

Research Article

Quantifying the ecological impact of invasive tunicates to shallow coastal water systems

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Abstract

Coastal ponds, due to their proximity to human activity, may be particularly vulnerable to invasions by non-native species. A number of invasive tunicate species have been documented in several of the coastal ponds on the island of Martha's Vineyard, Massachusetts. Tunicates are voracious filter feeders, thus our study attempted to examine the impact of their feeding on the normal food web in a coastal pond. In 2012 and 2013, we sampled Stonewall (high tunicate abundance) and Lagoon Ponds (tunicates absent) on Martha's Vineyard. We used quadrat sampling to quantify tunicate abundance, eelgrass shoot density and eelgrass canopy height. Fish, invertebrates and aquatic vegetation were collected via beach seine, minnow trap, crab traps or by hand. Water samples were run through a filter to collect phytoplankton. These biota samples were processed for carbon and nitrogen isotopic analysis. Temperature loggers were deployed in both ponds to collect water temperature. Detailed bathymetric readings were taken to generate an estimate of the volume of each pond. Tunicate filtration rates from published scientific literature, our volume estimate of Stonewall Pond and our measured tunicate abundance were used in a model to estimate the time needed by tunicates to filter a volume of water equal to Stonewall Pond. That time varied from less than an hour to over 17 hours. Isotopic analysis showed that tunicates were feeding on similar resources as the commercial shellfish species. There was broad overlap in the isotopic signatures between the biota from both ponds, suggesting that tunicates were not having a measurable impact to the food web. Tunicates exhibit significant seasonal abundance changes, with the peak occurring late summer into the early fall. The limited duration of this peak may not be sufficient to be reflected in the isotopic signature of resident biota. As water temperature continues to increase with climate change, the current assemblage of tunicates in these shallow water systems on Martha's Vineyard will likely change in response.

Key words: invasive tunicates, filtration rates, food webs, eelgrass, coastal salt pond

Introduction

Shallow coastal water systems are particularly susceptible to invasions by non-native species. Introductions of non-natives by ballast water transfer, aquaculture and the aquarium trade are well established in the scientific literature (Carlton and Geller 1993; Naylor et al. 2001; Padilla and Williams 2004). Changing ocean conditions related to climate change (e.g., water temperature, pH) may represent additional stressors that could

provide a competitive advantage to certain marine invasives over indigenous species (Anthony et al. 2009). Recently, U.S. Environmental Protection Agency (US EPA) divers have observed the increasing presence of non-native or invasive tunicates in coastal salt ponds (i.e., pond-like embayments connected to the ocean) on Martha's Vineyard and other locations in Massachusetts. These invasive tunicates are filter feeding organisms found primarily on hard substrate (rock, clam shells, docks, boat hulls, moorings), but are increasingly

found on vegetation (eelgrass, macroalgae). Their abundance varies seasonally, peaking in late summer/early fall. As the colonial tunicate species grow and spread, they tend to completely cover the substrate on which they are growing to the detriment of other organisms competing for the same space and food, and the leaf surface of vegetation which impedes photosynthesis (Morris Jr. et al. 2009; Wong and Vercaemer 2012). Wong and Vercaemer (2012) showed that excessive fouling of eelgrass by invasive tunicates can lead to shoot mortality.

The potential impact of invasive tunicates extends beyond the physical smothering of vegetation or hard substrate. Tunicates are industrious filter feeders, potentially filtering up to 10,000 ml/h (Fiala-Medioni 1978). Tunicate filtration rates can vary substantially with water temperature (Kjerulf Petersen and Riisgard 1992; Robbins 1983). The potential impact of large quantities of tunicates filtering large quantities of water is not yet understood. To date, studies on invasive tunicates have focused primarily on presence/absence, and the physical impact of their spread on fouling communities, shellfish and vegetation (Dijkstra et al. 2007; Morris et al. 2009; Wong and Vercaemer 2012). Kjerulf-Petersen and Riisgard (1992) attempted to quantify tunicate abundance in a Norwegian fjord and estimate the impact of tunicate grazing on phytoplankton. They speculated that tunicates may control phytoplankton communities in the late summer/early fall when water temperatures and filtration rates are at their highest.

The coastal ponds of Martha's Vineyard are important ecological communities that support many recreational activities (fishing, boating, swimming) and commercial fisheries (shellfish aquaculture, shellfish gathering, fishing). Their relatively small tidal range and semi-enclosed nature make them particularly susceptible to potential impacts from the filter feeding activities of large numbers of invasive tunicates.

The goal of this study was to determine if the filter feeding activities of invasive species of tunicates have the ability to disrupt the food web in the shallow coastal ponds of Martha's Vineyard. Little research currently exists on how invasive tunicates fit into the food web in New England's coastal waters (Dijkstra et al. 2007; Dijkstra and Harris 2007). We used stable isotopes to determine what native species may be competing for similar food resources as the invasive tunicates and thus be at greatest risk of potential impact.

