

## Research Article

## Assessing invasion risk of *Didemnum vexillum* to Atlantic Canada

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### Abstract

Aquatic invasive species are an ongoing economic and ecological problem in Atlantic Canada. To optimize management efforts of high-risk species, we must quantify risk of invasion at scales relevant to management efforts. Here we provide an updated and improved detailed-level risk assessment (DLRA) for *Didemnum vexillum* that uses new methods and tools to quantify and discriminate risk of invasion to the region. The screening level risk assessment framework CMIST (Canadian Marine Invasive Screening Tool) was used in a novel context to calculate uncertainty-adjusted invasion risk scores for 13 assessment zones in Atlantic Canada. Assessments were informed by 1) environmental niche modelling (MaxENT) to predict areas suitable for establishment; 2) source-based vector analysis to quantify potential for arrival and spread of *D. vexillum* via high-risk vectors (i.e., commercial vessels, ferries, fishing vessels, and aquaculture transfers); and 3) updated ecological data from the literature. Overall invasion risk, likelihood of invasion, and impact of invasion were highest in Bay of Fundy assessment zones and lowest in the most northern zones (St. Lawrence estuary, northern Gulf and the east coast of Newfoundland). Connectivity with source zones of *D. vexillum* via both natural (e.g., currents) and anthropogenic vectors (e.g., vessels) is highest in the Bay of Fundy due to proximity to established populations and high levels of vessel traffic. Potential for impacts is highest where vulnerable populations (e.g., scallops) and highly or moderately suitable areas for establishment exist. These areas are in the Minas Basin, Chignecto Bay, southwest New Brunswick, and southwest Nova Scotia with smaller areas in Mahone Bay and offshore on Western Bank and Sable Island Bank. Projections of environmental suitability for 2075 show a northeastward shift, with areas of high suitability retained in the Bay of Fundy and expanding into the Northumberland Strait. To reduce further local spread in the Bay of Fundy, bottom-disturbing activities, such as dredging and trawling where *D. vexillum* is present, should be addressed. In addition, movement of vessels between source areas and areas of high environmental suitability should be monitored, especially in anomalously warm years when populations are likely to be larger. Targeted monitoring of areas of current and future environmental suitability with high connectivity to source zones of *D. vexillum* should also be considered to improve early detection of new populations.

**Key words:** risk assessment, aquatic invasive species, environmental niche modelling, vector analysis, CMIST, colonial tunicate

### Introduction

In Atlantic Canada, aquatic invasive species (AIS) continue to be detected in new locations in the marine environment (McKenzie et al. 2016; Moore et al. 2014; Savoie and Saunders 2013; Simard et al. 2013) and established AIS continue to spread (Sephton et al. 2011; Lowen and DiBacco 2017). In the northeast US and Canada, AIS pose ecological and economic threats, having impacted native species and communities (e.g., Valentine et al. 2007a), altered fouling communities (Blum et al. 2007; Sephton et al. 2011),

and increased costs to the aquaculture industry (Carver et al. 2006; Daigle and Herbingier 2009). Effective prevention and mitigation of invasions requires understanding of the spatial and temporal scales of potential impacts to focus limited management resources in high risk areas. Risk assessments can inform such management efforts by providing an evaluation of invasion risk for the area of concern and have been performed worldwide using varying protocols for many years (Hewitt et al. 2004; Copp et al. 2005; Verbrugge et al. 2012). In Canada, detailed-level risk assessment (DLRA) is the most

comprehensive approach and is described in national DLRA guidelines (Mandrak et al. 2012) and referred to in Canada's rapid response framework for AIS (Locke et al. 2010).

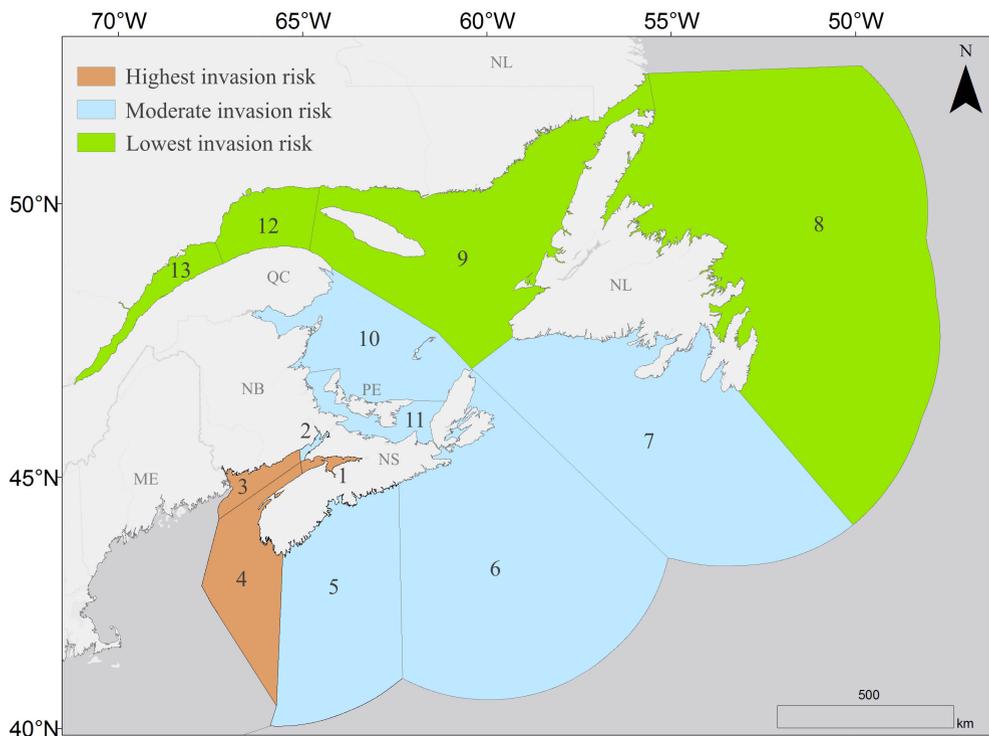
Previously, lack or low resolution of key data for evaluating AIS in Atlantic Canada created a reliance on expert opinion and limited DLRA at the coastal scale (e.g., Therriault and Herborg 2007; Therriault et al. 2008). In 2007, DLRA were performed for the Atlantic and Pacific coasts for five high-risk invasive tunicate species established in Canadian waters: *Ciona intestinalis* (Linnaeus, 1767), *Styela clava* (Herdman, 1881), *Botryllus schlosseri* (Pallas, 1766), *Botrylloides violaceus* (Oka, 1927), and *Didemnum* sp. (Therriault and Herborg 2007). These assessments established invasive tunicates as high-risk species and highlighted general areas and vectors of concern. However, risk could not be partitioned at a scale fine enough (i.e., ports, marinas, aquaculture sites, key habitats) for effective management. Now, improved data availability, quality, and analyses provide an opportunity to revisit previous AIS risk assessments with a new approach that increases objectivity and lends itself to discriminating risk within the region to improve AIS management strategies. AIS reassessments may be considered as part of a recommended review time-frame or on an ad hoc basis when significant new information becomes available that generates concern and requires advice (Mandrak et al. 2012). However, as DLRA are time-consuming, full reassessments may only be initiated when significant improvements can be made. For example, Mandrak et al. (2012) suggest reassessment when incorporation of new data could reduce uncertainty or alter risk scores.

