Some like it hot: temperature stress and juvenile development in the invasive colonial tunicate *Botrylloides violaceus*

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Abstract

The Salish Sea, a vital region for marine biodiversity in the Pacific Northwest, is predicted to increase ~1.5°C over the next century. Rising sea temperatures can change species distributions and may support the range expansion of invasive species with broader temperature tolerances or of tropical/subtropical origin. *Botrylloides violaceus* is a colonial ascidian native to the subtropical waters of the West Pacific and has successfully invaded the Salish Sea. In this study, acute temperature challenge experiments were conducted to understand how rising temperatures impact colony proliferation. Juvenile colonies were cultured in unfiltered seawater over three temperature ranges: 10-12°C, 14-16°C, and 18-20°C. Growth was assessed using counts of healthy, regressing, and budding zooids, while stress was inferred based on colony heartbeat and a qualitative health assessment. Production of healthy zooids and primary buds significantly increased with temperature and yielded larger colonies within 10 days. Heart rate significantly decreased in colonies that were maintained at 20°C, however, more baseline data are needed to relate this metric to overall colony health. These results indicate that rising temperatures in the Salish Sea are expected to increase the proliferation of *B. violaceus*, intensifying resource competition in benthic communities and potentially spelling danger for native populations. Funded by NSF grants MCB-2127517 and DBI-2149705.

Introduction

Anthropogenic climate change is a major threat to marine ecosystems, and is expected to bring warmer ocean temperatures – which have already increased by ~0.4°C since the 1950s (Doney et al., 2012). Many biological processes such as metabolic and respiration rate are temperature-dependent, but whether the impact of rising temperature on fitness is positive or negative is often organism-specific (Doney et al., 2012). Species that benefit from temperature increase may experience higher reproductive success and an increased survival rate, while species that experience this change as stressful experience the opposite (Dijkstra et al., 2011; Doney et al., 2012). Warming can open up new habitats for invasive species, creating a community shift towards species better adapted for warmer climates while pushing native species distributions poleward (Doney et al., 2012; Hellmann et al., 2008; Sagarin et al., 1999).

Longer, and more intense periods of warmth have coincided with the local dominance of invasive species, particularly annuals whose life cycle is dependent on temperature shifts (Dijkstra et al., 2011; Reitzel et al., 2004). In response to increased temperature, invasive invertebrate (especially fouling) species can exhibit increased recruitment compared to native species, causing a community state change (Dijkstra et al., 2011; Doney et al., 2012; Sagarin et al., 1999; Sorte et al., 2010; Stachowicz et al., 2002). Additionally, an extension of the growing season may prevent the seasonal senescence of annual species, possibly allowing invasive species to propagate unchecked (Tyrrell & Byers, 2007).

Ascidians (also called tunicates or urochordates) are known for being highly successful invaders, decreasing species richness and affecting biodiversity in ecosystems across the world (Aldred & Clare, 2014; Zhan et al., 2015). One such annual species that has dominated marine fouling communities is the botryllid ascidian *Botrylloides violaceus* (commonly known as the chain, or violet, tunicate). Now being found across several continents, *B. violaceus* is native to Japan, and likely spread via human travel between East Asia, Europe, and the Americas (Lambert et al., 2018). Examination of genetic similarities between populations on the East Coast of the U.S. concluded that anthropogenic vectors (movement of boats and shellfish stock) are likely more responsible for the spread of *B. violaceus* than any methods of natural dispersal (Bock et al., 2011). On the west coast of the U.S., *B. violaceus* occurs in large numbers from Ensenada, Mexico, to Alaska (Carver et al., 2006; Lambert & Lambert, 2003). Its presence was first recorded in California in the 1970s, but due to the morphological similarities between *B. violaceus* and *Botrylloides diagenesis* it may have been present much earlier – but was only correctly identified near the end of the 20th century (Bock et al., 2011; Carver et al., 2006; Lambert & Lambert, 2003).

