

Rare species detection and benthic recruitment across multiple scales of space and time with implications for early detection of marine invasive species

Thèse

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Résumé

Les activités anthropiques causent des invasions biologiques qui sont devenues un problème mondial susceptible de causer des dommages écologiques (p. ex., sur la biodiversité et l'habitat), économiques (sur les industries) et sociaux (sur le bien-être humain). La prévention et la détection précoce des nouvelles invasions sont des éléments essentiels pour la gestion des risques et des impacts sur les écosystèmes et les économies. Bien sûr, la prévention est préférable, mais la détection précoce est une étape cruciale pour enrayer la propagation ultérieure des espèces envahissantes, car elle offre la possibilité de les éradiquer avant les phases d'établissement de la population et de propagation. Bien qu'il s'agisse d'une option de gestion efficace en matière de coût et de temps, la détection précoce exige un effort d'échantillonnage considérable pour détecter les populations envahissantes aux tout premiers stades de leur invasion.

En utilisant le système benthique marin comme modèle, quatre études interdépendantes ont été menées pour identifier des stratégies d'échantillonnage susceptibles d'améliorer notre capacité à détecter des populations envahissantes rares et à comprendre les patrons et processus écologiques de recrutement benthique à multiples échelles spatiales et temporelles. Plus précisément, ces études expérimentales sur le terrain visaient à (1) évaluer la relation entre l'approvisionnement en larves et la fixation dans une population envahissante isolée, (2) déterminer la durée de l'échantillonnage et de la fréquence à l'aide de plaques de fixation pour la détection d'espèces rares, (3) déterminer l'importance relative aux sources de variations spatiales et temporelles du recrutement benthique, et (4) examiner l'effet de l'échelle spatiale de l'échantillonnage sur la détection des espèces en analysant les patrons de recrutement à de multiples échelles sur quatre ordres de grandeur allant de la dizaine de mètres à la dizaine de kilomètres.

Première étude : contrairement à l'hypothèse originale d'une relation étroite entre l'approvisionnement et la fixation initiale, l'approvisionnement en larves était plutôt un facteur déterminant de la fixation aux échelles moyennes. Ces résultats suggèrent que la force de cette relation s'affaiblit avec l'augmentation de l'échelle spatiale des observations de terrain. Néanmoins, un quart de la variation de la fixation à moyenne échelle peut encore être expliqué par l'approvisionnement sur des courtes échelles de temps (une semaine). Par

conséquent, cette relation confirme l'utilité des plaques de fixation en tant qu'outil efficace pour la détection précoce aux échelles moyennes dans une marina, car une faible densité de recrutement sur les plaques correspond à une faible abondance de propagules envahissantes dans la colonne d'eau.

Deuxième étude : des durées d'échantillonnage intermédiaires d'une à deux semaines (l'échelle des traitements allant d'un jour à un mois) étaient la durée optimale de déploiement de la plaque de fixation pour la détection des espèces « rares » (c'est-à-dire, des le début du recrutement). Une analyse au niveau de l'assemblage montre toutefois que l'augmentation de la durée et de la fréquence de l'échantillonnage augmentait logarithmiquement le nombre total d'espèces rares observées. Ces résultats espèce par espèce et au niveau de l'assemblage démontrent que la modification des éléments temporels de l'échantillonnage, tels que la durée et la fréquence, peut affecter considérablement la détection d'espèces.

Troisième étude : après avoir évalué plusieurs sources spatiales et temporelles (le site, la région, la saison, et l'année), le moment choisi pour le déploiement des plaques est apparu comme étant la plus grande source de variabilité du recrutement benthique d'espèces rares. En particulier, le moment optimal pour la détection précoce serait en automne (a) lorsque le recrutement saisonnier d'espèces envahissantes établies tend à atteindre un pic et (b) lorsque la détection au niveau du site d'espèces envahissantes rares tend à se produire.

Quatrième étude : l'échelle spatiale dominante dans le recrutement d'espèces rares est la plus petite (centaine de mètres). Cette échelle dominante peut être interprétée comme étant la bonne échelle spatiale pour la détection d'espèces rares. Une analyse plus poussée a montré que si l'échantillonnage a été structuré de manière aléatoire, l'échantillonnage à des échelles intermédiaires (millier de mètres) devient l'échelle optimale pour la détection d'espèces rares. Ces résultats élucident les différences de variabilité naturelle de la population benthique entre multiples échelles d'espace et de temps pour des espèces rares et communes.

Ces études écologiques font partie d'une boîte à outils de détection précoce nécessaire à la gestion des espèces envahissantes marines en renseignant sur la manière dont l'échantillonnage des espèces rares doit être faite à multiples échelles spatio-temporelles. Des expériences de terrain similaires optimisant la détection d'espèces rares (au-delà de l'utilisation de plaques de fixation pour détecter les organismes benthiques dans les provinces Maritimes canadiennes) devraient être réalisées pour d'autres taxons, régions,

et outils d’échantillonnage—en particulier, les envahisseurs à haut risque prévus, les invasions futures, et les outils récemment développés.

Abstract

As a consequence of anthropogenic activities, biological invasions have become a global problem that can cause ecological (e.g., biodiversity and habitat), economic (industries), and social (human wellbeing) harm. Prevention and early detection of new invasions are vital components of managing risks and impacts to ecosystems and economies. Prevention is, of course, preferred but early detection is a critical step that can ultimately stop future spread of invasive species because it provides an opportunity for eradication before population growth and spread. Despite being a cost- and time-effective management option, early detection requires considerably high sampling effort to detect incipient invasive populations at the early stages of their invasion.

Using the marine benthic system as a model, four inter-related studies were carried out to identify sampling strategies that could enhance our ability to detect rare invasive populations and to understand ecological patterns and processes of benthic recruitment across multiple scales of space and time. Specifically, these experimental field studies aimed to (1) evaluate the relationship between propagule supply and settlement in a closed invasive population, (2) determine the optimal sampling duration and frequency using settlement plates to detect rare species, (3) ascertain the relative importance of spatial and temporal sources of variation in benthic recruitment, and (4) examine how the spatial scale of sampling affects species detection by analyzing recruitment patterns at multiple scales across four orders of magnitudes ranging from tens of metres to tens of kilometres.

First study: Contrary to the expectation of a strong relationship between supply and initial settlement, larval supply was instead a limited determinant of settlement at mesoscales. This finding suggests that the strength of this relationship weakens as the spatial scale increased from previously reported small-scale field observations to mesoscales of the present study. Nonetheless, a quarter of the variation in settlement can still be explained by supply over short timescales (one week). Therefore, this relationship supports the utility of settlement plates as an effective tool for early detection at mesoscales within a marina because low densities of recruitment on plates correspond to low abundances of invasive propagules in the water column.

Second study: Intermediate sampling timescales of one to two weeks (duration treatments ranging from one day to one month) was the optimal plate deployment duration for "rare" species detection (i.e., during the seasonal onset of recruitment). An assemblage-level analysis, however, showed that increasing the duration and frequency of sampling logarithmically increased the total number of rare species observed. These species-by-species and assemblage-level findings demonstrate that modifying temporal aspects of sampling, such as duration and frequency, can substantially affect species detection.

Third study: The seasonal timing of plate deployment was determined to be the greatest source of variability in benthic recruitment of rare species after evaluating several spatial and temporal sources (i.e., site, region, season, and year). In particular, the optimal seasonal timing for early detection may be in the autumn (a) when the seasonal recruitment of established invasive species tends to peak and (b) when the site-level detection of rare invasive species tends to occur.

Fourth study: The dominant scale in rare species recruitment is at the smallest spatial scale (hundreds of metres). This dominant scale can be interpreted as being the correct spatial scale for rare species detection. Further analysis showed that if sampling was structured randomly, then forcing sampling at intermediate scales (thousands of metres) is the optimal scale for rare species detection. These results elucidate differences in the natural variability in benthic recruitment across multiple scales of space and time for both rare and common species.

These ecological studies are part of an early detection toolkit that can inform marine invasive species management with respect to how sampling for rare species should proceed across multiple spatiotemporal scales. Similar field experiments optimizing for rare species detection (beyond the use of settlement plates to detect benthic organisms in the Canadian Maritimes) should be done for other taxa, regions, and sampling tools—especially for forecasted high-risk invaders, regions susceptible to future invasions, and newly-developed tools.

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List of abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
BdOL	Bras d'Or Lake
eDNA	environmental deoxyribonucleic acid
GLM	Generalized linear model
GLMM	generalized linear mixed model
HSD	honestly significant difference
LOESS	local regression
MCMC	Markov chain Monte Carlo
NS	Nova Scotia
PCR	polymerase chain reaction
PEI	Prince Edward Island
POD	probability of detection
PVC	polyvinyl chloride
SCUBA	self-contained underwater breathing apparatus
S_R	random sampling strategy
S_{100}	small-scale sampling strategy by forcing sampling at scales ≥ 100s of m
S_{1000}	intermediate-scale sampling strategy by forcing sampling at scales ≥ 1000s of m
S_{10000}	large-scale sampling strategy by forcing sampling at scales = 10000s of m

Dedication

To my mom, Mei Tak Law. Thank you for always believing in me. And, in loving memory of my paternal grandfather, Ping Sum Ma, and my maternal grandmother, Ho Fook Sang.

本論文是獻給我的媽咪，羅美德。非常感謝你一直支持，鼓勵，和信任我。此外，本論文也是為了懷念我的爺爺，馬炳森，和我的婆婆，何福生，而作。



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A very special thank you to all my friends (in Vancouver, across Canada, and throughout the world) and to my entire family (including my parents, siblings, grandparents, aunts and uncles, cousins, and stepfamily members) who I often relied upon for moral support, companionship, and love—especially my grandmother (Lo Ngan Ying), my mother (Mei Tak Law), my father (Edward Ma), and my sister (Connie Ma).

Some of the career highlights during my doctoral studies consisted of (1) attending the *9th International Conference on Marine Bioinvasions* (Sydney, Australia) and the *12th International Temperate Reefs Symposium* (Hong Kong, China), (2) co-organizing the *44th Annual Benthic Ecology Meeting* (Quebec City, Canada), *Aquatic Invasive Species Knowledge Transfer Workshop* (Iqaluit, Canada), and *4th World Conference on Marine Biodiversity* (Montreal, Canada), (3) taking the *Taxonomy and Biology of Tunicates*

course (Bocas del Toro, Panama), and (4) participating in the scientific program of *Canada C3* expedition (sampling from the Maritimes to the Arctic) and the *Passamaquoddy Bay BioBlitz* (New Brunswick, Canada). Opportunities to participate in these scientific conferences, courses, and workshops have contributed greatly to my scientific training. These opportunities were made possible with the support (e.g., research fellowships, travel grants) from CAISN, Québec-Océan, Université Laval, Smithsonian Tropical Research Institute, Huntsman Marine Science Centre, North Pacific Marine Science Organization (PICES), Atlantic Reference Centre, student organisations at the university (ACCÉBUL, AEGSEG, and AELIÉS), and funding from my thesis supervisor and co-supervisor (L.E. Johnson and C.W. McKindsey).



Preface

This thesis consists of six sections, written in English, starting with an introduction (General introduction), followed by four chapters in the form of scientific articles (Chapter 1, Chapter 2, Chapter 3, and Chapter 4), and closing with a discussion (General discussion). I am the principal author responsible for all aspects of the research described in this thesis, including the formulation of research questions, design of field experiments, data collection, analyses, and preparation of manuscripts. My supervisor and co-supervisor, Ladd E. Johnson (Université Laval) and Christopher W. McKindsey (Fisheries and Oceans Canada), respectively, contributed to the identification and design of field studies. Co-authorship of any resulting publications from the work presented in all four chapters, unless otherwise requested, consists of L.E. Johnson, C.W. McKindsey. All supplemental tables and figures referred to in the body of this thesis can be found in Appendix 1. Research published as journal articles during my tenure as a doctoral candidate are appended in Appendix 2. The full citations of these articles are as follows:

Moore AM, Vercaemer B, DiBacco C, Sephton D, Ma KCK. 2014. Invading Nova Scotia: first records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013. *BioInvasions Records* 3: 225–234.

Ma KCK, Simard N, Stewart-Clark SE, Bernier RY, Nadeau M, Willis J. 2016. Early detection of the non-indigenous colonial ascidian *Diplosoma listerianum* in eastern Canada and its implications for monitoring. *Management of Biological Invasions* 7: 365–374.

Ma KCK, Goodwin C, Cooper JA. 2018. Second record of *Diplosoma listerianum* (Milne-Edwards, 1841) five years after and 280 kilometres from the site of the first record in Nova Scotia. *BioInvasions Records* 7: 159–163.

Ma KCK, Hawk HL, Goodwin C, Simard N. 2019. Morphological identification of two invading ascidians: new records of *Ascidiella aspersa* (Müller, 1776) from Nova Scotia and *Diplosoma listerianum* (Milne-Edwards, 1841) from New Brunswick and Quebec. *BioInvasions Records* 8: 50–64.

Carman MR, Colarusso PD, Neckles HA, Bologna P, Caines S, Davidson JDP, Evans NT, Fox SE, Grunden DW, Hoffman S, Ma KCK, Matheson K, McKenzie CH, Nelson EP,

Plaisted H, Reddington E, Schott S, Wong MC. 2019. Biogeographical patterns of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39º and 47º north latitude (New Jersey to Newfoundland). *Management of Biological Invasions* 10: 602–616.

Ma KCK, Glon HE, Hawk HL, Chapman CN. 2020. Reconstructing the distribution of the non-native sea anemone, *Diadumene lineata* (Actiniaria), in the Canadian Maritimes: Local extinction in New Brunswick and no regional range expansion in Nova Scotia since its initial detection. *Regional Studies in Marine Science* 34: 101049.

Some of the results described in this thesis has been previously presented at scientific conferences and departmental and network colloquia. The citations of my scientific contributions that were previously presented at international meetings are as follows:

Ma KCK, McKindsey CW, Johnson LE. 2019. *Optimal spatial scale of monitoring for marine invertebrates and its implications for early detection of invasive species*. 12th International Temperate Reefs Symposium, Hong Kong, China.

Ma KCK, Johnson LE, McKindsey CW. 2016. *Optimising the duration and frequency of settlement plate deployments*. 45th Annual Benthic Ecology Meeting, Portland, Maine, USA.

Ma KCK, Johnson LE, McKindsey CW. 2016. *Monitoring programmes for aquatic invasive species using larval recruitment plates can be modified for early detection*. 9th International Conference on Marine Bioinvasions, Sydney, Australia.

Ma KCK, McKindsey CW, Johnson LE. 2015. *Plenty of tunicates in the sea: Distribution of and relationship between larval supply and recruitment with respect to depth*. 44th Annual Benthic Ecology Meeting, Quebec City, Quebec, Canada.

General introduction

Invasive species and the invasion process

Invasive species—species living outside their native range and causing ecological or economic harm—are a global problem affecting the health and structure of invaded ecosystems (including biodiversity loss and, even, extinction of native species), the sustainability of local economies, and human wellbeing (Mack et al. 2000; Pimentel et al. 2000; Mooney and Cleland 2001; Bax et al. 2003; Molnar et al. 2008; Blackburn et al. 2019). Introductions, establishment, and spread (including range shifts) of invasive populations have been increasing at alarmingly high rates (Cohen and Carlton 1998; Perrings et al. 2002; Ricciardi 2007; Lambdon et al. 2008; Hulme 2009). However, the documentation of high numbers of biological invasions may, otherwise, be due to greater monitoring efforts through time and this rate of increase may, in fact, be more constant (Costello and Solow 2003). Furthermore, there is some evidence that the rate of new incursions may not be increasing at all when corrected for sampling effort relative to the speed of discovering new species, which includes the discovery of new native species (Coles et al. 1999).

The process of biological invasions consists of multiple steps: (1) transport or translocation, (2) introduction, (3) establishment, and (4) geographic spread (Richardson et al. 2000; Sakai et al. 2001; Williamson 2006; Theoharides and Dukes 2007; Figure 1). For some types of species introductions, two additional distinct steps include (i) uptake (or entrainment), when invasive propagules and the vector are associated prior to transport, and (ii) drop-off, when these propagules are disassociated with the vector and are released into a novel environment after transport (Lee and Chown 2009). However, these additional steps are sometimes not made for intentional introductions in which transport simply constitutes a single step (*sensu* Lee and Chown 2009). Although the legal framework for invasive species management typically uses political boundaries (e.g., national and subnational borders), these boundaries do not conform neatly with biological and geographic barriers that limit invasions (Richardson et al. 2000).

In aquatic systems, the movement of (a) ships (including ballast water and submerged surfaces), (b) ornamental species in the aquarium trade, and (c) aquaculture products and equipment are among some of the human-mediated vectors that transport marine invasive species from a donor to a recipient location (Ruiz et al. 1997; Padilla and Williams 2004; Lambert 2007; Clarke Murray et al. 2011; Pelletier-Rousseau et al. 2019). Despite preventive action to reduce the risk of invasions via ballast water exchange, shipping activities are predicted to be the primary force driving biological invasions by up to more than 1000% by the next 30 years (Sardain et al. 2019). If organisms survive the transport stage and viable propagules are released into a novel environment (i.e., drop-off), then the introduction of a non-indigenous species has occurred. The successful establishment of a nascent invasive population may ensue if environmental conditions at the recipient location are favourable for the survival and reproduction of introduced individuals and their descendants. However, only a low proportion of all introduced species become established and invasive (Williamson 1996). Those that do become established may later collapse as the invasive population modifies their habitat (Larson et al. 2019). Through time, geographic spread of an established invasive species may occur if (1) new areas within the range of natural or further human-assisted dispersal are susceptible to invasions (invasibility of a habitat) and (2) the invading species has characteristics that make it a fit, such as high fecundity and broad environmental tolerance (invasiveness of a species; Colautti et al. 2006; Richardson and Pyšek 2006).

Early detection and its challenges

Prevention and early detection of invasive species are vital components of managing risks and impacts because the probability of successful eradication decreases as the target invasive population increases in size and spatial extent (Myers et al. 2000; Rejmánek and Pitcairn 2002; Mehta et al. 2007). Prevention is, of course, preferred but when it fails, inevitably, invasive propagules are released. Therefore, once introduced, early detection can provide the initial warning of the presence of an introduction and an opportunity for timely eradication before population growth and range expansion (i.e., before establishment and geographic spread; Simberloff et al. 2005). Despite providing the highest return in terms of maximizing eradication success and minimizing costs in the long run, early detection (ideally followed by rapid response) is increasingly difficult with increasing rareness. As an emergent property of dispersal barriers and their ecology at the early stages of the invasion process, incipient invasive populations are, by their very nature,

rare in terms of (1) the low abundance or density of individuals and (2) the small spatial extent they colonize. Because of the low probability of encountering rare species, substantial resources are needed for early detection.

Despite high sampling effort, a sufficient population size or spatial extent of the targeted invasive population is required before their presence can be detected (i.e., *detection threshold*), which may be many years after their introduction (i.e., *lag phase*; Harvey et al. 2009; Lockwood et al. 2013; Figure 2). Although not an exhaustive list, the probability of rare species detection depends on the (1) number of individuals, (2) area or volume sampled, (3) effectiveness of sampling methods (also known as *detectability*), (4) number of sites sampled, (5) spatial and temporal distribution of the target species, and (6) design of the sampling process (Sutherland 1996; Hayes et al. 2005). Because humans necessarily handle samples, observer bias (i.e., observer error due to perception or visibility bias) will affect our ability to detect rare species. Therefore, (7) taxonomic expertise, (8) life history stage of the target species, and (9) size and morphology of the target species are additional factors that may affect the probability of species detection. Naturally, this applies to cases where specimens require species identification via conventional taxonomy based on morphological features (Stanislawczyk et al. 2018). Some ways to correct for this bias is to have multiple observers do the same data processing (either counting abundance or identifying specimens; Marsh and Sinclair 1989; Choquenot 1995). But observer bias, likewise, applies to scenarios where humans need to process individual specimens for identification via molecular approaches. Highly sensitive tools such as environmental DNA (eDNA) to detect a broad taxonomic range (i.e., both rare and common species) may have overcome some barriers limiting rare species detection (Deiner et al. 2018). Despite high sensitivity to detect a low number of individuals, sampling via eDNA is not exempt from all the other factors affecting the probability of detecting rare species such as the design of the sampling process.

Early detection, as a critical step for successful eradication and control, is a paradox for invasive species management because it is not possible until some detection threshold has been reached after some time has elapsed (Harvey et al. 2009). Unfortunately, early detection loses most of its value if there are not enough financial, logistical, and political resources for adequate monitoring activities and if early detection is not immediately followed by rapid response (e.g., eradication). The detection threshold is the combined effect of the current probability of detecting incipient invasive population (see above for factors affecting the

likelihood of detection). Thus, lowering the detection threshold would require adopting strategies to increase the probability of species detection. This can be achieved by (1) increased efficiency of the sampling method to detect rare species, (2) strategic allocation of sampling effort through space and time (e.g., enhanced sampling effort), and (3) particular consideration of the target species' ecology (Rew et al. 2006; Mehta et al. 2007; Harvey et al. 2009). Moreover, aquatic systems may have distinct challenges compared to terrestrial systems concerning early detection of invasive species. For instance, the vertical dimension (depth) is another spatial dimension in space that may substantially affect the probability of species detection if it is not correctly taken into consideration. Because water provides a relatively good medium for long-distance dispersal for a diverse group of species, monitoring invasions in remote aquatic environments (e.g., the deep sea, polar waters, remote coastal zones) is of paramount concern (Simkanin et al. 2019). However, there are numerous logistical challenges associated with accessing remote areas, such as (a) limited access due to a lack of infrastructure to launch and moor a boat and (b) prohibitively high cost of specialized training and equipment.

Pre-incursion tools, such as the identification of high-risk sites and predictive modelling, can strategically guide early detection and surveillance. To identify sites that are associated with a high risk of biological invasions (i.e., where invasive propagules are likely to be released) will depend on the target species and their primary vectors and pathways of introductions. For example, (a) seaports, harbours, and marinas, (b) airports, (c) devanning sites, and (d) natural habitats in the vicinity of these sites have all been implicated as high-risk sites for biological invasions (Brockerhoff et al. 2006; Barclay and Humble 2009, Ferrario et al. 2017, Leclerc et al. 2018). Consequently, the identification of these high-risk sites could guide early detection because the deployment of sampling stations (e.g., traps or baits) at these sites would increase the probability of detecting new incursions. Predictive modelling to evaluate the risk of invasions tends to be spatially explicit (e.g., environmental niche models) but temporal considerations can also be taken into account by incorporating future climate scenarios (Peterson and Robins 2003; Therriault and Herborg 2008). These models can inform invasive species management of geographic areas that are most susceptible to invasions by applying environmental tolerances determined from experiments done on invasive species or from data on their known range (i.e., presence data from their native and invaded range). Equipped with this information, sampling effort, subsequently, can be strategically concentrated at these areas. These models minimally require geo-referenced environmental and species' presence-only data to predict their potential distribution. For example, predictive models have mapped the habitat suitability for several prospective

marine invasive species in polar waters under current and future climate scenarios to build an effective early monitoring system (de Rivera et al. 2011; Byrne et al. 2016; Ware et al. 2016; Goldsmit et al. 2018). Ultimately, effective rapid response systems following early detection are needed to continually protect various marine ecosystems (biodiversity and habitat) and sustain maritime economies.

Where and when to sample

Because of the multiple spatial and temporal factors that can affect the probability of rare species detection, knowing where and when we wish to sample is a pivotal decision to make before any study, including monitoring, can proceed (Wiens 1989; Sutherland 1996). However, deciding where and when to sample is not a straightforward process because (a) space has multiple dimensions making sampling complex, (b) time is unidirectional (i.e., the *arrow of time*) with implications for temporal replication, and (c) relevant scales at which sampling is done across space and time may not be known in advance.

The number of samples or sample size, the size and shape of the sampling unit (*grain*), the spatial area or volume of the study area (*extent*), the sampling strategy or the layout in space (e.g., random, systematic, stratified), and the spatial distance among sampling units (*spatial lag*) are some fundamental spatial aspects in sampling that require decision-making on the part of the investigator (Fortin and Dale 2005). Ultimately, these aspects of sampling will affect the resulting spatial patterns that may be observed. Therefore, choosing the relevant size and shape of these aspects is crucial to the sampling design; otherwise, ecological trends may be missed altogether. However, there are many trade-offs among these aspects that might frustrate the investigator. For early detection specifically, these trade-offs need to be weighed against one another so that there is an overall increase in species detection and, consequently, an overall lowering of the detection threshold.

Temporal replication at a given point in space is incredibly difficult (perhaps, impossible depending on how a sampling area is defined), in part because time indiscriminately moves forwards. Therefore, pseudo-temporal replication is often employed in lieu of temporal replication in the strictest sense. A key assumption with pseudo-temporal replication is that the biotic and abiotic conditions at different moments of sampling are essentially the same. For example, the same studies carried out in different years but during the

same season might be an acceptable substitute for temporal replication because there is an expectation of stability over time (Vaughn and Young 2010). Similarly, the same studies carried out multiple times over the short-term when field conditions remain virtually unchanged might also be an acceptable substitute for temporal replication. Borne out of convenience, ecologists commonly use the *space-for-time substitution* technique as an alternative to long-term studies by inferring time across multiple sites (a) of different ages or (b) along a latitudinal gradient to predict the effects of climate change (Pickett 1989; Blois et al. 2013; but see Thomaz et al. 2012). This technique assumes that variation across space is the same as variation through time for a particular life stage of a given species. Perhaps, space-for-time substitution could be explicitly used as an alternative approach for temporal replication assuming that the different sites where the same studies are repeated are in essence the same (i.e., having no or negligible spatial variation).

Benthic and biofouling species

Invasive species are a ubiquitous part of biofouling assemblages. These assemblages, inhabited by many sessile and sedentary species and represented by a diverse range of taxa, are often unwanted by humans because they are often seen as a nuisance on wharf pilings, ship hulls, and submerged aquaculture equipment (e.g., Lambert 2007). The vase tunicate, *Ciona intestinalis*, and the zebra mussel, *Dreissena polymorpha*, are among the better-known biofouling species that have invaded Atlantic Canada and the Laurentian Great Lakes, respectively (Bossenbroek et al. 2007; Therriault and Herborg 2008; Ramsay et al. 2009; Ma et al. 2017a).

The life cycles of benthic invertebrates typically consist of a planktonic larval phase (for dispersal) and a sessile or sedentary adult phase (i.e., biphasic life history; Figure 3). Patterns of larval dispersal and settlement are predominantly influenced by several key factors, which include hydrodynamics, length of the larval period, and larval behaviour (Menge 2000; Pineda et al. 2007, 2010). At the end of the larval phase, larvae undergo metamorphosis and settlement as they transition to their adult phase (Pineda et al. 2007). Despite the liberal use of the term *settlement* and *recruitment* as synonyms in the literature, there are important distinctions to be made between these two terms. Settlement refers to the process where a larva encounters and attaches itself to a substrate (Caffey 1982). Furthermore, this process can be viewed in two steps: (1) search behaviour for a suitable substrate and (2) irreversible attachment to a substrate and

metamorphosis (Rodríguez et al. 1993). Recruitment, however, is the juvenile supply to the population as a result of initial settlement and post-settlement processes (Scheltema 1986; Thiébaut et al. 1998; Shanks 2009a).

A *settlement plate* is generally considered an efficient sampling tool to detect the presence of biphasic species (e.g., Crooks et al. 2011; Floerl et al. 2012; Marraffini et al. 2017; Tait et al. 2018). These plates consist of a hard surface, which is a substrate that larvae can encounter and settle on. Exposure of plates to the aquatic environment allows settling larvae to accumulate over time passively. Further, assemblages developing on settlement plates represent a wide diversity of taxa that closely match benthic assemblages that foul artificial structures in marinas and seaports (Marraffini et al. 2017; Tait et al. 2018). For a given study, settlement plates are often standardized in size and material to control for the effects attributed to where and when they are deployed or to experimental manipulations. However, in the literature, there exist many varieties of settlement plates (also called collector arrays, recruitment tiles, fouling panels, settling plates) consisting of different sizes (e.g., from 100 cm^2 to 1 m^2 in area) and different artificial (e.g., PVC, Plexiglas, Perspex, ceramic tiles, concrete, Correx, glass, fiberglass) and natural materials (Allen and Ferguson Wood 1950; Harms and Anger 1983; Hirata 1987; Glasby 2000; Tyrrell and Byers 2007; Darbyson et al. 2009; Valentine et al. 2016; Ma et al. 2017b, Kakkonen et al. 2019). In addition to size and material, there are myriad factors, such as orientation (horizontal *versus* vertical), roughness, and colour, that may influence larval settlement patterns, biotic interactions, and growth (Wisely 1959; Roberts et al. 1991; Thompson et al. 2002; Knott et al. 2004; Rius et al. 2010; Collin 2013; Chase et al. 2016; Ushiama et al. 2016; Tait et al. 2018).

Because settlement plates have been widely recommended for use in marine invasive species monitoring, techniques to facilitate settlement of a higher number of taxa and enhance rare species detection have been examined in many studies. Some enhancement techniques include (a) seasoning plates before deployment, (b) drilling holes or depressions into plates, and (c) roughening plate surfaces (Chase et al. 2016; Tait et al. 2016). Several studies suggest that the presence of biofilms may serve as a cue for larval settlement (Zobell and Allen 1935; Keough and Raimondi 1995, 1996; Johnson et al. 1997; Wieczorek and Todd 1997). Seasoning, where plates are immersed in filtered seawater, has been used prior to field deployment or laboratory manipulations to enhance larval settlement (Walters et al. 1999; Howes et al. 2007; Rius et al.

2010; Ma et al. 2017b). Further, holes and pits (sometimes with varying sizes and shapes) in plates provide additional microhabitats for species that prefer to recruit in crevices (Bergeron and Bourget 1986). This technique should augment the taxonomic richness (including, preferably, those that are rare) found on the plate and, perhaps, even increase the chances of survival of a higher number of species until plates are ultimately retrieved. Roughened surfaces are thought to facilitate larval settlement by providing a suitable tactile cue for attachment (Grégoire et al. 1996). However, in some species, settlement densities were reduced on roughened surfaces (Lemire and Bourget 1996). Regardless, increased surface roughness has been widely used in field studies to enhance settlement with short-term plate deployments (e.g., Hurlbut 1991a).

Objectives and thesis structure

The determination of the optimal sampling strategy to detect rare species requires an examination of as many factors in sampling that could minimize false negatives in species detection. Thus, the overarching objective is to provide a science-based toolkit on how sampling for rare species should proceed in the field with respect to detecting incipient invasive population as early as possible. This thesis addresses major themes related to early detection organized through four lines of research (across multiple scales of space and time), each corresponding to a chapter in this thesis (see Figure 4 for a global view of this thesis and key attributes associated with each chapter).

The first line of research (Chapter 1) serves to emphasize the importance of developing sampling tools that efficiently detect rare species at their early life-history stages (i.e., propagules and newly-settled recruits) when pre-settlement and initial settlement processes are taking place. In particular, this line of research aimed to evaluate the assumption that settlement reflects propagule supply (i.e., the availability of propagules; Keough and Downes 1982). Using an invasive ascidian as a model species, the supply–settlement relationship was examined in both the vertical (i.e., depth from the water surface) and horizontal dimensions (i.e., two-dimensional x–y Cartesian plane) to establish a predictive link between settlement rate and larval supply. This study demonstrates that densities of settlement on settlement plates reflect propagule supply at mesoscales within a marina.

Because temporal aspects when using passive sampling tools have not been comprehensively optimized, the second line of research (Chapter 2) explored the effect of sampling duration and sampling frequency on detecting rare species. A host of pre- and post-settlement processes, such as larval behaviour, metamorphosis, and juvenile mortality, are likely to affect the realized recruitment observed on plates (Keough and Downes 1982; Stoner 1990). In particular, seasoning and availability of space are two opposing processes that enhance and reduce the probability of larval settlement, respectively. Rare species are expected to be particularly sensitive to these processes and, therefore, determining the optimal sampling duration and frequency can enhance their detection. This line of research aimed to develop a novel experimental setup to optimize the duration and frequency of sampling for benthic species with settlement plates, at short timescales ranging from one day to about one month.

The penultimate line of research (Chapter 3) attempted to leverage seasonal variations in recruitment to inform the optimal timing for early detection. Specifically, this line of research aimed to ascertain whether the timing of peak recruitment might serve as a reliable proxy for the optimal time for early detection campaigns. Results from this field study were suggestive that high recruitment rates of marine invasive species dampen the recruitment of other species, which may have long-term impacts on the population dynamics of native species.

Finally, the fourth line of research (Chapter 4) analyzed recruitment patterns of both rare and common species at multiple spatial scales to evaluate multiple simulated sampling strategies. This line of research aimed to determine the optimal spatial scale for rare species detection, which can guide early detection. Also, findings from this study revealed the sources of spatial variation in recruitment and dominant scale of recruitment (i.e., the scale at which repeating patterns in recruitment was detected) for a wide range of marine species. Conventionally, sampling methods for invasive species monitoring are considered optimal when these methods have been maximized for taxonomic richness (e.g., Tait et al. 2018). However, this study demonstrated that the sampling strategy that maximized richness of all species (both rare and common) was not the same strategy that maximized richness of only rare species. This surprising conclusion challenges conventional optimization procedures because the resulting sampling strategies may unintentionally reduce the probability of detecting rare species.

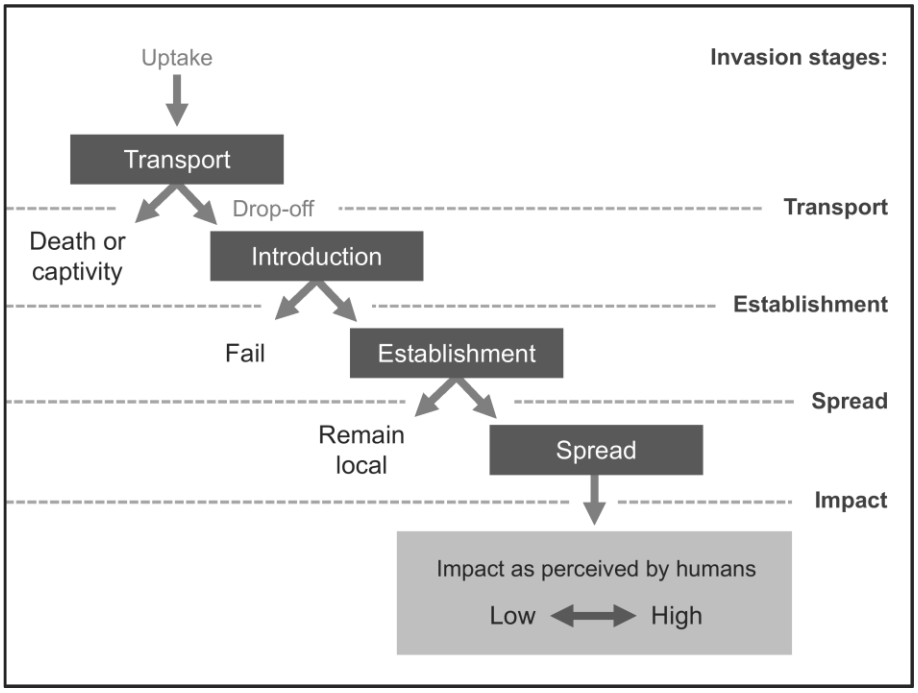


Figure 1 — Four stages of the invasion process that a species would need to undergo to become invasive and the alternative outcomes at each stage. Adapted from Lockwood et al. (2013).

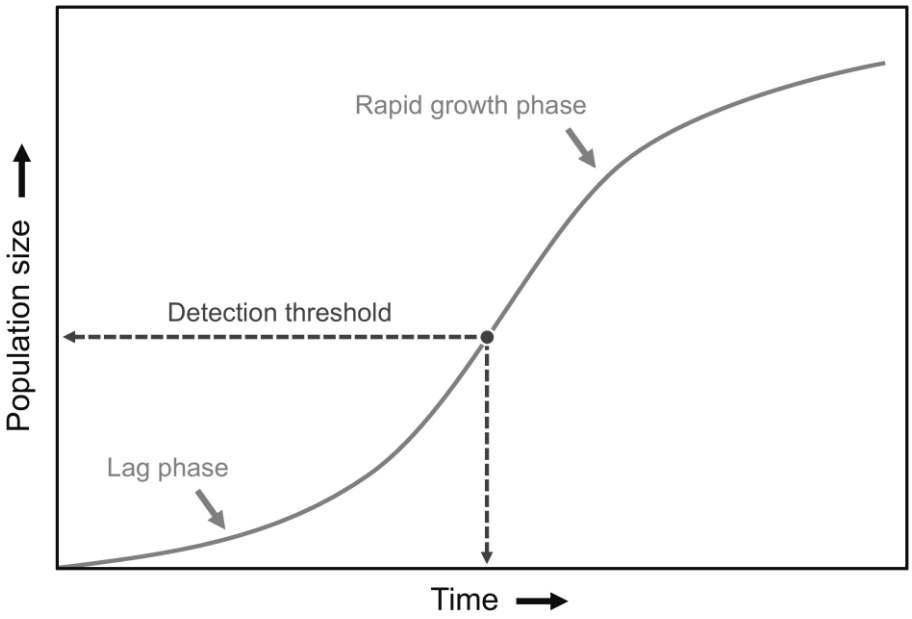


Figure 2 — Growth of an invasive population as a function of time since introduction and the threshold required for species detection. Adapted from Harvey et al. (2009).

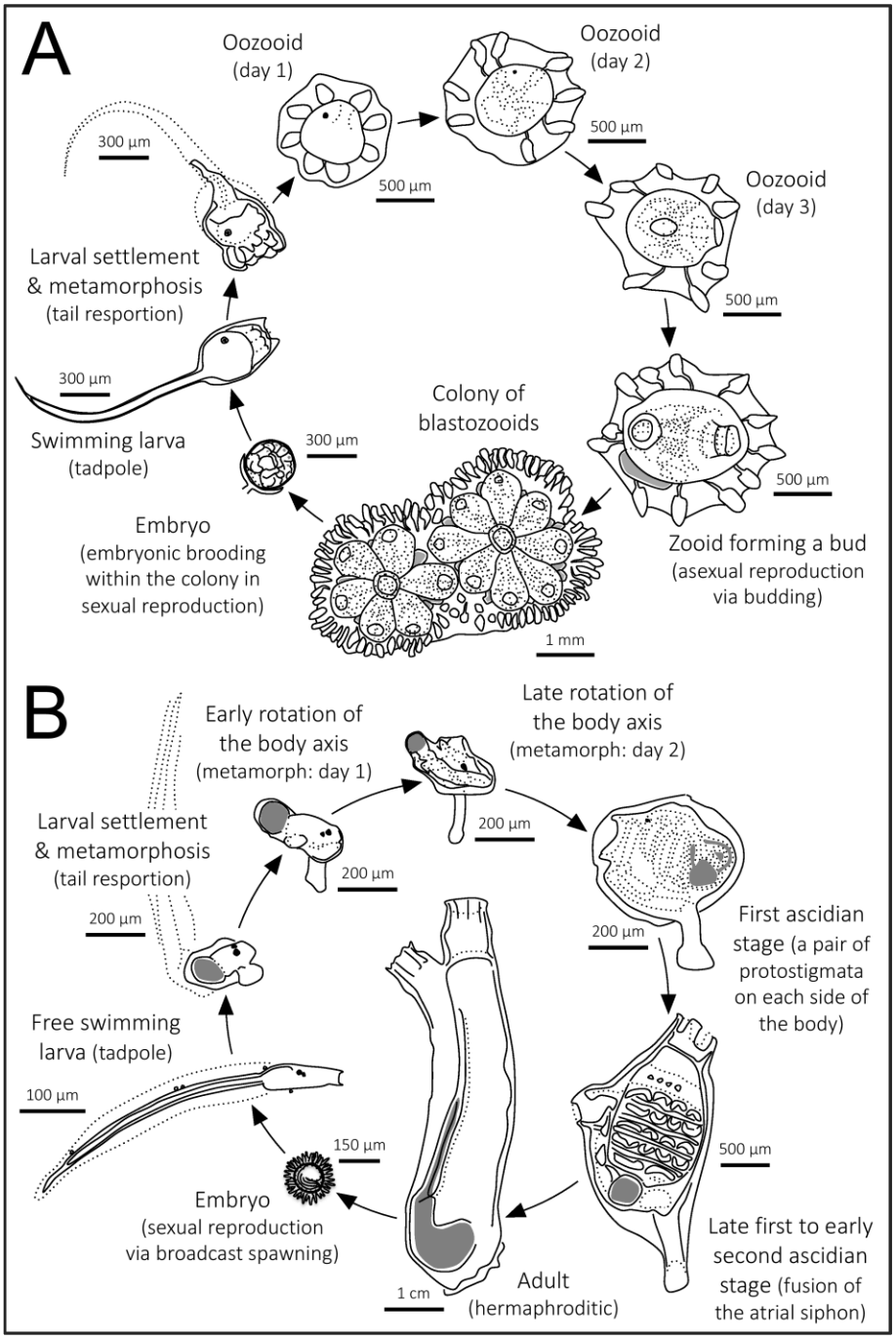


Figure 3 — The bentho-pelagic life cycles of (A) the colonial ascidian, *Botryllus schlosseri*, and (B) the solitary ascidian, *Ciona intestinalis*. Both life cycles consist of a planktonic larval phase and a benthic adult phase. See Milkman (1967) and Chiba et al. (2004) for more information on the ontogenic development of *B. schlosseri* and *C. intestinalis*, respectively. Drawings were done by K.C.K. Ma.

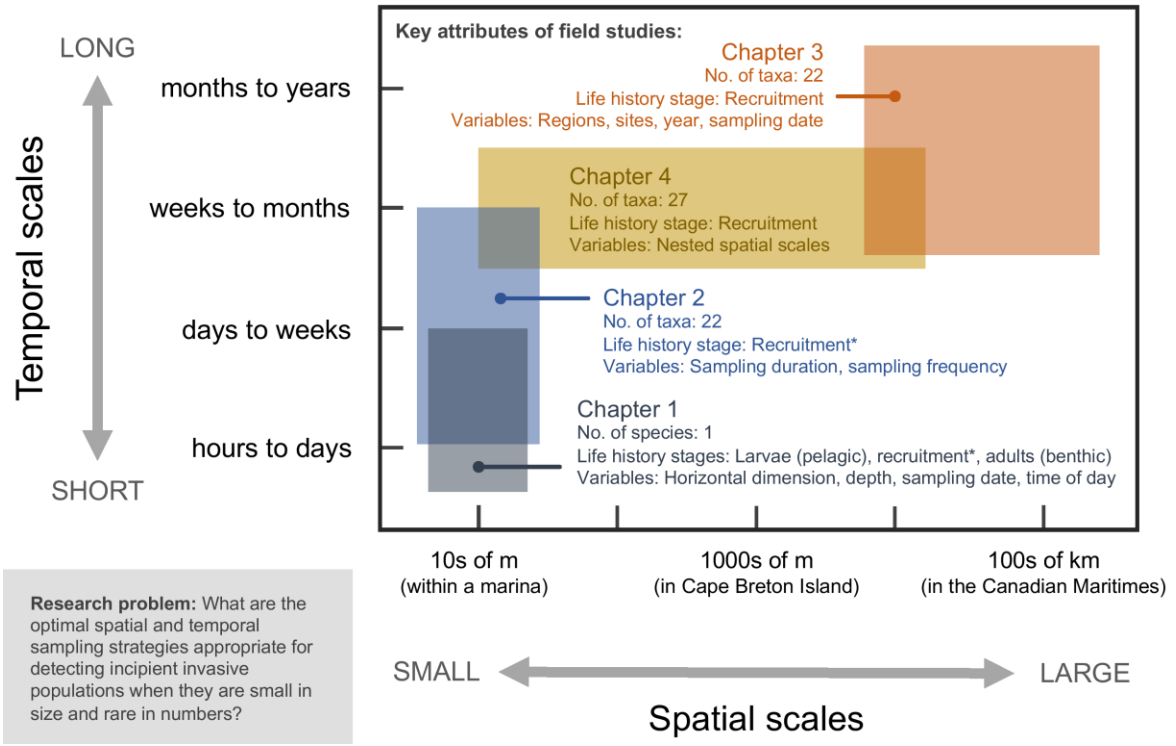


Figure 4 — Global view of the chapters (Chapter 1, Chapter 2, Chapter 3, and Chapter 4) as plotted along two dimensions: spatial scales in the x-axis and temporal scales in the y-axis. Key attributes—i.e., number of species, life history stage(s), and the focal variable(s)—that were treated for each field study (chapter) are itemized. These field studies span a broad range of relevant spatial scales, temporal scales, marine benthic taxa (representing a diversity of phyla), and life history stages to address the central research problem (as posed in this thesis). Note: Recruitment patterns observed at small timescales (* in figure) may be a better reflection of patterns of initial settlement.

Chapter 1. Larval supply is a limited determinant of settlement at mesoscales across an anthropogenic seascape

1.1 Résumé

La relation entre l'approvisionnement en larves et la fixation fait partie intégrante des processus démographiques des organismes marins benthiques qui déterminent leur répartition aux stades de vie ultérieurs. Chez les ascidies, une relation positive forte entre l'approvisionnement en larves et la fixation a déjà été documentée, mais seulement à de petites échelles spatiales (un emplacement) et à de courtes échelles de temps (moins d'un jour). Nous avons étudié comment cette relation pourrait se développer en échantillonnant l'abondance larvaire et la fixation d'une ascidie coloniale (*Botryllus schlosseri*) à travers les échelles moyennes (définies à quelques centaines de mètres) du paysage marin anthropique d'une marina de 1,5 ha. La fixation varie verticalement avec une diminution linéaire de la fixation avec une profondeur croissante. Ce patron correspond à la distribution verticale des larves le matin, quand elles sont les plus abondantes. Toutefois, l'abondance larvaire n'explique que 26% de la variation de la fixation lorsque les données sont examinées à l'échelle spatiale moyenne (c'est-à-dire, l'effet combiné des dimensions horizontale et verticale). Cet affaiblissement de la relation entre l'approvisionnement en larves et la fixation suggère que les deux processus peuvent se découpler à des échelles spatiales et temporelles plus grandes. Notre étude montre à quel point le changement de l'échelle de l'échantillonnage peut affecter notre compréhension des processus de dispersion et de fixation des larves.

1.2 Abstract

The relationship between larval supply and settlement is an integral part of the demographic processes of benthic marine organisms that determine their distribution at subsequent life stages. In ascidians, a strong (positive) relationship between larval supply and settlement has been previously documented, but only at small spatial scales (one location) and short time scales (less than one day). We investigated how this

relationship might scale up by sampling larval abundance and settlement of a colonial ascidian (*Botryllus schlosseri*) across mesoscales (defined as ca. 100s of m) of the anthropogenic seascape of a 1.5-ha marina. Settlement varied vertically with a linear decrease in settlement with increasing depth. This pattern corresponded with the vertical distribution of larvae in the morning when they were most abundant. However, larval abundance explained only 26% of the variation in settlement when the data were examined at the meso-spatial scale (i.e., the combined effect of the horizontal and vertical dimensions). This weakening of the relationship between larval supply and settlement suggests that the two processes may become decoupled at larger spatial and temporal scales. Our study underscores how changing the scales of sampling may affect our understanding of larval dispersal and settlement processes.

1.3 Introduction

Larval dispersal and settlement are key demographic processes for benthic marine organisms. For benthic invertebrates with biphasic life cycles (i.e., involving the dispersal of larval stages in the water column), recruitment of new individuals to the benthic adult population depends fundamentally on larval supply. However, until the 1980s, few studies had evaluated the processes and influence of the distribution and abundance of planktonic larvae on the dynamics of adult populations, i.e., supply-side ecology (Connell 1985; Lewin 1986; Roughgarden et al. 1987; but see Young 1987, 1990). Since then, further research has been fuelled by a keen interest in determining the role of larval supply on both recruitment rates and subsequent population dynamics of adult stages and benthic assemblages (Underwood and Fairweather 1989; Pineda et al. 2010). Much of this work has, however, focused on sessile species with planktotrophic larvae, which typically spend weeks in the plankton feeding and dispersing. Barnacles are by far the most well-studied group in this regard and between 75 and 87% of the variation in their recruitment has been explained by larval supply over many different spatial scales, including small spatial scales of < 10s of m (Miron et al. 1995), mesoscales of 10s to 100s of m (Gaines et al. 1985; Minchinton and Scheibling 1991; Yund et al. 1991), and large scales of 1s to 10s of km (Todd et al. 2006; Broitman et al. 2008).

In contrast to long-distance dispersing species such as barnacles, less is known about the relationship between larval supply and settlement in species with lecithotrophic (i.e., non-feeding) larvae, which spend a considerably shorter time in the plankton. Ascidians are the exception in part because larvae can be cultured

without much difficulty for laboratory experimentation (Pineda et al. 2012), and competency to settle may occur within hours of larval release (Millar 1971; Boyd et al. 1986; Svane and Young 1989; Marshall et al. 2003). Thus, *in situ* sampling for ascidian tadpole larvae should be a good approximation of larval supply just before settlement.

The supply–settlement relationship in ascidians (especially in brooding species) is also expected to be relatively strong because they are typically characterized as closed populations with short-distance dispersing larvae (Olson 1985, Davis and Butler 1989; Stoner 1990; Bingham and Young 1991; Petersen and Svane 1995; Yund and Stires 2002). Coupling of larval abundance and settlement should thus occur at small spatial (< 10s of m) and short temporal (< 1 day) scales. However, large-scale ecological surveys and monitoring activities, such as invasive species surveillance, are often done at substantially larger spatial scales (Sephton et al. 2011; McKenzie et al. 2016; Simkanin et al. 2016). Thus, it is critical to examine how well the supply–settlement relationship can be "scaled up" to meso- (and perhaps larger) spatial and temporal scales to reconcile small-scale observations with large-scale patterns (White and Caselle 2008). Increasing spatial and temporal scales is expected to increase variation in larval supply and settlement due to scale-dependent responses to biotic and abiotic processes (e.g., competition, local hydrodynamics; Menge 2000; Kinlan and Gaines 2003; Pineda et al. 2007, 2010). In ascidians, scale-dependent sources of variation on the supply–settlement relationship will affect a relatively narrow range of relevant scales due to their short larval life and dispersal ability. Hence, ascidians are more amenable for examinations of larval and settlement processes because contrasts among scales can be made across a smaller range of scales.

Surprisingly, it appears that only one study (Hurlbut 1991b, 1992) has evaluated the strength of the relationship between supply and settlement by measuring ascidian larval abundance and settlement simultaneously. In this study, 92% of the variation in settlement was explained by larval supply when sampling was done over a narrow range of depths at a single location over short temporal scales (< one day). Other studies have sampled for both ascidian larval abundance and settlement separately (Bourque et al. 2007), only larval abundance (Manríquez et al. 2016; Kanamori et al. 2017), or only settlement (Ma et al. 2017b; Casso et al. 2018). Further, most of these studies were done only at small (< 10s of m) spatial scales (but see Manríquez et al. 2016). Thus, it is not known if the strong relationship between larval abundance and settlement at small scales is equally applicable when increasing from the smallest spatial scale (i.e., one

sampling location) to meso-spatial scales of 10s and 100s of m and from short (i.e., every two hours) to meso-temporal scales of < one week.

Spatially, one also must distinguish between vertical and horizontal dimensions as processes affecting larvae differ between them (Pineda et al. 2007). For ascidians, larval behaviour and, in particular, settlement are likely to respond more strongly to cues that vary with depth (e.g., light attenuation; Stoner 1990, 1992; Manríquez and Castilla 2007), which could decouple larval supply from settlement. In contrast, local hydrodynamic conditions (e.g., currents, winds) are expected to influence horizontal variability in larval supply more than larval behaviour, especially in short-distance dispersing species such as ascidians (Johnson 1985; Olson 1985; Koehl 2007). Consequently, a stronger relationship between larval supply and settlement should be expected in the horizontal dimension than in the vertical dimension.

Here, we examined the relationship between supply and settlement in a closed population of the colonial ascidian *Botryllus schlosseri* over relevant vertical and horizontal scales, i.e. the scales over which the population is demographically closed. Spatially, we sampled for larvae and settlement such that each larval abundance datum was paired with a settlement rate datum. Given the strong supply–settlement relationship observed previously in ascidians and other invertebrate groups, we expected to find a similarly strong (positive) relationship at the mesoscales consisting of multiple sampling locations across an anthropogenic seascape. We expected that this relationship would be strongest in the horizontal dimension where larval behaviour would have less influence.

1.4 Materials and methods

1.4.1 Settlement

Settlement plates were used to document spatial patterns of *B. schlosseri* settlement in Ben Eoin marina (45.9818°N, 60.4314°W). The marina basin, approximately 1.5-ha with a maximum depth of 4 m, is located in East Bay of the Bras d'Or Lake, Nova Scotia, Canada (Figure 5). Settlement plates (14.6 × 14.6 cm, dark grey polyvinyl chloride [PVC] with one side roughened with a hand-held belt sander) were suspended

horizontally in the water column from floating docks at six depths (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 m below the water surface) with the roughened surface facing downward (Figure 6). Unlike many studies where plates are "stacked" in a depth series at each location (e.g., Ma et al. 2017b), only one plate was deployed at each location with the treatment *Depth* randomly assigned to ensure spatial independence of sampling locations. Each plate was attached to a brick (four small holes in the plate were used to attach it to a brick with cable ties) suspended at the end of a rope (Supplemental Figure S1). Plates were deployed on 24 September 2014 during the seasonal period of high settlement rates (K.C.K. Ma, *unpubl. data*; also see Chapter 3). There were 12 replicates for each depth for a total of 72 plates at 72 locations, distributed across the marina basin with a minimum 1-m separation between adjacent plates. Seven days after deployment, the number of *B. schlosseri* recruits on the downward facing surface of each plate was counted immediately after plate retrieval. Recruits were easily visible without magnification. Given the short deployment period and lack of any obvious source of mortality, recruitment patterns were assumed to reflect initial settlement patterns (e.g., Collin and Johnson 2014; but see Keough and Downes 1982); therefore, the recruitment of 7-d old *B. schlosseri* recruits (life stages: oozooids and first blastozooids) on plates are referred to as settlement in this study.

1.4.2 Larval abundance

Plankton samples were collected at half of the plate locations (time constraints prevented sampling at all locations) to document the spatial and temporal variation in larval abundance. 100-L seawater samples were collected using a 4-m long hose attached to a diaphragm pump. Sampling was conducted immediately adjacent (i.e., 20 to 30 cm in the horizontal dimension) to settlement plates at 1.0, 2.0, and 3.0 m depths so that larval abundance and settlement were sampled at almost the same location and depth (± 10 cm in the vertical dimension; Figure 6). After collection, water samples were filtered with a 74-µm mesh sieve, immediately preserved in 20% ethanol, and *B. schlosseri* larvae counted using a stereomicroscope (Figure 7). Sampling was done on three consecutive days (28 to 30 September 2014), to account for diel variability, with a total of 36 plankton samples collected each day between 10h30 and 19h15 (i.e., a total of 108 samples). The order of collecting plankton samples from the 36 locations was randomized each day to minimize potential bias of diurnal variation in larval release or behaviour.

1.4.3 Benthic adults

The abundance of *B. schlosseri* was estimated on the vertical sides of floating docks (hard plastic) and the undersides of berthed boats on 27 September 2014 while snorkelling in the upper one metre of the water column. The percent cover of colonies was determined semi-quantitatively (categories: not detected, < 5%, 5 to < 25%, 25 to < 50%, 50 to < 75%, and 75 to 100%) for 20 dock sections (area: approximately 0.5 m^2) and 20 boats (length ranging from 6 to 11 m).

1.4.4 Environmental data

Sensors (INW CT2X) were deployed at each of the six depth treatments to record seawater temperature and salinity values every 15 minutes on the same days when plankton samples were collected (28 to 30 September). All six sensors were attached to a single line hanging off a floating dock near the geographic centre of the marina basin. Sensors were calibrated prior to deployment. During the study period, water temperature and salinity displayed little variation (mean [range]: 16.3°C [15.6 to 17.0] and 20.2 psu [19.7 to 20.6], respectively). At any given time, differences among depths never exceeded 0.46°C or 0.39 psu.

1.4.5 Statistical analyses

Settlement and depth: the effect of depth *(Depth)* on settlement was evaluated using a one-way analysis of variance (ANOVA). A Tukey's honestly significant difference (HSD) *test* was performed to determine differences among depth treatments.

Larval abundance and depth: an analysis of covariance (ANCOVA, generalized linear model [GLM] procedure with Poisson error distribution) was used to evaluate the effects of depth (*Depth*), and day of sampling (*Date*) on larval abundance while accounting for the time of day when samples were taken (*Time of day*). A local regression (LOESS, span = 0.7) curve was applied to evaluate intra-diurnal patterns by showing trends in larval abundance over time of day (data pooled from all three depths and all three sampling days). The larval abundance dataset was then partitioned into three temporal categories (morning, afternoon, and

evening; n = 36 data points per time category), and the effect of *Depth* on larval abundance assessed for each of the partitioned datasets by an analysis of variance (ANOVA, GLM procedure with Poisson error distribution). A Tukey's HSD test was performed to determine differences among the three depth treatments. For colonial ascidian species, abundance in the water column is expected to peak after larval release earlier in the day (i.e., light induced larval release) and then to decrease over the course of the day as larvae undergo settlement or die (Svane and Young 1989; Hurlbut 1992; Forward et al. 2000). Given this expectation, the relationship between time of day and larval abundance was evaluated using linear regressions to show the predictive link between the two variables.

Larval abundance and settlement: the relationship between larval abundance and settlement (data pooled from all three depths and all three sampling days) was also assessed using linear regressions, but an additional non-parametric test (Spearman rank correlation) was used to evaluate the strength of the relationship while taking into account the non-normal distribution of larval data. To visualize the spatial distribution of deviations within the marina, the deviation values (percent deviation of recorded values from those predicted from the pooled regression) were plotted with a colour contour plot.

Horizontal distributions: horizontal variations in (i) settlement, (ii) larval abundance (mean over the three days of sampling), and (iii) benthic adults were visualized using colour contour plot graphs. Because settlement varied with depth (see Results below), settlement data for locations where plates were at 0.5, 1.5, 2.0, 2.5, and 3.0 m were normalized to those of the 1-m depth. ANOVAs and ANCOVAs (GLM procedures based on type III sums of squares) were computed with IBM SPSS (Version 25). All other statistical analyses were performed in the R programming environment (R Core Team 2019).

1.5 Results

1.5.1 Vertical variability in settlement

Settlement varied significantly as a function of depth, explaining about 31% of the variation in settlement (ANOVA, $F_{(5,66)} = 6.00$, $p < 0.001$). Over the 2.5 m depth range (from 0.5 to 3.0 m), settlement exhibited

ca. 35% decrease with increasing depth (Figure 8). Although the overall relationship appeared linear down to a depth of 2.5 m, settlement was only significantly greater at 0.5 m compared to the three deepest depths (2.0 to 3.0 m; Figure 8).

1.5.2 Temporal and vertical variability in larval abundance

Although larvae were detected at all times during the day during the sampling period, their intra-diurnal abundance varied significantly (i.e., significant covariate of *Time of day*; Table 1), such that they were more abundant in the morning (Supplemental Figure S2). Nevertheless, time of day explained only 15% of the variation in larval abundance ($R^2 = 0.15$, $F_{(1,106)} = 18.41$, $p < 0.001$). Overall, no statistically significant vertical or day-to-day variation in larval abundance was detected (ANCOVA GLM; Table 1), although some variation among days can be seen graphically (Supplemental Figure S3). However, larvae were more often detected (i.e., presence vs. absence) at shallower depths (81% of the samples taken at 1.0 m, 67% at 2.0 m, and only 56% at 3.0 m). Moreover, after partitioning into time categories (morning [between 10h30 and 13h10], afternoon [between 13h10 and 16h15], and evening [between 16h15 and 19h15]), larva abundance did exhibit significant vertical variability in the morning but not in the afternoon and evening (ANOVA GLM, $p = 0.026$; Table 2), when larval abundance was greater at depths of 1.0 and 2.0 m compared to 3.0 m (Figure 9).

1.5.3 Relationship between larval abundance and settlement

Larval abundance (mean values of the three sampling days) and settlement were positively correlated when data from all depths were pooled (Spearman rho = 0.52, $p = 0.001$, $n = 36$). However, larval abundance explained only 26% of the variation in settlement ($R^2 = 0.26$, $F_{(1,34)} = 12.25$, $p = 0.001$; Figure 10). The supply–settlement relationship was slightly stronger when larval abundance was $\log_{10}(x + 1)$ transformed ($R^2 = 0.30$, $F_{(1,34)} = 9.15$, $p = 0.005$). Further, a logarithmic regression on the untransformed data yielded similar results ($R^2 = 0.27$, $F_{(1,34)} = 12.29$, $p = 0.001$). Moreover, when separated by depth, there was only a significant linear relationship between supply and settlement at the 2.0-m depth ($R^2 = 0.37$, $F_{(1,10)} = 5.81$, $p = 0.037$, $n = 12$). Visual inspection of residual plots of untransformed data showed random patterns of

residuals, which indicates a good fit with a linear model. Notably, the weekly settlement on the plates (a mean of 176 recruits plate^{-1} or 0.83 recruits cm^{-2}) corresponded to the number of larvae that can be found in approximately 10 m^3 of water (i.e., 0.017 larvae L^{-1}).

1.5.4 Horizontal patterns within the marina

Settlement, larval abundance in the water column (mean over the three days of sampling), and percent cover of adults varied spatially across the marina (Supplemental Figure S4, Supplemental Figure S5, and Supplemental Figure S6). Locations of greater settlement rates (Supplemental Figure S4) and larval abundance (Supplemental Figure S5) were located in the western end of the marina basin (around Dock 1) with an average rate of 0.15 ± 0.02 recruits cm^{-2} d^{-1} (n = 18 settlement plates; ± one standard deviation) and 2.4 ± 2.5 larvae 100 L^{-1} (n = 30 plankton samples), compared to 0.12 ± 0.03 recruits cm^{-2} d^{-1} (n = 30) and 1.6 ± 1.7 larvae 100 L^{-1} (n = 39) around Dock 2 and 0.11 ± 0.03 recruits cm^{-2} d^{-1} (n = 24) and 1.2 ± 1.9 larvae 100 L^{-1} (n = 39) around Dock 3. However, a greater abundance of adults was concentrated on the southern portion of Dock 2 and the eastern end of the marina around Dock 3 (Supplemental Figure S6). Moreover, there appears to be an inverse relationship in the horizontal dimension between the spatial distributions of larval abundance and adults, such that larvae were found where adults were absent.

The spatial distribution of deviations between observed and predicted values of settlement (based on the linear regression shown in Figure 10) varied among docks. The western end of the marina around Dock 1 had an average of 16.1% more settlement than predicted (n = 10; Figure 11) whereas the eastern end (Dock 3) had an average of 13.5% fewer than predicted (n = 13). Dock 2, located in the middle had almost no deviation, had on average 0.6% more than predicted (n = 13). However, variation within each dock was observed but was most striking on Dock 3, where deviations ranged from 57.4% to –43.0%. Indeed, due to this variation, no significant relationship between larval abundance and settlement at finer spatial scales (i.e., < mesoscales of 100s of m) was found when the data were partitioned and analyzed by floating dock (i.e., Docks 1, 2, and 3).

1.6 Discussion

1.6.1 Vertical patterns

Our expectation that larval supply should influence the rate of settlement was observed at mesoscales within a marina. We found that larval supply explained only some of the variation in local settlement (i.e., only 26%) in this demographically-closed ascidian population. Although a positive relationship between larval supply and settlement was indeed observed at mesoscales, this finding suggests that larval supply has a more limited role as a predictor of small-scale and mesoscale settlement patterns than is widely believed or that changes to the spatial scale contributes to substantial variability on these patterns. This result is particularly surprising given that this ascidian species has a short larval period (and, consequently, short dispersal distance) and that the primary source of larvae originates from the adult population of the marina basin (i.e., essentially a closed population; Olson 1985; Davis and Butler 1989; Petersen and Svane 1995; Yund and Stires 2002; Collin et al. 2013).

