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Seasonal variability in a nascent population of a non-indigenous colonial ascidian (*Didemnum vexillum*) near Winchester Bay, Oregon

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Abstract The non-indigenous colonial tunicate *Didemnum vexillum* plagues many shellfish aquaculture operations around the world by smothering crop and gear and displacing juvenile bivalves. There are two populations of *D. vexillum* in Oregon, but little is known regarding their spatial extent, seasonal dynamics, annual variability, reproductive biology, or ecology. SCUBA divers monitored the seasonal variation and spatial extent of the nascent *D. vexillum* population in its current foothold at Umpqua Aquaculture, a longline oyster farm adjacent to Winchester Bay, Oregon. From May 2011 to November 2016, divers performed biannual subtidal surveys to measure the cover of *D. vexillum* on anchor lines. We found that this population of *D. vexillum* exhibited extensive fluctuation in colony during this period, especially between fall and spring. With one exception, cover was greater in the fall than the spring, though this fluctuation cannot be explained by our proxies for salinity or temperature. Seasonally, on average, *D. vexillum* covered the greatest proportion of line between 5.5 and 6.0 m depth in both the spring (37.5 ± 18.8%) and fall (45.1 ± 16.1%). During individual surveys, the depth of maximum percent *D. vexillum* cover was more variable, spanning ~ 4.5–6.5 m depth. Overall, we document a

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net increase in *D. vexillum* cover at Umpqua Aquaculture between the first and last surveys during the 5-year period. This study is the first published multi-year analysis of *D. vexillum* in Oregon and represents the longest continuous monitoring period of an in situ population of *D. vexillum* to date.

Keywords Colonial ascidian · Oyster aquaculture · Invasive species

Introduction

Non-indigenous and invasive species have dramatically altered the biodiversity, community structure, and function of marine ecosystems, particularly coastal and estuarine habitats (Grosholz 2002). It is estimated that the deleterious effects of invasive species cost an estimated 120 billion USD annually in the USA (Pimentel et al. 2005); preventing and mitigating these effects are priorities for federal and state-level coastal resource agencies (Daley and Scavia 2008; Rumrill et al. 2014). During this pivotal time, when many aspects of global change are anticipated to favor the establishment and success of invasive species (Dukes and Mooney 1999), it is critically important to gain an increased understanding about the factors associated with a shift from a low-level of detection to large-scale invasion for populations of non-indigenous and potentially invasive species.

Early detection and monitoring of nascent populations pose a particular challenge for the management and control of non-indigenous ascidians prior to an invasion in marine and estuarine habitats (Zhan et al. 2015; Drolet et al. 2016; McKenzie et al. 2016; Lins et al. 2018). As the reproductive output of the emerging population increases, propagule pressure builds to the point when recruitment becomes limited by the availability of suitable habitat rather than adequacy of larval supplies (Collin et al. 2013). Ecological conditions and intrinsic demographic factors that facilitate transformation of a nascent population into a biological invasion are understudied and poorly understood for non-indigenous ascidians and other invertebrates in the marine environment (Pereyra and Ocampo Reinaldo 2018).

The colonial tunicate *Didemnum vexillum* Kott, 2002 was first recorded on west coast of the USA in San Francisco Bay in 1993 (Bullard et al. 2007; Lambert 2009). Populations of *D. vexillum* are native to Japan (Stefaniak et al. 2012), but this species has become a global invader (Lambert 2009; Stefaniak et al. 2009), affecting shellfish aquaculture operations in New Zealand (Fletcher et al. 2013b), the Northwest Atlantic (Carman et al. 2010; Sephton et al. 2011; Moore et al. 2018; Auker 2019), the Northeast Pacific (Switzer et al. 2011), and the Mediterranean (Tagliapietra et al. 2012; Ordóñez et al. 2015; Casso et al. 2018). As epifouling compound ascidians, the colonies of *D. vexillum* are of particular concern for shellfish aquaculture because they can overgrow bivalves, smother crop and gear, reduce growth rates (Fletcher et al. 2013b), and deter larval settlement (Morris 2009). Conservative estimates put the global cost of biofouling control to the aquaculture industry at 1.5–3 billion USD annually (Fitridge et al. 2012). In New Zealand, *D. vexillum* caused 807,000 USD in damages to the green-lipped mussel (*Perna canaliculus*) aquaculture industry (Sinner and Coutts 2003).

