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Short Communication

Didemnum vexillum: invasion potential via harvesting and processing of the Pacific oyster (*Crassostrea gigas*) in British Columbia, Canada

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Abstract

Routine shellfish aquaculture practices in British Columbia (BC) result in cultured Pacific oysters (*Crassostrea gigas* (Thunberg, 1793)) being moved from tunicate infested culture areas to non-infested areas for processing, thereby posing a potential risk of spreading the colonial ascidian *Didemnum vexillum* Kott, 2002 and other nuisance species, to new areas. Three intervention points (IPs; i.e., stages of the processing regime) were identified in existing aquaculture practices where clusters of *C. gigas* received manipulation or stress that could alter the cover of epibionts, notably *D. vexillum*. These IPs were: IP1) harvesting, IP2) transportation (from harvesting areas to processing plants), and IP3) processing (shucking of the oysters). The percentage coverage of *D. vexillum* on oyster clusters was evaluated at each IP for products originating from two aquaculture sites in BC, Lemmens Inlet and Okeover Inlet. A total of 60 clusters were sampled from Lemmens Inlet and 46 from Okeover Inlet. Results demonstrated a significant loss ($P < 0.05$) of *D. vexillum* coverage on *C. gigas* clusters from IP1 to IP3 for both sites. Although variations existed between the sites, the mean percentage coverage decreased from 48% post-harvest to 30% post-transportation and 17% post-shucking. Since shucked oyster shells still have a substantial cover of *D. vexillum* on them and are disposed of in areas exposed to tidal waters of un-infested bays, the risk of secondary introduction related to shellfish aquaculture practices remains high. Based on these results, thresholds could be set to reduce the risk of introducing *D. vexillum* into new areas, and new research is recommended to determine the risk of dispersal of *D. vexillum* should it be inadvertently introduced via shellfish movements.

Key words: aquatic invasive species, vectors, Pacific oyster aquaculture, *Didemnum vexillum*, introduced ascidians

Introduction

Aquatic invasive species (AIS) are introduced to coastal regions of the world at a high frequency (Carlton 1989). Natural dispersal of AIS can occur via propagules in water currents or the movement of adults attached to floating objects (Carlton 1987). However, it is believed that anthropogenic dispersal is primarily responsible for the spread of many AIS, especially at a larger scale where natural dispersal is unlikely (Lambert 2005). Pacific oysters (*Crassostrea gigas* (Thunberg, 1793)) have been a mainstay of the shellfish aquaculture industry along the Pacific coast of North America (Verlaque 2001) with intentional introductions dating back nearly a century. However, this intentional introduction of oysters has had some unintentional consequences, most notably the introduction of over 100 non-indigenous invertebrate species to the coastal waters of the Northeast Pacific (Wonham and Carlton 2005).

In British Columbia (BC) specifically, there are several AIS of concern, including four tunicate species: the clubbed tunicate, *Styela clava* Herdman, 1881; the golden star tunicate, *Botryllus schlosseri* (Pallas, 1766); the violet tunicate, *Botrylloides violaceus* Oka, 1927 and the pancake batter tunicate or marine vomit, *Didemnum vexillum* Kott, 2002. *D. vexillum* has been identified on Pacific oyster farms on the Sunshine Coast (southern mainland coast of BC) and West Coast of Vancouver Island (Daniel and Therriault 2007; U.S. Geological Survey 2011; Cohen 2011). All oysters grown on Vancouver Island are processed on the East Coast of the island where *D. vexillum* has only been reported from a few locations (none near the processing plants), thus exposing this area to potential new incursions.

Over the past 20 years, there has been a steady increase in the global range of *D. vexillum* and to date it has been recorded in New Zealand, both coasts of North America, Europe, and the Western Mediterranean (Coutts and Forrest 2007; U.S. Geological Survey 2011; Ordóñez et al. 2015; Simkanin et al. 2016). Numerous factors may contribute to its successful spread and establishment. Anthropogenic disturbance, for example, creates a favourable habitat for AIS colonization (Cohen 2011; Valentine et al. 2007a). In areas where shellfish aquaculture has developed, *D. vexillum* can cover and overgrow bivalves to the point that their valve opening or siphons become occluded (Gittenberger 2007; Valentine et al. 2007a, 2007b; Cohen 2011; U.S. Geological Survey 2011) potentially leading to economic loss (Carman et al. 2010; Adams et al. 2011). This colonial ascidian also conjures high plasticity. It is able to adapt to a wide range of temperature in its lifecycle, contributing to success in its spread and establishment (Ordóñez et al. 2015).