In spite of this weak yet compelling relationship, given the day-to-day variability in the horizontal and diurnal distribution of larvae (Supplemental Figure S3), larval supply appears to play a role in determining settlement patterns with respect to depth. Conceptually, in the vertical dimension, the link between larval supply and settlement can be generalized into four models depending on the combined effects of the vertical distribution of larvae (stratified or non-stratified) and larval behaviour just prior to settlement (selective or non-selective). First, the *non-stratified-non-selective behaviour model* (Figure 12, Panel A) represents the pattern in which larvae inhabit the entire depth range without any vertical stratification in abundance and settle non-selectively on suitable substrata. This model is essentially the null expectation. Second, the *non-stratified-selective behaviour model* (Figure 12, Panel B) represents the pattern in which larvae inhabit the entire depth range without any vertical stratification in abundance but settle selectively on suitable substrata at specific depths. Third, the *stratified-non-selective behaviour model* (Figure 12, Panel C) represents the pattern in which larvae inhabit specific depths resulting in vertical stratification in abundance and settle non-selectively on suitable substrata. Finally, the *stratified-selective behaviour model* (Figure 12, Panel D) represents the pattern in which larvae inhabit specific depths resulting in vertical stratification in abundance but settle selectively on suitable substrata at other depths.

These models can be applied to our data, but they depend on the spatial or temporal scale at which the data are examined. When all data were combined, there was no significant effect of depth on larval abundance. Likewise, if the analysis was limited to settlement data for the three depths where larval abundance data were collected (i.e., 1.0, 2.0, and 3.0 m), there were no statistically significant differences in settlement among these depths. Thus, the pattern would correspond to the one described in the *non-stratified-non-selective behaviour model* (the null expectation). However, using the settlement data from all six depths (i.e., at a finer spatial scale), analysis of the data reveals a decrease in settlement with depth that is visually apparent over the depth range (Figure 8). This pattern corresponds better to the *non-stratified-selective behaviour model*. Finally, if the analysis is restricted to a finer temporal scale, certain situations (i.e., morning) occur when both larval supply and settlement are greater at shallower depths—a pattern corresponding to that of the *stratified-non-selective behaviour model*. This latter model represents the scenario such that one might expect and observe a positive supply–settlement relationship, i.e., the essential condition underlying the conceptual basis of supply-side ecology (Lewin 1986; Roughgarden et al. 1987). However, we were only able to observe this association in a limited set of our data. Interestingly, given the sampling design (i.e., larval abundance and settlement were measured at different temporal and spatial scales), it only explained approximately a quarter of the observed variation given spatial variation in larval supply and recruitment within the marina and temporal variation in larval supply among days.

Several aspects of this system support the idea that both larval supply and settlement should be higher near the water surface. Larvae should have initially been more abundant in shallower water as most adults were found growing in shallow depths on the undersides of floating docks and berthed boats (Supplemental Figure S6). Deeper populations were restricted to the chains used to anchor the docks or on the deeper rocks used for breakwaters. Outside the marina basin, *B. schlosseri* colonies were rare and only occasionally found encrusting eelgrass (K.C.K. Ma, *pers. obs.*). Larval behaviour should also contribute to a higher concentration of larvae in shallower depths (Hurlbut 1991b; Stoner 1992) as ascidian larvae are typically positively phototactic (and, for some species, negatively geotactic), at least immediately after their release (Grave and Woodbridge 1924; Crisp and Ghobashy 1971; Boyd et al. 1986; Svane and Young 1989). Further, the calm conditions usually found inside the marina (due to the protective breakwaters and the low-amplitude of tides in the Bras d'Or Lake; Desplanque and Mossman 2004) likely limited water column mixing, thereby preserving any vertical pattern created by the distribution of adults and larval behaviour.

Finally, there were no indications of abundant levels of ascidian predators (e.g., caprellid amphipods, fish, gastropods, sea stars) that might otherwise prey on larvae, new recruits, and adults (Millar 1971; Young and Chia 1984; Olson and McPherson 1987; Epelbaum et al. 2009; Whitlatch and Osman 2009; Collin and Johnson 2014; Rius et al. 2014).

If *B. schlosseri* settlement patterns were driven solely by larval supply, then a variable vertical distribution of larvae (i.e., the *stratified-non-selective behaviour model*) would be the simplest explanation for our observed pattern of greater settlement in shallower depths. However, the resolution of settlement patterns was much greater than that for larval abundance (six depths instead of three), and the decrease in settlement with depth occurred at depths between 0.5 and 2.0 m, where there were no differences in larval abundance (i.e., 1-m *versus* 2-m depths). At this finer scale of 0.5 m intervals, larval behaviour may be driving the higher settlement at shallower depths, including the very high settlement at the shallowest depth of 0.5 m. In particular, the general pre-settlement behaviour of ascidian larvae is negatively phototactic, not for swimming to greater depths, but rather to seek dark microhabitats (e.g., crevices, under ledges; Grave and Woodbridge 1924; Crisp and Ghobashy 1971; Young and Chia 1985; but see Forward et al. 2000). Larvae are sensitive to changes in light intensity (e.g., shadows), and abrupt changes may initiate settlement behaviour. For example, a light-to-dark transition has been shown to be a settlement cue for the solitary ascidian, *Ciona intestinalis*, as settlement patterns from an *in situ* experiment suggest that larvae swim upwards toward dark surfaces upon experiencing this cue (Collin 2013; also see Svane and Young 1989). Such shade-seeking behaviour should thus be more pronounced at shallower depths where light levels are higher, and thus contrasts are more dramatic when transitioning from light to dark (Olson 1983; Svane and Young 1989; Hurlbut 1993). In this study, our settlement plates were a distinct source of shade for larvae during the daytime. As light attenuation increases with increasing depth, the contrast between light and shade (under the plates) is likely to decrease with increasing depth, in turn, affecting the strength of light as a settlement cue. This mechanism would thus explain the observed linear decrease in settlement with increasing depth, especially at the shallowest depths.

1.6.2 Horizontal patterns

Horizontal variability in ascidian larval supply was observed at the mesoscale (i.e., among docks). While not surprising in of itself, this variation should be linked logically to both the underlying sources of larvae (i.e., the distribution of reproducing adults) and transport after release (i.e., hydrodynamic conditions; Olson 1985). Given the short ascidian larval period (typically less than a day; Grave and Woodbridge 1924; Miller 1971), the lack of pronounced tidal currents (i.e., relatively calm waters; Desplanque and Mossman 2004), and the semi-enclosed nature of the marina basin (i.e., a closed population), we expected horizontal variation to be driven by the distribution of the adult population. However, this does not appear to be the case as a visual examination reveals the highest adult abundance in the eastern portion of the marina, whereas the highest settlement occurred in the western portion. This spatial pattern could be explained by wind-forced hydrodynamics (e.g., Johnson 1985). For example, near-shore wind from the west drives the surface waters to transport larvae downwind in the easterly direction. Data from the nearest weather station (Eskasoni, about 13.5 km from the marina) show that the prevailing (morning) wind was blowing from the west for the first five days then from the east for the remaining two days of the 7-d study period (Environment and Climate Change Canada 2019). Thus, larvae would accumulate in the eastern side of the marina resulting in an uptick in settlement rate assuming that the influence of Ekman transport on larvae is small over these shallow depths (i.e., a small z value).

Ascidian settlement, and subsequent recruitment patterns, also exhibited spatial variability in the horizontal dimension (e.g., Yund and Stires 2002; Manríquez et al. 2016). This variation, according to supply-side ecology, should have been driven by larval abundance. However, the link between larval supply and settlement was likewise weak when examined over the larger horizontal dimensions within the marina basin. While these anomalies (both above and below expectations) occurred all across the basin, they were clustered towards the eastern side of the basin. The decoupling of settlement from larval supply may be a consequence of (1) sampled larvae not all being competent to settle, (2) a mismatch in the timing of our larval sampling (a 9-hr period of the day) that may have missed any early morning peaks, and or (3) the three days of larval sampling did not adequately represent the seven days of settlement activity on plates (see Pineda et al. [2010] for more on the causes of decoupling). The effects of local hydrodynamics and larval behaviour on dispersal are likely to play a role in the horizontal distribution of competent larvae in the water

column (Olson 1985; Pineda et al. 2010). Although it is not clear why observed settlement rates in certain areas in the marina deviated so greatly from values predicted from larval supply, it is likely that we would have found a stronger relationship between larval supply and settlement if our sampling had been done at smaller spatial and temporal scales as in earlier studies (Hurlbut 1991b, 1992). The observed spatial variation in larval supply and settlement at mesoscales within the marina suggests that certain patterns, such as vertical zonation in larval supply, are better observed at small scales.

Our results stand in contrast to the only other study examining the supply–settlement relationship in a colonial ascidian (Hurlbut 1991b, 1992), which documented a strong relationship between larval supply and settlement at the smallest scale possible—within a single sampling location—with intensive temporal sampling (every two hours at each of three depths; Hurlbut 1991b, 1992). In our study, the vertical range and number of data points (36 in this study *versus* 48 in Hurlbut [1991b, 1992]) used for the linear regression analysis were similar, but the horizontal and temporal scales were somewhat expanded. The increase in spatial scales likely contributed to a loss of predictability as the supply–settlement relationship weakened as the horizontal scale increased from small scales to mesoscales of ca. 100s of m. The weakened relationship may, however, merely be an artifact of differences between different species (*Botryllus schlosseri versus Didemnum candidum*) and differences between sites (Nova Scotia *versus* Hawaii). Likewise, low larval abundance could also contribute to a weakened supply–settlement relationship because the efficacy of sampling methods (e.g., tools, spatial scales of sampling, temporal scales of sampling) may be biased against detecting rare specimens (Pineda et al. 2010). Although we sampled during the seasonal peak of settlement, larval abundance was relatively low compared to other studies (maximal larval abundance can be up to 10 larvae 100 L^{-1} [Kanamori et al. 2017] or 56 larvae 100 L^{-1} [Bourque et al. 2007]). In particular, larval abundance (2 *versus* 28 larvae 100 L^{-1}) and near-surface settlement rate (0.15 *versus* 1.06 recruits cm^{-2} d^{-1}) were both an order of magnitude lower in our study compared to Hurlbut (1991b).

1.6.3 Conclusions

Larval supply and settlement exhibited vertical zonation at mesoscales across an anthropogenic seascape for this closed ascidian population. However, vertical zonation in larval abundance was not maintained throughout the day, and depending on the scale at which the data are viewed, the influence of larval supply

on settlement can be understood based on different combinations of vertical distribution patterns of larvae (e.g., stratified and non-stratified) and larval behaviours prior to settlement (e.g., selective and non-selective). Although these combinations of patterns (and the corresponding conceptual models) provide some insight on how larval supply and larval settlement behaviour affect settlement, we conclude that the larval supply–settlement relationship was still relatively weak in the vertical dimension. This relationship was likewise found to be weak in the horizontal dimension, which was inconsistent with our original assumption that a similarly strong supply–settlement relationship could be scaled up to mesoscales. This finding is particularly surprising for such a short-distance dispersing species in a semi-enclosed marina basin (i.e., essentially a closed population) because these conditions should logically promote a strong relationship between supply and settlement. Nevertheless, the supply–settlement relationship is evident given that three days of larval data could explain at least 26% of the variance in settlement determined at a larger temporal scale (seven days). This study also demonstrates that the short-term use of settlement plates might be a suitable method, and at best a coarse method, of estimating larval supply (and, further, a better tool to estimate the supply of competent larvae). Settlement plates are likely to substantially increase the probability of detecting *B. schlosseri* and other biphasic benthic invertebrates at low concentrations because settlement of recruits on plates is integrated over time. Further, the results of this study can guide early detection and surveillance of *B. schlosseri* and other invasive ascidians. For example, high vertical and horizontal variability in settlement at mesoscales within the marina suggests that plates should be suspended at shallow depths in the water column and spread spatially within the marina (and not clustered at just one part of the marina) to maximize the chances of species detection.

Table 1 — Results of an analysis of covariance (ANCOVA, GLM procedure with Poisson error distribution) testing the effects of *Depth*, *Date*, and *Depth* × *Date* on *Botryllus schlosseri* larval abundance with *Time of day* as the covariate. Statistically significant p values are **bolded**.

Source	df	Wald χ^2	p
(Intercept)	1	39.025	< **0.001**
Time of day	1	26.775	< **0.001**
Depth	2	3.360	0.186
Date	2	0.087	0.957
Depth × *Date*	4	7.144	0.128

Table 2 — Results of three partitioned analyses of variance (ANOVA, GLM procedure with Poisson error distribution) testing the effect of *Depth* on *Botryllus schlosseri* larval abundance in the morning, afternoon, and evening. Statistically significant p values are **bolded**.

Partitioned GLM	Source	df	Wald χ^2	p
Morning	(Intercept)	1	46.672	< **0.001**
	Depth	2	6.225	**0.044**
Afternoon	(Intercept)	1	3.997	**0.046**
	Depth	2	4.685	0.096
Evening	(Intercept)	1	0.124	0.725
	Depth	2	0.391	0.822



Figure 5 — Oblique aerial view of the marina in Ben Eoin, Nova Scotia, Canada. At that time of the study, the marina had a total of 75 berths and the entrance channel was ca. 30 m wide. Image source: http://www.beneoinmarina.com/.

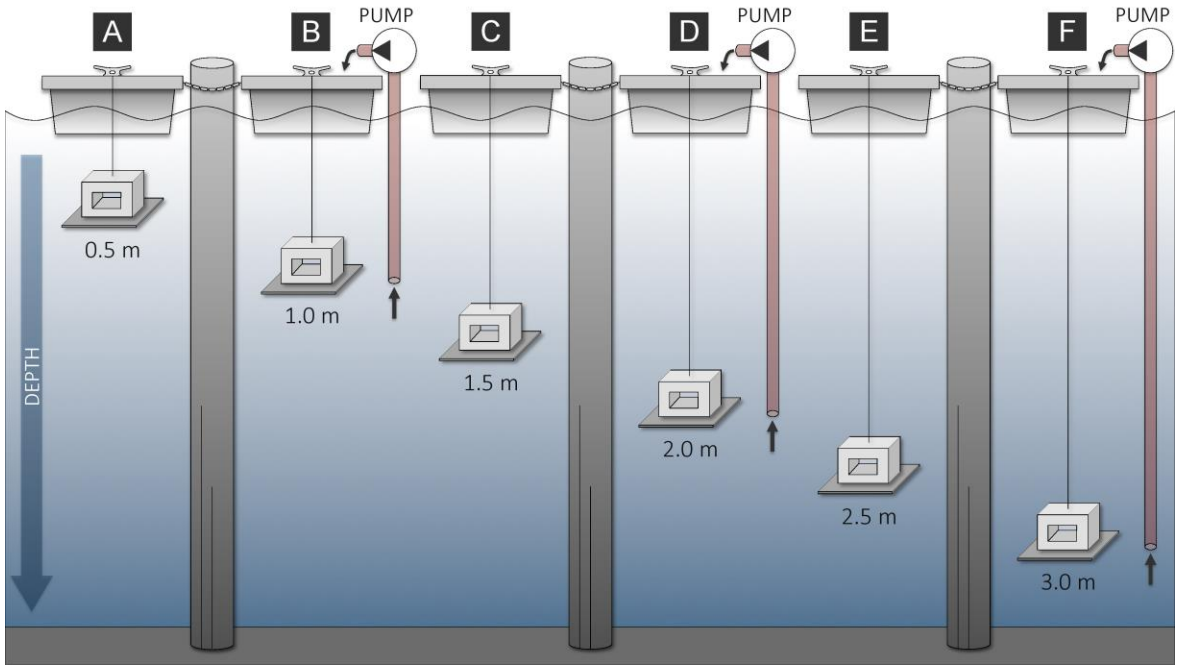


Figure 6 — Diagram of settlement plates suspended from floating dock structures and plankton samples collected via a pump at different depths. (A) Plate at 0.5 m below the water surface; (B) plate and plankton sample at 1.0 m; (C) plate at 1.5 m; (D) plate and plankton sample at 2.0 m; (E) plate at 2.5 m; and (F) plate and plankton sample at 3.0 m.

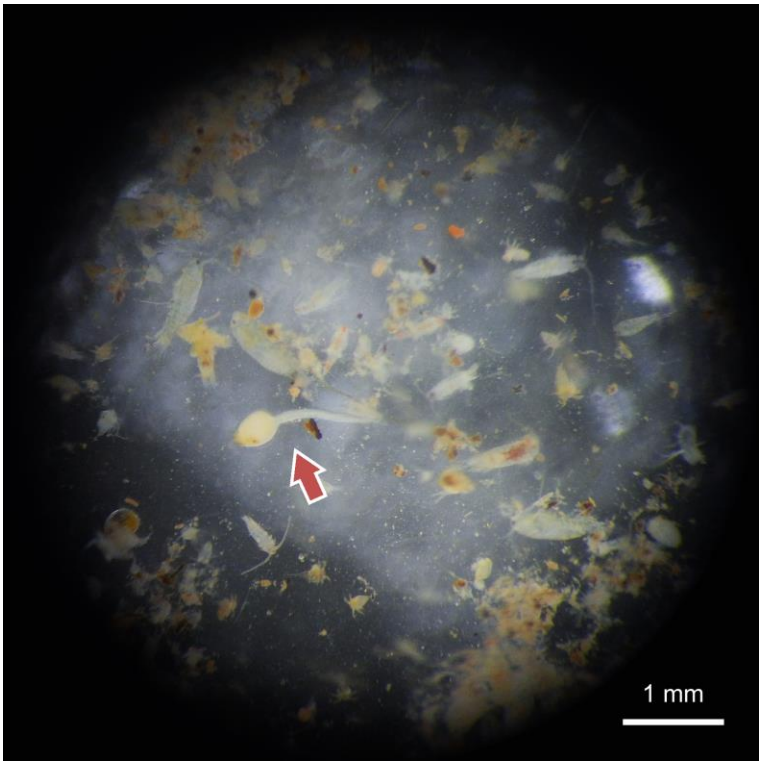


Figure 7 — A *Botryllus schlosseri* larva (red arrow) collected from a plankton sample, preserved in 20% ethanol, and viewed through a stereomicroscope.

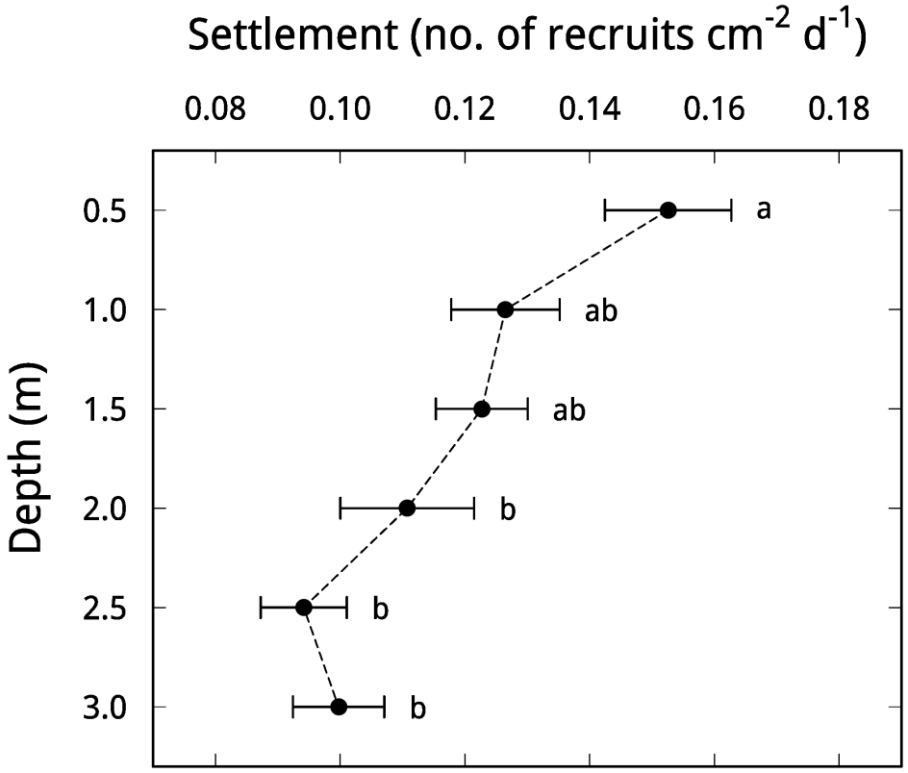


Figure 8 — Vertical distribution of *Botryllus schlosseri* settlement at six depths (bars = standard error; n = 12 per depth). Means labelled with the same letter are not significantly different (Tukey's HSD test).

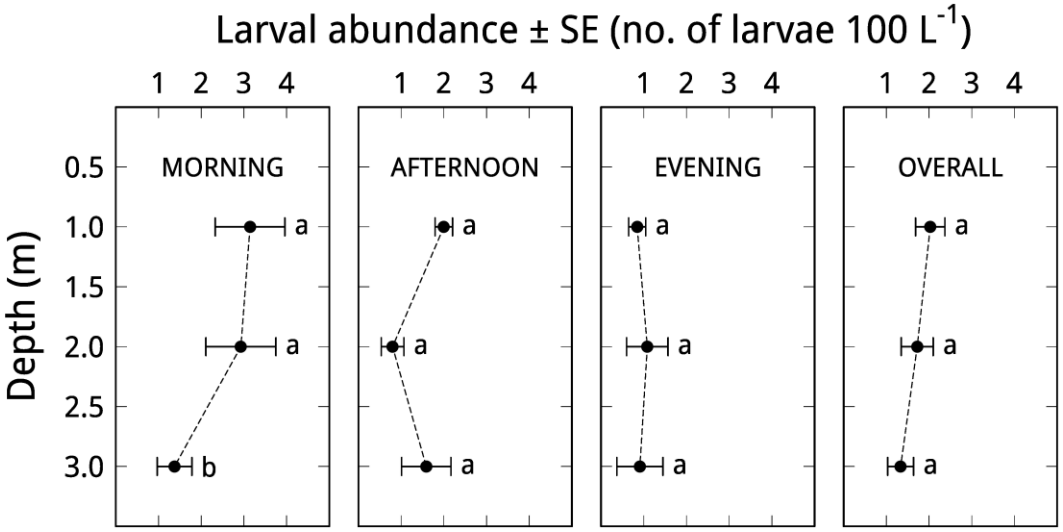


Figure 9 — Vertical distribution of *Botryllus schlosseri* larval abundance at three depth depths in the morning, afternoon, and evening (bars = standard error; n = 12 per depth) and for all times of the day combined ("overall"; n = 36 per depth). For each panel, the means labelled with the same letter are not significantly different (Tukey's HSD test).

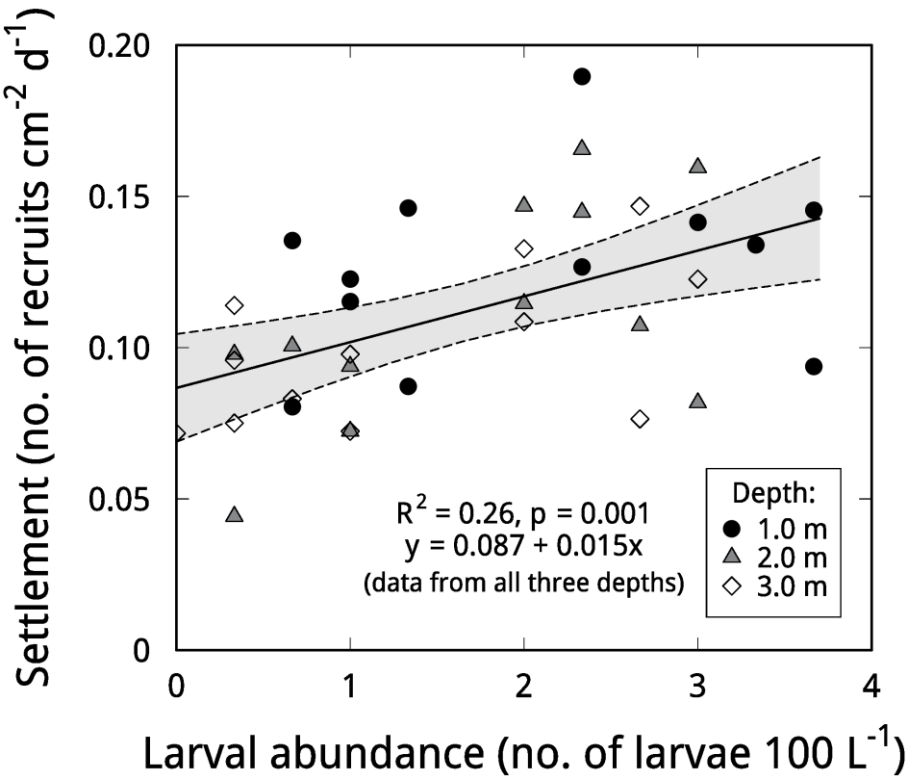


Figure 10 — Relationship between *Botryllus schlosseri* settlement rate and larval abundance (mean over three consecutive days of sampling); solid line = linear regression (data pooled from all three depths; N = 36); dashed line = 95% confidence limits of the linear regression.

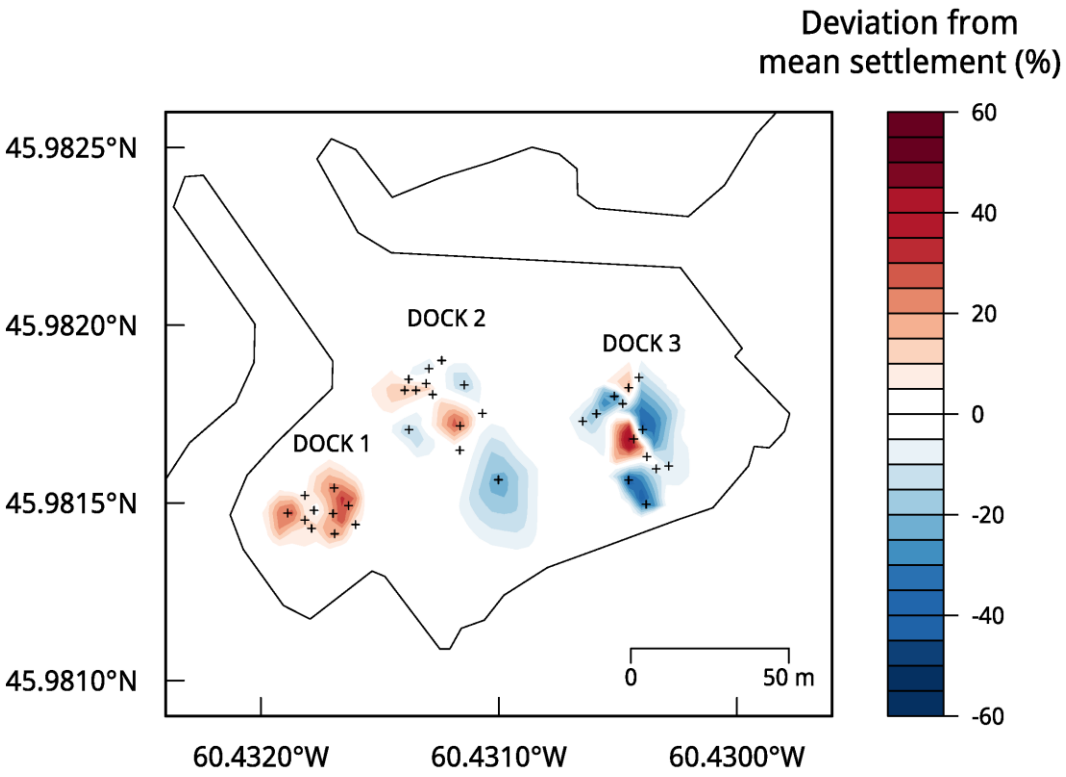


Figure 11 — Percent deviation of observed settlement values of *Botryllus schlosseri* from mean settlement of 0.12 recruits cm^{-2} d^{-1} inside the marina in Ben Eoin, Nova Scotia, Canada. N = 36 sampling stations (+).

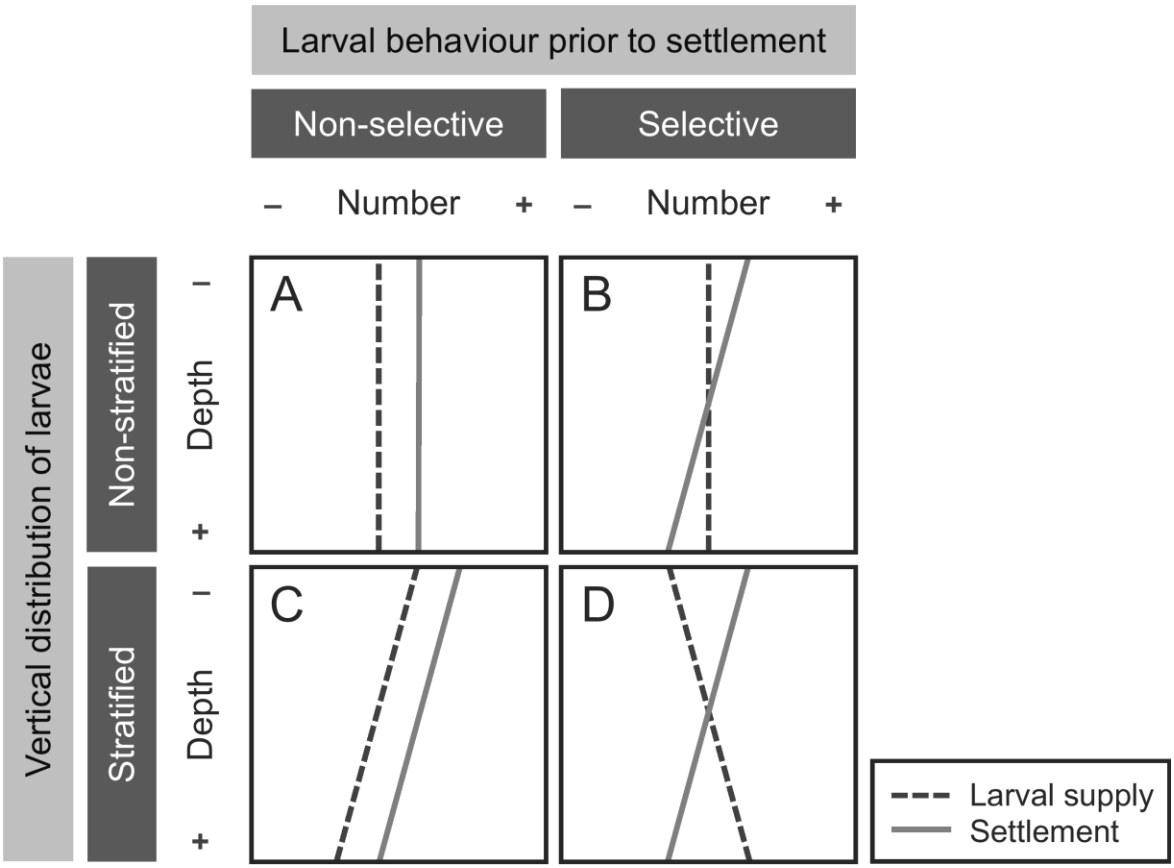


Figure 12 — Conceptualized models of the combined effects of the vertical distribution of larvae (stratified or non-stratified) and larval behaviour prior to settlement (selectively or non-selective settlement of competent): (A) *non-stratified-non-selective behaviour*; (B) *non-stratified-selective behaviour*; (C) *stratified-non-selective behaviour*; (D) *stratified-selective behaviour*.

Chapter 2. Optimal sampling duration and frequency using passive tools to detect rare species and to maximize taxonomic richness

2.1 Résumé

Les plaques de fixation sont couramment utilisées en tant qu'outil d'échantillonnage passif pour enquêter sur les espèces salissantes (y compris les invertébrés envahissants) et pour déterminer leurs patrons de recrutement. La durée et la fréquence d'échantillonnage optimales pour détecter les tendances pertinentes dans les expériences sur le terrain ou pour la surveillance peuvent être difficiles lorsque le temps et les ressources sont limités. L'étude vise à déterminer la stratégie d'échantillonnage optimale pour détecter les espèces rares (par exemple, les espèces au début du processus d'invasion alors que leur population est encore petite). Pour ce faire, nous avons déployé une série de plaques de fixation pour différentes durées (la gamme de durées : de 1 à 32 jours) et à des fréquences d'échantillonnage élevées (la gamme de fréquences : de 0,031 à 1 j^{-1}) au début du recrutement, une situation qui imite la faible pression de propagule pendant les premières étapes du processus d'invasion. Nous avons constaté qu'une durée d'échantillonnage intermédiaire d'une à deux semaines sous faible apport larvaire était optimale pour la plupart des espèces. Cependant, la combinaison d'une durée d'échantillonnage plus longue et d'une fréquence d'échantillonnage plus élevée constituait la meilleure stratégie pour maximiser la richesse taxonomique. Ces résultats peuvent guider des stratégies d'échantillonnage spécifiques à une espèce et au niveau de l'assemblage à l'aide de plaques de fixation. De plus, cette étude peut servir de méthodologie pratique pour optimiser d'autres outils d'échantillonnage et d'autres groupes taxonomiques qui n'ont pas été examinés dans la présente étude.

2.2 Abstract

Settlement plates are commonly used as a passive sampling tool to survey for fouling species (including invasive invertebrates) and to determine their recruitment patterns. The optimal sampling duration and sampling frequency to detect relevant patterns in field experiments or for monitoring can be challenging

when time and resources are limited. The study aims to determine the optimal sampling strategy to detect rare species (e.g., species early in the invasion process while their population size is still small). To do so, we deployed a series of settlement plates for different durations (range: one to 32 days) and at high sampling frequencies (range: 0.031 to 1 d^{-1}) during the seasonal onset of recruitment when larval supply is low, a situation that mimics low propagule pressure during the early stages of the invasion process. We found that an intermediate sampling duration of one to two weeks under low larval supply was optimal for most species. However, the combination of longer sampling duration and higher sampling frequency was the best strategy to maximize taxonomic richness. These results can guide species-specific and assemblage-level sampling strategies using settlement plates. Additionally, this study can serve as a practical methodology to optimize other sampling tools and other taxonomic groups that were not examined in the present study.

2.3 Introduction

Species in the natural environment are sampled to describe their distributional patterns (e.g., abundance, associations) and to experimentally test biological mechanisms and processes with hypotheses, i.e., measurative and manipulative studies, respectively (Tilman 1989; Underwood et al. 2000). Determining the appropriate temporal scale for sampling is difficult with major consequences if the wrong scales are selected to address a particular research question (Richmond and Seed 1991; Underwood 1994). There are many dimensions of time to consider, including timing (when samples are taken), sampling duration, sampling frequency, and time after a disturbance (Thompson et al. 2002; Langhans and Tockner 2006; Vaz-Pinto et al. 2014; Fletcher et al. 2018). Often, the appropriate temporal scale is chosen arbitrarily, based on trial and error (perhaps after many years of experience), or based on the literature (e.g., in deference to established but often unjustified practices). Sampling timescales are rarely optimized before initiating a study, whether it is an ecological field experiment or an environmental monitoring program.

Failure to determine the relevant temporal scale for sampling can have serious implications for protecting endangered species, monitoring global changes (biodiversity and habitat), and detecting and successfully eradicating invasive species. These crucial conservation and monitoring efforts are challenging given that limited resources need to be focused strategically to detect rare species all the while reducing the probability of false negatives (i.e., the conclusion that a species is not present when indeed it is). For biological invasions

specifically, populations at the earliest stages of the invasion process are by their very nature rare (number of individuals) and their spatial extent (geographically localized). Further, invasive species undergoing geographic spread (or native species including endemic and endangered species) tend to be rare at the edges of their distributional range, perhaps, because the environmental or habitat suitability is suboptimal for reproduction and growth (Canning-Clode et al. 2011; Cannizzo and Griffen 2019).

Decisions concerning sampling are particularly challenging when sampling for rare species because current sampling methods might not be sufficiently sensitive for species detection. To overcome the high probability of false-negatives, diversification of sampling tools and intensification of sampling effort over a short period of time are two techniques that are commonly used for invasive species management (Hayes et al. 2005; MacKenzie et al. 2005; Harvey et al. 2009; Tait et al. 2018). These techniques have been utilized to monitor for invasive species because early detection of nascent invasive populations (before they become established) followed by rapid response can drastically increase the probability of a successful eradication (Myers et al. 2000; Rejmánek and Pitcairn 2002; Mehta et al. 2007). For instance, a diverse combination of sampling tools that consisted of active sampling (e.g., SCUBA surveys), passive sampling (e.g., settlement plates), and molecular tools (e.g., PCR-based assays) have successfully detected marine invasive species at early stages of their invasion process many years before an established, self-staining population becomes evident (e.g., Chang et al. 2011; Moore et al. 2014; Ma et al. 2016; Kakkonen et al. 2019). Moreover, rapid assessments at locations deemed to have a high risk of invasions and bio-blitzes concentrate sampling effort over a short period of time, often with the assistance of taxonomic experts, that could substantially reduce the chances of false-negatives and misidentification of rare species (Arenas et al. 2006; Minchin 2007; Bishop et al. 2015; Collin et al. 2015).

Many marine invasive species are associated with fouling communities on human-made structures, which includes dock structures, submerged aquaculture equipment, and boat hulls (Arenas et al. 2006; Lambert 2007; Mineur et al. 2012; Pelletier-Rousseau et al. 2019). These anthropogenic structures are made of a variety of artificial materials, such as plastic and metal, which can be used to study invasive species (Gartner et al. 2016; Ma et al. 2017b; Tait et al. 2018). In particular, passive sampling tools, such as size-standardized artificial flat hard substrates known as *settlement plates*, are an effective tool to detect marine invasive species (predominantly invertebrate groups) because many of these species have biphasic life cycles, which

include a small planktonic stage that disperses widely (Roughgarden et al. 1988; Hurlbut 1992; Pineda et al. 2007; Floerl et al. 2012; Marraffini et al. 2017). This planktonic stage, typically spores and larvae, can then colonize a suitable habitat transitioning to the more sessile or sedentary adult stage of the life cycle (Pineda et al. 2007). And, over time, a community of fouling species develops on the bare surface of settlement plates (Allen and Ferguson Wood 1950; Chalmer 1982; Floerl et al. 2010).

Over short time scales, the number of propagules detected on settlement plates is an efficient estimate for propagule supply (i.e., the number of propagules in the water column; Gaines et al. 1985; Hurlbut 1991b, 1992; Michinton and Scheibling 1991; Yund et al. 1991; Miron et al. 1995; Todd et al. 2006). Therefore, short-term settlement plates can serve as a relatively sensitive sampling tool to detect competent propagules, even at low abundances, in marine systems (e.g., see Chapter 1). Of course, the presence and abundance of propagules may vary across space and time in response to abiotic and biotic processes, such as hydrodynamics and sources of mortality (Kinlan and Gaines 2003; Pineda et al. 2007, 2010). However, small-scale and short-term studies may substantially reduce variation in propagule supply because environmental variability influencing supply (e.g., temperature) is, in effect, minimized across space and time. Regardless, determining the appropriate timescales for sampling can be difficult without proper testing. For passive sampling tools, two major temporal aspects that require immediate decision-making before an ecological study can proceed are *duration* (which might be comparable to the grain of sampling in spatial ecology) and *frequency* of sampling (comparable to spatial lag; Thompson et al. 2002; Vaz-Pinto et al. 2014).

Assuming that the delivery of competent propagules is relatively constant over short time periods, longer sampling duration (i.e., deployment) of settlement plates can maximize the probability of detecting rare species by (1) allowing for the development of a biofilm (i.e., seasoning), which can act as a cue for settlement, (2) increasing the numbers of competent propagules encountering and settling on a plate, (3) accumulating more species over time, and (4) permitting organisms to grow to a larger size and, thus, become easier to identify (Keough and Raimondi 1996; Floerl et al. 2010; Vaz-Pinto et al. 2014; Tait et al. 2016). Thus, some studies season plates before they are deployed in the field or experimentally manipulated under laboratory conditions to enhance recruitment of propagules (Walters et al. 1999; Howes et al. 2007; Ma et al. 2017b). However, shorter durations may also encourage recruitment due to the availability of unoccupied space and can decrease the probability of juvenile mortality before plates are retrieved (Keough

and Downes 1982; Hunt and Scheibling 1997). Moreover, observer or visibility bias may also contribute to lower detectability of rare species. For instance, cryptic species and species with less distinctive features, such as striking colours and shapes, might decrease the chances of their detection (Stanislawczyk et al. 2018). Also, on longer duration plates, an observer could easily miss a small rare species hidden amongst a three-dimensional fouling community that has developed on a plate.

If longer sampling durations are expected to accumulate a higher number of recruits for a given species, then the arithmetic addition of recruitment on two shorter duration plates deployed one after another (i.e., *additive recruitment*) should be lower than the recruitment rate (number of recruits per unit area per unit time) from a single longer duration plate cumulated over the same temporal period (i.e., *cumulative recruitment*). The null expectation, however, would be the same rate regardless of sampling duration. The intuitive practice of converting recruitment rates to the same units of area and time for cross-study comparisons hides the effect of seasoning on these rates and may consequently be problematic for meta-analyses. Therefore, a method to calibrate these rates with respect to plate deployment duration would be invaluable for meta-analyses but this would require the formulation of a predictive relationship between rates (from low to high) and duration (from short to long).

Despite the advantages (and disadvantages) associated with seasoning, increasing sampling frequency is another temporal strategy that can also enhance rare species detection by (1) increasing temporal sensitivity of detecting species with short-lived propagules or species that infrequently release propagules, (2) providing more available bare surface area, through time, for settlement, and (3) reducing the effect of biotic resistance from a more developed community or from dominant species (e.g., Dafforn et al. 2009). High sampling frequency using settlement plates essentially resets ecological succession over time. However, all else being equal (e.g., sampling effort), lower sampling frequency uses fewer resources and is generally preferred, despite the constraints imposed by the temporal window of sampling. Therefore, trade-offs between short and long durations and between sampling effort per time point and sampling frequency require careful considerations and strategic optimization. The optimal strategy, however, depends on which criteria one chooses to use.

A sampling strategy may be considered optimal when the probability of rare species detection is maximal relative to other (suboptimal) strategies. However, to determine the optimal sampling strategy would require testing multiple strategies over time and then identifying the one that resulted with (i) the earliest timing of species detection. Some optimization studies may be unable to test these strategies over time and use the criteria of earliest timing of detection. Thus, strategies can simply be assessed by other criteria such as (ii) the greatest proportion of replicate samples with a positive detection, (iii) the highest recruitment density (i.e., number of recruits per unit area), and (iv) the highest recruitment rate (i.e., recruitment density per unit time). If a majority of these four criteria yield the same optimal strategy, then this strategy should be the most sensitive for rare species detection.

Different scenarios of propagule supply can be used to conceptualize strategies for the optimization of sampling duration and frequency. Given constant supply, a one-time deployment of a settlement plate would be all that is needed for species detection. However, one would need a longer sampling duration if propagule supply is low (Figure 13, Panel A). Conversely, shorter sampling duration would be sufficient if supply is higher (Figure 13, Panel B). Compared to constant (*pressed*) supply, a pulsed pattern of propagule delivery is more realistic (e.g., Hurlbut 1992). Unless one has prior knowledge of temporal patterns to guide timing of sampling (e.g., seasonal, lunar phasic, hourly patterns), increasing the frequency of sampling would increase the probability of species detection, regardless of the underlying temporal supply structure (Figure 13, Panels C and D). Few studies have evaluated how propagule supply dynamics influence sampling strategies using settlement plates (Simkanin et al. 2017).

The objectives of this study were three-fold. First, we evaluated relationships between recruitment rate (of marine invertebrates) and sampling duration. Specifically, we examined the relationship between additive and cumulative recruitment over a range of sampling durations. We focused on the short-term timescales of days to months (i.e., around the lower limit of about one week) because this is the temporal scale at which settlement of spores and larvae is expected to occur (Wahl 1989; Floerl et al. 2010). However, diurnal settlement patterns can be determined for some short distance dispersing species under high propagule supply conditions (e.g., Hurlbut 1992). Second, we evaluated the optimal sampling duration (based on four different criteria) for the detection of rare species (i.e., recruitment to plates under conditions of low propagule supply). Third, we evaluated the optimal sampling strategy (a combination of duration and

frequency) to maximize taxonomic richness of rare species, given that all species we focused our study on are assumed to be seasonally rare.

2.4 Materials and methods

2.4.1 Sampling protocol and experimental design

Settlement plates were used to access the presence of marine benthic populations (mostly invertebrates) within a marina habitat. Plates (dimensions: 14.6 × 14.6 cm) were cut from a 3-mm thick sheet of dark grey polyvinyl chloride (PVC) with one surface roughened with a handheld belt sander. Plates were suspended horizontally in the water column at 1 m below the water surface with the roughened surface facing downwards (Supplemental Figure S1). Each plate was suspended on a separate line from floating docks, such that the distance of any given plate to its nearest neighbouring plate was ca. 1s of m. The study site, Ben Eoin marina (45.9818°N, 60.4314°W; Figure 5), was located East Bay of the Bras d'Or Lake, Nova Scotia, Canada, and covered an area of approximately 1.5 ha with an average depth ranging from 3.5 to 4.0 m.

Settlement plates were deployed for varying durations and frequencies during a 32-d period (1 July to 2 August 2014) during the seasonal onset of recruitment for many of the fouling organisms found in the marina basin (see Chapter 3). Six duration treatments (1, 2, 4, 8, 16, and 32 d) and five frequency treatments (1, 0.5, 0.25, 0.125, and 0.031 d^{-1}) were created by deploying, retrieving, and replacing plates at different times during the experiment (Figure 14), ranging from the daily replacement of plates deployed for a single day to a single deployment of plates for 32 days. For each unique combination of sampling duration and deployment time-point, six replicate plates were used (N = 738 total). All plates were deployed and collected between 09h00 and 10h00. Supplemental settlement plates were also deployed to extend the experiment for an additional eight days. These supplemental plates consisted of 4, 8, and 16 d duration plates, which were deployed at a frequency of 0.25 d^{-1} (i.e., every four days) and retrieved on 6 and 10 August 2014.

Within one or two hours after retrieval, the entire downward-facing surface of each plate was examined under a stereomicroscope and any recruits counted. During the course of the experiment, a total of 22 marine

benthic species were observed and identified to the lowest taxonomic level possible (Supplemental Table S1).

2.4.2 Relationship between additive and cumulative recruitment

Nine marine benthic invertebrates (*Amathia gracilis*, *Botryllus schlosseri*, *Bougainvillia carolinensis*, *Conopeum tenuissimum*, *Crassostrea virginica*, *Crisularia turrita*, *Hiatella arctica*, *Lacuna vincta*, and *Mytilus* sp.) were used to examine the relationship between *additive* and *cumulative* recruitment. These species were selected because there were sufficient data for the analysis, i.e., each of these species had more than 100 recruits counted throughout the 32-d experiment.

Each pair of data consisted of recruitment determined from two shorter duration plates (additive recruitment) *versus* recruitment determined from one longer duration plate (cumulative recruitment) over the same temporal period (this period is hereafter referred to as *plate-days*). Due to the experimental design, there were a total of five possible plate-day conditions (2-, 4-, 8-, 16-, and 32-plate-days). Data from supplemental plates were also used to calculate additive and cumulative recruitment values. Any two plates used to calculate additive recruitment were deployed for the same length of time (duration) and during the same absolute period of time as the comparative single plate. For each species and plate-day condition, a linear regression was fitted to $\log_{10}(x + 0.01)$ transformed additive and cumulative recruitment rates.

2.4.3 Species-by-species optimization

The optimal sampling duration(s) were determined for each of the 22 taxa based on four criteria: (1) the earliest day (i.e., day of plate retrieval) of species detection, (2) the greatest proportion of colonized replicate plates (a proxy for the least spatially patchy), (3) the highest recruitment density (number of recruits plate^{-1}), and (4) the highest recruitment rate (number of recruits plate^{-1} d^{-1}). For each taxon and criteria combination, the six sampling durations (1 to 32 d) were ranked from optimal to least optimal (Supplemental Table S1). For each criterion, the optimal sampling duration for all taxa was tallied to identify the optimal duration for the greatest proportion of this species assemblage. A chi-square test was used to ascertain if there was a

significant difference between the expected distribution and the observed distribution of data among the four criteria.

2.4.4 Assemblage-level optimization

The cumulative species richness was tracked under 19 different sampling strategies to determine the optimal temporal strategy (i.e., combination of sampling duration and frequency) that could maximize richness by the end of the 32-d study period. These strategies consist of different combinations of sampling durations (1, 2, 4, 8, 16, 32 d) and frequencies (daily, every second day, every fourth day, every eighth day, and a one-time deployment). First, data used to construct species accumulation curves were parsed out to calculate cumulative species richness for lower sampling frequencies. Second, the relationship of each strategy was fitted to a logarithmic regression. Last, the number of taxa accumulated during the 32-d experiment, for each strategy, was plotted to evaluate the optimal sampling strategy. An analysis of variance (ANOVA, generalized linear model [GLM] procedure with Poisson error distribution) was used to evaluate the effects of *Duration*, *Frequency*, and *Duration* × *Frequency* on the number of taxa cumulated by the end of the 32-d experiment. Due to the unbalanced nature of the data, a GLM procedure (based on type III sums of squares) was applied to this ANOVA using IBM SPSS (Version 25). All other data manipulations and statistical analyses in this study were performed in the R programming environment (R Core Team 2019).

2.5 Results

2.5.1 Relationship between additive and cumulative recruitment

For all possible species-specific plate day conditions, positive relationships between additive and cumulative recruitment were found for a majority (6 out of 9) of marine benthic invertebrates (*A. gracilis*, *B. carolinensis*, *B. schlosseri*, *C. turrita*, *C. virginica*, and *L. vincta*), i.e., a good fit between the (log-transformed) data and the regression (Supplemental Table S2). However, the remaining three taxa that were tested (*C. tenuissimum*, *H. arctica*, and *Mytilus* sp.) had a weaker fit between the (log-transformed) data and the regression ($R^2 < 0.50$; Supplemental Table S2). By combining the data from all nine taxa, a positive

relationship with R^2 values ranging from 0.65 to 0.81 was found for each of the plate-day conditions (including the 32-plate-day condition; Supplemental Table S2 and Figure 15).

2.5.2 Species-by-species optimization

The intermediate durations of 8- and 16-d were identified as optimal based on the criteria used to evaluate multiple sampling durations ranging from 1- to 32-d. The use of 8-d sampling duration was optimal for the greatest proportion of taxa in terms of the earliest detection (64% of taxa), the detection of the highest recruitment density (41%), and the detection of the highest recruitment rate (27%; Figure 16). For 77% of the taxa, the use of the 16-d sampling duration was optimal for having the greatest proportion of colonized replicate plates (a proxy for the least spatially patchy; Figure 16). The distribution of the number of taxa as a function of optimal sampling duration was dependent on the criteria used to evaluate detectability ($\chi^2 = 130$; $df = 15$, $p < 0.001$).

2.5.3 Assemblage-level optimization

Species accumulation curves were derived for each sampling strategy (Supplemental Figure S7). No curve could be made for the one-time deployment of 32-d duration plates (i.e., 0.031 d^{-1}) because this strategy consisted of a single value, i.e., 19 taxa accumulated by the end of the 32-d study period. No strategy detected all 22 taxa by the end of the study, but two strategies detected 95% of the taxa: (1) 8-d duration at 1 d^{-1} sampling frequency and (2) 16-d duration at 0.25 d^{-1} frequency. However, the sampling effort between these two strategies was substantially different (i.e., 150 *versus* 30 plates). For each sampling strategy, a logarithmic regression was applied to derived data (i.e., Supplemental Figure S7) to determine the relationship between time elapsed and the cumulative number of species detected (Supplemental Table S3 and Supplemental Figure S8). Overall, longer sampling durations were more likely to detect more species by the end of the experiment than were shorter durations (Supplemental Figure S8). Also, higher sampling frequencies detected more species by the end of the experiment than were lower frequencies (Figure 17). However, within the range of duration and frequencies considered in this study, more species were detected by increasing sampling duration (up to ca. 500% more taxa) than by increasing sampling frequency (only up

to ca. 160%; Figure 17). There was significant variability in the number of taxa accumulated during the 32-d experiment among different sampling durations (ANOVA GLM, $df = 4$, $p < 0.001$) and among sampling frequencies ($df = 3$, $p = 0.015$; Table 3). There were no significant interactions between sampling duration and frequency. Based on this ANOVA, *Duration* and *Frequency* explained about 88% and 12% of the variation, respectively.

2.6 Discussion

2.6.1 Species-specific optimization

Efforts to optimize the sampling strategy can substantially improve species detection, especially for rare species (Tait et al. 2016). For passive sampling tools, such as settlement plates, sampling duration is one of many important temporal variables to optimize (Vaz-Pinto et al. 2014). There are valid arguments for and against long and short sampling durations (given constant delivery of competent propagules). Longer sampling duration (i.e., seasoning of settlement plate) allows more time for a biofilm to develop on the hard substrate, which may encourage settlement of propagules (Zobell and Allen 1935; Keough and Raimondi 1995, 1996; Johnson et al. 1997; Wieczorek and Todd 1997). Also, with longer sampling duration, the probability for a given individual propagule (e.g., spores or larvae) to encounter a plate would increase. Generally, a greater number of species accumulates on longer sampling duration plates (Vaz-Pinto et al. 2014). Long sampling duration can improve species detection by permitting organisms to grow to a larger size and, thus, to become more easily identified by trained taxonomists (Floerl et al. 2010). Conversely, short sampling duration can improve species detection by making available space for propagules to settle and by detecting newly settled recruits before increasing the probability of juvenile mortality (Keough and Downes 1982; Hunt and Scheibling 1997).

Based on the four criteria used in this study, our results clearly show that an intermediate sampling duration between one and two weeks is optimal for species detection using settlement plates under low propagule supply. Ideally, temporal strategies, such as sampling duration and frequency, should be tested over time and evaluated based on when rare species are detected (i.e., earlier is better). The general consensus that the optimal duration is an intermediate one to two weeks as reached by this study, however, suggests that

optimization tests based on other criteria, such as the highest recruitment densities or rates, are appropriate methods to determine optimal conditions for sampling. Further, the enhanced species detection via intermediate duration (as identified in this study) has practical implications for both the design of ecological studies and monitoring for rare species using settlement plates. In these studies, settlement plates can be used to quantify recruitment rate of targeted fouling species or to monitor the development of fouling assemblages after deploying plates for a typical duration of four weeks to several months (Floerl et al. 2010; Ma et al. 2017b; Marraffini et al. 2017; Tait et al. 2018). Thus, the intermediate sampling duration would be the minimum or lower limit necessary for species detection. For example, this is important to detect recruitment during the seasonal onset and end of reproduction when propagule supply would be expected to be low.

Additionally, the biological response (recruitment) using the targeted species' optimal sampling duration would also be associated with the least amount of temporal variation in environmental conditions. Monitoring for rare species (e.g., endangered species, nascent populations of invasive species) using settlement plates can be achieved by intensive sampling at small spatial scales; however, optimizing for sampling duration could have the desired outcome with less sampling effort (assuming that the optimal seasonal window for sampling is known, which may vary by species). The identification of the optimal sampling duration has important implications for early detection of invasive species, since any time saved by using a shorter sampling duration would directly contribute to even earlier species detection and, thus, allow for rapid response (Harvey et al. 2009). And, if early detection at the timescales of days and weeks truly matters, then shorter durations and, therefore, earlier plate retrieval relative to longer durations will potentially translate to an earlier time of (a) species identification and (b) reporting of new records of invasive species. The enhanced sensitivity of shorter durations to detect rare species when sampling with settlement plates opens up the possibility of applying this passive sampling tools for invasive species and biodiversity surveys in remote areas and for rapid assessment surveys (including bio-blitzes). The temporal window for sampling is limited. Thus, if a sampling window of one to two weeks is logistically feasible, then settlement plates can be utilized for both survey scenarios.

Optimal strategies for species detection may entail a sampling design with different combinations of sampling duration, i.e., using long and short durations concurrently. Although we did not explore this line of research, we provide some ecological insights into the effect of different durations on recruitment rates and

the relationship between additive and cumulative recruitment. Ecological studies and monitoring using settlement plates may not necessarily use equal sampling duration throughout the entire study period due to unforeseen circumstances such as extreme weather events or logistic constraints (Ma et al. 2017b; Sokolowski et al. 2017). Recruitment data can be standardized as a rate value as part of the data processing and analysis. However, this process of standardization assumes that any biological or physical process acting upon settlement plates and fouling organisms is constant over time, and can be problematic for data interpretation if the sampling duration (and, similarly, frequency) vary tremendously within a study. Our results reveal that it is conceptually possible to recalibrate recruitment rates among different short-term sampling durations (e.g., shown simplistically in this study as the relationship between additive and cumulative recruitment). We recommend due care in the implementation of a sampling strategy to be taken to properly standardize biological response derived from different sampling durations, for instance, for meta-analyses.

2.6.2 Assemblage-level optimization

Throughout this experiment, increasing sampling duration increased the number of species detected over time. Unlike species-specific optimization, no intermediate sampling duration was found, but the species accumulation curve appeared to plateau by the end of the study period after one month has elapsed. This plateau suggests that there is a diminishing return on maximizing taxonomic richness with longer sampling duration. Although longer durations tend to detect more species than shorter durations, some taxa are not detected on the longest sampling durations. There could be a couple of explanations for this. First, some species may settle on the plates and then leave the substratum. This case is plausible for mobile species such as sea stars, which may fall off the plate once they grow to a larger size. Second, observer or visibility bias may make it difficult to detect small and rare species that can take advantage of the three-dimensional structure of the fouling community and evade detection. In this study, this is a plausible scenario for the free-living flatworms (Platyhelminthes: Turbellaria), which their detection on shorter duration plates was made easier when the human observer can see them moving against the background of the bare settlement plate. Third, the net effect of inter-specific interactions, such as competition for space, may take out or else substantially reduce the abundance of certain species over time.

Our data demonstrate that increasing sampling frequency increases the number of the species detected. Increasing sampling frequency can improve the detection of rare species by simply spreading high sampling effort over time and providing finer temporal coverage between sampling time points. Furthermore, the combination of high sampling frequency and long sampling duration allows for multiple trajectories of the ecological succession, each with a different temporal starting point. The structure of the fouling assemblage depends on the seasonal timing and, to a lesser extent, year of plate deployment because different species recruit at different times and these initial recruits inevitably shape the development of the community over the long-term (Sutherland 1974; Otsuka and Dauer 1982). This stochastic outcome is consistent with our results that a sampling strategy involving longer durations and high frequencies can maximize taxonomic richness. The main drawback of increasing sampling frequency is the increasing cost associated with producing and handling more settlement plates. Notably, changing sampling duration does not have this same drawback. Perhaps, a better study to experimentally test the effect of sampling frequency would require separate series of plates for each frequency, but this would require a substantial number of plates to achieve independence of samples.

2.6.3 Temporal sampling strategies to detect rare species

Temporal variation in propagule supply (and settlement) likely affects how sampling should be designed to optimize the detection of rare species. Four temporal sampling scenarios concerning sampling duration and sampling frequency were conceptualized and introduced under different supply patterns (Figure 13). Although the timing of this study was temporally restricted to the seasonal onset of reproduction when propagule supply is expected to be low, findings from this study provide support on how to optimize temporal aspects of sampling (see Figure 13). When viewed from a coarse timescale, supply and settlement rates exhibit seasonal variability such that the onset (and end) of the reproductive season is associated with low propagule supply and settlement rates. At finer scales, pulsed supply and local settlement patterns could be resolved for some species if the right durations and frequencies are used in the sampling design to detect these patterns. In this study, we observed the seasonal onset of settlement onto plates and, for some species, evidence of pulsed recruitment over short temporal periods, which corresponds to the scenario shown in Panel C of Figure 13. The results from both the species-specific and assemblage-level optimization analyses indicate that short sampling durations are not optimized under the condition of low propagule supply

(i.e., rarity). Interestingly, the optimal sampling duration for a majority of species, when analyzed species-by-species, was an intermediate duration (in the order of weeks). However, when analyzed at the assemblage-level, the optimal duration was the longer duration of about a month. In this study, we only analyzed the effect of sampling frequency on species detection at the assemblage-level and found that the optimal frequency was the higher frequency of daily sampling. This finding is likely due to temporal variability related to seasonal variability in propagule supply and diel variability in release of propagules (e.g., larvae; Hurlbut 1992; Bourque et al. 2007; Manríquez et al. 2016; also see Chapter 1). Moreover, the combination of short sampling durations and low sampling frequencies is the least optimal strategy for the detection of rare species.

2.6.4 Conclusions

Initial settlement patterns and rare species are difficult to observe in nature because of the inherent challenges in designing sampling strategies that are sensitive enough to detect their presence (MacKenzie et al. 2005; Tait et al. 2016). In many ways, different sampling durations represent different sampling methods; therefore, an investigator should take appropriate steps to calibrate their data when comparing recruitment rates determined from plates with different immersion times (e.g., comparing of rates within a study or among studies). Such calibrations may be possible by using the relationship between additive and cumulative recruitment that we investigated for several fouling invertebrate species. Moreover, our unique experimental design allowed for a better comparison of *in situ* sampling duration. In practice, comparing data among sampling durations is complex and difficult because the investigator cannot control for both sampling start and end dates, which confounds the interpretation of the results. Arguments for long sampling durations for species detection seem to outweigh arguments for short durations. However, our findings support the use of an intermediate sampling duration over a chosen temporal sampling window (e.g., see Chapter 3 on the optimal seasonal timing for early detection) to detect rare species within a short timescale of about a month. Whether an intermediate sampling duration remains the optimal duration when tested over longer timescales (e.g., six months) is difficult to postulate. Thus, further experiments are needed to determine if this generalization can scale-up. We propose a conceptual framework to optimize temporal sampling strategies (a combination of duration and frequency) to different scenarios of propagule supply (see Figure 13). As predicted, our results were consistent with the expectation that a sampling strategy involving longer sampling

duration and higher sampling frequency would maximize detection (e.g., taxonomic richness) under a temporally varied (e.g., pulsed) low propagule supply scenario, which best represents the reality of this study (seasonal onset of reproduction).

Table 3 — Results of an analysis of variance (ANOVA, GLM procedure with Poisson error distribution) testing the effects of *Duration*, *Frequency*, and *Duration* × *Frequency* on the number of species accumulated during the 32-d experiment. Statistically significant p values are **bolded**.

Source	df	Wald χ^2	p
(Intercept)	1	3066.676	< **0.001**
Duration	4	50.078	< **0.001**
Frequency	3	10.470	**0.015**
Duration × *Frequency*	10	0.762	1.000

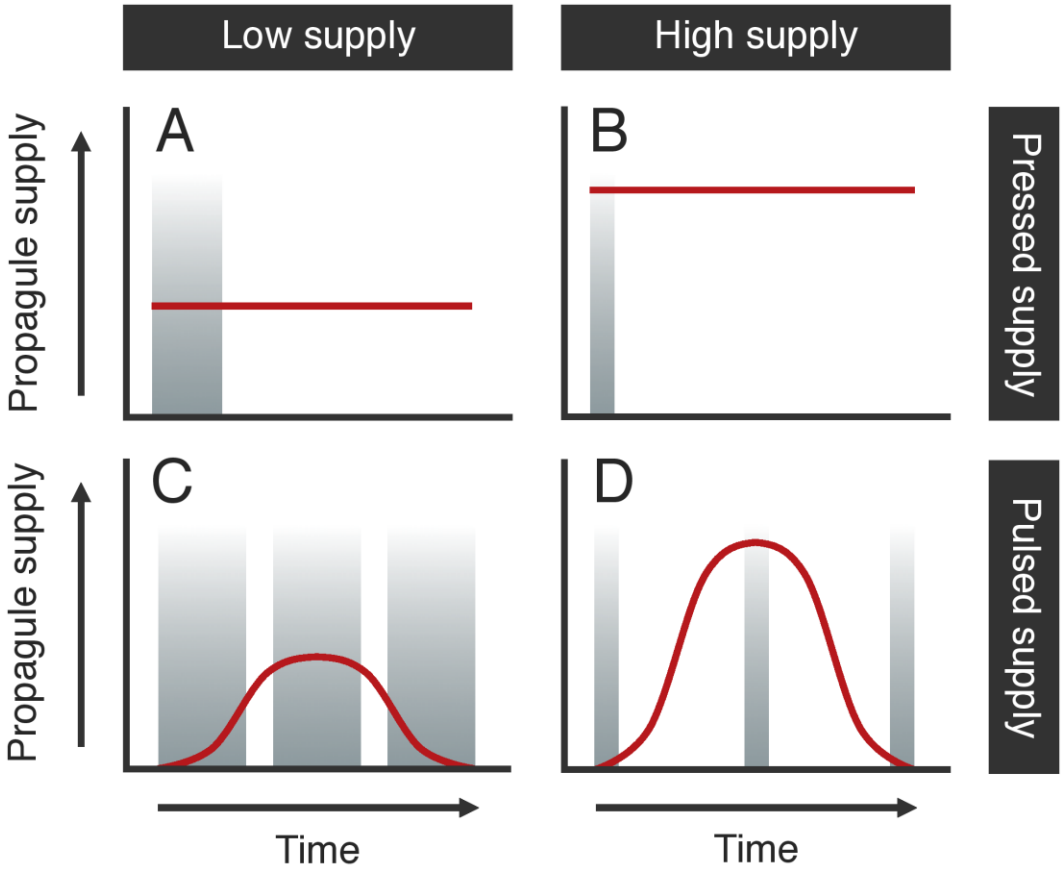


Figure 13 — Temporal strategies for the optimization of sampling duration and sampling frequency under different scenarios of propagule supply (red line). (A) Pressed low supply; (B) pressed high supply; (C) pulsed low supply; (D) pulsed high supply. Width of shaded periods represent sampling duration (short *versus* long) and number of shaded periods represent sampling frequency (low *versus* high).