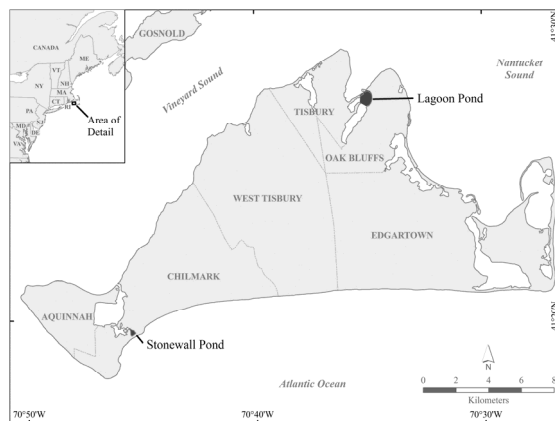


Figure 1. Location of Lagoon Pond (41.458° N, 70.587° W) and Stonewall Pond (41.326° N, 70.757° W), on Martha's Vineyard.

Methods

Site Descriptions: Stonewall Pond is a shallow (<3 m deep) coastal pond that is part of the larger Menemsha Pond complex located on the southwestern end of Martha's Vineyard (Figure 1). There is limited surface freshwater flow into the pond, so it is marine/estuarine in nature. There is limited development around the Menemsha Pond complex and these houses are on septic systems and thus contribute nitrogen to the groundwater. A low bridge greatly limits boat access to Stonewall Pond, thus curtailing human usage of the pond. The entrance to Stonewall Pond is long and narrow. We refer to this section of the pond as the "neck". Prior to our study, large quantities of tunicates had been observed on both hard substrate and the extensive eelgrass meadow in the center of Stonewall Pond.

The northern end of Lagoon Pond was chosen because it was the part of the pond that best matched Stonewall Pond in size and shape and lacked the presence of tunicates on eelgrass. Lagoon Pond is located on the northeastern shore of the island in the towns of Oak Bluffs and Tisbury (Figure 1). It is a large shallow pond with a single connection point to the ocean. Freshwater sources to Lagoon Pond are Upper Lagoon Pond, Mud Creek and Brush Pond (Poole 1988). Land use along the immediate shoreline of Lagoon Pond is 79% residential, 15% open space or public land and 6% commercial (Poole 1988). Human usage of Lagoon Pond includes recreational boating, and recreational and commercial fishing and shellfish gathering.

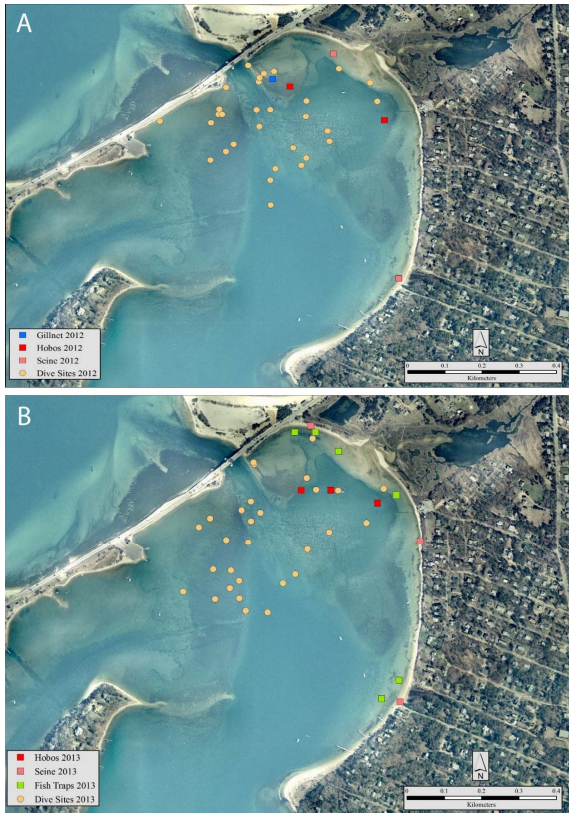


Figure 2. Sample locations within Lagoon Pond in 2012 (A) and 2013 (B).

Eelgrass/Tunicate Sampling: Based on sampling we conducted in these ponds in 2011 and 2012, and consistent with observations by others in New England waters (Valentine et al. 2007; Valentine et al. 2009), we determined that tunicate peak abundance occurred late summer into the early fall. Thus, sampling for this study occurred from September 11 to 13, 2012 and from September 9 to 12, 2013. Sample locations for eelgrass and tunicate samples were selected using a stratified random design. Thirty sampling locations were chosen for each pond, with Stonewall location divided between the “neck” area (10 sites) and the pond area (20 sites) (Figures 2 and 3).

At the sample sites, 0.0625 m² quadrats were dropped haphazardly to the bottom. If the bottom was unvegetated and no tunicates were present, there was no further sampling at that location. If the sample location contained hard substrate and tunicates were present, tunicates were removed as carefully as possible by gently peeling or scraping everything within the quadrat and placing the collected material in plastic Ziploc bags.

