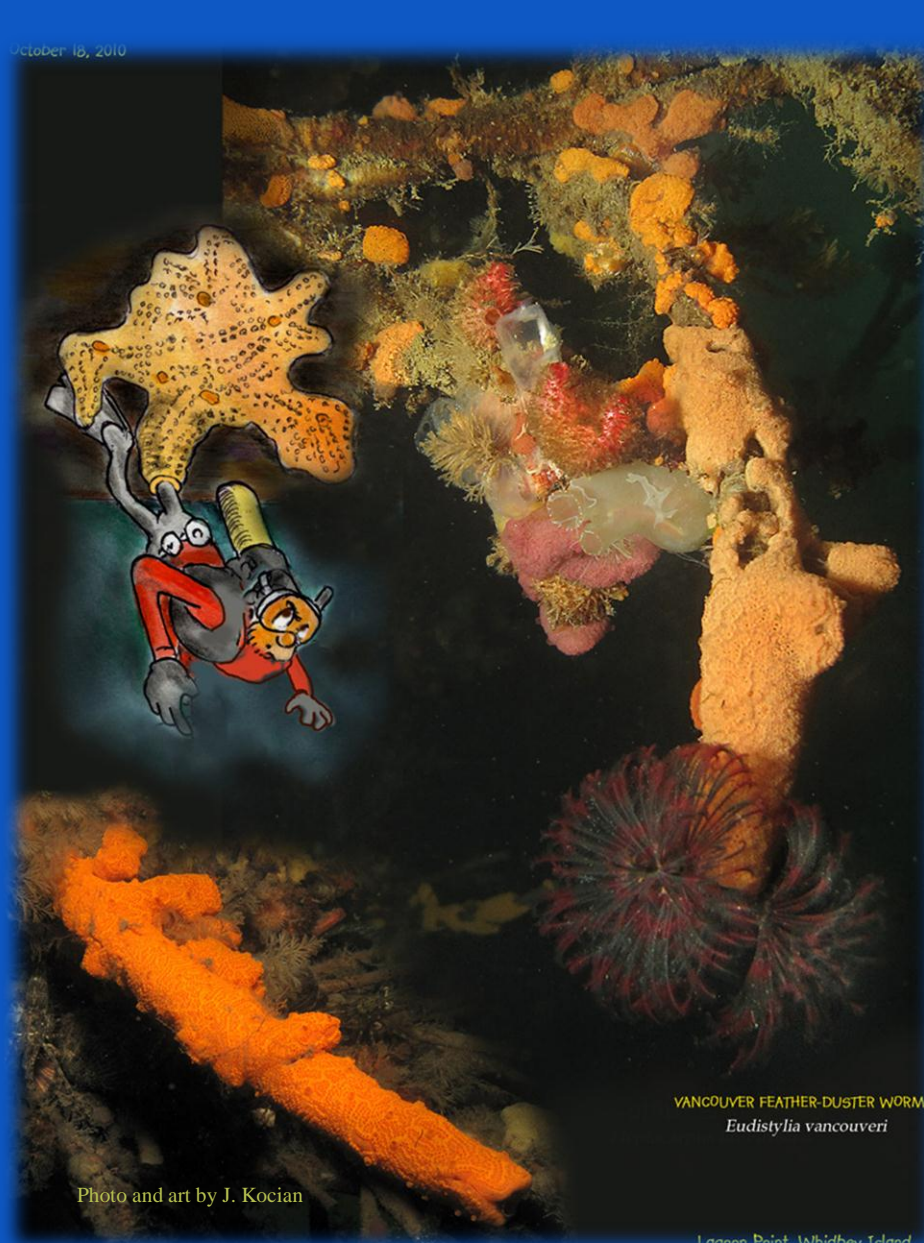


EVALUATING EFFECTS OF INVASIVE TUNICATES IN PUGET SOUND

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Introduction

At least four species of non-native tunicates have become established in the Salish Sea. These species can tolerate a variety of environmental conditions and have spread to numerous locations in the region. Researchers and shellfish growers from other regions report that several of these species may have economic impacts due to fouling of cultured shellfish and aquaculture equipment, and by decreasing growth in cultured mussels.

Because they are aggressive competitors for space, the introduced tunicates may also alter native communities. Relatively few studies have rigorously examined these community-level effects and in aggregate they have shown mixed results including negative, positive, and neutral effects. In addition, most of these studies were conducted on the Atlantic coast of North America.

We conducted a preliminary study that focused on the impacts of four non-indigenous tunicates in the Salish Sea: the colonial species *Didemnum vexillum* and *Botrylloides violaceus*, and the solitary species *Ciona savignyi* and *Styela clava*. During the period of late June through mid-October 2010 we collected samples with and without non-indigenous tunicates at six marinas and two mussel culture facilities in Puget Sound.

