemnum vexillum* and *Botrylloides violaceus*, to exhibit higher oxygen consumption rates than the native species, *Aplidium glabrum* (Figure 1; $F = 3.426$, $df = 2$, $p = 0.0516$), though the differences were not statistically significant. *Didemnum vexillum* generated more than twice as much NH_4^+ waste per unit time than *A. glabrum* and *B. violaceus* ($F = 14.228$, $df = 2$, $p < 0.001$; Figure 2).

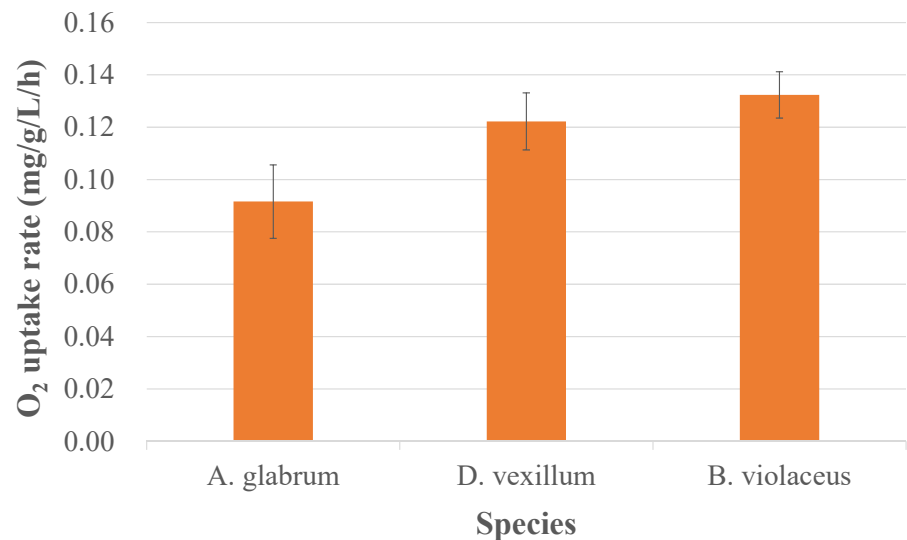


Figure 1. Oxygen uptake rates of *Aplidium glabrum*, *Didemnum vexillum*, and *Botrylloides violaceus* measured at the beginning of Experiment 1 (mean \pm SE). Oxygen consumption was not significantly different across 3 species ($F = 3.426$, $df = 2$, $p = 0.0516$).