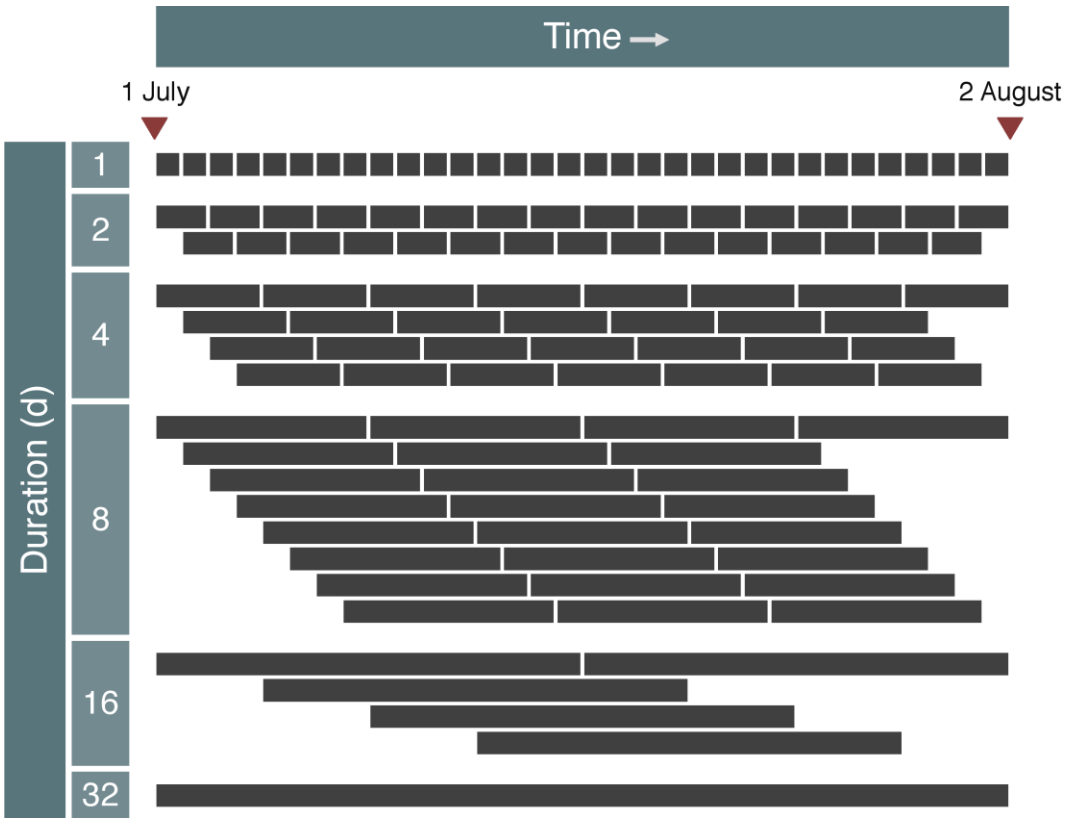


Figure 14 — High-frequency sampling program using settlement plates with six different sampling durations ranging from 1 to 32 days. The experiment began on 1 July 2014 and terminated on 2 August 2014. Sampling program of supplemental settlement plates is not shown.

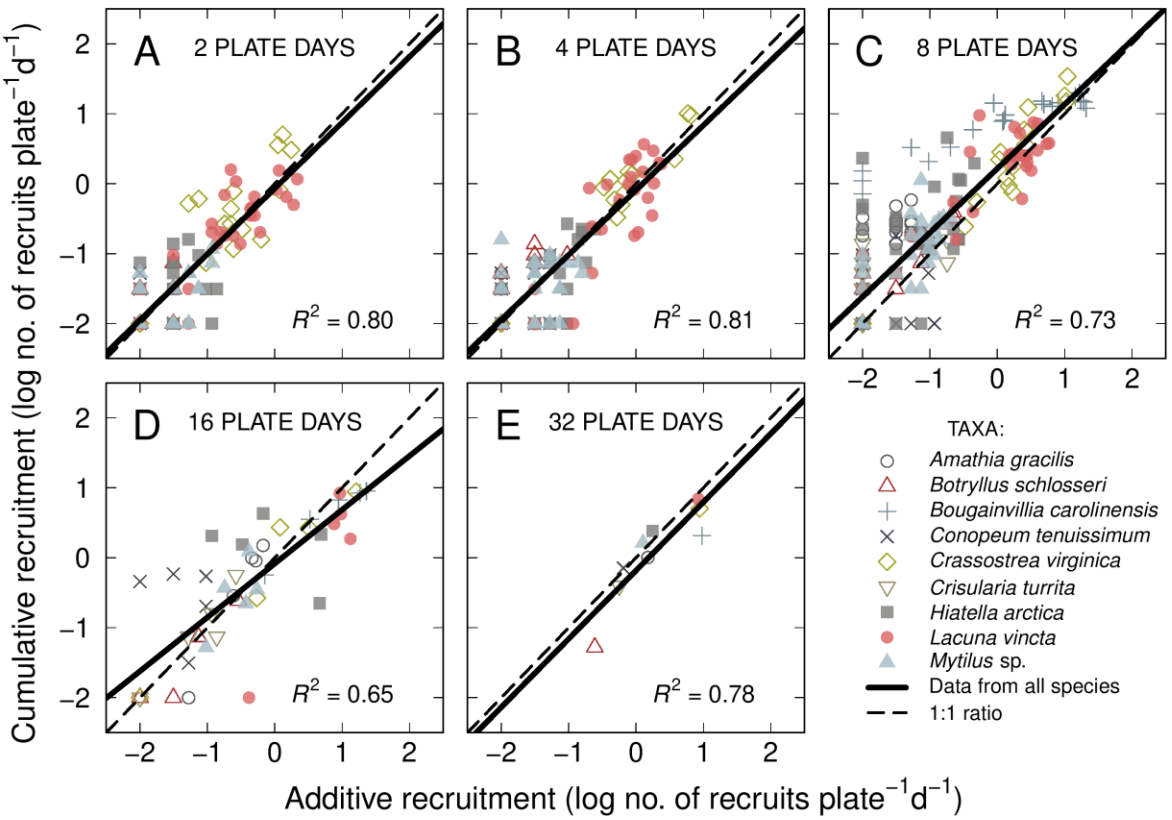


Figure 15 — Relationships between additive and cumulative recruitment evaluated for five plate-day conditions. Black solid line (—) = linear regression based on $\log_{10}(x + 0.01)$ transformed data for nine marine benthic species combined; black dashed line (---) = 1:1 ratio for visual reference. (A) 2-plate-days; (B) 4-plate-days; (C) 8-plate-days; (D) 16-plate-days; and (E) 32-plate-days. No data for *Amathia gracilis* for the 2-plate-day condition and for *Bougainvillia carolinensis* and *Crisularia turrita* for 2- and 4-plate-day conditions.

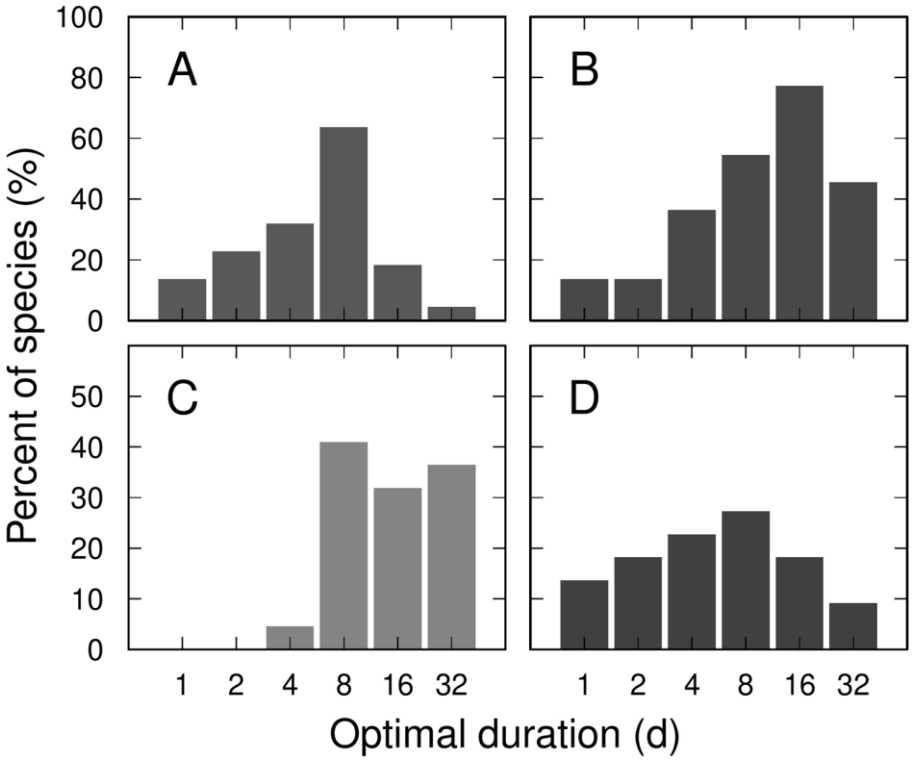


Figure 16 — Distribution of optimal sampling durations for an assemblage of 22 marine benthic species under low propagule supply evaluated using four criteria. Criteria: (A) earliest day of species detection; (B) greatest proportion of colonized replicate plates; (C) highest recruitment density; and (D) highest recruitment rate. For each criterion, percentages do not add up to 100 due to instances (of a given species) where multiple durations were tied for the optimal position.

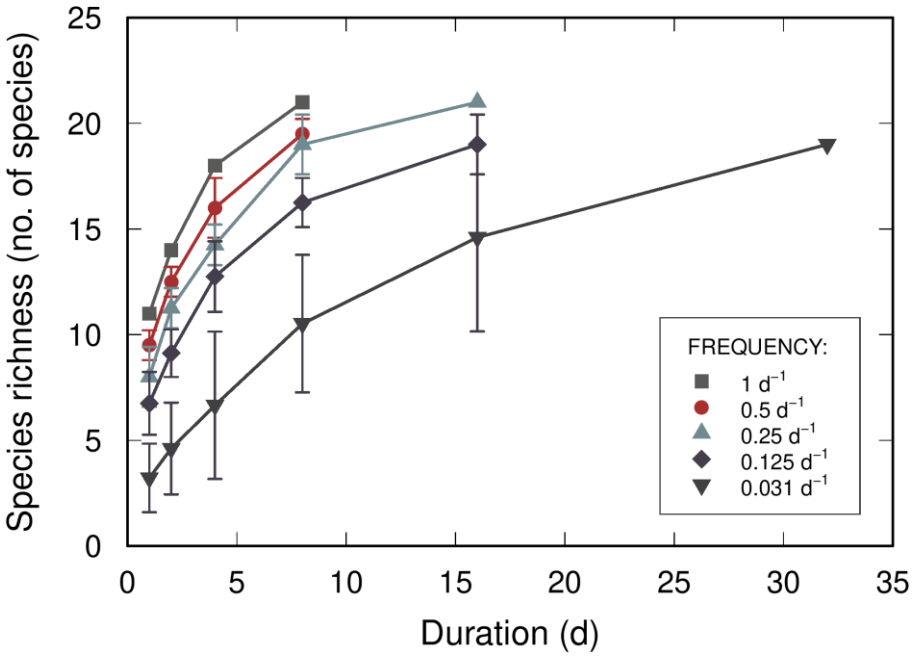


Figure 17 — Species richness accumulated by the final day of the 32-d experiment as a function of sampling duration (plate submergence time). Sampling frequency refers to the rate of deploying a new set of replicate plates over the course of the experiment. Each data point is the mean value for each sampling strategy. Bars represent ± one standard deviation. See Supplemental Figure S7 for the original data used to calculate each data point.

Chapter 3. Relative importance of spatial and temporal coverage for rare species sampling and the optimal seasonal timing for early detection of marine invasive species

3.1 Résumé

Les invertébrés benthiques démontrent une grande variation spatiale et temporelle du recrutement. Déterminer comment les variations changent dans l'espace et dans le temps peuvent guider la détection précoce et la surveillance des espèces envahissantes et des ressources naturelles d'importance économique (p. ex., les huîtres et les moules). Pour les invasions biologiques en particulier, nous proposons que le moment du pic de recrutement (quand les espèces sont susceptibles d'être les plus abondantes) puisse servir de moment optimal pour la détection des espèces envahissantes aux premiers stades du processus d'invasion, lorsque ces espèces sont, par nature, rares (quand la probabilité de détection est la plus élevée même si cette probabilité est globalement faible). Les patrons saisonniers pour un large éventail de taxons d'invertébrés benthiques (n = 22) ont été déterminés à l'aide de plaques de fixation à court terme (durée d'échantillonnage d'environ un mois) et à haute résolution temporelle (fréquence d'échantillonnage d'environ toutes les deux semaines) qui ont été déployées pendant la saison de reproduction de 2012 à 2014 sur 14 sites côtiers des Maritimes canadiennes. La dimension spatiale (entre les sites) était la principale source de variation du recrutement pour les espèces répandues et communes et la dimension temporelle (parmi les dates d'échantillonnage) pour les espèces moins communes et les espèces rares. Le moment du pic de recrutement suggère que des taux de recrutement élevés de certaines espèces envahissantes pourraient réduire le recrutement d'autres espèces, peut-être en raison de la concurrence pour un espace limité sur des plaques à court terme. Cette interaction biotique indique que les espèces envahissantes établies pourraient avoir des conséquences importantes à court et à long terme sur les espèces indigènes et la dynamique de leurs populations. De manière remarquable, le moment saisonnier du pic de recrutement des populations établies d'espèces envahissantes correspond à peu près au moment de la détection d'espèces envahissantes rares dans des localités où les populations ne sont pas connues pour être autosuffisantes (c'est-à-dire, la détection précoce spécifique au site).

3.2 Abstract

Benthic invertebrates exhibit large variation in recruitment across space and time. Determining how variation changes over space and time can guide early detection and monitoring of both invasive species and economically-important natural resources (e.g., oysters and mussels). For biological invasions specifically, we propose that the seasonal timing of peak recruitment (i.e., when species are likely to be most abundant) can serve as an informative proxy for the optimal time for detecting invasive species at early stages of the invasion process when these species are, by their very nature, rare (i.e., when the probability of detection is highest even though that probability is small overall). Seasonal patterns for a broad range of benthic invertebrate taxa (n = 22) were determined using short-term (duration: ca. one month) and high temporal resolution (frequency: ca. every two weeks) settlement plates deployed during the reproductive season from 2012 to 2014 at 14 coastal field sites across the Canadian Maritimes. The spatial dimension (among sites) was the main source of variation in recruitment for widespread and common species and the temporal dimension (among sampling dates) for less common and rare species. Seasonal timing of peak recruitment suggests that high recruitment rates of some invasive species may reduce the recruitment of other species, perhaps as a result of competition for limited space on short-term plates. This biotic interaction indicates that established invasive species could have substantial short- and long-term consequences on native species and their population dynamics. Remarkably, the seasonal timing of peak recruitment of established populations of invasive species roughly corresponded with the timing of detecting rare invasive species at localities where populations were not known to be established and self-sustaining (i.e., site-specific early detection).

3.3 Introduction

Coastal monitoring for marine invasive species is costly because it requires political commitment and stable sources of funding to dedicate resources for (a) early detection, (b) mitigation of negative ecological and economic impacts, and (c) eradication campaigns (Wotton and Hewitt 2004; Sephton et al. 2011; Minchin and Nunn 2013; Collin et al. 2015; McKenzie et al. 2016). Ideally, preventing biological invasions (e.g., open-ocean ballast water exchange to reduce risk of species introduction) should be the favoured management option, but detection of nascent population at early stages of the invasion process (i.e., after propagules are released and before a population is locally established or has spread too far) can substantially

increase the probability of successful eradication of a non-indigenous species (Myers et al. 2000; Rejmánek and Pitcairn 2002; Mehta et al. 2007). However, given the extensive length and complex geometry of most coastlines, early detection in coastal systems requires strategic sampling procedures that are optimized to detect nascent populations, which are, by their very nature, rare (Harvey et al. 2009; Welk 2004; Liebhold and Tobin 2006), and an understanding of how rare species vary across space and time can inform the design of invasive species monitoring programs (Hauser et al. 2006; Tait and Inglis 2016).

In marine benthic systems where most invasions occur on hard substrates, monitoring fouling species is a common practice that often involves immersing settlement plates (i.e., artificial hard substrates) in the water and periodically returning to monitored sites to examine plates for fouling organisms (Ruiz et al. 2011; Clarke Murray et al. 2014; Marraffini et al. 2017; Tait and Inglis 2016; Tait et al. 2018). This method is a practical approach to detect marine invasive species that have biphasic life cycles with planktonic larvae and benthic adults, including representatives of a majority of invertebrate phyla (Maughan and Barnes 2000; Marraffini et al. 2017). Despite the ease of using settlement plates, trade-offs between high spatial coverage (e.g., spatial extent) and high temporal coverage of sampling (e.g., annual surveys of sentinel sites; Sephton et al. 2017) can be necessary, especially when faced with finite resources. Tools to strategically guide managers where and when to sample for invasive species include ecological risk assessments, predictive modelling (e.g., environmental niche models), and identification of high-risk vectors and pathways (Padilla and Williams 2004; Clarke Murray et al. 2014; Foster et al. 2016; Lowen et al. 2016; Ware et al. 2016; Goldsmit et al. 2018). Although these tools cannot predict how species spread over time (i.e., the order that sites will be invaded), limited resources can be strategically concentrated across space and time to maximize the probability of detecting new invasions. Over time, particular sites strongly linked with high rates of biological invasions have been identified as sentinel sites for monitoring based on the collective experience gained by managers and researchers. Although not explicitly stated, the prioritization of these sites (i.e., sentinel sites) might assume that they are undergoing invasional meltdown (i.e., the process by which invasion and interactions of non-indigenous species facilitate the invasion of future non-indigenous species), which would increase the probability of detecting future invasions (Simberloff and Von Holle 1999; Jeschke et al. 2012). Moreover, the creation of sentinel sites permits much-needed long-term monitoring of ecological change (Magurran et al. 2010).

Although the conventional emphasis on high spatial coverage is still prevalent, the practice of establishing sentinel sites for long-term monitoring may be because high temporal coverage in the form of consistent, annual sampling is crucially lacking in the design and implementation of a monitoring program. For invasive species monitoring, other aspects of time, such as the seasonal timing of sampling, sampling duration, and sampling frequency (or intensity), are key parameters in sampling designs to optimize (Hauser et al. 2006; Jenkins and Martins 2010; but see Fletcher et al. 2018). In practice, increasing the frequency of sampling, which effectively increases the temporal resolution of monitoring, may be challenging (and, thus, a moot point) because visitations of field sites are constrained by a plethora of ephemeral circumstances such as sudden changes in management priorities, weather, and human resources. Theoretically, introductions may be occurring at all times (and, perhaps, accelerating) as long as the vectors and pathways exist and are operational (Cohen and Carlton 1998; Ricciardi and MacIsaac 2000; Ricciardi 2006). Vectors (e.g., aquaculture activities and recreational boating) and pathways can exhibit strong seasonal patterns (e.g., Ma et al. 2019; also see Appendix 2). Further, there appears to be a logistical tendency of prioritizing resources on increasing spatial coverage (number of sites) rather than increasing temporal coverage (number of visitations per site through time). For instance, in eastern Canada, monitored sites are typically visited three times a year: at the beginning of the field season around May or June, in the middle around August, and at the end around September or October (Sephton et al. 2011; McKenzie et al. 2016; Sephton et al. 2017). However, not all sites are monitored each year.

Environmental conditions vary seasonally in temperate waters and can drive ecological patterns in benthic assemblages (e.g., Coma et al. 2000). For example, inter-annual variability in winter conditions can affect the magnitude of recruitment of marine invertebrates in the subsequent reproductive season (Stachowicz et al. 2002a; Terwin et al. 2002; Chang et al. 2017). Seasonal fluctuations in environmental conditions have implications for the survival of invasive propagules, especially at early stages of the invasion process. Because of the low probability of propagules surviving in a novel environment, favourable abiotic conditions overcoming any physiological and reproductive barriers and high propagule pressure can greatly increase the likelihood of a successful introduction (Williamson 1996; Lockwood et al. 2009; Blackburn et al. 2011). In fact, the development and survival of larvae of invasive invertebrates have been shown to be more sensitive to environmental variations than is the adult phase (Pineda et al. 2012). Thus, fouling species hitchhiking on boat hulls may release propagules via spawning of gametes or release of larvae only under the right environmental conditions (Minchin and Gollasch 2003). Consequently, any phenological processes that

enhance the detection of invasive propagules can inform when to target early detection of nascent invasive populations. For example, the seasonal timing of the first records for a majority of invasive ascidians in eastern Canadian provinces tend to occur during the autumn (Ma et al. 2016), which might correspond well with their timing of peak recruitment and their timing of detection by human observers. If this temporal relationship can be demonstrated, then seasonal patterns of established invasive species could serve as a proxy for the timing of detecting new biological invasions (i.e., early detection), especially if these species share similar life-history strategies.

During the growing season, fouling species on space-limited settlement plates proliferate (e.g., Stachowicz et al. 1999). This rapid development of the fouling assemblage in size and abundance eventually (and sometimes rapidly) decreases the probability of detecting rare species through (1) high competition for bare space/suitable substrate such that the odds of propagules of a rare invasive species successfully settling onto a plate with a developed benthic community are low, (2) unfavourable interspecific interactions (e.g., predation or overgrowth) resulting in mortality, or (3) human observational bias such that rare or cryptic species are missed during plate processing such that plates with a high diversity of species can cause rare species to be less distinct in form and colour (Dafforn et al. 2009; Collin and Johnson 2014; Stanislawczyk et al. 2018). These issues of biotic resistance and sampling biases with respect to the optimal season for early detection of marine invasive species remain poorly examined.

We aim to ascertain the relative importance of spatial and temporal scales affecting observations of recruitment of marine invertebrates by examining the following sources of variability: (1) large spatial scales (regional scales of 100s of km), (2) small spatial scales (site-level scales of < 100s of km), (3) long temporal scales (years), and (4) short temporal scales (time of deployment). Sources of variability that are associated with the high variability in observations of rare species can inform the design of the sampling procedure specifically for the early detection of rare invasive species (e.g., how limited resources for sampling should be prioritized in space and time). Given that temporal considerations in sampling are also integral for rare species detection, the seasonal timing of peak recruitment of all native and invasive species per site is examined to understand the temporal dynamics at each field site to evaluate how the timing of peak recruitment is partitioned over time (i.e., evidence of temporal niche partitioning; Hutchinson 1958; Stachowicz and Byrnes 2006; Reinhardt et al. 2013). We expect that peak recruitment of native species

(relative to that of invasive species) will dampen or suppress the recruitment of invasive species because high settlement of native species may inhibit settlement of invasive species (evidence for biotic resistance). Conversely, we expect that higher rates (i.e., around the timing of peak recruitment) of invasive species recruitment will substantially impact the recruitment of native species (evidence for negative impact on native assemblages). Further, we aimed to determine whether the seasonal timing of peak recruitment could serve as a meaningful and robust proxy for the optimal timing for detecting rare invasive species (i.e., implications for early detection).

3.4 Materials and methods

3.4.1 Recruitment patterns

Artificial settlement plates were used to determine the *de novo* recruitment rates of 22 marine benthic (invertebrate) taxa (see Table 4). Plates were constructed of grey PVC (area: 14.6 × 14.6 cm), suspended in the water column in the horizontal position at 1 m below the water surface, and hung off of floating docks with polyethylene rope (i.e., one plate per line; Supplemental Figure S1). The bottom-facing surface was roughened using a handheld belt sander. Half of a cored clay brick (ca. 0.85 kg) was fixed to the top-facing surface of the plate to keep the line taut.

From 2012 to 2014, time-series recruitment data were collected from a total of 14 coastal harbours (sites) across the Canadian Maritimes in Nova Scotia and Prince Edward Island (map: Figure Figure 18). In 2012, sampling was done at seven sites in Charlottetown (46.2299°N, 63.1247°W), Halifax (44.6810°N, 63.6105°W), Lunenburg (44.3757°N, 64.3110°W), Mahone Bay (44.4477°N, 64.3740°W), Montague (46.1650°N, 62.6467°W), St. Peter's (45.6611°N, 60.8760°W), and Sydney (46.1389°N, 60.1972°W). In 2013, field sites were at only five sites in Cape Breton Island, Nova Scotia: Baddeck (46.0993°N, 60.7482°W), Ben Eoin (45.9818°N, 60.4314°W), Orangedale (45.9007°N, 61.0879°W), St. Peter's (see above), and Whycocomagh (45.9684°N, 61.1125°W). In 2014, three additional field sites in Cape Breton Island were included in the study: Bras d'Or (46.2518°N, 60.3022°W), Dundee (45.7002°N, 61.0917°W), and New Harris (46.2371°N, 60.4999°W).

Plates were deployed at the beginning of summer in May or June before or during the seasonal onset of sexual reproduction for many of the studied taxa. A set of six replicate plates, clustered near the geographic center of the site, was deployed for four weeks (mean of 28.5 days) at each site. Once the study is underway, a new set of plates was deployed in the vicinity of the previous set of plates (i.e., essentially a repeated measure) every two weeks until the end of the field season. In 2012, the study started between the third and last week of June and ended between the third and last week of October for a total of eight deployments. In 2013, the study started on the last week of May and ended in mid-August for a total of five deployments. In 2014, the study started in mid-May and ended on the second week of October for a total of ten deployments. Although not the main focus of this study, the first set of plates was deployed at an earlier start date for each successive year to determine the timing of the seasonal onset of recruitment.

Benthic recruits on the downward facing plate surface were identified to the lowest possible taxonomic level. Recruitment rates were standardized per unit area and time (number of recruits cm^{-2} d^{-1}). Soft-bodied species (e.g., ascidians) were counted live immediately after plate retrieval (e.g., see Figure 19). After enumerating soft-bodied species, plates were air-dried and hard-bodied species (e.g., barnacles, bivalves, bryozoans) were identified and counted at a later date using a stereomicroscope.

For each taxon, a generalized linear mixed-effects model (GLMM with Poisson error distribution) was used to analyze variability in recruitment rates (number of recruits cm^{-2} d^{-1}) among regions (i.e., Cape Breton Island, Prince Edward Island, and South Shore of Nova Scotia; random effect), sites (random effect), years (random effect), and dates of plate deployment (random effect). For each source of variation (*Region*, *Site*, *Year*, *Date of deployment*) the variance components (estimated with GLMM) were converted to percentages (%) to examine the distribution of variance. Additionally, three-way GLMMs (*Region* × *Site* × *Date of deployment*) and (*Site* × *Year* × *Date of deployment*) on recruitment data were computed for each taxon to examine the robustness of the four-way GLMM (*Region* × *Site* × *Year* × *Date of deployment*). Notably, the results of the four-way GLMMs were robust despite data collection across the three different regions were done a single year (2012) and data collection across the three different years were done in a single region (Cape Breton Island).

Local regressions (LOESS) were fitted to the recruitment data, which were either $\log_{10}(x)$ or $\log_{10}(x + 0.001)$ transformed, to visualize overall temporal trends. For example, LOESS was computed to compare the temporal dynamics of maximal recruitment for native and invasive species (for each site and all sites combined). LOESS was also used to generalize the seasonal recruitment patterns for each invasive species with sufficient data (and, in the supplement, for other native species). All statistical analyses and graphical outputs were done in the R programming environment (R Core Team 2019). The GLMMs were fitted to the data using the lme4 package in R.

3.4.2 Seasonal timing of detection of rare invasive species

Among the assemblage of marine benthic (invertebrate) taxa found recruiting onto settlement plates, seven species were invasive (non-native and or cryptogenic) in the region: *Ascidiella aspersa*, *Botrylloides violaceus*, *Botryllus schlosseri*, *Caprella mutica*, *Ciona intestinalis*, *Diplosoma listerianum*, and *Membranipora membranacea*. Of these species, *A. aspersa* and *D. listerianum* were discovered for the first time in Nova Scotia, and their early detection likely represented an ongoing biological invasion in the region. In collaboration with other research and monitoring groups, the early detection and subsequent invasion history of these two ascidians in the Canadian Maritimes have been documented in a series of papers (Moore et al. 2014; Ma et al. 2016, 2018, 2019; also see Appendix 2).

In addition to settlement plate monitoring, (a) attached eelgrass (*Zostera marina*) shoots, (b) freshly detached eelgrass shoots floating on the water surface, and (c) rocks from shallow waters were haphazardly collected and examined for the presence of invasive species from sites surveyed in 2013 and 2014 (Figure 20). Attached eelgrass was sampled from the same patch of eelgrass meadow at each site (i.e., repeated measure) every second week using the metal blade of a bow rake attached to a 4-m-long PVC handle. Depending on the site, floating eelgrass shoots were collected near dock structures, near recreational boats, and/or from the shore.

At many of our field sites, the populations of invasive species are considered established such that recruitment patterns are discernible (also see Sephton et al. 2011, 2017). These sites are thus categorized as "established sites" (*sensu* Falk-Petersen et al. 2006). In contrast, "non-established" sites were ones where

these invasive species were detected at low abundances (i.e., rare with less than ten individuals detected per site per season) and distributed sporadically in time (temporally disjunct). The combination of low abundance and disjunct in temporal distribution is suggestive that the species is having establishing a self-sustaining reproductive population. The timing of detecting invasive species at non-established sites was superimposed on the seasonal window of peak recruitment determined from the data at established sites to evaluate whether the period of peak recruitment is the optimal time to sample for rare (invasive) species.

3.5 Results

3.5.1 Phenological patterns and variability in recruitment

For most of the taxa in this study (n = 13; 59% of the taxa), the source to which most of the variation in recruitment rates can be attributed to was the local (*Site*) spatial scale (Figure 21). Most variation in recruitment for seven taxa (32%) was attributed to short temporal scale (*Date of deployment*; Figure 21). Large spatial scale (*Region*) was the greatest source of variation in recruitment for one species (*C. virginica*) and long-term temporal scale (*Year*) for another species (*C. fornicata*; Figure 21). There are no apparent relationships between these groupings and taxonomic groups or species status (i.e., native or invasive). However, there is a significant difference in mean total abundance (i.e., the sum of all recruits enumerated on plates) between (a) widespread, commonly encountered species on our experimental settlement plates (i.e., mean total abundance of 25,266 recruits) and (b) rarely encountered species (mean of 356 recruits; unequal variance two-sample t-test: $p = 0.041$). Taxa in the former group (widespread, commonly encountered species) were associated with high spatial variability while those in the latter group (rarely encountered species) were associated with high temporal variability.

Overall, seasonal recruitment windows (e.g., the seasonal onset of recruitment) and seasonal recruitment patterns (i.e., timing of site- and year-integrated peak recruitment) varied substantially among study taxa (Supplemental Figure S9 and Supplemental Figure S10). However, among these taxa, seasonal recruitment patterns for the colonial ascidian, *D. listerianum*, and the encrusting bryozoan, *Schizoporella* sp., are not known due to a dearth of data spanning the sampling season. For most taxa, there was a distinct timing of peak recruitment (unimodal peak). Taxa with early peaks in recruitment occurring in July or early August

included *Asterias rubens*, *Ectopleura larynx*, *Electra pilosa*, *Crassostrea virginica*, *Cryptosula pallasiana*, *Halichondria panicea*, *Hiatella arctica*, and *Mytilus* sp. Species with mid-season peaks in recruitment occurring in August included *C. intestinalis*, *Conopeum tenuissimum*, and *Crepidula fornicata*. Taxa with late seasonal peaks in recruitment (early September through October) included *A. aspersa*, *B. schlosseri*, *B. violaceus*, *C. mutica*, *Cribrilina* sp. *Crisularia turrita*, *M. membranacea*, and *Molgula manhattensis*. A distinct recruitment peak was not apparent in *Amphibalanus improvisus*, which showed high rates of recruitment between August and September. Among the study taxa, only *C. tenuissimum* recruitment exhibited two distinct seasonal peaks (bimodal): first in July and the second in August. However, recruitment for some taxa, such as *C. turrita* and *Cribrilina* sp., appear to have an earlier yet weaker peak in July.

3.5.2 Temporal dynamics of peak recruitment

The timing of maximal recruitment rates for each taxon at each site was plotted and examined in Figure 22 (but also see the entire seasonal recruitment data for each taxon in Supplemental Figure S11). At several sites, native species tended to peak earlier in the season (i.e., between June and August) and invasive species later in the autumn. Sites exhibiting this "temporal offset" pattern were Baddeck, Ben Eoin, Mahone Bay, and New Harris. The most obvious instance of temporal offset between native and invasive species was found in Ben Eoin and New Harris. In Bras d'Or, recruitment of invasive species peaked during periods when maximal recruitment of native species tended to be at relatively lower rates. This "high–low contrast" pattern between the peak recruitment rates of native and invasive species is also observed in other sites such as Lunenburg. However, in Lunenburg, this high–low contrast pattern changed over time. Earlier in the season (summer), the maximal recruitment rate of an invasive species was higher than the maximal recruitment of native species. The pattern reversed later in the season (autumn) where the maximal rates of recruitment of native species were higher than the maximal recruitment of invasive species. In Halifax, the high–low contrast pattern changed from relatively higher recruitment of native species earlier in the season (summer) to higher rates of invasive species later in the season (autumn). Finally, there are no differences in the seasonal trajectory ("coexistence" pattern) of the timing of maximal recruitment between native and invasive species in Charlottetown, Dundee, and St. Peter's (and, perhaps, also in Montague and Sydney). By pooling the data from all 14 sites, the temporal trend visually exhibited an inverse relationship between the overall timing of peak recruitment between native and invasive assemblages (Figure 23). Maximal

recruitment rates in native species were greatest in mid-July and late August or early September. However, these rates were greatest in invasive species (including the cryptogenic species, *C. intestinalis*) in late June, early August, and late September.

3.5.3 Invasive species at established sites

Established populations of six invasive species were found at multiple field sites, and data from these sites were used to describe seasonal patterns of recruitment. *Ascidiella aspersa* appeared to be established in Lunenburg, based on the high rates of recruitment over multiple sampling time points. Similarly, Charlottetown and Lunenburg were sites where populations of *B. violaceus* were established. Although a modest level of *C. mutica* recruitment was detected in New Harris, the high number of individuals fouling polyethylene rope over time suggested that a population was established at this site. Established populations of *C. intestinalis* were evident at the following six sites: Bras d'Or, Halifax, Lunenburg, Mahone Bay, Montague, and Sydney. *Membranipora membranacea* was established at five sites (Halifax, Lunenburg, Mahone Bay, New Harris, and Sydney). *Botryllus schlosseri* was the most common and widespread invasive species in the study with established populations at 11 sites in Baddeck, Ben Eoin, Bras d'Or, Charlottetown, Dundee, Halifax, Lunenburg, Mahone Bay, New Harris, Orangedale, and St. Peter's. The presence and absence of these invasive species reported in this study are consistent with the monitored sites (e.g., Baddeck, Ben Eoin, Halifax, Lunenburg, Mahone Bay, St. Peter's, and Sydney) surveyed by the federal government between 2012 and 2015 (Sephton et al. 2017). Additional observations of *A. aspersa* in Lunenburg in 2013 have been reported, which suggest that the local population survived the winter (Ma et al. 2019). Early records of *C. intestinalis* from Charlottetown and Montague—between the years of 2008 to 2010 and 2004 to 2006, respectively (i.e., prior to this study)—have been documented in the literature (Lutz-Collins et al. 2009; Ramsay et al. 2008, 2009; Kanary et al. 2011).

3.5.4 Site-specific early detection of invasive species at non-established sites

Sixteen records of site-specific early detection (i.e., new records for the site) were documented for six species at six field sites between 2012 and 2014 (Figure 24). Three recruits of *D. listerianum* were detected on

settlement plates in Lunenburg on 16 October 2012 (date of plate retrieval). This observation constituted one of the earliest records of this non-native species in Nova Scotia (Moore et al. 2014; Ma et al. 2016; also see Appendix 2). Similarly, *B. schlosseri* recruitment was not observed via plates in Whycocomagh yet this species recruited onto larger settlement plates (area: 20 × 20 cm) deployed off of nearby buoys for a separate study (detection on these larger plates are hereafter referred to as buoy stations) at two time points: 17 September and 9 October 2013. During all three years, low numbers of *C. intestinalis* recruits were detected on plates in St. Peter's on 27 September 2012, 16 August 2013, 8 August 2014, and 9 October 2014. Further, detection of this species in St. Peter's was temporally disjunct over the sampling season for both years, suggesting that a population of *C. intestinalis* was not established. Occasionally, few recruits of *C. intestinalis* were observed concurrently on nearby buoy stations. Although recruits of *B. violaceus* and *C. intestinalis* were not detected on any of our plates in New Harris, small colonies of *B. violaceus* were found on rocks (pictured in Figure 20) and floating eelgrass shoots on 15 September 2014, and a single *C. intestinalis* recruit was observed on a nearby buoy station on 28 September 2014. In 2012, several *M. membranacea* recruits were observed on plates in Charlottetown on 30 July and 9 October 2012. Between 2013 and 2014, a single *M. membranacea* recruit was detected on plates at three sampling time points in Baddeck (21 July 2013, 8 and 17 October 2014) and once in Whycocomagh (8 October 2014). Although short-term settlements plates may not be entirely suitable for their detection, low numbers of recruits of *C. mutica* were observed on plates at three sites: Charlottetown on 9 October 2012 (n = 1 recruit), Lunenburg on 16 October 2012 (n = 1), and Baddeck on 17 October 2014 (n = 2). Consistent with the claim that these observations can be treated as site-specific early detections, government monitoring in Baddeck and Lunenburg did not detect *C. mutica* during the same years in 2012 and in 2014 and, moreover, the preceding and following years from 2011 to 2015 (Sephton et al. 2015; Sephton et al. 2017). Although first detected from Brudenell River in Prince Edward Island in 1998, no records of *C. mutica* from Charlottetown were known prior to 2007 (Locke et al. 2007) and, to our knowledge, between 2007 and 2012.

3.5.5 Seasonal timing of peak recruitment as a proxy for early detection

Upon visual inspection, the seasonal timing of early detection of invasive species at non-established sites temporally aligned with the seasonal timing of peak recruitment derived from established sites for *B. schlosseri* and *B. violaceus*, but only for some detections of *C. intestinalis* (Figure 23). For *C. mutica* and

M. membranacea, a majority of the site-specific early detections were observed in October, which roughly corresponded with the timing of peak recruitment from established sites in September (Figure 23). Overall, a majority of site-specific early detections (n = 13; 76%), including that of *D. listerianum*, occurred in either September or October (Figure 23). The remaining four records of site-specific early detection were for *M. membranacea* (n = 1) and *C. intestinalis* (n = 3), which occurred in July and August, respectively. For both these species, the timing of these mid-summer detections coincided when recruitment rates (as determined from established sites) were seasonally low.

3.6 Discussion

Since the advent of programs to monitor for marine invasive species, most programs seemed to have prioritized spatial coverage (both in terms of geographic extent and small-scale spatial resolution) over temporal coverage (both sampling duration and frequency; e.g., Sephton et al. 2017). In some cases, monitored sites are not revisited each year, likely due to constraints on finite resources. In contrast to point sampling, some passive monitoring tools (e.g., settlement plates) are deployed over various lengths of time (duration), which permit species to accumulate over time (Hirata 1987; Tait et al. 2016; Marrafini et al. 2017). Although longer immersion of settlement plates may increase temporal coverage and overall taxonomic richness, it may not be the optimal duration for rare species detection as bare hard substrate for larval settlement decreases over time (see Chapter 2). This study demonstrates that for widespread, common species, the spatial dimension is the main source of variation in recruitment on settlement plates (consistent with Broitmann et al. [2008]). However, for rare species, the temporal dimension is the main source of variation in recruitment. Therefore, if the goal of monitoring programs is to detect new incursions as early as possible when the invading species are likely to be rare in terms of population size, then it would be critical to prioritize temporal aspects of sampling (e.g., monitoring throughout the season and conducting annual surveys at a given site) over spatial aspects (e.g., increasing the number of sites).

In highly seasonal systems such as in temperate and polar waters, knowing the seasonal onset of recruitment can inform managers on the earliest time in the year to commence monitoring activities (also see similar seasonal data from Osman [1977], Harms and Anger [1983], and Terwin et al. [2002]). Further, it would be an ineffective use of the sampling tool if monitoring occurred when no propagules could potentially be in the

water column or when vectors and pathways transporting invasive species are not active. In 2012, high recruitment of the invasive ascidian, *B. violaceus*, in June in Lunenburg, suggests that the onset of recruitment for marine benthic invertebrates in the Canadian Maritimes can start even earlier (e.g., seasonal onset of fertilization could be as early as April; Westerman et al. 2009). Although we did not return to Lunenburg in subsequent seasons, we moved the start of sampling earlier in May in 2013 and 2014 to ascertain the seasonal onset of recruitment for other fouling species settling on plates. Despite these efforts at our field sites, the seasonal onset of recruitment for several species (namely *A. aspersa*, *A. rubens*, *B. violaceus*, and *H. panicea*) was not resolved in this study. However, other seasonal studies on *A. aspersa*, for instance, in temperate waters have previously documented (1) recruitment commencing either as early as mid-June (Stachowicz et al. 2002a) or substantially later in August (Harms and Anger 1983) and (2) the presence of larvae in the water column as early as June (Kanamori et al. 2017). Moreover, the onset of *B. violaceus* recruitment has been observed in early June from temperate waters (Stachowicz et al. 2002a), but may be earlier if fertilization is in April (Westerman et al. 2009).

Each species would need a strategy to ensure the long-term reproductive fitness of the population or occupy a specific temporal niche (Hutchinson 1958; Schoener 1974; Tokeshi 1986; Reinhardt et al. 2013). Thus, the seasonal peak of recruitment for a given species is likely influenced by the other species recruiting at the same time, especially when the timing of their peak recruitment overlaps, because they are competing for limited space on hard substrate. Further, high diversity of resident species can act as a form of biotic resistance against the successful establishment of an invasive species (Elton 1958; Stachowicz et al. 2002b; Levine et al. 2004; Freestone et al. 2013). Thus, high recruitment rates of the assemblage of native species should make it difficult for an invasive species to recruit to settlement plates, especially if recruitment of invasive and native species occurred at the same time. From this study, an example of this form of recruitment dampening or suppression by native species (a form of biotic resistance) may be occurring in the later part of the season (from late August to September) in Lunenburg. However, in the long-term, high rates of invasive species recruitment might, in turn, dampen the rate of recruitment or shift the temporal niche of native species (Harrington et al. 2009). This dampening–suppressive pattern by invasive species may be impacting the native assemblage in Baddeck, Bras d'Or, Halifax (in August and September), and Lunenburg (in June). Combining the data from all the sites in the Canadian Maritimes reveals a possible high–low contrast pattern in the timing peak recruitment between native and invasive species over the season where the timing of higher peak invasive recruitment corresponded with slightly depressed peak native recruitment.

Over evolutionary time, peak recruitment for native species is expected to occupy specific temporal niches and, therefore, the timing of these peaks would be spaced out in time (Reinhardt et al. 2013). Unfortunately, we were unable to study a natural system without any introduced species and anthropogenic impacts, because every site is an urban space that has been impacted by at least one marine invasive species. The "temporal offset" and "high–low contrast" patterns in peak recruitment observed at about half of the sites in this study provide some, albeit site-specific, evidence that an introduced invasive species can encounter some biotic resistance from high recruitment rates of native species. However, once established, high recruitment rates of invasive species could exert substantial ecological pressure (e.g., competition for space) that could, in turn, dampen the natural recruitment rates of native species. Pattern switching (i.e., when peak recruitment of invasive species was greater than that of native species) was observed at a few of our field sites (e.g., Bras d'Or, Halifax), which hints at the possibility of invasive species dampening the recruitment of native species. Further research is needed to better understand how these interspecific patterns (e.g., temporal offset, high–low contrast, coexistence) arise as species invade a site over time.

The unimodal and bimodal recruitment patterns of invasive species may be an artifact of their evolutionary history and/or constraints imposed by abiotic factors in their invaded range (Stachowicz et al. 2002a; Ma et al. 2017b). Previous studies documented a unimodal and bimodal seasonal recruitment pattern in a population of the invasive colonial ascidian, *B. schlosseri*, in Newfoundland and in a population of the cryptogenic solitary ascidian, *C. intestinalis*, in Nova Scotia, respectively (Harris et al. 2017; Ma et al. 2017a). Consistent with the Newfoundland study, the seasonal recruitment pattern of *B. schlosseri* at the larger spatial scale of this study is suggestive of a unimodal pattern. Moreover, the recruitment pattern of *C. intestinalis* is clearly unimodal at the larger scale of the Canadian Maritimes. Although *C. intestinalis* exhibited unimodal recruitment patterns at some sites, a bimodal pattern has been more commonly reported in Nova Scotia (Carver et al. 2006; Harris et al. 2017; Howes et al. 2007; Vercaemer et al. 2011). This intra-specific variability indicates that recruitment data of multiple populations might average out at larger spatial scales and obscure important single-population fine-scale patterns.

Describing the seasonal and inter-annual recruitment patterns of established populations of targeted invasive species is considered an important step towards assessing the ecological impact of a biological invasion

(Osman 1977; Watson and Barnes 2004). Further, ascertaining this phenological information could guide management concerning the timing of eradication and control measures (Deibel et al. 2014). However, studies investigating long-term recruitment patterns tend to be carried out on local populations (preferably, a closed population) and it may not be appropriate to assume that temporal dynamics from a single population (1) are the same for other populations or (2) represent overall patterns across larger spatial scales. Given the large spatial variability among sites, caution should be exercised when applying conclusions drawn from small-scale temporal data for monitoring, including early detection, across large spatial scales. Also, these long-term population dynamic studies tend to be focused on particular species; thus, it is difficult to generalize for all invasive species across different taxa for management purposes. These shortcomings were addressed in this long-term (multi-year), large-scale (multi-site), multi-species study using short-term and high temporal resolution passive sampling, which generalized seasonal recruitment patterns for both native and invasive marine species across the Canadian Maritimes.

Overall seasonal (site- and year-integrated) recruitment patterns of invasive species might be an effective approach to guide bio-invasion management and policy decisions. In particular, as clearly shown in this study, these general recruitment patterns can provide insights into the optimal timing of efforts aimed at early detection of invasive species. Further, the seasonal timing of peak recruitment appears to correspond to the optimal timing for early detection of most invasive species during the autumn months. For (invasive) biphasic benthic (invertebrate) species, this temporal correspondence could be a result of the same abiotic and biotic cues affecting the release of larvae (propagules) and their settlement onto hard substrates. For instance, fouling organisms transported by ship hulls are likely to be biologically primed to release their gametes and larvae during their seasonal timing of peak recruitment, especially if they encounter suitable environmental conditions for settlement and recruitment as the ship moves through different water bodies (Minchin and Gollasch 2003). Thus, for a fouling invasive species, this timing would also correspond to the optimal seasonal timing of their detection at uncontaminated sites when the probability of their detection is the highest. From this study, the case of the early detection the invasive ascidian *D. listerianum* demonstrates how the timing of peak recruitment can inform the timing of detecting newly introduced species before they become established. Upon discovery in Nova Scotia in 2012, the identity of this invasive ascidian was immediately verified by expert confirmation (digital photographs) and molecular methods (Moore et al. 2014; Ma et al. 2016; also see Appendix 2). Notably, the timing of its detection in October corresponded to the timing of peak recruitment of the majority of marine invasive ascidians that were observed in this study

(in autumn). At least for the ascidian taxa, this suggests that the phenology of established invasive ascidians could inform the seasonal timing of early detection of rare invasive ascidians. Future studies can take this approach to determine the optimal timing of early detection for other invasive taxa beyond that of invasive ascidians; however, more studies would be required to adapt this approach in the face of a changing climate because seasonal patterns will likely change correspondingly.

In addition to detecting rare invasive species, this study has generated a wealth of ecological time-series data that can inform temporal and spatial recruitment patterns for native species, including economically-important species such as oysters and mussels. This information not only offers substantial insights into their population dynamics but also dynamics at the assemblage-level (Osman 1977; Broitmann et al. 2008). Beyond the temporal aspect of early detection of invasive species, this application of rare species detection can be applied to monitor the range expansion of marine species, such as poleward shifts in species range in the context of global change (e.g., climate change; Sorte et al. 2010; Yamano et al. 2011; Renaud et al. 2012). Similar to rare invasive species at the invasion front, these spreading species are likely to be rare at the limits of their range where they may be experiencing suboptimal environmental conditions (Canning-Clode et al. 2011; Cannizzo and Griffen 2019).

Table 4 — Occurrence of 22 marine benthic taxa on settlement plates in 14 sites across Prince Edward Island (PEI) and Nova Scotia (NS). BA = Baddeck; BE = Ben Eoin; BO = Bras d'Or; CH = Charlottetown; DU = Dundee; HA = Halifax; LU = Lunenburg; MB = Mahone Bay; MO = Montague; NH = New Harris; OR = Orangedale; SP = St. Peter's; SY = Sydney; WH = Whycocomagh.

Taxon	Authority	PEI		South Shore (NS)			Cape Breton Island (NS)									
		CH	MO	HA	LU	MB	BA	BE	BO	DU	NH	OR	SP	SY	WH	
Porifera, Demospongiae																
Halichondria panicea	(Pallas, 1766)					×								×		
Cnidaria, Hydrozoa																
Ectopleura larynx	(Ellis & Solander, 1786)	×	×		×		×									
Arthropoda, Amphipoda																
Caprella mutica	Schurin, 1935	×			×		×				×					
Arthropoda, Cirripedia																
Amphibalanus improvisus	(Darwin, 1854)	×	×	×			×	×	×	×	×	×	×	×	×	
Mollusca, Bivalvia																
Crassostrea virginica	(Gmelin, 1791)						×	×	×	×	×	×	×		×	
Hiatella arctica	(Linnaeus, 1767)			×			×	×		×	×		×		×	
Mytilus sp.	Linnaeus, 1758	×	×	×	×		×	×	×	×	×	×	×	×	×	
Mollusca, Gastropoda																
Crepidula fornicata	(Linnaeus, 1758)	×					×	×	×	×	×	×	×		×	
Echinodermata, Asteroidea																
Asterias rubens	Linnaeus, 1758	×		×	×	×	×	×		×	×	×	×	×	×	
Bryozoa, Gymnolaemata																
Conopeum tenuissimum	(Canu, 1908)			×			×	×	×	×	×	×		×	×	
Cribrilina sp.	Gray, 1848	×			×	×	×		×		×			×		
Crisularia turrita	(Desor, 1848)				×	×	×	×					×	×	×	
Cryptosula pallasiana	(Moll, 1803)	×		×	×		×		×					×		
Electra pilosa	(Linnaeus, 1767)	×		×	×	×	×	×	×	×	×	×	×	×	×	
Membranipora membranacea	(Linnaeus, 1767)	×		×	×	×	×				×			×	×	
Schizoporella sp.	Hincks, 1877									×					×	
Chordata, Ascidiacea																
Ascidiella aspersa	(Müller, 1776)				×											
Botrylloides violaceus	Oka, 1927	×			×											
Botryllus schlosseri	(Pallas, 1766)	×		×	×	×	×	×	×	×	×	×	×			
Ciona intestinalis	(Linnaeus, 1767)		×	×	×	×			×				×	×		
Diplosoma listerianum	(Milne Edwards, 1841)				×											
Molgula manhattensis	(De Kay, 1843)	×					×		×			×				
Total number of taxa:		13	4	10	14	8	16	10	11	10	12	9	10	11	11	

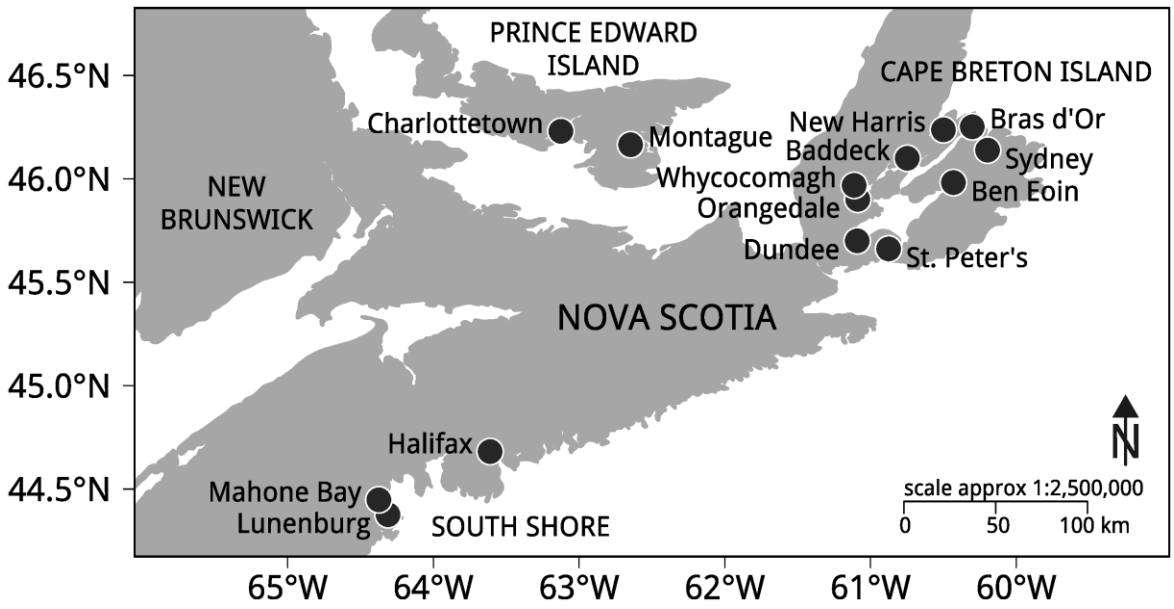


Figure Figure 18 — Map of the fourteen field sites in the Canadian Maritimes.

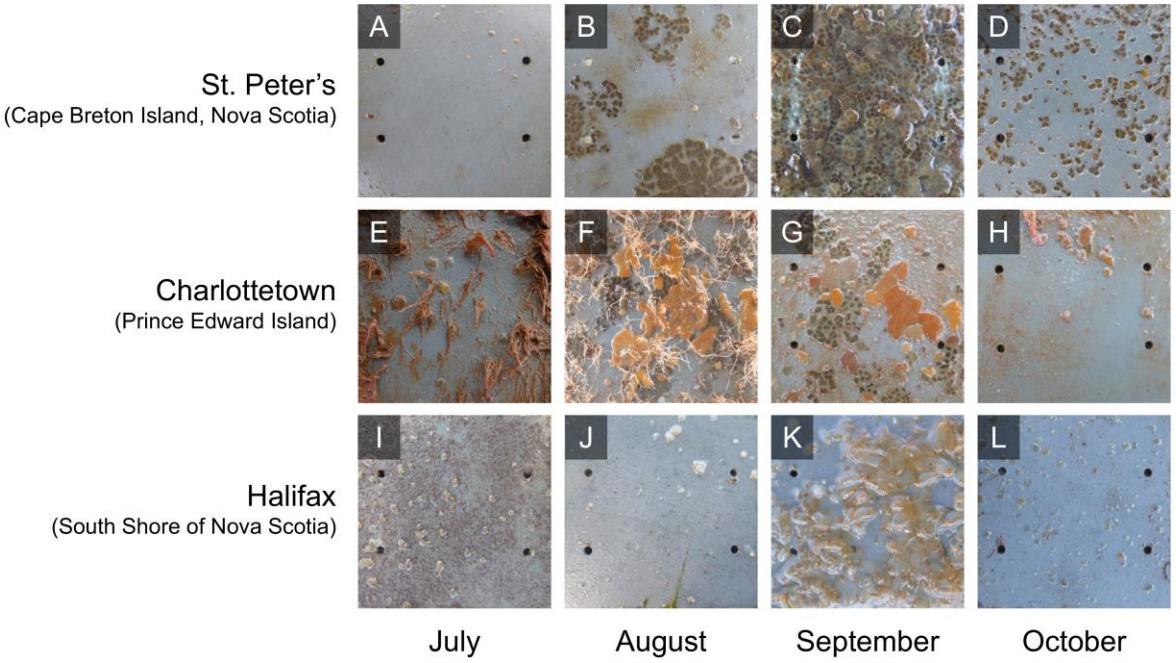


Figure 19 — Examples of settlement plates upon retrieval after a sampling duration of approximately four weeks in July, August, September, and October. These example plates are from three field sites representing three different regions in the Canadian Maritimes: (A–D) St. Peter's, Cape Breton Island, Nova Scotia; (E–H) Charlottetown, Prince Edward Island; (I–L) Halifax, South Shore of Nova Scotia. Area of a plate: 14.6 × 14.6 cm.

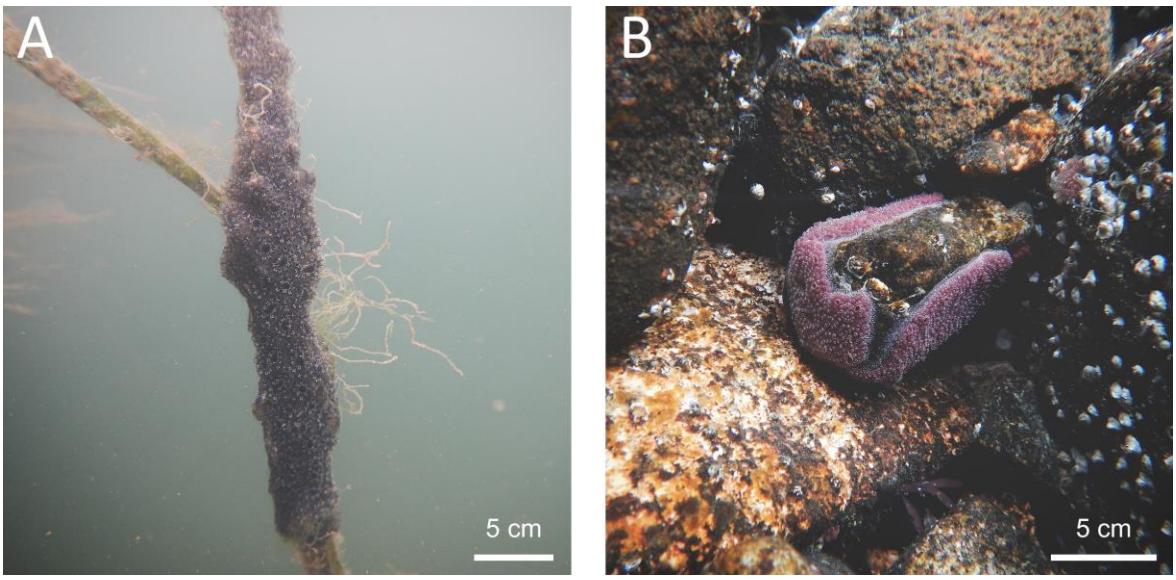


Figure 20 — Examples of marine invasive species fouling (A) eelgrass shoots (a *Botryllus schlosseri* colony from an established population in St. Peter's, Nova Scotia, Canada) and (B) rocks from shallow waters (a site-level early detection of a *Botrylloides violaceus* colony in New Harris, Nova Scotia, Canada).

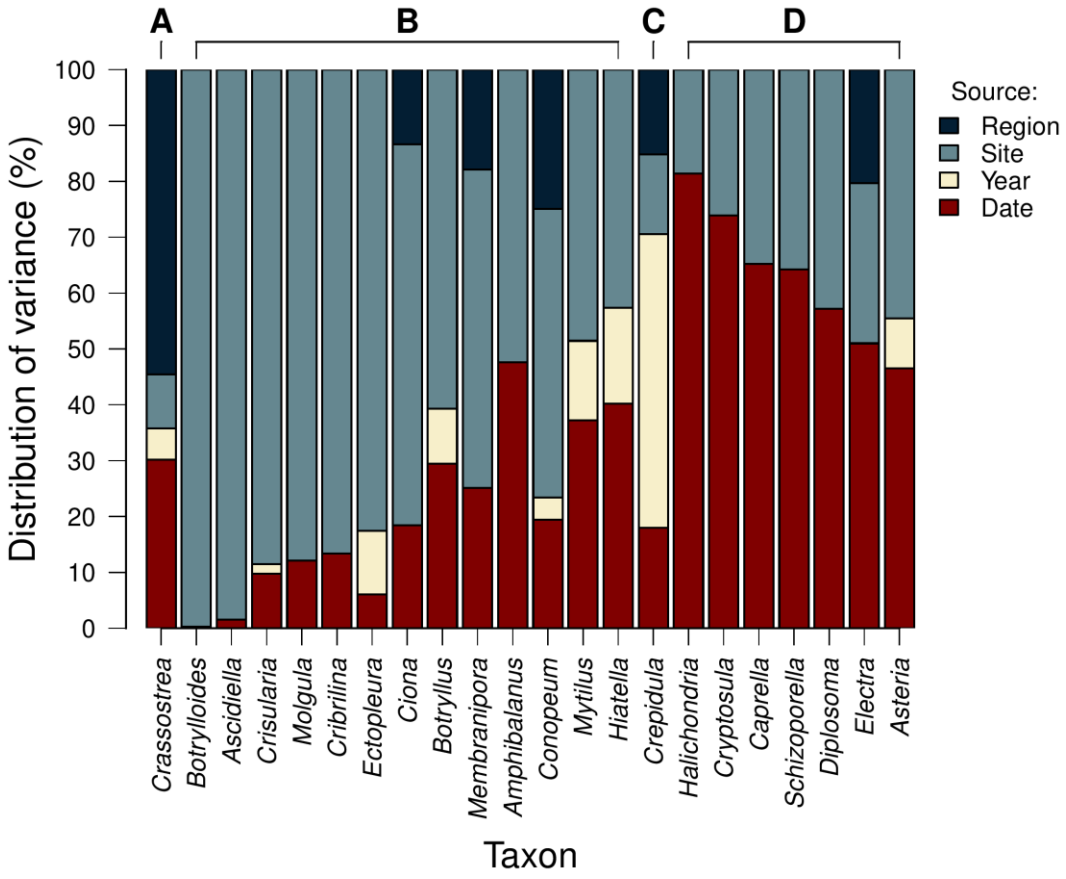


Figure 21 — Distribution of variance in recruitment on settlement plates estimated with generalized linear mixed-effects model (GLMM) for 22 marine benthic taxa (species are referred to by their genus). Species are grouped by the source that was associated with the most variance: (A) *Region* (n = 1 species); (B) *Site* (n = 13); (C) *Year* (n = 1); (D) *Date of deployment* (n = 7).