Because effects of invasive tunicates on other sessile fouling organisms are relatively well studied, we concentrated mainly on other ecologically important organisms associated with the fouling community. These included larger organisms such as amphipods and polychaete worms and smaller ones such as harpacticoid copepods.



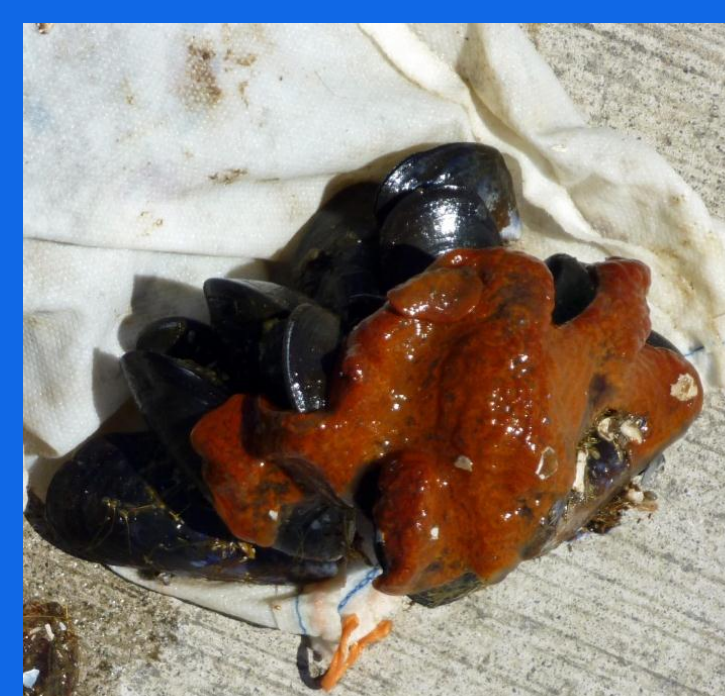
Ciona savignyi



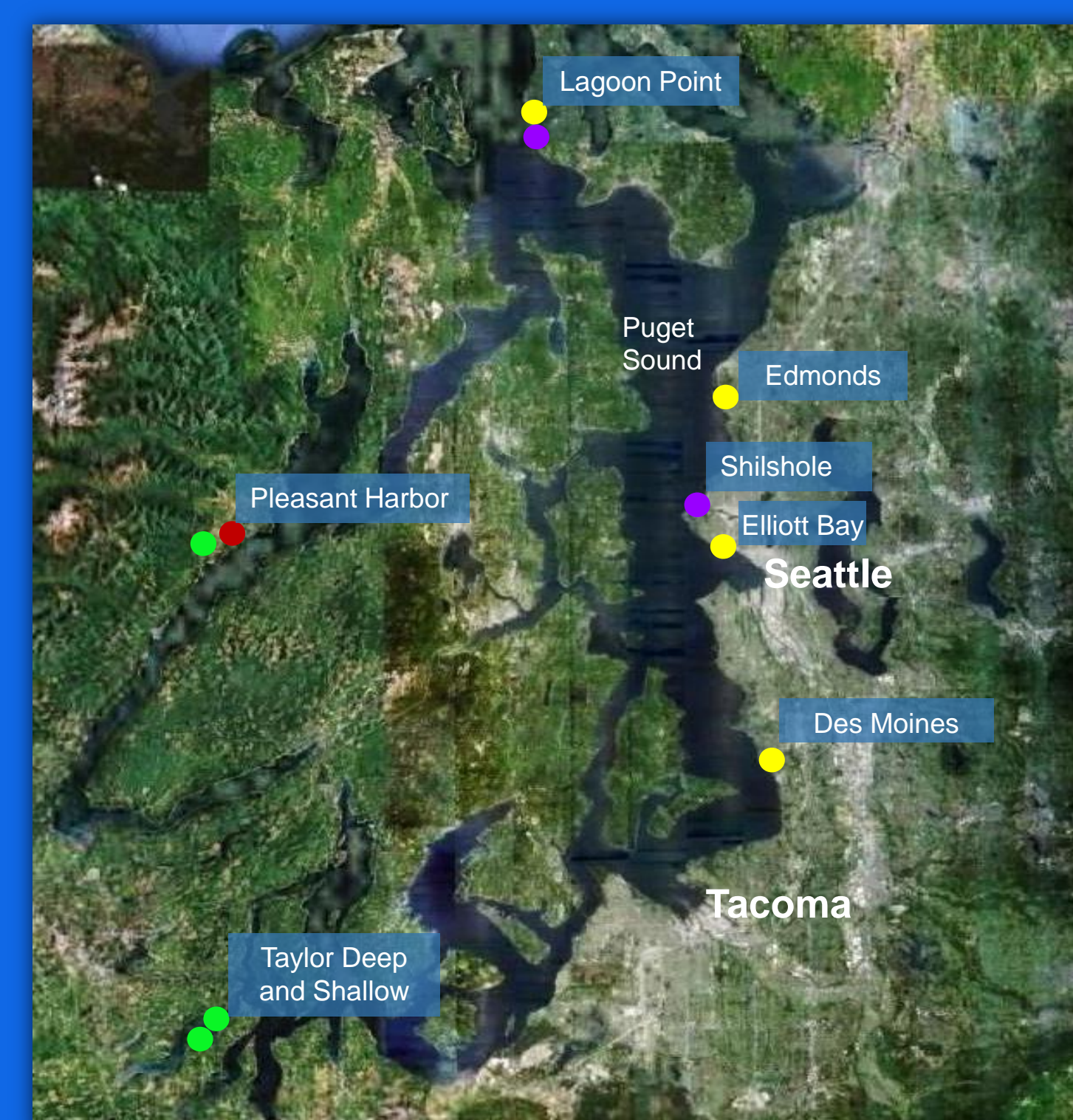
Didemnum vexillum



Styela clava



Botrylloides violaceus



Google Earth map of study sites. The two southern-most sites are mussel aquaculture facilities; the remainder are marinas.

Methods

Organisms were removed from samples (n=5) of mussels, oysters or the tube worm *Eudistylia vancouveri* with and without non-indigenous tunicates and were screened into macro (500 µm) and meiofauna (106 µm) fractions. In several cases, tunicate and non-tunicate samples were taken from standardized areas on dock or piling substrata. Densities were standardized by total shell or tube area of the substrate-forming organism.