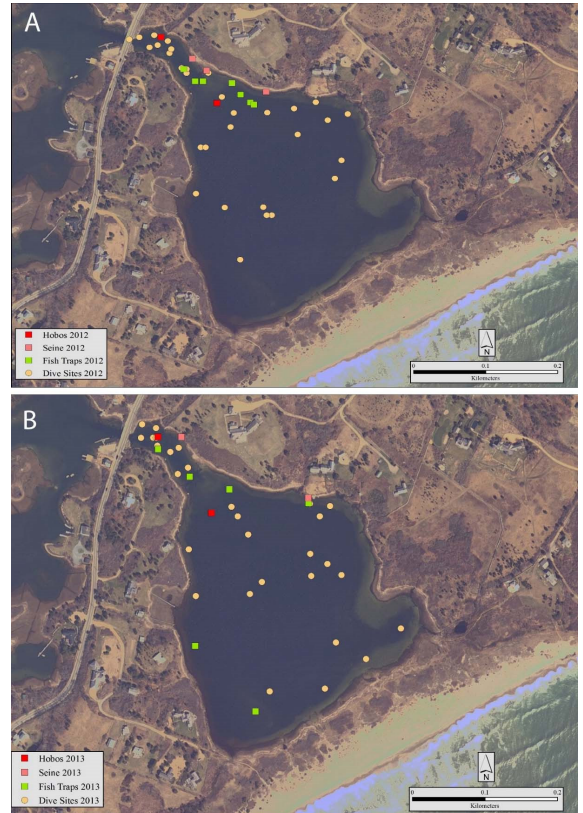


Figure 3. Sample locations for Stonewall Pond in 2012 (A) and 2013 (B).

If eelgrass was present, all uprights shoots within the quadrat were cut at the sediment/water interface and placed in mesh collection bags. Eelgrass shoot density was determined by counting the number of meristems per sample bag. This value was multiplied by 16 and the resulting product yielded the number of shoots per m². Eelgrass canopy height was determined by randomly selecting 10 shoots per sample and measuring from the base of the meristem to the tip of the longest leaf and multiplying these values by 0.8 (Short and Coles 2001). Tunicate coverage of eelgrass surface area per shoot was estimated visually and classified as 0–25%, 26–50%, 51–75% or 76–100%.

Tunicate abundance was determined by counting the number of individuals of each tunicate species in each sample. For colonial species, enumeration required the aid of a microscope to count each individual colony. After enumeration, tunicates were processed for biomass by placing them in a drying oven for 24 hours at 60° C and then weighing them on an analytical balance.

Table 1. Model scenarios.

Scenario	Tunicate abundance	Filtration rate (ml/h)	Tidal stage
1	2012	2,000	Low
2	2012	5,000	Low
3	2012	10,000	Low
4	2013	2,000	Low
5	2013	5,000	Low
6	2013	10,000	Low
7	2012	2,000	High
8	2012	5,000	High
9	2012	10,000	High
10	2013	2,000	High
11	2013	5,000	High
12	2013	10,000	High

Bathymetric mapping: A Garmin GPSMAP 421S mounted on an approximately 5.5 m boat was utilized to collect bathymetric data along track lines throughout the tide cycle. Water depths were corrected for tide stage to generate a bathymetric map.

Water temperature: Two HOBO pendant sensors (Onset Corp.) were deployed in each pond approximately 1 m off the bottom in water that was 1.8–2.4 m deep (Figures 2 and 3). Water temperature was recorded every 15 minutes. In 2012, HOBOs were deployed from September 10 to October 5. In 2013, HOBOs were deployed from August 20 to September 12.

Salinity: Salinity was not specifically measured, but both ponds are true coastal lagoons with relatively limited freshwater inflow, compared to the tidal exchange. Salinities have been measured in Lagoon Pond in the past by other researchers and were consistently found in the low 30 ppt range. Vegetation, fish and invertebrate species composition are reflective of a typical New England marine system.

Tunicate Impact Analysis: The potential impact of tunicates on the food web was only assessed for Stonewall Pond, as the northern end of Lagoon Pond did not have any measurable amount of tunicates. Published data on tunicate filtration rates is relatively limited, and consists primarily of solitary species. Rosa et al. (2013) report filtration rates for colonial species in milliliters/g dry weight of tunicate. Hourly filtration rates for colonial tunicates was calculated by using the filtration values from Rosa et al. (2013) and the dry weight measurements of colonial tunicates from this study. Thus, three filtration rates (2,000 ml/h, 5,000 ml/h, 10,000 ml/h) were selected for our analysis that spanned the values found in the published literature (Fiala-Medioni 1978; Kjerfulf Petersen and Riisgard 1992, Kim and Moon 1998; Yahel et al. 2005; Rosa et al. 2013). Tunicate

abundance was calculated using the mean number of tunicates from the quadrat samples and extrapolating to the area sampled within the pond. Tunicate abundance in the “neck” portion and the main body of the pond were summed to give an abundance estimate for the entire pond. Abundance estimates were derived separately for 2012 and 2013. Pond volume was calculated from the bathymetric survey. The quantity of time it would take for tunicates to filter a volume of water equivalent to the entire pond was calculated using a variety of tunicate abundance estimates, filtration rates and pond volumes (low vs. high tide). The specific combinations are outlined in Table 1.