The goal of this study was to evaluate the risk of incursion of *D. vexillum* from infested *C. gigas* culture sites to non-infested processing areas. The first objective was to assess the level of infestation from source to destination. The second objective was to evaluate the change in level of infestation at three critical intervention points in the processing pipeline, based on the Hazard Analysis Critical Control Points (HACCP) principles (dos Santos 2002; Canadian Food Inspection Agency 2012). This information would thereafter assist in providing advice on key management practices in aquaculture: (1) identify critical points where AIS can be controlled, (2) monitor critical control points, and (3) establish corrective actions to prevent AIS introductions (Gunderson and Kinnunen 2004). This approach, which is being used by the blue mussel industry on Prince Edward Island (PEI) in relation to the tunicate *Ciona intestinalis* (Linnaeus, 1767) (D. Bourque [Fisheries and Oceans Canada, Gulf Region, Moncton, NB] pers. comm.), was tested with the oyster industry on Vancouver Island. Intervention points (IPs), where fouling dislodgement is known to occur, were assessed as critical control points to evaluate the infestation level of *D. vexillum* on clusters of *C. gigas* from harvest to final processing. These IPs aid in the identification of areas where invasive tunicate fouling is reduced or eliminated from the aquaculture product, thereby reducing the potential to inadvertently introduce this invader to new locations.

Materials and methods

Oyster (*Crassostrea gigas*) culture sites fouled with *D. vexillum* and their corresponding processing plants were identified after site visits in January 2009. These visits lead to the identification of two inlets with a significant amount of fouling, Lemmens Inlet (near Tofino, BC (49.203405; −125.866204)) and Okeover Inlet (near Powell River, BC (49.978624; −124.688819)). Intervention points (IPs) for the reduction in coverage of *D. vexillum* were identified for the shucked oyster market: 1) harvesting, 2) transportation (from the harvesting area to the processing plant), and 3) processing (shucking of the oysters). Each IP was defined as a harvesting/processing practice where the aquaculture product received a manipulation or a stress that could alter the coverage of the tunicate on the clusters.

Assessing Didemnum vexillum coverage

At each IP, we estimated the percent cover of *D. vexillum* on oyster clusters. A total of 60 clusters were sampled from Lemmens Inlet and 46 from Okeover Inlet. Fewer observations were recorded from Okeover Inlet, so that sampling did not impede the fast paced harvesting process at this site. Since the clusters are not the same size, percent cover was used instead of weight. Although weights would have given a finite number for each cluster, the additional processing time on a moving boat would have impeded the harvesting method thereby rendering the assessment invalid. With the assistance of a handler, a single observer (recorder) estimated the percentage coverage of *D. vexillum* on each cluster used in the trial using 4 categories. The clusters were transferred from the oyster growers to the handler. Clusters with maximum cover were targeted, as a higher amount would give a better representation of the proportion of *D. vexillum* that would fall off. But, some clusters that were assessed had a lower cover due to the natural variability of tunicate fouling on shellfish aquaculture sites and the fast paced harvesting. The recorder collected the data and associated a tag number with each cluster. The handler placed the assessed cluster in an identified mesh bag, which was then handed back to the crew members who returned them with the other cluster in large nets. Each cluster was followed and assessed at the three Intervention Points.

Site variation within the IPs

We assessed site variation in % cover of *D. vexillum* at each of the three IPs. For each IP, % coverage analysis was done after each process. That is, % coverage analysis was conducted post-harvest, post-transportation, and post-shucking.

Harvesting (IP1)

The initial assessment of the clusters was conducted at the time of harvest. The site in Lemmens Inlet used a long line system with clusters grown on a double twine rope. At harvest, the lines were pulled onto the boat and cut between each cluster. The grower at this site manually removed excess epibionts, including *D. vexillum*, and dead shells to reduce the weight per shipment to the processing plant. These clusters were then dumped in mesh bags for shipment.

The site in Okeover Inlet also used a long line system, but clusters were grown on PVC pipes (French tubes). Harvesting at this site differed from the Lemmens' site. Here, ropes were cut and the pipes were loaded onto a tray. The clusters on the pipes were removed via a sloughing device. The device pushes a slightly larger diameter pipe over the end of the smaller diameter pipe used as a substrate to grow the oysters, which allows the clusters to be removed. A conveyor belt catches the oysters and dumps them into a mesh bag for shipment. No manual cleaning was done using this harvesting procedure.