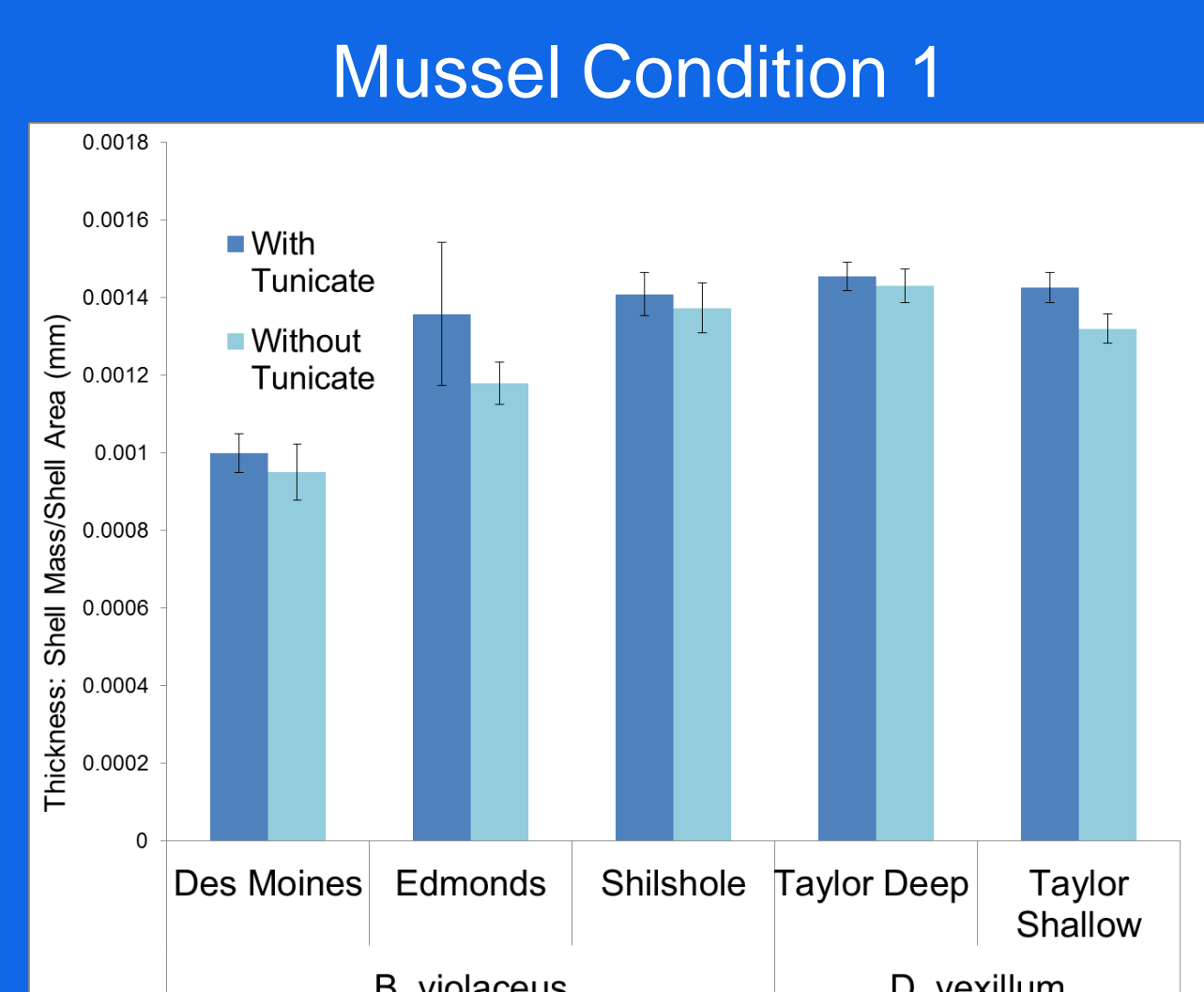
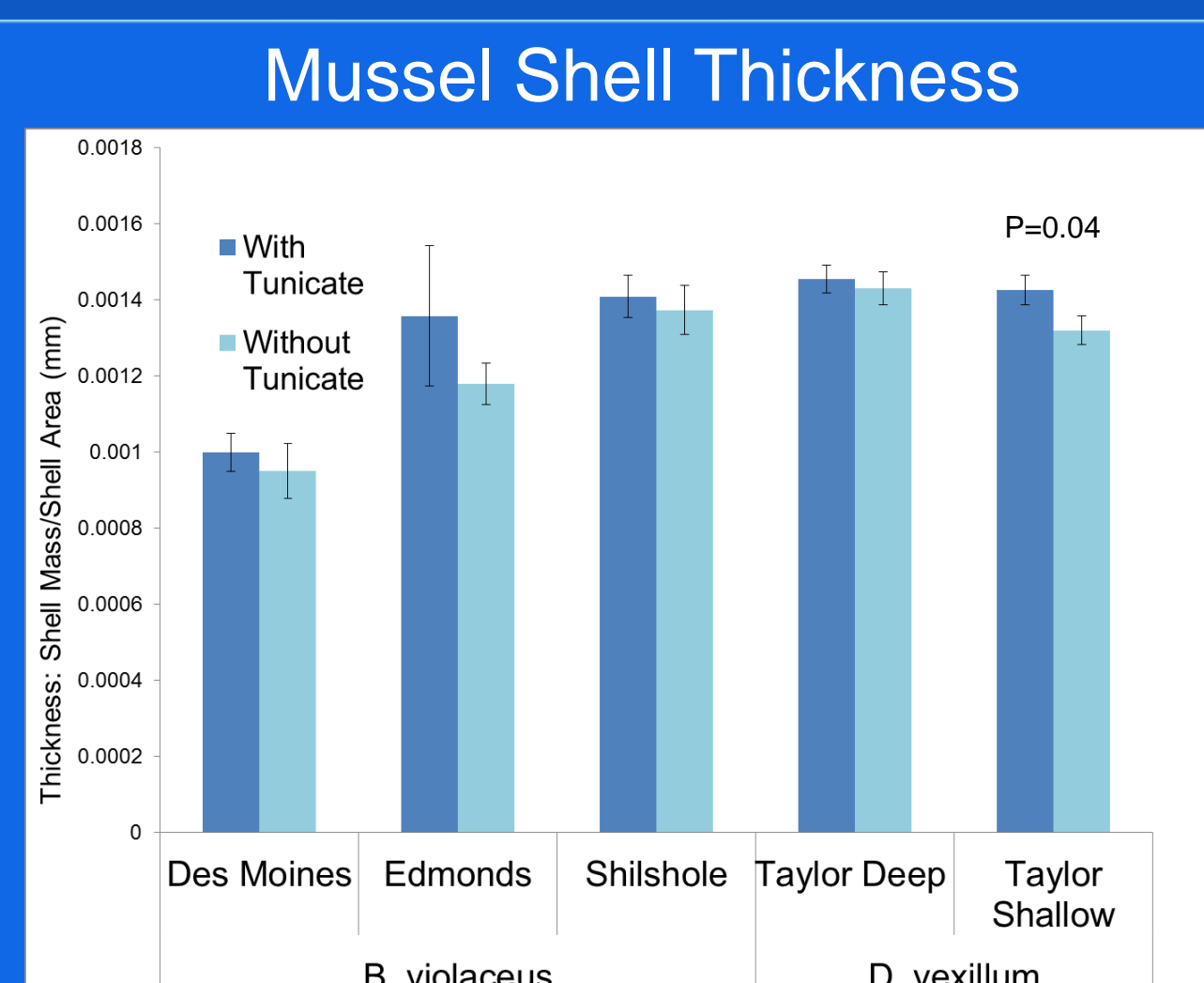
We analyzed the effects of tunicate presence on the shell thickness and condition of mussels at five sites; two with *Didemnum vexillum* and three with *Botrylloides violaceus*. Five aggregations of mussels with and five without tunicates were sampled at each site. Dry body weight, dry shell weight, and shell dimensions were measured for the mussels in each group. We used indices of shell thickness and condition that have been previously developed. Shell thickness was estimated as shell mass/shell surface area. Condition was estimated in two ways: (1) dry body mass/dry shell mass and (2) dry body mass/shell surface area.