Here we revisit the DLRA for *Didemnum* sp. (now confirmed as *Didemnum vexillum* Kott, 2002) on the Atlantic coast of Canada, which scored high for overall ecological risk with low uncertainty in the original assessment 10 years ago (Therriault and Herborg 2007). *D. vexillum* is a risk to Atlantic Canada based on both climate match and availability of a shipping vector (Locke 2009). Its presence and impacts on both natural (e.g., scallop beds) and artificial habitats (e.g., aquaculture leases) have been of concern for the region since at least 2006. The instigation for reassessment came from its identification in Atlantic Canada for the first time in 2013 (Moore et al. 2014) and concerns for its spread to areas of dense aquaculture in the Gulf of St. Lawrence and Placentia Bay, NL. However, to understand invasion risk at that scale, a new DLRA would have to include recent data on its ecology and improve in three areas: environmental niche modelling (ENM) to predict areas suitable for establishment, vector analysis to quantify potential for arrival and spread via high-risk

vectors, and risk assessment method to better partition risk and uncertainty within the region.

ENMs are increasingly being used to understand and predict distributions of marine species (Robinson et al. 2011; Tyberghein et al. 2012). ENM uses species occurrence data and relevant environmental and geographic variables to predict potential species distributions under assumptions that 1) the species' distribution is strongly determined by climate and 2) the species is in equilibrium with the climate (Araujo and Peterson 2012). In the initial DLRA, GARP (Genetic Algorithm for Rule Set Production) was used to generate an ENM for the Atlantic Coast using distribution data from *D. vexillum* on the Pacific Coast and local temperature and salinity tolerance data (Therriault and Herborg 2007). The authors suggested that higher resolution environmental data would greatly improve future analyses and that the GARP modelling approach may also need to be revisited given expected development in the field. Recently, MaxENT (Maximum Entropy Modelling) has been used to develop new ENMs for *D. vexillum* that project both current and future environmental suitability for establishment (Lowen and DiBacco 2017). These models use more proximate distribution data for *D. vexillum* (i.e., from its distribution in the northwest Atlantic) and higher resolution seasonal temperature and salinity layers (Lowen et al. 2016) and agree very well with observed species distributions (Lowen and DiBacco 2017). MaxENT ENMs present improvements over previous models such as GARP, which is prone to overprediction (Elith and Leathwick 2006), with newer models more effectively able to delineate potential areas for establishment based on a suite of internal and external validation metrics (see Lowen et al. 2016).

Quantifying movement of AIS vectors along high-risk pathways from source areas to areas of concern is critical for effective AIS management. Ports and marinas are known hubs for introduction and spread of AIS (Carlton 1996; Minchin and Sides 2006) and tunicates in particular (Lacoursière-Roussel et al. 2012; Darling et al. 2012). Pathway risk assessments (e.g., Drake and Lodge 2004; Simard et al. 2017) explore connectivity between these endpoints in source and assessment areas; however, existing analyses in DLRA in Canada have not due to lack of access to high resolution vessel traffic data. For *D. vexillum*, prior vector analyses have used overall (i.e., non-directional and non-source-related) density of high-risk vectors (i.e., vessels) or endpoints (i.e., aquaculture leases, marinas, ports) as either unweighted (Therriault and Herborg 2007) or weighted proxies (Herborg et al. 2009). Both pathway assessments and vector density maps are generally



**Figure 1.** Invasion risk for assessment zones 1 to 13 used in the CMIST detailed-level risk assessment for *Didemnum vexillum* in Atlantic Canada. ME = Maine; NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; PE = Prince Edward Island; QC = Quebec.

useful for identifying high-frequency routes; however, only vectors and endpoints with *D. vexillum* represent real risk of introduction and spread.

Risk scores and associated uncertainty are the main products from risk assessments used to make decisions on AIS management for a given assessment area. Scoring in the previous DLRA for *D. vexillum* was qualitative (Therriault and Herborg 2007) as recommended in Mandrak et al. (2012), which relies heavily on expert opinion. Newer risk assessment tools for marine invertebrates, such as the Marine Invertebrate Invasive Scoring Kit (MI-ISK) (Copp et al. 2009) and the Canadian Marine Invasive Screening Tool (CMIST) (Drolet et al. 2016), use semi-quantitative scoring, which can be more objective and facilitate comparisons and prioritization of AIS. Drolet et al. (2016) further simplify scoring by integrating uncertainty into final risk scores using Monte Carlo simulation. Although *D. vexillum* is not being compared to other species in this DLRA, semi-quantitative scoring with integrated uncertainty could be used to compare risk within Atlantic Canada. By considering the assessment at a sub-Atlantic scale, invasion risk may be effectively partitioned to identify low and high risk areas,

including areas of specific concern. A similar approach has been taken for the recreational boating vector in Atlantic Canada (Simard et al. 2017).

The objectives of this study were (1) to conduct a DLRA for *D. vexillum* in Atlantic Canada at a finer scale than the previous assessment to provide updated recommendations for management and mitigation and (2) to recommend an improved approach for assessments of other AIS. Overall, we better discriminate invasion risk within Atlantic Canada using improved ENMs, vector analysis, and scoring method to produce an easier to interpret and useful product for managers.

## Methods

### Assessment zones

Canada's Atlantic Coast was sub-divided into 13 assessment zones for this DLRA, encompassing both nearshore and offshore areas (AZ1–AZ13) (Figure 1). As no zonation was used in the previous DLRA, assessment zones were defined in consideration of environmental cohesiveness (e.g., see Figure 2 in DiBacco et al. 2016) and natural biogeographical subdivisions, marine ecoregions (DFO 2009), and

applicability for management. For example, given the higher density of *D. vexillum* in the Bay of Fundy (Figure 2) and its overall prevalence in scallop habitat (Valentine et al. 2007a; Morris et al. 2009), assessment zones in the Bay of Fundy were based on boundaries of Scallop Production Areas (DFO 2016a).