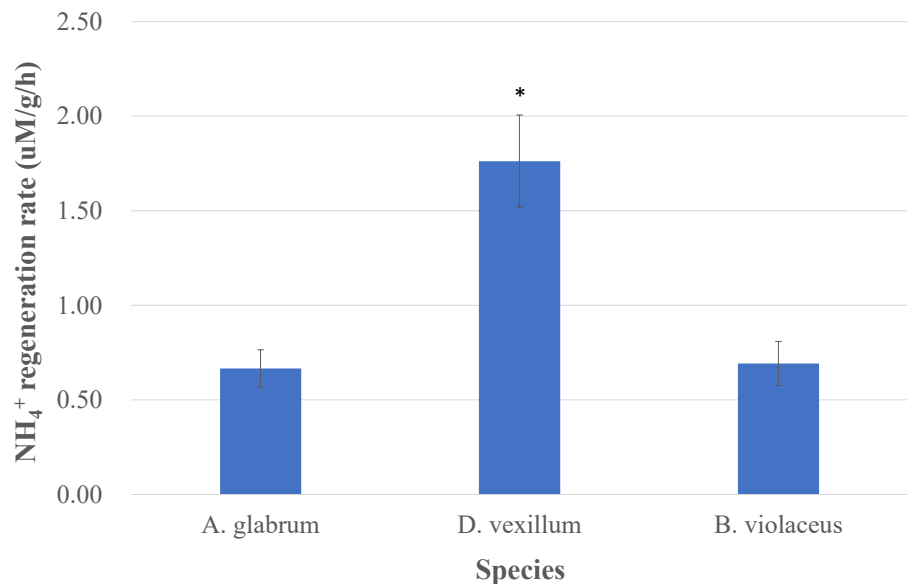


Figure 2. Ammonium regeneration rate of *Aplidium glabrum*, *Didemnum vexillum*, and *Botrylloides violaceus* measured at the beginning of Experiment 1 (mean \pm SE). Ammonium regeneration rate was significantly different across 3 species ($F = 14.228$, $df = 2$, $p < 0.001$). The asterisk indicates *D. vexillum* had significantly higher NH₄⁺ regeneration rate than the other two species.

Experiment #2: Algal filtration rates and food source dependence of mussels and tunicates

The filtration rates of individual blue mussels (standardized to biomass) were not correlated with their percent coverage by tunicates and bryozoans ($R^2 = 0.07$, $p = 0.3387$; Figure 3). The filtration rates of the epifauna were consistently around 5 $\mu\text{g/L/h}$ (not adjusted for epibiont biomass) even though percent coverage varied from near 0 to 70%, while the blue mussel filtration rate varied between 0.5–5 $\mu\text{g/g/L/h}$ (Figure 4). Based on the results of the stable isotope analysis, blue mussels and invasive tunicates had

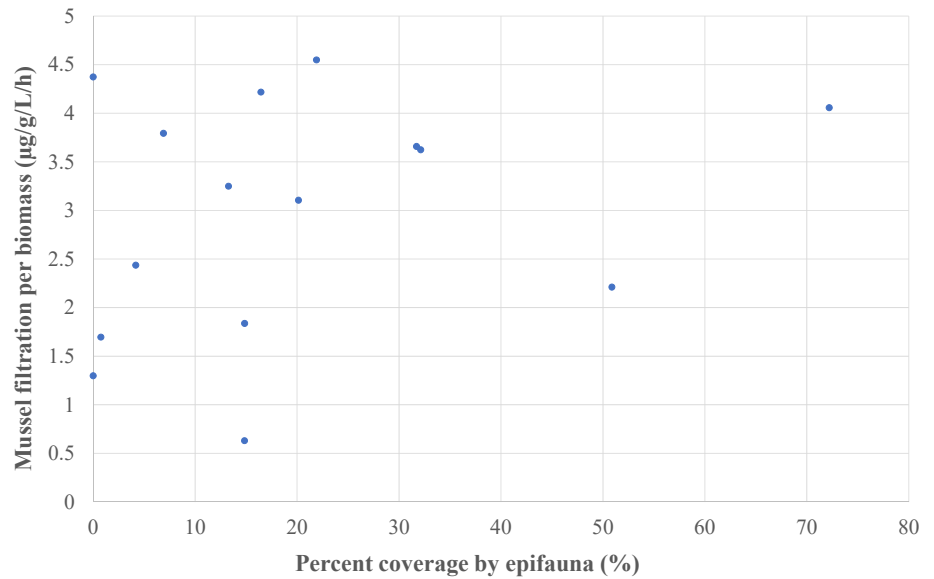


Figure 3. Net filtration rate of mussels that had different shell area coverage (%) by tunicates and bryozoans. In all cases, the coverage did not extend to the shell margins and did not limit the gape opening of mussels ($R^2 = 0.07$, $p = 0.3387$).