We used t-tests to compare total densities of organisms with and without tunicates and to look for specific impacts on species that are known to be important prey for juvenile salmon and other fish. The multivariate test ANOSIM was used to compare community assemblages with and without tunicates. We then used a multivariate SIMPER analysis to determine the top five taxa driving the differences indicated by significant ANOSIM results.

Impacts on Mussel Size and Condition

Previous studies have shown that fouling organisms such as algae and sponges can decrease the growth and condition of mussels. There is concern among shellfish growers that colonial tunicates present a similar problem because they are capable of spreading rapidly and completely overgrowing large areas of substrate.

Fouling by tunicates has been shown to affect the settling, maintenance and harvest of farmed shellfish including mussels and oysters. However, these impacts may vary based on aquaculture techniques and shellfish species. For example, at the two mussel culture facilities where we sampled *Didemnum vexillum*, the growers have not experienced problems with growth or harvest.



Results and Discussion

Although mussel condition factor 1 was higher at four of the five sites for mussels that did not have tunicates, none of the differences were statistically significant. Shell thickness was greater at both sites in the presence of *Didemnum vexillum* but t-tests showed this result was significant only at the Taylor Shallow site.

These results are different from those recently reported in a study of the impacts of *D. vexillum* on the growth and condition of *Mytilus edulis* in the Gulf of Maine. In that study, mussels were sampled every three months over a year. The tunicate had significant negative impacts on *M. edulis* shell thickness during one sample month and on the ratio of dry tissue mass to shell volume in two months. Some of the impacts were correlated with monthly differences in the abundance of *D. vexillum* (i.e., less mussel tissue during maximum tunicate growth). However, the overall results did not identify a consistent or predictable impact of tunicate overgrowth on mussels. These and our results suggest that tunicate effects on mussels can be site- and time-dependent and that at a given site sampling throughout the tunicate growth cycle may yield a clearer picture of tunicate effects on mussels.

Diversity

Communities of smaller invertebrates associated with fouling communities in the Salish Sea have not been well studied. In this study, we found that diversity of these organisms was high both in the presence and the absence of non-native tunicates. On average the taxa richness was very similar between "with tunicate" and "without tunicate" samples.

In the meiofauna fraction we found 96 different taxa, made up largely of harpacticoid copepods. Seven of the copepod species have not been previously recorded from the Pacific coast of North America.

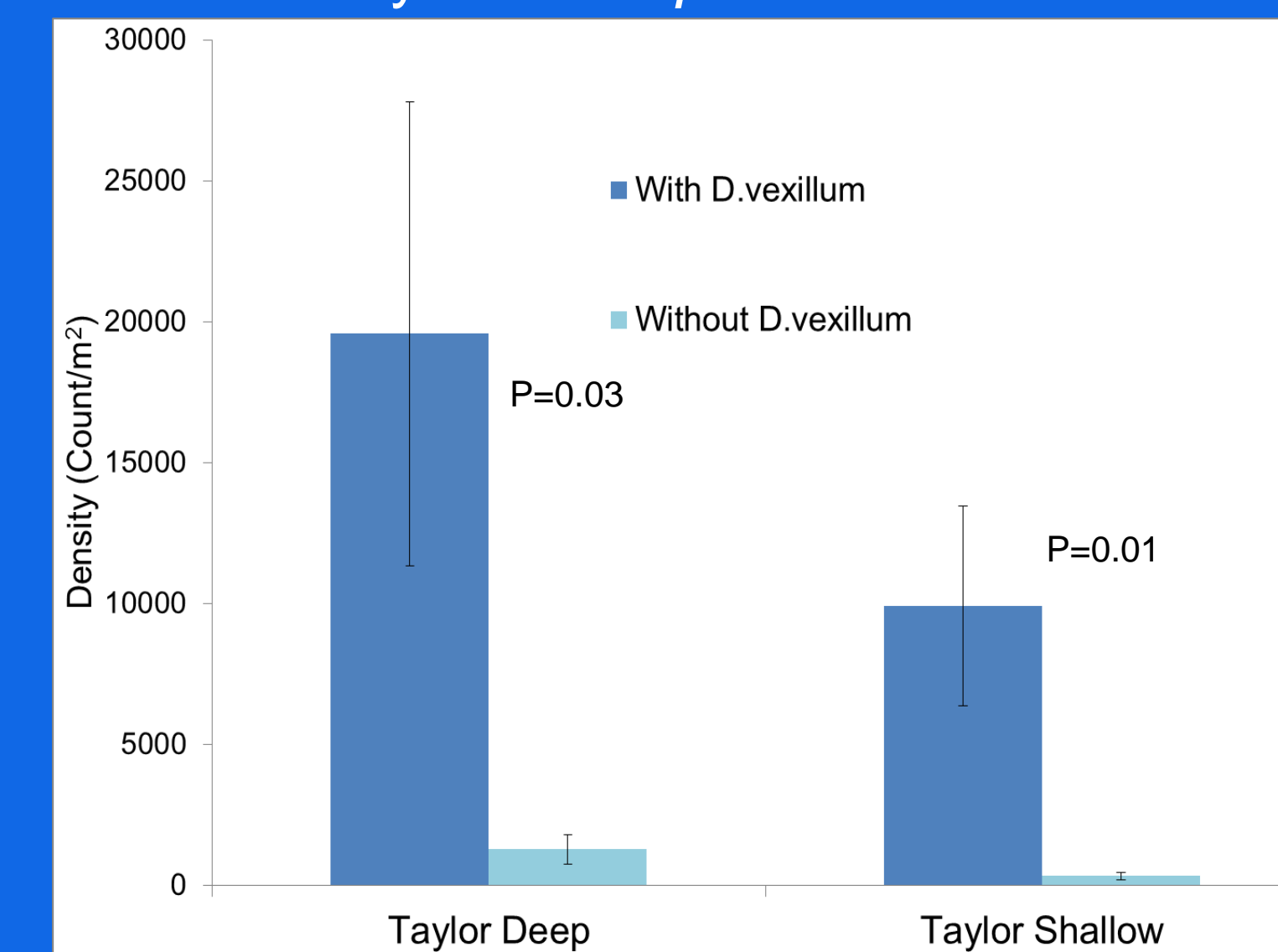
Of the 262 taxa found in the macrofauna samples, the polychaete worms were the most diverse group. The family Spionidae, in particular, was represented by eleven different species.