Populations of *D. vexillum* have recently become established within several estuaries and marinas on the NE Pacific coast, from southeast Alaska (Cohen et al. 2011) to southern California (Lambert 2009; Tracy and Reyns 2014; McKenzie et al. 2017). Two known populations of *D. vexillum* occur in Oregon (Coos estuary, Charleston Marina; and Umpqua estuary, Winchester Bay) where both populations were first observed in 2010 (Rumrill et al. 2014). Pacific oysters (*Crassostrea gigas*) are cultivated at the Winchester Bay site, and it is likely that *D. vexillum* arrived via oyster transfers, which are a known vector for the introduction of numerous invasive species (Mineur et al. 2007). In contrast, commercial oyster mariculture operations do not occur in the immediate vicinity of the *D. vexillum* population at the Charleston Marina where it is likely that other vectors (i.e. recreational and commercial vessel traffic) may have played a role in establishment (Clarke Murray et al. 2011; Roche et al. 2015). Little is known regarding the spatial extent, seasonal dynamics, annual variability, reproductive biology, or ecology of these nascent populations of *D. vexillum* in the Oregon estuaries (Rumrill et al. 2014). Assessment of the risks posed by invasive species is a key conservation issue outlined in the Oregon Conservation Strategy (Oregon Department of

Fish and Wildlife 2016), and *D. vexillum* is listed as one of the top 100 most dangerous invasive species in Oregon (Oregon Invasive Species Council 2016). Furthermore, state officials recently rated *D. vexillum* as a 'High Risk' species due, in part, to concern that *D. vexillum* colonies would soon generate sufficient propagule pressure to colonize new sites in the Oregon estuaries and spread to other bays that also contain appropriate habitat (Rumrill et al. 2014).

Colonies of *Didemnum vexillum* are considered as ecosystem engineers (Wallentinus and Nyberg 2007); they frequently fill diverse niches because they survive and thrive in a wide range of environmental conditions. For example, colonies tolerate a wide range of seawater temperatures (− 2 to 24 °C; Valentine 2009), salinities (10–36‰; Bullard and Whitlatch 2009; Gröner et al. 2011), depths (0–81 m; Valentine et al. 2007), and habitats (estuarine to outer coast; Bullard and Whitlatch 2009). In addition, the short-lived tadpole larvae of *D. vexillum* readily settle and complete metamorphosis on a wide diversity of substrata, including artificial structures (Daley and Scavia 2008; Stefaniak and Whitlatch 2014; Fletcher et al. 2018), loose cobble (Valentine et al. 2007), and eelgrass (Carman and Grunden 2010). *D. vexillum* can thus become established as epifoulers within healthy communities of benthic invertebrates (Bullard and Whitlatch 2009; Carman et al. 2019; Cottier-Cook et al. 2019). During the winter, *D. vexillum* colonies are known to exhibit regression, but not complete death (Valentine et al. 2007). In other studies, this regression pattern has been strongly correlated to seasonal fluctuations in temperature and salinity (Gröner et al. 2011), but resistance to these fluctuations varies across populations (Valentine 2009; Fletcher and Forrest 2011; Gröner et al. 2011).

The primary objective of this study was to characterize the seasonal variation and spatial extent of the *Didemnum vexillum* population at its current foothold in Winchester Bay, Oregon. We hypothesized that, in congruence with other studies, *D. vexillum* cover would be greater in fall than in spring, and that variability in spatial cover is directly correlated with changes in salinity (Gröner et al. 2011) and temperature (Valentine 2009; Fletcher and Forrest 2011; Fletcher et al. 2013a). To test these hypotheses, SCUBA divers completed a series of subtidal surveys to measure the cover by *D. vexillum* in Winchester Bay biannually from 2011 to 2016. This study is the first multi-year analysis of *D. vexillum* population dynamics in Oregon, and represents the longest continuous monitoring period of an in situ population of *D. vexillum* to date.

Methods

Monitoring occurred in the triangular South Jetty at the mouth of, but separated from, the Umpqua River in Winchester Bay, Oregon (43° 39′ 54.5″ N 124° 12′ 40.3″ W; Fig. 1), within and adjacent to an active Pacific oyster mariculture farm. Umpqua Aquaculture grows Pacific oysters (*Crassostrea gigas*) using suspended lines attached to floats, allowing for constant submersion regardless of tidal fluctuation. The depth of the study area is ∼ 9 m. SCUBA divers performed subtidal surveys biannually in May and October from 2011 to 2016. Umpqua Aquaculture uses a standard subtidal longline configuration in which several vertical anchored float lines support a horizontal surface line. A number of vertical oyster culture lines are attached to the horizontal surface line, evenly spaced several feet apart. Even at low tide, the oyster culture lines are suspended well above the bottom substrate. During each sample event, divers followed subtidal vertical anchored float lines (May 2011, $n = 11$; October 2011, $n = 14$; May 2012, $n = 14$; October 2012, $n = 12$; May 2013, $n = 20$; October 2013, $n = 17$; May 2014, $n = 22$; October 2014, $n = 17$; May 2015 = 21; October 2015, $n = 23$; May 2016, $n = 18$; November 2016, $n = 23$) from the bottom to the surface, along which they counted and measured *D. vexillum* colonies. To choose survey sites, divers used random systematic sampling, where at each sampling event, they enumerated the float lines, randomly selected a beginning line, then divers surveyed each third line. The in situ diver-based survey approach minimized disturbance to the gear and product owned by Umpqua Aquaculture. To account for the multi-dimensional structure of any additional fouling organisms and the *D. vexillum* colonies encrusting them, divers measured along the vertical contours of clumps, rather than following a linear path. While surveying, divers measured each line and colony to the nearest centimeter, and recorded the depths at which the *D. vexillum* cover began and ended, as well as some other intermittent depths, from their dive computers.