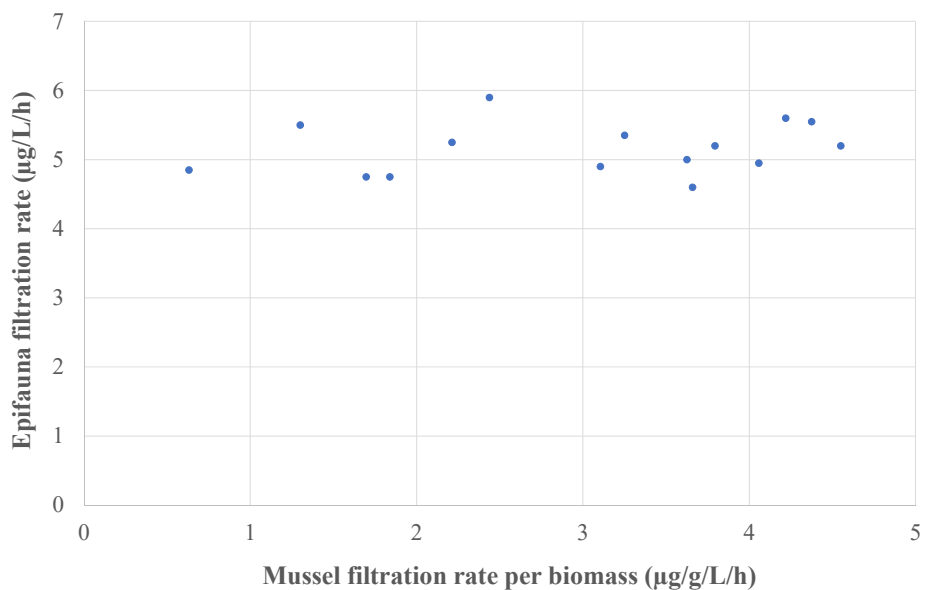


Figure 4. Comparison between epifauna algal filtration rate and blue mussel filtration rate. Filtering rate was measured as chlorophyll depletion over a two-hour incubation. No significant correlation was found between epifauna and mussel filtration rates ($R^2 = 0.034$, $p = 0.5106$).

similar $\delta^{13}\text{C}$ values between -22‰ and -18‰ , which are in the typical range for phytoplankton (Figure 5; Deegan and Garritt 1997; Nelson et al. 2015). Blue mussels had slightly higher $\delta^{15}\text{N}$ values than tunicates, which might be due to the consumption of zooplankton as a part of their diet (Wong and Levinton 2004).

Discussion

Native and invasive tunicates were metabolically different. The generally higher oxygen demands of *Didemnum vexillum* and *Botrylloides violaceus*

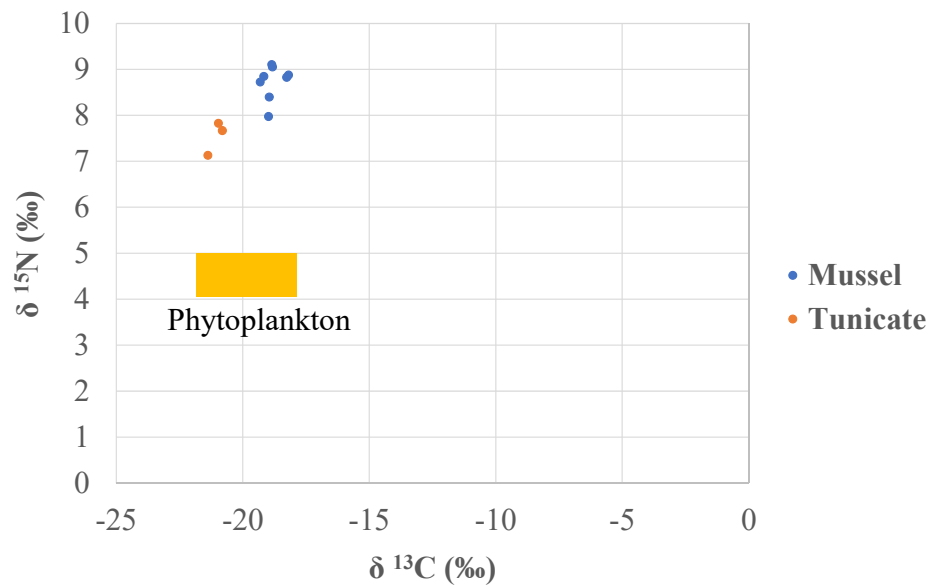


Figure 5. Measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of blue mussels and tunicates. The yellow box represents isotopic values of phytoplankton retrieved from Deegan and Garritt 1997 and Nelson et al. 2015.