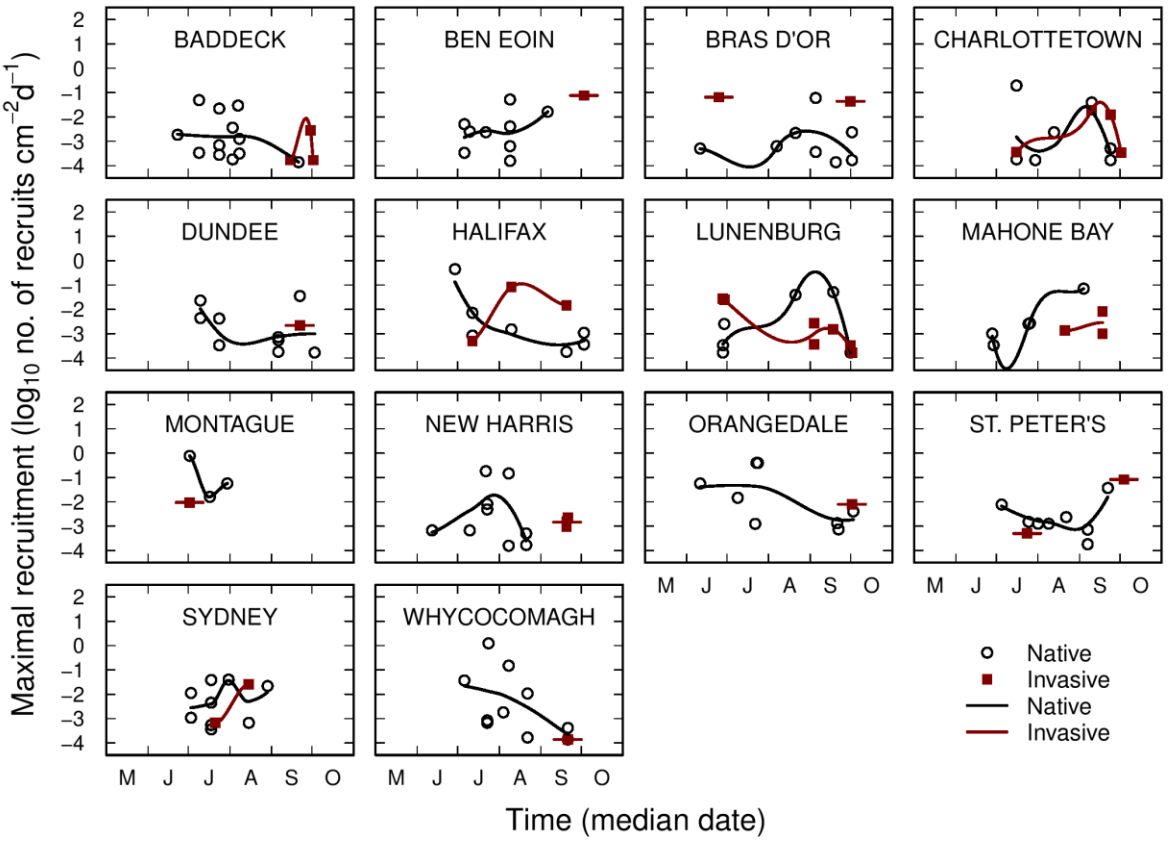


Figure 22 — Seasonal timing of maximal recruitment on settlement plates for 15 native (open black circles) and seven invasive (closed red squares) species detected as each site. Local regression (LOESS, span = 1) was fitted to the data to reveal temporal maximal recruitment trends of native and invasive taxa. LOESS not available for sites if (1) only one invasive species was present, (2) invasive species were temporally clustered (i.e., New Harris), or (3) only two invasive species were present and temporally disjunct (i.e., Bras d'Or, St. Peter's). Invasive species include both non-native and cryptogenic species. Maximal recruitment rates were $\log_{10}(x)$ transformed. See Supplemental Figure S11 for seasonal recruitment patterns of native and invasive taxa for each site.

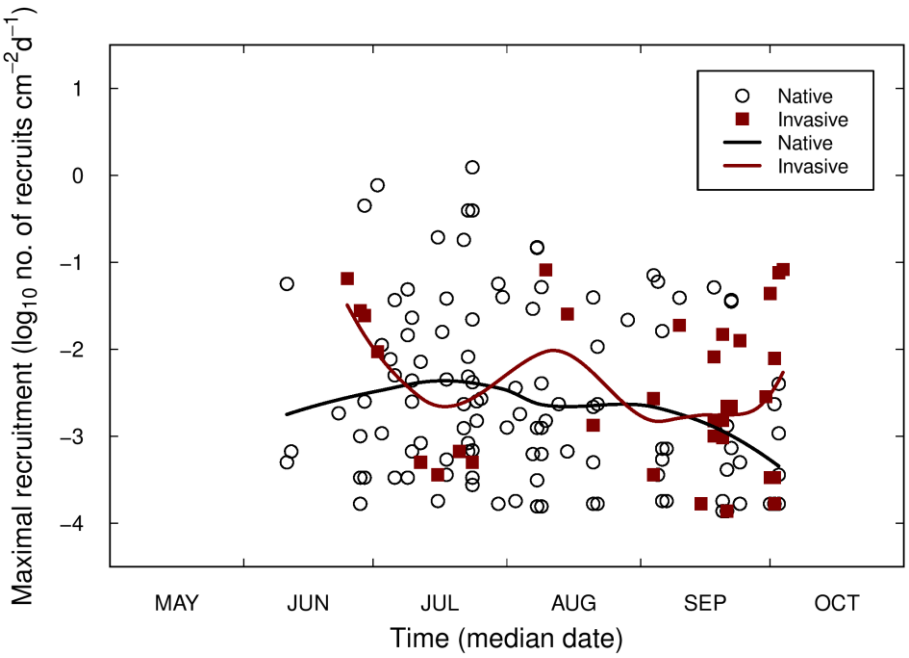


Figure 23 — Seasonal timing of maximal recruitment on settlement plates for 15 native (open black circles) and seven invasive taxa (closed red squares) detected across the Canadian Maritimes (site- and year-integrated). Local regression (LOESS, span = 0.7) was fitted to the data to reveal temporal maximal recruitment trends of native and invasive taxa. Invasive taxa include both non-native and cryptogenic species. Maximal recruitment rates were $\log_{10}$ (x) transformed.

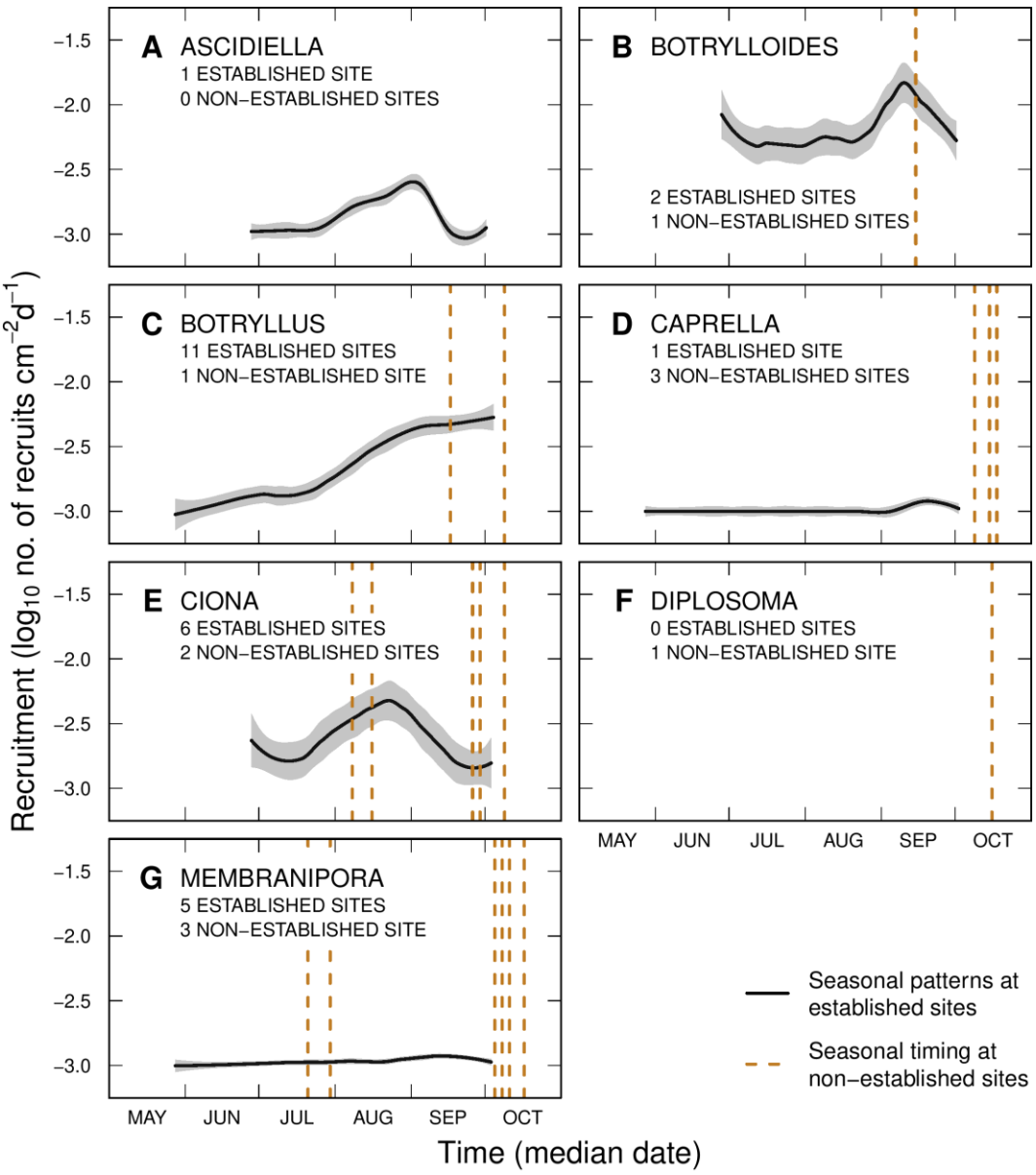


Figure 24 — Seasonal recruitment patterns of seven marine invasive species (species are referred to by their genus) at established sites and seasonal timing of species detection at non-established sites: (A) *Ascidiella aspersa*; (B) *Botrylloides violaceus*; (C) *Botryllus schlosseri*; (D) *Caprella mutica*; (E) *Ciona intestinalis*; (F) *Diplosoma listerianum*; (G) *Membranipora membranacea*. Solid black line = local regression (LOESS, span = 0.5) fitted to $\log_{10}(x + 0.001)$ transformed recruitment rates. Gray shaded area = 95% confidence limits of the LOESS curve. Dashed orange line = date of observation (but overlapping dates are shifted ± 2 days for better visualization of the data).

Chapter 4. Multi-scale sources of spatial variation in recruitment, dominant scales of recruitment, and optimal sampling strategy for rare species detection

4.1 Résumé

L'échantillonnage d'espèces introduites (envahissantes) rares au moyen—par exemple, de l'échantillonnage pour la biodiversité, de la détection aux premières phases du processus d'invasion—nécessite de concentrer les ressources limitées pour maximiser la probabilité de détection. Dans le cas d'une détection précoce, l'incapacité à détecter les espèces envahissantes et à réagir rapidement pourrait entraîner leur établissement et éventuellement, leur propagation. Dans les systèmes côtiers marins, l'échelle spatiale actuelle pour le monitorage des espèces envahissantes correspond à celle des ports de plaisance, des ports de mer et des sites d'aquaculture (environ 10 km ou plus dans les Maritimes canadiennes). Cette étude évalue comment l'échelle spatiale à laquelle l'échantillonnage est réalisé (de quelques dizaines de mètres à quelques dizaines de kilomètres) influe sur la détection d'espèces rares et communes. Dans le lac Bras d'Or, en Nouvelle-Écosse, au Canada, le recrutement d'espèces d'invertébrés marins salissants a été déterminé à l'aide de plaques de fixation en PVC. Les plaques ont été déployées selon un plan d'échantillonnage imbriqué (hiérarchique) en juillet et à nouveau en août 2014. Les échelles spatiales associées à la plus grande variance du recrutement et l'échelle spatiale dominante du recrutement (c'est-à-dire, l'échelle avec des patrons répétitifs détectables) ont été identifiées pour chaque espèce. En ré-échantillonnant les données de terrain et en supposant que l'ensemble des données représente une connaissance complète de la distribution des espèces dans le système, la probabilité de détection des espèces et la richesse en espèces cumulatives ont été calculées selon quatre stratégies d'échantillonnage simulées différentes : (1) l'échantillonnage aléatoire, (2) l'échantillonnage adaptatif en forçant l'échantillonnage à de petites échelles spatiales (≥ centaines de mètres), (3) l'échantillonnage adaptatif en forçant l'échantillonnage à des échelles spatiales intermédiaires (≥ milliers de mètres), et (4) l'échantillonnage adaptatif en forçant l'échantillonnage à de grandes échelles spatiales (dizaines de milliers de mètres). À égalité d'effort d'échantillonnage, des stratégies d'échantillonnage spatialement explicites peuvent considérablement augmenter la probabilité de détection d'espèces rares (jusqu'à 250% dans un cas). De plus, forcer l'échantillonnage à l'échelle spatiale la plus

grande maximisait la richesse en espèces. Cependant, cette stratégie d'échantillonnage a diminué la détection d'espèces rares. Ces résultats mettent en évidence le problème potentiel de l'utilisation de procédures d'échantillonnage optimisées pour augmenter la richesse taxonomique, ce qui peut réduire de manière contre-intuitive la probabilité de détecter des espèces rares.

4.2 Abstract

Sampling for rare introduced (invasive) species—e.g., biodiversity surveys, detection at the early phases of the invasion process—requires focusing limited resources to maximize the probability of detection. In the case of early detection, failure to detect invasive species early and respond rapidly could result in their establishment and, possibly, spread. In marine coastal systems, the current spatial scale for invasive species monitoring is at the scale where marinas, seaports, and aquaculture sites are located (e.g., approximately 10s of km or greater in the Canadian Maritimes). This study assesses how the spatial scale at which sampling is done, ranging from 10s of m to 10s of km, affects detection of rare and common species. In the Bras d'Or Lake, Nova Scotia, Canada, the recruitment of fouling marine invertebrate species was determined using PVC settlement plates. Plates were deployed in a nested (hierarchical) sampling design in July and, again, in August of 2014. The spatial scales associated with the most variance in recruitment and the dominant spatial scale in recruitment (i.e., scale with detectable repeating patterns) were identified for each species. By resampling the field data and assuming that the full dataset represents a complete knowledge of species' distribution in the system, the probability of species detection and the cumulative species richness were calculated under four different simulated sampling strategies: (1) random sampling, (2) adaptive sampling by forcing sampling at small spatial scales (≥ 100s of m), (3) forcing sampling at intermediate spatial scales (≥ 1000s of m), and (4) forcing sampling at large spatial scales (10000s of m). Given equal sampling effort, spatially-explicit sampling strategies can substantially enhance the probability of detection of rare species (up to 250% in one instance). Moreover, forcing sampling at the largest spatial scale maximized species richness; however, this sampling strategy decreased rare species detection. These findings highlight the potential problem of using sampling procedures optimized for increasing taxonomic richness, which may counter-intuitively reduce the probability of detecting rare species.

4.3 Introduction

The detection of rare species in natural systems is a vexing challenge and an immense undertaking when dedicated resources for sampling are finite. Despite this challenge, there are applications across different fields of biology where rare species detection is crucial for effective management (Venette et al. 2002). For example, detection of (1) endangered or endemic species that might become extinct, (2) range expansions, such as poleward shifts, where species are in environmentally suboptimal limits of their distribution, (3) low densities of invasive species at the end of an eradication campaign to minimize the risk of Lazarus effect (i.e., the rapid increase of an invasive population when eradication is prematurely terminated), and (4) new incursions of invasive species when there are few individuals in the environment (Seymour et al. 2005; Canning-Clode et al. 2011; Thomsen et al. 2012; Lockwood et al. 2013; Cannizzo and Griffen 2019). Although there are promising and highly-sensitive detection tools available, such as environmental DNA, sampling requires sufficient understanding of the ecology of target species to maximize the probability of detection without exhausting limited resources. Simply selecting where and when samples are taken from the field (i.e., sites and dates) are not the only decisions an investigator needs to make for an ecological study or a monitoring program, but the spatial and temporal scales must also be carefully considered. Put differently, sampling the wrong places at the wrong time and at the wrong scales could hamper management efforts and needlessly increase the chances of false negatives in species detection.

For biological invasions specifically, preventive efforts to stop the transport of introduced (invasive) species to uncontaminated localities will occasionally fail, and invasive propagules will be released into the environment (Lockwood et al. 2013). Although prevention is preferred, the best management options involve eradication and control once invasive species are introduced (Simberloff 2003a, 2003b, 2009; Finnoff et al. 2007; Howald et al. 2007). However, successful eradication and control necessitate early detection when the incipient invasive population is small (rare in numbers) and localized (small in spatial extent), followed by rapid response, because eradication success decreases dramatically as the targeted invasive population increases in size and spreads (Myers et al. 2000; Rejmánek and Pitcairn 2002; Harvey et al. 2009; Bodey et al. 2010; Vander Zanden et al. 2010; Drolet et al. 2014). Although there could be a bias towards publishing successful over unsuccessful cases, eradication of small and localized invasive populations has been well documented (Sharov and Liebhold 1998; Olson and Roy 2005; Liebhold and Bascompte 2003). However,

small and localized nascent populations are difficult to detect, and thus rapid response may not be possible. Furthermore, the probability of detecting an invasive species depends on (1) the number (or density) of introduced individuals, (2) the area (or volume) surveyed, (3) the effectiveness of the survey method, (4) the skill and experience of observers, (5) the number of localities surveyed, (6) the spatial and temporal distribution of the target species, and (7) the design of the survey (Hayes et al. 2005; Mehta et al. 2007).

Paradoxically, successful eradication and control of invasive species are dependent on early detection, but that is usually not possible until some time after colonization (i.e., lag time; Harvey et al. 2009). Also, detection of a rare species occurs only once the population size or spatial extent exceed a detection threshold. There are few ways that detection thresholds can be lowered: (1) increased efficiency of surveying methods, (2) strategic allocation of monitoring efforts, (3) particular consideration of a target species' biology, or (4) enhanced sampling intensity (Rew et al. 2006; Mehta et al. 2007; Harvey et al. 2009; Lockwood et al. 2013). For many of these approaches, lowering the detection threshold requires calibrations to determine the optimal search effort. For example, enhancement of species richness (Marraffini et al. 2017; Deiner et al 2018; Tait et al. 2018), human observational or visibility bias (Stanislawczyk et al. 2018), sampling effort (Ram et al. 2014; Evans et al. 2017; Grey et al. 2018), and survey methods (Trebitz et al. 2009; Britton et al. 2011; Simmons et al. 2016) have been studied and optimized in aquatic systems.

Because it is difficult, in practice, to carry out field surveys in ecological studies at multiple spatial scales simultaneously, careful consideration is required to select the appropriate scales for sampling (Wiens 1989; Sutherland 1996; Bellehumeur and Legendre 1998; He and Gaston 2000; Rahbek and Graves 2000; Hill and Hamer 2004). Of course, the "appropriate" scales would depend on the purpose of the study and an understanding of how key biological processes generate patterns over multiple spatial scales (Levin 1992). Moreover, how spatial scale affects the targeted ecological response is often species-specific (e.g., Hill and Hamer 2004). For instance, settlement patterns, and subsequently recruitment, may vary markedly among marine species at different spatial scales depending on local hydrodynamics, mode of larval development, and larval dispersal behaviour (e.g., Doherty 1987; Fowler et al. 1992).

The fundamental problem of choosing the appropriate spatial scale for sampling is urgently needed in applied ecology such as resource management and management of biological invasions (Thrush et al. 1999).

Moreover, spatial scale remains a largely understudied aspect of invasion ecology and invasive species management (*sensu* Mehta et al. 2007). Various spatial aspects have been investigated that could inform early detection of aquatic invasive species; e.g., how spatial resolution affects the predictive ability to forecast invasion success and the optimal spatial scale required to discern important spatial patterns in an invasive species' distribution (Lowen et al. 2016). Simulation models can also assist managers on how to allocate monitoring effort across a landscape (or seascape; Cacho et al. 2009).

The spatial scale of sampling for early detection and monitoring of marine invasive species is currently done at the scale of the distribution of marinas, seaports, and aquaculture sites (e.g., approximately 10s of km or greater), which may not be sufficiently sensitive to detect rare invasive species. Here, we analyzed fouling species recruitment data collected across multiple spatial scales using settlement plates, which are commonly used as a passive sampling tool to monitor marine invasive species, to examine the effect of spatial scale on rare species detection (Sephton et al. 2011; McKenzie et al. 2016, Tait and Inglis 2016). We investigated the benthic marine assemblage because the recruitment of fouling species is tractable using settlement plates and it is typically comprised of many marine invasive species. The scales under-investigation include scales that are increasingly smaller than those typically used in the monitoring of marine invasive species (i.e., $\leq$ 10s of km). The focus on smaller scales (instead of larger scales) could substantially increase the chances of identifying the appropriate scale for early detection and surveillance since it is more likely that 10s of km is underestimating the detection of marine invasive species than otherwise. Moreover, plates have been successfully used to detect incipient invasive populations (Moore et al. 2014; Ma et al. 2016; also see Appendix 2). In this paper, our objectives are to compare rare and common species by (1) identifying the spatial scale that is the main source of variability in recruitment rate, (2) determining the dominant spatial scale, i.e., the scale at which repeating patterns in recruitment were detected, and (3) evaluating different simulated survey designs to determine the scale that maximizes (a) the probability of detection for each species and (b) species richness for the entire assemblage (i.e., a combination of rare and common species) and for rare species only.

4.4 Materials and methods

4.4.1 Study system

This study was carried out in the Bras d'Or Lake (BdOL), Nova Scotia, Canada (geographic centre: 45.95°N, 60.80°W). BdOL is an inland brackish sea with salinities ranging from 20 to 25 psu and > 1000 km of coastline. Notably narrow, the maximum tidal range in the BdOL is 30 cm with a mean range of approximately 10 cm (Desplanque and Mossman 2004).

4.4.2 Buoy sampling stations

Settlement plates oriented in the horizontal position were immersed in the water column to provide a suitable hard substrate for larvae to encounter and recruit onto over time. Plates were constructed of dark grey and non-toxic polyvinyl chloride (PVC) with a standardized area of 20 × 20 cm. The downward facing surface of each plate was roughened using a hand-held random orbital sander. Each plate was fixed to a polyethylene rope, positioned at 1 m below the water surface, and hung from a Styrofoam buoy (i.e., one plate per buoy station). A counterweight attached to the same rope, below the plate, kept the line taut. The entire buoy sampling station was anchored to the seafloor with a cinderblock, which was connected to the counterweight via a slack line such that the plate remains perpendicular to the water surface regardless of the tidal height (Figure 25, Panel A).

4.4.3 Spatial scales and temporal replication

In the BdOL, buoy sampling stations were deployed nearshore at multiple spatial scales across four orders of magnitude (10s of m, 100s of m, 1000s of m, and 10000s of m) and at two sampling periods (July and August) for temporal replication (Hurlbert 1984). These scales were selected to cover a broad range of larval dispersal abilities of short-distance (e.g., ascidians and bryozoans) and long-distance dispersing benthic species (e.g., barnacles and bivalves; Shanks et al. 2003; Shanks 2009b). At the smallest scale, a "site"

consisted of three buoy “stations” spaced 10 m apart along the shore. The cross-shore distance of each buoy station was about 20 m. A "location" consisted of three sites spaced 100 m apart (along-shore). A "sector" consisted of three locations spaced 1000 m apart (along-shore). At the largest scale, the "system" consisted of seven (in July 2013) and eight (in August 2013) sectors spaced 10000s of m apart. Minimum and maximum distances among sectors were 10 km and 90 km (Figure 25, Panel B).

Each of the eight sectors was associated with a marina that had a boat ramp to allow access to the water (map: Figure 26). In sequential order of deployment and sampling, these marinas were located in Whycocomagh (45.9684°N, 61.1125°W), Baddeck (46.0993°N, 60.7482°W), New Harris (46.2371°N, 60.4999°W), Bras d’Or (46.2518°N, 60.3022°W), Ben Eoin (45.9818°N, 60.4314°W), St. Peter’s (45.6611°N, 60.8760°W), Dundee (45.7002°N, 61.0917°W), and Orangedale (45.9007°N, 61.0879°W). However, none of the buoy sampling stations were deployed inside any marinas. The first (N = 189 buoy stations) and second (N = 216 stations) deployment commenced in early July and mid-August, respectively.

Plates in both deployment periods—hereafter referred to as “July” and “August”—were immersed in the water for a duration of about six weeks. Due to the limited availability of bare space on settlement plates, the short plate duration ensured that recruitment reflects larval settlement before three-dimensional development of the fouling community and substantial biotic interactions (Dafforn et al. 2009). Also, short durations are expected to maximize the probability of detecting rare species by reducing visibility bias, because these species may be easily missed during processing of settlement plates if they are not distinctive in shape and colour (Stanislawczyk et al. 2018). The Ben Eoin sector was added in August because this area became the site of other related field experiments shorter thereafter (see Chapter 1 and Chapter 2). Although all 189 (July) or 216 (August) plates were not deployed on the same day due to the spatial extent of the BdOL, plates were deployed within an 8-d window (i.e., a sector d^{-1}). Similarly, all plates were retrieved within an 8-d window. Only one buoy sampling station was lost in each sampling period. However, they were found and replaced shortly after (approximately one week) the start of the experiment. Consequently, the analyses contained no missing data.

4.4.4 Data processing

Recruited species on the downward facing surface of the plate were identified to the lowest possible taxonomic level and the presence and absence and recruitment rates of 27 marine benthic (invertebrate) taxa were determined across all plates. Recruitment rates were standardized per unit area and time (i.e., number of recruits cm^{-2} d^{-1}). Soft-bodied organisms (e.g., ascidians) were counted live upon plate retrieval (e.g., see Figure 27). After counting soft-bodied organisms, plates were air-dried and hard-bodied organisms (e.g., barnacles, bivalves, bryozoans) were identified and enumerated at a later time using a stereomicroscope.

4.4.5 Data analyses

Since rarity is an emergent property of many ecological processes, a practical definition of "rare species" is expressly required for the analyses in this study. Correspondingly, a quartile definition, *sensu* Gaston (1994), is used in this study such that rare species are defined as the top 25% of the rarest species in the benthic assemblage. Of course, other approaches in defining rarity and commonness can be found in the literature—such as defining an arbitrary threshold in the frequency in occurrence or basing on a combination of species geographic distribution, habitat selection, and local density (Rabinowitz et al. 1986; Galand et al. 2009).

Spatial variation of recruitment: For each species, a nested generalized linear mixed-effects model (GLMM with Poisson error distribution) using the lme4 package in R was fitted to the recruitment data to assess spatial variability for each of the four scales: *Station* (10s of m), *Site* (100s of m), *Location* (1000s of m), and *Sector* (10000s of m). All four scales were treated as random effects. For the GLMMs, the source of variation at the smallest spatial scale (*Station*) was assumed to have a value of one because there are no replicates at this scale given the nested sampling design. For each source of variation (*Station*, *Site*, *Location*, *Sector*) the variance components (estimated with GLMM) were converted to percent values to examine the distribution of variance and allow for comparisons to be made across species. In addition to this nested GLMM approach, a Bayesian approach using a Markov chain Monte Carlo (MCMC) generalized linear mixed model (GLMM) was performed with the MCMCglmm package in R to estimate variance components (also converted to

percentages) for each of the 27 taxa. Both the nested GLMM and Bayesian MCMC GLMM approaches were used to estimate spatial variation in recruitment because the former is a more well-known method and the latter is better suited for estimating the variance at the smallest spatial scale of the study (*Station*) and the variance of rare species (i.e., datasets with lots of zero values).

Dominant scales of recruitment: The dominant scale of recruitment for each taxon was evaluated using wavelet analysis. A wavelet is an oscillating mathematical function that can reveal signal details and trends as a function of temporal scale and may be adapted for spatial datasets (i.e., processing spatial distances instead of time-series; Bradshaw and Spies 1992, Dale 1999, Fortin and Dale 2005). For each taxon, a one-dimensional signal consisted of $\log_{10}(x + 1)$ transformed recruitment rates in sequential order beginning with the first sampled plate (in Whycocomagh) and terminating with the last sampled plate (in Orangedale). The wavelet power spectrum was computed by applying the Morlet wavelet (n = 100 simulations). Scale-averaged wavelet power was used to identify the power at scales of 10s of m (period = 1), 100s of m (period = 3), 1000s of m (period = 9), and 10000s of m (period = 27). The dominant scale of recruitment for each taxon was identified as the spatial scale with the highest wavelet power (i.e., the scale with the most pronounced repeating pattern). This analysis was performed using the WaveletComp package in the R programming environment (R Core Team 2019, Roesch and Schmidbauer 2018).

Species-by-species simulated sampling strategies: For each taxon, presence and absence field data were resampled (and assuming that the full complement of the field data represent complete knowledge of species distribution) using a bootstrap procedure (1000 iterations) to estimate the *probability of detection* (POD) as a function of sampling effort (number of plates). The bootstrap (resampling) procedure was computed for four different "simulated" sampling strategies: (1) random sampling (hereafter referred to as S_R), (2) adaptive sampling by forcing sampling at scales $\geq$ 100s of m (S_{100}), (3) forcing sampling at scales $\geq$ 1000s of m (S_{1000}), and (4) forcing sampling at scales = 10000s of m (S_{10000}).

The simulated S_R strategy consisted of randomly sampling the field data without replacement starting with one plate up to the full complement (i.e., all 189 and 216 plates for July and August, respectively). The simulated S_{100} strategy required sampling the field data randomly without replacement but with the added constraint that the next plate must be randomly selected from a previously unsampled “site” until all sites

have been sampled, before a sampled site can be selected from again. The simulated S_{1000} strategy is similar to S_{100} except that the next plate must be randomly selected from a previously unsampled "location" until all locations have been sampled, before a sampled location can be selected from again. Finally, random sampling for the simulated S_{10000} strategy specified that the next plate must be randomly selected from a previously unsampled "sector" until all sectors have been sampled, before a sampled sector can be selected from again. To determine which simulated sampling strategy was the best for a given taxon, the percent change in POD between each pair of strategies was calculated. Given that S_R was consistently the most suboptimal strategy, the percent change was evaluated between each of the strategies with S_R as a function of sampling effort: (1) S_{100} *versus* S_R, (2) S_{1000} *versus* S_R, (3) S_{10000} *versus* S_R. The simulated sampling strategy (S_{100}, S_{1000}, or S_{10000}) with the greatest percent change from S_R was considered optimal.

Assemblage-level simulated sampling strategies: The presence and absence field data were resampled using a bootstrap procedure (iterations = 1000 times) to estimate the cumulative number of taxa as a function of sampling effort. The same four simulated sampling strategies (described above) were tested on (1) all the taxa (N = 24 and N = 25 taxa in July and August, respectively) and (2) on only the upper 25% rarest species (N = 6 taxa in July and August). The percent change was again evaluated between the S_{100}, S_{1000}, and S_{10000} strategies and S_R (given that the S_R strategy was the most suboptimal strategy) as a function of sampling effort, and the simulated sampling strategy (S_{100}, S_{1000}, or S_{10000}) with the greatest percent change from S_R was considered optimal.

4.5 Results

4.5.1 The fouling assemblage

The fouling assemblage on settlement plates (e.g., see Figure 27) consisted of 27 marine benthic (invertebrate) taxa, including one hydrozoan (Tubulariidae), four amphipods (*Caprella mutica*, Corophiidae, *Gammarus* sp., and Ischyroceridae), one barnacle (*Amphibalanus improvisus*), one echinoderm (*Asterias rubens*), four bivalves (*Anomia simplex*, *Crassostrea virginica*, *Hiatella arctica*, and *Mytilus* sp.), two gastropods (*Bittiolum alternatum* and *Crepidula fornicata*), ten bryozoans (*Conopeum tenuissimum*, *Cribrilina annulata*, *Cribrilina punctata*, *Crisularia turrita*, *Cryptosula pallasiana*, *Einhornia crustulenta*,

Electra pilosa, *Escharella* sp., *Membranipora membranacea*, and *Schizoporella* sp.), and four ascidians (*Botrylloides violaceus*, *Botryllus schlosseri*, *Ciona intestinalis*, and *Molgula manhattensis*; Table 5). Among these taxa, *B. schlosseri*, *B. violaceus*, *C. mutica*, and *M. membranacea* are considered non-native species to the region (Sephton et al. 2011, 2017; Ma et al. 2017a). Although known as a new invasion in nearby areas, such as insular Newfoundland, *C. intestinalis* is considered a cryptogenic species in eastern Canada (Sargent et al. 2014; Ma et al. 2017a).

Taxa with a total of more than ten thousand recruits observed during the July and August deployments combined (i.e., abundance), *A. improvisus*, *B. alternatum*, *B. schlosseri*, *C. virginica*, and *Mytilus* sp. were the most abundant and widespread in this study (Table 5). The rarest taxa were *C. intestinalis*, *C. pallasiana*, *E. crustulenta*, *Escharella* sp., *M. membranacea*, *Schizoporella* sp., and Tubulariidae, each having a total of < 20 recruits observed in July and August (Table 5). For any given taxon, abundance varied between the two sampling time points such that 14 taxa decreased and 13 taxa increased in total abundance between July and August (Table 5). The coarse scale (sector-level) distributions of all taxa in this study are presented in Supplemental Figure S12 to show their general distribution within the BdOL in July and August.

4.5.2 Spatial variability in recruitment

Recruitment varied spatially across the sampling stations (Supplemental Figure S13). In most cases, the results of the nested GLMM and Bayesian MCMC GLMM were in agreement in estimating variance, especially for common and widespread species (Supplemental Table S4). Using only the results from the Bayesian approach, the source associated with most of the recruitment variability was at the largest spatial scale of the study (*Sector*) for most taxa (n = 16 taxa in July [67%] and n = 12 in August [48%]; Supplemental Figure S14 and Supplemental Figure S15). The intermediate spatial scale (*Location*) was the greatest source of recruitment variability for seven and nine taxa in July (29%) and August (36%), respectively (Supplemental Figure S14 and Supplemental Figure S15). The smallest spatial scale (*Site*) was the greatest source of variability for only one taxon in July (4%) and four taxa in August (16%; Supplemental Figure S14 and Supplemental Figure S15). For all spatial scales analyzed, no relationships were found between the variance in benthic recruitment rates (i.e., variance component estimated from the MCMC GLMM) and species' abundance (i.e., the total number of recruits counted) and between the variance

and proportion of plates colonized (Supplemental Figure S16). Despite this lack of relationship, for a majority of rare taxa (*A. simplex*, *C. intestinalis*, *Escharella* sp., *Gammarus* sp., *M. manhattensis*), the greatest source of variance was the largest spatial scale (*Sector*) during the July deployment (Supplemental Figure S14). The intermediate spatial scale (*Location*) was the greatest source of variance for only one rare species (*M. membranacea*) in July. During the August deployment, the largest spatial scale (*Sector*) was the greatest source of variance for two rare taxa (*M. membranacea*, *Schizoporella* sp.; Supplemental Figure S15), the intermediate scale (*Location*) for one rare species (*E. crustulenta*), and the smallest scale (*Site*) for three rare species (*C. annulata*, *C. intestinalis*, *C. pallasiana*).

4.5.3 Dominant scales in recruitment

For a given taxon, wavelet analysis on one-dimensional recruitment signals (see Supplemental Figure S13) revealed that either the smallest (100s of m) or the largest spatial scales (10000s of m) exhibited the highest scale-averaged wavelet power (i.e., the dominant scale associated with repeating patterns). Overall, the smallest spatial scale was the dominant scale for 12 taxa in the July deployment (50%) and 11 taxa in the August deployment (44%; Figure 28). The intermediate scale (1000s of m) was the dominant scale for only one taxon (4%) and four taxa (16%) in July and August, respectively (Figure 28). The largest scale was the dominant scale for 11 taxa in both July (46%) and August (44%; Figure 28).

Overall, the dominant scale for a given taxon is related to their abundance and distribution such that the dominant scale for rare taxa tended to be at small spatial scales and for common taxa at large spatial scales (Figure 29). In particular, at small spatial scales, scale-averaged wavelet power increased with increasing rarity (i.e., low abundance and low proportions of plates colonized; Figure 29, Panels A and B). Surprisingly, no correlation between scale-averaged wavelet power and abundance or distribution was found at the intermediate spatial scales (Figure 29, Panels C and D). However, at large spatial scales, scale-averaged power increased with increasing abundance and distribution (Figure 29, Panels E and F).

4.5.4 Simulated sampling strategy: Species-by-species optimizations

All simulated sampling strategies showed that, for a given probability of species detection, rarer taxa required greater sampling effort compared to widespread, common species (Supplemental Figure S17). For each taxon, the mean percent change in POD was computed to evaluate the small-scale S_{100}, intermediate-scale S_{1000}, and large-scale S_{10000} simulated sampling strategies (Figure 30). However, the percent change for any given taxon varies substantially as a function of sampling effort and, in some instances, adaptive sampling strategies (i.e., S_{100}, S_{1000}, S_{10000}) could lower the POD when compared to the random sampling strategy (Figure 30). Thus, the mean percent change in POD, integrating sampling effort, was used to evaluate the optimal sampling strategy for each taxon (Supplemental Figure S18). Common and widespread taxa (i.e., *A. improvisus*, *B. alternatum*, *B. schlosseri*, *C. virginica*, *H. arctica*, and *Mytilus* sp.) tended to have substantially smaller mean percent change in POD (± 0.0045%). The small-scale S_{100} sampling strategy was the optimal strategy for seven taxa in both the July (29% of the species) and August (28%; Supplemental Figure S18) deployments. The intermediate-scale S_{1000} sampling strategy was the optimal strategy for nine taxa in July (38%) and ten in August (40%; Supplemental Figure S18). The large-scale S_{10000} sampling strategy was the optimal strategy for eight taxa in both the July (33%) and August (32%) deployments (Supplemental Figure S18).

The optimal strategy for a majority of rare taxa (83%), i.e., the top 25% of the rarest taxa in the benthic assemblage (quartile definition), during the July deployment was the intermediate-scale S_{1000} sampling strategy. For the August deployment, the optimal strategy for most of the taxa (67%) was instead the small-scale S_{100} sampling strategy. The optimal strategies for the five invasive species (i.e., non-native and cryptogenic) in this study were either the intermediate-scale S_{1000} or the large-scale S_{10000} sampling strategies. For instance, the encrusting bryozoan *C. pallasiana*, which is a relatively rare species in this study (only five recruits observed), exhibited the greatest mean percent change in POD under the small-scale S_{100} strategy (mean: 12.5%) and a percent change of up to 39.5% if sampling effort consisted of a minimum of 72 plates (Figure 30 and Supplemental Figure S18).

Common taxa, i.e., the top 75% of the most abundant taxa in the benthic assemblage (quartile definition), tended to reach maximal change in POD (up to about 50% change relative to the random S_R sampling

strategy) with a sampling effort of approximately 25 settlement plates (Supplemental Figure S19). Despite substantial variability in both sampling effort and maximal percent change compared to common taxa, rare species were more likely to benefit from adaptive sampling strategies than more common species (up to 250% change relative to the random S_R sampling strategy; Supplemental Figure S19). However, in several cases, rare taxa require greater sampling effort before attaining maximal percent change (Supplemental Figure S19). Linear regressions relating abundance and proportion of plates colonized with mean percent change relative to the random S_R sampling strategy clearly demonstrated that rarer taxa were associated with the greatest mean percent changes for all simulated sampling strategies (Figure 31). This positive relationship was the strongest (up to $R^2 = 0.90$) for simulated strategies as the spatial scale of sampling increased from small-scale S_{100} to large-scale S_{10000} sampling strategy (Figure 31).

4.5.5 Simulated sampling strategy: Assemblage-level optimization

The species accumulation curves computed for the entire assemblage (or "all taxa") and for "rare taxa only" appeared to exhibit a plateau with increasing sampling effort (Supplemental Figure S20). Although differences in species accumulation among the different simulated sampling strategies may seem difficult to detect visually, up to approximately 7% and 11% change relative to the random S_R sampling strategy was found for the "all taxa" and "rare taxa only" analyses, respectively (Figure 32). However, by calculating the mean percent change (essentially the area under the curve), the intermediate-scale S_{1000} sampling strategy was the optimal assemblage-level sampling strategy to detect the greatest number of taxa (means: 0.8% in July and 0.7% in August). For rare taxa, the optimal strategies were the intermediate-scale S_{1000} sampling strategy in July (mean: 1.7%) and the small-scale S_{100} sampling strategy in August (1.6%; Figure 32).

4.6 Discussion

In this study, a number of approaches were used to determine the optimal spatial scale to detect rare species such as incipient invasive populations at the earliest stages in the invasion process when they are, by their very nature, rare. Rarity is an emergent property of many biotic and abiotic processes that structure the underlying adult distribution (and the configuration of the benthic community) and affect larval settlement patterns (Connell 1972; Lewin 1986; Roughgarden et al. 1987; Sebens 1991). In fact, rarity is not uncommon

in nature, i.e., many species are rare in natural communities (*sensu* Cao et al. 2001). Although the causes of rarity in the species examined in this paper are not known (e.g., the distribution of adults), we gained insights into strategies to sample for rare species that could be widely adopted in applied ecology. First, we identified the spatial scale associated with the most variance in recruitment rates for rare and, separately, for common species because these scales are likely to be the scales at which processes are driving patterns in distribution and abundance. Second, we determined the dominant scale, i.e., the scale at which repeating patterns in recruitment rates were detected, for both rare and common species because these scales are likely to be most influential scales despite several concurrent processes may be operating across multiple scales. Third, we evaluated different sampling strategies by resampling the field data at different spatial scales to (a) compare between rare and common species and (b) test which strategy maximized taxonomic richness.

It is common for ecological studies to evaluate the spatial scale at which ecological phenomena, such as recruitment of marine species, varies because it may provide insights into the processes that drive patterns across multiple scales or inform sampling design (e.g., Fowler et al. 1992; Underwood and Chapman 1996; Bellehumeur and Legendre 1998). Most studies have identified the smallest scale to be the source with the highest variability in abundance (Kristensen et al. 1985; Morrisey et al. 1992; Thompson et al. 1996; Underwood and Chapman 1996). Presumably, the spatial scale with the highest variance in recruitment would be the targeted optimal scale for rare species detection because it should correspond to the scale with a range of both low and high values (including absences). However, in this study, no relationships were found between either abundance or proportion of plates colonized and the distribution of variance in benthic recruitment that was estimated from the MCMC GLMM (Supplemental Figure S16). This apparent lack of generality suggests that spatial variability in recruitment is highly species-specific or, perhaps, even specific to a place and time. This finding is markedly different from another study that examined multiscale sources of variability (range: from 0.1s to 1000s of m) in gastropod densities, in which Chapman and Underwood (2008) reported that rare species had greater variability in densities at smaller scales and common species at larger scales.

Previous studies have found it difficult to predict the spatial distributional range size in marine organisms using dispersal ability (Kinlan et al. 2005; Lester et al. 2007). Despite this lack of predictability, there is an expectation that propagule dispersal ability can have substantial implications for the spatial design of early

detection and monitoring programs. If sampling stations are spaced too far apart, then short-dispersing species may not be detected. Conversely, if the spatial domain of sampling (extent) is too small, then long-dispersing species (i.e., originating from afar) may not be detected. This concern of dispersal ability has guided other disciplines of applied ecology. For example, marine protected areas (or ecological reserves) have been recommended to be sufficiently large (4 to 6 km in diameter) for short-distance propagules to disperse within and spaced sufficiently close together (10 to 20 km apart) to receive propagules from nearby protected areas or reserves (Shanks et al. 2003). This spatial design ensures that these marine protected areas are effective refuges for local biodiversity.

The dominant scale in recruitment provides better insights into the optimal spatial scale for rare species detection because patterns can be generalized. Although the multi-scale sources of variability in recruitment appear to be species-specific, there are distinct differences between rare and common species in relation to their dominant scale. By testing a range of three scales from hundreds of metres to tens of kilometres, our analysis clearly demonstrates that the dominant scale for benthic recruitment tends to occur at smaller spatial scales as abundance and the proportion of plates colonized decreases (i.e., for species that are relatively rarer in abundance of recruits and more localized in their distribution). Conversely, the dominant scale for benthic recruitment tends to be at larger spatial scales as abundance and distribution increase (as species become increasingly common). Moreover, if rare species tend to exhibit repeating recruitment patterns at the smallest scale of the study, then the optimal scale to sample for rare species should occur at or above this scale (100s of m). Unsurprisingly, the intermediate (1000s of km) scales lacks any predictability in relation to abundance and distribution as the wavelet power–abundance and wavelet power–distribution relationships changed from negative at small spatial scales to positive at large spatial scales. Perhaps, relative stability at small scales (low spatial structure) and the stochasticity observed at intermediate scales (high spatial structure) suggests that these scales coincide with the lower and upper bounds of the appropriate spatial scale for rare species detection.

Spatially-explicit simulated sampling strategies (e.g., S_{100}, S_{1000}, S_{10000}) show that species detection improved substantially compared to just random sampling (i.e. S_R) as species' abundance and distribution decrease (i.e., as species become increasingly rare). Overall, a majority of rare species exhibited enhanced detectability by forcing sampling at scales greater or equal to 1000s of m (the intermediate-scale S_{1000}

sampling strategy). This enhanced scale in detecting rare species could be the result of the combined effects of advantages and disadvantages at both the smallest and largest spatial scales.

Of course, targeting a single invasive species at a time for invasive species monitoring can be impractical and logistically unrealistic. Instead, early detection and monitoring programs have tended towards developing strategies that can maximize the detection of a broad range of species (Deiner et al. 2018). Although the large-scale S_{10000} sampling strategy can enhance taxonomic richness by up to ca. 7% (i.e., peak percent change), we found that the intermediate-scale S_{1000} sampling strategy was the net optimal sampling strategy to maximize taxonomic richness (i.e., sampling effort-integrated percent change). Results maximizing only rare species were similar to the results maximizing taxonomic richness for all species. For rare species, we found that the optimal strategy changed from the intermediate-scale S_{1000} strategy in July (peak: ca. 11%; mean: 1.7%) to the small-scale S_{100} sampling strategy in August (peak: ca. 9%; mean: 1.6%). These values represent a modest yet persistent improvement in the probability of species detection and demonstrate that the outcomes of spatially-explicit sampling strategies can be distinctly better than random sampling. Further, the temporal shift in optimal sampling strategy (i.e., from intermediate- to small-scale) can be attributed to the fact that rare species in August were even rarer (in abundance and proportion of plate colonized) than those in July, which suggests that the optimal scale to sample for rare species may be at small spatial scales (i.e., following the optimal strategy in August when species were rarer, relative to July).

Our study shows that sampling strategies that are optimal for rare species could be substantially different from those that are best for common species, which should be integrated into the design of sampling protocols and tools. Consequently, this study highlights the potential problem of assuming that maximizing taxonomic richness is the optimal sampling strategy, which can counter-intuitively reduce the probability of detecting rare species. This finding has broad implications for early detection and surveillance of incipient populations of marine invasive species when their population sizes are small in number and spatial extent but also, for example, biodiversity surveys, monitoring of rare endangered species, and biogeographic studies of species at the limits of their distributional range where they may be rare and increasingly difficult to detect.

In summary, the results from the three approaches provide insights into some of the potential errors when attempting to determine the optimal spatial scale for rare species detection. Although the source of variance

in recruitment for rare species varied from small- to large-scales depending on the species and further generalizations in relation to other biological traits, such as larval dispersal ability, may require more research. However, (1) identifying the dominant scale in recruitment and (2) simulating sampling strategies at multiple scales have the predictive power to guide early detection and monitoring efforts. Both these approaches clearly indicate that sampling at smaller spatial scales improves rare species detection because (1) rare species tended to exhibit stronger repeating patterns in recruitment at these smaller scales and (2) forcing sampling at small- and intermediate-scales (i.e., at spatial scales smaller than 10000s of m) tended to enhance their simulated detection. Hence, studies and monitoring should sample for rare species at both small and larger spatial scales but focus resources to sample at smaller scales at and near specific localities that are, for example, identified by environmental niche models, highly susceptible to new invasions (e.g., near marinas and ports), and actively undergoing an invasive species eradication campaign.

Table 5 — List of 27 marine benthic taxa and their invasion status, abundance (total number of recruits counted), proportion of plate colonized (in square brackets), percent change in abundance between July and August, and percent change in proportion. NA = not applicable.

Taxon	Authority	Invasion status	Abundance in July [Proportion of plates]		Abundance in August [Proportion of plates]		Percent change in abundance [Percent change in proportion]	
Cnidaria, Hydrozoa								
Tubulariidae	Goldfuss, 1818	NA	12	[0.048]	0	[0]	–100.0	[–100.0]
Arthropoda, Amphipoda								
Caprella mutica	Schurin, 1935	Non-native	78	[0.090]	338	[0.083]	333.3	[–7.8]
Corophiidae	Leach, 1814	NA	11	[0.053]	200	[0.125]	1,718.2	[135.8]
Gammarus sp.	Fabricius, 1775	NA	9	[0.042]	14	[0.065]	55.6	[54.8]
Ischyroceridae	Stebbing, 1899	NA	156	[0.164]	119	[0.194]	–23.7	[18.3]
Arthropoda, Cirripedia								
Amphibalanus improvisus	(Darwin, 1854)	Native	171,692	[0.704]	101,691	[0.815]	–40.8	[15.8]
Mollusca, Bivalvia								
Anomia simplex	Mabille, 1895	Native	11	[0.026]	59	[0.065]	436.4	[150.0]
Crassostrea virginica	(Gmelin, 1791)	Native	28,875	[0.878]	3,241	[0.690]	–88.8	[–21.4]
Hiatella arctica	(Linnaeus, 1767)	Native	1,178	[0.730]	52	[0.157]	–95.6	[–78.5]
Mytilus sp.	Linnaeus, 1758	NA	1,149,156	[0.974]	162,994	[0.931]	–85.8	[–4.4]
Mollusca, Gastropoda								
Bittiolum alternatum	(Say, 1822)	Native	13,986	[0.841]	259	[0.315]	–98.1	[–62.5]
Crepidula fornicata	(Linnaeus, 1758)	Native	15	[0.069]	20	[0.093]	33.3	[34.8]
Echinodermata, Asteroidae								
Asterias rubens	Linnaeus, 1758	Native	225	[0.302]	10	[0.042]	–95.6	[–86.1]
Bryozoa, Gymnolaemata								
Conopeum tenuissimum	(Canu, 1908)	Native	636	[0.307]	313	[0.199]	–50.8	[–35.2]
Cribrilina annulata	(O. Fabricius, 1780)	Native	18	[0.053]	5	[0.023]	–72.2	[–56.6]
Cribrilina punctata	(Hassall, 1841)	Native	28	[0.074]	71	[0.111]	153.6	[50.0]
Crisularia turrita	(Desor, 1848)	Native	26	[0.048]	141	[0.106]	442.3	[120.8]
Cryptosula pallasiana	(Moll, 1803)	Native	0	[0]	5	[0.014]	NA	[NA]
Einhornia crustulenta	(Pallas, 1766)	Native	0	[0]	5	[0.005]	NA	[NA]
Electra pilosa	(Linnaeus, 1767)	Native	58	[0.190]	35	[0.134]	–39.6	[–29.5]
Escharella sp.	Gray, 1848	NA	3	[0.016]	0	[0]	–100.0	[–100.0]
Membranipora membranacea	(Linnaeus, 1767)	Non-native	7	[0.026]	9	[0.032]	28.6	[23.1]
Schizoporella sp.	Hincks, 1877	NA	0	[0]	2	[0.009]	NA	[NA]
Chordata, Ascidiacea								
Botrylloides violaceus	Oka, 1927	Non-native	576	[0.095]	440	[0.083]	–23.6	[–12.6]
Botryllus schlosseri	(Pallas, 1766)	Non-native	2,339	[0.481]	15,707	[0.856]	571.5	[78.0]
Ciona intestinalis	(Linnaeus, 1767)	Cryptogenic	4	[0.021]	1	[0.005]	–75.0	[–76.2]
Molgula manhattensis	(De Kay, 1843)	Native	1	[0.005]	78	[0.088]	7,700.0	[16.6]

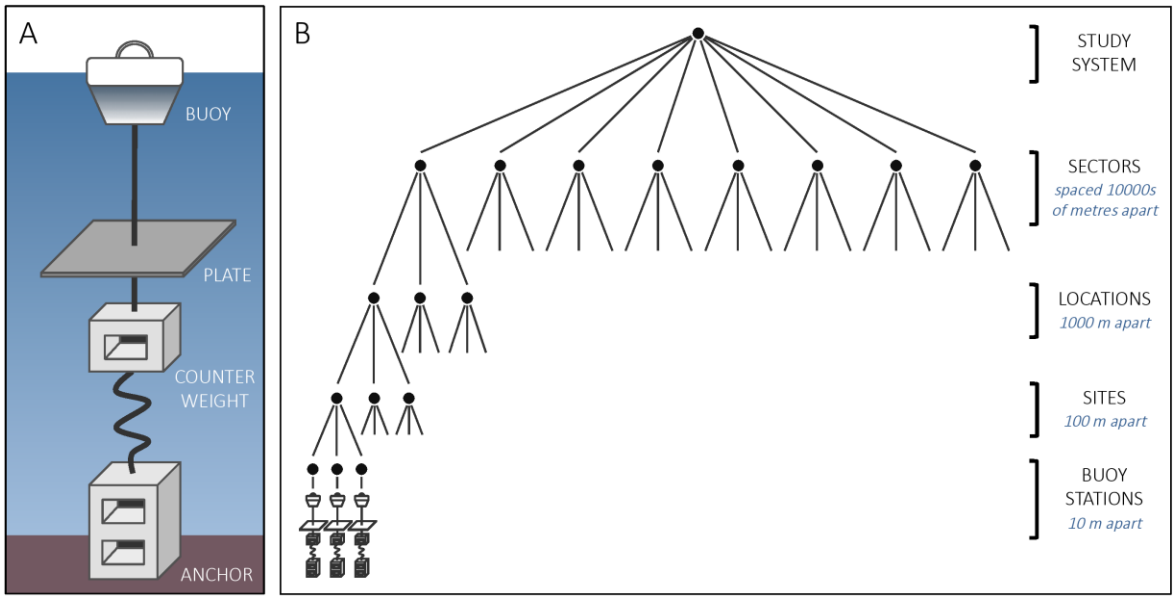


Figure 25 — Diagram showing the design of (A) the buoy sampling station with the settlement plate, positioned at 1 m below the water surface, attached to a taut line and a cinderblock anchoring the station to the seafloor, and (B) the hierarchical nested sampling such that scales ranged from 10s of m up to 10000s of m, over four orders of magnitude. N = 27 buoy sampling stations per sector.

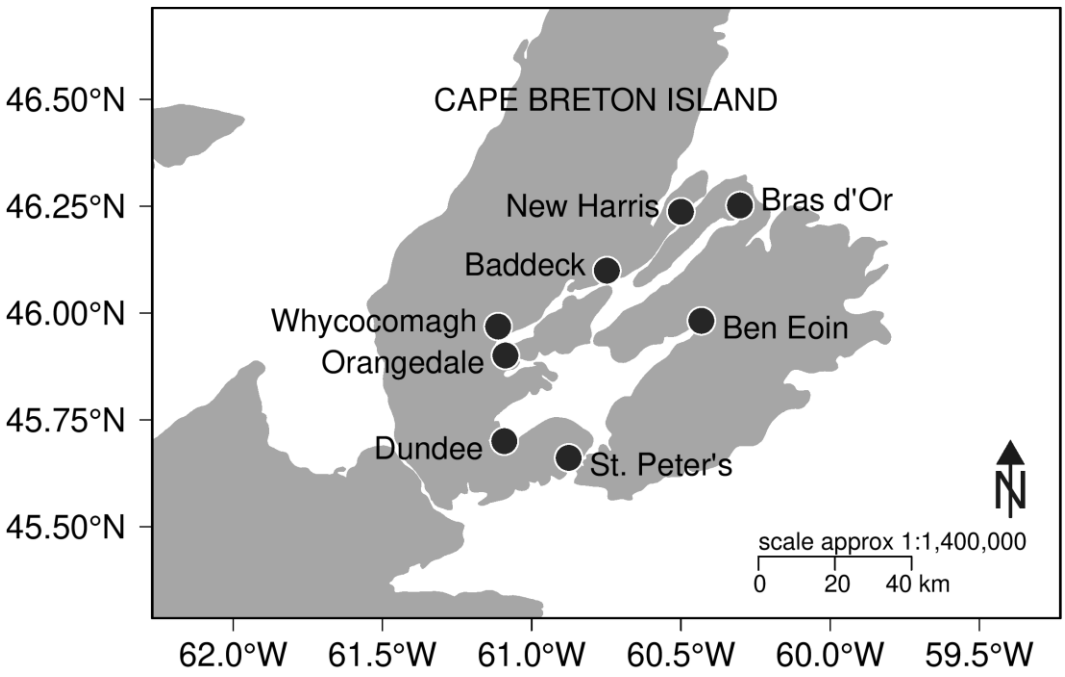


Figure 26 — Map of the eight sectors around the Bras d'Or Lake in Cape Breton Island, Nova Scotia, Canada.

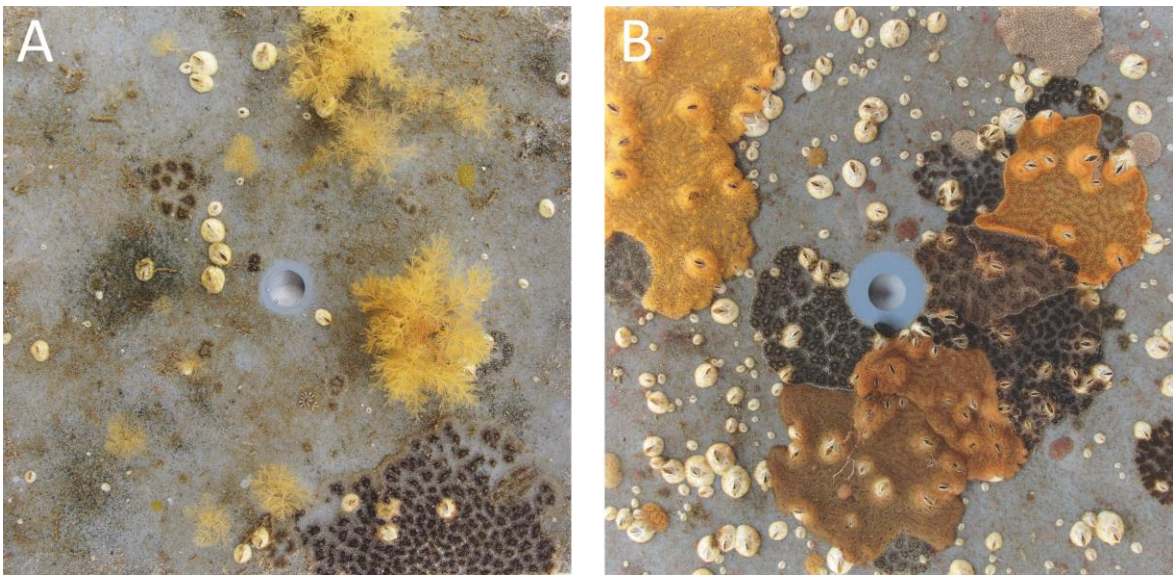


Figure 27 — Examples of settlement plates upon retrieval after a sampling duration of approximately six weeks. Some visible and conspicuous marine benthic species included: (A) the bay barnacle (*Amphibalanus improvisus*), the golden star tunicate (*Botryllus schlosseri*), and the bushy bryozoan (*Crisularia turrita*); (B) *A. improvisus*, *B. schlosseri*, and the violet tunicate (*Botrylloides violaceus*). Area of a plate: 20 × 20 cm.

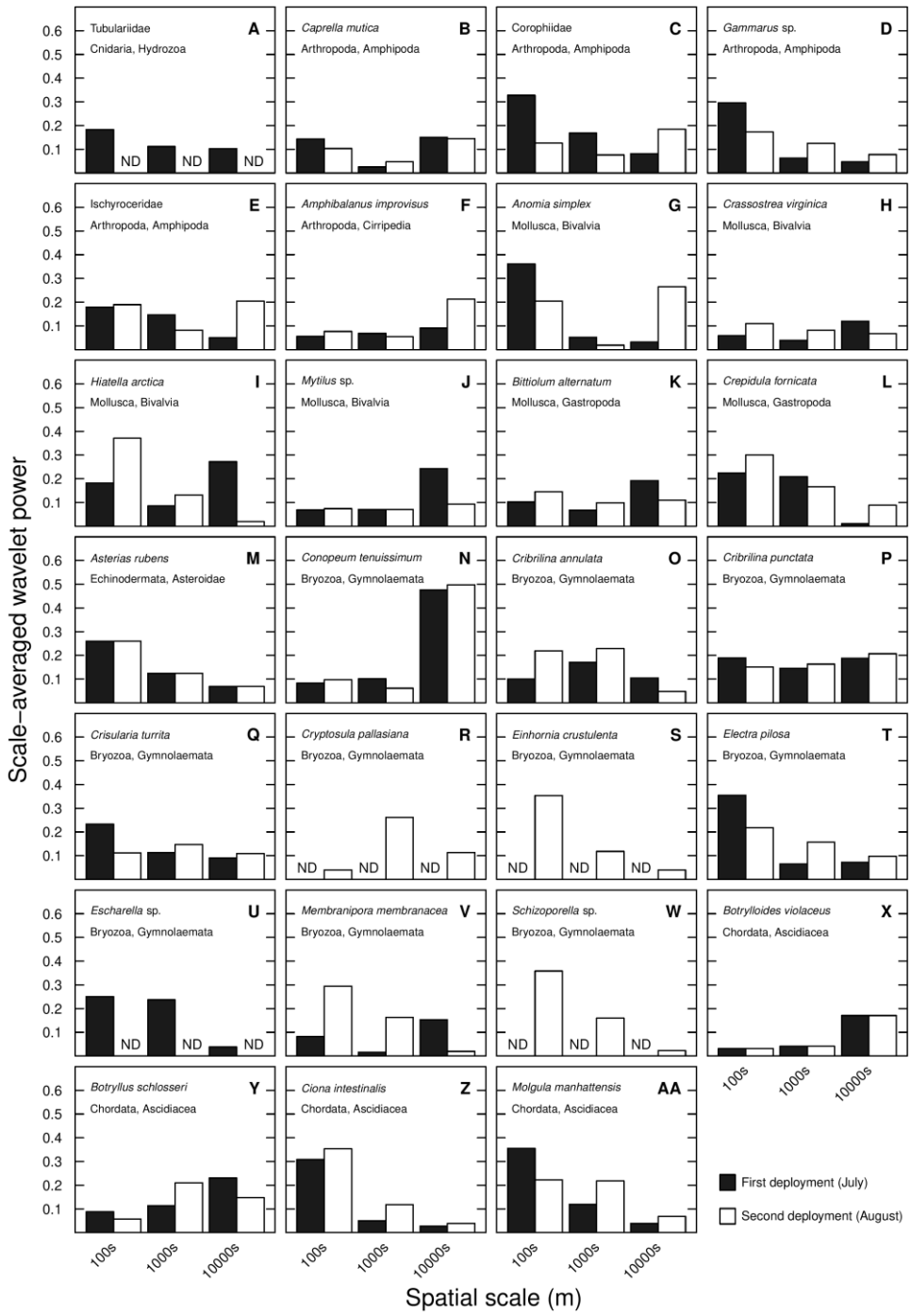


Figure 28 — Scale-averaged wavelet power as a function of spatial scale for $\log_{10}(x + 1)$ transformed recruitment rates of 27 marine benthic taxa (species are ordered by their higher taxonomic rank). Spatial scales ranged from small (100s of m), intermediate (1000s of m), to large (10000s of m). (A) Tubulariidae; (B) *Caprella mutica*; (C) Corophiidae; (D) *Gammarus* sp.; (E) Ischyroceridae; (F) *Amphibalanus improvisus*; (G) *Anomia simplex*; (H) *Crassostrea virginica*; (I) *Hiatella arctica*; (J) *Mytilus* sp.; (K) *Bittiolum alternatum*; (L) *Crepidula fornicata*; (M) *Asterias rubens*; (N) *Conopeum tenuissimum*; (O) *Cribrilina annulata*; (P) *Cribrilina punctata*; (Q) *Crisularia turrita*; (R) *Cryptosula pallasiana*; (S) *Einhornia crustulenta*; (T) *Electra pilosa*; (U) *Escharella* sp.; (V) *Membranipora membranacea*; (W) *Schizoporella* sp.; (X) *Botrylloides violaceus*; (Y) *Botryllus schlosseri*; (Z) *Ciona intestinalis*; (AA) *Molgula manhattensis*. ND = no data.