#### *Risk assessment tool*

The Canadian Marine Invasive Scoring Tool (CMIST) (Drolet et al. 2016; DFO 2015) was used to assess invasion risk by *D. vexillum* in Atlantic Canada. CMIST has been tested, peer-reviewed, and recommended for use with marine invertebrates, including tunicates (DFO 2015). CMIST assessments involve 8 questions for scoring *likelihood of invasion* (i.e., present status, rate of introduction, survival, establishment, and spread) and 9 questions for scoring *impact of invasion* (i.e., on population growth, communities, habitat, ecosystem function, at-risk or depleted species, fished species, genetics) to be completed by an informed assessor using a combination of expert opinion and best-available data. Risk scores for each question range from 1 (low) to 3 (high) and are accompanied by uncertainty scores that range from 1 (low certainty) to 3 (high certainty). *Likelihood of invasion* and *impact of invasion* scores are multiplied together into an overall risk score (CMIST score) that incorporates uncertainty using a Monte Carlo simulation (Drolet et al. 2016). Scores in this study were determined by consensus by the three authors using information from a literature review as well as the environmental niche model and vector analysis presented below. CMIST assessments were performed for each assessment zone. Results were compared both among assessment zones and with the CMIST SLRA score for *D. vexillum* on the Scotian Shelf (DFO 2015) to determine relative invasion risk within the region and assess the utility of the tool for DLRAs.

#### *Environmental niche modelling*

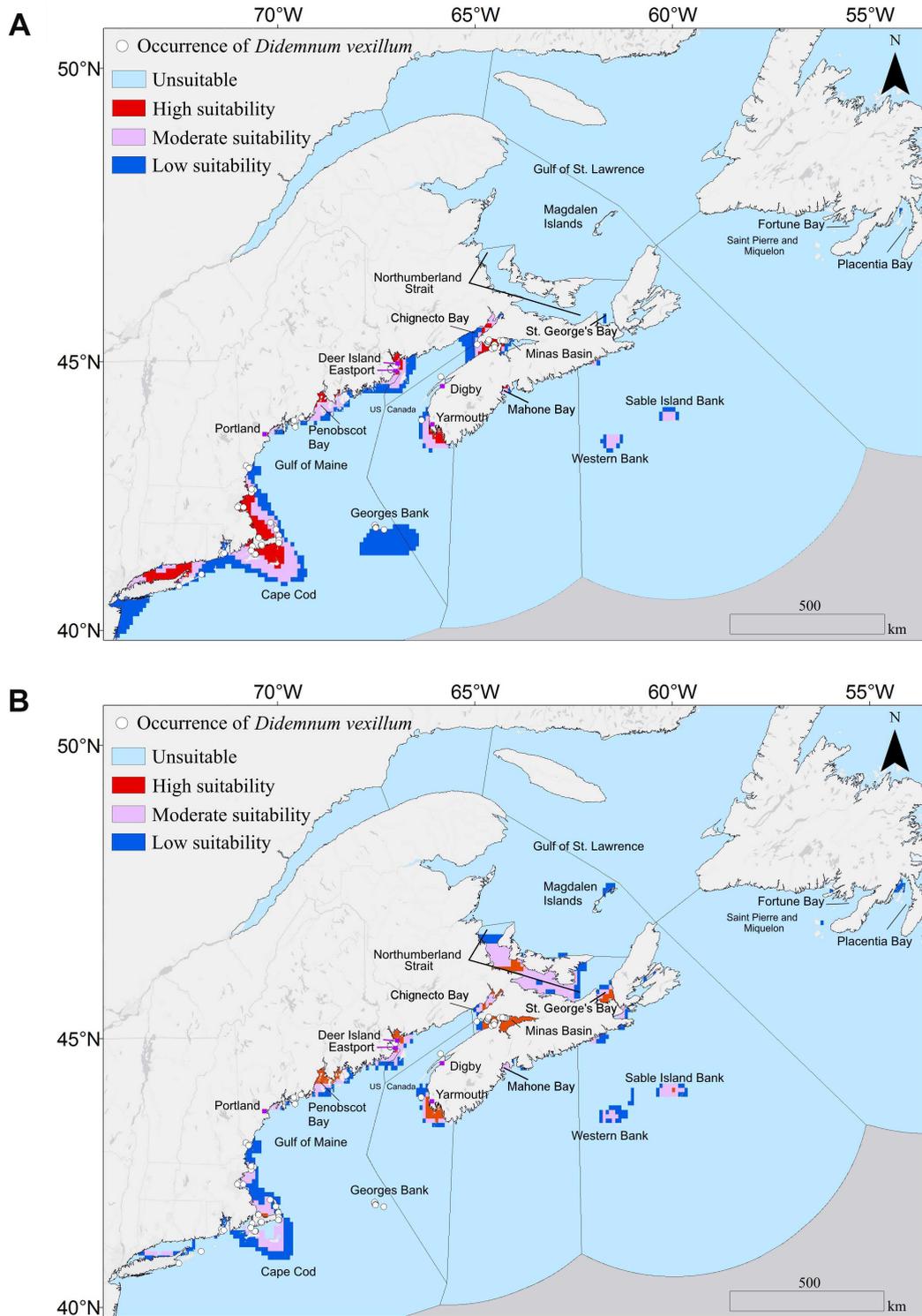
Environmental suitability was predicted using MaxENT (Phillips et al. 2004). Models were calibrated with occurrence data from the northeast US (USGS 2004–2014) and Atlantic Canada (Moore et al. 2014; Vercaemer et al. 2015; Sephton et al. 2017), encompassing the species range in the northwest Atlantic until 2015 from approximately 40.84 to 45.35 °N. High resolution surface and bottom temperature and salinity data were converted to four seasonal (Jan–Mar; Apr–Jun; Jul–Sep; Oct–Dec) climatological data layers (2002–2012) and used as model predictors. Sea surface temperature (SST) was

assembled from Level 3 SST climatological satellite data compiled by DFO; sea surface salinity (SSS) was assembled from global oceanographic climatological SSS composites (Tyberghein et al. 2012). Benthic temperature and salinity climatological data were assembled from a numerical climatological model (GLORYS2V1) adapted to the northwest Atlantic by DFO. Future environmental suitability (i.e., 2075) was projected from climatological seasonal temperature and salinity data layers derived from projected monthly anomalies in the northwest Atlantic (NEMO RCP8.5-2075; Brickman et al. 2016). The cut-off for areas predicted suitable for establishment was estimated at a logistic threshold of 0.34 (Lowen and DiBacco 2017). The logistic probability distribution was reclassified at equal intervals: low (0.34–0.49), medium (0.5–0.65), and high suitability (> 0.65). Only the current scenario model was used to directly inform scoring in CMIST. The future scenario model was used to identify areas of future concern to further inform recommendations to management (see Discussion).

Although geographic variables, such as bottom type and currents are relevant to the distribution of *D. vexillum* and can be included in MaxENT models, these layers were not available at a resolution comparable to temperature and salinity layers. As there is close agreement between species distributions projected for numerous other tunicates, including *D. vexillum*, based on temperature and salinity, this was considered a realistic proxy for abiotic habitat. Bottom type and currents were considered qualitatively based on expert knowledge and literature review in answering CMIST questions.