Food Web Analysis

Sample collection: Multiple species were collected from each site to represent the trophic levels; primary producers, and primary and secondary consumers. Three or more individuals of each species were collected from Lagoon and Stonewall Ponds each year. Collection methods included a beach seine (15 m shore seine with 6.4 mm mesh), standard minnow traps, crab traps, and collection by hand in shallow water (Figures 2 and 3). For complete list of species analyzed see Appendix 1.

Tunicates were carefully removed from the substrate they had colonized, identified, and cleaned of any associated plant, algae or invertebrates using a dissecting microscope. All samples were placed in labeled plastic bags, and held on ice and returned to the laboratory where they were frozen at -20°C until analyzed.

Seawater samples were collected in replicate to examine the isotopic composition of particulate organic matter from each pond during 2012 and 2013. Two liters of water were collected at mid-depth of the water column (< 1 m). Samples were filtered in the field using Whatman GF/F glass fiber filters (Whatman International Ltd), removed and wrapped in aluminum foil and kept on ice until returned to the laboratory where they were frozen until analyzed.

Sample preparation for isotopic analysis: Samples for isotopic analysis were thawed and were rinsed in distilled water. Algae and tunicates were further cleaned under a dissecting microscope. For species of small size, multiple individuals were needed to achieve sufficient biomass for isotopic analysis. Muscle tissues from crabs were extracted from the carapace, including chelipeds, for analysis. Two whole shrimp were used per sample. Snails were extracted from their shell;

the operculum was removed and discarded. Three snails were used per sample. One whole fish of each species was used for analysis, except for *Menidia* sp. where we used 3 individuals if they were < 35 mm total length. All samples were dried at 60°C for 2–4 days. Dried samples were then ground into a homogeneous powder using a mortar and pestle. Eelgrass samples were ground with a Wiley Mill.

Isotopic Analysis: Isotopic analysis for nitrogen and carbon ratios was conducted in continuous flow mode using an EA 3000 Elemental Analyzer (Euro Vector Instruments and Software, Milan, Italy) interfaced to an isotope-ratio mass spectrometer (IRMS, GVI-Isoprime, Elementar, Hanau, Germany). Standard procedures for analyzing samples were followed according to Pruett et al. (2006).

Statistical analysis: Statistical differences between size classes of scallops, silversides and killifish were examined using a one-way analysis of variance (ANOVA) (Zar 1984) in Excel. Visualization of the trophic levels was accomplished using the statistical program Systat (<http://www.systat.com>, 2004). Statistical differences in eelgrass shoot density and canopy height between ponds were examined using a one-way ANOVA (Zar 1984) in Excel.

Results

Eelgrass: In 2012, eelgrass shoot density and canopy height was not significantly different ($p>0.05$) between Stonewall and Lagoon Pond (Figure 4). In 2013, shoot density was significantly greater ($p=0.004$) in Lagoon than Stonewall, while canopy height was not different between the ponds (Figure 5). Eelgrass areal coverage was not quantified, but the number of quadrats with eelgrass more than doubled from 2012 to 2013 in Lagoon Pond. In 2012, the highest shoot density measured in Lagoon Pond was $192/m^2$, while in Stonewall Pond it was $268/m^2$. In 2013, the highest shoot density in Lagoon Pond was $928/m^2$, while the highest in Stonewall was $320/m^2$.

Tunicates: Sampling in the northern end of Lagoon Pond in 2012 and 2013 did not detect tunicates. In 2012, six species of tunicates (*Asciidiella aspersa* (D.F.Müller, 1776), *Botrylloides violaceus* (Okra, 1927), *Botryllus schlosseri* (Pallas, 1766), *Didemnum albidum* (Verrill, 1871), *Didemnum vexillum* (Kott 2002), and *Diplosoma listerianum* (Milne-Edwards, 1841)) were found in Stonewall Pond. In 2013, seven species of tunicates (the six previously found and *Molgula*

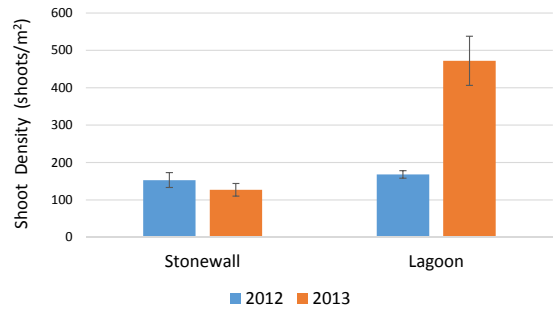


Figure 4. Eelgrass shoot density.