Transportation (IP2)

The second assessment was conducted after transportation to the processing plant on the East Coast of Vancouver Island. The shipment from Lemmens Inlet was sent to the processing plants via land with a 5-hour transit time. The Lemmens Inlet clusters were shucked 2 days after their arrival at the processing plant. The shipment from Okeover Inlet was sent to the processing plants via boat with a 6-hour transit time. These clusters were also shucked 2 days after their arrival at the processing plant. The transit times represent the typical time needed for shipments between growers and processors.

Processing (IP3)

The third assessment was conducted after the shucking of the oysters. At the processing plant, mesh bags were emptied into an exterior chute bringing clusters into the shucking room. After being shucked, the shells and fouling were pushed aside in a wheelbarrow. Once filled, the wheelbarrows were wheeled out to the shell pile located in the intertidal zone outside the processing plants. No variation in the shucking and disposal method was noticed between the two sites.

Statistical analysis

Percent coverage data were organized into four categories: ≤ 25%, > 25% but ≤ 50%, > 50% but ≤ 75%, and > 75%. We initially attempted an ordinal logistic regression on the four categories described above (Dohoo et al. 2009) to test for effects of site and IP, but sparse data for the highest category (> 75%) led to computational issues. As such, we conducted a two-part analysis with different outcomes to test for effects of site and IP on percent coverage of *D. vexillum*: i) logistic regression for percent coverage outcomes of > 75% *vs.* ≤ 75%, and ii) ordinal logistic regression for percent coverage outcomes of ≤ 25%, > 25% but ≤ 50%, and > 50%. For the ordinal logistic regression, the assumption of proportional odds (PO) was violated. Therefore, the model was run without this assumption for all parameters, and PO was tested specifically for each site comparison at the IPs (effects are presented either separately or in combination, depending on whether the PO assumption was held or not). All statistical analyses were conducted using Stata v. 14 with a significance threshold of $\alpha < 0.05$; ordinal logistic regression was conducted using the gologit2 add-on command (Williams 2006).

Results

The percent coverage of *D. vexillum* on clusters of *C. gigas* decreased during the harvesting and processing procedure used at both sites (Figure 1). A decrease in *D. vexillum* coverage was observed between each step of the processing pipeline, with an average accumulative decrease in % coverage of approximately 15% between each IP (Figure 1). At IP1, percent cover of *D. vexillum* was 47.96% ± 3.46 SE, while percent cover only reached 32.56% ± 3.32 SE and 20.48% ± 2.56 SE at IPs 2 and 3, respectively.

Logistic regression (for percent coverage outcomes of > 75% *vs.* ≤ 75%) revealed a significant interaction between site and IP, with sites only differing significantly at IP 1 (Table 1). At IP 1, the odds that the percent coverage of *D. vexillum* on clusters of *C. gigas* was > 75% (*vs.* ≤ 75%) was almost 10 times (9.852) higher at Okeover Inlet than at Lemmens Inlet (Table 1). In contrast, the odds that the percent coverage of *D. vexillum* on clusters of *C. gigas* was > 75% (*vs.* ≤ 75%) was ~3.5 times higher (3.537) at

Table 1. Estimated effects of logistic regression analysis with robust standard error method adjusting for tag cluster effects for high percent coverage (> 75%) versus low percent coverage (≤ 75%).

Variable	Coefficient estimate	Robust SE	*P*-value	Odds ratio	95% CI for odds ratio
Site					
Okeover	Reference		<0.001#		
Lemmens	2.288	0.601		9.852	3.035, 31.98
IP					
1	Reference		<0.001#		
2	-0.728	0.520		0.483	0.174, 1.338
3	-0.728	0.520		0.483	0.174, 1.338
Site x IP					
Site 2 x IP 1	Reference		0.019#		
Site 2 x IP 2	-1.025	0.684			
Site 2 x IP 3	-16.53	n/a			
Site comparisons					
IP 1	2.288	0.601	<0.001	9.853	3.035, 31.98
IP 2	1.263	0.865	0.144	3.537	0.649, 19.28
IP 3	-14.24	n/a	0.129	0.000	n/a

Overall *P*-value for group effects

Table 2. Estimated effects of ordinal logistic regression analysis for the ordered dependent variable categories fall in the percent coverage: ≤ 25%, > 25% but ≤ 50%, and > 50%.