#### *Vector analysis*

A source-based vector analysis was selected to characterize likelihood of introduction and spread of *D. vexillum* based on its known worldwide distribution in 2015 (USGS 2004–2014; Moore et al. 2014; Vercaemer et al. 2015). Seven source zones of *D. vexillum* (SZ1–SZ7) (Table 1) were identified. Outside of Atlantic Canada, source zones encompassed climatically similar regions within which *D. vexillum* has been observed (e.g., northeast US, northwest Europe). Large source regions were used to ensure that any vectors within these zones would be considered, consistent with the precautionary approach used by managers. Within Atlantic Canada, two smaller source zones (SZ1 and SZ2) were defined. These source zones correspond with assessment zones AZ1 and AZ4, respectively, and were isolated to facilitate better concordance with the risk assessment.



**Figure 2.** Areas in Atlantic Canada of low, moderate, and high suitability for establishment of *Didemnum vexillum* based on MaxENT environmental niche modelling using (A) current and (B) 2075 climate scenarios. Circles are occurrences of *D. vexillum* (USGS 2004–2014; Moore et al. 2014; Vercaemer et al. 2015) used to calibrate the model. Environmentally suitable areas are overlain by boundaries for 13 assessment zones used in the CMIST detailed-level risk assessment.

**Table 1.** Source zones of *Didemnum vexillum* used in CMIST assessments.

Source zone	Name	Description
SZ1	AZ1	Minas Basin
SZ2	AZ4	Nova Scotia Lower Bay of Fundy
SZ3	Northeast US	North of Cape Hatteras including Georges Bank
SZ4	Northwest Europe	Spain north to United Kingdom and to entrance of Baltic Sea; Mediterranean Sea east from Spain to Adriatic Sea
SZ5	West coast US and Canada	California to southeast Alaska
SZ6	Japan	All
SZ7	New Zealand	All

Connectivity between source zones and assessment zones was explored for anthropogenic vectors identified as high risk for transfer of *D. vexillum* and for which sufficiently high-resolution data on departure and destination locations were available from 2010 to 2015. Generally, biofouling is considered the highest risk anthropogenic mechanism for spread of tunicates, via vessels or aquaculture (e.g., Herborg et al. 2009; Minchin and Sides 2006). In an expert survey for tunicates moderate and high-risk anthropogenic and natural vectors included large, small, and slow-moving vessels, aquaculture transfers, commercial fishing, natural larval dispersal, and natural adult dispersal by drift (Herborg et al. 2009). Here we identified four vectors with sufficient data for a quantitative source-based analysis: commercial vessels, ferries, fishing vessels, and aquaculture transfers.

Data on movements of commercial vessels to and within Canada from 2010 to 2014 were obtained from the Canadian Coast Guard (VTMIS-INNAV 2014); 2015 data were not available at the time of analysis. Departure and destination locations for each trip were assigned to source zones and assessment zones, but only trips originating in source zones were retained. Number of annual arrivals to each assessment zone from each source zone was then determined and averaged from 2010 to 2014. Ferry traffic was not included in the commercial dataset, so vessel routes and estimated frequency were determined from publicly available schedules for ferries operating at any time between 2010 to 2015. Total number of annual arrivals to each assessment zone from each source zone was then estimated. Fishing vessel data were obtained from the Fisheries and Oceans Canada (DFO) Maritimes Vessel Monitoring System database for 2011 to 2015. As fishing data are not trip based, connectivity was approximated using the number of vessels in common between zones as a proxy. Number of fishing vessels in common between source and assessment zones was tabulated for each year from 2011 to 2015 and then averaged. Departure and destination locations of approved transfers of aquatic organisms to

Atlantic Canada were obtained for 2010 to 2015 from DFO's Introductions and Transfers Committee (ITC). Only transfers of shellfish, including spat, seed, and adults for non-research purposes were included to confine the analysis to highest risk activities. Note that these data do not include movements of aquaculture gear or product between leases, processing facilities, and points of sale, which are also vectors of high risk. The number of transfers between source and assessment zones was calculated from 2010 to 2015 then averaged. Results from each analysis were considered collectively to answer CMIST questions. Where applicable, other moderate or high risk anthropogenic and natural vectors were considered qualitatively.

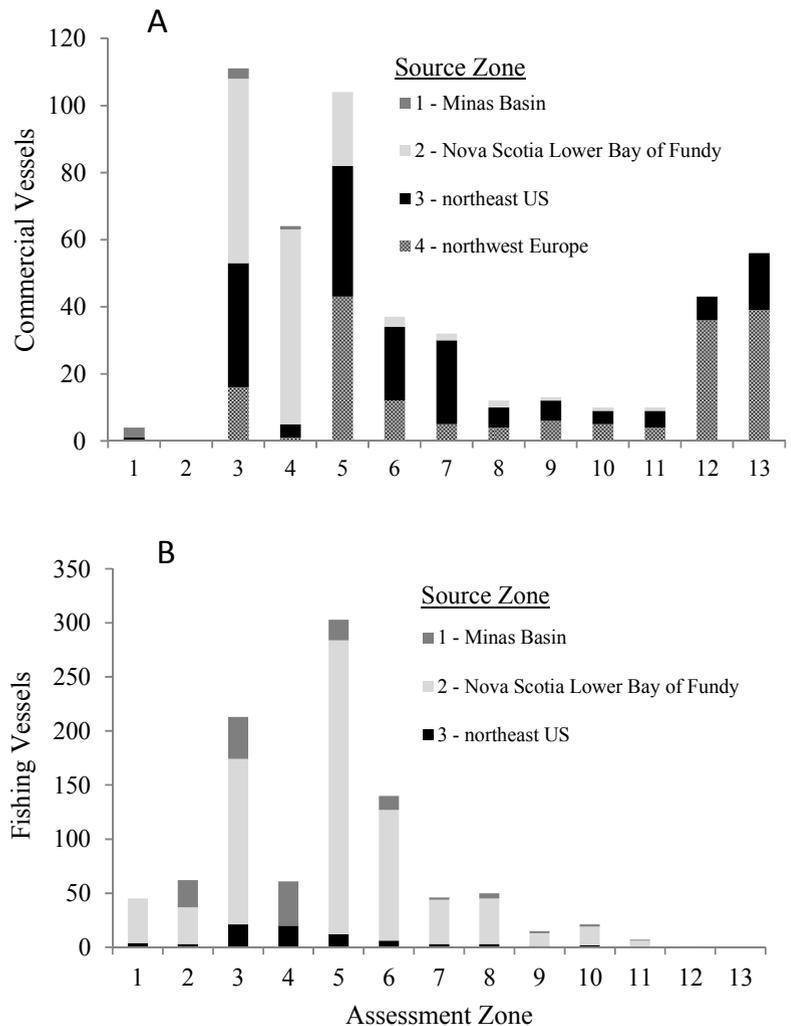
## Results

### *DLRA approach*

#### Environmental niche modelling

Models were cross-validated and had a high TSS (> 0.85), high sensitivity (> 0.85), and low false positive rate (< 10%), implying high accuracy of projections (Franklin and Miller 2010). Of the environmental predictors, temperature and salinity were fairly equal contributors at 56% and 44% respectively. Summer temperature (43%), spring salinity (28%), and summer salinity (16%) were the main seasonal contributors. Lower tolerance limits for establishment based on these seasonal temperature and salinity tolerances were 4 °C, 8 °C, 7 °C, and 3 °C for spring, summer, fall, and winter and an overall salinity of 25.