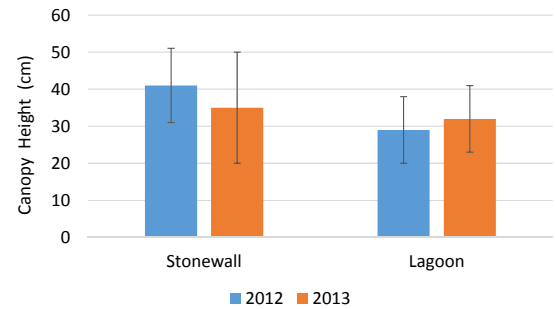


Figure 5. Eelgrass canopy height.

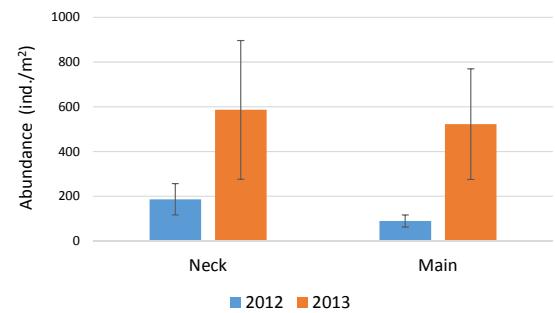


Figure 6. Tunicate abundance in Stonewall pond.

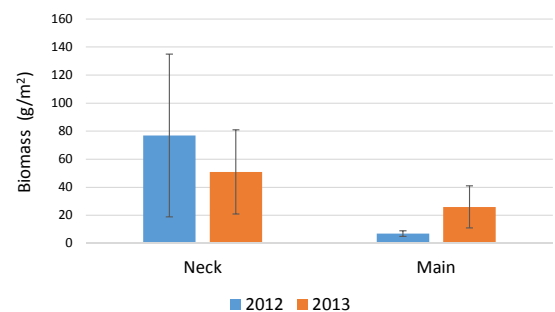


Figure 7. Tunicate biomass in Stonewall pond.

Table 2. Tunicate abundance, biomass and mean biomass by species for Stonewall Pond.

Species	Life form	Native/non-native	# individuals	biomass (g)	mean biomass/individual (g)
<i>Ascidia aspersa</i> (Müller, 1776)	Solitary	non-native	14	23.05	1.65
<i>Botryllus schlosseri</i> (Pallas, 1766)	Colonial	non-native	31	13.57	0.44
<i>Botrylloides violaceus</i> (Oka, 1927)	Colonial	non-native	192	43.06	0.22
<i>Didemnum albidum</i> (Verrill, 1871)	Colonial	native	61	39.33	0.65
<i>Didemnum vexillum</i> (Kott, 2002)	Colonial	non-native	43	119.83	2.79
<i>Diplosoma listerianum</i> (Milne-Edwards, 1841)	Colonial	non-native	20	5.62	0.28
<i>Molgula manhattensis</i> (De Kay, 1843)	Solitary	native	81	14.91	0.18

Table 3. Time in hours needed by tunicates to filter entire volume of Stonewall Pond.

Model scenario	Tunicate abundance	Filtration rate (ml/hr)	Tidal stage	Time needed to filter entire pond (hrs)
1	4,628,576 (2012)	2,000	High	17.82
2	4,628,576	5,000	High	7.13
3	4,628,576	10,000	High	3.56
4	4,628,576	2,000	Low	9.69
5	4,628,576	5,000	Low	3.87
6	4,628,576	10,000	Low	1.94
7	9,494,333 (2013)	2,000	High	8.69
8	9,494,333	5,000	High	3.48
9	9,494,333	10,000	High	1.74
10	9,494,333	2,000	Low	4.72
11	9,494,333	5,000	Low	1.89
12	9,494,333	10,000	Low	0.94

manhattensis (DeKay, 1843)) were detected in Stonewall Pond.

Within the quadrat samples, substrates that tunicates were attached to included *Zostera marina* (Linnaeus, 1753), *Codium fragile* (Suringar) Hariot, 1889, other macro-algae and rocks. Of the seven species attached to marine plants, five are colonial forms (*B. violaceus*, *B. schlosseri*, *D. vexillum*, *D. listerianum*, *D. albidum*) and two are solitary forms (*A. aspersa*, *M. manhattensis*). Two species are classified as native species (*D. albidum*, *M. manhattensis*), and five as non-native species (*A. aspersa*, *B. violaceus*, *B. schlosseri*, *D. vexillum*, *D. listerianum*). The most common tunicate was *B. violaceus*.

Tunicate abundance in Stonewall Pond tended to be higher in the “neck” section of the pond (Figures 6 and 7). In 2012, mean tunicate abundance in the “neck” section was 186 individuals/m² (Figure 6) and mean biomass was 77 g/m² (Figure 7). In 2013, mean tunicate abundance in the “neck” section was 586 individuals/m² (Figure 6) and mean biomass was 51 g/m² (Figure 7). Tunicate

species diversity peaked at 4 with *A. aspersa*, *B. violaceus*, *B. schlosseri* and *D. listerianum* all found attached to eelgrass in one sample.