Colonial tunicates like *B. violaceus* consist of several individuals (zooids) embedded in a common tunic; reproduction can occur sexually through internal fertilization and asexually through blastogenesis (commonly referred to as 'budding') (Lambert et al., 2018; Figure 1). Unlike larval production, budding is synchronized across systems within a botryllid colony – each generation grows to replace the previous in a process known as takeover (Dijkstra et al., 2017; Nourizadeh et al., 2021). *B. violaceus* relies exclusively on budding for colony growth and expansion, but can induce so-called "vascular" budding (where new buds are formed from vascular tissue, unconnected to existing zooids) in response to injury to perform whole-body regeneration (WBR)

(Nourizadeh et al., 2021). Colonial ascidians are more susceptible to predation than solitary ascidians, but are able to recover almost completely using WBR (Hiebert et al., 2019). To survive injury and retain precious space on the substratum, the timing of budding and number of buds produced in each generation must be competitive with those of surrounding species, and are thus linked to colony success (Sorte et al., 2010).

While rising temperatures in the Gulf of Maine favored the increased reproduction and proliferation of *B. violaceus*, reproductive cycles can differ between colonies and populations, prompting investigation of the effect of increased seasonal temperatures on botryllid populations in other regions (Dijkstra et al., 2017; Grosberg, 1988). The Salish Sea of the Pacific Northwest is one of the most significant places for biodiversity conservation in the region, but is greatly threatened by climate change – coastal ocean temperatures are expected to increase by ~1.5°C by the end of the century based on current climate change models – and so may be vulnerable to shifts in species composition as a result (Khangaonkar et al., 2019; Kraus & Hebb, 2020; Simon et al.,

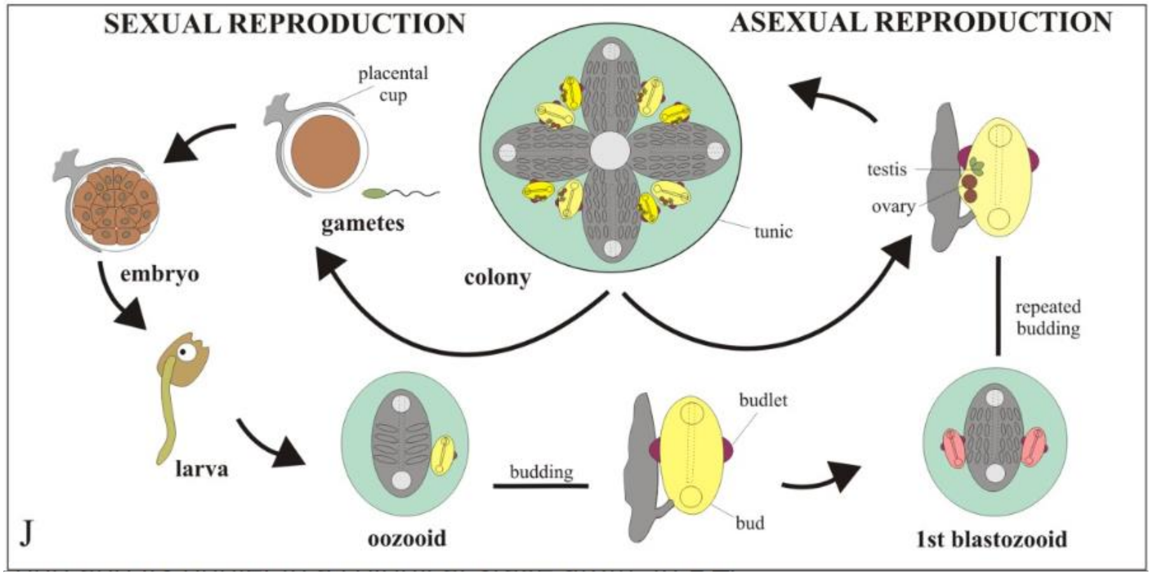


Figure 1. Life cycle of B. schlosseri (Gasparini et al., 2015) which is analogous to B. violaceus due to the similarity of botryllid ascidians (Berrill, 1947). Focus will be from the larval stage to the 1st blastozooid and its repeated budding.

2022). This region is also key to shellfish aquaculture, so predicting the response of such a prolific invasive fouling botryllid is necessary for maintaining native and farmed populations of shellfish that are important for both cultural and economic reasons (Carver et al., 2006; Epelbaum, Herborg, et al., 2009).

To better predict the response of *B. violaceus* populations in the Salish Sea to ocean warming, we measured overall growth and health of juvenile colonies of *B. violaceus* at temperatures representative of predicted increases associated with climate change. The number of buds produced by a given colony, the length of the blastogenic cycle, and colony heart rate were used as proxies for health, in addition to qualitative observations.