Present-day suitability for establishment of *D. vexillum* exists in the Bay of Fundy and along the outer coast of Nova Scotia up to Newfoundland (AZ1–AZ7) and in the Northumberland Strait (AZ11) (Figure 2A) with the largest areas of suitability in the Bay of Fundy (AZ1–AZ4). In these zones, areas of high environmental suitability generally occurred in nearshore shallow habitats with moderate and low suitability zones extending outwards (i.e., Minas Basin, southwest Nova Scotia, southwest New Brunswick,



**Figure 3.** (A) Number of commercial vessel arrivals to assessment zones from source zones of *Didemnum vexillum* with  $n > 5$  (annual mean 2010–2014) (B) Number of fishing vessels in common between assessment zones and source zones of *D. vexillum* with  $n > 5$  (annual mean 2011–2015).

and Mahone Bay). Other areas of moderate suitability were offshore in AZ6 on Western Bank and Sable Island Bank. Areas of low suitability occurred in St. George’s Bay, NS (AZ11) and Placentia Bay, NL (AZ7) as well as offshore on Georges Bank (AZ4).

The projected (2075) distribution map shows a general northeastward shift in the range of *D. vexillum*. Low suitability area contracts in the Bay of Fundy (AZ1–AZ4), Georges Bank, and northeast US yet areas of high suitability remain. Expansion of suitable area is evident in southern Newfoundland to St. Pierre and Miquelon (AZ7) and the Magdalen Islands (AZ10) but is focused in the Northumberland Strait (AZ11), where expansion covers most of the zone and includes two centres of high suitability in St. George’s Bay and across the eastern part of the Northumberland Strait (Figure 2B).

Vector analysis

Commercial vessel traffic from source zones was identified for all assessment zones except Chignecto Bay (AZ2) (Figure 3A). Highest number of total arrivals were to NB Lower Bay of Fundy (AZ3) ( $n = 112$ ) from NS Upper and Lower Bay of Fundy (SZ1; SZ2), northeast US (SZ3), and northwest Europe (SZ4) and to the south shore of Nova Scotia (AZ5) ( $n = 104$ ) from the Minas Basin (SZ2), the northeast US (SZ3), and northwest Europe (SZ4). Within Atlantic Canada, arrivals from source zones in the Bay of Fundy were greatest to other zones within the Bay of Fundy (AZ1; AZ3; AZ4). Outside Atlantic Canada, arrivals to all assessment zones except Chignecto Bay (AZ2) were identified. The highest number of arrivals from the east coast US (SZ3)

were to the south shore of Nova Scotia (AZ5) (n = 39) and NB Lower Bay of Fundy (AZ3) (n = 37). The highest number of arrivals from northwest Europe (SZ4) were to the south shore of Nova Scotia (AZ5) (n = 45), the St. Lawrence estuary (AZ13) (n = 39), and the northern Gulf of St. Lawrence (AZ12) (n = 36). Arrivals from the west coast of Canada and the US (SZ5), Japan (SZ6), and New Zealand (SZ7) were negligible.

Ferry traffic from source zones was identified from Eastport, ME to Deer Island, NB (SZ3 to AZ3) (seasonal, when operating; n = 1050), from Portland, ME to Yarmouth, NS (SZ3 to AZ4) (seasonal, when operating; n = 105), and from Digby, NS to Saint John, NB (SZ2 to AZ3) (year-round; n = 506).

Connectivity via fishing vessels was extensive, with some level of connectivity between the Minas Basin (SZ1), NS Lower Bay of Fundy (SZ2), and northeast US (SZ3) and all assessment zones, except the northern Gulf of St. Lawrence and estuary (AZ12; AZ13) (Figure 3B). Overall connectivity with source zones was highest for the south shore of Nova Scotia (AZ5) (n = 303) and NB Lower Bay of Fundy (AZ3) (n = 213), driven by a high number of vessels in common with NS Lower Bay of Fundy (SZ2) (n = 153 and n = 272, respectively).

Number of aquaculture transfers from source zones to assessment zones was very low, with only four approved transfers of shellfish for aquaculture purposes identified. Two approvals were from within Atlantic Canada, from NS Lower Bay of Fundy (SZ2) to elsewhere in Nova Scotia (AZ6) (*Crassostrea virginica* Gmelin, 1791) and to the southern Gulf of St. Lawrence (AZ10) (*Mytilus edulis* Linnaeus, 1758). Two approvals were from outside Atlantic Canada, from northeast US (SZ3) to Nova Scotia (AZ6) (*C. virginica*) and from Europe (SZ4) to the southern Gulf of St. Lawrence (AZ10) (*C. virginica*).

#### DLRA for *D. vexillum*

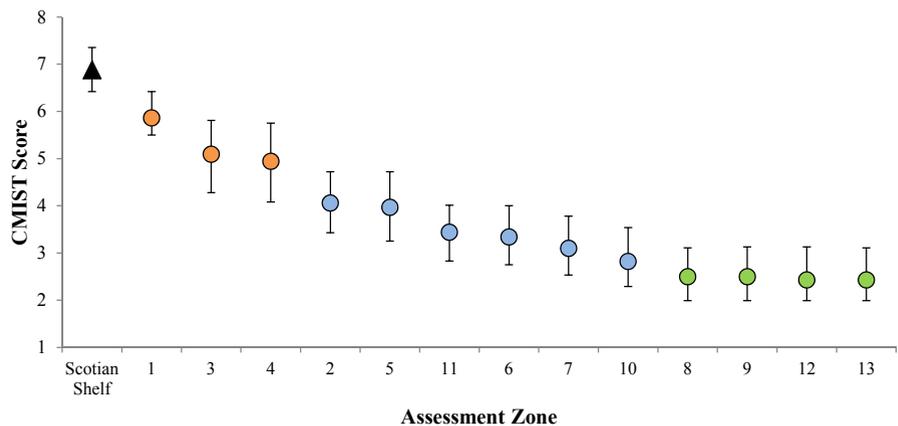
##### Synopsis

*D. vexillum* was first detected off Parrsboro, NS in 2013 (Moore et al. 2014) and has since been observed throughout the Minas Basin and in two other locations off the coast in the Bay of Fundy (see Figure 2; Vercaemer et al. 2015). In the northeast US, populations of *D. vexillum* border Canadian waters in Eastport, ME (Martin et al. 2011) and Georges Bank (Valentine et al. 2007a) as well as further south along the coast to south of Cape Cod (Bullard et al. 2007). Other populations exist in northern Europe, the west coast of North America, Japan, and New Zealand (USGS 2004–2014). In Atlantic Canada, suitable environmental conditions for establishment