One isopod, *Ianiropsis serricaudis*, was almost exclusively associated with *Didemnum vexillum* at mussel farms in south Puget Sound. This species is native to Japan and Korea and has been reported as a non-indigenous species in San Francisco Bay. *Ianiropsis serricaudis* is also likely a non-indigenous introduced species in Puget Sound. It is not known if this species was introduced with *D. vexillum* or by some other route.



Asian isopod *Ianiropsis serricaudis*

Density of *Ianiropsis serricaudis*



Community Level Effects

Numerous studies and observations by shellfish growers have reported the rapid growth and spread of non-native tunicates, and several studies have demonstrated that these invasions have led to decreases in other sessile fouling organisms by overgrowing or outcompeting them for space. However, results of quantitative studies of community-level effects of invasive tunicates are mixed, showing both higher and lower densities of organisms in the presence of the introduced species and often showing no tunicate effect. For example, in San Francisco Bay large populations of *Ciona intestinalis* usually resulted in decreased species richness, but were also correlated with increased abundances of certain taxa.

Most studies of community effects caused by invasive tunicates have focused on sessile fouling species. While other types of macrofaunal organisms such as amphipods and polychaete worms are very abundant in fouling communities, these non-sessile organisms have been much less studied, and effects on meiofauna such as harpacticoid copepods have not been investigated. Because of this lack of information, we focused on these organisms.

Assemblage Analysis

For each site with significant ANOSIM results the SIMPER analysis identified the species driving the assemblage differences. The table below summarizes how many of the top five taxa at each of these sites were more abundant with and without non-indigenous tunicates.