Methods:

Adult and Larval Collection

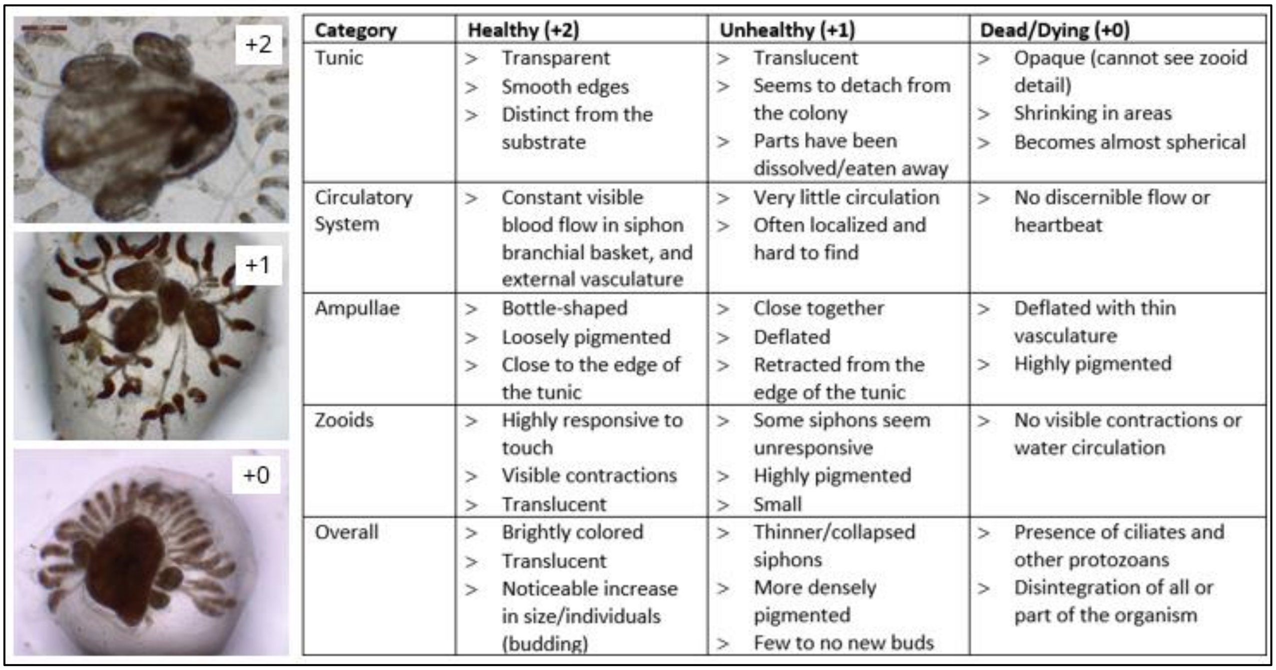
Colonies of *B. violaceus* were collected from the floating docks in Snug Harbor, WA (48° 34' 15.6" N, 123° 10' 1.2" W) using a scientific collection permit from University of Washington Friday Harbor Laboratories. While *B. violaceus* is the only species of *Botrylloides* that occurs in the Salish Sea, colonies were only collected if they exhibited a clearly visible “chain” pattern of zooids and displayed commonly observed colors (e.g., orange, rust, etc.) to ensure consistency in species identification (Simon et al., 2022). All colonies were collected from the plastic dock floats (10-20 cm below the surface) in July 2022, a month when daily air temperatures reached over 23 ℃. After collection, colonies were immersed in an unfiltered flow-through seawater system (a “sea table”) which provided any particulates needed for feeding.

Collected colonies were torn into large fragments (approximately 5 cm by 7 cm) all fragments were placed in a plastic kitchen colander within a sea table. Adult colonies produced batches of larvae in the late morning that over time floated to the surface and metamorphosed. Once they settled on the surface, the oozooids were transferred to extra-large microscope slides (2" x 3") using a small paintbrush (Epelbaum et al., 2009b; Swalla, 2004). After 24 hours, slides containing settled juveniles were added to the appropriate temperature treatment.