of *D. vexillum* are focused in the Upper Bay of Fundy, southwest New Brunswick, southwest Nova Scotia, Western Bank and Sable Island Bank, and small areas along the southern coast of Nova Scotia, Northumberland Strait, and Newfoundland (Figure 2A). Under future climate conditions, there is a northeastward shift in suitable area for establishment; most of the Northumberland Strait becomes suitable for establishment and there is a contraction in the northeast US, Georges Bank, and Bay of Fundy (Figure 2B). Lower seasonal tolerance limits for establishment of *D. vexillum* estimated in the model presented here reflect 3 °C and 25 salinity minima (Lowen and DiBacco 2017; Miller 2016); warmer summer temperatures will help support a longer growing season in which development can be completed to establish self-sustaining populations. Many potentially suitable areas in Atlantic Canada have been monitored for *D. vexillum*, in particular southwest Nova Scotia and southwest New Brunswick (Sephton and Vercaemer 2015; Martin et al. 2011), but colonies have yet to be widely detected using PVC collectors deployed for approximately 5 months in the nearshore at 1 m depth. Currently, *D. vexillum* has only been detected in Atlantic Canada waters after monitoring natural subtidal substrates, resulting in the detection of *D. vexillum* in areas that were predicted suitable (Vercaemer et al. 2015). *D. vexillum* may not currently be able to establish in all locations despite high suitability; however, short-term or seasonal survival is likely given anecdotal temperature tolerance (−2 °C to 24 °C) (Bullard et al. 2007) and salinity tolerance (20 to at least 30) (Gröner et al. 2011; Bullard and Whitlatch 2009).

Internationally, *D. vexillum* has been a prolific invader and has ongoing potential to arrive in Atlantic Canada via natural and anthropogenic vectors. In Atlantic Canada, it has only been reported on natural subtidal substrates (Vercaemer et al. 2015); however, in other source zones, *D. vexillum* occurs on hard substrates, both anthropogenic (e.g., vessel hulls, pilings, aquaculture gear) and natural (e.g., rocks, scallops, and shell hash) in subtidal and intertidal areas (USGS 2004–2014). *D. vexillum* may be transferred by multiple vectors from those substrates, including biofouling of vessels, aquaculture transfers, and naturally via reattachment of fragments (Morris and Carman 2012; Valentine et al. 2007b). Risk of introduction along this complex network of potential pathways has not been fully characterized. The majority of commercial vessel traffic from source zones of *D. vexillum* come from the northeast US and northwest Europe (Figure 3A), although there is connectivity between ports along the Atlantic coast as well (pers. obs.). Recreational

**Figure 4.** CMIST scores for *Didemnum vexillum* in 13 assessment zones in Atlantic Canada (●) compared to the SLRA for *D. vexillum* in the Scotian Shelf marine ecoregion (▲) (DFO 2015). Shown are overall CMIST scores adjusted for uncertainty. Colours represent invasion risk: highest (orange), moderate (blue), lowest (green). Error bars indicate 95% confidence intervals.



vessel movements are predominantly local, but long trips can also connect distant marinas (Simard et al. 2017). This is similar to fishing vessels, which can act both as fishing and recreational vessels connecting offshore (i.e., bottom) and coastal endpoints. More comprehensive surveys of recreational boating activity are needed before this vector can be assessed more effectively. Bottom-activities, both anthropogenic (i.e., scallop dredges, ground fish trawls) and natural (i.e., currents) present a risk of fragmenting colonies, which could spread and create more disparate populations in the Bay of Fundy. Transfer via aquaculture imports is regulated and not likely to be a major factor; however, transfer of gear between local sites and product does occur within provinces in Atlantic Canada and has not been well described. If *D. vexillum* reaches marinas, recreational boats and fishing vessels would become key vectors for mediating spread.

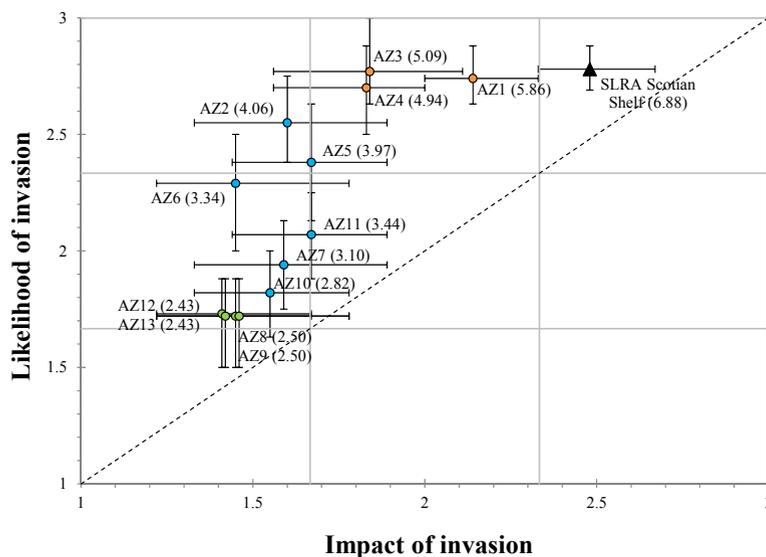
There have been no significant or noticeable impacts from *D. vexillum* in Atlantic Canada to date. However, multiple natural and anthropogenic habitats and associated species may be vulnerable, primarily scallop beds and eelgrass habitats, which are important ecological communities and fishing grounds. *D. vexillum* exists in large mats that overgrow sessile species on the seafloor, including scallops (Valentine et al. 2007a), eelgrass (Carman and Grunden 2010) as well as epibenthic flora and fauna (Lengyel et al. 2009). In the US portion of Georges Bank, settlement area available to larval recruits and shelter for juvenile fish may have been reduced by *D. vexillum*, also reducing availability of benthic prey to demersal fish (Valentine et al. 2007a). There is also evidence of community changes, including a shift in polychaete communities (Lengyel et al. 2009) and positive asso-

ciation of some benthic invertebrates with *D. vexillum* (Smith et al. 2014). In eelgrass beds, growth of polychaetes and tanaids on eelgrass blades was facilitated by the presence of *D. vexillum* (Long and Grosholz 2015), whereas other colonial tunicates have been shown to reduce eelgrass growth (Wong and Vercaemer 2012).

Vulnerable anthropogenic habitats include aquaculture leases, ports, and marinas. *D. vexillum* can overgrow mussels in fouling communities (Dijkstra et al. 2007), on monitoring plates (Auker and Oviatt 2008), and in aquaculture operations (Bullard et al. 2007; Coutts and Forrest 2007). Impacts to aquaculture leases would primarily be economic via fouling organisms and gear, which has been a major problem in Atlantic Canada (McKindsey et al. 2007). Economic impacts may be compounded by a new species with different biology, as most mitigation measures in aquaculture operations (i.e., mussel line cleaning protocols and power washing) are targeted to solitary tunicates not encrusting colonial tunicates. Invasive tunicates are already dominant in many fouling communities, and *D. vexillum* would be expected to compete for space and food with the other filter feeders.