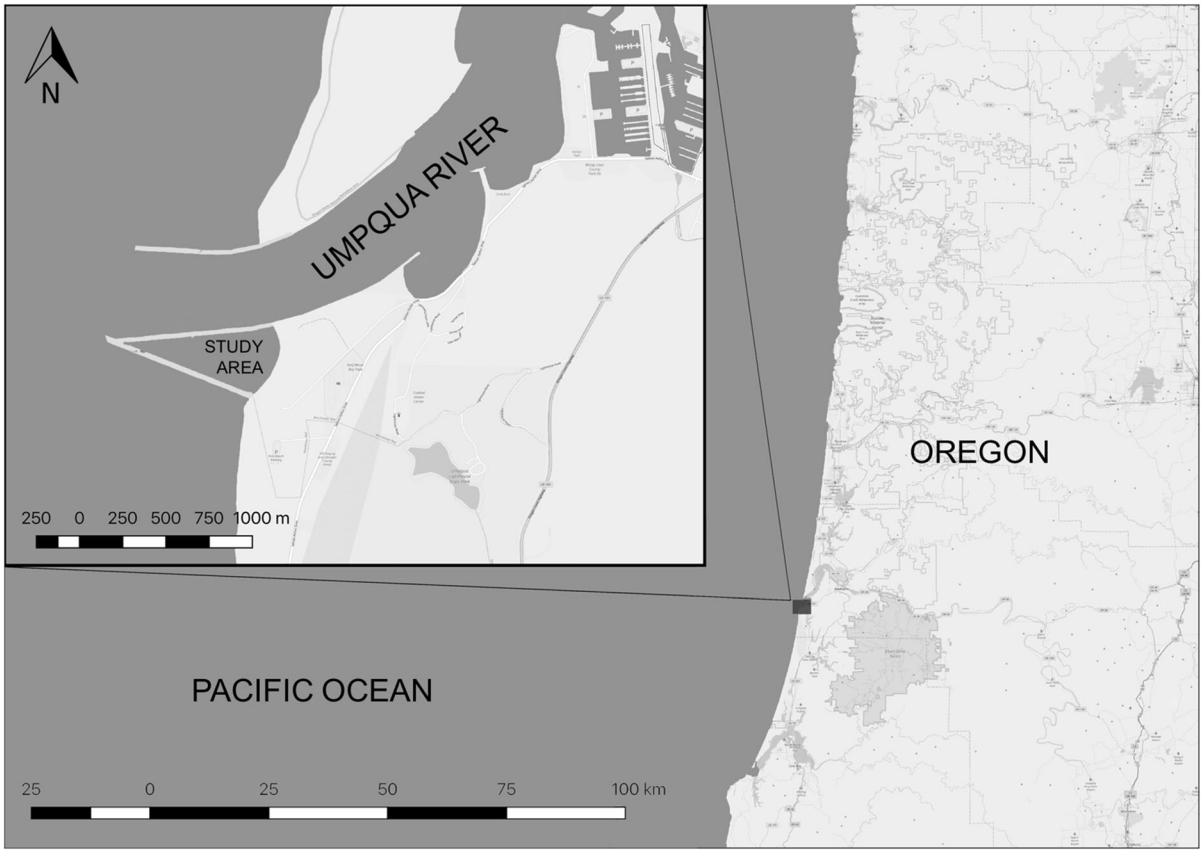

Fig. 1 Map of study area, Umpqua Aquaculture, which sits at the interface of the Pacific Ocean and the mouth of the Umpqua River in Winchester Bay, Oregon and is surrounded by two triangular jetty walls; dark grey represents inlayed map area

There is not a permanent continuous water quality monitoring asset within Umpqua Aquaculture or adjacently in Winchester Bay, which is situated at the mouth of an estuary and the outfall of the Umpqua River, a moderately large river that drains 12,103 km^2 of Southwestern Oregon (Wallick et al. 2011). However, HOBO® (Onset Computer Corporation, Massachusetts) probes collected temperature and salinity data at Umpqua Aquaculture from January 2012 to January 2013. One probe was located at the base of the jetty wall, and another hung at approximately $\sim$ 1 m depth using an oyster line. We pulled the probes after a year because a collaborator brought it to our attention that those particular HOBO® data loggers were not accurate in the wide and highly variable range of estuarine conditions.