Temperature Gradient Set-up

Temperature stress was tested over three ranges: 10-12°C (control) – the natural temperatures of the sea table; 14-16°C (medium) to mimic potential warming temperatures after extreme global change; and 18-20°C (high) to mimic the temperatures to which *B. violaceus* is native (W. J. Lambert et al., 2018). All temperature treatments were housed in the same sea table within 15L plastic containers (16 3/4" L x 11 7/8" W x 7" H), with the flow-through of incoming seawater acting as a coolant. As the water temperature of the sea table naturally fluctuates throughout the day, temperature treatments were maintained within a range of 2°C rather than at one constant temperature, and ranges were chosen to allow daily fluctuations of water temperature in the harbor. The medium and high temperature conditions were heated using a 500W heater inside the plastic container and corresponding controller (Finnex), which kept the detected water temperature within ± 2 °F (± ~1.12 °C) of the desired temperature. Manual water changes were performed approximately every 48 hours.

Figure 2. Anatomy of two example B. violaceus colonies under compound microscopy. Growth metrics are indicated by the following annotations: az (adult zooid) varies in length and is identified in the context of the larger colony; blue arrow (regressing zooid); orange arrow (primary bud); green arrow (secondary bud/budlet). The yellow arrow identifies one of the ampullae.



Category	**Healthy (+2)**	**Unhealthy (+1)**	**Dead/Dying (+0)**
Tunic	> Transparent > Smooth edges > Distinct from the substrate	> Translucent > Seems to detach from the colony > Parts have been dissolved/eaten away	> Opaque (cannot see zooid detail) > Shrinking in areas > Becomes almost spherical
Circulatory System	> Constant visible blood flow in siphon branchial basket, and external vasculature	> Very little circulation > Often localized and hard to find	> No discernible flow or heartbeat
Ampullae	> Bottle-shaped > Loosely pigmented > Close to the edge of the tunic	> Close together > Deflated > Retracted from the edge of the tunic	> Deflated with thin vasculature > Highly pigmented
Zooids	> Highly responsive to touch > Visible contractions > Translucent	> Some siphons seem unresponsive > Highly pigmented > Small	> No visible contractions or water circulation
Overall	> Brightly colored > Translucent > Noticeable increase in size/individuals (budding)	> Thinner/collapsed siphons > More densely pigmented > Few to no new buds	> Presence of ciliates and other protozoans > Disintegration of all or part of the organism

Figure 3. Qualitative rubric for colony health based on condition of major aspects of *B. violaceus* anatomy. Top left – example of healthy system, all categories worth 2 points. Middle left – example of unhealthy system, all categories worth 1 point. Bottom left – example of dead/dying system, all categories worth +0 points.

Larval Development

Eleven *B. violaceus* oozooids were exposed to each of the three temperature conditions, 10-12°C (control), 14-16°C (medium), and 18-20°C (high). Organisms were imaged daily and monitored over a period of 10 days for the following endpoints: number and size of zooids, buds (primary), and budlets (secondary) to approximate growth and blastogenesis, heart rate to approximate stress level, and overall health of the colony based on a qualitative rubric (Figure 2, 3; Brunetti et al., 1980; J. Dijkstra et al., 2008).

Statistical Analyses

A two-factor (temperature, time) analysis of variance (ANOVA) was performed on all endpoints using SPSS v. 28.0 (IBM). All metrics were analyzed for significance across time and temperature and pairwise comparisons were made between each of the temperature treatments. Data were evaluated for normality and homogeneity of variance using Levene and Shapiro-Wilk tests, respectively. If either test failed, a non-parametric equivalent (Kruskal-Wallis H test) was performed. Post-hoc multiple comparisons were performed using Tukey HSD. The significance threshold was set at $\alpha = 0.05$.

Results:

Number of healthy zooids

The number of healthy zooids significantly increased across both time ($p < 0.001$) and increase in temperature ($p= 0.007$), with the high temperature treatment resulting in the largest average number of healthy zooids (Figure 4a). Post-hoc pairwise comparisons of temperature showed significance between the high and control treatments ($p = 0.002$) and the high and medium treatments ($p = 0.023$), but not between the control and medium treatments ($p = 0.376$) (Figure 4a).

Number of regressing zooids

The number of regressing zooids was found to be significant across time ($p = 0.021$) but not across temperature ($p = 0.638$), and no significance was found between different temperatures after performing post-hoc analysis (Figure 4b).

Number of primary buds

The number of primary buds was found to be significant over time ($p = 0.003$) and temperature ($p = 0.004$), and post-hoc analysis revealed significance between the medium temperature and the other two treatments ($p_{\text{medium-control}} = 0.010$, $p_{\text{medium-high}} = 0.003$), but not between the control and the high treatments ($p = 0.692$) (Figure 4c).