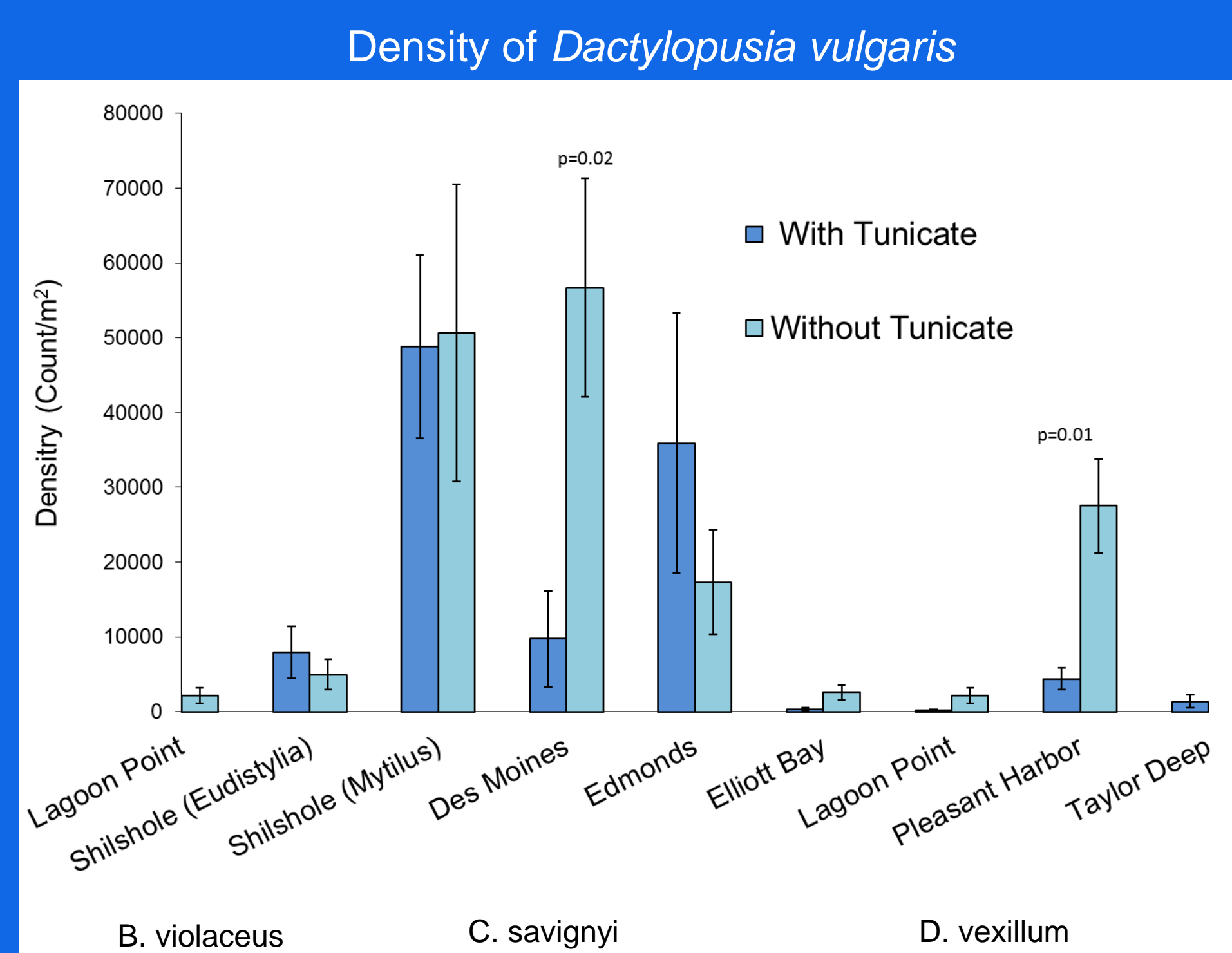
| | Site/Species | # of taxa more abundant with tunicate | # of taxa more abundant without tunicate |
|------------|--------------------------------------|---------------------------------------|--|
| Meiofauna | Lagoon Point <i>B. violaceus</i> | 4 | 1 |
| | Taylor Deep <i>D. vexillum</i> | 5 | 0 |
| | Taylor Shallow <i>D. vexillum</i> | 4 | 1 |
| Macrofauna | Lagoon Point <i>C. savignyi</i> | 2 | 3 |

Meiofauna

There were no significant differences in total meiofauna densities between samples with and without tunicates. Harpacticoid copepods were significantly more abundant when *Didemnum vexillum* was present at the Taylor Deep and Shallow sites. ANOSIM results indicated that there were significant assemblage differences at three sites.