In the absence of continuous environmental data collected within the study area over the entire study period, we turned to other nearby long-term continuous water quality stations as proxies: NOAA Buoy 139's (National Data Buoy Center 2017) sea surface temperature values for temperature, and USGS Station #14321000's Umpqua River output (U.S. Geological Survey 2018) for salinity. Because the triangular jetty walls surrounding Umpqua Aquaculture are expected to limit the complete exchange of water during every tidal cycle (Fig. 1), we accounted for longer-term water overturn by using the prior 15-day averages of sea surface temperature and river output as our proxy values. We used a 15-day lag because other studies that tested *D. vexillum*'s response to salinity lasted only two weeks (Bullard and Whitlatch 2009; Gröner et al. 2011).

To confirm previous reporting that our proxies were valid (Rumrill et al. 2014), we paired the 2012 daily average salinity values collected within the study area with the respective corresponding daily averages of our proxies. Indeed, salinity data were inversely

related to Umpqua River output during the same time frame ($r^2 = 0.27$, $p < 0.001$; Online Resource 1a). Further, the temperature fluctuations recorded during the HOBO® probe deployment period were comparable to the sea surface temperatures recorded further offshore ($r^2 = 0.38$, $p < 0.001$; Online Resource 1b; Rumrill et al. 2014).

We used RStudio (v. 1.1.414; RStudio Team 2018) for all statistical analyses. First, we performed nonparametric ANOVAs (Mann–Whitney *U* tests; base R) to compare the proportions of line covered (%), and pre-survey 15-day average Umpqua River discharge ($m^3\ s^{-1}$) and offshore sea surface temperature (°C) by season. We then visualized the proportion of line covered over time, as well as vertical colony distribution using the depth and cover distance data in point and whisker plots. We fit the variable to a linear model, proportion of line covered (%; $n = 12$), and colony was not normally distributed, nor was its residuals. Therefore, we used R package "TeachingDemos" to Box-Cox transform the data:

$$f(y) = (y^{\lambda} - 1)/\lambda \qquad \text{(Box and Cox 1964)}$$

where y is the dependent variable and λ is the transformation parameter that best normalizes the dependent variable's distribution. We regressed these normalized data against the salinity and temperature proxies, as well as season, and summarized these results in Table 2.

Results

In situ environmental data

Data collected by the HOBO® loggers from February to December 2012 indicated that surface (~ 1 m depth) temperatures ranged from 7.5 to 15.7 °C, with a mean observed temperature of 11.2 °C, while surface salinities ranged from 5.75 to 28.6 psu and averaged at 22.1 psu. Bottom temperatures and salinities ranged from 8.23 to 14.2 °C (mean 10.6 °C) and 6.67–29.4 psu (mean 22.4 psu), respectively (Table 1). The differences in surface and bottom salinities was seasonal: from February to mid-August, surface salinity was up to 15 psu less than bottom salinity, and from mid-August to December, surface salinity was up to 15 psu greater than bottom salinity (Online Resource 2a). Temperature was also strongly seasonal. In the late spring through early fall, surface temperatures were 1–3 °C warmer than bottom temperatures; and in the late winter, early spring, and late fall, surface temperatures were between 1 °C cooler and 1 °C warmer than bottom temperatures (Online Resource 2b).

Table 1 Minimum, maximum, and mean temperature (°C) and salinity (psu) values measured in situ from February 24 to December 12, 2012 at the surface and bottom of the study area

	Minimum	Maximum	Mean
Surface temperature (°C)	7.52	15.7	11.2
Bottom temperature (°C)	8.23	14.2	10.6
Surface salinity (psu)	5.75	28.6	22.2
Bottom salinity (psu)	6.67	29.4	22.4

Overall seasonal patterns

There were significant seasonal differences between the surveys for proportion of line covered (%; $p < 0.001$), as well as pre-survey 15-day average Umpqua River discharge ($m^3\ s^{-1}$; $p < 0.001$) and sea surface temperature (°C; $p < 0.001$; Table 2). The temperature proxy was higher in the fall surveys than the spring surveys, and spring river discharge, the salinity proxy, was lower in the fall than the (Online Resource 3). The May 2015 discharge was low relative to the other spring surveys, and the November 2016 discharge was high relative to the other fall surveys (Online Resource 3). Across the study, the pre-survey 15-day Umpqua River discharge was 30.0–199 $m^3\ s^{-1}$, or when transformed according to the in situ salinity versus discharge relationship, 21.8–23.3 psu (Online Resource 1a). The pre-survey 15-day mean temperature at NOAA Buoy 139 was 9.85–17.2 °C, or approximately 9.89–13.3 °C when transformed according to the in situ temperature versus buoy temperature relationship (Online Resource 1b).