Variable	Coefficient estimate	SE	*P*-value	Odds ratio	95% CI for odds ratio
Site	n/s	n/s	<0.001	n/s	n/s
IP	n/s	n/s	<0.001	n/s	n/s
Site x IP	n/s	n/s	0.034	n/a	n/a
Site comparisons					
IP 1	1.309	0.408	0.001	3.703	1.666, 8.234
IP 2 (threshold 0#)	-0.124	0.197	0.527	0.883	0.600, 1.300
IP 2 (threshold 1##)	0.472	0.221	0.033	1.604	1.040, 2.473
IP 3	-0.485	0.467	0.299	0.616	0.247, 1.537

n/s = not shown
= Odds comparison of % coverage > 25% *vs* ≤ 25%
= Odds comparison of % coverage > 50% *vs* ≤ 50%

Okeover Inlet at IP 2, and 0 times higher (0.000) at IP 3 (Table 1).

Similarly, ordinal logistic regression (for percent coverage outcomes of ≤ 25%, > 25% but ≤ 50%, and > 50%) revealed a significant interaction between site and IP (Table 2). At IPs 1 and 3, the assumption of proportional odds was not violated, so comparisons were the same at both thresholds (i.e., > 25% [Threshold 0] and > 50% [Threshold 1]); PO was violated at IP 2 and the comparison between the two sits was dependent on the threshold. At IP 1, the estimated odds of being in the higher percent coverage category at Okeover Inlet was significantly greater (3.703 times) than the odds of being in the higher percent coverage category at Lemmens Inlet (Table 2). Conversely, at IP 3 the estimated odds of being in the higher percent coverage category at Okeover Inlet was not significantly greater (0.616 times) than the odds of being in the higher percent coverage category at Lemmens Inlet (Table 2). At IP 2, the estimated odds of percent coverage at Okeover Inlet being > 50% (as opposed to ≤ 50%) was significantly higher than the odds of percent coverage at Lemmens Inlet being

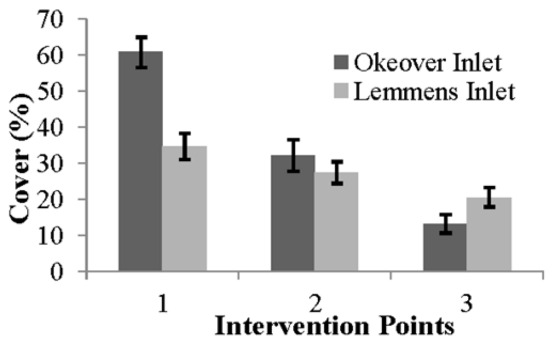

Figure 1. Cover of *D. vexillum* on oyster clusters throughout the various processes, (1) post-harvest, (2) post-transportation and (3) post-shucking. The standard errors are displayed.

> 50% (Table 2; Threshold 1). In contrast, the estimated odds of percent coverage at Okeover Inlet being > 25% (as opposed to ≤ 25%) was not significantly higher than the odds of percent coverage at Lemmens Inlet being > 25% (Table 2; Threshold 0).

Discussion

IP Variations

This observational study found a significant decrease in *D. vexillum* coverage on *C. gigas* clusters from post-harvest to post-shucking for two shellfish aquaculture sites in BC. Although variations existed between the sites, mean tunicate cover decreased from 48% post-harvest to 30% post-transportation and 17% post-shucking. Possible causes for this decrease included physical removal during each stage of the process or fall-off due to mortality. Tunicate mortality could be caused by exposure to air or sunlight during transportation to processing plants. Katayama and Ikeda (1987) noticed mortality in *Didemnum moseleyi* (Herdman, 1886) (later confirmed as *D. vexillum* by Lambert 2009) after 5 hours or more of air drying in direct sunlight, although the magnitude of the effect was dependent on ambient temperature.

It is possible that the air flow that resulted from traveling decreased the temperature and removed humidity from the oyster clusters during overland transport to the processing plant. However, clusters that were transported in nets were covered by a tarpaulin. This canvas blocks direct sunlight, but keeps humidity and temperature high in the core of the shipment. The effect from the wind would reduce the perimeter temperature and humidity as a function of the length of travel, thus possibly having a mitigating effect on *D. vexillum* by desiccation. However, tunicates located in the centre of the cluster mass would be expected to encounter conditions more amenable to survival. It remains unknown what specific transit-mediated conditions would be required to ensure complete mortality in the protected and more protected interior layers of oyster shipments.