suggest that these two invasive tunicates might be growing at faster rates than the native tunicate *Aplidium glabrum*, which is consistent with the trend of declining native tunicate abundance due to the competition from invasive tunicates (Carman et al. 2010). The high ammonium regeneration rate of *D. vexillum* could be attributed to its flow through digestive system in which food is taken in via a single siphon by filter feeding, moves quickly through the animals and excess water and waste products are expelled via a common atrial siphon (Ruppert et al. 2004). The expulsion of water and waste products together can lead to what has been called a “leaky” digestive system in which ammonium and other waste products leave the body quickly.

The blue mussel filtration rate was not negatively correlated with percent coverage of the tunicates and bryozoans. This was probably because the epifauna only occupied the surface of blue mussel shells in our samples and not the margins. If the tunicates had overgrown the margins of the shells, the mussel filtration might have been inhibited, decreasing mussel filtration rate. The similar $\delta^{13}\text{C}$ values of invasive tunicates and blue mussels indicate that the organisms are indeed both supported by the phytoplankton food web. Additionally, the whole-organism filtration rates of epifauna over a wide range of shell surface covering were in the same magnitude as those of blue mussels (Figures 3 and 4). These two findings imply that there could be competition between blue mussels and their associated tunicate epifauna to acquire the same food. When tunicates start to actively grow and reproduce during summer, blue mussels could be locally outcompeted. Furthermore, it would be helpful to examine the size of particles that were consumed by native, invasive tunicates, and blue mussels to gain a better understanding of their trophic dynamics.

Invasive tunicates might be more resilient to changes in temperature and pH conditions that are likely to occur under climate change. Elevated seawater temperatures are changing the distribution patterns of tunicate populations, giving some introduced species a competitive advantage (Epelbaum et al. 2009), especially those species that have a range of environmental tolerances (Lambert and Lambert 2003). As ocean temperatures increase, native species will likely decrease in abundance, and introduced species are likely to increase in this system (Sorte et al. 2010). The metabolic rate doubled at experimentally controlled, warmer temperatures for the solitary tunicate *Styela plicata* (Lesueur, 1823) (Montalto et al. 2017). The results of our metabolic tests indicate that temperature may be a stronger driver to changes in metabolism than pH (unpublished data). There might be a synergistic effect of warming and acidification on the oxygen consumption rate for *D. vexillum*. However, this hypothesis needs to be tested. Although our study has provided some insight on the unique ecology of invasive tunicates, future studies may keep investigating on the difference in filtration rate and growth and survivorship response to environmental drivers between native and invasive tunicates. Our comparisons are limited, because replication levels were too low when samples were divided into different temperature and pH treatment groups (hence unpublished). We suggest that these hypotheses be tested in the summer, when tunicates exist in high abundance and are thus easily accessible.

Acknowledgements

This project was part of the MBL Semester in Environmental Sciences. Special thanks to Rich McHorney, Helena McMonagle, Madeline Gorchels, and Leena Vilonen, for their guidance and assistance in the laboratory; to Michelle Woods and Olivia Bispo for their help in the field; to Anne Giblin for providing the oxygen probes; to Marshall Otter for running stable isotope samples; to Dave Remsen, Janice Simmons, and Katie Dever for providing frozen algae paste; to Dave Grunden for assistance with blue mussel collection at Martha's Vineyard. The authors would also like to thank the two anonymous reviewers for providing comments and critiques to help improve the manuscript.