Number of secondary buds

The number of secondary buds per colony was not found to be significant across time or temperature, and post-hoc analysis showed no significance between temperatures. However, comparing the control and high temperature treatments, the number of secondary buds approached significance, with a p-value of 0.051 (Figure 4d).

Colony heartbeat

Average colony heartbeat was found to be significant across time ($p = 0.011$) and temperature ($p < 0.001$) with colonies in the high temperature treatment exhibited significantly lower heart rates than colonies in the other two treatments (Figure 4e).

Qualitative observations

After assessing colony health over time using a qualitative rubric (Figure 3), each temperature treatment had a distinct trend. Colonies in both the control and medium temperature treatments tended to decrease in health over time. In contrast, colonies in the high temperature treatment tended to increase in health over time and showed less variability.

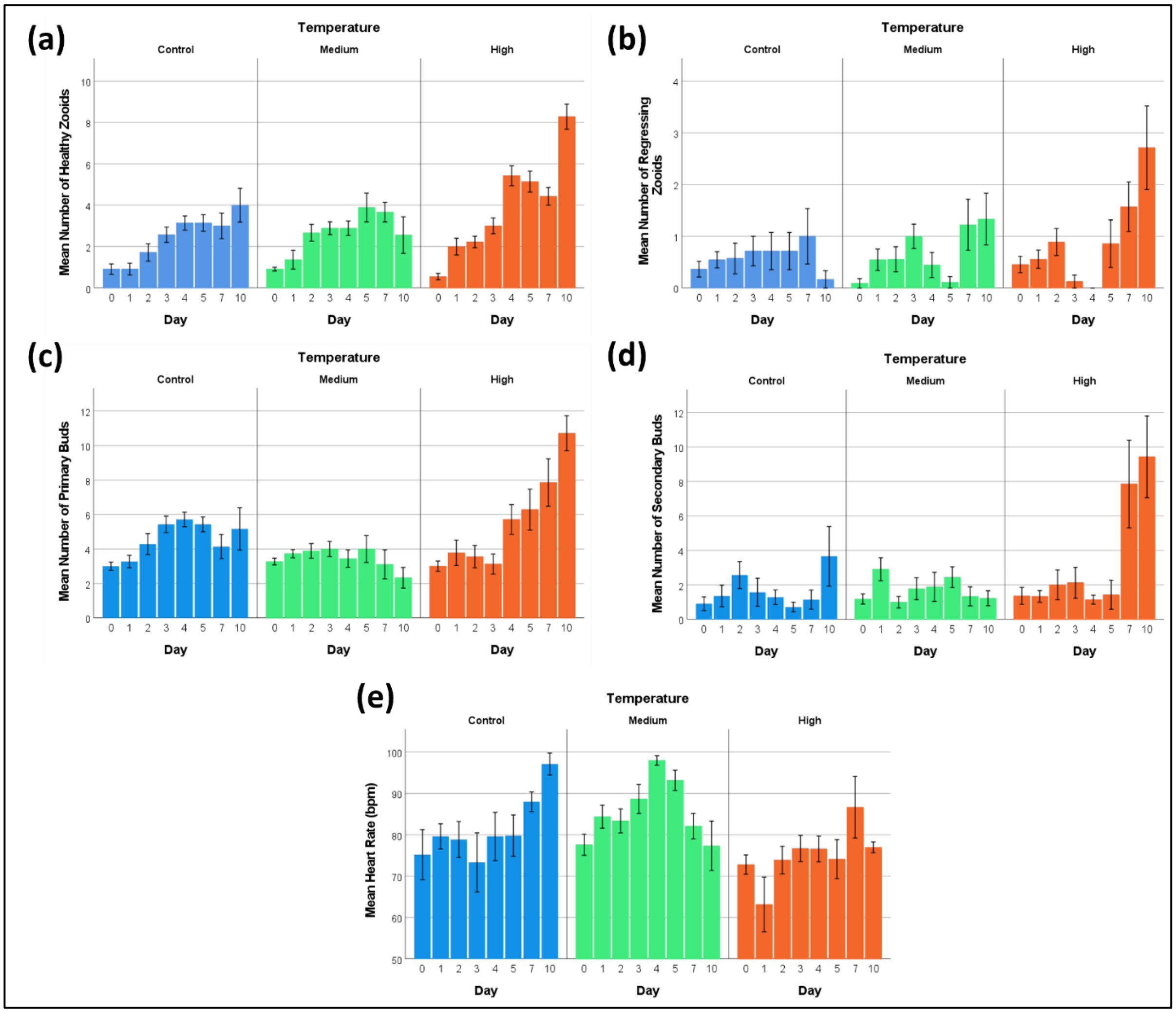


Figure 4. Graphed averages of growth and stress metrics. All error bars represent standard error. a) Mean number of healthy zooids graphed over temperature and time. b) Mean number of regressing zooids graphed over time and temperature. c) Mean number of primary buds graphed over time and temperature. d) Mean number of secondary buds graphed over time and temperature. e) Average colony heartbeat graphed over time and temperature.

Discussion

Many of the results from these temperature challenge experiments aligned with our expectations for the temperature tolerance and preferences of *B. violaceus*. Colonies in higher temperatures produced more healthy zooids than colonies in lower temperatures. These results agree with our qualitative observations that colonies in the higher temperature treatment were generally healthier over time than those in the other treatments, and supports *B. violaceus* being more successful at temperatures closer to its native range. Rising temperatures are predicted to positively impact reproductive rate of this species and promote growth and colony proliferation, as the length of the blastogenic cycle in botryllid ascidians is inversely correlated with seawater temperature (Dijkstra et al., 2017; Grosberg, 1988; Westerman et al., 2009). Our results for production of primary buds, while significant, demonstrated a different trend than expected. Instead of increasing linearly with temperature, the medium temperature treatment showed significantly fewer buds produced than either the control or the high, resulting in a U-shape response curve.

Neither the number of regressing zooids nor the number of secondary buds was found to be significant across temperatures. Due to the nature of asexual growth, the number of individuals in *B. violaceus* colonies increases exponentially, but the acute nature of this experiment only allowed time for a small number of blastogenic cycles (Berrill, 1947; Nourizadeh et al., 2021). As such, the average juvenile colony contained fewer than 3 healthy zooids and 4 secondary buds until the very last day of observation (Day 10) (Figure 4a). The average number of regressing zooids never surpassed 4 zooids (Figure 4b). For both these parameters, the sample size may have resulted in low statistical power. This indicates that counting these regressing zooids and secondary buds requires a longer period of observation before a trend appears, and they may be better parameters in a more chronic experiment.