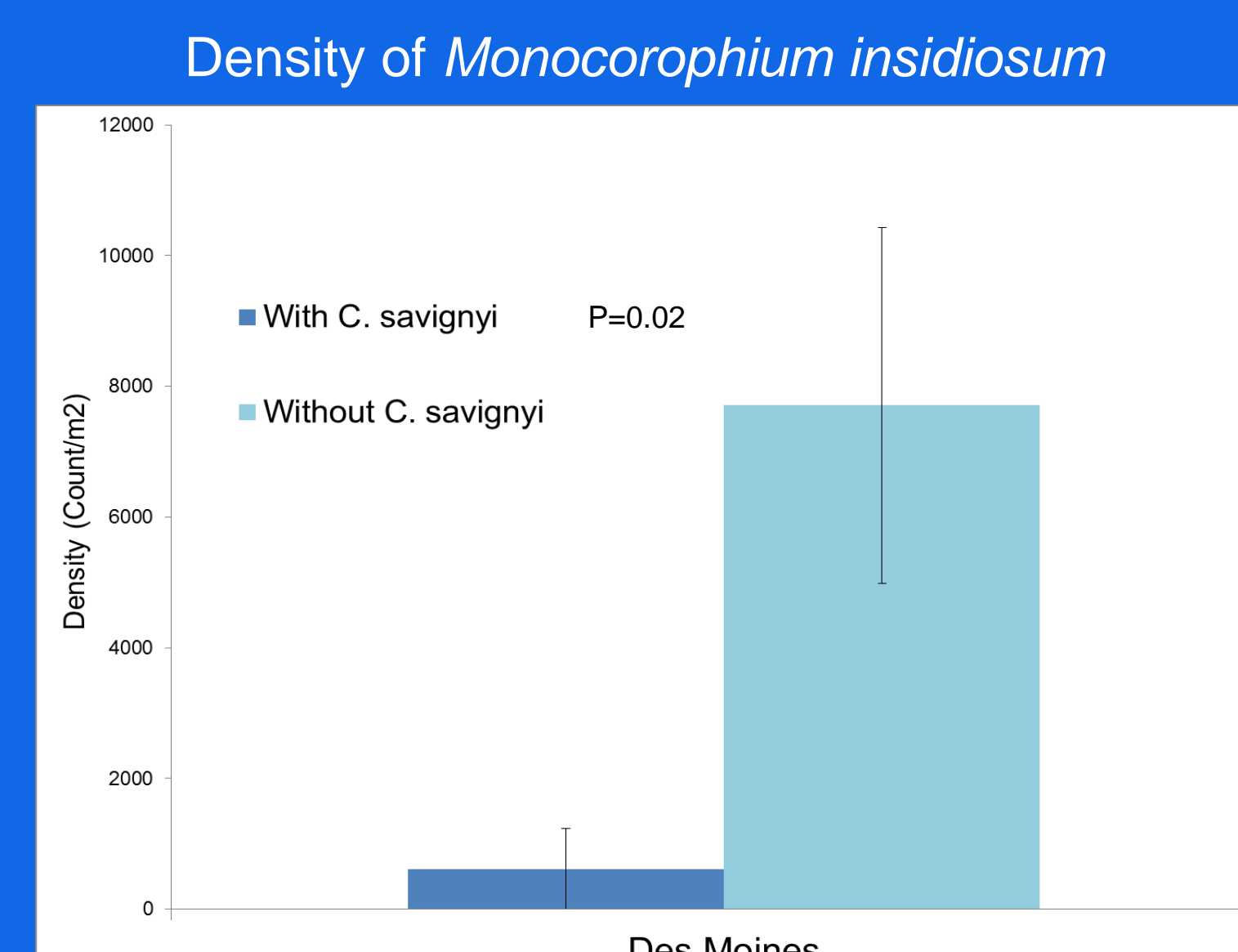
Tisbe sp. and *Dactylopusia vulgaris*, two harpacticoids consumed by juvenile salmon, together represented 20% of all harpacticoids. T-tests comparing their densities with and without tunicates showed that:

- Densities of *D. vulgaris* were significantly lower with *Ciona savignyi* at the Des Moines Marina and with *D. vexillum* at Pleasant Harbor.
- There were no differences in *Tisbe sp.* abundances between tunicate and non-tunicate samples.



Macrofauna

There were no significant differences in the total densities of organisms at any of the sites. Amphipods were significantly more abundant at Des Moines Marina in the absence of *Ciona savignyi*. This difference was driven mostly by one species, *Monocorophium insidiosum*.



Lagoon Point was the only site at which ANOSIM identified significant community differences in the macrofauna. A native solitary tunicate, *Corella willmeriana*, was the species that contributed most to community differences between tunicate and non-tunicate samples at this site. The native tunicate was more abundant in the samples without *Ciona savignyi*.



Corella willmeriana, photo by Janna Nichols

Conclusions

In general, we did not find that any of the four tunicate species had large impacts on the non-fouling assemblages that we studied. Instances of negative associations (e.g. *Dactylopusia vulgaris*) or positive associations (e.g. *Ianiropsis serricaudis*) did not occur consistently across sites and in many instances were not statistically significant. Also, taxa richness was similar between tunicate and non-tunicate samples. These results are similar to those from many previous studies in which there were not consistent overall trends toward greater or fewer organisms in the presence of the tunicate.

Mussels had thicker shells when they were overgrown by *Botrylloides violaceus* and *Didemnum vexillum*, but this difference was only significant at one of the *D. vexillum* sites. Also, there were no significant effects on mussel condition. These findings are at odds with some recent research from the Atlantic coast, and also with the perception of many shellfish growers concerned about deleterious effects of the tunicates on their harvests. However, the mussel growers at the facilities where we sampled *D. vexillum* had not experienced problems with growth or harvest, and it remains unknown whether or not invasive tunicates will affect aquaculture in the Salish Sea.

Further Research

Our results suggest that community effects of invasive tunicates in the Salish Sea may be site- and time-dependent. It would be informative to pick several sites that showed different results based on our one-time survey, and track community dynamics across time.

Likewise, previous studies that have documented negative effects of invasive tunicates on mussels have found that the effects were not consistent across times and locations. Thus, understanding of tunicate effects on mussels in the Salish sea would benefit from controlled experimental studies that take these factors into account.

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