References

- Adams CM, Shumway SE, Whitlatch RB, Getchis T (2011) Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. *Journal of the World Aquaculture Society* 42: 242–252, <https://doi.org/10.1111/j.1749-7345.2011.00460.x>
- Anthony A, Atwood J, August P, Byron C, Cobb S, Foster C, Fry C, Gold A, Hagos K, Heffner L, Kellogg DQ, Lellis-Dibble K, Opaluch JJ, Oviatt C, Pfeiffer-Herbert A, Rohr N, Smith L, Smythe T, Swift J, and Vinhateiro N (2009) Coastal lagoons and climate change: ecological and social ramifications in US Atlantic and Gulf coast ecosystems. *Ecology and Society* 14: 8, <https://doi.org/10.5751/ES-02719-140108>
- Auker LA, Majkut AL, Harris LG (2014) Exploring biotic impacts from *Carcinus maenas* predation and *Didemnum vexillum* epibiosis on *Mytilus edulis* in the Gulf of Maine. *Northeastern Naturalist* 21: 479–494, <https://doi.org/10.1656/045.021.0314>
- Bone Q, Carre C, Chang P (2003) Tunicate feeding filters. *Journal of the Marine Biological Association of the United Kingdom* 83: 907–919, <https://doi.org/10.1017/S002531540300804Xh>
- Bullard SG, Carman MR (2009) Current trends in invasive ascidian research. In: Wilcox CP, Turpin RB (eds), *Invasive Species: Detection, Impact and Control*, Nova Science Publishing, Hauppauge, NY, pp 57–59
- Buschbaum C, Saier B (2001) Growth of the mussel *Mytilus edulis* L. in the Wadden Sea affected by tidal emergence and barnacle epibionts. *Journal of Sea Research* 45: 27–36, [https://doi.org/10.1016/S1385-1101\(00\)00061-7](https://doi.org/10.1016/S1385-1101(00)00061-7)