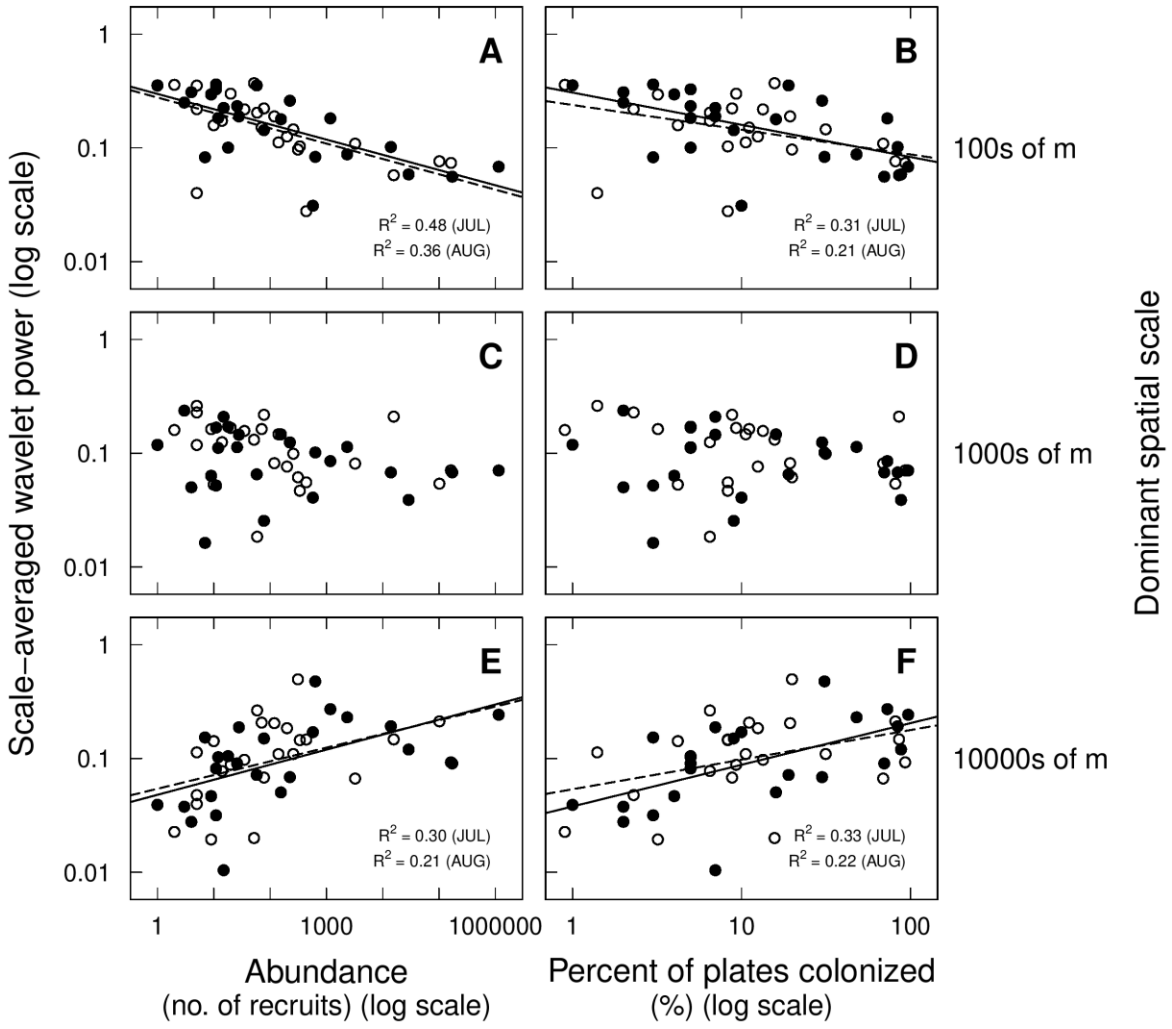


Figure 29 — Relationship between $\log_{10}(x)$ transformed scale-averaged wavelet power and $\log_{10}(x)$ transformed abundance data and between $\log_{10}(x)$ transformed scale-averaged wavelet power and $\log_{10}(x)$ transformed proportion of plates colonized at different spatial scales ranging from small (100s of m; A, B), intermediate (1000s of m; C, D), to large (10000s of m; E, F). Abundance = total number of recruits counted. N = 24 marine benthic taxa during the first deployment period in July (closed circles; ●) and N = 25 taxa during the second period in August (open circles; ○). Only significant linear regressions are shown (solid [—] and dashed [---] lines for July and August deployments, respectively).

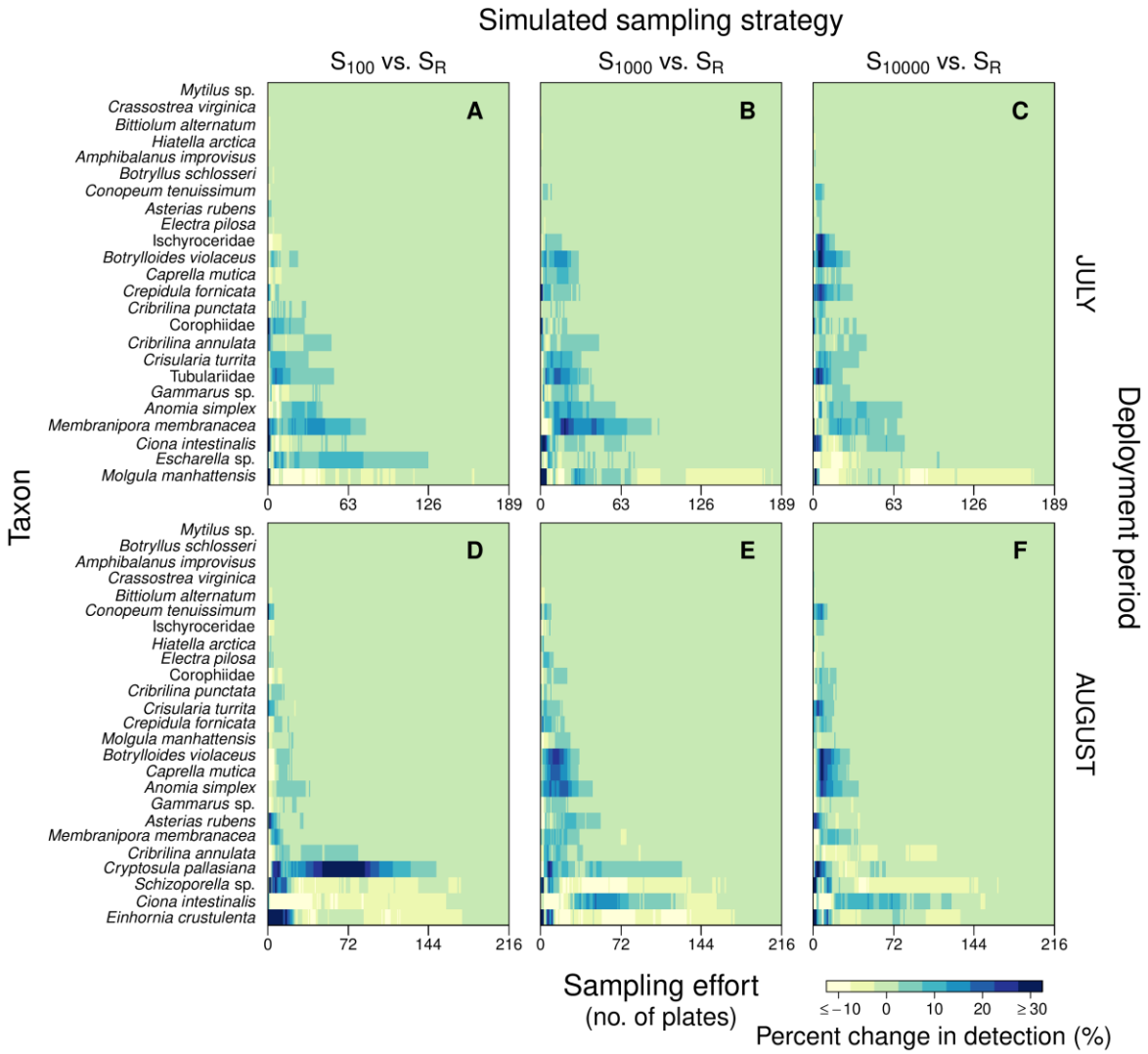


Figure 30 — Percent change in the probability of detection between simulated sampling strategies. (A–C) N = 24 marine benthic taxa during the first deployment period in July and (D–F) N = 25 taxa during the second deployment in August. Taxa are ordered from the most widespread and abundance (top) to the rarest (bottom). (A, D) Percent change from random sampling (S_R) to adaptive sampling by forcing sampling at scales ≥ 100s of m (S_{100}); (B, E) percent change from S_R to forcing sampling at scales ≥ 1000s of m (S_{1000}); (C, F) percent change from S_R to forcing sampling at scales = 10000s of m (S_{10000}).

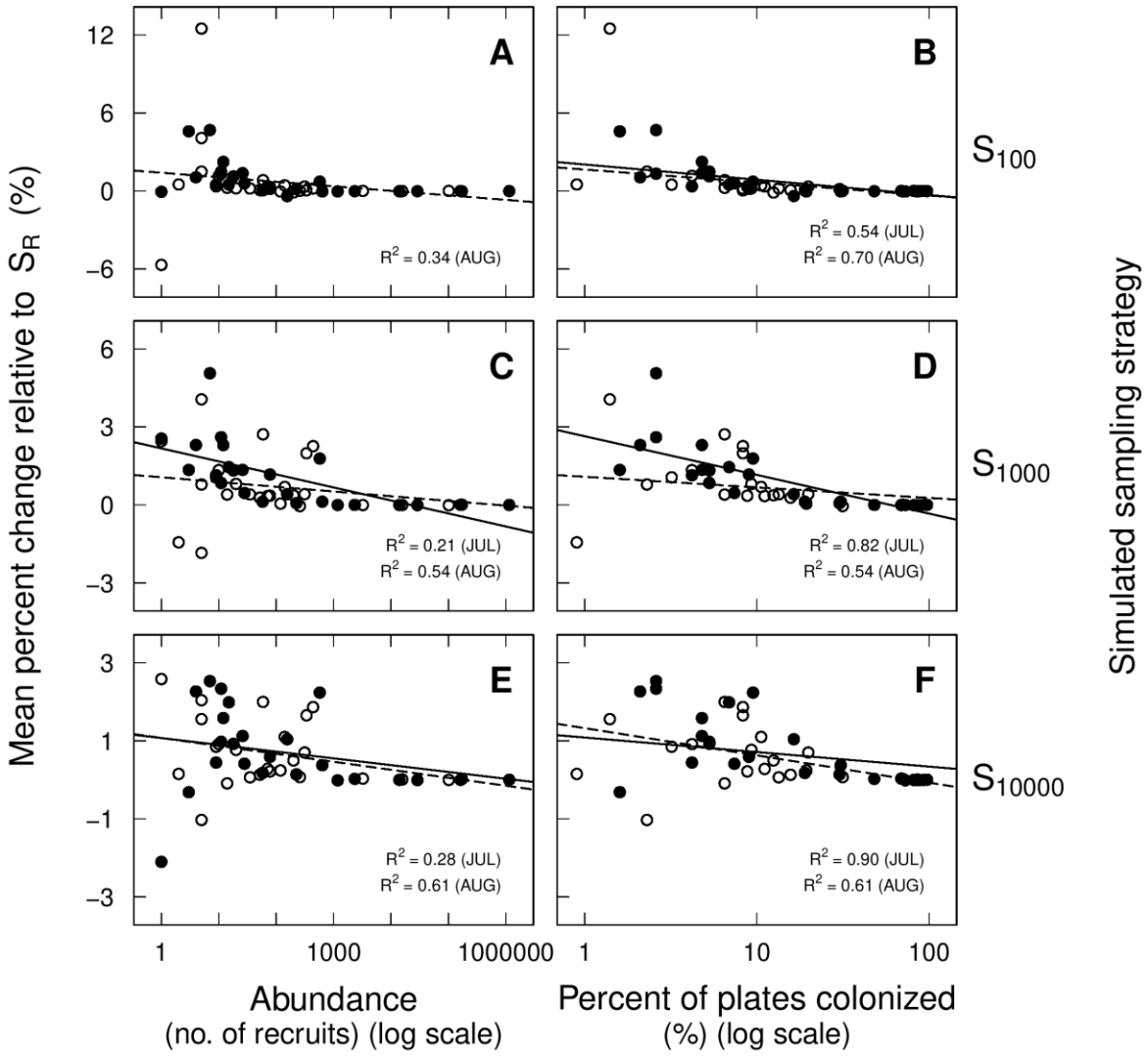


Figure 31 — Relationship between mean percent change relative to S_R and $\log_{10}$ (x) transformed abundance data and between mean percent change relative to S_R and $\log_{10}$ (x) transformed proportion of plates colonized for different simulated sampling strategies: (A, B) forcing sampling at scales ≥ 100s of m (S_{100}), (C, D) forcing sampling at scales ≥ 1000s of m (S_{1000}), and (E, F) forcing sampling at scales = 10000s of m (S_{10000}). Abundance = total number of recruits counted. N = 24 marine benthic taxa during the first deployment period in July (closed circles; ●) and N = 25 taxa during the second period in August (open circles; ○). Only significant linear regressions are shown (solid [—] and dashed [---] lines for July and August deployments, respectively).

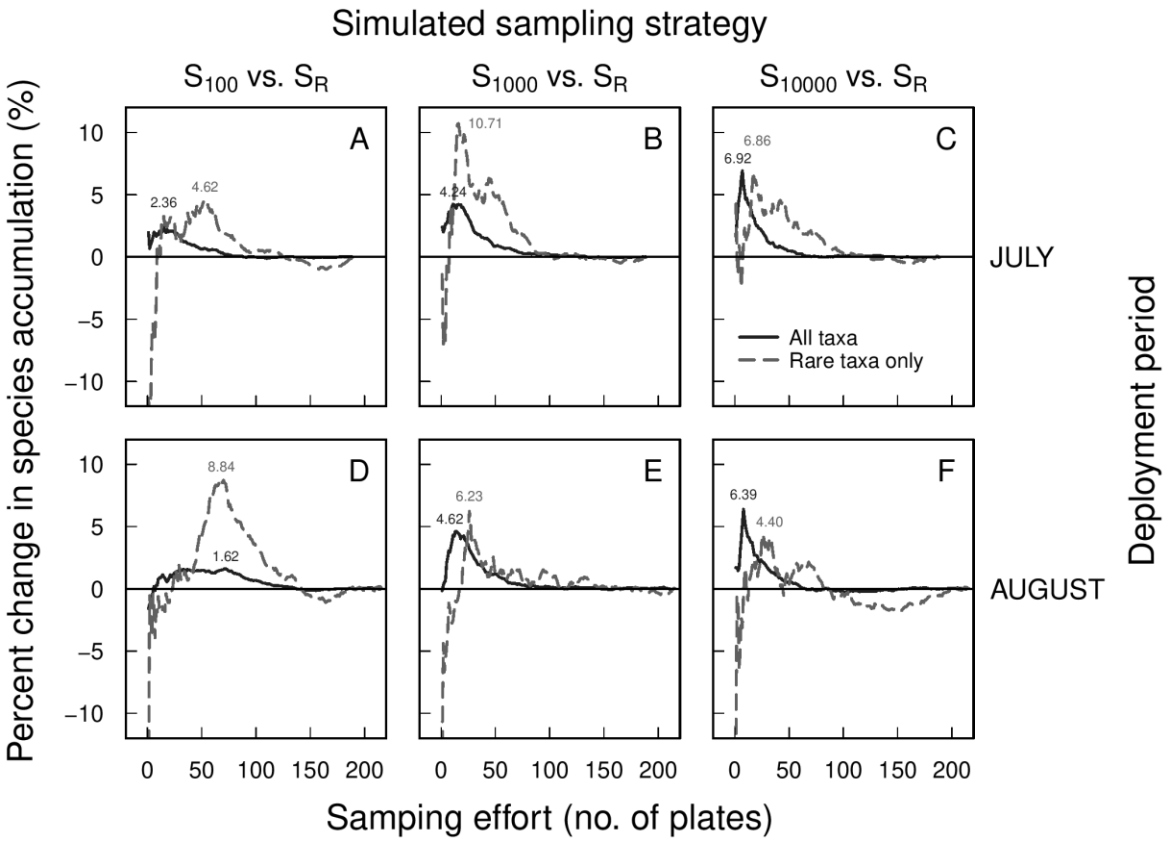


Figure 32 — Percent change in species accumulation (all taxa and rare taxa only) between simulated sampling strategies. (A–C) "All taxa" curves (solid lines [—]) consisted of N = 24 marine benthic taxa during the first deployment period in July and (D–F) N = 25 taxa during the second deployment in August. (A–F) "Rare taxa only" curves (dashed lines [---]) consisted of the upper 25% rarest taxa in the assemblage. (A, D) Percent change from random sampling (S_R) to adaptive sampling by forcing sampling at scales ≥ 100s of m (S_{100}); (B, E) percent change from S_R to forcing sampling at scales ≥ 1000s of m (S_{1000}); (C, F) percent change from S_R to forcing sampling at scales = 10000s of m (S_{10000}).

General discussion

Implications for early detection

Early detection of marine invasive species is an emerging scientific endeavour in invasion ecology that would benefit greatly from science-based evidence to guide effective sampling of incipient invasive populations. Despite its considerable value for effective management, early detection has been overlooked because rapid response (e.g., eradication) is not always an option due to a lack of a clear response framework. In marine benthic systems, with widespread application of low-technology tools such as settlement plates (passive sampling) and promising development in high-technology such as molecular tools (point sampling) to survey the aquatic world, it has become necessary to think of where and when to sample strategically to detect small populations of incipient invasive species. An understanding of how species disperse and distribute in space and time can be appropriately exploited to guide the design of the sampling procedure. In particular, most of the research described in this thesis aimed to contrast rare and common scenarios to ensure that the sampling procedures are truly capable of enhancing rare species detection.

Although the first chapter (Chapter 1) examined the relationship between propagule supply and settlement in the context of spatial scale, it also laid out the case for the utility of settlement plates as an effective sampling tool for early detection at mesoscales within a marina. At mesoscales, propagule supply was a limited determinant of settlement. Despite the weaker than expected relationship, at least a quarter of the variation in settlement was explained by supply over short timescales of a week. Further, this supply–settlement relationship can be applied to understand rare species detection (such as early detection of incipient invasive populations) because the rare end of the rare-cum-common continuum is suggestive of the limits of species detection using our sampling methods. At the rarer extreme of this continuum, lowest values of propagule abundance (supply) ranged from 0 to 0.3 larvae 100 L^{-1} and the corresponding low values of settlement ranged from approximately 0.05 to 0.10 recruits cm^{-2} d^{-1}. These low values suggest that short-term passive sampling for newly-settled recruits is just as or more sensitive than point sampling for propagules in the water column at mesoscales within a marina. This study supports the use of settlement plates to detect

benthic marine species and it is reassuring given that the smallest spatial scale for aquatic invasive species monitoring occurs typically at these mesoscales (e.g., several sampling stations within a commercial harbour, a recreational marina, or an aquaculture site). The use of settlement plates continues to be an effective method for detecting aquatic invasive species. For instance, the enhanced settlement of marine invasive species was observed on settlement plates attached to mobile structures in shallow waters (namely floating docks) compared to those in deeper waters and those on fixed structures (Dafforn et al. 2009). More recently, surveys using a combination of various sampling methods, including settlement plates, have drastically expanded the number of known marine invaders from five to more than 50 species (an approximately ten-fold increase) in the Galápagos Islands, Ecuador (Carlton et al. 2019).

The different sampling methods used in Chapter 1 to sample for larvae and newly-settled recruits (i.e., early life history stages) provide important insights into early detection of marine invasive species. These early life history stages represent (1) the life history stage(s) at the initial moment of the drop-off of invasive propagules and (2) the dispersing phase of an invasive species' life cycle. Sampling for larvae or newly-settled recruits (i.e., both settlement and recruitment) can, even for a brief moment in time, substantially increase the geographic extent of detecting the species without needing to search the benthos for adults. First, if early detection truly matters, then the ability to detect invasive propagules as they are released or disassociate with the vector (i.e., drop-off) represents the earliest moment in time that early detection could occur. In aquatic systems, this process is primarily via (a) the discharge of contaminated ballast water at the recipient location (containing invasive spores or larvae from donor location) or (b) fouling species attached to ship hulls becoming reproductively mature and spawning gametes, spores, or larvae (i.e., invasive propagules) into a novel environment. Second, the seasonal reproductive cycles of an invasive species (even nascent populations) generate *de novo* cohorts of invasive propagules, which actively or passively disperses in space. For a small population, this process of larval dispersal suddenly, even for a short time, expands the spatial extent of the population until all the propagules settle or die. The sudden increase in the spatial size of the population permits the sampling to occur (i) in the pelagic zone of a body of water, instead of just surveying the benthos, and (ii) at the slightly larger spatial scale of the larval population, instead of the smaller scale of the benthic adult population. Nonetheless, benthic surveys have been successful in detecting new invasive species but usually in deeper waters or relatively remote coastal regions where monitoring using settlement plates is absent, e.g., the detection of the invasive ascidians *Didemnum vexillum* by

recreational divers in Minas Basin, Nova Scotia, Canada, and *Diplosoma listerianum* by government divers in Brier Island, Nova Scotia, Canada (Moore et al. 2014; Ma et al. 2018; also see Appendix 2).

The second chapter (Chapter 2) exploited the phenological circumstance that common species are rare during the seasonal onset of reproduction and settlement to experimentally test different temporal sampling strategies. In this chapter, we took a closer look at how sampling duration (the length of time that the settlement plate was immersed in the water) and frequency (the number of times a new set of plates were deployed over time) affect species detection. By testing a range of sampling durations from one day to 32 days, an intermediate duration of one to two weeks was determined to be the optimal timescales for a majority of benthic species. This finding supports the expectation that there are advantages and disadvantages of both short and long sampling durations. Unless a sampling design can employ both short and long sampling durations, trade-offs between these advantages and disadvantages rendered the intermediate duration as the optimal temporal strategy. The wealth of data generated from this study also permitted the evaluation of how different combinations of sampling durations and frequencies (N = 23 combinations) affected the detection of cumulative species richness. Within a 32-d sampling window, increasingly longer sampling durations and higher frequencies maximized the number of the benthic species that were detected on settlement plates. However, none of the 23 combinations, on their own, detected all the species that were observed in the study. In fact, the best combination associated with the least sampling effort was an intermediate duration of 16 days and a high frequency of sampling every fourth day. A closer examination of the data further revealed that, within the range of durations and frequencies considered, more species were detected by increasing duration than by increasing frequency (i.e., 500% *versus* 160% more species). This study suggests that an intermediate sampling duration, likely a consequence of sufficient seasoning (enhancing settlement over time) and availability of space (allowing settlement over time), coupled with high sampling frequency (increases temporal coverage) are integral temporal aspects to consider when designing and implementing a sampling procedure to detect rare species.

Because limited resources for sampling will need to be strategically allocated in space and time, the third chapter (Chapter 3) investigated the relative importance of four sources of variation in benthic recruitment: small-spatial scale (site-level), large-spatial scale (regional), small-temporal scale (date of plate deployment or seasonal timing), and large-temporal scale (year). Intriguingly, seasonal timing of sampling was the main

source that was associated with the highest variation in rare species recruitment among all the benthic species in the study. From this large-scale (i.e., 14 coastal sites throughout the Canadian Maritimes) and multi-year (i.e., 2012 to 2014) study, the relative importance of seasonal timing for rare species detection has implications for early detection of incipient invasive populations when they are rare in terms of abundance and spatial extent. An in-depth look at the data also revealed an important seasonal pattern linked to marine invasive species detection. Both seasonal timing of peak recruitment at sites where these invasive species were established (i.e., a self-sustaining population) and timing of local detection at sites where they were not considered established tend to occur during the autumn months (i.e., September and October). These findings suggest that invasive species management should consider the relative importance of time in their sampling design for surveys that are aimed to detect invasive species when they are expected to be rare, for example, (a) for early detection of new invasive species, (b) when monitoring the distributional limits of a rapidly spreading invader (i.e., invasion fronts), and (c) at the end of an eradication campaign when the number of individuals are increasingly small (Seymour et al. 2005; Canning-Clode et al. 2011; Thomsen et al. 2012; Lockwood et al. 2013; Cannizzo and Griffen 2019). Furthermore, it would be interesting to consider how a decadal timescale of sampling affect rare species detection given that such patterns at larger temporal scales influence the structure of benthic assemblages (i.e., abundance and distribution; Chang et al. 2017).

Building on the extensive scientific interest in elucidating the effects of scale on spatial patterns of species abundance and distribution (Morrissey et al. 1992; Thrush et al. 1994; Chapman et al. 1995; Underwood and Chapman 1996), the work described in the fourth chapter (Chapter 4) is among the few studies that explicitly identified the scales relevant for rare species (Chapman and Underwood 2008). The degree of recruitment variability for rare benthic species did not correlate with any of the spatial scales investigated in this study (from tens of metres to tens of kilometres). However, past studies on rare gastropods found that the smallest scale of the study was the largest source of variability in density (Chapman and Underwood 2008). A similar finding (i.e., the smallest scale) was found by identifying the dominant scale for rare species recruitment (i.e., detection of repeating patterns). For rare species, the dominant scale is at the smallest spatial scale (range of scales tested: hundreds of metres to tens of kilometres). If rare species tend to exhibit repeating recruitment patterns at the smallest scale of our study, then the optimal scale to sample for rare species should occur at or above this scale (hundreds of metres). A powerful approach to test different sampling strategies on actual field data was to simulate these different strategies by resampling the data multiple times using a bootstrap procedure. Within the fixed spatial extent of the study system, this analysis clearly

demonstrates that detection of rare species benefit, more so than common species, from sampling strategies that force sampling to be carried out with certain spatial structure instead of sampling randomly. If the goal is to detect as many species as possible (i.e., maximizing species richness), then the simulated sampling strategies show that more species will be detected with greater sampling effort and by forcing sampling at the largest spatial scale (tens of kilometres). However, the outcome changed to forcing sampling at the intermediate scale (ones of kilometres) when the goal is to, instead, detect as many rare species as possible (the bottom quartile of species in terms of abundance). This information provides much-valued guidance for early detection. Thus, resource permitting, the relevant spatial scale for rare species detection should be at ones of kilometres.

Broadly, this thesis highlights the importance of determining the optimal life history stage (Chapter 1), temporal aspects (Chapter 2 and Chapter 3), spatial aspects (Chapter 3 and Chapter 4), taxonomic aspects (Chapter 2, Chapter 3, and Chapter 4) when sampling for rare species. Although new technologies, such as environmental DNA (eDNA), may be highly sensitive in detecting rare species in aquatic systems (Evans et al. 2017; Deiner et al. 2018) and could readily replace settlement plates as the most effective sampling tool for incipient invasive populations, issues regarding where and when to sample remain unknown. Further, applying molecular tools (e.g., eDNA) in the field and any other types of sampling tools will require the same optimization procedures across space and time as done with plates (Chapter 2, Chapter 3, and Chapter 4). The design of field experiments described in this thesis set an example of how similar studies can be adapted to enhance the probability of detecting other rare aquatic species (beyond the marine benthic species) and to customize the sampling strategies for other aquatic regions around the world. Further, sampling strategies that maximize taxonomic richness may not necessarily be the most appropriate strategy for rare species detection (Chapter 4). Counter-intuitively, these strategies may be biased towards the detection of a high number of common species at the expense of detecting rare species. More research is needed to better understand the differences between rare and common species and the processes driving their distributional patterns because strategies that have been developed and tested on non-rare conditions can have adverse implications for early detection of rare invasive species and other applied fields that manage rare species.

Early detection toolkit across space and time

Although the nearshore benthic marine system in the Canadian Maritimes was the focal system in this thesis, many of the key research questions (Figure 33) concerning sampling for rare species apply to other aquatic and, perhaps, terrestrial systems around the world (e.g., remote polar regions) and the use of other sampling tools (e.g., molecular methods). Broadly, this toolkit contains a series of field experiments with the goal of identifying sampling strategies that targets (1) the correct life history stages, (2) the optimal spatial and temporal scales, and (3) rare species in the assemblage (see Figure 33). For the marine benthic system specifically, the application of this toolkit has been successful in refining the sampling design to detect rare species. The key fundamental research questions posed in this thesis are as follows:

1. Do settlement rates of an invasive marine benthic species reflect its propagule supply over relevant vertical and horizontal scales within a marina?
2. What is the optimal sampling duration to detect rare species?
3. What is the optimal sampling strategy, a combination of sampling duration and frequency, to maximize taxonomic richness of rare species?
4. What is the relative importance between spatial and temporal components of sampling for rare species detection?
5. What is the optimal seasonal timing to detect rare species?
6. What is the optimal spatial scale to detect rare species?
7. Is the strategy that maximizes taxonomic richness the same strategy that maximizes richness of rare species?

The findings described in this thesis provide some concrete answers to these research questions, which also address the overarching objective of informing how sampling should proceed across space and time to detect incipient invasive populations (Figure 33). For instance, settlement rates of an invasive marine benthic species reflect its propagule supply at mesoscales within a marina. Since there is a relationship between settlement and supply at this spatial scale in both the horizontal and vertical dimensions, the utility of settlement plates targeting newly settled recruits is, therefore, supported as an appropriate method to detect early life history stages of a dispersing benthic marine species. For passive sampling tools over short time

scales, an intermediate sampling duration was the optimal duration to detect rare species based on four different criteria (i.e., earliest day of species detection, greatest proportion of colonized replicate plates, highest recruitment density, and highest recruitment rate). At the assemblage-level, an intermediate sampling duration coupled with high sampling frequency maximized taxonomic richness after an approximately one-month sampling period when propagule supply was assumed to be low (i.e., the seasonal onset of recruitment). The temporal dimension (and not the spatial dimension) was found to be relatively important source for rare species detection. Although this does not discount the importance of spatial coverage in the design of an early detection campaign, it does, however, suggest that more thought should be paid to the temporal component (especially seasonal timing) of the sampling design. Upon closer examination, the optimal seasonal timing for several marine invasive invertebrate species occurred in the autumn months of September and October, coincident with the seasonal timing of peak recruitment for a majority of the invasive species that were investigated in the Canadian Maritimes. After assessing multiple scales, the optimal spatial scale to detect rare species tends to involve sampling at smaller spatial scales. Further, simulations evaluating different spatially-explicit sampling strategies show that forcing sampling at the smallest spatial scales generally enhances rare species detection. Finally, it is important to recognize that a sampling strategy designed to maximize species richness can be altogether a different strategy for maximizing an assemblage of rare species only. This distinction is of paramount importance depending on the sampling objective. If the goal were to develop a single sampling method that can ascertain the local biodiversity, then selecting the method that maximizes species richness would surely be appropriate. However, for many other types of applications that require sampling for rare species, this method may counter-intuitively reduce the probability of detecting these species.

The toolkit to optimize for early detection of invasive species is a much-needed effort to enhance the probability of detecting invasive species in the waters around the Canadian Maritimes. However, similar optimization studies are crucially required to target (1) the breadth of taxonomic groups, beyond the marine benthic organisms examined in this thesis and (2) the many different regions around the world, such as distant and inaccessible regions in polar waters and the deep sea. This toolkit is far from being complete. Future work is vital to build a strong science-based foundation to guide early detection efforts at local, regional, and global scales.

Early detection toolkit: Next steps

Time spent in the field has resulted in the discovery of several new records of marine invasive species (see Appendix 2). Although their detections were not the primary goal of the fieldwork, the collection, identification, and documentation of these invaders provided direct insights into the circumstances leading to their early detection. Undoubtedly, (1) collaborations among different research groups (including citizen scientists), (2) application of multiple sampling methods, and (3) high sampling effort across space and time (assuming that resources are not a limiting factor) are some of the prerequisites for successful early detection campaigns. Optimization of many relevant spatial and temporal aspects of early detection sampling has been explored in this thesis and these studies form the basis of the early detection toolkit. However, future research directions that can broaden this toolkit include (1) evaluating the effects of observer bias (i.e., observer error due to perception or visibility bias) on the probability of species detection and (2) quantifying the dispersal of invasive propagules from a newly colonized source population. Preliminary studies were carried out to determine observer bias and the spatial extent of larval dispersal of a marine invasive species from a newly constructed and colonized marina.

Observer bias: Our ability to visually detect rare species (e.g., individuals or small nascent populations of invasive species) is influenced by a number of factors, including sampling effort, taxonomic expertise of the identifier, and/or the number of individuals in a given sample. Settlement plates are a standard tool for measuring recruitment strength in ecological studies and detecting fouling invasive species, yet small (and/or young) individuals may be missed during sample processing. The probability of detection is hypothesized to be proportional to the number of individuals in a sample. Here, the relationship between the probability of detecting marine invertebrates on settlement plates and the number of individuals on plates was examined. To do so, volunteer assistants ($n = 5$), who were previously trained in invertebrate identification, recorded the presence and absence of eight taxa (colonial ascidians, solitary ascidians, encrusting bryozoans, erect bryozoans, barnacles, oysters, skeleton shrimps, and sea stars) on 82 settlement plates. These plates were chosen from among more than 600 of those that were deployed for approximately six weeks and had between zero and at least 20 individuals (or colonies) of each taxon per plate. For each taxon, the relationship between the probability of detection and the number of individuals (or colonies) was best described by a logarithmic function. These findings have implications for the accuracy and precision of detecting species with low

abundances on settlement plates. In particular, this preliminary study highlights the possible underestimation of species with abundances less than or equal to 20 individuals (or colonies) per sample in marine ecological studies and aquatic invasive species monitoring activities.

Spatial extent of larval dispersal from a newly constructed and colonized marina: Ports and marinas are hotspots for biological invasions because they are often locations of species introductions and provide habitat to shelter aquatic invasive species (e.g., marina as a local refuge). A new marina was constructed in Bras d'Or Lake, Nova Scotia, Canada, in 2012. This 1.5-ha marina is an artificially created enclosed basin with a ca. 30-m-wide entrance to the brackish lake. In 2013 and 2014, a large, reproductive population of an invasive ascidian, *Botryllus schlosseri*, was observed to have extensively colonized artificial structures within the marina, including hulls of many resident boats. This study examines the larval dispersal of this species immediately outside the marina basin. Larval dispersal was determined by deploying buoys with settlement plates at multiple depths (one per metre from 1 to 6 m) and distances of 50 to 150 m from the marina entrance. Results show horizontal (cross-shore distance) and vertical variability of larval settlement (a proxy for larval abundance in the water column). This preliminary study documents the rapid colonization of a new marina by an invasive ascidian and the spatial extent of ascidian larval dispersal (10s of metres), suggesting that hull fouling is likely more important than natural dispersal for secondary introductions.

Closing perspectives

The science of enhancing rare species detection (e.g., early detection of invasive species) can greatly benefit from experimental field studies, such as those presented in this thesis (quantitative approach), in addition to documenting the circumstances of rare species observations (qualitative approach). Instead of waiting for the discovery a rare species (e.g., the introduction of a new invasive species to a locality; Chang et al. 2011; Ma et al. 2016), our ecological studies can directly investigate (a) patterns and processes that are unique to rare species and not to common ones and (b) factors that affect the probability of rare species detection. One of the most pressing challenges to rare species detection is determining the appropriate spatial and temporal scale for sampling, which has been examined at smaller spatial and temporal scales (see Chapter 1 and Chapter 2) and at larger spatial and temporal scales (see Chapter 3 and Chapter 4). These chapters spanned

many scales across space and time that are believed to be relevant for the detection of benthic marine species (see a global view of the chapters in Figure 33 as plotted along two dimensions of space and time).

The research described in this thesis could have optimized various components of sampling individually. However, we undertook the difficult task of examining multiple variables. For instance, in Chapter 1, instead of investigating the influence of propagule supply and initial settlement with respect to the vertical and, separately, the horizontal dimension, they were explored concurrently. Also, in Chapter 2, the effect of sampling duration and frequency on rare species detection was combined. In Chapter 3, different scales of both space and time on the variability of benthic recruitment were evaluated in the same analysis to determine the relative importance of spatial and temporal components of sampling. Although Chapter 4 focused on just the spatial dimension, it explored a broad range of scales (i.e., four orders of magnitude from 10s of m to 10000s of m) through multiscale analyses instead of evaluating the probability of species detection (and also the spatial structure of benthic recruitment) for each scale individually. Consequently, this thesis (as an early detection toolkit) provides a good foundation for a quantitative approach to develop and optimize sampling strategies for rare species detection with considerable emphasis on spatiotemporal aspects of sampling instead of statistical (e.g., increased sampling effort), economic (e.g., calculating trade-offs between costs and management outcomes), and technological (e.g., testing the effectiveness of various sampling tools) aspects of sampling.

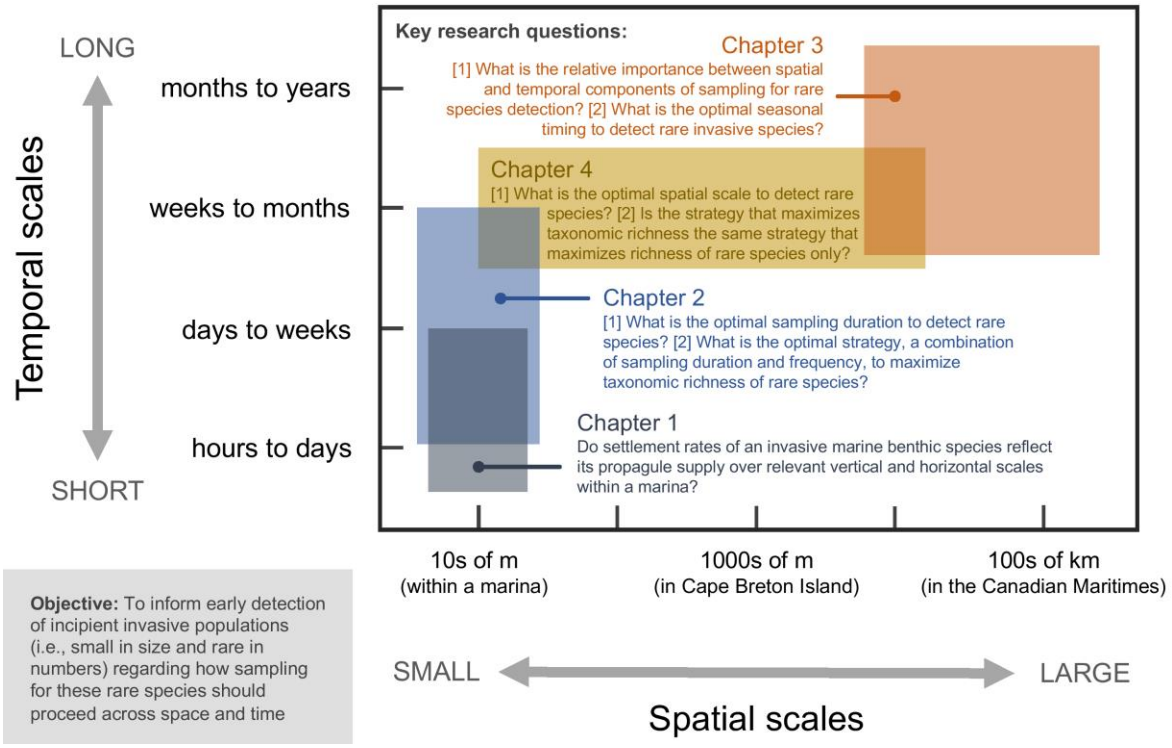


Figure 33 — The overarching objective of the thesis and key research questions posed by each chapters (Chapter 1, Chapter 2, Chapter 3, and Chapter 4). These chapters are plotted along two dimensions: spatial scales in the x-axis and temporal scales in the y-axis.

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Appendix 1. Supplemental tables and figures

Supplemental Table S1 — First day of observation of 22 marine benthic taxa and their optimal sampling durations (1, 2, 4, 8, 16, and 32 d) ranked according to (A) earliest day (day of plate retrieval) of species detection; (B) greatest proportion of colonized replicate plates; (C) highest density (recruits plate^{-1}); and (D) highest rate (recruits plate^{-1} d^{-1}). Optimal sampling duration is **bolded**. Ties are shown with an equal sign (=).

Taxon	Authority	First day of observation	Ranking of sampling duration (d)			
			(A) Earliest	(B) Least patchy	(C) Densest	(D) Highest rate
Ciliophora, Heterotrichea						
Folliculina sp.	Lamarck, 1816	July 6	**4**>1=2>9>16>32	**4=8=16**>32>2>1	**16**>8>32>4>2>1	**8**>16>4>32>1>2
Ochrophyta, Bacillariophyceae						
Periphytic diatoms	—	July 2	**1**>2>4>8>16>32	**1=2=4=8=16**>32	**8**>4>16>1>2>32	**1**>2>4>8>16>32
Cnidaria, Anthozoa						
Actiniaria	—	July 18	**2=8**>4=16>32	**16**>32>8=4=2	**32**>8>16>2=4	**8**>32>2=16>4
Cnidaria, Hydrozoa						
Bougainvillia carolinensis	(McCady, 1859)	July 16	**8**>16>4>32	**4=8=16=32**	**8**>4>16>32	**4**>8>16>32
Platyhelminthes, Turbellaria						
Anaperus gardineri	(Graff, 1911)	July 5	**1**>2>4=8>16	**4=8**>1=2>16	**8**>4>1=2>16	**1**>2>8>4>16
Sterreria psammicola	(Sterrer, 1970)	July 12	**8**>2	**8**>2	**8**>2	**2**>8
Annelida, Polychaeta						
Nereididae	Blainville, 1818	July 16	**8**>16>32	**32**>16>8	**32**>8=16	**32**>8>16
Spionidae	Grube, 1850	July 17	**16**>8>32	**16=32**>8	**32**>16>8	**16**>32>8
Arthropoda, Amphipoda						
Gammarus sp.	Fabricius, 1775	July 7	**4**>8>2>16>1>32	**8**>4=16>1=2=32	**16**>4>2>8>32>1	**2**>4>16>1>8>32
Mollusca, Bivalvia						
Crassostrea virginica	(Gmelin, 1791)	July 20	**1=2=4=8**>16>32	**1=2=4=8=16=32**	**8**>4>16>32>2>1	**8**>4>2>1>16>32
Hiatella arctica	(Linnaeus, 1767)	July 12	**4=8**>1=2>16>32	**4=8=16**>32>2>1	**8**>16>32>4>2>1	**8**>16>1>32>2>4
Mytilus sp.	Linnaeus, 1758	July 8	**2**>8>4>1>16>32	**16=32**>8>4>1=2	**32**>16>8>4>2>1	**8**>16>1>2>32>4
Mollusca, Gastropoda						
Bittiolum alternatum	(Say, 1822)	July 19	**2=4=8**>16>1>32	16>8>2=4=32>1	**32**>16>8>4>2>1	**2**>16>1=8>4>32
Crepidula formicata	(Linnaeus, 1758)	July 23	**8**>4>16	16>8>4	**16**>4=8	**4**>8>16
Lacuna vincta	(Montagu, 1803)	July 5	**2**>8>1>4>16>32	**1=2=4=8=16=32**	**8**>16>32>4>1>2	1>8>4>2>16>32
Littorina sp.	Férussac, 1822	August 2	**4=8=16=32**	**16=32**>4=8	**16**>32>8>4	**16**>4>8>32
Tenellia fuscata	(Gould, 1870)	July 29	**16**>32	**32**>16	**32**>16	**32**>16
Echinodermata, Asteroidea						
Asterias rubens	Linnaeus, 1758	July 29	**8=16**>4>32	**4=8=16=32**	**4=8=16=32**	**4**>8>16>32
Bryozoa, Gymnolaemata						
Amathia gracilis	(Leidy, 1855)	July 14	**8**>4>16>2>32	**8=16**>32>4>2	**16**>32>8>4>2	**16**>8>32>2=4
Conopeum tenuissimum	(Canu, 1908)	July 14	**8**>16>4>2>1>32	**16=32**>8>4>2>1	**32**>16>8>4>2>1	**16**>8>32>1=2=4
Crisularia turrita	(Desor, 1848)	July 18	**8**>16>4>32	**16**>8=32>4	**16**>32>4>8	**4**>16>8>32
Chordata, Ascidiacea						
Botryllus schlosseri	(Pallas, 1766)	July 25	**4=8**>1=16>2>32	**8=16**>4>1=2=32	**8**>16>4>2>32>1	**2=4=8**>1>16>32

Supplemental Table S2 — Linear regressions modelled for nine marine benthic taxa and five plate day conditions (2, 4, 8, 16, and 32 d) on the relationship between $\log_{10}(x + 0.01)$ transformed additive and cumulative recruitment rates. ND = no data. Statistically significant p values are **bolded**.

Taxon	2 plate days			4 plate days			8 plate days			16 plate days			32 plate days		
	$F_{(1,29)}$	R^2	p	$F_{(1,27)}$	R^2	p	$F_{(1,25)}$	R^2	p	$F_{(1,3)}$	R^2	p	F	R^2	p
Cnidaria, Hydrozoa															
Bougainvillia carolinensis	—	—	ND	—	—	ND	62.31	0.71	**< 0.001**	46.83	0.94	**0.006**	—	—	ND
Mollusca, Bivalvia															
Crassostrea virginica	230	0.89	**< 0.001**	1606	0.98	**< 0.001**	1172	0.98	**< 0.001**	58.87	0.95	**0.005**	—	—	ND
Hiatella arctica	11.19	0.28	**0.002**	25.97	0.49	**< 0.001**	5.99	0.19	**0.022**	0.88	0.23	0.417	—	—	ND
Mytilus sp.	14.13	0.33	**< 0.001**	6.20	0.19	**0.019**	21.14	0.46	**< 0.001**	3.35	0.53	0.165	—	—	ND
Mollusca, Gastropoda															
Lacuna vincta	105	0.78	**< 0.001**	96.66	0.78	**< 0.001**	69.83	0.74	**< 0.001**	32.19	0.91	**0.011**	—	—	ND
Bryozoa, Gymnolaemata															
Amathia gracilis	—	—	ND	0.62	0.02	0.437	17.37	0.41	**< 0.001**	368	0.99	**< 0.001**	—	—	ND
Conopeum tenuissimum	0.21	0.01	0.651	5.54	0.17	**0.026**	2.22	0.08	0.149	0.19	0.06	0.693	—	—	ND
Crisularia turrita	—	—	ND	—	—	ND	4.86	0.16	**0.037**	23.06	0.88	**0.017**	—	—	ND
Chordata, Ascidiacea															
Botryllus schlosseri	24.34	0.46	**< 0.001**	53.75	0.67	**< 0.001**	259	0.91	**< 0.001**	23.80	0.88	**0.016**	—	—	ND
	$F_{(1,184)}$	R^2	p	$F_{(1,201)}$	R^2	p	$F_{(1,241)}$	R^2	p	$F_{(1,43)}$	R^2	p	$F_{(1,7)}$	R^2	p
All taxa	731	0.80	**< 0.001**	856	0.81	**< 0.001**	663	0.73	**< 0.001**	81.48	0.65	**< 0.001**	25.08	0.78	**0.002**

Supplemental Table S3 — Logarithmic regressions modelled on the relationship between time elapsed and each of the 18 sampling strategies: a combination of sampling duration (1, 2, 4, 8, or 16 d) and frequency (1, 0.5, 0.25, or 0.125 d^{-1}). Statistically significant p values are **bolded**.

Duration (d)	Frequency (d^{-1})	Equation	$F_{(df)}$	R^2	p
1	1	$y = -2.7 + 8.1 \log_{10} x$	$164.5_{(1,30)}$	0.85	**<0.001**
1	0.5	$y = -1.7 + 6.5 \log_{10} x$	$110.4_{(1,30)}$	0.79	**<0.001**
1	0.25	$y = -0.1 + 4.7 \log_{10} x$	$69.3_{(1,30)}$	0.70	**<0.001**
1	0.125	$y = -1.1 + 3.5 \log_{10} x$	$59.4_{(1,30)}$	0.66	**<0.001**
2	1	$y = -6.5 + 13.1 \log_{10} x$	$269.5_{(1,29)}$	0.90	**<0.001**
2	0.5	$y = -4.6 + 11.0 \log_{10} x$	$247.1_{(1,29)}$	0.90	**<0.001**
2	0.25	$y = -2.5 + 8.6 \log_{10} x$	$164.9_{(1,29)}$	0.85	**<0.001**
2	0.125	$y = -0.02 + 5.8 \log_{10} x$	$67.7_{(1,29)}$	0.70	**<0.001**
4	1	$y = -14.2 + 20.5 \log_{10} x$	$321.4_{(1,27)}$	0.92	**<0.001**
4	0.5	$y = -12.2 + 18.4 \log_{10} x$	$344.9_{(1,27)}$	0.93	**<0.001**
4	0.25	$y = -9.6 + 15.8 \log_{10} x$	$197.1_{(1,27)}$	0.88	**<0.001**
4	0.125	$y = -5.8 + 12.6 \log_{10} x$	$118.1_{(1,27)}$	0.81	**<0.001**
8	1	$y = -23.1 + 29.8 \log_{10} x$	$755.6_{(1,23)}$	0.97	**<0.001**
8	0.5	$y = -20.5 + 27.4 \log_{10} x$	$296.5_{(1,23)}$	0.93	**<0.001**
8	0.25	$y = -15.9 + 23.5 \log_{10} x$	$162.0_{(1,23)}$	0.88	**<0.001**
8	0.125	$y = -10.1 + 19.0 \log_{10} x$	$90.1_{(1,23)}$	0.80	**<0.001**
16	0.25	$y = -38.0 + 39.5 \log_{10} x$	$27.2_{(1,3)}$	0.90	**0.014**
16	0.125	$y = -19.0 + 25.7 \log_{10} x$	$3.3_{(1,3)}$	0.52	0.168

Supplemental Table S4 — Distribution of spatial variance in recruitment across multiple scales (from small- to large-scales: *Sites* [100s of m], *Locations* [1000s of m], and *Sectors* [10000s of m]) estimated using generalised linear mixed-effects models (GLMM) and Markov chain Monte Carlo generalized linear mixed models (MCMC GLMM) for 27 marine benthic taxa. CI = confidence interval; VC = variance component. The scale (excluding the residual error term) with the most variance is **bolded**.

Taxon	Source	First deployment period (July)						Second deployment period (August)					
		GLMM		MCMC GLMM				GLMM		MCMC GLMM			
		Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI	Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI
Cnidaria, Hydrozoa													
Tubulariidae	*Sector*	0	0	0.1	1.6	< 0.1	0.6						
	Location	4.3	18.2	0.6	8.9	< 0.1	4.8						
	Site	**18.1**	**77.5**	**6.2**	**89.1**	**2.7**	**11.1**						
	Station	1.0	4.3	< 0.1	0.3	< 0.1	0.1						
Arthropoda, Amphipoda													
Caprella mutica	*Sector*	5.3	11.0	27.6	33.9	< 0.1	142.4	7.0	6.5	31.5	10.2	< 0.1	172.8
	Location	**41.1**	**85.7**	**52.3**	**64.2**	**< 0.1**	**193.0**	**97.0**	**89.7**	**272.6**	**88.5**	**< 0.1**	**846.1**
	Site	0.6	1.2	< 0.1	0.1	< 0.1	0.2	3.2	3.0	0.5	0.2	< 0.1	3.2
	Station	1.0	2.1	1.5	1.8	0.3	3.3	1.0	0.9	3.3	1.1	1.1	6.7
Corophiidae	*Sector*	**2.9**	**71.7**	**7.0**	**92.0**	**< 0.1**	**26.3**	**2.2**	**34.0**	**5.4**	**38.8**	**< 0.1**	**21.1**
	Location	0.1	3.2	0.3	4.5	< 0.1	3.3	1.6	24.0	3.9	28.2	< 0.1	11.9
	Site	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	1.7	26.6	0.2	1.6	< 0.1	1.4
	Station	1.0	25.1	0.3	3.4	< 0.1	1.6	1.0	15.3	4.3	31.4	1.4	8.2
Gammarus sp.	*Sector*	**7.6**	**70.6**	**83.5**	**95.8**	**< 0.1**	**272.1**	0	0	0.2	19.7	< 0.1	1.0
	Location	2.2	20.1	2.9	3.4	< 0.1	17.4	< 0.1	< 0.1	0.1	13.5	< 0.1	0.4
	Site	0	0	0.2	0.3	< 0.1	1.9	**13.3**	**93.0**	**0.6**	**61.4**	**< 0.1**	**2.5**
	Station	1.0	9.3	0.5	0.6	< 0.1	2.3	1.0	7.0	0.1	5.4	< 0.1	0.3
Ischyroceridae	*Sector*	**15.7**	**86.0**	**87.1**	**97.7**	**< 0.1**	**362.2**	**2.3**	**39.8**	2.8	31.8	< 0.1	12.5
	Location	0.7	3.9	0.6	0.7	< 0.1	2.5	1.9	32.5	**4.6**	**52.2**	**0.3**	**11.0**
	Site	0.8	4.6	0.8	0.9	< 0.1	2.2	0.6	10.2	< 0.1	0.1	< 0.1	< 0.1
	Station	1.0	5.5	0.6	0.7	< 0.1	1.7	1.0	17.5	1.4	15.8	0.5	2.4
Arthropoda, Cirripedia													
Amphibalamus improvisus	*Sector*	**24.3**	**81.9**	**51.8**	**90.2**	**4.7**	**146.2**	**8.2**	**75.6**	**13.4**	**86.8**	**2.0**	**32.6**
	Location	3.5	11.8	4.6	8.0	1.2	9.7	0.9	8.4	1.2	7.6	0.3	2.4
	Site	0.9	3.0	0.8	1.5	0.4	1.4	0.7	6.8	0.6	4.1	0.3	1.0
	Station	1.0	3.4	0.2	0.3	0.1	0.2	1.0	9.2	0.2	1.6	0.2	0.3

Supplemental Table S4 (continued)

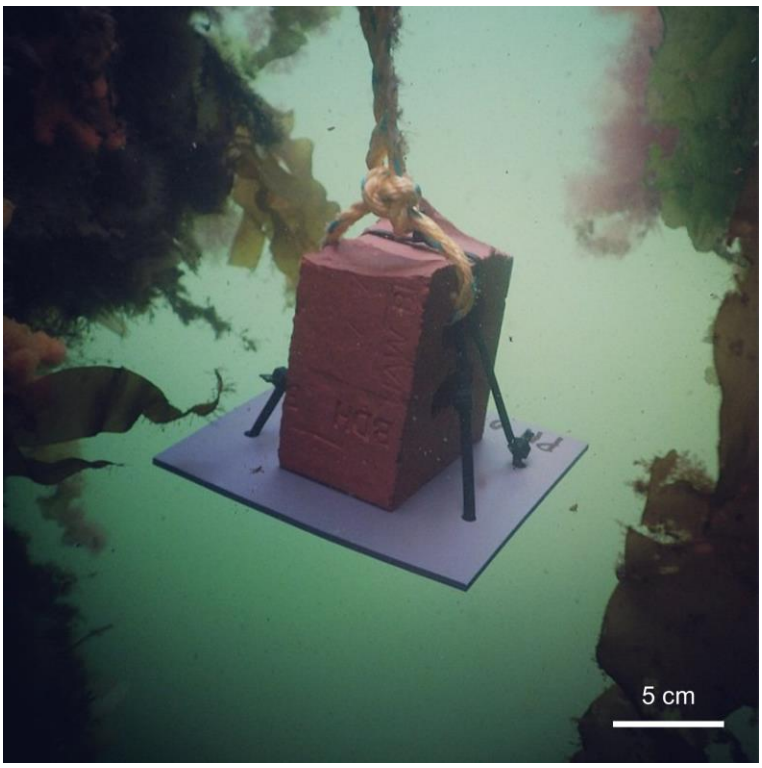
Taxon	Source	First deployment period (July)						Second deployment period (August)					
		GLMM		MCMC GLMM				GLMM		MCMC GLMM			
		Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI	Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI
Mollusca, Bivalvia													
Anomia simplex	*Sector*	**60.4**	**95.5**	**662.5**	**98.7**	**< 0.1**	**3164.9**	12.6	12.5	**15.9**	**82.2**	**< 0.1**	**39.1**
	Location	1.8	2.9	1.4	0.2	< 0.1	4.4	**84.6**	**84.2**	0.2	0.9	< 0.1	1.0
	Site	< 0.1	< 0.1	0.1	< 0.1	< 0.1	0.2	2.3	2.3	3.2	16.3	< 0.1	6.6
	Station	1.0	1.6	7.3	1.1	< 0.1	22.6	1.0	1.0	0.1	0.6	< 0.1	0.7
Crassostrea virginica	*Sector*	**2.8**	**42.8**	**3.6**	**46.9**	**< 0.1**	**11.4**	**3.8**	**62.6**	**6.5**	**80.6**	**0.9**	**16.4**
	Location	2.0	31.4	3.3	42.3	0.8	7.5	0.8	12.5	0.9	10.8	0.1	1.9
	Site	0.7	10.2	0.5	6.8	0.2	0.9	0.5	8.6	0.5	6.8	0.2	0.9
	Station	1.0	15.5	0.3	3.9	0.2	0.4	1.0	16.3	0.1	1.8	0.1	0.2
Hiatella arctica	*Sector*	0.3	10.5	0.1	4.0	< 0.1	0.7	0.6	22.2	0.2	7.7	< 0.1	1.2
	Location	**1.0**	**32.9**	**1.6**	**56.8**	**0.4**	**2.9**	**0.9**	**35.8**	**2.2**	**86.1**	**< 0.1**	**4.2**
	Site	0.7	23.9	0.1	5.2	< 0.1	0.6	0.1	4.0	0.0	1.3	< 0.1	0.0
	Station	1.0	32.7	0.9	34.0	0.6	1.3	1.0	38.0	0.1	4.9	< 0.1	0.8
Mytilus sp.	*Sector*	1.8	31.0	1.3	20.6	< 0.1	5.9	**4.4**	**50.7**	**6.3**	**59.0**	**< 0.1**	**16.5**
	Location	**2.1**	**36.9**	**3.9**	**63.2**	**0.9**	**7.4**	2.2	25.7	3.1	28.4	0.8	7.1
	Site	0.8	14.5	0.8	12.3	0.4	1.2	1.0	12.1	0.9	8.2	0.4	1.4
	Station	1.0	17.5	0.2	4.0	0.2	0.3	1.0	11.5	0.5	4.4	0.3	0.6
Mollusca, Gastropoda													
Bittiolum alternatum	*Sector*	**1.6**	**30.3**	1.8	28.6	< 0.1	6.5	1.1	20.3	0.6	9.6	< 0.1	3.1
	Location	1.4	26.0	**2.6**	**41.6**	**< 0.1**	**5.8**	**2.5**	**46.2**	**4.3**	**70.7**	**1.1**	**8.8**
	Site	1.4	25.4	1.2	18.7	0.5	2.1	0.8	14.6	0.4	6.2	< 0.1	1.3
	Station	1.0	18.4	0.7	11.1	0.5	0.9	1.0	18.8	0.8	13.5	0.2	1.5
Crepidula fornicata	*Sector*	**4.4**	**78.2**	**9.3**	**99.6**	**0.7**	**26.0**	**0.9**	**31.3**	**0.4**	**53.0**	**< 0.1**	**2.2**
	Location	0	0	< 0.1	0.1	< 0.1	< 0.1	0.8	28.8	0.3	41.3	< 0.1	1.3
	Site	0.2	4.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	4.0	< 0.1	0.5	< 0.1	0.0
	Station	1.0	17.7	< 0.1	0.3	< 0.1	0.2	1.0	35.9	< 0.1	5.2	< 0.1	0.2
Echinodermata, Asteriodae													
Asterias rubens	*Sector*	**3.6**	**66.3**	**9.4**	**86.0**	**0.6**	**28.4**	< 0.1	< 0.1	0.7	1.8	< 0.1	1.9
	Location	< 0.1	< 0.1	< 0.1	0.2	< 0.1	0.1	**10.8**	**91.5**	**36.4**	**98.0**	**0.8**	**84.1**
	Site	0.8	15.3	0.2	1.9	< 0.1	1.0	0	0	< 0.1	< 0.1	< 0.1	0.0
	Station	1.0	18.4	1.3	12.0	0.4	2.2	1.0	8.5	0.1	0.2	< 0.1	0.5

Supplemental Table S4 (continued)

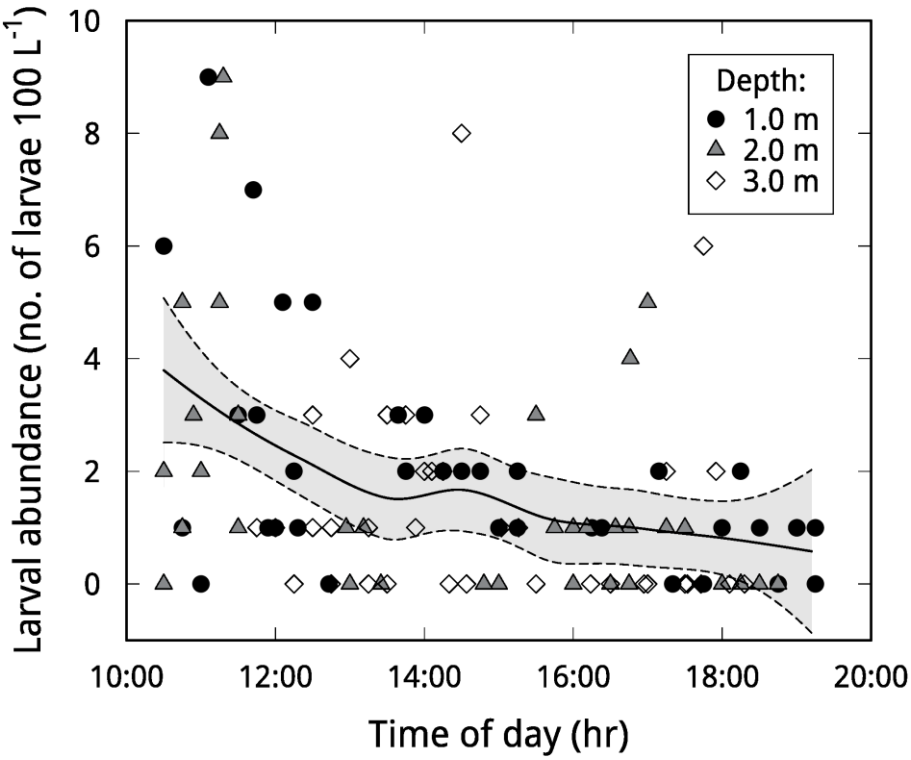
Taxon	Source	First deployment period (July)						Second deployment period (August)					
		GLMM		MCMC GLMM				GLMM		MCMC GLMM			
		Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI	Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI
Bryozoa, Gymnolaemata													
Conopeum tenuissimum	*Sector*	**5.3**	**66.7**	**13.0**	**85.8**	**< 0.1**	**35.8**	**5.4**	**51.6**	6.2	43.9	< 0.1	25.1
	Location	1.0	12.3	1.2	7.8	< 0.1	3.2	3.7	35.5	**7.5**	**52.9**	**0.5**	**18.7**
	Site	0.7	8.6	1.0	6.4	0.2	2.3	0.4	3.4	0.4	2.5	< 0.1	0.9
	Station	1.0	12.5	< 0.1	< 0.1	< 0.1	< 0.1	1.0	9.5	0.1	0.7	< 0.1	0.4
Cribrilina annulata	*Sector*	0	0	**39.5**	**80.0**	**< 0.1**	**184.6**	0	0	< 0.1	1.4	< 0.1	0.3
	Location	8.3	17.5	8.2	16.5	< 0.1	32.7	0	0	0.3	9.6	< 0.1	2.8
	Site	**38.3**	**80.4**	1.6	3.2	< 0.1	6.8	**53.1**	**98.2**	**2.6**	**77.2**	**< 0.1**	**6.1**
	Station	1.0	2.1	0.1	0.2	< 0.1	0.6	1.0	1.8	0.4	11.8	< 0.1	3.3
Cribrilina punctata	*Sector*	0.2	0.5	**5.1**	**53.7**	**< 0.1**	**16.2**	**55.0**	**93.1**	**8.8**	**82.6**	**< 0.1**	**25.6**
	Location	4.3	12.1	0.7	7.4	< 0.1	4.7	1.1	1.9	< 0.1	0.2	< 0.1	0.1
	Site	**30.2**	**84.6**	3.6	38.0	< 0.1	8.7	2.0	3.3	1.8	17.0	0.6	3.7
	Station	1.0	2.8	0.1	0.8	< 0.1	0.4	1.0	1.7	< 0.1	0.2	< 0.1	0.1
Crisularia turrita	*Sector*	0	0	0.8	3.3	< 0.1	3.2	**4.3**	**47.6**	**19.7**	**76.1**	**< 0.1**	**70.7**
	Location	3.0	9.7	**16.0**	**69.3**	**< 0.1**	**62.5**	0.9	10.0	1.7	6.4	< 0.1	7.3
	Site	**27.0**	**87.1**	0.3	1.4	< 0.1	2.4	2.8	31.3	0.9	3.4	< 0.1	5.1
	Station	1.0	3.2	6.0	26.0	0.1	17.4	1.0	11.1	3.7	14.1	0.7	7.6
Cryptosula pallasiana	*Sector*							0	0	0.6	4.3	< 0.1	3.8
	Location							0	0	0.8	5.4	< 0.1	4.9
	Site							**125.7**	**99.2**	**13.1**	**89.5**	**3.0**	**26.6**
	Station							1.0	0.8	0.1	0.8	< 0.1	0.6
Einhornia crustulenta	*Sector*							0	0	82.2	9.6	< 0.1	435.8
	Location							0	0	**297.2**	**34.8**	**< 0.1**	**378.1**
	Site							**125.7**	**99.2**	5.5	0.6	< 0.1	1.7
	Station							1.0	0.8	469.1	54.9	2.7	2165.8
Electra pilosa	*Sector*	**2.4**	**56.7**	**16.1**	**90.1**	**< 0.1**	**78.5**	< 0.1	< 0.1	0.1	5.9	< 0.1	0.4
	Location	0.8	19.4	1.2	6.7	< 0.1	3.4	**0.7**	**31.2**	**1.0**	**79.4**	**< 0.1**	**2.3**
	Site	0	0	0.1	0.3	< 0.1	0.5	0.5	23.7	0.1	6.6	< 0.1	0.6
	Station	1.0	23.8	0.5	2.9	< 0.1	1.6	1.0	45.1	0.1	8.1	< 0.1	0.6
Escharella sp.	*Sector*	< 0.1	< 0.1	**6.4**	**49.8**	**< 0.1**	**23.2**						
	Location	0	0	5.0	38.8	< 0.1	15.1						
	Site	**56.1**	**98.2**	1.4	10.8	< 0.1	3.4						
	Station	1.0	1.8	0.1	0.6	< 0.1	0.6						