These results have implications for benthic, and more specifically ascidian, biodiversity in the Salish Sea as climate change progresses. *B. violaceus* shows significant increase in growth in response to rising temperatures, indicating that it will proliferate more rapidly as the environment warms, potentially allowing this species to expand into new habitat. Ascidians are some of the most successful invaders, having made their way to nearly every continent using both natural and anthropogenic forms of dispersal (Bock et al., 2011; Zhan et al., 2015).

Depending on the severity of the invasion, introduced ascidians may alter community structure by outcompeting and overgrowing other benthic organisms (Tracy & Reyns, 2014). Both *B. violaceus* and its close relative *Botryllus schlosseri* have been observed to overgrow oysters and other bivalves, occasionally increasing mortality through local food depletion and physical smothering (Carver et al., 2006). Dense mats of *Didemnum vexillium* can prevent benthic-pelagic species interactions by inhibiting planktonic predators foraging efficiency in benthic and fouling communities, and functioning as physical barriers to geochemical cycling of nutrients (Mercer et al., 2009; Tracy & Reyns, 2014; Zhan et al., 2015). Increased proliferation of an organism like *B. violaceus* may improve water clarity due to increased particle removal, however, this could jeopardize food resources for other resident filter feeders, such as bivalves and zooplankton (Carver et al., 2006). Based on the trends observed in this study, rising temperatures in the Salish Sea may allow for increased growth and proliferation of *B. violaceus,* potentially threatening not only native ascidians, but native species of other taxa that compete for space and resources within benthic communities.

To better determine the impact of climate change on ascidian species distribution in the Salish Sea, it is necessary to conduct similar temperature challenge experiments with native ascidians that cohabitate with *B. violaceus* such as *Distaplia occidentalis*, *Aplidium solidum,* or *Displosoma listerianum*. Given what is known about the immediacy of climate change and its expected impacts on ocean temperatures in the Salish Sea region of the Pacific Northwest, species distributions will likely change as the region warms to favor tunicates both invasive and native (Dijkstra et al., 2011; Khangaonkar et al., 2019). Future work will also need to consider competition, and the availability of different substrates, as *B. violaceus* is known to prefer artificial over natural surfaces (Tyrrell & Byers, 2007). This could threaten regional biodiversity by displacing native species, and increasing competition for resources – most notably the already limited space on natural and artificial substrates that is vital for the formation of benthic communities (Carver et al., 2006; Mercer et al., 2009).