- Carlton JT, Geller JB (1993) Ecological roulette: the global transport of non-indigenous marine organisms. *Science* 261: 78–82, <https://doi.org/10.1126/science.261.5117.78>
- Carman MR, Morris JA, Karney RC, Grunden DW (2010) An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. *Journal of Applied Ichthyology/Zeitschrift Fur Angewandte Ichthyologie* 26: 8–11, <https://doi.org/10.1111/j.1439-0426.2010.01495.x>
- Carman MR, Colarusso PD, Nelson EP, Grunden DW, Wong MC, McKenzie C, Matheson K, Davidson J, Fox S, Neckles H, Bayley H, Schott S, Dijkstra JA, Stewart-Clark S (2016a) Distribution and diversity of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39° and 47° north latitude (New Jersey to Newfoundland). *Management of Biological Invasions* 7: 51–57, <https://doi.org/10.3391/mbi.2016.7.1.07>
- Carman MR, Lindell S, Green-Beach E, Starczak VR (2016b) Treatment 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>
- Colarusso P, Nelson E, Ayvazian S, Carman MR, Chintala M, Grabbert S, Grunden, D (2016) Quantifying the ecological impact of invasive tunicates to shallow coastal water systems. *Management of Biological Invasions* 7: 33–42, <https://doi.org/10.3391/mbi.2016.7.1.05>
- Deegan LA, Garritt RH (1997) Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* 147: 31–47, <https://doi.org/10.3354/meps147031>
- Dijkstra J, Sherman H, Harris LG (2007) The role of colonial ascidians in altering biodiversity in marine fouling communities. *Journal of Experimental Marine Biology and Ecology* 342: 169–171, <https://doi.org/10.1016/j.jembe.2006.10.035>
- Epelbaum A, Herborg LM, Therriault TW, Pearce CM (2009) Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. *Journal of Experimental Biology and Ecology* 369: 43–52, <https://doi.org/10.1016/j.jembe.2008.10.028>
- Hellmann JJ, Byers JE, Bierwagen BG, Dukes JS (2008) Five potential consequences of climate change for invasive species. *Conservation Biology: The Journal of the Society for Conservation Biology* 22: 534–543, <https://doi.org/10.1111/j.1523-1739.2008.00951.x>
- Keller RP, Perrings C (2011) International policy options for reducing the environmental impacts of invasive species. *Bioscience* 61: 1005–1012, <https://doi.org/10.1525/bio.2011.61.12.10>
- Lambert G (2007) Invasive sea squirts: A growing global problem. *Journal of Experimental Marine Biology and Ecology* 342: 3–4, <https://doi.org/10.1016/j.jembe.2006.10.009>
- Lambert CC, Lambert G (2003) Persistence and differential distribution of non-indigenous ascidians in harbors of the Southern California Bight. *Marine Ecology Progress Series* 259: 145–161, <https://doi.org/10.3354/meps259145>
- Lutz-Collins V, Ramsay A, Quijon P, Davidson J (2009) Invasive tunicate fouling mussel lines: evidence of their impact on native tunicates and other epifaunal invertebrates. *Aquatic Invasions* 4: 213–220, <https://doi.org/10.3391/ai.2009.4.1.22>
- Mckenzie CH, Matheson K, Caines S, Wells T (2016) Surveys for non-indigenous tunicate species in Newfoundland, Canada (2006 - 2014): a first step towards understanding impact and control. *Management of Biological Invasions* 7: 21–32, <https://doi.org/10.3391/mbi.2016.7.1.04>
- Montalto V, Bagarella R, Rinaldi A, Sara G, Mirto S (2017) Thermal adaptation and physiological responses to environmental stress in tunicates. *Aquatic Biology* 26: 179–184, <https://doi.org/10.3354/ab00685>
- Nelson JA, Deegan LA, Garritt R (2015) Drivers of spatial and temporal variability in estuarine food webs. *Marine Ecology Progress Series* 533: 67–77, <https://doi.org/10.3354/meps11389>
- Petersen J (2007) Ascidian suspension feeding. *Journal of Experimental Marine Biology and Ecology* 342: 127–137, <https://doi.org/10.1016/j.jembe.2006.10.023>
- Rahel FJ, Olden JD (2008) Assessing the Effects of Climate Change on Aquatic Invasive Species. *Conservation Biology* 22: 521–533, <https://doi.org/10.1111/j.1523-1739.2008.00950.x>
- Ruppert EE, Fox RS, Barnes RD (2004) *Invertebrate Zoology*, 7th edition. Cengage Learning, 945 pp
- Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) (2007) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp 12–17
- Solorzano L (1969) Determination of Ammonia in Natural Waters by the Phenolhypochlorite Method. *Limnology and Oceanography* 14: 799–801, <https://doi.org/10.4319/lo.1969.14.5.0799>
- Sorte CJB, Williams SL, Zerebecki RA (2010) Ocean warming increases threat of invasive species in a marine fouling community. *Ecology* 91: 2198–2204, <https://doi.org/10.1890/10-0238.1>
- Stefaniak L, Lambert G, Gittenberger A, Zhang H, Lin S, Whitlatch RB (2009) Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. *Aquatic Invasions* 4: 29–44, <https://doi.org/10.3391/ai.2009.4.1.3>
- Valigra, L (2005) Sea squirt threatens native marine life. *The Gulf of Maine Times*. <http://www.gulfofmaine.org/times/summer2005/squirt.html> (accessed 6 November 2018)
- Wong WH, Levinton JS (2004) Culture of the blue mussel *Mytilus edulis* (Linnaeus, 1758) fed both phytoplankton and zooplankton: a microcosm experiment. *Aquaculture Research* 35: 965–969, <https://doi.org/10.1111/j.1365-2109.2004.01107.x>
- Yund PO, Collins C, Johnson SL (2015) Evidence of a Native Northwest Atlantic CO1 Haplotype Clade in the Cryptogenic Colonial Ascidian *Botryllus schlosseri*. *Biological Bulletin* 228: 201–216, <https://doi.org/10.1086/BBLv228n3p201>