#### DLRA scores

The highest CMIST risk score was 5.86 for the Minas Basin (AZ1) and the lowest was 2.43 for northern Gulf of St. Lawrence and estuary (AZ12; AZ13). Remaining assessment zones fell in between these scores with no clear breaks in rankings (Figure 4). However, in a heat map, *likelihood of invasion* scores had a wider range (1.72–2.77) and were generally higher than *impact of invasion* scores (1.41–2.11) and groupings were more evident. The Bay of



**Figure 5.** Overall CMIST scores (in brackets) and *likelihood of invasion* and *impact of invasion* scores for *Didemnum vexillum* in 13 assessment zones (AZ) in Atlantic Canada (●) and the Scotian Shelf marine ecoregion (▲) from the SLRA (DFO 2015). Colours represent invasion risk: highest (orange), moderate (blue), lowest (green). Error bars indicate 95% confidence intervals. Shown in parentheses are overall CMIST scores adjusted for uncertainty. Hatched line shows 1:1 line.

of Fundy, except Chignecto Bay, (AZ1; AZ3; AZ4) had highest scores for both *likelihood of invasion* and *impact of invasion* and the northern Gulf of St. Lawrence and estuary and east and west coasts of Newfoundland (AZ8; AZ9; AZ12; AZ13) had lowest scores (Figure 5). Remaining assessment zones had similar *impact of invasion* scores (1.45–1.67), therefore most differentiation among zones was from *likelihood of invasion* scores (1.82–2.55), which had a greater range. All assessment zone risk scores were lower than the SLRA risk score reported for the entire Scotian Shelf ecoregion (CMIST score = 6.88) (Figure 4; Figure 5). This was driven by a much higher *impact of invasion* score (2.48) as *likelihood of invasion* score (2.78) was comparable to scores for assessment zones in the Bay of Fundy (AZ1; AZ3; AZ4) (Figure 5).

## Discussion

### *DLRA for D. vexillum in Atlantic Canada*

#### Overall risk

Overall, invasion risk is highest in the Minas Basin where the species has already been detected in multiple locations, the environment is highly suitable for establishment, and potential impacts to scallop beds are high. Invasion risk is moderate in adjacent Chignecto Bay, where introduction by natural dispersal and fishing vessels is particularly likely and has high habitat suitability. Risk of spread within the Bay of Fundy is also high (Figure 3), where bottom-disturbing activities such as dredges, and strong bottom currents

may lead to uptake on fishing vessels or fragmentation, respectively. Risk of new introductions and secondary spread remains, especially to the lower Bay of Fundy where vectors (i.e., vessel traffic) and suitable environment for establishment exist. There is no significant amount of commercial traffic from source zones to areas of suitable conditions in Atlantic Canada because endpoints for commercial vessels are in larger ports (Halifax, Port Hawkesbury, Sydney), which do not appear to have high environmental suitability. However, in warm years, the risk would be higher, especially to the Northumberland Strait as populations experience more favourable conditions for settlement, reproduction, and establishment. Although Georges Bank is currently an environmentally suitable area, it is projected to become unsuitable with climate change, whereas more northern Western Bank and Sable Island Bank will experience greater risk. Risks associated with aquaculture imports at this scale are low; however, movement of aquaculture product within assessment zones is high and would promote secondary spread via both product and gear.

#### Monitoring and mitigation recommendations

Results from this risk assessment are being incorporated into ongoing monitoring efforts by DFO (A. Silva, DFO, pers. comm.) to help target ongoing monitoring efforts for *D. vexillum* more effectively. AIS monitoring in coastal habitats should continue to focus on marinas and nearshore areas in southwest New Brunswick, southwest Nova Scotia, and Mahone

Bay, where environmental suitability is high (Figure 2A). Despite not being detected in these locations thus far by regular monitoring or rapid assessments (Martin et al. 2011; Sephton and Vercaemer 2015), these areas are still at risk and would represent new hubs for spread by small vessels. Additional collectors, more appropriate collectors (e.g., subtidal), or more frequent and comprehensive rapid assessments and field surveys may also be advised in these areas to increase the likelihood of early detection (Moore et al. 2014). As *D. vexillum* has only been detected subtidally in Atlantic Canada from 4 m to 66 m depth (Vercaemer et al. 2015) and extensive areas of environmental suitability exist offshore, these areas should be regularly sampled to monitor persistence and rate of spread. Currently, this is achieved through collaboration with annual commercial scallop stock assessment surveys, which were levied to delineate the species' distribution post-detection (see Vercaemer et al. 2015). These surveys cover the spectrum of environmental suitability in the Bay of Fundy and Scotian Shelf, including high-risk areas in the Minas Basin and Georges Bank. This regular, systematic sampling would improve early detection of *D. vexillum*, help identify high-risk areas by detecting seasonal population changes, and improve future models via groundtruthing. In addition, as samples are only taken incidentally and preserved for subsequent genetic identification, there is no significant disruption to the industry workflow. New techniques, specifically eDNA sampling and analysis, might also be considered in both nearshore and offshore monitoring to confirm existence of propagule pressure (Ma et al. 2016) and increase the likelihood of early detection (e.g., Goldberg et al. 2013). Finally, model projections may guide development of preemptive and targeted AIS monitoring and mitigation strategies (Lowen and DiBacco 2017). Results from climate projections here could be used to plan mitigation measures to prevent the secondary transfer of propagules to disjunct, suitable areas via recognized vectors, especially vessel traffic. For example, monitoring in the Northumberland Strait and Canso Canal area in warmer years could identify the extent of propagule pressure in the system, assist in groundtruthing models, and provide a baseline for assessing future changes and impacts on mussel aquaculture and scallop fishing industries.

Mitigation measures to reduce the likelihood of secondary introductions and range expansion of *D. vexillum* in Atlantic Canada require a vector-based approach. Although, *D. vexillum* is a controlled species in Canada's *Aquatic Invasive Species Regulations* under the *Fisheries Act*, no national regulations on biofouling exist. Ideally, biofouling controls would

be instituted for vessels moving from the northeast US to Atlantic Canada, particularly southwest New Brunswick and Nova Scotia, where invasion risk is particularly high from both natural and anthropogenic vectors. The recreational vessel pathway is not well characterized in the region and new work should focus on identifying high-frequency routes linking source areas to endpoints in environmentally suitable areas, particularly those that may be amenable to implementation of inspections or boat washing stations. In the absence of an applicable regulatory framework, any controls or mitigations measures would be on a voluntary basis in the short term. Reduction of spread from within Atlantic Canada should focus on scallop fishing vessels active in the Bay of Fundy. Scallop vessels may transfer *D. vexillum* large distances by dumping contaminated dredge material in distant locations from where it was brought on board (J. Sameoto, DFO, pers. comm.). Discouraging this practice or identifying areas within the Bay of Fundy where dumping would present minimal risk of establishment and spread are required. Communication with fishery operators regarding changes in distribution of *D. vexillum* has been ongoing (DFO 2016b), and requesting voluntary compliance at annual stock assessment meetings would be an effective approach, especially if recommendations are informed by previous year's sampling to identify areas of concern. Further restrictions may be considered for anthropogenic vectors between source populations and the Gulf of St. Lawrence, where an invasion could represent significant impacts to aquaculture operations in the region and invasion by natural vectors is currently unlikely.