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References

Aldred, N., & Clare, A. S. (2014). Mini-review: Impact and dynamics of surface fouling by solitary and compound ascidians. *Biofouling*, *30*(3), 259–270. https://doi.org/10.1080/08927014.2013.866653

Berrill, N. J. (1947). *The Developmental Cycle of Botrylloides*. 15.

Bock, D. G., Zhan, A., Lejeusne, C., MacISAAC, H. J., & Cristescu, M. E. (2011). Looking at both sides of the invasion: Patterns of colonization in the violet tunicate Botrylloides violaceus. *Molecular Ecology*, *20*(3), 503–516. https://doi.org/10.1111/j.1365-294X.2010.04971.x

Brunetti, R., Beghi, L., Bressan, M., & Marin, M. G. (1980). Combined Effects of Temperature and Salinity on Colonies of Botryllus schlosseri and Botrylloides leachi (Ascidiacea) from the Venetian Lagoon. *Marine Ecology Progress Series*, *2*(4), 303–314.

Carver, C. E., Mallet, A. L., & Vercaemer, B. (2006). *Biological Synopsis of the Colonial Tunicates (Botryllus Schlosseri and Botrylloides Violaceus)*. https://www.academia.edu/15812239/Biological_Synopsis_of_the_Colonial_Tunicates_Botryllus_Schlosseri_and_Botrylloides_Violaceus_

Dijkstra, J. A., Dutton, A., Westerman, E., & Harris, L. (2008). Heart rate reflects osmostic 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, J. A., Westerman, E. L., & Harris, L. G. (2011). The effects of climate change on species composition, succession and phenology: A case study. *Global Change Biology*, *17*(7), 2360–2369. https://doi.org/10.1111/j.1365-2486.2010.02371.x

Dijkstra, J. A., Westerman, E. L., & Harris, L. G. (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*(10), 1182–1192. https://doi.org/10.1111/ddi.12604

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N., Polovina, J., Rabalais, N. N., Sydeman, W. J., & Talley, L. D. (2012). Climate Change Impacts on Marine Ecosystems. *Annual Review of Marine Science*, *4*(1), 11–37. https://doi.org/10.1146/annurev-marine-041911-111611

Epelbaum, A., Herborg, L. M., Therriault, T. W., & Pearce, C. M. (2009). 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*(1), 43–52. https://doi.org/10.1016/j.jembe.2008.10.028

Epelbaum, A., Therriault, T. W., Paulson, A., & Pearce, C. M. (2009). Botryllid tunicates: Culture techniques and experimental procedures. *Aquatic Invasions*, *4*(1), 111–120. https://doi.org/10.3391/ai.2009.4.1.12

Gasparini, F., Manni, L., Cima, F., Zaniolo, G., Burighel, P., Caicci, F., Franchi, N., Schiavon, F., Rigon, F., Campagna, D., & Ballarin, L. (2015). Sexual and asexual reproduction in the colonial ascidian Botryllus schlosseri. *Genesis*, *53*(1), 105–120. https://doi.org/10.1002/dvg.22802

Grosberg, R. K. (1988). Life-History Variation Within a Population of the Colonial Ascidian Botryllus schlosseri. I. The Genetic and Environmental Control of Seasonal Variation. *Evolution*, *42*(5), 900–920. https://doi.org/10.2307/2408907

Hellmann, J., Byers, J., Bierwagen, B., & Dukes, J. (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

Hiebert, L. S., Vieira, E. A., Dias, G. M., Tiozzo, S., & Brown, F. D. (2019). Colonial ascidians strongly preyed upon, yet dominate the substrate in a subtropical fouling community. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1899), 20190396. https://doi.org/10.1098/rspb.2019.0396

Khangaonkar, T., Nugraha, A., Xu, W., & Balaguru, K. (2019). Salish Sea Response to Global Climate Change, Sea Level Rise, and Future Nutrient Loads. *Journal of Geophysical Research: Oceans*, *124*(6), 3876–3904. https://doi.org/10.1029/2018JC014670