Tunicate coverage within the quadrat samples was generally in the 0–25% range, with occasionally up to the 50–75% range. Colonial tunicates varied in size from small patchy colonies to large, encapsulating colonies. Tunicate biomass varied by species (Table 2). Of the five colonial tunicate species, *D. vexillum* was, on average, heavier than the other colonial species with *B. violaceus* having the smallest biomass. Of the two solitary tunicate species, *A. aspersa* was generally heavier than *M. manhattensis*.

Bathymetric Mapping: Bathymetric soundings were corrected for tidal stage in order to calculate pond volume. The volume of Stonewall Pond is estimated at 89,666 m³ and 164,988 m³ during low and high tide respectively. This represents a tidal exchange of 75,322 m³.

Water Temperature: Water temperatures in both ponds were very similar. Temperatures peaked at 25°C and showed a declining slope to the end of

the measurement period in October. Minimum temperature measured in October was 18°C.

Tunicate Impact Analysis: The time required for tunicates to filter a volume of water equivalent to the entire pond varied from about 2 hours (at highest tunicate abundance and filtration rate at low tide) to a maximum of 17.8 hours (at lowest tunicate abundance and filtration rate at high tide) (Table 3). Based on a sensitivity analysis, tunicate filtration rate had the largest effect on model output, causing it to vary by a factor of five. Tunicate abundance had the second largest effect on model output, causing model output to vary by a factor of 2.04. Pond volume, as represented by tidal stage, caused the model output to vary by a factor of 1.83.

Food Web Analysis: The field collections during 2012 produced 24 species in Lagoon Pond and 22 species in Stonewall Pond. Similarly, in 2013 there were 26 species collected in Lagoon Pond and 27 species from Stonewall Pond. During 2013 we were able to achieve greater replication of the samples with more sampling effort than in the 2012 collections. For plants, tunicates and sessile invertebrates, we were able to collect up to six individuals in both years; while the numbers of more mobile fauna, including macroinvertebrates and fishes was variable between years.

A comparison of the nitrogen and carbon isotopic signatures between taxonomic groups (where $N \geq 3$ individuals) collected from Lagoon Pond and Stonewall Pond for 2012 and 2013 demonstrates higher $\delta^{15}\text{N}$ values for each group collected at Lagoon Pond than Stonewall Pond, while the $\delta^{13}\text{C}$ values are approximately the same between taxonomic groups at both ponds for both years (Figure 8A and B).

The average nitrogen and carbon isotopic ratios from tissue samples for each species (where $N=3$ individuals) collected from Lagoon Pond and Stonewall Pond during 2013, illustrate the relative position of each species in relation to other species in that taxonomic group (Figure 8A and B). Within both ponds, fin fish species had the highest average $\delta^{15}\text{N}$ ratios, with Lagoon Pond needlefish and sticklebacks and Stonewall Pond alewives had $\delta^{15}\text{N}$ values greater than 12. In both ponds, particulate organic matter (POM), algae and eelgrass had the lowest average $\delta^{15}\text{N}$ ratios. The eelgrass and brittle stars were the most enriched and the POM was the most depleted in average $\delta^{13}\text{C}$. There is considerable overlap in the average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures for invertebrate species. The bivalve species produced a more depleted average $\delta^{13}\text{C}$ ratio than the snails, shrimp

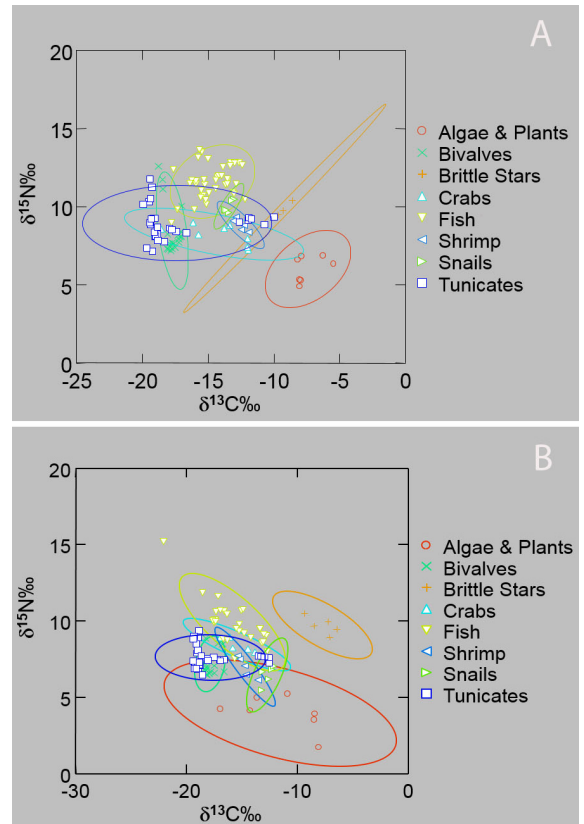


Figure 8. A and B: Individual isotope data for Lagoon and Stonewall Ponds for 2013 by class (for species with $N = 3$ or more individuals). A standard deviation 95% confidence interval is drawn around the samples.