#### *Approach for DLRAs*

##### Environmental niche modelling

The map of environmental suitability for establishment presented here (Figure 2A) agrees better with observed species distributions than Therriault and Herborg's (2007) GARP model, which shows a more widespread distribution, likely a result of overprediction (Elith and Leathwick 2006). Occurrences of *D. vexillum* (Figure 2A) were generally associated with high suitability areas, except off Digby, NS, (AZ4) which was an unsuitable area. Similar differences in species distributions exist between model projections for other tunicate species assessed in both Therriault and Herborg (2007) and Lowen and DiBacco (2017). These differences underline the need to update models (as done here with a more refined machine learning algorithm, updated occurrence data, and high resolution environ-

mental layers) to increase the resolution of projections and focus AIS management resources. Further improvements could include the incorporation of geophysical parameters, such as bottom type to refine projections.

Although model projections for 2075 were not considered directly in answering CMIST questions, they are useful in considering future AIS management measures due to the close approximation of climate to recent anomalously warm years (Lowen and DiBacco 2017). For example, 2012 was the warmest for Atlantic Canada since 1981 with an average normalized annual temperature anomaly of +2.8 standard deviation in some locations (Johnson et al. 2013) and was followed by another warm year in 2013 (Hebert et al. 2014). Detections of new AIS also increased in 2012 and 2013 (Moore et al. 2014), three of which, *Ascidella aspersa* (Müller, 1776), *Diplosoma listerianum* (Milne Edwards, 1841), and *D. vexillum*, were observed in predicted areas of future environmental suitability (Lowen and DiBacco 2017). As present-day proxies for future projections, anomalously warm years can be used to pre-emptively groundtruth future projections for *D. vexillum*, focusing monitoring in the Northumberland Strait, where there is significant aquaculture activity and potential for economic impacts.

### Vector analysis

The vector analysis presented here improves on previous analyses by distinguishing relative pressure from certain high-risk vectors using simple quantitative methods that focus on transfers from known source areas of *D. vexillum*. Not all key pathways were quantified here, such as recreational boating due to the lack of comprehensive data across our study region; however, quantification of a portion of vessel traffic significantly improves the objectivity of the arrival component of the risk assessment. Also, movement of aquaculture product and gear remains a poorly understood but high risk pathway that may best be examined at a higher resolution more focused on secondary spread. Characterization of departure and destination locations for fishing vessels, recreational vessels, aquaculture gear and product, and natural dispersal would provide a more comprehensive perspective that would facilitate multi-vector analyses as recommended in Williams et al. (2013). Higher resolution analyses at the port, marina, or aquaculture facility level would improve risk assessments further by isolating highest risk locations as in Drake and Lodge (2004), who showed some ports were more at risk of introduction by ballast water than others by number of visits from

source sites. However, caution and a conservative approach must still be used when identifying source sites as not all locations of *D. vexillum* are known. Generally, source-based analyses are useful for management as they identify all levels of connectivity, including low-frequency pathways, which can still lead to invasions. This is particularly relevant for *D. vexillum*; a likely vector for introduction to the Minas Basin from the northeast US is documented here via commercial and fishing vessels (Figure 3A), which was not evident in Therriault and Herborg (2007).

### Risk assessment method

Risk scores for *D. vexillum* from this DLRA were lower than reported by Therriault and Herborg (2007) and DFO (2015), most likely due to the finer scale of assessment. Both prior assessments evaluated risk at a large scale, Atlantic Canada (Therriault and Herborg 2007) or Scotian Shelf (DFO 2015), where there is high variability in habitat and vectors. At that scale, risk appears to be conflated to the entire assessment area, including low risk areas, which results in more conservative (higher) risk scores. Those approaches are thus more useful for screening or prioritizing species than for targeted management, and to be most effective, the scale of assessment should be comparable to the desired scale of management. Here, smaller assessment zones allowed invasion risk to be allocated more appropriately within Atlantic Canada to provide a more realistic representation of risk that is more practical for targeted management purposes to prioritize mitigation efforts. Further division of assessment zones into smaller areas would facilitate incorporation of higher resolution vector data (i.e., ports versus marinas) which would likely further partition risk among suitable areas both coastal and offshore.

Overall, CMIST functioned well as a DLRA tool and has several advantages over the general framework in Mandrak et al. (2012). First, it is a more objective approach that reduces inter-assessor variability (Drolet et al. 2016). In addition, the use of semi-quantitative scoring instead of qualitative scoring is more effective for ranking and comparing multiple assessment areas as it can be represented in integrated visual displays (Figures 4 and 5). The incorporation of uncertainty into a single risk score with error bars enhances interpretation and usability by management (DFO 2015). Risk assessment tools, including CMIST (Drolet et al. 2017), have been shown to be over-parameterized. Here, 5 of 17 questions were scored identically for all assessment zones and were thus uninformative for partitioning risk. These questions were mostly species-specific (Q6: natural control agent,

Q13: diseases and parasites, Q14: genetic effects, Q15: impacts on at-risk or depleted species, Q17: invasiveness elsewhere) and essentially independent of assessment area. In the original application of CMIST for multiple species in multiple assessment areas, no scores were identical for all assessments; however, accuracy was improved when 8 of 17 questions were removed and remaining questions weighted. CMIST scores were not optimized and weighted in this application, but could be over-parameterized beyond the 5 non-informative questions. Although not all CMIST questions contribute to risk partitioning or accuracy, risk scores, uncertainty scores, and rationales are still informative for management and important to understanding the overall risk of the species.

CMIST has already received traction as a peer-reviewed tool and is being used in multiple contexts due to these features, for example, in assessing high-risk ballast water AIS in Arctic environments (K. Howland, DFO, pers. comm.) and AIS associated with debris from the 2011 tsunami off the coast of Japan to the west coast of North America (T. Therriault, DFO, pers. comm.). In addition, CMIST is flexible and assessments can be readily updated to integrate new information on impacts, vectors of introduction, environmental suitability, and distribution as data becomes available and according to management needs.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** CMIST questions, risk scores, and uncertainty scores for Atlantic Canada assessment zones.

This material is available as part of online article from:

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