Oyster lines were proportionally more covered with *D. vexillum* in the fall than the spring ($p < 0.001$; Table 1), with one notable exception: percent cover decreased, though not significantly, from May 2011 ($25.9 \pm 10.0\%$) to October 2011 ($16.0 \pm 4.2\%$; Fig. 2). In May 2015, the percent cover was lowest across all surveys ($8.9 \pm 2.1\%$), followed by

Table 2 Mann–Whitney *U* tests comparing fall and spring seasonal measurements

	U	*p*
Proportion of line covered (%)	4252	< 0.001*
Pre-survey 15-day average Umpqua River discharge (m^3s^{-1})	7579	< 0.001*
Pre-survey 15-day average sea surface temperature (°C)	1135	< 0.001*

*p values significant at the $\alpha < 0.05$ level

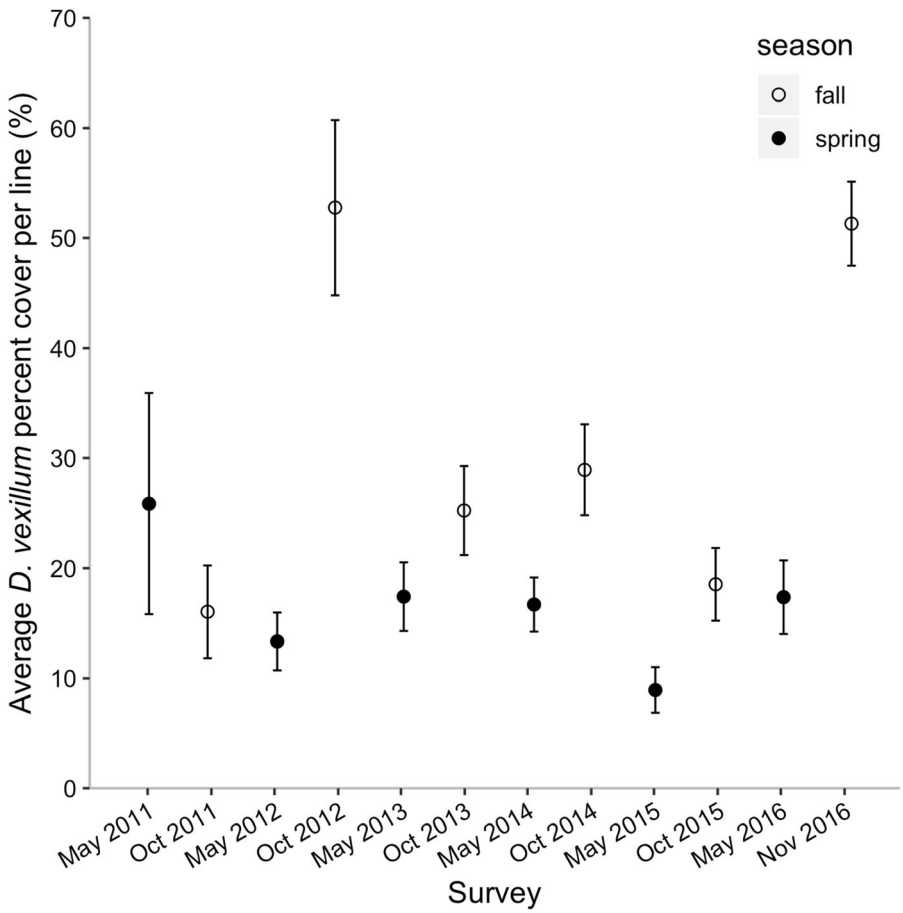

Fig. 2 Mean *D. vexillum* percent cover (%) per oyster longline for biannual surveys conducted at Umpqua Aquaculture from May 2011 to May 2016 (May 2011, $n = 11$; October 2011, $n = 14$; May 2012, $n = 14$; October 2012, $n = 12$; May 2013, $n = 20$; October 2013, $n = 17$; May 2014, $n = 22$; October 2014, $n = 17$; May 2015 = 21; October 2015, $n = 23$; May 2016, $n = 18$; November 2016, $n = 23$); space between points is proportional to time between surveys; error bars are ± 1 SE (propagated)

significantly greater percent cover measured in October 2012 (52.8 ± 8.0%). The May 2013, 2014, and 2015 surveys (17.4 ± 3.1, 16.7 ± 2.5%, and 8.9 ± 2.1%, respectively) both had lower proportional cover than their successive October 2013, 2014, and 2015 surveys (25.3 ± 4.1, 28.9 ± 4.1%, and 18.5 ± 6.0%, respectively); percent cover for the October 2015 and May 2016 surveys did not differ (18.5 ± 3.3 and 17.4 ± 3.3%, respectively). Percent cover did not differ for five of the six spring surveys (i.e., all but May 2015), though it did for the fall surveys (Fig. 2).

D. vexillum did not grow near the surface. The overall mean fall and spring cover of *D. vexillum* colonies at 0-2.5 m depth did not exceed 20%. There are two exceptions to this broad pattern: in the October 2012 and November 2016 surveys, cover exceeded 20% as shallow as 0.5-1.0 m and 2.0-2.5 m, respectively (Fig. 3). Similarly, mean colony cover generally did not exceed 20% at depths past 7.5 m (but see October 2012, where cover was 12.3 ± 12.3% at 7.5-8.0 m depth). Seasonally, on average, *D. vexillum* covered the greatest proportion of line between 5.5 and 6.0 m depth in both the spring (37.5 ± 18.8%) and fall (45.1 ± 16.1%). Across individual surveys, however, the colonies covered the greatest proportion of line between ∼ 4.5 and 6.5 m depth. The centers of the depth-based (i.e., vertical) spatial distributions are depicted in Fig. 3.