and brittle stars. The brittle stars also possessed a greater average $\delta^{15}\text{N}$ ratio than other invertebrate species in Lagoon Pond (Figure 8A). The average nitrogen and carbon isotopic signatures for the tunicate species from both ponds are clustered with a depleted average $\delta^{13}\text{C}$ ratio of approximately -18 and an average $\delta^{15}\text{N}$ ratio between 7 and 12‰. The isotopic signature for most of the tunicate species overlaps that of the scallop, quahog and jingle shell. The exception is *Didemnum albidum* whose $\delta^{13}\text{C}$ signature is less depleted and in line with shrimp and crabs.

Isotopic values for individuals collected from each pond during 2013, have been arranged by broad taxonomic grouping and with 95% confidence intervals computed. What is most noticeable is the overlap between groups within each pond (Figures 8A and B). The only non-overlapping group is the brittle stars in Stonewall Pond.

Assigning a trophic level to the tunicates collected in this study indicates they are most closely aligned with other primary consumers, particularly bivalves (scallops and quahogs; Figure 8A and B).

A comparison of average nitrogen and average carbon isotopic signatures of tunicates collected during 2013 shows a consistently greater average nitrogen isotope value at Lagoon Pond compared to Stonewall Pond; a variable average nitrogen signature and an average carbon signature of approximately -19 for most tunicate species from both ponds; with *D. vexillum* and *D. albidum* more enriched in their average carbon isotopic ratio than other tunicate species. There was no pattern in isotopic signatures for tunicate species classified as native and non-native. Solitary tunicates have less variability in $\delta^{13}\text{C}$ signature than colonial tunicates.

There was a wide spread of data reported for the %C by primary consumer species at both ponds. The values ranged from over 15% to approximately 42%. The values were highly consistent between the ponds. There were no differences noted between solitary and colonial tunicates or native versus non-native tunicates. In 2012 the bivalve species had at least as great %C as the tunicate species and in 2013 the %C values were similar (Figure 8A and B).

Discussion

Our model results suggest tunicates have the potential to filter a volume of water equivalent to that of Stonewall Pond in less than a day and depending on the assumptions used, less than a tidal cycle. Our model considered high and low tide volume of Stonewall Pond, tunicate abundance in Stonewall in 2012 and 2013 and filtration rates for tunicates reported in the scientific literature. Tides on Martha's Vineyard are generally in the 3-foot range, and as a result water volume had the smallest effect on model output. Estimates of tunicate abundance in the pond did vary year to year, but tunicate filtration rate was the factor that had the greatest effect on model results. Published data on tunicate filtration rates is currently mainly focused on solitary species, which highlights the need for research on filtration rates in colonial species.

Similar to many filter feeders, temperature is a significant factor controlling tunicate feeding rate. Kjerfulf Petersen and Riisgard (1992) measured filtration rates in *Ciona intestinalis* (Linnaeus, 1767) and found a linear increase in filtration rate with

temperature up to 21°C, and >21°C filtration rate declined dramatically. Ribes et al. (1998) measured the *in situ* feeding rate of the temperate ascidian *Halocynthia papillosa* (Linnaeus, 1767) and found the feeding rate increased up to 22° C, before declining. Kim and Moon (1998) found the filtration rate in *Styela clava* Herdman, 1881 increased until declining at a point between 28° and 29°C. Limited data exists on filtration rates in colonial tunicates, with Rosa et al. (2013) the only published source we could find. This lone study was not designed to examine filtration rate as a function of temperature in colonial tunicates. This lack of information points to a significant need for additional research.

Tunicate growth and reproduction follow seasonal patterns that are controlled by water temperature. Lopez-Legntil et al. (2013) studied resource allocation in the temperate colonial ascidian *Didemnum fulgens* (Milne-Edwards, 1841) in relation to water temperature. They found the timing of gonadal development, larval release and growth rates all correlated well with water temperature. Gonadal maturity peaked between 17°C and 19°C and quickly declined at temperatures above 19°C. They found growth rates were inversely correlated with temperature. Maximum growth occurred in the winter and spring and growth minimums occurred in the summer and fall.

Cape Cod is a geomorphological dividing point for marine species with temperate species found predominantly to the north and subtropical species to the south. This dividing point is ecologically interesting as both temperate and subtropical species occur close to their thermal limits. Thus, small changes in water temperature can result in large latitudinal shifts for some species. During our measurement period, water temperatures in Stonewall Pond peaked at 25° C and exceeded 22° C for much of the measurement period. Based on the literature, 22° C represents a critical threshold where the filtration rate, growth and gonadal development for temperate species is reduced (Kjerfulf Petersen and Riisgard 1992; Ribes et al. 1998; Lopez-Legntil et al. 2013). As water temperatures continue to rise in response to global climate change, the period of time water temperatures exceed 22° C will likely increase as well. Eventually, this trend could result in a competitive shift away from temperate species to more subtropical ones like *Styela clava*, whose optimal filtration temperature was not exceeded during our measurement periods.