Supplemental Table S4 (continued)

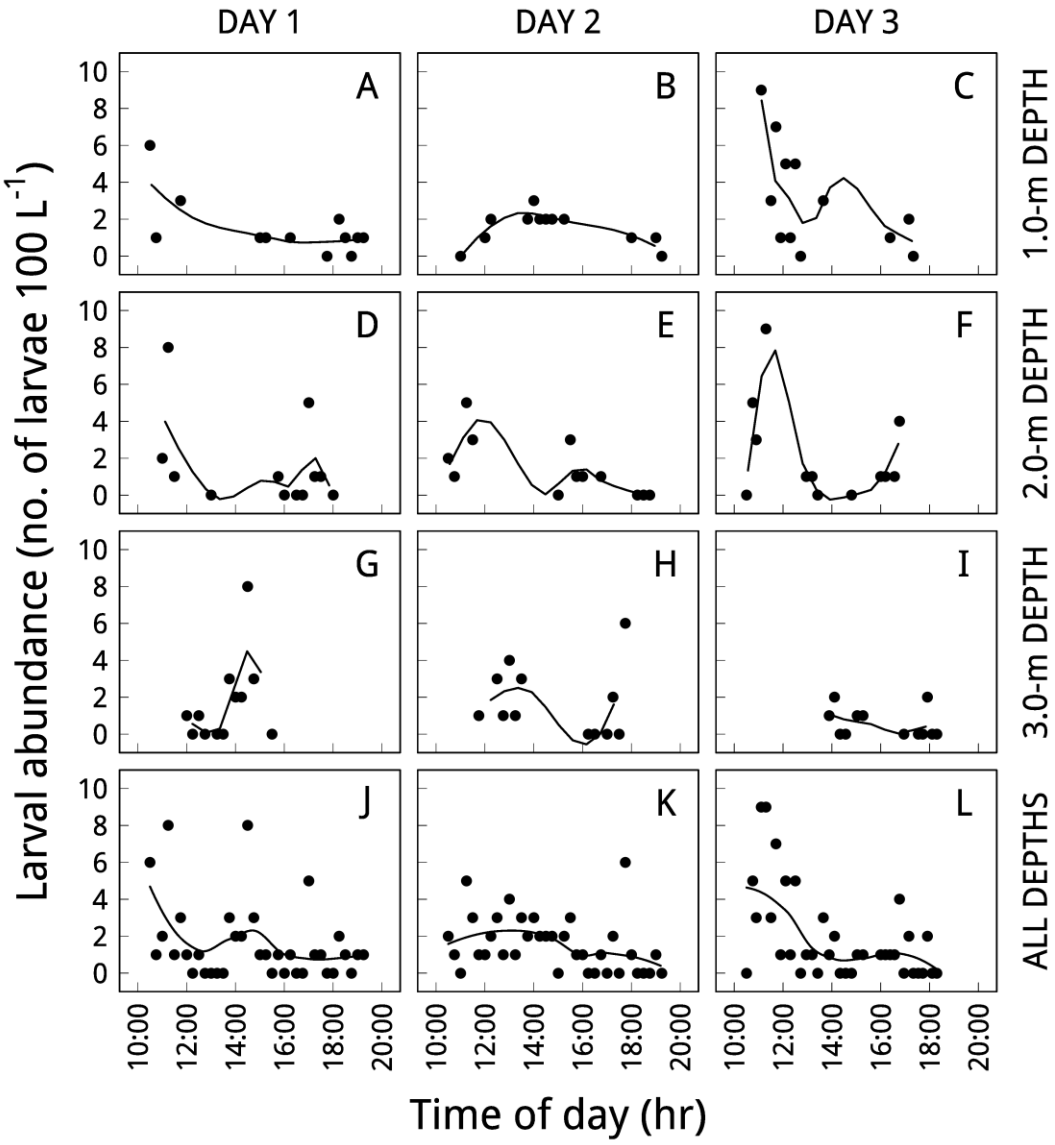
Taxon	Source	First deployment period (July)						Second deployment period (August)					
		GLMM		MCMC GLMM				GLMM		MCMC GLMM			
		Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI	Variance (mean)	VC (%)	Variance (mean)	VC (%)	Lower 95% CI	Upper 95% CI
Bryozoa, Gymnolaemata													
Membranipora membranacea	*Sector*	< 0.1	< 0.1	1.6	4.6	< 0.1	8.2	**14.6**	**88.2**	**70.4**	**96.9**	**< 0.1**	**277.5**
	Location	**98.2**	**99.0**	**33.4**	**94.5**	**2.0**	**72.4**	0	0	< 0.1	0.1	< 0.1	0.1
	Site	0	0	< 0.1	0.1	< 0.1	0.0	0.9	5.7	0.1	0.1	< 0.1	0.1
	Station	1.0	1.0	0.3	0.8	< 0.1	1.7	1.0	6.1	2.1	2.9	< 0.1	7.8
Schizoporella sp.	*Sector*							0	0	**0.1**	**20.3**	**< 0.1**	**0.1**
	Location							**< 0.1**	**< 0.1**	< 0.1	1.2	< 0.1	0.0
	Site							0	0	< 0.1	0.6	< 0.1	0.0
	Station							1.0	100.0	0.5	77.9	< 0.1	5.5
Chordata, Ascidiacea													
Botrylloides violaceus	*Sector*	14.5	8.0	36.7	20.7	< 0.1	192.3	17.0	9.2	6.3	26.5	< 0.1	29.9
	Location	**166.5**	**91.4**	**140.7**	**79.1**	**1.6**	**393.2**	**166.4**	**89.9**	**16.7**	**69.9**	**0.6**	**40.2**
	Site	0.2	0.1	0.1	< 0.1	< 0.1	0.3	0.7	0.4	0.8	3.5	0.2	1.8
	Station	1.0	0.5	0.3	0.2	0.1	0.6	1.0	0.5	0.0	0.1	< 0.1	0.1
Botryllus schlosseri	*Sector*	**7.5**	**57.6**	**16.6**	**73.0**	**< 0.1**	**49.1**	**3.7**	**56.7**	**5.8**	**75.1**	**0.9**	**14.5**
	Location	2.5	18.9	3.3	14.6	< 0.1	9.0	0.8	12.5	0.6	7.3	< 0.1	1.8
	Site	2.1	15.8	2.3	10.2	0.9	4.6	1.0	15.3	1.2	15.4	0.5	1.9
	Station	1.0	7.7	0.5	2.2	0.3	0.7	1.0	15.5	0.2	2.3	0.1	0.2
Ciona intestinalis	*Sector*	**2.1**	**57.0**	**0.8**	**39.6**	**< 0.1**	**4.3**	0	0	< 0.1	6.3	< 0.1	< 0.1
	Location	0.6	15.3	0.3	13.1	< 0.1	1.9	0	0	< 0.1	5.2	< 0.1	< 0.1
	Site	0	0	0.7	33.4	< 0.1	6.5	**< 0.1**	**< 0.1**	**< 0.1**	**6.9**	**< 0.1**	**< 0.0**
	Station	1.0	27.7	0.3	14.0	< 0.1	1.7	1.0	100.0	< 0.1	81.6	< 0.1	0.1
Molgula manhattensis	*Sector*	0	0	**83.5**	**95.8**	**< 0.1**	**272.1**	< 0.1	< 0.1	**11.6**	**42.7**	**< 0.1**	**46.7**
	Location	0	0	2.9	3.4	< 0.1	17.4	8.6	27.3	7.5	27.7	< 0.1	39.3
	Site	**< 0.1**	**< 0.1**	0.2	0.3	< 0.1	1.9	**21.8**	**69.5**	7.2	26.7	< 0.1	17.8
	Station	1.0	100.0	0.5	0.6	< 0.1	2.3	1.0	3.2	0.8	2.9	< 0.1	2.3



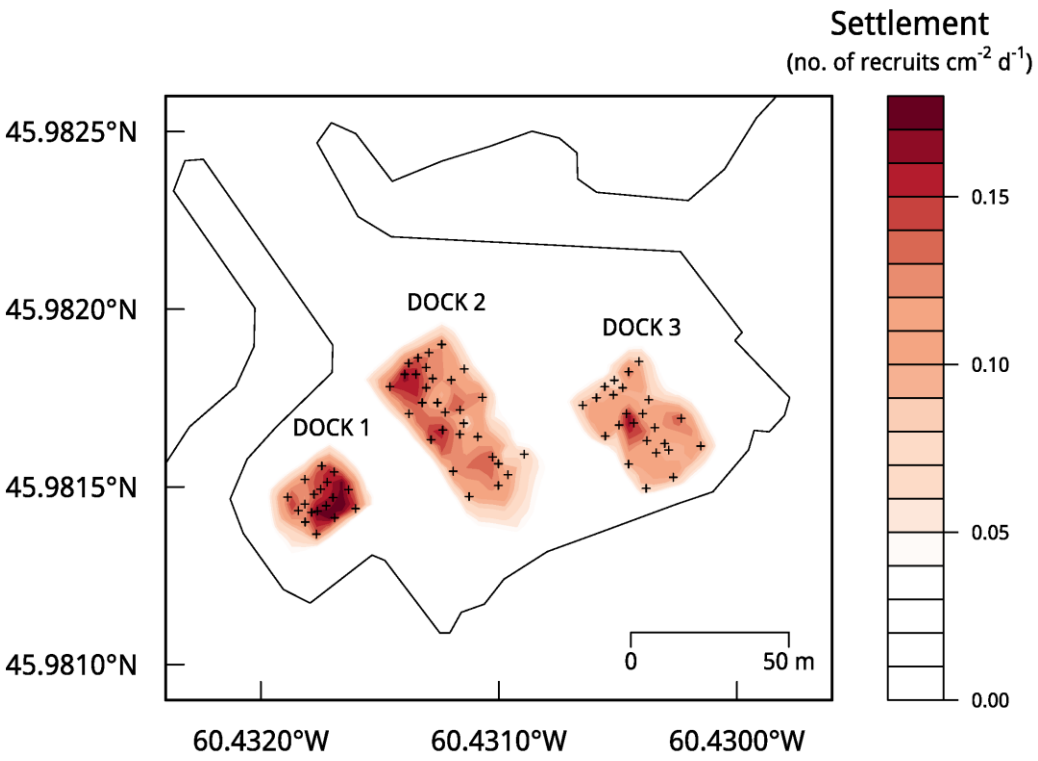
Supplemental Figure S1 — An example of a 14.6 × 14.6 cm PVC settlement plate suspended horizontally in the water column from a floating dock structure with polyethylene rope.



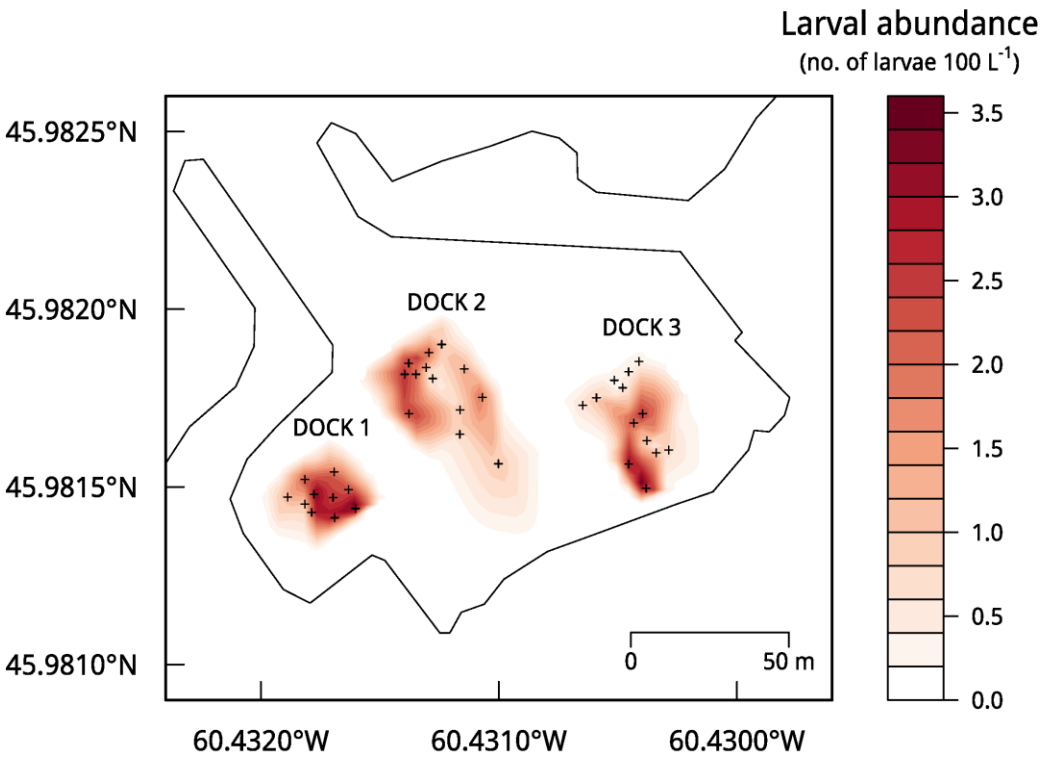
Supplemental Figure S2 — *Botryllus schlosseri* larval abundance as a function of time of day; solid line = local regression (LOESS, span = 0.7) curve to show temporal trends in larval abundance (data pooled from all three depths and from all three consecutive days of sampling; N = 108); dashed line = 95% confidence limits of the LOESS curve.



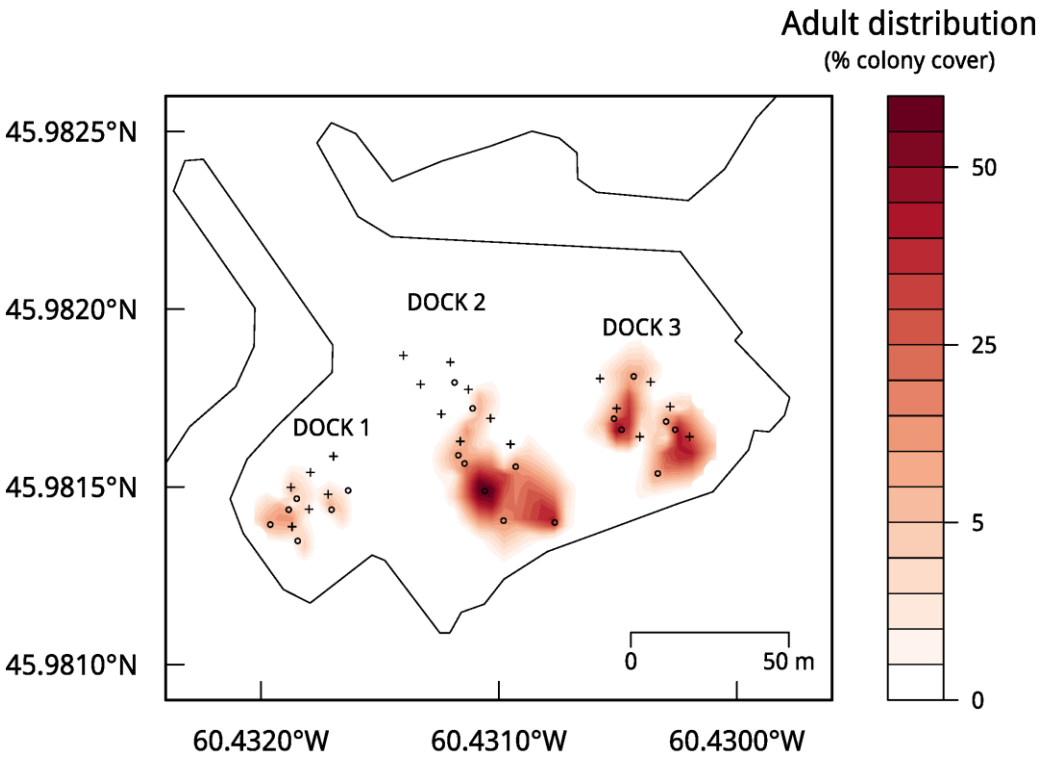
Supplemental Figure S3 — *Botryllus schlosseri* larval abundance as a function of time of day for different days (Day 1, 2, and 3) and for different depths (1.0 m, 2.0 m, 3.0 m, and all depths); solid line = local regression (LOESS, span = 0.7) curve to show temporal trends in larval abundance.



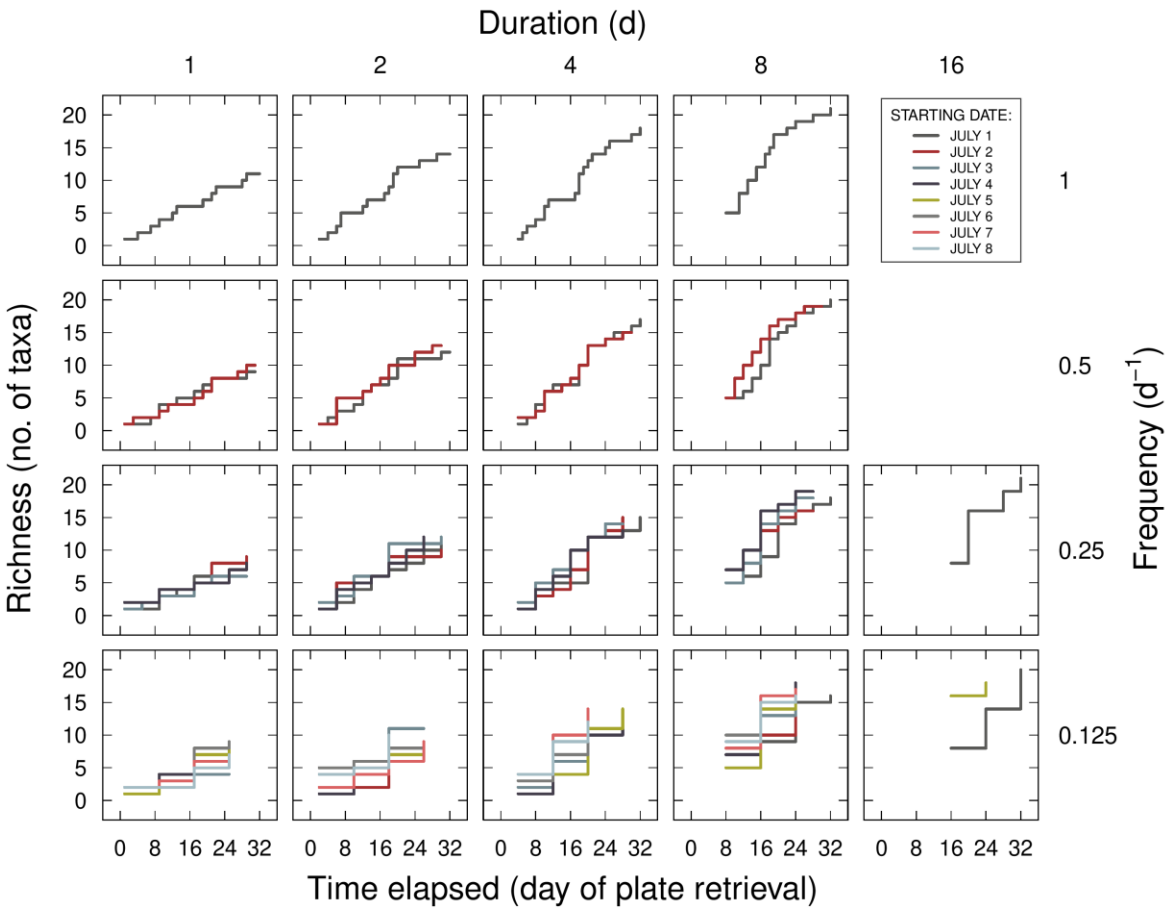
Supplemental Figure S4 — Surface distribution of depth-corrected settlement rates of *Botryllus schlosseri* inside the marina in Ben Eoin, Nova Scotia, Canada. N = 72 sampling stations (+).



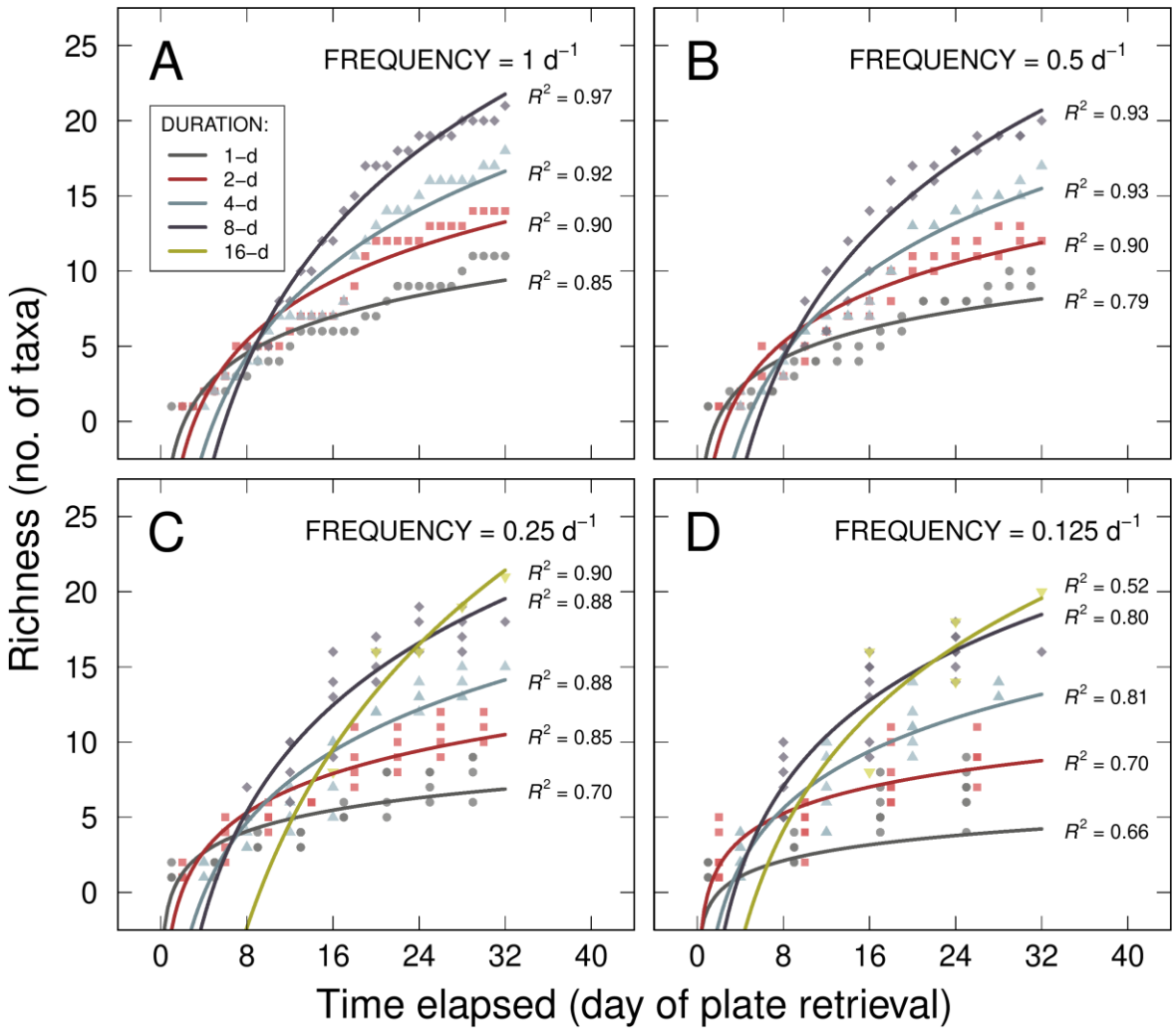
Supplemental Figure S5 — Distribution of larval abundance of *Botryllus schlosseri* (mean over three days) inside the marina in Ben Eoin, Nova Scotia, Canada. N = 36 sampling stations (+).



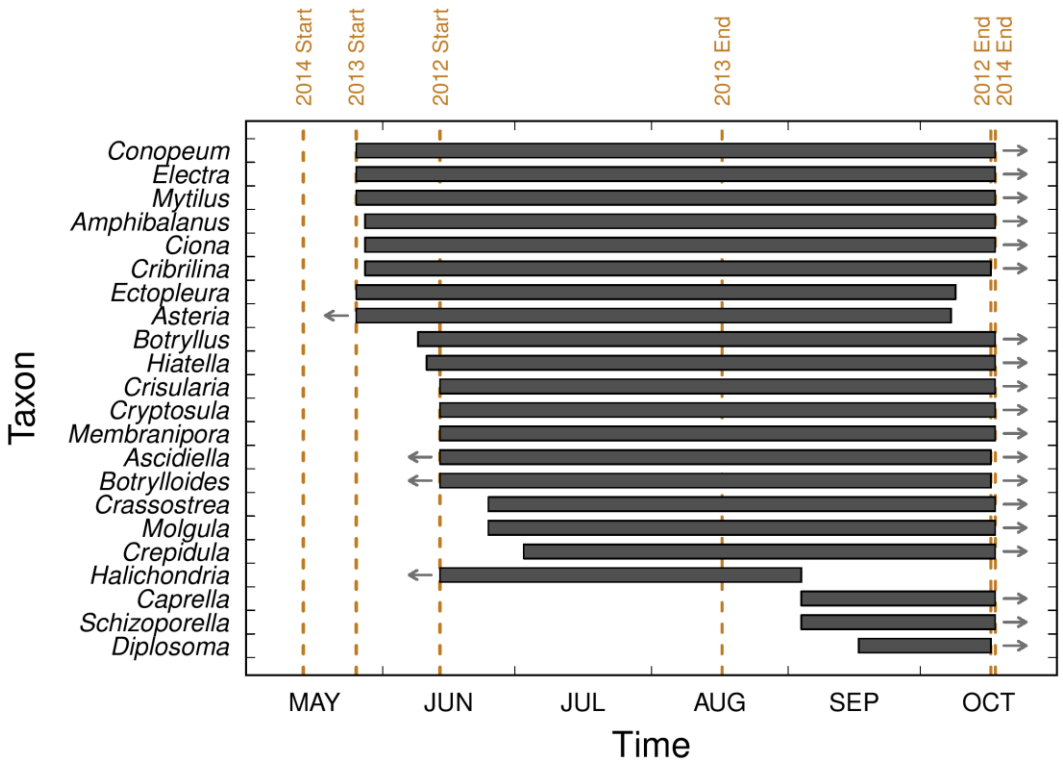
Supplemental Figure S6 — Surface distribution of adult colonies of *Botryllus schlosseri* (semi-quantitative values of percent cover on surveyed dock structures and berthed boats) inside the marina in Ben Eoin, Nova Scotia, Canada. N = 20 dock sections (+); N = recreational boats (○).



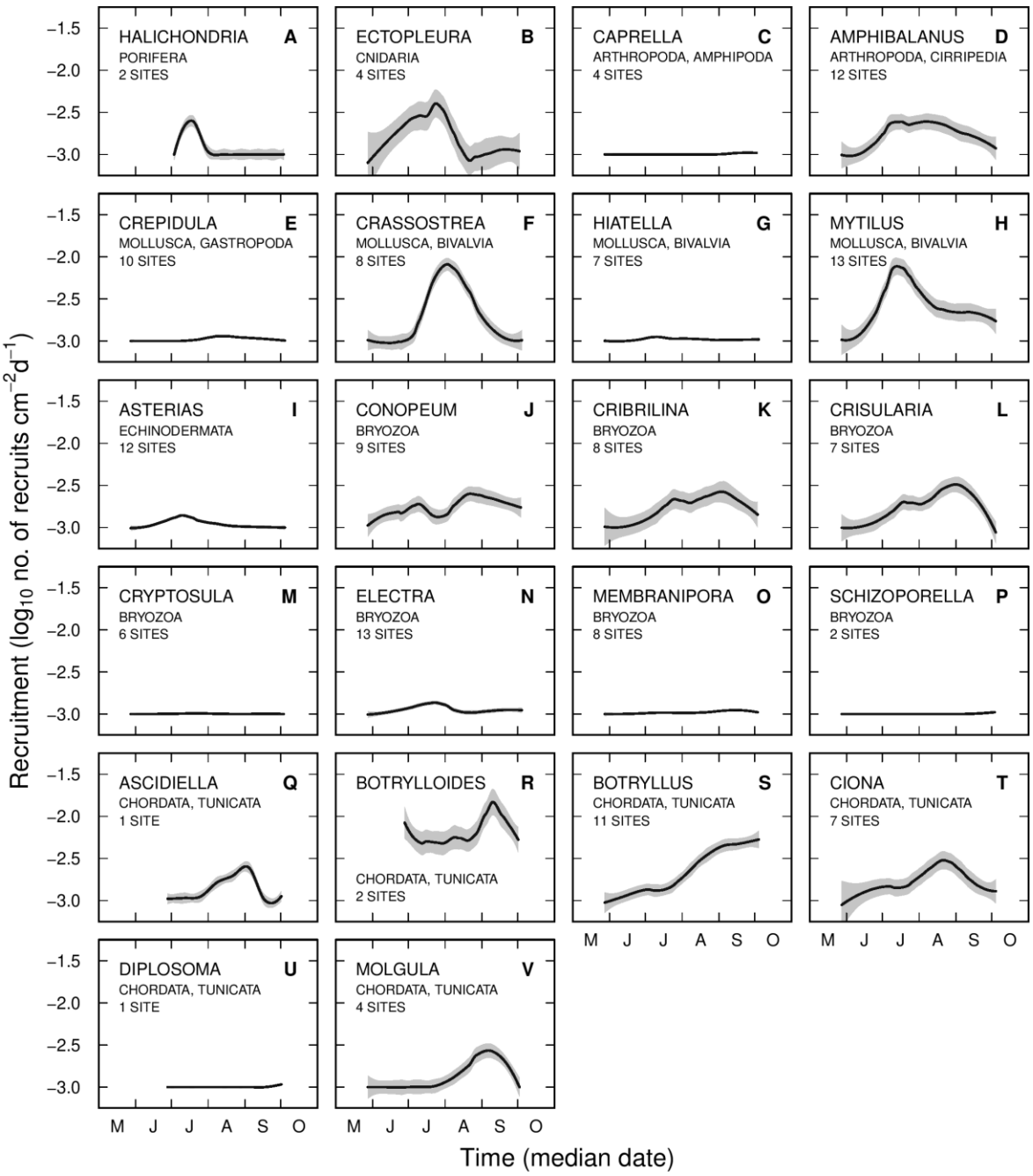
Supplemental Figure S7 — Richness as a function of time elapsed for 18 different sampling strategies, a combination of sampling duration (1, 2, 4, 8, or 16 d) and sampling frequency (1, 0.5, 0.25, or 0.125 d^{-1}).



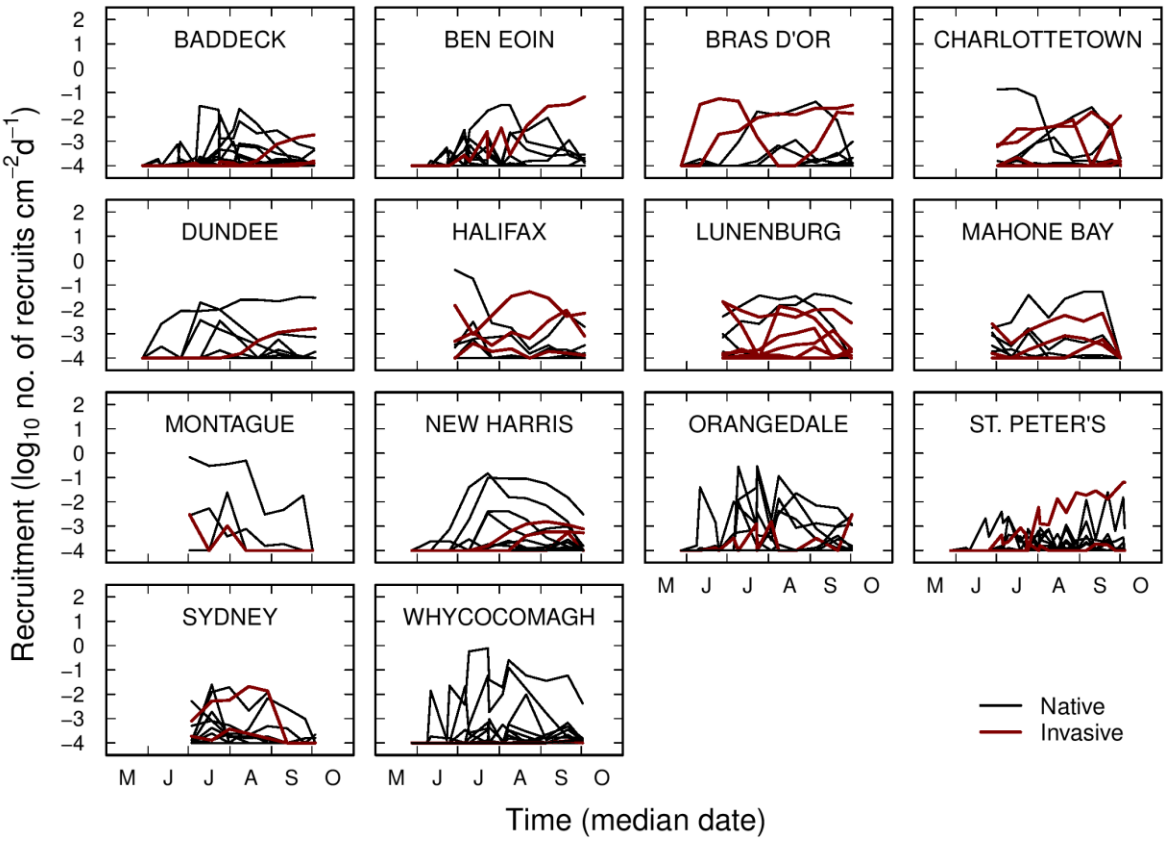
Supplemental Figure S8 — Logarithmic regressions of richness and time elapsed for 18 different sampling strategies, a combination of sampling duration (1, 2, 4, 8, or 16 d) and sampling frequency. Frequencies: (A) 1 d^{-1}; (B) 0.5 d^{-1}; (C) 0.25 d^{-1}; (D) 0.125 d^{-1}.



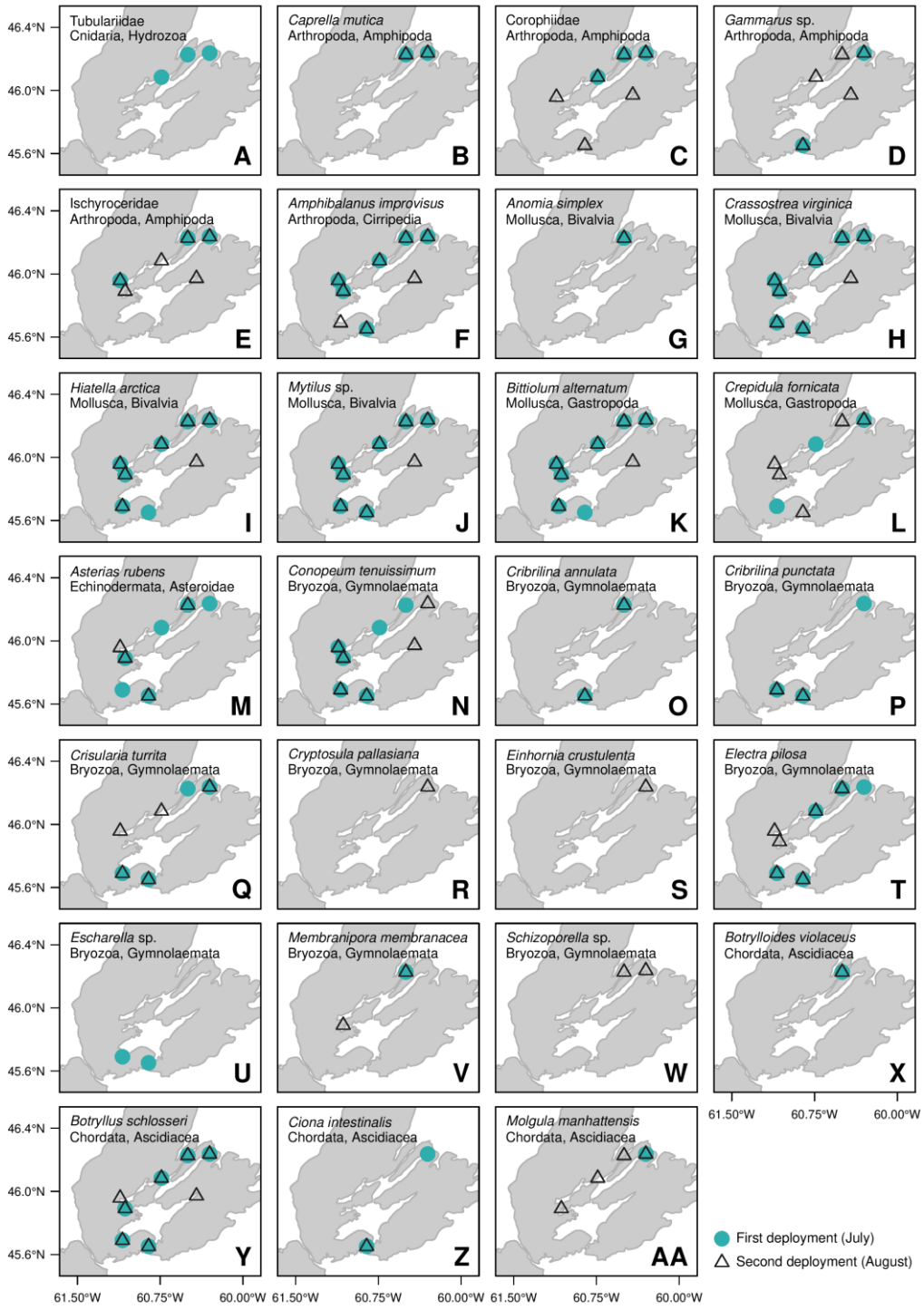
Supplemental Figure S9 — Overall seasonal window of recruitment (site- and year-integrated) as determined with settlement plates for 22 marine benthic taxa (taxa are referred to by their genus) ranked from top to bottom by the length of the seasonal window. Gray arrows indicate uncertainty of the seasonal onset and end of recruitment. Dashed orange lines indicate the start and end of the sampling season in 2012, 2013, and 2014.



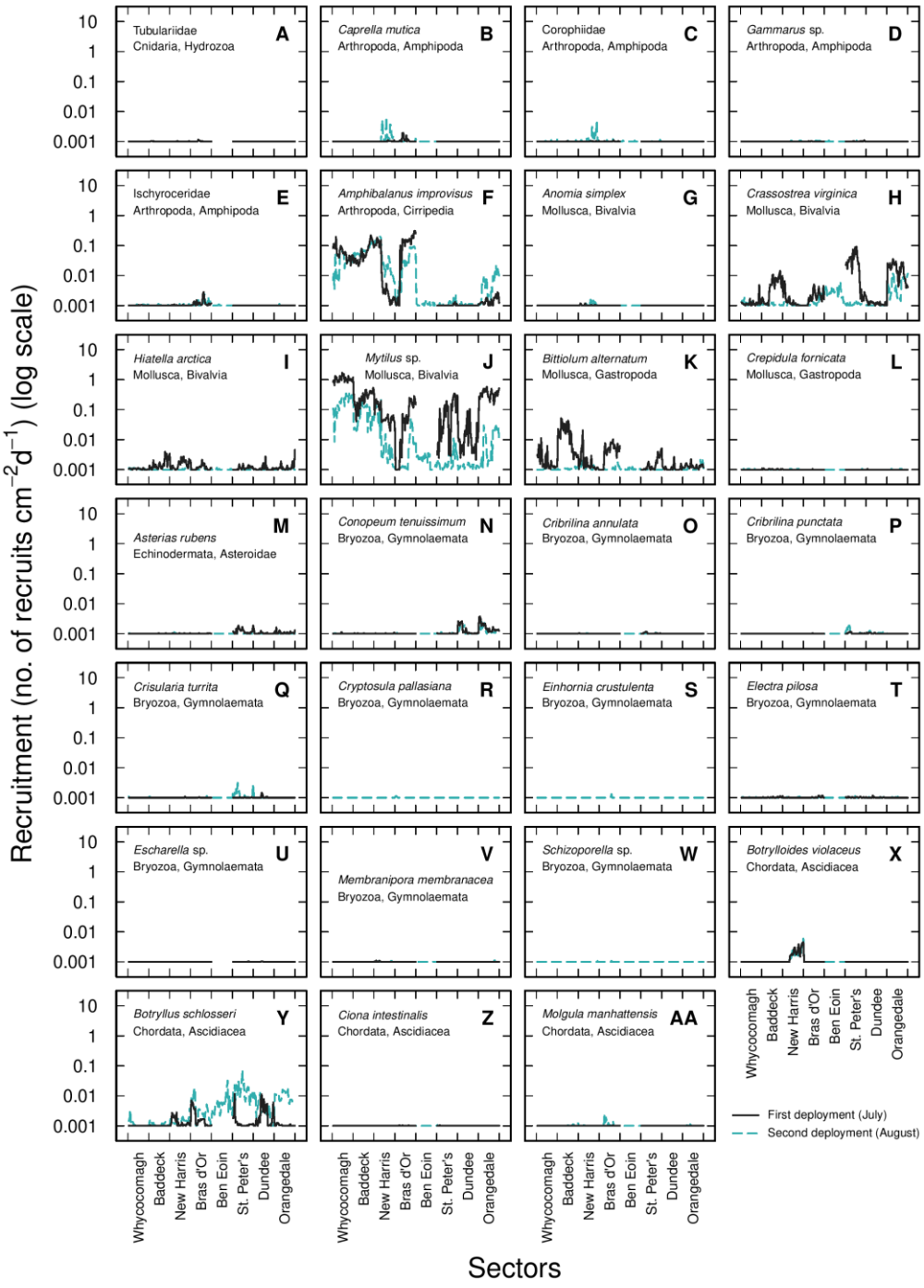
Supplemental Figure S10 — Seasonal *de novo* recruitment patterns for 22 marine benthic taxa (taxa are referred to by their genus; ordered by their higher taxonomic rank) detected on settlement plates: (A) *Halichondria panicea*; (B) *Ectopleura larynx*; (C) *Caprella mutica*; (D) *Amphibalanus improvisus*; (E) *Crepidula fornicata*; (F) *Crassostrea virginica*; (G) *Hiatella arctica*; (H) *Mytilus* sp.; (I) *Asterias rubens*; (J) *Conopeum tenuissimum*; (K) *Cribrilina annulata*; (L) *Crisularia turrita*; (M) *Cryptosula pallasiana*; (N) *Electra pilosa*; (O) *Membranipora membranacea*; (P) *Schizoporella* sp.; (Q) *Ascidiella aspersa*; (R) *Botrylloides violaceus*; (S) *Botryllus schlosseri*; (T) *Ciona intestinalis*; (U) *Diplosoma listerianum*; (V) *Molgula manhattensis*. Solid black line = local regression (LOESS, span = 0.5) fitted to $\log_{10}(x + 0.001)$ transformed recruitment rates. Gray shaded area = 95% confidence limits of the LOESS curve.



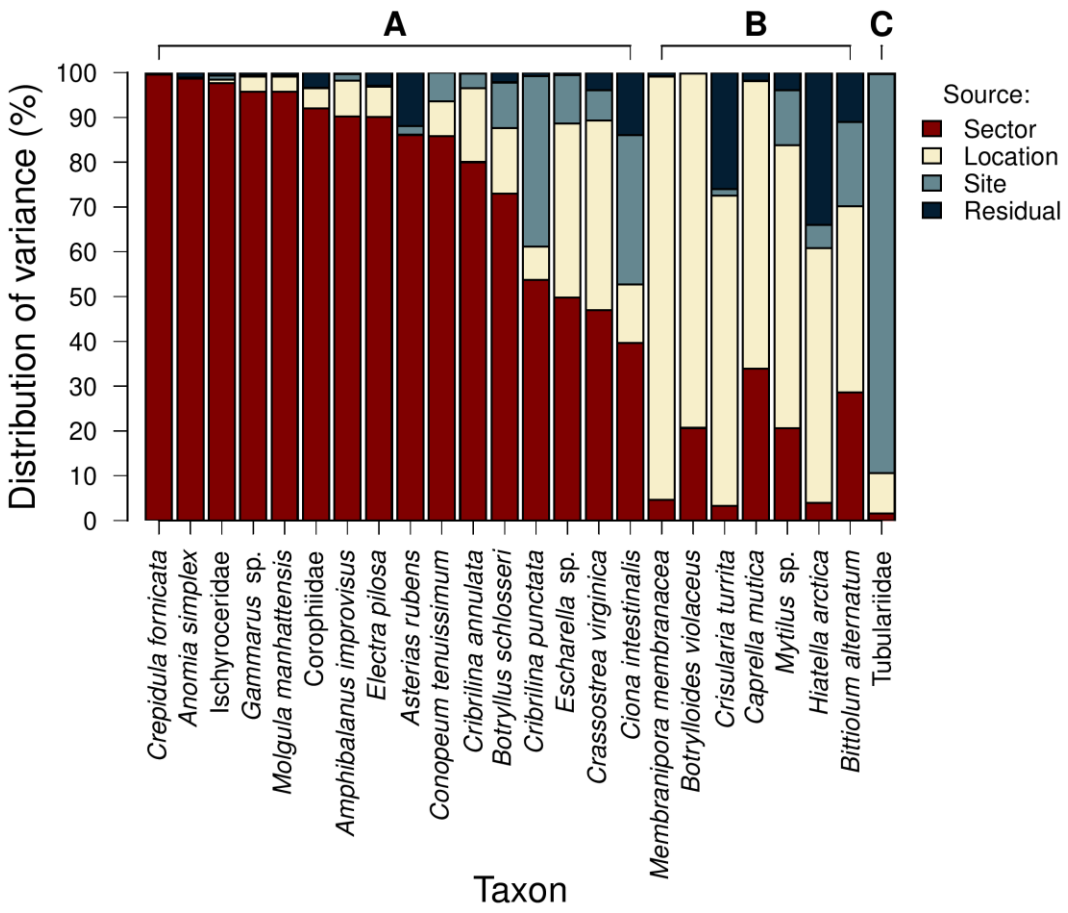
Supplemental Figure S11 — Seasonal *de novo* recruitment patterns on settlement plates for 15 native (solid black lines) and seven invasive (solid red lines) species at each site. Recruitment rates were $\log_{10}(x + 0.0001)$ transformed.



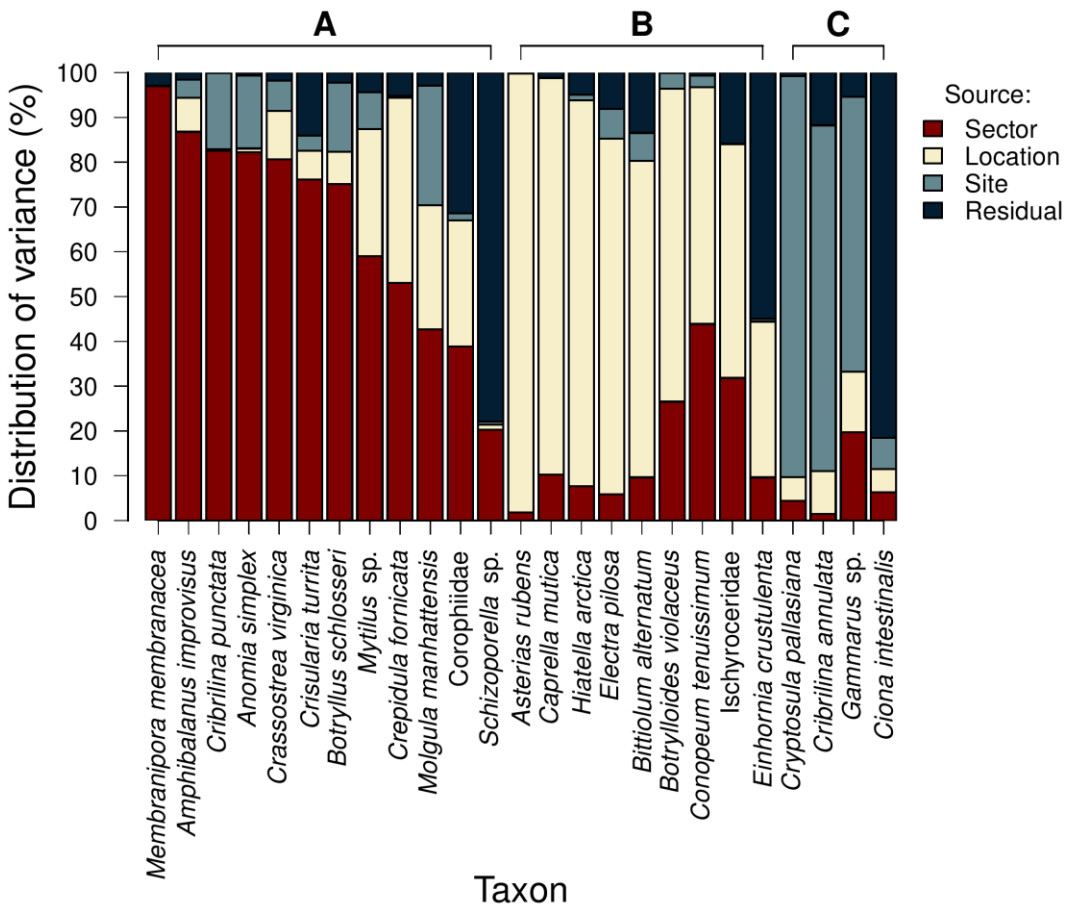
Supplemental Figure S12 — Coarse scale (sector-level) distribution of 27 marine benthic taxa in the Bras d'Or Lake, Nova Scotia, Canada. (A) Tubulariidae; (B) *Caprella mutica*; (C) Corophiidae; (D) *Gammarus* sp.; (E) Ischyroceridae; (F) *Amphibalanus improvisus*; (G) *Anomia simplex*; (H) *Crassostrea virginica*; (I) *Hiatella arctica*; (J) *Mytilus* sp.; (K) *Bittiolum alternatum*; (L) *Crepidula fornicata*; (M) *Asterias rubens*; (N) *Conopeum tenuissimum*; (O) *Cribrilina annulata*; (P) *Cribrilina punctata*; (Q) *Crisularia turrita*; (R) *Cryptosula pallasiana*; (S) *Einhornia crustulenta*; (T) *Electra pilosa*; (U) *Escharella* sp.; (V) *Membranipora membranacea*; (W) *Schizoporella* sp.; (X) *Botrylloides violaceus*; (Y) *Botryllus schlosseri*; (Z) *Ciona intestinalis*; (AA) *Molgula manhattensis*.



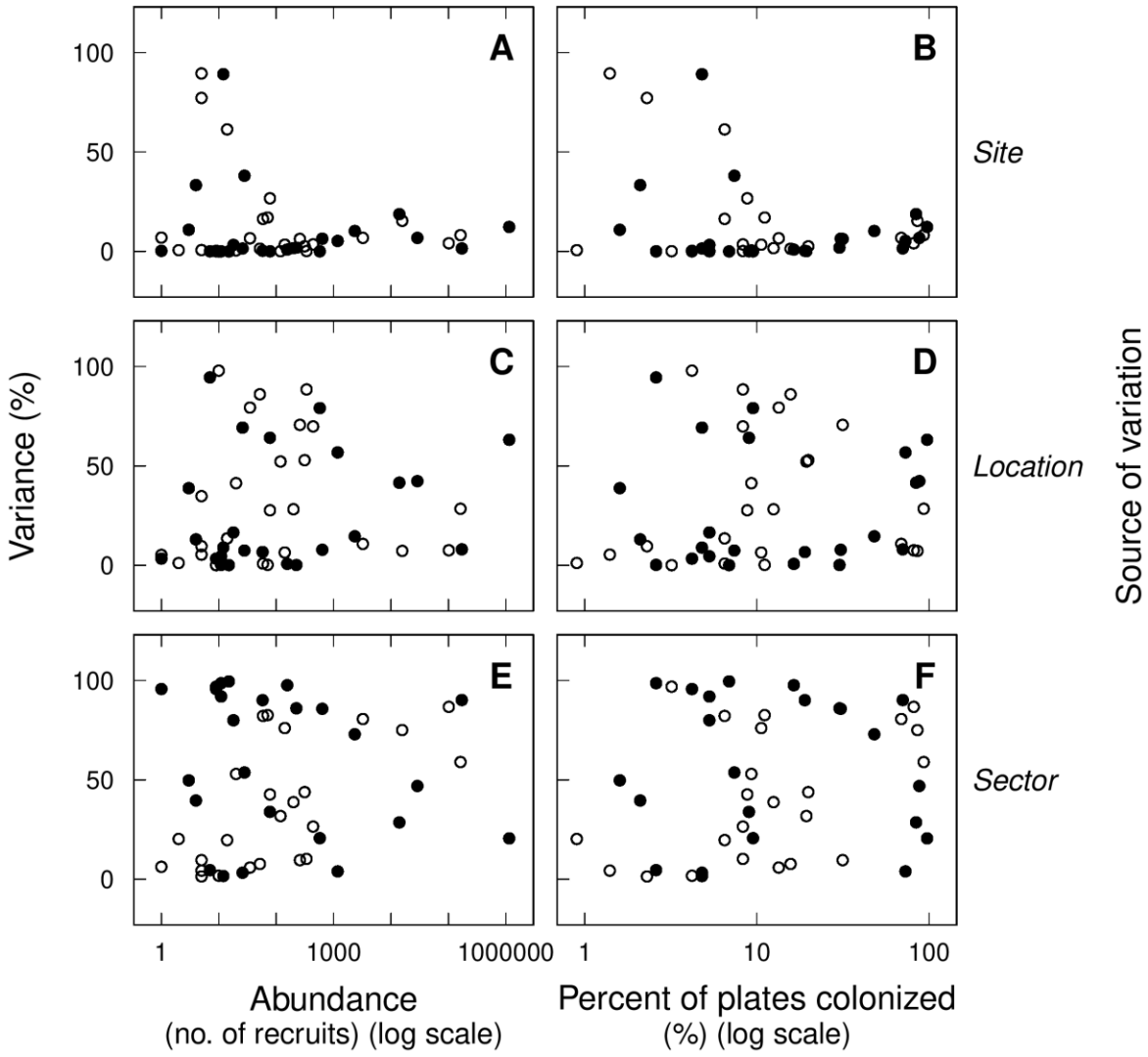
Supplemental Figure S13 — Recruitment rates of 27 marine benthic taxa—the sampling station order indicates sampling order. Data were $\log_{10}(x + 0.001)$ transformed. N = 189 buoy stations during the first deployment period in July (solid line) and N = 216 during the second deployment in August (dashed line). (A) Tubulariidae; (B) *Caprella mutica*; (C) Corophiidae; (D) *Gammarus* sp.; (E) Ischyroceridae; (F) *Amphibalanus improvisus*; (G) *Anomia simplex*; (H) *Crassostrea virginica*; (I) *Hiatella arctica*; (J) *Mytilus* sp.; (K) *Bittiolum alternatum*; (L) *Crepidula fornicata*; (M) *Asterias rubens*; (N) *Conopeum tenuissimum*; (O) *Cribrilina annulata*; (P) *Cribrilina punctata*; (Q) *Crisularia turrita*; (R) *Cryptosula pallasiana*; (S) *Einhornia crustulenta*; (T) *Electra pilosa*; (U) *Escharella* sp.; (V) *Membranipora membranacea*; (W) *Schizoporella* sp.; (X) *Botrylloides violaceus*; (Y) *Botryllus schlosseri*; (Z) *Ciona intestinalis*; (AA) *Molgula manhattensis*.



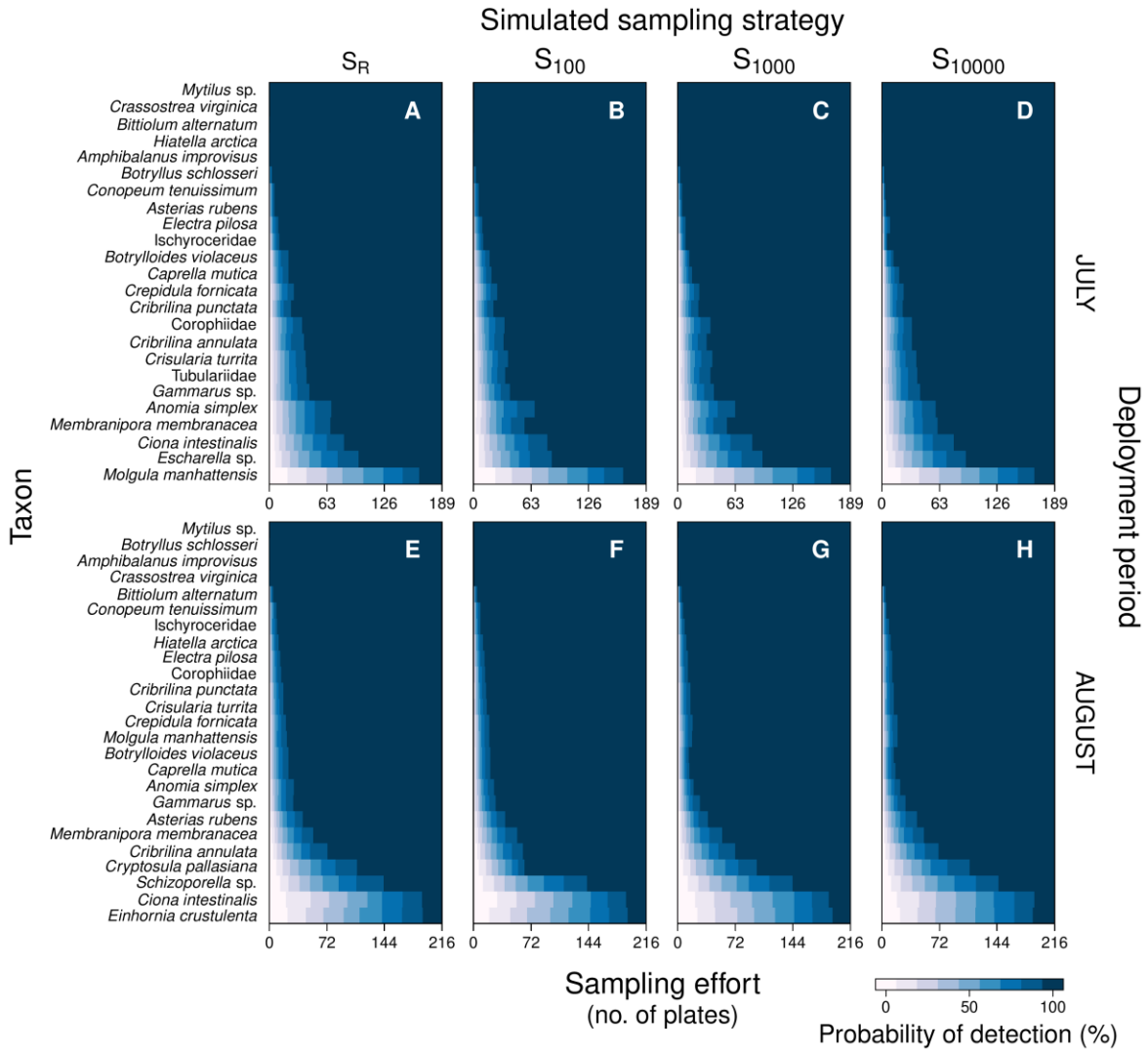
Supplemental Figure S14 — Distribution of variance in recruitment during the first deployment period in July estimated with Markov chain Monte Carlo generalized linear mixed model (MCMC GLMM) for 24 marine benthic taxa. Taxa are grouped by the source (excluding the residual error term) that was associated with the most variance: (A) *Sector* (10000s of m; n = 16 taxa); (B) *Location* (1000s of m; n = 7); (C) *Site* (100s of m; n = 1).