Linear regression models

The optimal normalization factor (λ) for the transformed response (i.e., percent cover) data was -0.28, and the skewness and kurtosis factors were 0.029 and 2.37. Umpqua River discharge did not predict variance in the dependent variable of percent *D. vexillum* cover per line (r^2 = 0.007, p = 0.789; Table 2). Sea surface temperature also did not predict the variance in cover per line or percent cover per line (r^2 = 0.002), nor were the slopes from these linear regression models significant (p = 0.886). A multiple linear regression combination of discharge, temperature, and season yielded a model that explained the variance of percent *D. vexillum* cover (r^2 = 0.599), but did not have a slope significantly different than zero (p = 0.052; Table 2). There were no significant serial correlations within any of these models.

Discussion

Our subtidal surveys of *Didemnum vexillum* on oyster longlines in Winchester Bay, OR, revealed a persistent population that exhibited erratic seasonal fluctuations in spatial cover. The average *D. vexillum* percent cover per line (%) from May 2011 to November 2016 was seasonally variable (Figs. 2, 3). with the percent cover per line is significantly greater in the fall than the spring (Table 1). Percent colony cover was greatest at

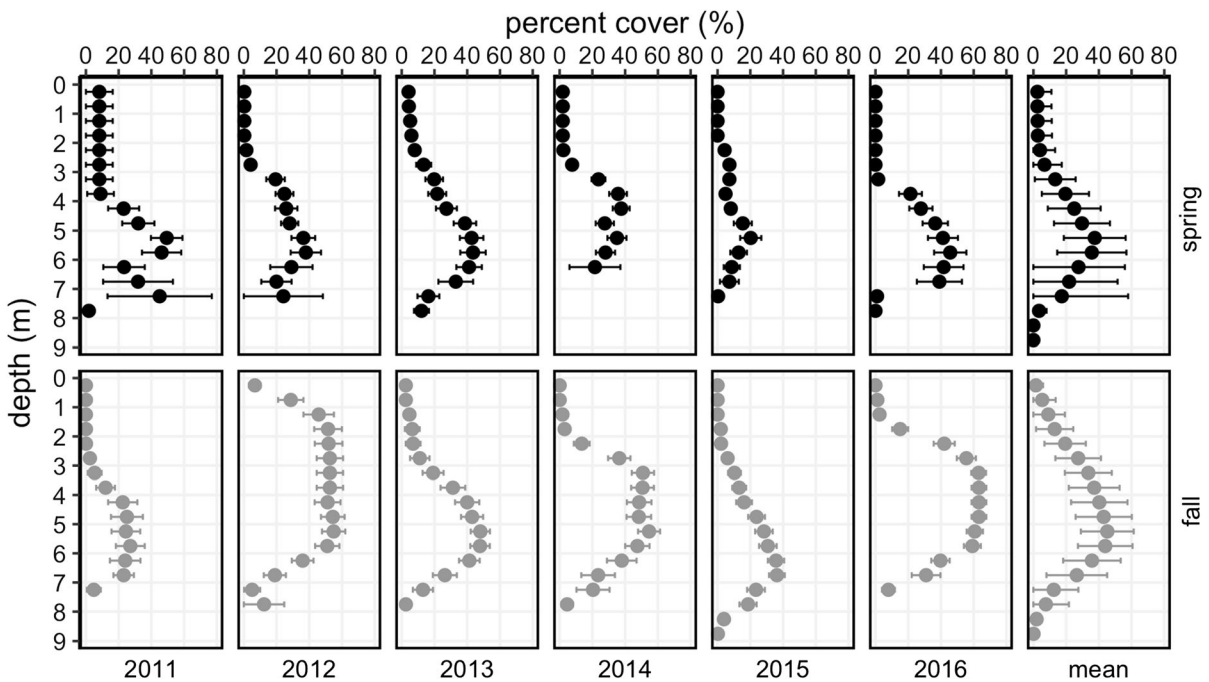

Fig. 3 Seasonal survey profile of percent *D. vexillum* colony cover distributed along subtidal oyster longlines. Points represent 0.5 m sections of line (e.g., a point at 6.25 is the mean percent cover at 6–6.5 m depth); error bars are ± 1 SE (spring and fall means propagated)

depths of ~ 4.5–6.5 m depth (Fig. 3). *D. vexillum*'s lack of growth toward the bottom likely reflects colony aversion to sedimentation that may occur as a result of wave action. The mean Umpqua River discharge ($m^3\ s^{-1}$) for the 15 days prior to each survey—the proxy for salinity trends within the Winchester Triangle in absentia of those data—was also significantly different between fall and spring. Discharge was significantly greater in the spring (meaning that salinity was lower), and temperature was lower in the spring than the fall. Based on this evidence, we found no support for our a priori null hypothesis that *D. vexillum* colony cover is the same across seasons.