Kjerfulf-Petersen and Riisgard (1992) showed that the filtration potential of tunicates in their

Norwegian fjord varied by a factor of 10 throughout the year, due to seasonal changes in abundance and changes in filtration rates with temperature. They speculated that tunicates were controlling phytoplankton populations in the fjord in late summer when estimated tunicate filtration rates equaled the volume of the fjord in a 24-hour period. Our model generated similar results with tunicate filtration rates equaling the volume of Stonewall Pond in less than 24 hours for all model scenarios. This rapid filtration of the pond would likely significantly influence phytoplankton population numbers.

Our isotopic analysis shows tunicates are feeding on similar resources as other filter feeders, particularly the bivalves (scallops and quahogs). The isotopic analysis also shows invasive tunicates and native tunicates feeding on similar resources as colonial and solitary species. Thus, invasive tunicates are competing with native filter feeding species for food resources, which may have detrimental effects on native species during the time of year (late summer/early fall) of peak invasive tunicate abundance.

Isotopic analysis of the biological communities in Lagoon and Stonewall Pond did not reveal any differences in carbon content. The potential of invasive tunicates to exert top-down control through their grazing is likely a short term phenomena, only occurring when tunicate abundance numbers and filtration rates are at their peak in late summer/early fall. Carbon flow through the food web, is operating on a much longer time scale and thus isotopic analysis may not reflect short term changes, such as the seasonally high abundance of tunicates.

The success of many invasive species is in part attributed to a lack of predators in their new environments. Dijkstra and Harris (2007) tested predation on invasive solitary and colonial tunicates by species common to Great Bay, New Hampshire. They found that cunner (*Tautologlobus adspersus* (Walbaum, 1792)) and several crab species (*Carcinus maenas* (Linnaeus, 1758), *Cancer irroratus* Say, 1817, *Cancer borealis* Stimpson, 1859) would consume solitary ascidians, but not colonials. They documented predation on colonial tunicates by the sea star *Henricia sanguinolenta* (O.F. Müller, 1776). All of these predators can occur on Martha's Vineyard, but our sampling either did not detect them or suggests their abundance in Stonewall and Lagoon Ponds are limited. Other crustacean species (*Callinectes sapidus* Rathbun, 1896, *Panopeus herbstii* H. Milne Edwards, 1834) are more numerically dominant.

Our isotopic analysis shows these crustaceans are feeding at a trophic level above tunicates and bivalves, which means it is possible that tunicates comprise part of their diet. The brittle star (*Ophioderma brevispina* (Say, 1825)) was the only sea star species found in either pond, and they were very common. They are primarily detritivores or carrion feeders, so it is unlikely they would feed on colonial tunicates.

Comparison of the nitrogen isotopes between the ponds showed that the primary producers were enriched for nitrogen in Lagoon Pond compared to Stonewall Pond. This reflects the more urban nature of the watershed around Lagoon Pond compared to Stonewall Pond.

The colonization of eelgrass by tunicates is also a seasonal phenomenon peaking in late summer and fall. This is likely due to a nexus of factors involving the growth of both eelgrass and the tunicates. Eelgrass growth is predominantly controlled by available light (Dennison and Alberte 1985). During peak growth periods, eelgrass will lose and replace leaves in several days to a week (Duarte 1991). This rapid turnover of leaves helps the plant control epiphytic growth, including the colonization by tunicates. As day length declines later in the summer into the fall, the leaf turnover rate declines. Simultaneously, water temperatures become more favorable for growth and reproduction of many of the invasive colonial tunicates. The combination of these 2 factors allow for the extensive colonization of eelgrass by tunicates. Late in the fall, eelgrass will shed much of its aboveground biomass. Much of that biomass ends up on the wrack line of beaches after storms, but some is dispersed into the open ocean. Associated epiphytes, including tunicates, may be widely dispersed using floating eelgrass leaves as a vector (Worcester 1994).

Our study shows the filtration capacity of invasive tunicates during the late summer/early fall may be sufficient to influence phytoplankton abundance in the shallow coastal ponds of Martha's Vineyard. This large filtration capacity is a short-term phenomena which is seasonal in nature and coincides with peak tunicate abundance and filtration rates. Due to the short-term nature of this large filtration capacity, our isotopic analysis did not detect any differences in carbon flow between the 2 ponds. Increasing water temperature as a result of global climate change may result in temperate tunicate species being pushed beyond their temperature preferences within the coastal ponds of Martha's Vineyard.

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The following supplementary material is available for this article:

Appendix 1. Summary of all organisms collected and analyzed for stable isotopes.

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