Kraus, D., & Hebb, A. (2020). Southern Canada’s crisis ecoregions: Identifying the most significant and threatened places for biodiversity conservation. *Biodiversity and Conservation*, *29*(13), 3573–3590. https://doi.org/10.1007/s10531-020-02038-x

Lambert, C., & Lambert, G. (2003). Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. *Marine Ecology Progress Series*, *259*, 145–161. https://doi.org/10.3354/meps259145

Lambert, W. J., Dijkstra, J. A., Clark, E., & Connolly, J. (2018). Larval exposure to low salinity compromises metamorphosis and growth in the colonial ascidian Botrylloides violaceus. *Invertebrate Biology*, *137*(4), 281–288. https://doi.org/10.1111/ivb.12226

Mercer, J. M., Whitlatch, R. B., & Osman, R. W. (2009). *Potential effects of the invasive colonial ascidian (Didemnum vexillum Kott, 2002) on pebble-cobble bottom habitats in Long Island Sound, USA*. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.555.926&rep=rep1&type=pdf

Nourizadeh, S., Kassmer, S., Rodriguez, D., Hiebert, L. S., & De Tomaso, A. W. (2021). Whole body regeneration and developmental competition in two botryllid ascidians. *EvoDevo*, *12*(1), 15. https://doi.org/10.1186/s13227-021-00185-y

Reitzel, A., Miner, B., & McEdward, L. (2004). Relationships between spawning date and larval development time for benthic marine invertebrates: A modeling approach. *Marine Ecology Progress Series*, *280*, 13–23. https://doi.org/10.3354/meps280013

Sagarin, R. D., Barry, J. P., Gilman, S. E., & Baxter, C. H. (1999). Climate-Related Change in an Intertidal Community Over Short and Long Time Scales. *Ecological Monographs*, *69*(4), 465–490. https://doi.org/10.1890/0012-9615(1999)069[0465:CRCIAI]2.0.CO;2

Simon, A., Adamczyk, E., Basman, A., Chu, J., Gartner, H., Fletcher, K., Gibbs, C., Gibbs, D., Gilmore, S., Harbo, R., Harris, L., Humphrey, E., Lamb, A., Lambert, P., McDaniel, N., Scott, J., & Starzomski, B. (2022). Toward an atlas of Salish

Sea biodiversity: The flora and fauna of Galiano Island, British Columbia, Canada. Part I. Marine zoology. *Biodiversity Data Journal*, *10*, e76050. https://doi.org/10.3897/BDJ.10.e76050

Sorte, C. J. B., Williams, S. L., & Zerebecki, R. A. (2010). Ocean warming increases threat of invasive species in a marine fouling community. *Ecology*, *91*(8), 2198–2204. https://doi.org/10.1890/10-0238.1

Stachowicz, J. J., Terwin, J. R., Whitlatch, R. B., & Osman, R. W. (2002). Linking climate change and biological invasions: Ocean warming facilitates nonindigenous species invasions. *Proceedings of the National Academy of Sciences*, *99*(24), 15497–15500. https://doi.org/10.1073/pnas.242437499

Swalla, B. J. (2004). *Development of Sea Urchins, Ascidians, and Other Invertebrate Deuterostomes: Experimental Approaches*. Elsevier.

Tracy, B. M., & Reyns, N. B. (2014). Spatial and temporal patterns of native and invasive ascidian assemblages in a Southern California embayment. *Aquatic Invasions, 9*(4), 441–455. http://dx.doi.org/10.3391/ai.2014.9.4.03

Tyrrell, M. C., & Byers, J. E. (2007). Do artificial substrates favor nonindigenous fouling species over native species? *Journal of Experimental Marine Biology and Ecology*, *342*(1), 54–60. https://doi.org/10.1016/j.jembe.2006.10.014

Westerman, E., Whitlatch, R., Dijkstra, J., & Harris, L. (2009). Variation in brooding period masks similarities in response to changing temperatures. *Marine Ecology Progress Series*, *391*, 13–19. https://doi.org/10.3354/meps08107

Zhan, A., Briski, E., Bock, D. G., Ghabooli, S., & MacIsaac, H. J. (2015). Ascidians as models for studying invasion success. *Marine Biology*, *162*(12), 2449–2470. https://doi.org/10.1007/s00227-015-2734-5