Because we found no significant linear relationship between freshwater output (i.e., salinity; $r^2 = 0.007$; $p = 0.789$) and the transformed mean *D. vexillum* cover per line (m; Table 2), the available data do not lend evidence to support the hypothesis that salinity is one of the key environmental factors driving *D. vexillum*'s regression between fall and spring. This finding contrasts the well-established notion that short-term salinity changes are a primary control for *D. vexillum* and other colonial ascidians (Brunetti et al. 1980; Valentine et al. 2007; Bullard and Whitlatch 2009; Epelbaum et al. 2009a; Gröner et al. 2011), but this may be a function of the limitations of our proxy data. Further, sea surface temperature did not explain any variance in percent *D. vexillum* cover as measured (Table 2). Multiple linear regression models combining the Umpqua River discharge and season successfully explained the variances in mean *D. vexillum* cover and percent cover ($r^2 = 0.517$; $p = 0.038$); however, season is driving the significance of this model, as that variable alone described ~ 40% of the variance ($r^2 = 0.389$; $p = 0.028$) and also pulls the multiple linear regression combinations of sea surface temperature and season and river discharge, sea surface temperature, and season further toward statistical significance ($r^2 = 0.436$, $p = 0.076$ and $r^2 = 0.599$, $p = 0.052$, respectively) (Table 3).

It is probable that the available proxies for salinity and temperature in this analysis were tenuous because of how the rip-rap walls of the survey area (Fig. 1) likely limit water exchange from the river and the open ocean. Further, sea surface temperature data may not be a good metric for organisms that exhibit a clear depth preference. Vertical temperature profiles of the water column through time would be more appropriate—especially considering that the 2012 in situ temperature data revealed that the differences in surface and bottom temperatures was seasonal (Online Resource 2b). Since *D. vexillum* salinity-driven mortality has been shown to be duration-dependent, not intensity-dependent (Gröner et al. 2011), the time frame for which we evaluated freshwater discharge may have been too long or too short, and could in part explain the lack of a clear relationship. It is also possible that another environmental factor that we did not account for (e.g., food quality and availability, substrate availability, ocean hydrodynamics; Bates 2005) more strongly describes the observed trends in *D. vexillum* cover. *D. vexillum* can thrive on the pseudofeces of oysters (Mazouni et al. 2001), and that ability may be exacerbated in an enclosed area like Umpqua Aquaculture. Moreover, Grosberg (1988) demonstrated that food availability directly impacted egg production rates in another colonial tunicate, *Botryllus schlosseri*. The growth rate of *B. schlosseri* is lower in the laboratory than the field, which previous studies attribute to the frequency of food delivery and diversity of food types (Brunetti and Copello 1978; Chadwick-Furman and Weissman 1995). Food therefore may also explain *D. vexillum*'s population dynamics, especially considering the substantial impact coastal upwelling has on nutrient availability to Oregon's coastal habitats (Barth et al. 2007).

Despite the lack of a strong linear relationship between salinity and mean *D. vexillum* percent cover across the entire study, the survey profiles of *D. vexillum*'s mean proportional distribution along subtidal oyster longlines (Fig. 3) lend additional evidence of growth being salinity-driven. Tidal fluctuation and freshwater input induce a stratified water column in estuarine and near-coast habitats (Simpson et al. 1990), wherein a vertical gradient of fresher water forms at the water's surface. Indeed, *D. vexillum* observed during the October 2012 survey exhibited growth near the top of the water column (Fig. 3); because we have continuous in situ salinity data from 2012, we can infer that this growth may be related to the fact that in the preceding 2 months before the October 2012 survey, surface salinities were 0-10 psu higher than bottom salinities. Moreover, in examining the mean spring and fall depth distributions of *D. vexillum* (Fig. 3), colonies covered a greater proportion of shallower depths in the fall than they did the spring.

Table 3 Summary of simple and multiple linear regression statistics for Box-Cox transformed percent cover (%)

	F	*df*	r^2	*p*
River discharge[a]	0.075	1.10	0.007	0.789
Temperature[b]	0.022	1.10	0.002	0.886
Season	6.6	1.10	0.398	0.028*
River discharge + temperature	0.034	2.9	0.007	0.967
River discharge + season	4.81	2.9	0.517	0.038*
Temperature + season	3.48	2.9	0.436	0.076
River discharge + temperature + season	3.99	3.8	0.599	0.052

*p values significant at the $\alpha < 0.05$ level

[a]Prior 15-day average Umpqua River discharge (m^3 s^{-1})

[b]Prior 15-day average temperature (°C) recorded at NOAA Buoy 139

While the findings presented here do not capture the relationships of salinity- and temperature-driven fluctuations of growth in *D. vexillum* and other colonial tunicates (McCarthy et al. 2007; Valentine 2009; Fletcher et al. 2013a), it is known that *D. vexillum* exhibits measurable interregional and interpopulation variation in behavior and sensitivity to environmental conditions (Lambert 2009). Such variation is apparent across seasons within the present dataset. Indeed, it is likely that the population widely fluctuates within the months between the surveys—particularly between late June and October, when environmental factors are most favorable to *D. vexillum* reproduction and population growth. The methods performed in these surveys only capture a snapshot of the population through time. Only more frequent sampling would elucidate the nuances of this population's growth and recession; nevertheless, these 12 survey snapshots provide valuable insight to this *D. vexillum* population.