Site variation within the IPs

Post-harvest cover of *D. vexillum* before transportation (IP1) was significantly greater in Okeover Inlet (60.8%) compared to Lemmens Inlet (34.5%). The difference between these two sites was likely due to the removal of epibionts by the grower in Lemmens Inlet. The significant difference in post-harvest cover between sites was followed by smaller differences at later stages (4.74% for post-transportation and 7.46% for post-shucking). This demonstrated an abrupt decrease of *D. vexillum* cover on the clusters from Okeover Inlet relative to Lemmens Inlet but confirmed the potential for *D. vexillum* to be moved from culture sites in both cases.

Differences in both transit time and transportation may account for the observed difference in cover from post-harvest to post-transportation between the two sites. However, due to the same nets and method of covering the clusters, the additional hour of transport and water-borne transportation mode was not likely the determining factor in this reduction of coverage in Okeover Inlet. Because we were tracking *D. vexillum*, this highlighted that there is a loss of tunicates between the two geographical locations resulting in possible spread. For this sampling, the loss of tunicate was kept in the mesh bags.

The IPs identified and monitored in this study show potential points where the introduction of *D. vexillum* could occur in the harvesting procedure of *C. gigas*. *D. vexillum* has not been reported in the waters surrounding the processing plants, even though harvesting and processing have been occurring for a number of years. It is unclear why this is the case. Perhaps *D. vexillum* does not survive the handling procedures during processing or experiences high mortality during overland transport. If *D. vexillum* colonies are dead before they leave the processing plants, the species will not become established in the surrounding areas. Since establishment can occur even from small fragments of living colonies (Bullard et al. 2007; Daniel and Therriault 2007; Valentine et al. 2007a; Morris Jr. and Carman 2012; Carman et al. 2014), all *D. vexillum* colonies would have to be dead before they are released into the environment. Because it is unclear if portions of colonies remain alive, and because substantial amounts of *D. vexillum* are present on shucked shells deposited in the intertidal zone (13% and 20% cover), this represents a potential invasion risk. More work is needed to fully evaluate the risk associated with these processing procedures to ensure proper establishment of corrective actions to prevent the introduction of this colonial tunicate.

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References

Adams CM, Shumway SE, Whitlatch RB, Getchis T (2011) Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. *Journal of the World Aquaculture Society* 42: 242–252, https://doi.org/10.1111/j.1749-7345.2011.00460.x

Bullard SG, Sedlack B, Reinhardt JF, Litty C, Gareau K, Whitlatch RB (2007) Fragmentation of colonial ascidians: differences in reattachment capability among species. *Journal of Experimental Marine Biology and Ecology* 342: 166–168, https://doi.org/10.1016/j.jembe.2006.10.034

Canadian Food Inspection Agency (2012) Hazard Analysis Critical Control Point (HACCP). http://www.inspection.gc.ca/about-the-cfia/newsroom/food-safety-system/haccp/eng/1346306502207/1346306685922 (accessed 01 June 2017)

Carlton JT (1987) Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science* 41: 452–465

Carlton JT (1989) Man's role in changing the face of the ocean: biological invasions and implications for conservation of near-shore environments. *Conservation Biology* 3: 265–273, https://doi.org/10.1111/j.1523-1739.1989.tb00086.x

Carman MR, Morris JA, Karney RC, Grunden DW (2010) An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. *Journal of Applied Ichthyology* 26: 8–11, https://doi.org/10.1111/j.1439-0426.2010.01495.x

Carman MR, Grunden DW, Ewart D (2014) Coldwater reattachment of colonial tunicate *Didemnum vexillum* fragment to natural (eelgrass) and artificial (plastic) substrates in New England. *Aquatic Invasions* 9: 105–110, https://doi.org/10.3391/ai.2014.9.1.09

Cohen AN (2011) The Exotics Guide: Non-native Marine Species of the North American Pacific Coast. Center for Research on Aquatic Bioinvasions, Richmond, CA, and San Francisco Estuary Institute, Oakland, CA. Revised September 2011. http://www.exoticsguide.org (accessed 22 February 2012)