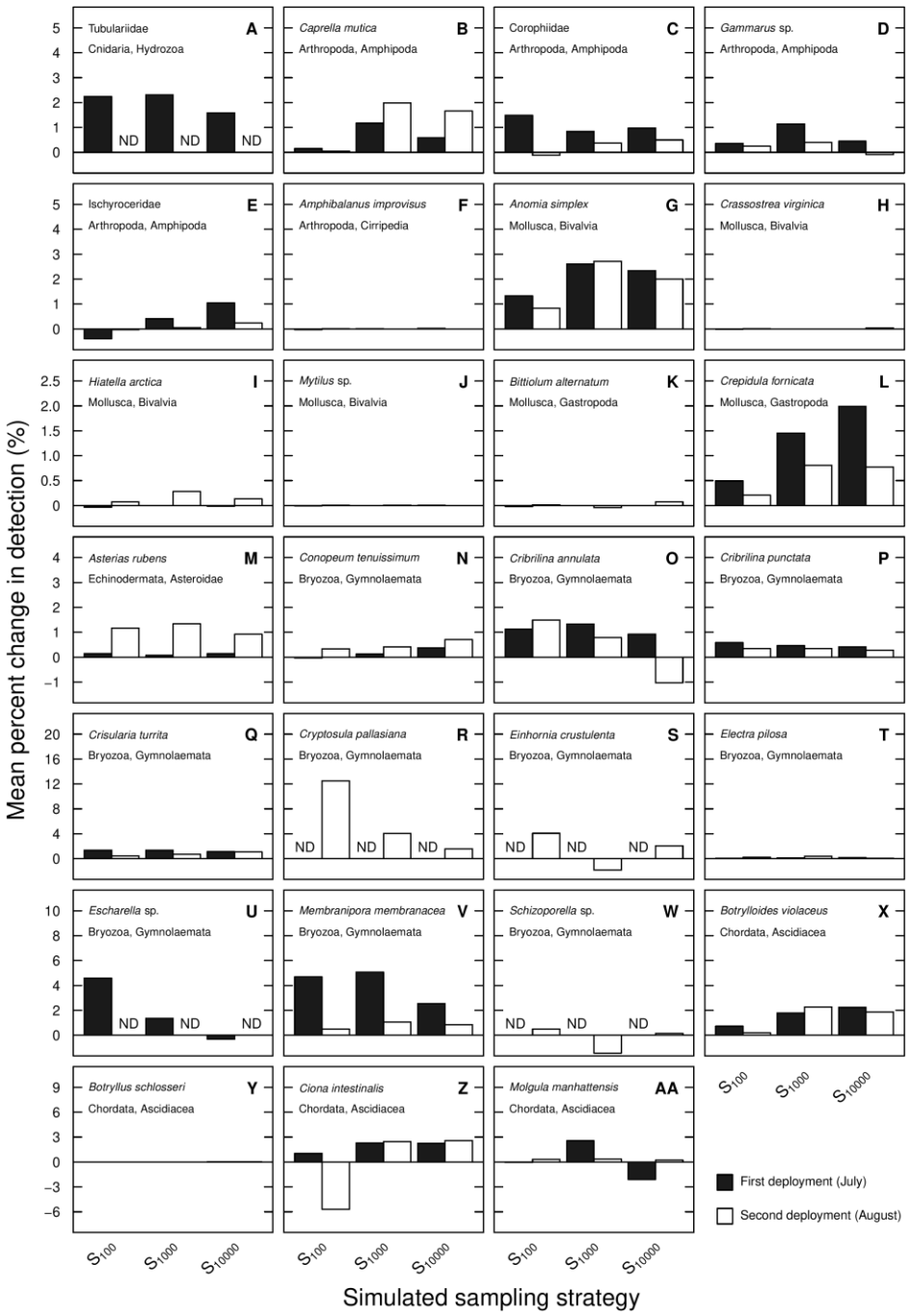
Supplemental Figure S15 — Distribution of variance in recruitment during the first deployment period in August estimated with Markov chain Monte Carlo generalized linear mixed model (MCMC GLMM) for 25 marine benthic taxa. Taxa are grouped by the source (excluding the residual error term) that was associated with the most variance: (A) *Sector* (10000s of m; n = 12 taxa); (B) *Location* (1000s of m; n = 9); (C) *Site* (100s of m; n = 4).



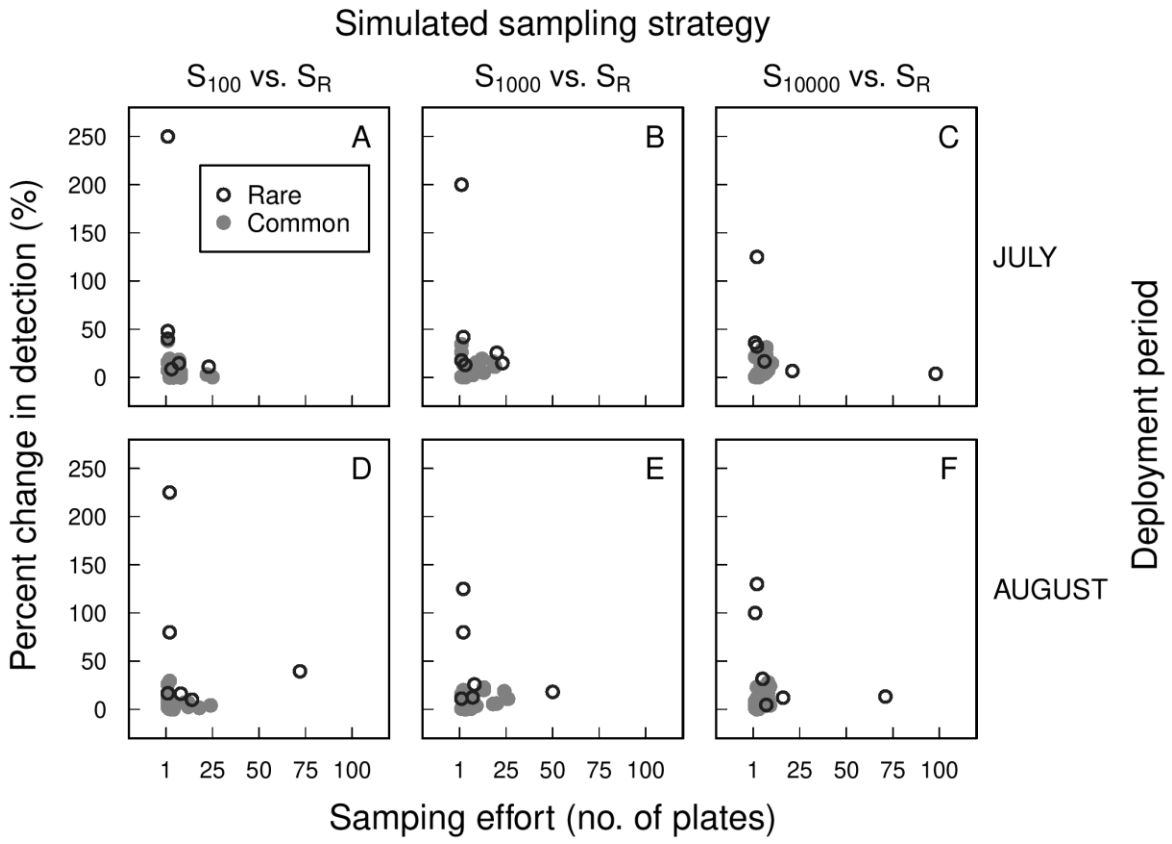
Supplemental Figure S16 — Relationship between variance in recruitment rates and $\log_{10}$ (x) transformed abundance data and between variance and $\log_{10}$ (x) transformed proportion of plates colonized for different source of variation: (A, B) *Site*, (C, D) *Location*, and (E, F) *Sector*. Abundance = total number of recruits counted. N = 24 marine benthic taxa during the first deployment period in July (solid points; ●) and N = 25 taxa during the second period in August (open points; ○). No significant relationships were found.



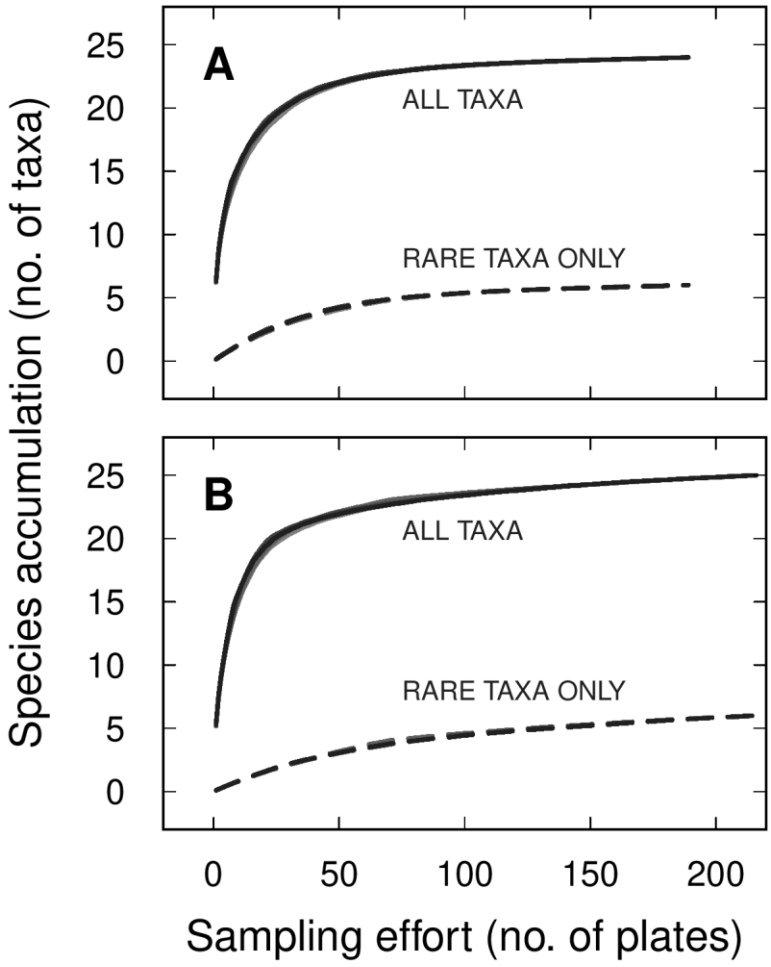
Supplemental Figure S17 — Probability of detection estimated from a bootstrap procedure on presence and absence data as a function of sampling effort under different simulated sampling strategies. (A–D) N = 24 marine benthic taxa during the first deployment period in July and (E–H) N = 25 taxa during the second deployment in August. Taxa are ordered from the most widespread and abundance (top) to the rarest (bottom). (A, E) Random sampling (S_R); (B, F) adaptive sampling by forcing sampling at scales ≥ 100s of m (S_{100}); (C, G) forcing sampling at scales ≥ 1000s of m (S_{1000}); (D, H) forcing sampling at scales = 10000s of m (S_{10000}).



Supplemental Figure S18 — Evaluation of sampling strategies based on the mean percent change (mean for all 189 and 216 sampling conditions under different sampling effort in July and August, respectively) in the probability of detection (POD) for 27 marine benthic taxa. Percent change in POD from random sampling (S_R) was computed for small-scale (S_{100}), intermediate-scale (S_{1000}), and large-scale (S_{10000}) simulated sampling strategies. (A) Tubulariidae; (B) *Caprella mutica*; (C) Corophiidae; (D) *Gammarus* sp.; (E) Ischyroceridae; (F) *Amphibalanus improvisus*; (G) *Anomia simplex*; (H) *Crassostrea virginica*; (I) *Hiatella arctica*; (J) *Mytilus* sp.; (K) *Bittiolum alternatum*; (L) *Crepidula fornicata*; (M) *Asterias rubens*; (N) *Conopeum tenuissimum*; (O) *Cribrilina annulata*; (P) *Cribrilina punctata*; (Q) *Crisularia turrita*; (R) *Cryptosula pallasiana*; (S) *Einhornia crustulenta*; (T) *Electra pilosa*; (U) *Escharella* sp.; (V) *Membranipora membranacea*; (W) *Schizoporella* sp.; (X) *Botrylloides violaceus*; (Y) *Botryllus schlosseri*; (Z) *Ciona intestinalis*; (AA) *Molgula manhattensis*. ND = no data.



Supplemental Figure S19 — Maximal percent change in the probability of species detection and the required sampling effort for the upper 75% of the most abundant taxa (i.e., common taxa; closed circles) and the bottom 25% of the taxa in terms of abundance (i.e., rare taxa; open circles). (A–C) N = 24 marine benthic taxa during the first deployment period in July and (D–F) N = 25 taxa during the second deployment in August. (A, D) Percent change from random sampling (S_R) to adaptive sampling by forcing sampling at scales ≥ 100s of m (S_{100}); (B, E) percent change from S_R to forcing sampling at scales ≥ 1000s of m (S_{1000}); (C, F) percent change from S_R to forcing sampling at scales = 10000s of m (S_{10000}).



Supplemental Figure S20 — Species accumulation curves of marine benthic taxa estimated from a bootstrap procedure on presence and absence data as a function of sampling effort under different simulated sampling strategies (S_R, S_{100}, S_{1000}, and S_{10000}). (A) Species accumulation during the first deployment period in July; (B) species accumulation during the second deployment period in August. Each of the four "all taxa" curve (solid lines [—]) consisted of N = 24 marine benthic taxa in July and N = 25 taxa in August. Each of the four "rare taxa only" curve (dashed lines [---]) consisted of the top 25% of the rarest taxa in the benthic assemblage.

Appendix 2. Published journal articles

Attached to this appendix are articles that appeared in (1) open access journals published by the Regional Euro-Asian Biological Invasions Centre (REABIC) where the authors retain the copyright of their articles and (2) a subscription journal published by Elsevier B.V. where authors retain the right to share their article for personal and scholarly purposes. The attached copies are provided for non-commercial research and educational use, and may be disseminated by the authors under the terms of the Creative Commons Attribution License (Attribution 4.0 International: CC-BY and CC-BY-NC-ND). These articles represent work complementing the research presented in this thesis. In chronological order of publication, the full citations of these contributions are as follows:

Moore AM, Vercaemer B, DiBacco C, Sephton D, Ma KCK. 2014. Invading Nova Scotia: first records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013. *BioInvasions Records* 3: 225–234.

Ma KCK, Simard N, Stewart-Clark SE, Bernier RY, Nadeau M, Willis J. 2016. Early detection of the non-indigenous colonial ascidian *Diplosoma listerianum* in eastern Canada and its implications for monitoring. *Management of Biological Invasions* 7: 365–374.

Ma KCK, Goodwin C, Cooper JA. 2018. Second record of *Diplosoma listerianum* (Milne-Edwards, 1841) five years after and 280 kilometres from the site of the first record in Nova Scotia. *BioInvasions Records* 7: 159–163.

Ma KCK, Hawk HL, Goodwin C, Simard N. 2019. Morphological identification of two invading ascidians: new records of *Ascidiella aspersa* (Müller, 1776) from Nova Scotia and *Diplosoma listerianum* (Milne-Edwards, 1841) from New Brunswick and Quebec. *BioInvasions Records* 8: 50–64.

Carman MR, Colarusso PD, Neckles HA, Bologna P, Caines S, Davidson JDP, Evans NT, Fox SE, Grunden DW, Hoffman S, Ma KCK, Matheson K, McKenzie CH, Nelson EP, Plaisted H, Reddington E, Schott S, Wong MC. 2019. Biogeographical patterns of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39º and

47º north latitude (New Jersey to Newfoundland). *Management of Biological Invasions* 10: 602–616.

Ma KCK, Glon HE, Hawk HL, Chapman CN. 2020. Reconstructing the distribution of the non-native sea anemone, *Diadumene lineata* (Actiniaria), in the Canadian Maritimes: Local extinction in New Brunswick and no regional range expansion in Nova Scotia since its initial detection. *Regional Studies in Marine Science* 34: 101049.

Article 1. Invading Nova Scotia: first records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013

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Research Article

Invading Nova Scotia: first records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013

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Abstract

Research and monitoring efforts led to the discovery of four new non-indigenous tunicates and one new non-indigenous anemone in Nova Scotia in 2012 and 2013. In this report, we summarize and interpret the circumstances leading up to and actions taken following the first Nova Scotian records of the high impact invasive tunicate *Didemnum vexillum* Kott, 2002 as well as the tunicates *Styela clava* Herdman, 1881, *Ascidiella aspersa* (Müller, 1776), and *Diplosoma listerianum* (Milne-Edwards, 1841) and the anemone *Diadumene lineata* (Verrill, 1869). While dockside research and monitoring were responsible for detections of four species, the possibility that *Didemnum vexillum* was present was first reported by a member of the public, through Fisheries and Oceans Canada's Aquatic Invasive Species Monitoring program.

Key words: *Styela clava*, *Ascidiella aspersa*, *Diplosoma listerianum*, *Diadumene lineata*, invasive species, Canada, monitoring

Introduction

Introductions and range expansions of non-indigenous marine invertebrates, especially tunicates, have caused substantial ecological impacts to fouling communities and economic impacts on bivalve aquaculture in the northeastern United States and Atlantic Canada (Blum et al. 2007; Dijkstra et al. 2007; Carman et al. 2010; Thompson and MacNair 2004; Lutz-Collins et al. 2009; McKindsey et al. 2007). Hull fouling is often considered the likeliest vector for the introduction of many non-native fouling species (Coutts et al. 2003), and Nova Scotia is particularly susceptible. Halifax, Nova Scotia was the top shipping arrival port in Atlantic Canada in 2000 and 2002 with 1215 (of 2061) and 836 (of 1225) international arrivals, respectively. Another 12 Nova Scotia ports received at least 3 visits in either of those years, including Port Hawkesbury with 80 in 2000 (Kelly 2004). Halifax and many other locations around the province, such as Lunenburg, also have high recreational boating traffic in the summer months. In addition, southern Nova Scotia and the Bay of Fundy are adjacent to and influenced by the prevailing oceanographic currents from the United States. They would, therefore, receive the greatest propagule pressure from established populations of non-indigenous species in eastern waters of the United States undergoing northward range expansions by natural processes.

In 2006, Fisheries and Oceans Canada (DFO) initiated a monitoring program to study the spread of aquatic invasive species (AIS) in Atlantic Canada and to detect and help mitigate new invasions. In Nova Scotia, a key aspect of the AIS Monitoring program targeted fishing harbours, marinas, and aquaculture leases for deployment of collector arrays to determine the presence of two invasive tunicate species of concern: *Didemnum vexillum* Kott, 2002 and *Styela clava* Herdman, 1881 and the extent of three other species already present in the province: *Ciona intestinalis* (Linnaeus, 1767), *Botryllus schlosseri* (Pallas, 1766), and *Botrylloides violaceus* Oka,

1927 (see Sephton et al. 2011 for details). In 2009, Locke published a "watch list" of 17 invasive tunicates likely to successfully invade Atlantic Canada (hereafter referred to as the Atlantic Canada watch list) but, until 2012, there were no detections of new non-indigenous tunicates in Nova Scotia.

Methods

In 2012, several new research projects aimed at monitoring the effect of AIS on the structure and function of native communities increased the overall monitoring effort in Nova Scotia. During 2012 and 2013, there were two AIS monitoring efforts in Nova Scotia, summarized here as AIS Monitoring and AIS Research.

AIS Monitoring

The DFO Maritimes Region AIS Monitoring program in Nova Scotia and southern New Brunswick deployed collector arrays in marinas, public wharves, and near aquaculture leases across Nova Scotia (Sephton et al. 2011). Annually since 2006 in late May/early June, four collector arrays, each consisting of three downward-facing 10 cm × 10 cm sanded PVC plates, are deployed from floating docks at approximately 1 m depth. In mid-August, two of these arrays are retrieved and replaced with new ones. All four remaining arrays are retrieved in late October. Upon retrieval, arrays are examined visually for presence and percent cover of invasive species and photographed for reference. In 2012 and 2013, arrays were deployed at 70 and 75 targeted monitoring stations, respectively, along approximately 7,000 km of coastline. At some stations, an inverted plastic saucer housing three Petri dishes was added to the collector arrays to increase material choice and surface area for invertebrate settlement.

In parallel with collector-based monitoring, DFO conducted rapid assessments in targeted areas using a combination of dockside, underwater, and boat-sampling techniques. Dockside techniques used were underwater video and visual surveys of docks, ropes, and underwater structures as well as water sampling for genetic analysis. Underwater techniques used were SCUBA and snorkel surveys of docks and underwater structures. Boat-sampling techniques used were visual inspection and underwater video of small buoys, channel markers, and their associated ropes and chains. Different combinations of these techniques were used in different situations to suit the purpose of the investigation: monitoring, rapid response, or population delineation.

The AIS monitoring program also informed harbour authorities, marina managers, fishermen, aquaculture site staff, the inhabitants of coastal communities, and the general public about non-indigenous species and when and how to report suspect specimens.

AIS Research

AIS Research in 2012 examined recruitment and fouling community succession in harbours with and without tunicates. There were two experimental designs:

1) Twenty sanded PVC collector plates (14.6 cm × 14.6 cm) were deployed horizontally from floating docks at 1 m depth at 16 sites in Nova Scotia from late May to late October. Every two weeks, non-indigenous tunicate settlers were counted and removed from 10 collector plates per site. All plates were then photographed for further analysis (i.e., percent-cover time series) and returned to the water.

2) A six-plate collector array was deployed every two weeks from floating docks at between 1 and 2 m depth at four sites in Nova Scotia starting in early June and ending in late October. After one month of exposure, collector arrays were retrieved and the plates were photographed for further analysis.

AIS Research in 2013 was on a smaller spatial scale, taking place on one dock in Halifax Harbour, but was more intense in terms of plates deployed (n = 50) and sampling intensity. It involved deploying collector plates at 1 m depth from the floating dock starting in mid-July. Plates were photographed weekly until late November.

Results

The combined monitoring and research programs detected three new non-indigenous tunicates in Nova Scotia in 2012: *Styela clava*, *Ascidiella aspersa* (Müller, 1776), and *Diplosoma listerianum* (Milne-Edwards, 1841). In 2013, a further two non-indigenous invertebrate species were identified: the tunicate *Didemnum vexillum* and the anemone *Diadumene lineata* (Verrill, 1869) (Figure 1 and Table 1). The following outlines methods that led to the species discoveries, actions taken post-discovery, the species' current status and extent in Nova Scotia as well as implications for future monitoring.

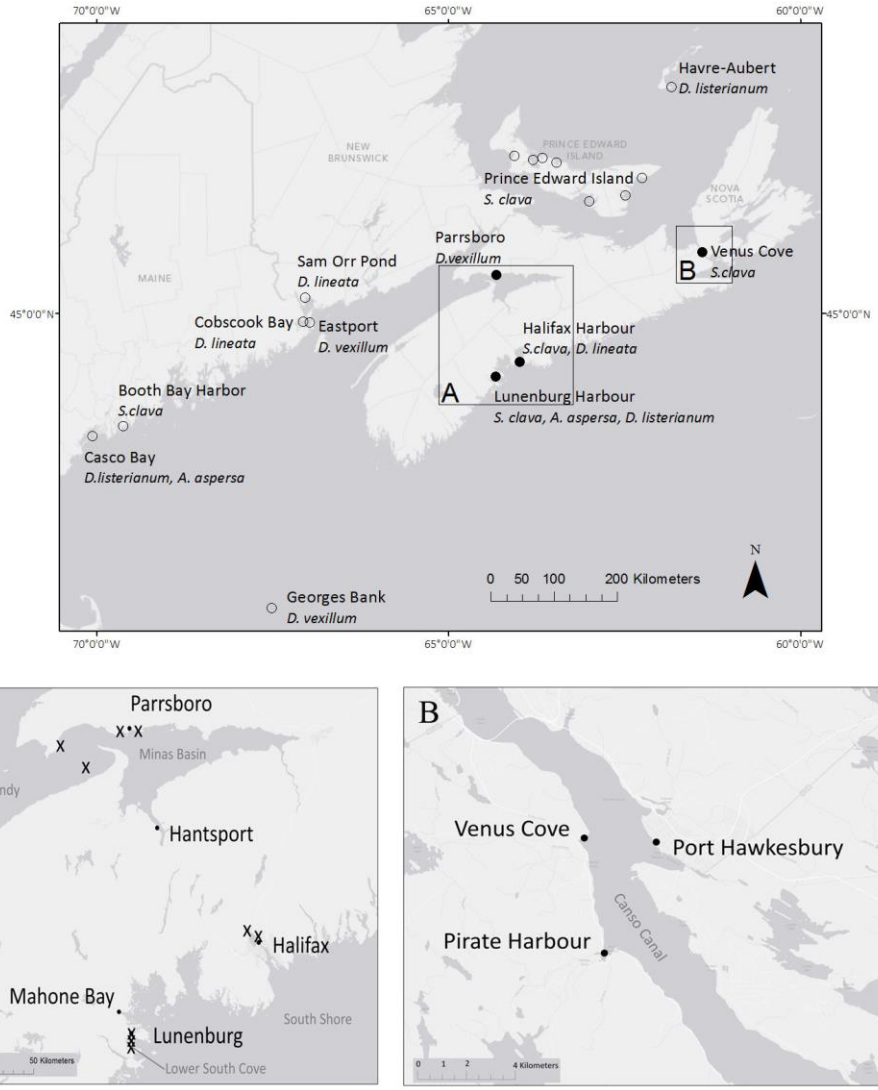


Figure 1. Map of central Atlantic Canada centred on Nova Scotia with confirmed locations of first records (solid circles) and nearest known records (open circles) of five non-indigenous invertebrate species: *Styela clava, Ascidiella aspersa*, *Diplosoma listerianum, Didemnum vexillum* and *Diadumene lineata*. A) Map of the Minas Basin and the South Shore of Nova Scotia. General locations of follow-up surveys are indicated with an x. B) Map of the portion of the Canso Canal where *S. clava* was observed.

Table 1. Summary table of the initial observation and identification of AIS in coastal Nova Scotian waters, including the exposed southern coast of Nova Scotia and Bay of Fundy

Species	Location	Lat.	Long.	Date Identified	Earliest Known Date Present	Substrate	Method[a]	Confirmation[b]
Styela clava	Lunenburg	44.3755	-64.3105	Oct 2012	Oct 2012	PVC plate	R	EV (specimen)
	Halifax	44.6810	-63.6109	Oct 2012	Oct 2012	PVC plate	M, R	EV (specimen)
	Pirate Harbour	45.5871	-61.3883	Jul 2012	Jul 2012	floating dock	A	unconfirmed
	Venus Cove	45.6153	-61.3901	Aug 2013	Aug 2013	wharf	M	EV (specimen)
Ascidiella aspersa	Lunenburg	44.3755	-64.3105	Oct 2012	Jul 2012	PVC plate	M, R	EV(photo)
Diplosoma listerianum	Lunenburg	44.3755	-64.3105	Oct 2012	Oct 2012	PVC plate	R	genetic
Didemnum vexillum	Parrsboro	45.3761	-64.2770	Oct 2013	Oct 2013	rock	A	genetic
Diadumene lineata	Halifax	44.6810	-63.6101	Feb 2014	Nov 2013	PVC plate	R	EV (photo)

[a]Method: R = research, M = monitoring, A = amateur
[b]Confirmation: EV = expert visual

Styela clava

Styela clava is a solitary, stalked, club-shaped tunicate with a brown, leathery tunic covered in tubercules. Sexually mature individuals typically range in size from 90 to 160 mm (See Millar 1970 for a full description). *S. clava* is indigenous to the NW Pacific Ocean but has invaded temperate waters worldwide including both North American coasts, the United Kingdom, Europe, Australia, and New Zealand. It has been present in New England since 1970 and was first documented in Atlantic Canada in 1998 in the Brudenell River, Prince Edward Island (Clarke and Therriault 2007). Since 1998, *S. clava* has spread to other estuaries on Prince Edward Island but had not been detected beyond that province's waters until 2012.

Styela clava was discovered on 2 October 2012 on collector plates in Lunenburg Harbour and in Halifax Harbour (Figure 1; Table 1). Specimens of *S. clava* were removed, preserved in ethanol, and positively identified. In both locations, only a few juvenile individuals of *S. clava* were observed, although one larger individual (36 mm in length) was present in Lunenburg Harbour (Figure 2). In response to these detections, DFO conducted surveys in Halifax Harbour Basin and in Lunenburg Harbour (Figure 1A) in November 2012 using snorkelling and small buoy inspections (Vercaemer et al. 2012; Vercaemer and Sephton 2014). Underwater video revealed moderate populations of *S. clava*, including mature individuals on ropes and chains of buoys in both locations.



Figure 2. *Styela clava* (length 36 mm) attached to a brick in Lunenburg Harbour, NS. Photograph by A.M. Moore.

In 2012 and 2013, there were also anecdotal reports of *S. clava* from Pirate Harbour, in the Canso Canal (S. Sorowka, Department of Biology, St. Francis Xavier University, pers. comm.) and the Strait of Canso Yacht Club (SCYC) in Port Hawkesbury, Nova Scotia (N. Filip, Department of Biology, St. Francis Xavier University, pers. comm.) (Figure 1B). Dockside inspections at Pirate Harbour uncovered no *S. clava* individuals; however, several individuals were present on the underside of floating docks at nearby Venus Cove, which has been monitored annually since 2008 (except 2012). Both the SCYC and Venus Cove were collector-based monitoring stations in 2014.

Styela clava has likely been present in Nova Scotia since at least late 2011. However, given the distribution and extent of current populations, the size of individuals, and the timing of detection, *S. clava* could have been present in Nova Scotia years earlier and simply gone undetected due to insufficient monitoring efforts and/or low population abundance.

Ascidiella aspersa

Ascidiella aspersa is a solitary tunicate, ovate in shape, with a clear-grey, papillate, tunic sometimes tinged pink around its two prominent siphons. It resembles *Ciona intestinalis* especially in its early growth stages (< 10 mm in length); however, its tunic is firm to the touch unlike that of *C. intestinalis,* which is soft and gelatinous. *Ascidiella aspersa* is indigenous to the Mediterranean Sea and coastal waters throughout Europe and the British Isles but has become abundant in Australia and New Zealand since arriving in the early 1900s (Kott 1998). It was first detected in New England in the 1980s, presumably spread by hull fouling or ballast water, and is currently established in Massachusetts (Fofonoff et al. 2003) and present at least as far north as Casco Bay, Maine. *Ascidiella aspersa* was included on the Atlantic Canada watch list in 2009.

Ascidiella aspersa was first recognized and positively identified in Nova Scotian waters on 2 October 2012 from specimens on collector plates in Lunenburg Harbour (Figure 1; Table 1). This is the first record of *A. aspersa* in Canada. Using photographic time series from months previous, we determined that this species was actually present as early as 5 July 2012 on collector plates from three different docks in Lunenburg Harbour. Individuals were present in densities up to 20 individuals per plate, ranging in length from 10 to 40 mm by early October (Figure 3). In response to the detection (and others, see *Styela clava* and *Diplosoma listerianum*), DFO conducted a rapid assessment survey of the Lunenburg area (Figure 1A) in November 2012 using snorkelling and small buoy inspections (Vercaemer et al. 2012). The survey revealed extensive populations of *A. aspersa* on docks, pilings and buoys in Lunenburg Harbour and as far south as Lower South Cove.

Ascidiella aspersa was likely present in 2011, perhaps before, having successfully overwintered to spawn for the first time in detectable numbers in early 2012 when it developed multiple large, dense, and widespread populations in multiple bays near Lunenburg, Nova Scotia.



Figure 3. Multiple specimens of *Ascidiella aspersa* (large, ovate solitary organisms) on a collector plate from Lunenburg Harbour, NS. Photograph by A.M. Moore.

Diplosoma listerianum

Diplosoma listerianum is a colonial tunicate, transparent and grayish in colour with pigmented zooids. Its tunic is gelatinous to the touch, unlike other non-indigenous colonials *Botryllus schlosseri* and *Botrylloides violaceus*, which are firmer. *Diplosoma listerianum* is cryptogenic, although it is presumed to be indigenous to northern Europe as it was first described by Millar (1952) as one of the region's more common ascidians. It was first detected in the Gulf of Maine in the Isles of Shoals in 1993 and is now considered established from Cape Cod, Massachusetts to Casco Bay, Maine, although its distribution is temporally variable (Dijkstra 2007). In Canada, *Diplosoma listerianum* was first collected in 2008 from Havre-Aubert, Quebec, which is located in the Magdalen Islands (Figure 1) (Simard et al. 2013). However, no colonies have been found since, despite targeted surveys and despite *Diplosoma listerianum* DNA being detected via targeted PCR-based assays in plankton samples from Havre-Aubert in 2010 and 2011 and Cap-aux-Meules (also in the Magdalen Islands) in 2011 (Simard et al. 2013). *Diplosoma listerianum* was included on the Atlantic Canada watch list in 2009.

Diplosoma listerianum was initially found in Nova Scotia in small patches on collector plates in Lunenburg Harbour on 2 October 2012 (Figure 1; Table 1). Specimens were collected and preserved in ethanol. Identification was confirmed by G. Lambert (Marine Biological Consultants, Seattle, Washington, USA) via digital photographs and later using the PCR-based assay developed by Willis et al. (2011) (S. Stewart-Clark, Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada, unpubl. data). Colonies were present on both newly deployed plates (deployed for approximately four weeks) and plates with fully developed communities (deployed for approximately four months) (Figure 4). Photographs from the beginning of the experiment were re-examined to see if *Diplosoma listerianum* could have been present earlier, but no colonies could be positively identified. In response to the detection (and others, see *Ascidiella aspersa* and *Styela clava*), DFO conducted a rapid assessment survey of the Lunenburg area (Figure 1A) in November 2012 using snorkelling and small-buoy inspections (Vercaemer et al. 2012). No additional specimens of *Diplosoma listerianum* were observed; however, visibility was poor during the survey, and colonies, with their transparent tunic, could have been overlooked.

Previous detections of *Diplosoma listerianum* in Atlantic Canada have not led to established populations (Simard et al. 2013). However, given that there have been several detections of *Diplosoma listerianum* in Atlantic Canada both visually and from DNA in water samples (Simard et al. 2013), there could be as yet undiscovered populations in Atlantic Canada.

Didemnum vexillum

Didemnum vexillum is a colonial ascidian, usually creamy white or tan in colour, that forms a firm gelatinous layer on natural and artificial substrates. The species sometimes has darker-coloured lines (cloacal canals of the shared excurrent system) that run between groupings of zooids (1 mm long) just beneath the surface and calcareous spicules embedded at the surface of the matrix. Originally from Japan (Stefaniak et al. 2012), the species has been reported in temperate and cold waters of both coasts of North America, in Europe, and in Australia. In Canada, *Didemnum vexillum* has been reported in British Columbia, mainly on artificial structures, such as aquaculture cages. The northernmost coastal location from which it has been reported

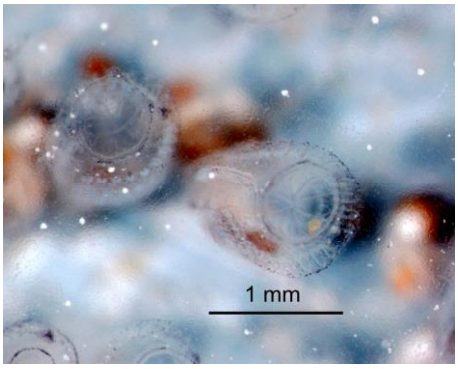


Figure 4. *Diplosoma listerianum* on a collector plate from Lunenburg Harbour, NS. Photograph by K.C.K. Ma.



Figure 5. *Didemnum vexillum* colonies on rocks from the upper Bay of Fundy, NS. Photograph by B. Vercaemer.



Figure 6. *Diadumene lineata* on a collector plate from Halifax Harbour. Photograph by A.M. Harris.

on the eastern shore of the US is Eastport, Maine, where it was first observed in 2003 (Figure 1) (Bullard et al. 2007). This species also occurs in deeper waters on Georges Bank off New England (Valentine et al. 2007) where it covered an area of approximately 230 km^2. *Didemnum vexillum* was included on the Atlantic Canada watch list in 2009, and there was an intense public awareness campaign regarding its appearance and likely habitat. Rapid assessment surveys conducted in Passamaquoddy Bay, New Brunswick, and areas near Eastport, Maine, since 2009 (Martin et al. 2010), as well as in southwestern Nova Scotia in 2013, did not detect this species.

A specimen thought to be *Didemnum vexillum* was collected in October 2013 by a recreational diver off the coast of Parrsboro, in the Minas Basin, at a depth of approximately 10 m (Figure 1; Table 1). Microscopic examination of spicules and subsequent molecular identification (S. Stewart-Clark, unpublished data) confirmed the sample as *Didemnum vexillum*. In April 2014, in response to the detection, DFO conducted a rapid assessment targeting 30 stations in four areas of the Minas Basin and Minas Channel (Figure 1A). The assessment used underwater video to identify potential patches of *Didemnum vexillum* that were then sampled by scallop dredge and sent for genetic analysis. Numerous colonies were observed overgrowing rocks, bivalve shells, and seaweeds (Figure 5). Samples from 22 of the 30 stations were confirmed to be *Didemnum vexillum* by a PCR-based assay (S. Stewart-Clark, unpublished data). Colonies were in a wintering state, but were healthy and likely to grow and spread in the spring and fall. Because initial observations on the east coast of the USA documented isolated and small populations that underwent a rapid population expansion within a decade (Bullard et al. 2007), DFO has initiated an assessment to delineate the current distribution of *Didemnum vexillum* throughout the Bay of Fundy as well as regions of the southern Scotian Shelf, including Georges Bank, German Bank, and Browns Bank. This assessment will establish a baseline to monitor the populations' abundance changes and rate of range expansion.

Although first recorded in 2013, *Didemnum vexillum* could have been present in Nova Scotia as early as 2011, when a recreational diver first noticed and photographed patches of a white organism on the benthos off the coast of Parrsboro. Unfortunately, samples were not obtained until 2013; consequently this initial report is unconfirmed.

Diadumene lineata

Diadumene lineata is a small greenish anemone with distinctive vertical orange or tan stripes around its body. Native to northeastern Asia including Japan, China, and Hong Kong, *D. lineata* was first recorded on the Atlantic coast (Connecticut and Massachusetts) of North America in 1892 (Verrill 1898) and is now established as far north as Cobscook Bay, Maine (Figure 1) (Trott 2004). In Canada, *Diadumene lineata* was found in Sam Orr's Pond on the southern coast of New Brunswick on the Bay of Fundy in 2009, possibly introduced on substrates (e.g., shells) transported by the invasive alga *Codium fragile fragile* (Suringar) Hariot, 1889, which can float to new locations (Saunders et al. 2013). This species can reproduce asexually and sexually, although sexually reproducing populations of *Diadumene lineata* are rare, if not absent, outside of its native range in Asia (Ting and Geller 2000).

In Nova Scotia, *Diadumene lineata* was first detected in February 2014 during photographic analysis of collector plates deployed at a dock in Halifax Harbour (Figure 1; Table 1). The photographs were taken on 19 November 2013, and one specimen was retrieved from a collector plate on 3 July 2014 (Figure 6). In October 2014, an extensive population of *Diadumene lineata* was observed along the same dock, consisting of numerous individuals interspersed amongst mussels near the water's surface. No further attempt at determining the species' extent is underway as the species is not perceived to be a major threat to indigenous communities or aquaculture operations.

Discussion

The Atlantic Canada watch list, using shipping and climate zone filters, included three tunicate species found in Nova Scotia in 2012 and 2013: *Ascidiella aspersa*, *Diplosoma listerianum,* and *Didemnum vexillum*. *Styela clava, Ciona intestinalis*, *Botryllus schlosseri*, *Botrylloides violaceus*, and *Molgula manhattensis* (De Kay, 1843) were excluded from the watch list because they already were present in Atlantic Canada. All of the new non-indigenous invertebrates reported in this study, except *Didemnum vexillum,* were found near high traffic shipping (Halifax and Port Hawkesbury) or recreational (Lunenburg) ports, where other similar non-indigenous species (i.e., *Ciona intestinalis*, *Botryllus schlosseri,* and *Botrylloides violaceus*) are already present. *Didemnum vexillum*

was found seemingly far from a major port city; however, until late 2011, the nearby town of Hantsport shipped gypsum, with 119 gypsum ships docking there in 2000, many of which arrived from the United States (Carver and Mallet 2003) where *Didemnum vexillum* is established.

Earlier first records of detections have also occurred in the high traffic port of Mahone Bay (Figure 1). Only 11 km from Lunenburg, Mahone Bay shares Lunenburg's high recreational boat traffic and is the first reported location for four other non-indigenous species that have hull fouling or ballast as a likely vector: *Codium fragile fragile*, a green alga, discovered in 1989 (Bird et al. 1993), *Membranipora membranacea* (Linnaeus, 1767), an encrusting bryozoan, found in 1992 (Scheibling et al. 1999), *Botrylloides violaceus* found in 2001 (Carver et al. 2006), and *Heterosiphonia japonica* Yendo, 1920, a red alga discovered in 2012 (Savoie and Saunders 2013).

All of these first records support the idea that primary introductions to Nova Scotia are occurring via either shipping or boating activities (DiBacco et al. 2012). Monitoring for new non-indigenous species, including the 14 tunicates remaining on the Atlantic Canada watch list, should therefore be focused on high traffic locations, particularly those with other non-indigenous species and those with a history of first records. While it is possible that *Didemnum vexillum* could have been introduced by hull fouling or ballast in 2011, it has not yet been detected in ports in the area. Instead, its introduction into the Bay of Fundy could have been a range expansion from the United States via rafting on eelgrass (Carman and Grunden 2010) or fragmentation caused by physical disturbance (e.g., current and wave action) of natural substrates (Reinhardt et al. 2012). Because of the uncertainty around the introduction of *Didemnum vexillum*, monitoring should also continue to focus on all potential vectors with public education programs targeting boaters, fishermen, and divers who regularly observe the substrates suitable for colonization.

Monitoring a large area to detect species soon after they invade is a difficult and costly task. By collaborating and sharing information, the AIS research and monitoring programs in Nova Scotia were able to detect and delineate five new non-indigenous species in a short time that would not have been possible independently. On its own, the AIS Monitoring program would have directly detected only two of the new species: *Styela clava* in Halifax Harbour (not Lunenburg Harbour) and *Ascidiella aspersa* in Lunenburg Harbour with indirect detections of *Styela clava* in Pirate Harbour as well as *Didemnum vexillum* via the public outreach program. Conversely, the AIS Research program would have observed all species but *Didemnum vexillum*, but would not have been able to perform follow-up inspections to delineate local populations or plan and execute subsequent monitoring. Because of the success of this collaboration, we examined the reasons for differential detection patterns between the research and monitoring programs to identify potential improvements.

The main differences between the research and monitoring programs were spatial coverage of collectors, renewal interval of settlement space, and monitoring frequency. The main AIS Research project had 20 collectors deployed along one or more docks with a total of 4,263 cm^2 of settlement area per site, whereas the AIS Monitoring Group deployed four collectors on one or more docks with a total of only 1,200 cm^2 of settlement area per station. While both groups spread collectors out to account for spatial variability of larval settlement, the AIS Research group had 3.5 times the surface area available for settlement, increasing the likelihood of detecting a species if present, especially rare species. In addition, the 2012 AIS Research projects involved bi-weekly removal of tunicates from some plates or the monthly replacement of collectors. Both of these methods facilitated the detection of rare species (indigenous or non-indigenous) by consistently providing settlement space and examining that space before dominant fouling species could obscure detections. The AIS Monitoring group visits its stations twice post-deployment, in August and October, and although two collector arrays are replaced mid-season, at stations with heavy fouling, common in high traffic marinas, new species present in small numbers could easily be prevented from settling or be overgrown and obscured on plates by the end of the season and remain undetected. Ultimately, more surface area and more frequent collections, especially in high traffic areas, would increase the likelihood of detecting new non-indigenous species early in the invasion process.

The reports leading to the detection of *Didemnum vexillum* in Parrsboro and *Styela clava* in Venus Cove are a reflection of public outreach efforts of the DFO Maritimes AIS Monitoring program. In Nova Scotia, public education is a fundamental part of the AIS Monitoring program, and outreach efforts take the form of signage, presentations, sample collection, interviews with the media, and even monitoring itself (which increases interaction

with the public). Members of the public are encouraged to learn, identify, and report suspected non-native species, which helps cover a territory that is too large to adequately monitor through collector plates or rapid assessments alone. The detection of the high-impact invader *Didemnum vexillum* by a knowledgeable recreational diver underlines the importance of continued public outreach programs to educate the general public about AIS in general, but especially for high risk species.

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Research Article

Early detection of the non-indigenous colonial ascidian *Diplosoma listerianum* in eastern Canada and its implications for monitoring

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Abstract

In eastern Canada, the initial suspected discovery of the non-indigenous colonial ascidian *Diplosoma listerianum* in Quebec was followed by species identification and increased awareness for its detection in the neighbouring provinces of Nova Scotia and Prince Edward Island. A phylogenetic analysis was conducted based on sequences generated from the 18S ribosomal DNA gene isolated from tissues of two Canadian samples (Quebec and Nova Scotia) and samples from several species belonging to the *Diplosoma* genus, which confirmed the species identity of the eastern Canadian specimens. Colonies of *D. listerianum* found in Quebec (2008) and Nova Scotia (2012) are currently the only tissue records of this species in eastern Canada. Water samples of concentrated plankton collected in Quebec (2010 and 2011) and in Prince Edward Island (2011) tested PCR positive for *D. listerianum* DNA, but colonies were not observed concurrently. As a case study for early detection, this paper documents how *D. listerianum* was detected in eastern Canada by different groups (government monitoring, academic research) and with different methods (dive surveys, genetic analysis of water samples, collector plates). This case study suggests that the co-operation between groups and the breadth of the methods used contributed to the early detection of this species in three eastern Canadian provinces. In particular, we show that (i) partnerships between government, academia, and industry, (ii) a national capacity for species-specific molecular detection tools, and (iii) the recognition that the effectiveness and limitations of different monitoring methods may be site-specific can have positive early detection and monitoring outcomes.

Key words: early detection, monitoring, ascidians, *Diplosoma listerianum*, eastern Canada, invasive species

Introduction

The introduction and spread of aquatic invasive species (AIS), such as some ascidian species (subphylum Tunicata, class Ascidiacea), is a global ecological and economic concern (Lambert 2007; Zhan et al. 2015). Ecologically, the introduction of invasive ascidians can alter the biodiversity of benthic communities by competing with indigenous species for space and food, through predation, and by carrying exotic diseases that harm indigenous species (Harms and Anger 1983; Blum et al. 2007; Dijkstra et al. 2007; Lutz-Collins et al. 2009). Economically, invasive ascidians can damage the real and/or perceived value of marine resources and elevate costs associated with processing aquaculture products (Davis and Davis 2009, 2010), for example, by fouling bivalve aquaculture products and equipment (Lutz-Collins et al. 2009; Rocha et al. 2009).

Non-indigenous ascidians may be introduced to non-infested areas by fouled ship and boat hulls and aquaculture equipment (Ramsay et al. 2008; Davis and Davis 2009) but may only survive and reproduce if biotic and environmental conditions are suitable. This is of concern given that invasive ascidians are generally associated with broad environmental tolerance, rapid growth and sexual maturity, and high fecundity (Lambert 2002). Following establishment,

regional spread to non-infested areas may occur via recreational boats (Clarke Murray et al. 2011; Lacoursière-Roussel et al. 2012) and commercial activities (Coutts et al. 2003; Coutts and Taylor 2004; Davidson et al. 2009; Frey et al. 2014). In Canada, public awareness, monitoring, and rapid response programmes have been established to detect invasive ascidians and other AIS incursions at early stages of the invasion process to minimise their secondary spread.

In the last decade, populations of four invasive ascidian species have established and spread widely in eastern Canada: *Botrylloides violaceus* Oka, 1927, *Botryllus schlosseri* (Pallas, 1766), *Ciona intestinalis* (Linnaeus, 1767), and *Styela clava* Herdman, 1881 (Thompson and McNair 2004; Ramsay et al. 2008, 2009; Locke et al. 2009; Sephton et al. 2011; Simard et al. 2013; Deibel et al. 2014). Using a screening procedure based on shipping and climate zone filters, Locke (2009) identified a "watch list" of 17 ascidian species considered to be most likely to successfully invade eastern Canada. *Ascidiella aspersa* (Müller, 1776), *Didemnum vexillum* Kott, 2002, and *Diplosoma listerianum* (Milne-Edwards, 1841) were included on this list prior to their discovery in eastern Canada (Simard et al. 2013; Moore et al. 2014). Of these species, this paper focuses on the early detection of *D. listerianum* in eastern Canada.

The colonial ascidian *D. listerianum* (order Enterogona, family Didemnidae) is a sessile marine filter-feeder that has a semi-cosmopolitan distribution (Goodbody 2003; Marins et al. 2010; Mackenzie 2011; Pérez-Portela et al. 2013). In North America, this species is considered cryptogenic on the west, southeast (south of North Carolina), and Gulf of Mexico coasts (Rocha and Kremer 2005; Carman et al. 2011). *D. listerianum* was first observed in the northeast United States in 1993 and is considered non-indigenous from Maine to Massachusetts (Harris and Tyrrell 2001; Agius 2007; Dijkstra et al. 2007; Carman and Grunden 2010). There have been no historic observations of *D. listerianum* in any records from eastern Canada (see review in Ma [2012]) and thus it is considered non-indigenous in eastern Canada. *D. listerianum* colonies are thin, translucent, and fleshy with hermaphroditic zooids (2 mm in length) arranged around multiple common cloacae. Larvae are brooded and released into the water column upon maturity. The larva is non-feeding with a relatively short life-span of a few hours before settlement and metamorphosis (Lane 1973; Mackenzie 2011). Colonies of *D. listerianum* exhibit reduced surface area and mortality under experimental low salinity conditions of 20 and 27 psu but not under ambient conditions of 34 psu (Gröner et al. 2011) and colonies disappear when early March seawater temperatures are below ca. 2 °C (Osman and Whitlatch 2007).

D. listerianum is an AIS that has been targeted for monitoring in eastern Canada, which involves partnerships among government, academia, and industry. Although the first records of *D. listerianum* in the provinces of Quebec and Nova Scotia have been previously reported by Simard et al. (2013) and Moore et al. (2014), respectively, the objective of this paper is to synthesise the available information leading to its initial detection and monitoring efforts following its discovery in eastern Canada into one report, including the first report of this species in the province of Prince Edward Island (PEI). As a case study, this paper provides insights into the early detection of an AIS and to document the known geographic distribution during the early stages of its invasion of eastern Canada. Also, this paper describes the methods used to identify the species of the specimens, and a phylogenetic analysis to further confirm species identity of the Canadian specimens.

Materials and methods

Detection via monitoring programme

The federal Department of Fisheries and Oceans (DFO) implemented an AIS monitoring programme in eastern Canada in 2006. The programme's objectives are (a) to detect new AIS as early as possible, to document their spread, and to identify potential vectors of primary and secondary spread, and (b) to minimise the secondary spread of these species through awareness and rapid response. Between the years 2006 and 2015, the programme monitored approximately 350 sites in the Gulf of St. Lawrence, insular Newfoundland, and the Maritime Provinces (Martin et al. 2011; Sephton et al. 2011; Simard et al. 2013; McKenzie et al. 2016); however, only a subset of these sites was monitored annually. Monitoring efforts at high-risk AIS introduction sites, such as marinas, commercial ports, fishing ports, and aquaculture sites, are targeted by DFO with the co-operation of industry partners and provinces. Region-specific methodologies are outlined below.

Quebec

The primary AIS monitoring tools used by DFO in Quebec were: (i) employing divers to conduct underwater surveys (hereafter referred to as dive surveys), (ii) sampling for plankton in the water column for genetic analysis (water samples), and (iii) deploying settlement plates (collector plates).

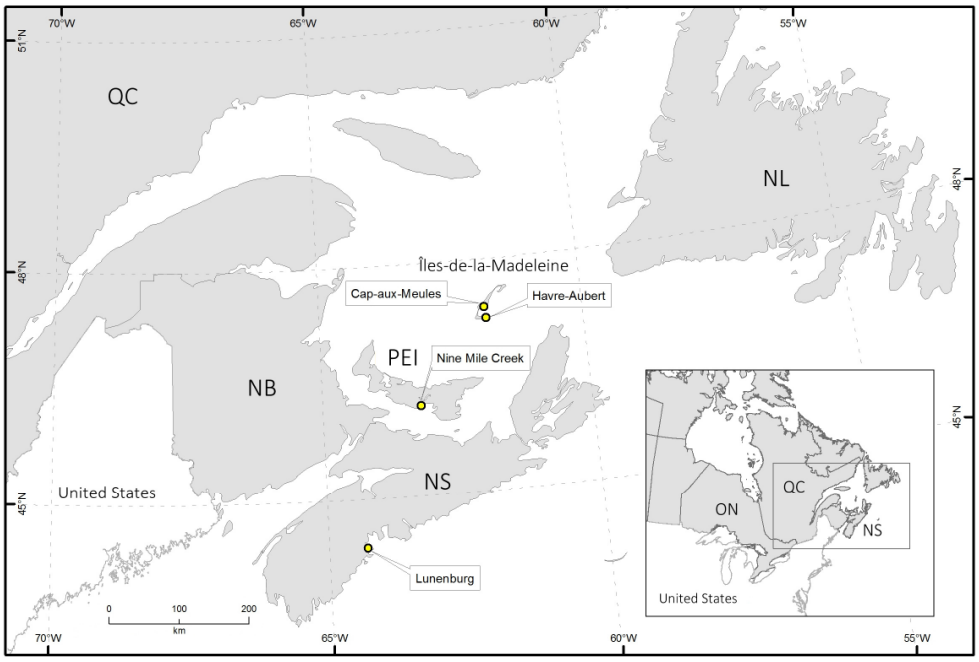


Figure 1. Map of eastern Canada and the currently known distribution of *Diplosoma listerianum* based on collection of colonies (Simard et al. 2013; Moore et al. 2014) and analysis of water samples for *D. listerianum* DNA (present study). NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; ON = Ontario; PEI = Prince Edward Island; QC = Quebec.

As part of the programme, a total of 39 sites in Quebec were monitored between 2006 and 2015 (Simard et al. 2013). Dive surveys were conducted at marinas, fishing docks, and aquaculture sites (Simard et al. 2013). At each site (depth < 3 m) that was monitored, two divers searched for AIS present on the sea bottom and artificial structures (e.g., floating and permanent docks, aquaculture socks, ropes, and buoys) for approximately two hours. All AIS were noted, photographed, and geographically referenced. Specimens of unknown species were collected, preserved in ethanol, and transported to the laboratory for identification.

In Îles-de-la-Madeleine, Quebec, water samples were collected at three sites (10s of metres apart) in a marina in Havre-Aubert and three sites—one site in a marina, one in a fishing port, and one in a commercial port—in Cap-aux-Meules (Figure 1) in August (2010–2014) and October (2010–2012). At each site, a water sample was collected by pumping 150 L of seawater through a 64-micron mesh. The inflow hose connected to the pump was lowered through the water column to ensure that samples contained water from various depths between 1–3 m below the water surface. For each sample, concentrated plankton accumulated on the mesh was then transferred into DNA-free tubes. The samples were kept on ice during the transport from the field to the laboratory, and then stored at −80 °C until they were analysed. A PCR-based assay targeting *D. listerianum* was used to analyse the water samples (Willis et al. 2011).

Collector plates were arranged in a sampling device consisting of three Petri dish and three roughened PVC plates (10 cm × 10 cm; see Simard et al. 2013 for methodology). For each of the years between 2007 and 2015, collector plates were either anchored or attached to existing structures at sites deemed to be of high-risk for AIS introductions, including Havre-Aubert and Cap-aux-Meules, for about five months from June to October.

In addition to the AIS monitoring programme, a separate DFO study on biodiversity consisted of dive surveys to characterise benthic fouling communities on dock pilings at targeted ports in Îles-de-la-Madeleine (N. Simard, unpublished data). Samples

of fouling benthic species were preserved in a formaldehyde solution and transported to the laboratory for identification.

Prince Edward Island

As part of DFO's (Gulf Region) monitoring programme in PEI, AIS were monitored at 39 sites between 2006 and 2015. Nine Mile Creek, PEI, is an area of concern for the presence of AIS since it is a commercially important mussel-producing site on the southern shore of the island. For this reason, DFO received requests from the internal Introductions and Transfers sub-committee to conduct rapid assessment surveys within this area. Rapid assessments were conducted in Nine Mile Creek in 2009 and yearly from 2011 to 2015. Crop, seed, and anchor lines of mussel leases were inspected visually by divers and by lifting mussel socks onto boats. Floating docks and permanent structures were examined by filming underwater with a video camera and analysing the video footage. Water samples were collected from four sites in Nine Mile Creek on October 19, 2011, and from five sites on August 22, 2012, using the same methods as those used in Quebec.

Nova Scotia

Approximately 120 sites were monitored by DFO (Maritimes and Gulf Regions) for AIS in Nova Scotia from 2006 to 2015 (D. Sephton and R. Bernier, personal communication). On November 1, 2012, the programme collected three water samples at the same location where colonies of *D. listerianum* were discovered by Moore et al. (2014) in Lunenburg, Nova Scotia (Figure 1). Each water sample was collected by pumping 300 L of seawater. The inflow hose of the pump was slowly lowered to just above the seafloor (4.6 m below the water surface) and then returned to the surface. The methods of storing and analysing the water samples were the same as those collected in Quebec and PEI.

Genetic identification of Canadian samples

PCR positive tissue samples of different species belonging to the *Diplosoma* genus were analysed by amplifying and sequencing a 522 bp section of the 18S ribosomal DNA (rDNA) gene in both directions using the universal CASIS CAS2 primer set developed by Le Roux et al. (1999). To ensure that the species identity of Canadian samples (GenBank accession number: HM641906.1) was correct, samples of several species belonging to the *Diplosoma* genus were also sequenced in the 18S rDNA region. Samples of *Diplosoma aggregatum* Hirose and Hirose, 2009 (HM641903.1), *D. listerianum* (HM641905.1), *Diplosoma gumavirens* Hirose E., Oka and Hirose M., 2009 (HM641900.1), *Diplosoma ooru* Hirose and Suetsugu, 2005 (HM641902.1), *Diplosoma variostigmatum* Hirose and Oka, 2008 (HM641901.1), and *Diplosoma watanabei* Hirose E., Oka and Hirose M., 2009 (HM641904.1) were obtained from Ishigaki Island, Okinawa Prefecture, Japan, in 2007–2008, and identified by E. Hirose. The identities of generated sequences were confirmed by BLAST analysis of the GenBank database (Altschul et al. 1997). Multiple sequence alignments were done using the Clustal W application in MEGA (Larkin et al. 2007; Tamura et al. 2007) with these Canadian and Japanese samples and with the 18S rDNA sequences of *D. ooru* (AB211100.1), *Diplosoma simile* (Sluiter, 1909) (AB211104.1), *Diplosoma simileguwa* Oka, Suetsugu and Hirose, 2005 (AB211108.1), *Diplosoma* sp. (AB211120.1), and *Diplosoma virens* (Hartmeyer, 1909) (AB211114.1). Neighbour-joining (NJ) Kimura 2-parameter model with gaps and missing data handled by complete deletion and maximum parsimony (MP) phylogenetic trees were constructed using the 18S rDNA sequences with MEGA version 4.1 (Tamura et al. 2007; Kumar et al. 2008). The validity of constructed NJ and MP tree topologies was evaluated by using the bootstrap (re-sampled 1000 times) test of phylogeny (Felsenstein 1985). *Echinorhinus cookei* Pietschmann, 1928 (M91181.1) was used as an outgroup species for the 18S rDNA tree.

Results

Colonies of Diplosoma listerianum

D. listerianum colonies were detected by a diver in the Havre-Aubert marina (47.2360ºN, 61.8343ºW), Îles-de-la-Madeleine, Quebec, on October 23, 2008 (Simard et al. 2013). Based on morphology, the identification of these specimens was confirmed by G. Lambert (Marine Biological Consultants, United States) and M. R. Carmen (Woods Hole Oceanographic Institute, United States). Specimens were adult colonies growing on mussels (*Mytilus* sp.) on marina docks at depths between 1 and 2 m. A single *D. listerianum* colony was also collected during the biodiversity study in Havre-Aubert on September 11, 2008. This specimen was initially misidentified as *Aplidium constellatum* (Verrill, 1871) and then correctly identified as *D. listerianum* by G. Lambert on October 5, 2009. No colonies have been observed in subsequent dive surveys (2009–2015) in Îles-de-la-Madeleine. Additionally, colonies of *D. listerianum* were not observed on any of the collector plates deployed as part of DFO's AIS monitoring programme (2006–

Table 1. Known records of *Diplosoma listerianum* in eastern Canada.

Date	Location	Method	Reference
September 11, 2008	Havre-Aubert, Quebec	Underwater survey via SCUBA	N. Simard, unpublished data
October 23, 2008	Havre-Aubert, Quebec	Underwater survey via SCUBA	Simard et al. 2013
October 13, 2010	Havre-Aubert, Quebec	PCR-based assay on water sample	Present study; see Table 2
August 23, 2011	Havre-Aubert, Quebec	PCR-based assay on water sample	Present study; see Table 2
August 24, 2011	Cap-aux-Meules, Quebec	PCR-based assay on water sample	Present study; see Table 2
October 12, 2011	Cap-aux-Meules, Quebec	PCR-based assay on water sample	Present study; see Table 2
October 13, 2011	Havre-Aubert, Quebec	PCR-based assay on water sample	Present study; see Table 2
October 19, 2011	Nine Mile Creek, Prince Edward Island	PCR-based assay on water sample	Present study; see Table 2
October 2, 2012	Lunenburg, Nova Scotia	Specimen on collector plate	Moore et al. 2014

2015) in Îles-de-la-Madeleine (Simard et al. 2013; N. Simard, unpublished data).

To date, no colonies of *D. listerianum* have been observed in PEI through DFO's extensive monitoring efforts (R. Bernier, unpublished data). However, juvenile colonies (maximum age: four weeks) of *D. listerianum* were found on experimental collector plates hanging from a floating dock in Lunenburg, Nova Scotia, on October 2, 2012 (Moore et al. 2014). Identification was confirmed by G. Lambert (via digital photographs) and later by S. E. Stewart-Clark using the PCR-based assay developed by Willis et al. (2011). Following the detection of *D. listerianum*, an underwater rapid assessment was performed in Lunenburg but no specimens were found on natural nor on artificial structures in the harbour (Vercaemer et al. 2012). In addition, no colonies of *D. listerianum* were observed on any of DFO's collector plates on nearby docks in Lunenburg in the same year. All currently known records of *D. listerianum* in eastern Canada are tabulated in Table 1 (see supplementary Table S1 for more information).

Water samples

Water samples from Havre-Aubert in 2010 and 2011, Cap-aux-Meules in 2011, and Nine Mile Creek in 2011, tested positive for *D. listerianum* DNA (Tables 1 and 2). Between 2012 and 2014, water samples from all locations (Îles-de-la-Madeleine [2012–2014], Nine Mile Creek [2012], and Lunenburg [2012]) tested negative for *D. listerianum* (Tables 2 and S1).

Phylogenetic analysis

18S rDNA sequences generated from Quebec and Nova Scotia samples had 100% sequence similarity but had a consecutive 41 base deletion in the sequence compared to the sequence from the *D. listerianum* positive control sample from Japan. Phylogenetic analysis shows that the sequences generated from the Quebec and Nova Scotia locations group together with the Japanese *D. listerianum* sample in both NJ and MP phylogenetic trees (Figure 2), as confirmed using the bootstrap test, which also confirmed the species identity of the two Canadian samples as *D. listerianum*. The sequences generated from the three *D. listerianum* samples group together and separate from all other *Diplosoma* species analysed in this study. This reveals that the 18S rDNA gene is a good species-specific marker for differentiating *D. listerianum* from other species classified under the *Diplosoma* genus.

Discussion

Invasion history and implications of Diplosoma listerianum

The presence of *D. listerianum* colonies in Quebec (Mackenzie 2011; Simard et al. 2013) and Nova Scotia (Moore et al. 2014) and *D. listerianum* DNA in PEI (present study) represent the first records of this species in each of their respective provinces in eastern Canada as well as a continuing trend of biological invasions by non-indigenous ascidians. Despite extensive monitoring efforts (primarily analyses of water samples) following its discovery in each of the three provinces, there are no known established populations of *D. listerianum* in eastern Canada. However, given the number and breadth of occurrences, these sporadic observations (in space and time), as reported in this paper, suggest widespread propagule pressure at early stages of the invasion process throughout eastern Canada. The vectors and the pathways of the *D. listerianum* detected in Quebec, PEI, and Nova Scotia are unknown. Fouling of ship hulls and niche areas, aquaculture equipment, and aquaculture products are suspected vectors for the introduction of non-indigenous ascidians (Dijkstra

Table 2. Summary of the detection of *Diplosoma listerianum* using a PCR-based assay on water samples collected in Quebec, Prince Edward Island, and Nova Scotia, from 2010 to 2014 (summer = July and August; autumn = September, October, and November).

Location	Sampling description	Autumn of 2010	Summer of 2011	Autumn of 2011	Summer of 2012	Autumn of 2012	Summer of 2013	Autumn of 2013	Summer of 2014
Havre-Aubert, Quebec	Three sites within a marina were sampled	One of three samples tested positive	One of three samples tested positive	Two of three samples tested positive	All three samples tested negative	All three samples tested negative	All three samples tested negative	No samples collected	All three samples tested negative
Cap-aux-Meules, Quebec	A marina, a fishing dock, and a commercial dock were sampled	All three samples tested negative	Of the three samples, only the sample from the fishing dock tested positive	Of the three samples, only the sample from the commercial dock tested positive	All three samples tested negative	All three samples tested negative	All three samples tested negative	No samples collected	All three samples tested negative
Nine Mile Creek, Prince Edward Island	Several sites within a mussel lease and one site at a wharf were sampled	No samples collected	No samples collected	All samples (three from the mussel lease and one from the wharf) tested positive	All samples (four from the mussel lease and one from the wharf) tested negative	No samples collected	No samples collected	No samples collected	No samples collected
Lunenburg, Nova Scotia	Three sites within the port were sampled	No samples collected	No samples collected	No samples collected	No samples collected	All three samples tested negative	No samples collected	No samples collected	No samples collected

et al. 2007). Shipping was identified as the potential vector for the transport of *D. listerianum* to the north-eastern United States, where it was first detected in Cape Cod, Massachusetts, in 1993 (Dijkstra et al. 2007).