Despite finding that the *D. vexillum* population varied significantly throughout the duration of this study, perhaps our most interesting finding is that percent cover in November 2016 was nearly double the percent cover measured in May 201 l, and nearly triple of that observed in May 2016. This observation is critical because it shows that the *D. vexillum* population at Umpqua Aquaculture has the capacity to expand rapidly. However, a similar expansion of the population occurred between May and October of 2012, after which the population quickly declined. This erratic and episodic pattern suggests that the invasion of *D. vexillum* in Oregon may not be as severe relative to what has been reported and forecasted for other systems. To date, most literature on the invasion ecology of *D. vexillum* has emphasized the extensive threats the species poses to quickly taking over nearshore ecosystems and aquaculture operations (e.g., Bullard et al. 2007; Daley and Scavia 2008). Nevertheless, the fact that we found such erratic fluctuations in *D. vexillum* cover with no clear increasing trend during the study period at this site in Oregon does not mean it does not pose a future risk. The existing colonies maintain the potential to spread and grow, should a future environmental condition trigger an outbreak from this population's current foothold. As of July 2017, *D. vexillum* still exists at Umpqua Aquaculture (Knorek pers. obs.), though it had receded from the jetty rocks it reportedly colonized in earlier surveys (Rumrill et al. 2014).

The broad population decline between October 2012 and May 2016 in Winchester Bay parallels that of the recent decline in Oregon's only other known *D. vexillum* population in Charleston, 36 km south of the study area in Winchester Bay. Divers conducted *D. vexillum* presence/absence surveys of the Charleston Marina within the same general time frame of this study. Upon encountering *D. vexillum* in the Charleston Marina, divers removed the colonies and dropped them into the soft sediment substrate. They did not attempt such eradication methods in the Winchester Triangle, as doing so could have adversely impacted Umpqua Aquaculture's apparatus. During the most recent survey in January 2017, the divers found relatively few colonies (Hansen pers. obs.). During a 2019 Oregon Institute of Marine Biology-led BioBlitz,

no *D. vexillum* was found in the Charleston Marina, and the salinity there was also low. With caution, we can state that some confluence of environmental variables unfavorable to *D. vexillum* have at the very least, resulted in significant progress toward eradication, or at least ecologically significant slowing of Oregon's *D. vexillum* population in Charleston Harbor. However, given our finding that *D. vexillum* can expand rapidly in a matter of months (e.g., from May to October 2012 and 2016; Fig. 2), regular surveys of both Charleston Harbor and the Winchester Triangle should continue to ensure either population does not expand out of control or the current physical confines.

Curiously, populations of *D. vexillum* on the NE Pacific coast are now retained within protected harbors or other artificial structures. This observation contrasts with the east coast, where *D. vexillum* has carpeted 50–90% of a 230 km^2 area of the Georges Bank benthos (Valentine et al. 2007). There are numerous reasons for which this dichotomy may occur, including localized genetic adaptations to tolerate extreme temperature (Grosholz 2001) and salinity (Renborg et al. 2014), or large-scale oceanographic phenomena (e.g., coastal upwelling, which drives temperature and nutrient availability). Predation on *D. vexillum* may also play a role, but this interaction is still poorly studied (but see Epelbaum et al. 2009b; Dijkstra et al. 2013; Stefaniak 2017). Simkanin et al. (2013) showed that the predation of another invasive colonial tunicate, *Botrylloides violaceus*, by native predators limits its spread from marinas to nearby rocky reefs in British Columbia. Further, Forrest et al. (2013) provided compelling evidence for predatory biotic resistance to *D. vexillum*'s establishment in cobble habitats of New Zealand. However, another experiment showed its limited control via predation by small *Littorina* sp. snails (Carman 2009), which could be another indication that *D. vexillum* has no chemical anti-predator. As *D. vexillum* has exhibited high reproductive plasticity elsewhere (Ordóñez et al. 2015) and environmental conditions on the west coast allow for year-round survival and—potentially—reproduction, it is possible that more west coast *D. vexillum* populations will eventually establish reproductively viable populations in more natural habitats, as they have in and around Bodega Bay, California. Moreover, *D. vexillum*'s demonstrated ability to raft on eelgrass blades (Carman et al. 2014) and reproduce by fragmentation in suspension (Morris and Carman 2012), justifies prioritizing its regular monitoring in Oregon and along the west coast.

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