Coutts ADM, Forrest BM (2007) Development and application of tools for incursion: Lessons learned from the management of pest *Didemnum vexillum*. *Journal of Experimental Marine Biology and Ecology* 342: 154–162, https://doi.org/10.1016/j.jembe.2006.10.042

Daniel KS, Therriault TW (2007) Biological Synopsis of the Invasive Tunicate *Didemnum* sp. Canadian Manuscript Report of Fisheries and Aquatic Sciences 2788, 53 pp

Dohoo I, Martin W, Stryhn H (2009) Veterinary Epidemiologic Research, Second edition. VER Inc., Prince Edward Island, Canada, 865 pp

dos Santos CAML (2002) HACCP and aquaculture. In: Jahancke M, Spencer-Garnett Em Reilla A, Martin R (eds), Public, animal and environmental health issues in aquaculture, 1st edition. John Wiley and Sons Inc., New York, USA, pp 103–120

Gittenberger A (2007) Recent population expansions of non-native ascidians in the Netherlands. *Journal of Experimental Marine Biology and Ecology* 342: 122–126, https://doi.org/10.1016/j.jembe.2006.10.022

Gunderson JL, Kinnunen RE (2004) Aquatic invasive species − Hazard analysis and critical control point training curriculum. Second Edition. Minnesota Sea Grant Publications Number: MNSG-F11. Michigan Sea Grant Publications Number: MSG-00-400, 83 pp

Katayama K, Ikeda Z (1987) Tolerance of fresh water, hot water, and sun-drying by *Didemnum moseleyi*, fouling organisms attached to culture oyster. *Bulletin of the Fisheries Experiment Station, Okayama Prefecture* 2: 104–106

Lambert G (2005) Ecology and natural history of the protochordates. *Canadian Journal of Zoology* 83: 34–50, https://doi.org/10.1139/z04-156

Lambert G (2009) Adventures of a sea squirt sleuth: unravelling the identity of *Didemnum vexillum*, a global ascidian invader. *Aquatic Invasions* 4: 5–28, https://doi.org/10.3391/ai.2009.4.1.2

Morris Jr JA, Carman MR (2012) Fragment reattachment, reproductive status, and health indicators of the invasive colonial tunicate *Didemnum vexillum* with implications for dispersal. *Biological Invasions* 14: 2133–2140, https://doi.org/10.1007/s10530-012-0219-8

Ordóñez V, Pascual M, Fernández-Tejedor M, Pineda MC, Tagliapietra D, Turon X (2015) Ongoing expansion of the worldwide invader *Didemnum vexillum* (Ascidiacea) in the Mediterranean Sea: high plasticity of its biological cycle promotes establishment in warm waters. *Biological Invasions* 17: 2075–2085, https://doi.org/10.1007/s10530-015-0861-z

Simkanin C, Fofonoff PW, Larson K, Lambert G, Dijkstra JA, Ruiz GM (2016) Spatial and temporal dynamics of ascidian invasions in the continental United States and Alaska. *Marine Biology* 163: 163, https://doi.org/10.1007/s00227-016-2924-9

U.S. Geological Survey (2011) Marine Nuisance Species. http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/index.htm (accessed 22 February 2012)

Valentine PC, Collie JS, Reid RN, Asch RG, Guida VG, Blackwood DS (2007a) The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat — ecological observations and potential effects on groundfish and scallop fisheries. *Journal of Experimental Marine Biology and Ecology* 342: 179–181, https://doi.org/10.1016/j.jembe.2006.10.038

Valentine PC, Carman MR, Blackwood DS, Heffron E J (2007b) Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. *Journal of Experimental Marine Biology and Ecology* 342: 109–121, https://doi.org/10.1016/j.jembe.2006.10.021

Verlaque M (2001) Checklist of the macroalgae of Thau Lagoon (Hérault, Fance), a hot-spot of marine species introduction in Europe. *Oceanologica Acta* 24: 29–49, https://doi.org/10.1016/S0399-1784(00)01127-0

Williams R (2006) Generalized ordered logit/partial proportional odds models for ordinal dependent variables. *The Stata Journal* 6: 58–82

Wonham M, Carlton J (2005) Trends in marine biological invasions at local and regional scales: the northeast Pacific Ocean as a model system. *Biological Invasions* 7: 369–392, https://doi.org/10.1007/s10530-004-2581-7