Investigations of the intra-specific diversity and structure of genetic markers could reconstruct the invasion history of an AIS and identify the putative source population of an introduced population (Wares et al 2005). In fact, a global analysis of sequences of the mitochondrial gene cytochrome *c* oxidase subunit I suggests that *D. listerianum* is a species complex that consists of four monophyletic clades (Pérez-Portela et al. 2013). Unfortunately, the 18S rDNA sequences from this study cannot be compared with the mitochondrial sequences analysed by Pérez-Portela et al. (2013) to assess which clades are invading eastern Canada.

Invasive ascidians are associated with negative ecological and economic impacts, such as outcompeting other ascidian species for space (Schmidt and Warner 1986; Altman and Whitlatch 2007; Vance et al. 2009), fouling benthic communities, including eelgrass (Blum et al. 2007; Carman and Grunden 2010), and fouling bivalve aquaculture sites (Gittenberger 2009; Lutz-Collins et al. 2009; Rocha et al. 2009). *D. listerianum* has had notable ecological impacts in both the Gulf of Maine (United States) and the United Kingdom. In the Gulf of Maine, colonies of *D. listerianum* appeared for a short time on experimental collector plates made of Plexiglas and at low abundances relative to other non-indigenous colonial ascidian species (Dijkstra et al. 2007) but reached a maximum cover of 64% determined from SCUBA dive surveys of rocky sub-tidal sites in November of 1995 (Harris and Tyrrell 2001). Additionally, this species was more abundant on sub-tidal rocks in deeper waters (e.g., 20% cover at 20 m depth; Harris and Tyrrell 2001; Dijkstra et al. 2007). In the United Kingdom, Vance et al. (2009) documented that *D. listerianum* exhibited rapid growth and became dominant in a fouling community, quickly occupying up to about 50% of PVC recruitment panels and encrusting many species in the process. Although this species has not been found at any Canadian aquaculture sites, colonies of *D. listerianum* were previously reported on mussel lines at contaminated aquaculture sites in The Netherlands (Gittenberger 2009) and in Brazil (Rocha et al. 2009). Hence, if populations of *D. listerianum* do establish in eastern Canada, this could have ecological and economic consequences.

The results of the present study show that the application of different monitoring methods strengthened the ability to detect *D. listerianum* early in the invasion process in eastern Canada. As of 2015, dive surveys found *D. listerianum* colonies in Quebec, PCR-based assays detected DNA of this species in Quebec and PEI, and collector plates detected colonies in Nova Scotia. This shows that not all methods were always successful at every site, which suggests that the successful application of a particular method may be site-specific even for the same species. Therefore, it is important for future AIS

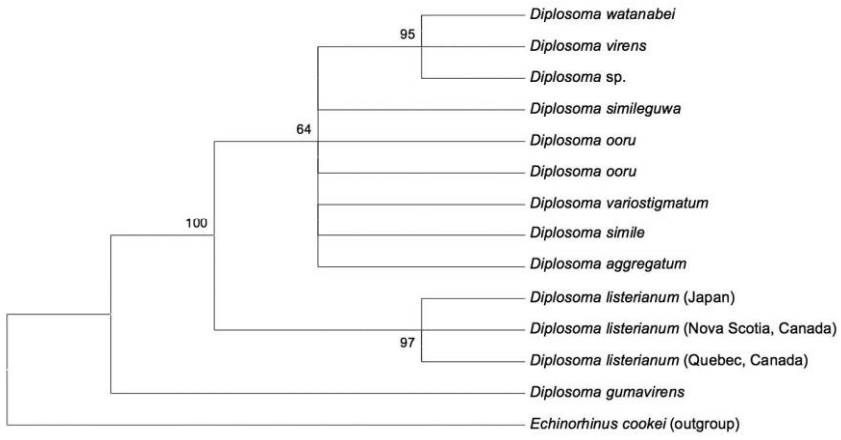


Figure 2. Condensed phylogenetic tree (50% bootstrap cut-off value) constructed from 18S rDNA sequences of *Diplosoma* species using neighbour-joining method with *Echinorhinus cookei* as an outgroup.

monitoring to have different monitoring tools available and, perhaps, to use a combination of different methods (despite being costly and time consuming) for a given site while recognising that the effectiveness and limitations of monitoring methods may be site-specific. Future research can further explore why the success of certain monitoring methods might be site-specific for the same species and how to optimise available methods to match local environmental conditions (e.g., temperature and salinity), hydrological patterns (e.g., wave action), and type of site (marina, port, aquaculture site, etc.) while reducing costs and detecting invasive species as early as possible.

Patterns associated with the early detection of invasive ascidians

In the broader context of biological invasions of eastern Canada by seven ascidian species (*A. aspersa*, *B. schlosseri*, *B. violaceus*, *C. intestinalis*, *D. listerianum*, *D. vexillum*, and *S. clava*), we reviewed the literature and summarised the first records for each species by province in Table 3 to better understand the circumstances that led to their initial discoveries. Presently, there are six non-indigenous ascidians that have been detected in Nova Scotia, five in PEI, five in Quebec, three in Newfoundland, and two in New Brunswick (total of 21 cases; Table 3). A majority of these first records were detected in the autumn (47.6%) and fewer in the summer (23.8%). The remainder of the records (28.6%) did not mention the month or season of initial discovery. Most of these first records (42.9%) were detected through government monitoring programmes, 23.8% by industry stakeholders, 19.0% by research activities, 4.8% by a member of the public, and 9.5% by unknown or uncategorised groups. In regards to the method of discovery, 28.6% were detected by anecdotal observations of specimens (mostly on hard substrates and aquaculture products), 28.6% via SCUBA, 23.8% via collector plates, 9.5% by molecular probes, and 9.5% by unknown methods.

The above summary suggests that early detection and monitoring of non-indigenous ascidians can benefit from different methods and from the involvement of different groups and stakeholders. However, this benefit for early detection should not be interpreted as an optimal approach for detecting and monitoring of non-indigenous ascidians. However, there are a couple patterns that may offer insights into the detection of non-indigenous ascidians in eastern Canada. Firstly, Table 3 indicates that detection occurs mostly in autumn (between September and October), perhaps corresponding to the maturity (i.e., larger size) of individuals that were introduced earlier in the year or to greater reproductive activity (i.e., greater density of larvae and eDNA in the water column) that may be detected by means of molecular probes. Secondly, DFO's monitoring programme has the advantage over local interest groups (e.g., recreational divers, aquaculturists, etc.) to detect AIS because the programme, in its current form, has a larger geographic coverage (national versus local

Table 3. First records of non-indigenous ascidian species by province in eastern Canada. NA = information not available.

Species	Date	Province[1]	Method of initial discovery	Group	Reference
Ascidiella aspersa	July 5, 2012	NS	Specimen on collector plate	Academia	Moore et al. (2014)
Botrylloides violaceus	2001	NS	Anecdotal observation	Industry[2]	Carver et al. (2006)
	2002	PEI	Underwater survey via SCUBA	Government	Locke et al. (2009)
	September 6, 2006	NB	Anecdotal observation; specimen on a buoy	Government	Bernier et al. (2009), Locke et al. (2009)
	October 2007	NL	Underwater survey via SCUBA	Academia	Callahan et al. (2010)
	October 10, 2010	QC	Underwater survey via SCUBA	Government	Simard et al. (2013)
Botryllus schlosseri	Before the 1980s	NB	NA	NA	Brinkhurst et al. (1975), Linkletter et al. (1977)
	Before the 1980s	NS	NA	NA	Carver et al. (2006)
	1945	NL	Specimen on collector plate	Government	US Navy (1951)
	Summer of 2001	PEI	Anecdotal observation	Industry	Locke et al. (2009)
	July 2006	QC	Anecdotal observation; specimen on dock structures	Industry[3]	Simard et al. (2013)
Ciona intestinalis[4]	October 2004	PEI	Anecdotal observation	Industry	Locke et al. (2009)
	August 3, 2006	QC	Specimen on collector plate	Government	Simard et al. (2013)
	September 19, 2012	NL	Underwater survey via SCUBA	Government	Sargent et al. (2013)
Didemnum vexillum	October 2013	NS	Underwater survey via SCUBA	Public	Moore et al. (2014)
Diplosoma listerianum	September 11, 2008	QC	Underwater survey via SCUBA	Government	Mackenzie (2011), Simard et al. (2013), N. Simard, unpublished data
	October 19, 2011	PEI	PCR-based assay on water sample	Government	Present study
	October 2, 2012	NS	Specimen on collector plate	Academia	Moore et al. (2014)
Styela clava	1997	PEI	Anecdotal observation; specimen on aquaculture products	Industry	Locke et al. (2009)
	August 24, 2011	QC	PCR-based assay on water sample	Government	N. Simard, unpublished data
	October 2, 2012	NS	Specimen on collector plate	Academia	Moore et al. (2014)

[1] NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; PEI = Prince Edward Island; QC = Quebec

[2] Claire Carver is an industry stakeholder

[3] Species was detected by Merinov

[4] Molecular and morphological evidence (Brunetti et al. [2015]) suggest that *Ciona intestinalis* is two distinct species: *Ciona robusta* (formerly *Ciona intestinalis* type A) and *Ciona intestinalis* (formerly *Ciona intestinalis* type B); global molecular analysis (Bouchemousse et al. [2016]) indicates that *Ciona intestinalis* is distributed in eastern Canada; in the present paper, we considered *Ciona intestinalis* as a cryptogenic species in eastern Canada but a non-indigenous species in Îles-de-la-Madeleine, insular Newfoundland, and Prince Edward Island based on the absence of historical records of any *Ciona* sp. on these islands

interests) and over research activities because it tends to have a larger temporal coverage (2006 to present versus the typical life-span of a research project). Greatly expanding the role of the public and industry stakeholders to report AIS has the potential advantage of larger geographic and temporal coverage matching or exceeding the scale of DFO's monitoring programme.

Conclusion

Early detection and monitoring for AIS along an extensive and complex coastline, as is the case in eastern Canada, is costly and time consuming. The continued use of molecular tools in monitoring activities and rapid assessments may assist in the early detection of invasive ascidian species (Stewart-Clark et al. 2009, 2013; Willis et al. 2011). Species-specific molecular probes, as used in this study, are thus critical to the identification of an AIS by means of water samples. Hence, a national capacity for the development of species-specific (e.g., PCR-based assays) and non-targeted (e.g., DNA [meta] barcoding) molecular detection tools is advocated. In recent years, there are several examples of citizen reporting and collaborations among government, academia, and industry that resulted in the early detection of non-indigenous ascidian species (Sargent et al. 2013; Deibel et al. 2014; Moore et al. 2014). Additionally, the conscientious reporting of unknown organisms and fouled barges by industry stakeholders to the government contributed to the initial discoveries of several non-indigenous ascidians in PEI (Locke et al. 2009). Therefore, we advocate for the continued public AIS awareness and partnership among government, academia, and industry to co-ordinate monitoring efforts and allocate resources efficiently.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Location of the studied sites (records of *Diplosoma listerianum*).

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2016/Supplements/MBI_2016_Ma_etal_Supplement.xls

Article 3. Second record of *Diplosoma listerianum* (Milne-Edwards, 1841) five years after and 280 kilometres from the site of the first record in Nova Scotia

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Rapid Communication

Second record of *Diplosoma listerianum* (Milne-Edwards, 1841) five years after and 280 kilometres from the site of the first record in Nova Scotia

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Abstract

Five samples of *Diplosoma listerianum*, a non-indigenous colonial ascidian species complex, were collected via SCUBA between August 27 and September 28, 2017, from four sites in the coastal waters (depth ranging between 3.1 and 18.0 metres) around Brier Island, Nova Scotia, Canada. These samples constitute the second record of *D. listerianum* in Nova Scotia—five years after the first discovery and 280 kilometres (by sea) from the site of the first record. Semi-quantitative abundance of *D. listerianum* from Brier Island was determined and was found to be frequent or common in three of these four sites that were surveyed via SCUBA. The occurrence at several sites, coupled by the presence of eggs, ovaries, and larvae in some tissue samples suggest that there is presently an established population of *D. listerianum* off of Brier Island.

Key words: invasive species, eastern Canada, Didemnidae, Ascidiacea, Tunicata

Introduction

Diplosoma listerianum (Milne-Edwards, 1841) is a colonial ascidian species complex (Tunicata: Ascidiacea) that has a cosmopolitan distribution in marine waters and is non-indigenous to the coastal waters of northeast North America (Mackenzie 2011; Pérez-Portela et al 2013). The first record of this species from northeast North America was from the Isles of Shoals, Maine, in 1993 followed by an additional record from Portsmouth Harbour, New Hampshire in 1999 (Dijkstra et al. 2007). In eastern Canada the species was detected for the first time at Îles de la Madeleine, Quebec, in 2008, followed by records from Nine Mile Creek, Prince Edward Island (2011), and Lunenburg, Nova Scotia (2012; Mackenzie 2011; Simard et al. 2013; Moore et al. 2014; Ma et al. 2016). Additionally, suspected colonies of *D. listerianum* have been found in 2016 in southwest New Brunswick (Sephton et al. 2017). *D. listerianum* has been detected by multiple methods, including *in situ* (SCUBA), molecular probes, and settlement plates.

In Nova Scotia, despite targeted searches for *D. listerianum* after its initial detection (*i.e.,* first record; Moore et al. 2014), the absence of further evidence of this species suggests that its introduction has failed in Lunenburg (Sephton et al. 2017). In this report, the second record of *D. listerianum* is documented five years after the discovery of and 280 kilometres (by sea) from the site of the first record in Nova Scotia.

Material and methods

Five colonial ascidian samples were collected via SCUBA in August and September 2017 at four sites from the coastal waters around Brier Island, Nova Scotia, Canada (Table 1; Figure 1). Samples were collected during a baseline semi-quantitative habitat survey of Brier Island, following a modified Marine Nature Conservation Review protocol (Connor and Hiscock 1996). Most species were identified *in situ* but for more difficult groups, samples were collected to confirm identification. These samples are retained

Table 1. Date, location, and habitat description of samples of *Diplosoma listerianum* from Brier Island, Nova Scotia, Canada. Abundance of *D. listerianum* at each surveyed site was classified according to the semi-quantitative SACFOR scale, which classifies abundance in six categories from rare to super-abundant (Connor and Hiscock 1996).

ARC museum specimen no.	Date (dive no.)	Site name	Latitude, longitude	Depth (m)	Temperature (°C)	Salinity (ppt)	Abundance	Habitat description
ARC 81413	August 27, 2017 (170827-01)	Peter's Island	44.2586, –66.3367	3.1–6.1	11.58	32.41	Rare	Kelp forest (*Saccharina latissima* and *Laminaria digitata*) on a steep slope of cobbles and large boulders.
ARC 81414 and ARC 81415	August 28, 2017 (170828-03)	East side of Whipple Point	44.2337, –66.3894	4.8–7.8	11.93	32.41	Frequent	Large bedrock steps dominated by coralline and foliose red algae.
ARC 81416	August 30, 2017 (170830-01)	Gull Rock	44.2095, –66.3855	3.5–15.5	12.67	32.17	Frequent	Vertical bedrock wall with *Laminaria digitata* kelp forest on upper parts.
ARC 81417	September 28, 2017 (170928-01)	South side of Brier Island	44.2446, –66.3531	13.5–18.0	13.21	32.59	Common	Bedrock and boulder wall. Cover of kelp and foliose red algae. Large areas of *Diplosoma listerianum* encrusting over other fauna and flora.

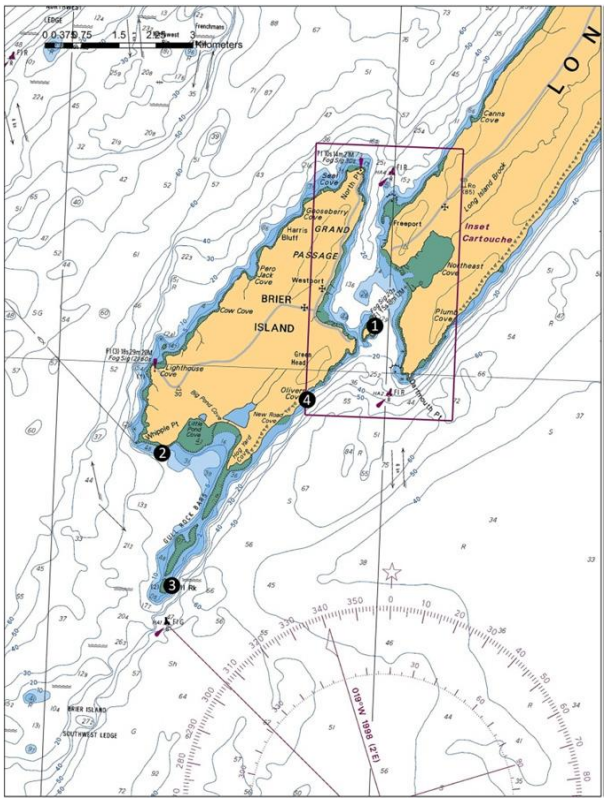


Figure 1. Locations of samples of *Diplosoma listerianum* collected from the coastal waters around Brier Island, Nova Scotia, Canada. 1 = Peter's Island; 2 = east side of Whipple Point; 3 = Gull Rock; and 4 = south side of Brier Island. Chart reproduced with permission from the Canadian Hydrographic Service.

in the collections of the Atlantic Reference Centre (ARC), Huntsman Marine Science Centre, New Brunswick, Canada. Samples were relaxed in seawater with the addition of menthol crystals for 10 to 14 hours (usually overnight) and then preserved in 10% seawater formalin buffered with marble chips (and a subsample was preserved in 95% ethanol). After fixation in formalin for several days the samples were transferred into 50% isopropyl for long-term storage.

In October 2017, samples were examined for identification and imaging. The taxonomic keys to ascidian identification by Rocha et al. (2012) and by

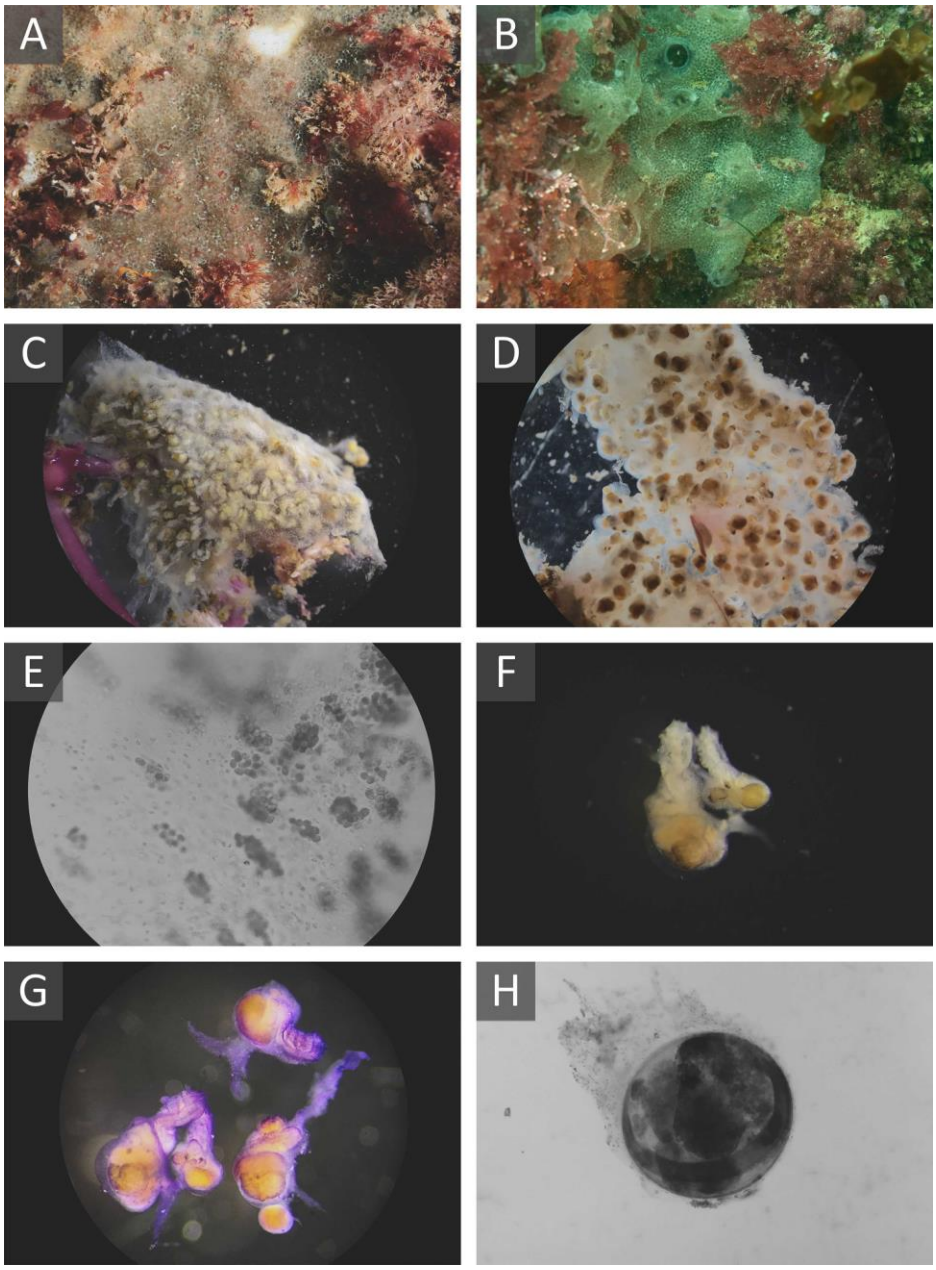


Figure 2. *Diplosoma listerianum* from coastal waters around Brier Island, Nova Scotia, Canada. (A, B) Live colonies photographed in the field; (C-D) preserved colonies illuminated under a stereomicroscope; (E) fragment of the tunic with granulations; (F) zooids; (G) zooids stained with haematoxylin; and (H) larva. The colour of the colony in panel (B) is not accurate due to camera lighting malfunction. Scale bar not available. Length of zooids ranged from 1 to 2 mm.

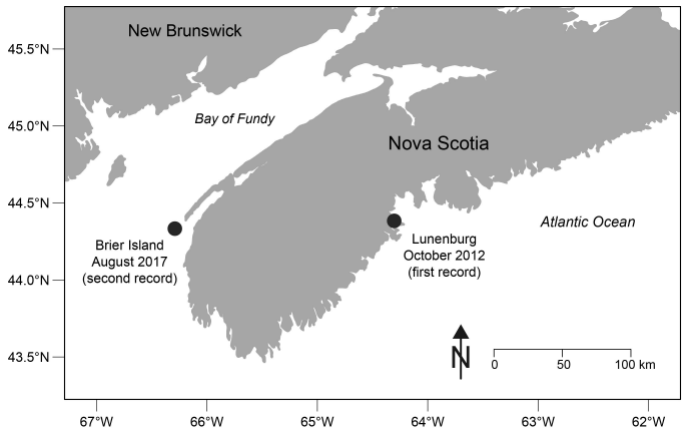


Figure 3. Locations of the first and second records of *Diplosoma listerianum* in Nova Scotia, Canada. The distance between the two locations is about 280 kilometres by sea.

Brunetti and Mastrototaro (2017) were used to identify specimens based on morphological features to genus and to species, respectively. Taxonomic descriptions in Van Name (1945) were also consulted. Additionally, Joyce A. Teixeira of Universidade Federal do Paraná (Brazil) examined digital photographs of the specimens for visual confirmation of species identity.

Results and discussion

A colonial mode of life, zooids completely embedded in the tunic, vertical arrangement of zooids, cloacal cavities, division of the body into thorax and abdomen, posterior position of the alimentary canal to the pharynx, four pharyngeal rows of stigmata, embryos and larvae in the tunic, and larvae with three adhesive papillae arranged in a line attribute these specimens to the family Didemnidae (Figure 2). Thin encrusting colonies with a soft tunic, lack of spicules, and white granulations (approximately 0.02 mm in diameter) on the tunic attribute these specimens to the genus *Diplosoma* (Figure 2). Translucent tunic, zooids ranging from 1 to 2 mm in length, thorax-abdomen ratio of 1:0.7, brown colouration around the siphons and on the abdomen, yellow colouration of organs, a two-lobed testis, and a straight sperm duct are characteristics that indicate they belong to the *D. listerianum* species complex (Figure 2).

The samples collected from Brier Island and described in this report constitute the second record of *D. listerianum* observed in Nova Scotia. This non-indigenous species was initially documented in 2012 in Lunenburg (Moore et al. 2014). This species has not been detected in Lunenburg since 2013 (Sephton et al. 2017), which suggests that the introduction event in 2012 had failed and a population did not establish. The second record documented in this report being five years after and 280 kilometres from the site of the first record contribute to the understanding of the invasion history of *D. listerianum* in Nova Scotia (Figure 3).

The identification of possible vectors and pathways requires further investigation. Due to the limited natural dispersal ability of *D. listerianum*, spread of this species is likely to be anthropogenic (*e.g.,* the movement of fouled boats, aquaculture products and gear, *etc*.; Mackenzie 2011; Pérez-Portela et al. 2013).

The abundance of *D. listerianum* was categorised as frequent or common in three of the four sites (Table 1). The presence of eggs, ovaries, and larvae in some of the samples from Brier Island indicates that the population is able to reach sexual maturity. One of the samples that was examined for this report was found encrusting the tunic of a native solitary tunicate, *Boltenia echinata* (Linnaeus, 1767). Other samples were found on either encrusting directly on bedrock and boulders or overgrowing turf algae and fauna. The occurrence at several sites, coupled by the presence of eggs, ovaries, and larvae in tissue samples suggest that there is an established population of *D. listerianum* in the coastal waters around Brier Island.

Globally, the *D. listerianum* species complex consists of at least four monophyletic clades (Pérez-Portela et al. 2013). Investigations into the species complex can help clarify the worldwide invasion pattern of *D. listerianum*. In eastern Canada, the clade(s) that the Canadian samples may belong in is presently unknown. To determine the clade(s) of the

eastern Canadian samples, an analysis of the mitochondrial sequences (*e.g.*, the cytochrome *c* oxidase subunit I) is required (Pérez-Portela et al. 2013). Furthermore, a more comprehensive genetic analysis of eastern Canada samples could provide important insights into the patterns of *D. listerianum* introductions; for instance, are all the introductions throughout eastern Canada coming from the same point of origin? The determination of donor site(s), vector(s), and pathway(s) associated with *D. listerianum* introductions in Nova Scotia can be informative in preventing future introductions before populations of this species become established and undergo secondary spread throughout the region. Additionally, long-term (multi-year) and large-scale (large geographic extent) monitoring of coastal waters for *D. listerianum* and other non-indigenous ascidian species in eastern Canada can be an effective approach to detect these species at early stages of the invasion process.

Acknowledgements

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Article 4. Morphological identification of two invading ascidians: new records of *Ascidiella aspersa* (Müller, 1776) from Nova Scotia and *Diplosoma listerianum* (Milne-Edwards, 1841) from New Brunswick and Quebec

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Research Article

Morphological identification of two invading ascidians: new records of *Ascidiella aspersa* (Müller, 1776) from Nova Scotia and *Diplosoma listerianum* (Milne-Edwards, 1841) from New Brunswick and Quebec

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Abstract

We report new observations of two invasive ascidian species, the solitary *Ascidiella aspersa* and the colonial *Diplosoma listerianum*, from Eastern Canada, which include some records from new sites. Because these species share many superficial characteristics with native and non-native species in the region, identifications based on detailed taxonomic studies of internal structures—and not only on external features—are essential and can complement molecular methods. In this report, specimens of *A. aspersa* collected from Nova Scotia in 2013 and *D. listerianum* from New Brunswick and Quebec in 2017 were described morphologically to confirm species identity. Specimens of both were collected one year after initial discoveries in Nova Scotia and New Brunswick, respectively. Specimens of *D. listerianum* from Quebec were collected after a six-year hiatus. This report contributes a new record of *A. aspersa* 30 km (by sea) north of the site of the first record in Canada. It also confirms the first record of *D. listerianum* and documents its rapid spread in New Brunswick. This report is the first time that *A. aspersa* from Eastern Canada and *D. listerianum* from New Brunswick and the second time that *D. listerianum* from Quebec was explicitly identified based on an examination of internal features. Species confirmation of these easily overlooked or misidentified taxa contributes a time-stamp on current invasion fronts and suggests that the risk of further introductions and spread of these invasive species is present at un-invaded sites throughout Eastern Canada.

Key words: invasive species, monitoring, Canada, Ascidiidae, Didemnidae, Ascidiacea, Tunicata

Introduction

Aquatic invasive species can impact ecosystem functioning and harm local economies, for instance, by disrupting shellfish aquaculture operations (Carman et al. 2010; Adams et al. 2011). Various ascidian species are considered aquatic pests around the world, and they are commonly found fouling underwater structures in ports, marinas, and aquaculture sites. As of 2018, seven species of invasive ascidians have been reported in the

waters of Eastern Canada (Ma et al. 2017a): *Ascidiella aspersa* (Müller, 1776), *Botrylloides violaceus* Oka, 1927, *Botryllus schlosseri* (Pallas, 1766), *Ciona intestinalis* (Linnaeus, 1767), *Didemnum vexillum* Kott, 2002, *Diplosoma listerianum* (Milne-Edwards, 1841), and *Styela clava* Herdman, 1881 (*e.g.*, Moore et al. 2014, Sephton et al. 2017). In Eastern Canada, the current distributions of *D. listerianum* (first detected in 2008), *A. aspersa* (in 2012), and *D. vexillum* (in 2013) are geographically localised relative to the other five invasive ascidian species (Moore et al. 2014; Ma et al. 2016; Ma et al. 2018; Simard et al. 2013; Vercaemer et al. 2015; Sephton et al. 2017).

The native range of *D. listerianum* is unknown and the species is considered cryptogenic in parts of the world where it is already established (Rocha and Kremer 2005). In the northwest Atlantic Ocean (including Eastern Canada), *D. listerianum*—considered an invasive species—was first reported in Maine in 1993 and again in New Hampshire in 1999 (Dijkstra et al. 2007). In Eastern Canada, *D. listerianum* was initially reported in the Quebec province in 2008 and in Nova Scotia in 2012 (Simard et al. 2013; Moore et al. 2014). Molecular traces of *D. listerianum* (*i.e.*, larvae and eDNA) were detected in water samples collected in Quebec and in Prince Edward Island in 2010 and 2011 (Ma et al. 2016). As larval dispersal in colonial ascidians is limited (< 10 m; Grosberg 1987; Olson and McPherson 1987), detection therefore indicated that there were established populations nearby. The primary method of detection for this invasive species was by a species-specific 18S rDNA PCR-based assay (Willis et al. 2011; Moore et al. 2014; Ma et al. 2016) with only one record using morphology (Ma et al. 2018).

Ascidiella aspersa is native to European waters and it is considered invasive in other regions around the world, including Eastern Canada (Mackenzie 2011; Moore et al. 2014). On the eastern coast of North America, this species was first reported in New England in the 1980s and has since been found as far north as Casco Bay, Maine (Mackenzie 2011; Moore et al. 2014). The first recorded observation of *A. aspersa* in Eastern Canada was in 2012 in Lunenburg Harbour, Nova Scotia (Moore et al. 2014). Identification of these first specimens was performed by expert examination of external features in digital photographs. The methods of identification used for subsequent records of *A. aspersa* on the southern shore of Nova Scotia have not been published even though this species was detected through recent monitoring efforts (Sephton et al. 2017). To date, no confirmed records of *A. aspersa* have been reported from sites outside of the southern shore of Nova Scotia (Ma et al. 2017a; Sephton et al. 2017).

Our report describes specimens of *A. aspersa* collected in Nova Scotia and *D. listerianum* collected in New Brunswick one year after initial discoveries in their respective provinces. In addition, it presents new records of *D. listerianum* collected from Îles-de-la-Madeleine, Quebec, after

Table 1. Distribution and percent cover (semi-quantitative abundance opportunistically estimated from the underside of floating docks) of *Diplosoma listerianum* from harbours and marinas in southwest New Brunswick between September and October 2017.

Site	Latitude (ºN)	Longitude (ºW)	Percent cover
Saint John	45.2723	66.0657	Not detected
Lorneville	45.1923	66.1486	Not detected
Five Fatham Hole	45.1867	66.2575	Not detected
Chance Harbour	45.1226	66.3514	Not detected
Dipper Harbour	45.0943	66.4180	Not detected
Boynes Cove	45.1309	66.4979	Not detected
Blacks Harbour	45.0558	66.7943	Not detected
Back Bay	45.0559	66.8632	Not detected
L'Etete	45.0513	66.8954	Not detected
Stuart Town	45.0190	66.9377	Not detected
St. Andrews Biological Station	45.0825	67.0848	Not detected
St. Andrews Harbour	45.0714	67.0544	0–5%
Beaver Harbour	45.0688	66.7387	0–5%
Lords Cove	45.0060	66.9466	5–25%
Leonardville	44.9717	66.9527	5–25%
Fairhaven	44.9640	67.0080	25–50%
North Head	44.7630	66.7494	25–50%
Ingalls Head	44.6626	66.7563	25–50%
Seal Cove	44.6491	66.8385	25–50%

a six-year hiatus. The species identities of these specimens were determined by examining multiple internal structures in addition to external features. As the first confirmed record of *D. listerianum* in New Brunswick, this report also documents its rapid spread in the Bay of Fundy, and the presence of this invasive species fouling the hull of a recreational boat in the Passamaquoddy Bay.

Materials and methods

Collection and preparation of specimens

In September 2013, 18 individuals of *A. aspersa* were collected from the undersides of floating docks and submerged stabiliser chains at the Lunenburg Yacht Club, located in the far southwest corner of Mahone Bay, Nova Scotia (44.4108°N; 64.3217°W). Concurrently, two specimens were collected from wooden piers in Lunenburg Harbour (44.3750°N; 64.3083°W), near the initial 2012 collection site of Moore et al. (2014). Lunenburg Harbour and the Lunenburg Yacht Club are on opposite sides of a peninsula and are separated by approximately 30 km of coastline. Although tissue relaxation steps (*e.g.*, Stefaniak and Heupel 2016) are commonly used to prevent the distortion of muscles for viewing after preservation, specimens at these locations were large and numerous so that relaxation was not employed and fresh specimens were merely preserved in 95% ethanol.

Between September and October 2017, 19 harbours and marinas in southwest New Brunswick were opportunistically surveyed for the presence of *D. listerianum* (see Table 1 for a list of sites). The percent cover of *D. listerianum* as part of the fouling community growing on the underside of floating docks was determined semi-quantitatively. Submerged sections



Figure 1. Detection of colonies of *Diplosoma listerianum* fouling the underside of a recreational boat during the end-of-season haul-out at Bayside, New Brunswick, Canada, on 9 October 2017. (A) One of the recreational boats in the process of being hauled out of the water; (B) the underside of a boat fouled by *D. listerianum* and another invasive colonial ascidian *Botrylloides violaceus*; (C) a colony of *D. listerianum* that was detached from the boat hull. Photo credit: K.C.K. Ma.

of 20 different 1-m wide dock sections, as viewed from the surface, were each designated one of six percent cover levels (not detected, < 5%, 5 to 25%, 25 to 50%, 50 to 75%, and 75 to 100%). At sites where *D. listerianum* were present, entire colonies were collected from kelp blades (*Laminaria* spp.) that were growing on the undersides of floating docks. In addition, the undersides of 20 recreational boats were visually inspected while out of the water at Bayside, New Brunswick (45.0672°N; 67.0353°W), during the end of season haul-out of boats (Figure 1). Colonies of *D. listerianum* found fouling these boats were collected. Finally, a single colony of *D. listerianum* was collected via SCUBA on a herring weir from Sandy Island (44.9714°N; 66.9138°W) as part of a bio-blitz surveying the Passamaquoddy Bay region in May 2017. This site is a disused weir and the specimen was collected from the surface of the wooden weir piling. Immediately after collection, all specimens (from kelp blade, boat, and weir substrates) were relaxed in seawater with added menthol crystals before being preserved in 95% ethanol (Stefaniak and Heupel 2016).

In October 2017, several colonies of *D. listerianum* were collected from the permanent dock via SCUBA and from collector plates in the Havre-Aubert marina, Îles-de-la-Madeleine, Quebec (47.2360°N; 61.8343°W). At the site, ten collector arrays were deployed such that each array consisted

of one PVC plate (dimensions: 10 × 10 cm) and three Petri dishes (diameter: 24 cm). This sampling strategy to monitor for marine invasive species was adopted starting in 2016 (see Simard et al. 2013 for a description of the sampling strategy used between 2006 and 2015 in Îles-de-la-Madeleine). Similar to the protocol used in New Brunswick, all specimens were relaxed in a seawater bath with menthol crystal immediately after collection and then preserved in 10% solution of formaldehyde. Different methods of ascidian relaxation and preservation were used (*e.g.*, ethanol without relaxation, ethanol with relaxation, formaldehyde with relaxation) because collection events were part of different investigations; however, all specimens, regardless of method, were in suitable condition for dissection. The percent cover of *D. listerianum* was estimated from a video made of the permanent dock and floating docks on 28 November 2017. For the entire site, the percent cover on the permanent dock (n = 240 underwater wooden planks around the perimeter of the structure) and on the undersides of floating docks was estimated semi-quantitatively (see above) from the video sequence.

Taxonomic identification

Preserved specimens of *D. listerianum* and *A. aspersa* were photographed and an initial identification was made using external features. Next, specimens were dissected by removing the tunic. The body of the *A. aspersa* was cut ventrally from the posterior to the anterior. For *D. listerianum*, individual zooids and larvae were isolated from the tunic matrix. Haematoxylin was used to stain tissue samples to illuminate important morphological features (such as tentacles and vessels). Ascidian taxonomic references and species descriptions were used to target morphological features that distinguish these two species from similar taxa in Canada and around the world (Van Name 1945; Rocha et al. 2012; Brunetti and Mastrototaro 2017).

Results and discussion

Species identification

Solitary ascidians collected from Lunenburg, Nova Scotia were confirmed as *A. aspersa*. The specimens ranged from 0.5 to 3 cm (mean: 1.57 cm) in length (Figure 2A), and the tunics were firm, translucent, papillated, and (in life) greyish-blue in colour with pink spots around the siphons (Figure 2B). The oral and atrial siphons each had six to eight lobes (Figure 2B). These specimens presented an undivided ovate body (Figure 2C), a pharynx wall without any folds (Figure 2D), a continuous and finely toothed dorsal lamina (Figure 2E), 16 to 18 simple oral tentacles, and a single aperture at the C-shaped dorsal tubercle, which was turned towards the anterior (both horns rolled inwards; Figure 2F). The alimentary canal

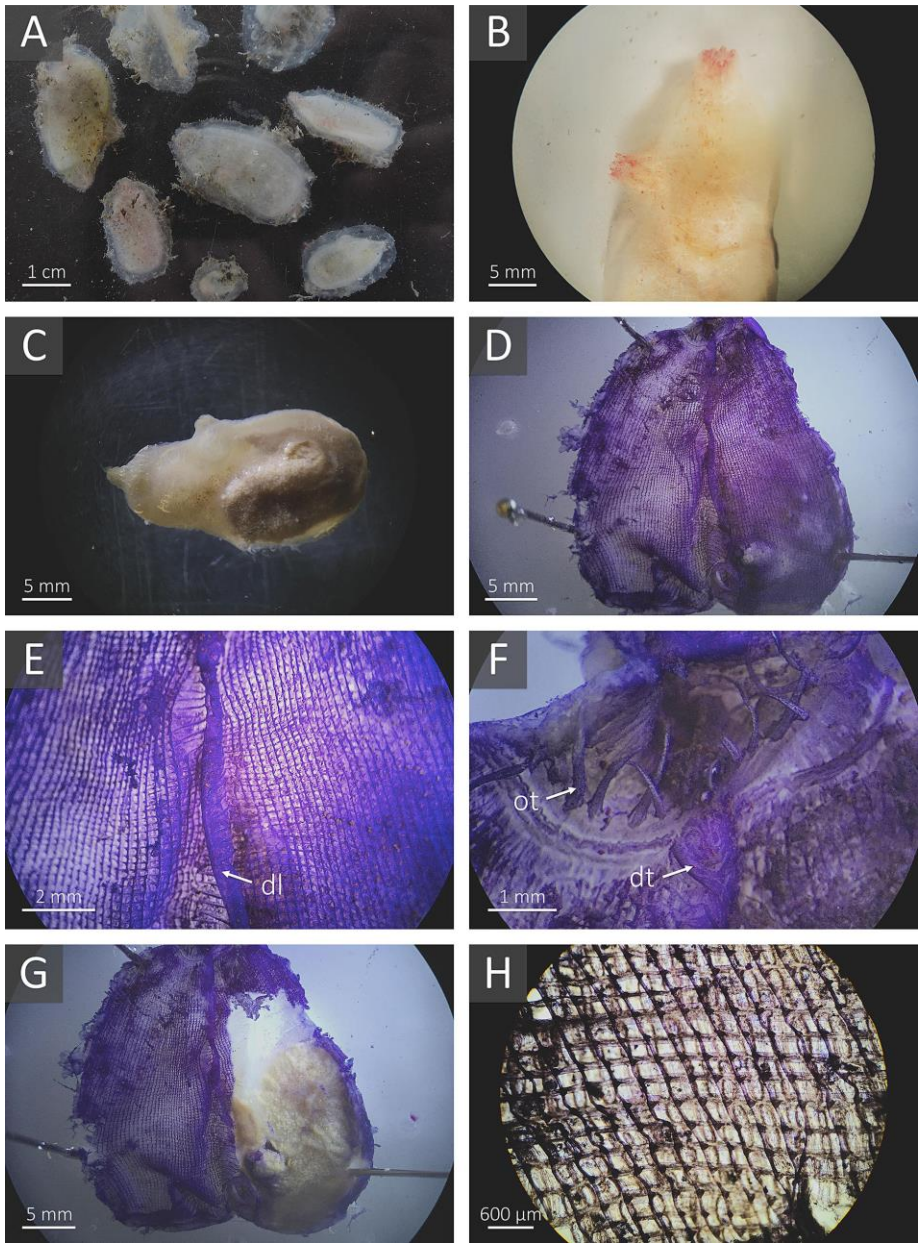


Figure 2. *Ascidiella aspersa* from Lunenburg, Nova Scotia. (A) Whole, live individuals immediately following collection; (B) right side of live individual with two pigmented siphons; (C) left side of the body of a preserved individual with the tunic removed; (D) dissected body stained with haematoxylin; (E) stained pharynx and dorsal lamina (dl); (F) stained oral tentacles (ot) and dorsal tubercle (dt); (G) view of the alimentary canal and gonads on the left side of the body after removing a section of the pharynx; and (H) stained section of the pharynx. Photo credit: K.C.K. Ma.

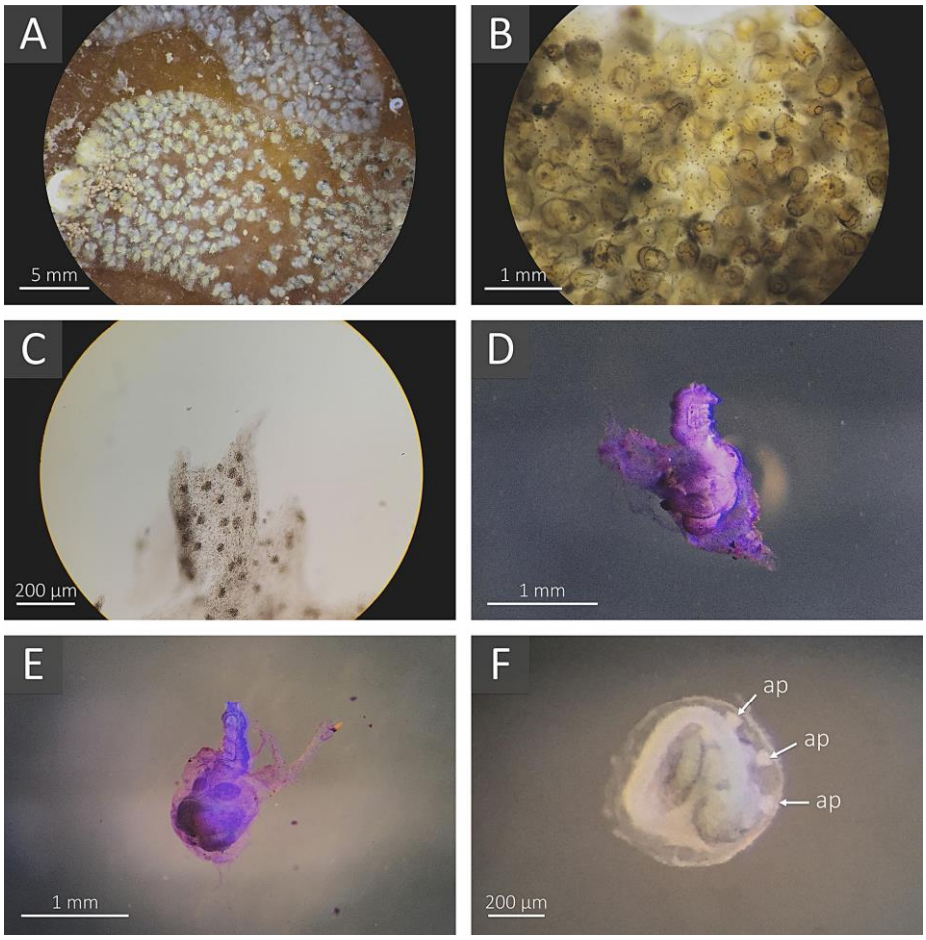


Figure 3. *Diplosoma listerianum* from coastal waters of southwest New Brunswick, Canada. (A) Live colonies on a kelp blade; (B) close-up of a live colony; (C) fragment of the tunic with granulations; (D, E) zooids stained with haematoxylin; and (F) unstained larva and adhesive papillae (ap). Photo credit: K.C.K. Ma.

was located on the left side of the body and gonads were attached to the intestinal wall (Figure 2G). Pharyngeal papillae supported 60 to 62 complete longitudinal vessels without projecting into the lumen (lack of intermediate branchial papillae and lack of secondary papillae). Pharyngeal stigmata were straight (Figure 2H), arranged in 50 to 110 pharyngeal rows. Finally, consistent with one of the most distinguishing characters of *A. aspersa,* there were more longitudinal vessels than tentacles.

Ascidian colonies collected from coastal waters of southwest New Brunswick (Figure 3) and from Havre-Aubert, Quebec (Figure 4), were confirmed as *D. listerianum*. These specimens presented a colonial mode of

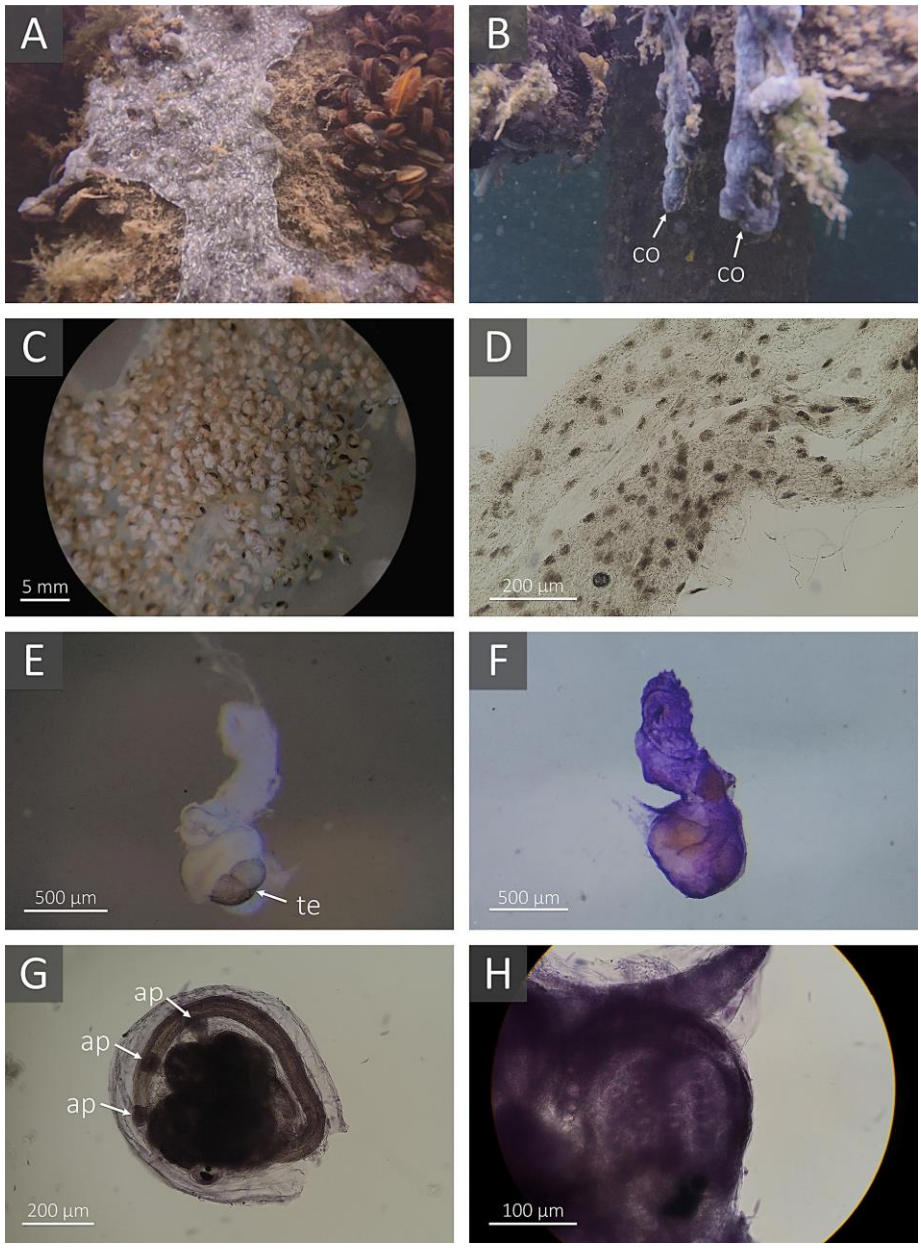


Figure 4. *Diplosoma listerianum* from Havre-Aubert, Îles-de-la-Madeleine, Quebec, Canada. (A) Live colony on dock structure; (B) live colonies (co) with lobate growth form; (C) preserved colony; (D) fragment of the tunic with granulation; (E) unstained zooid and its two-lobed testis (te); (F) zooid stained with haematoxylin (G) stained larva and adhesive papillae (ap); and (H) stained oozooid with four rows of stigmata. Images in panels A and B were extracted from video footage. Scale bars are not available for images in panels A and B. Photo credit: N. Simard (A, B), K.C.K. Ma (C–H).

life (sheet-like and lobate growth forms), zooids (1.2 to 1.4 mm in length) that were completely embedded in the tunic, atrial siphons that were opened into a cloaca system, and brown colouration of organs (Figures 3A, B; 4A–C). The greyish tunic was soft and translucent, no spicules were present in the tunic, and white granulations were observed in the tunic (Figures 3C and 4D). The zooid body was divided into two parts (thorax and abdomen; ratio of 1.0 to 0.7), the atrial aperture was wide (not tubular), the testis was two-lobed, and the sperm duct was straight (Figures 3D, E and 4E, F). There were four rows of straight pharyngeal stigmata. Brooding embryos and larvae were found in both the New Brunswick and Quebec specimens. The larva exhibited three adhesive papillae arranged in a line (Figures 3F and 4G) and an oozooid with four rows of stigmata (Figure 4H).

Invasion of the south shore of Nova Scotia by Ascidiella aspersa

Lunenburg Harbour was the site of the first record of *A. aspersa* in Nova Scotia in 2012. As part of the government's monitoring program, this species has been observed in waters in Lunenburg Harbour in subsequent years (2013 and 2014) but not in 2015 (Sephton et al. 2017). In 2013 and 2014, the species was also found on moorings and lines at an aquaculture lease in Corkum's Island, approximately 3.5 km south of Lunenburg Harbour. Although *A. aspersa* was not detected at the Lunenburg Yacht Club (approximately 30 km north of Lunenburg Harbour by sea) by the government's monitoring program in 2013 and in 2015 (Sephton et al. 2017), we detected this species in both Lunenburg Harbour and the Lunenburg Yacht Club in 2013. The detection of *A. aspersa* at these two sites suggests that recreational boats (largely pleasure sailing and recreational fishing) are the most likely vector responsible for its regional spread within the south shore of Nova Scotia (*e.g.*, Simard et al. 2017). In fact, the connectivity of the harbour and Mahone Bay regions by recreational boating activities appears to be extensive (Simard et al. 2017).

The lack of detection of *A. aspersa* in Lunenburg Harbour and vicinity in 2015 suggests that local populations may have failed (Sephton et al. 2017), but it is yet unknown whether local populations are reseeded by boats passing among connected regional sites. In the summer of 2016, suspected individuals of *A. aspersa* were reported for the first time in Shelburne, Nova Scotia, which is approximately 140 km (by sea) south of Lunenburg (Sephton et al. 2017). As of spring 2018, there have been no additional records of *A. aspersa* in other waters of Eastern Canada, and this report is the first confirmation of *A. aspersa* in Eastern Canada using detailed morphological identifications (Supplementary material Table S1).

Rapid invasion of southwest New Brunswick by Diplosoma listerianum

Although colonies of *D. listerianum* had previously been reported from nine of the 14 monitored sites (locations of these sites were not recorded) for

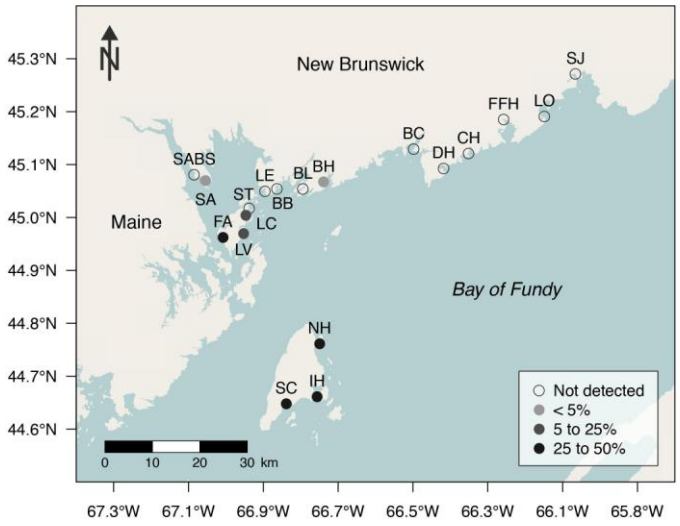


Figure 5. Distribution and percent cover of the fouling community on the underside of floating docks (semi-quantitative abundance) of *Diplosoma listerianum* in southwest New Brunswick in the autumn of 2017, one year after its initial discovery. BB = Back Bay; BC = Boynes Cove; BH = Beaver Harbour; BL = Blacks Harbour; CH = Chance Harbour; DH = Dipper Harbour; FA = Fairhaven; FFH = Five Fathom Hole; IH = Ingalls Head; LC = Lords Cove; LE = L'Etete; LO = Lorneville; LV = Leonardville; NH = North Head; SA = St. Andrew's Harbour; SABS = St. Andrew's Biological Station; SC = Seal Cove; SJ = Saint John; ST = Stuart Town. This map does not include records of *D. listerianum* collected from the underside of a boat in Bayside and from the herring weir in Sandy Island.

the first time in the New Brunswick coast of the Bay of Fundy, the identification of these specimens was not confirmed by morphological examination (Sephton et al. 2017). Consequently, colonies presented here in this report are the first confirmed records of *D. listerianum* in New Brunswick based on an examination of internal structures (Table S1).

The invasion of New Brunswick by this species was considerably rapid. Within a single year since its first detection, it increased its geographic extent by approximately 800 km^2 (estimated based on occurrence records). In 2017, this invasive colonial ascidian was present at 50% of sampled sites (Table 1). Abundance tended to be greater from sites on Deer Island and Grand Manan Island, which are more southerly (Figure 5). Surveys were not conducted on Campobello Island due to logistic constraints. Within our study area, no colonies were observed at sites north of Beaver Harbour (Figure 5). We hypothesise that a greater abundance of *D. listerianum* represents an older introduction event under the assumptions that (1) at a given site, population size is a function of time elapsed since initial introduction event, and (2) a greater abundance on recruitment plates is a proxy for a larger population size. Hence, the latitudinal pattern in *D. listerianum* abundance that was observed in our report is suggestive of

northward spread. These findings are consistent with the known distribution of *D. listerianum* in neighbouring waters: (1) established populations have been reported from coastal waters immediately south of New Brunswick in Maine, USA, and (2) geographically and temporally disjunct detection of this species early in the invasion process from other neighbouring Canadian provinces (Quebec, Nova Scotia, and Prince Edward Island). Notably, the detection of this species in other Canadian provinces can be interpreted as separate introduction events based on the disjunct distribution in space and time revealed through the relatively high spatial and temporal resolution of monitoring in Eastern Canada (Ma et al. 2016).

We found several colonies of *D. listerianum* on only one of the 20 inspected recreational boats (Figure 1). All surveyed boats were hauled out for the winter of 2016, placed in the water in May or June of 2017, moored in St. Andrews Harbour (New Brunswick), and hauled out in September or October for the winter of 2017. Anecdotally, these boats remained within the Passamaquoddy Bay for the entire 2017 season (KCK Ma, *pers. observ.*). Movement of fouled maritime vessels, aquaculture equipment, and aquaculture products are the main vectors for the introduction of invasive ascidians (Lambert and Lambert 1998; Carman et al. 2010). Ascidians foul boats by the attachment of settling larvae on hulls, rudders, sea chests, etc. (presumably when boats are berthed). Once fouling ascidians are attached to the hull, they can often survive the transit through water from the site of entrainment (donor location) to the site of introduction (recipient location). Introduction occurs at a previously uncontaminated site when individuals attached to hulls (1) reproduce and release gametes and or larvae at the recipient location and or (2) propagate by viable fragments dislodged from fouled hulls (Clarke Murray et al. 2012). The presence of *D. listerianum* fouling boat hulls provides evidence that the movement of recreational boats is a vector for the spread of *D. listerianum* in the Passamaquoddy Bay. Finally, our detection of *D. listerianum* attached to the Sandy Island weir suggests that the local spread of this species may be associated with recent boating activities and the availability of artificial substrate (*e.g.*, Locke et al. 2007) because, even though the weir is now disused for fishing, it remains a popular destination for recreational boaters. Leonardville and Lords Cove are less than five kilometres away and are the closest sites to the weir with confirmed detections of *D. listerianum* (see Table 1). This short distance, in theory, is well within the range of local boat traffic.

Invasion of Îles-de-la-Madeleine, Quebec, by Diplosoma listerianum

Îles-de-la-Madeleine (Quebec) is a group of islands located in the Gulf of St. Lawrence, geographically close to Prince Edward Island. Colonies of *D. listerianum* were initially discovered from Havre-Aubert on Îles-de-la-Madeleine in September and October 2008 (first records); a taxonomist

identified these samples by morphology (Simard et al. 2013; Ma et al. 2016). Prior to the present report, the last confirmed detection of *D. listerianum* from Havre-Aubert (and, overall, from Îles-de-la-Madeleine) occurred in 2011 (Ma et al. 2016). As a result of recent monitoring efforts described in the present report, *D. listerianum* was detected again from Havre-Aubert in 2017 after a 6-year hiatus (Table S1).

During the SCUBA video survey of Havre-Aubert in 2017, the permanent dock structures were estimated from video footage to have between 5 and 25% coverage by *D. listerianum* (primarily by large colonies), and at least 60% of the individual wooden planks were colonised. Colonies were also detected on the undersides of floating docks but with lower coverage (0 to 5%). This moderate yet homogeneous coverage is within the range that has been previously observed for another invasive ascidian species in Eastern Canada during its peak growth season (Ma et al. 2017b).

Although *D. listerianum* was not detected between 2011 and 2016, the population size of this species could have been so small that it was simply below the threshold of detection. The analysis of collector arrays used for monitoring during 2016 and 2017 is currently ongoing (N. Simard, *unpubl. data*). The re-appearance of *D. listerianum* in Îles-de-la-Madeleine in 2017 could be due to (1) a recent introduction event, (2) increased survival and growth as a response to inter-annual climate variation (for instance, Osman and Whitlatch [2007] has linked local disappearance to mean winter temperatures below approximately 4 °C), (3) increases in population size as a result of new artificial substrate available for rapid colonisation (*i.e.*, the wooden planks of the permanent dock in Havre-Aubert were replaced with new ones in 2016), or, theoretically, (4) improved sampling effort (however, this is unlikely because SCUBA surveys were done annually, except in 2016, in Havre-Aubert since its initial discovery). The anticipated results of annual monitoring using collector arrays might provide additional information to interpret long-term patterns of *D. listerianum* detection in Îles-de-la-Madeleine.

The detection of *D. listerianum* late in the season in October (by SCUBA divers) in Havre-Aubert is consistent with the tendency of initial discoveries of invasive ascidians in Eastern Canada to be during the autumn months (Ma et al. 2016). Also around the same time, small colonies were observed on a few collector arrays retrieved in October 2017 as part of the government's monitoring program from the same site (N. Simard, *unpublished data*). Once this species becomes firmly established in the Havre-Aubert marina, the population will likely exhibit seasonal variability in abundance.

Identification methods used for Canadian ascidian records

Three methods of species identification have been accepted by peer-reviewed journals in reporting records of invasive ascidians, each with benefits,

limitations, and sources of error. First, visual examination of external features, often from *in situ* photographs, is the method most likely to lead to misidentification (*e.g.*, being mistaken for other congeners) due to overlapping morphological features or distinguishing features hidden behind debris. Nevertheless, the first record of *A. aspersa* in Eastern Canada (Moore et al. 2014) was based solely on identification of specimens from field photographs. Second, PCR-based assays targeting species from environmental samples are rapid and sensitive detection tools (*e.g.*, Willis et al. 2011). In fact, PCR-based assay contributed the first record of *D. listerianum* from coastal waters of Prince Edward Island (Ma et al. 2016). However, detection from environmental samples, particularly when physical specimens have yet to be observed, is susceptible to false positives due to PCR contamination (Darling and Mahon 2011). The third method for invasive ascidian identification in Eastern Canada is the evaluation and description of key morphological features; however, the reporting of identifications using this method has not been standardised and sometimes absent (*e.g.*, Callahan et al. 2010; Sargent et al. 2013). Until now, few early records of invasive ascidians have provided detailed taxonomic descriptions of internal structures as conclusive evidence of detection (*e.g.*, Ma et al. 2018). In our report, we provided photographs and descriptions of our taxonomic examinations of key internal structures of two invasive ascidian species. Therefore, we advocate that morphological identification and reporting of invasive ascidian species should not be limited to external features, particularly since ascidians have wide variation in morphology and features are commonly veiled by sediment or epibionts. Furthermore, the best practice is to utilise multiple (if not all three) methods to provide evidence of species identification. The detection of *D. vexillum* in Nova Scotia (Moore et al. 2014; Vercaemer et al. 2015) is a prime example of the use of multiple methods (*i.e.*, examination of spicules and PCR-based assays of morphologically identified specimens) to confirm the identity of a potentially high-impact invasive species.

Conclusions

Historical records and the circumstances leading to the early detections of invasive species are invaluable for re-constructing biological invasions. Therefore, it is critical to confirm the identities of invasive species as early as possible to avoid the possibility of misidentification and mismanagement of invasive species. An invasive species should be properly identified to reliably distinguish it from similar species from anywhere in the world (*e.g.*, congeners). For instance, there are three species belonging to the *Ascidiella* genus and 43 species belonging to the *Diplosoma* genus (WoRMS Editorial Board 2018). Furthermore, species confirmations of early detections using the best available tools is a vital step to rapidly responding to biological invasions, *e.g.*, the discovery of *D. vexillum* in

Eastern Canada (Moore et al. 2014). We provide species confirmations based on the morphology of two invasive ascidians (*A. aspersa* and *D. listerianum*) that are in the process of invading Eastern Canada. In particular, a more rigorous approach of species identification is critically missing for *A. aspersa* from this region. Our report provides a snapshot of the rapid invasion of *D. listerianum* into the southwest region of the Bay of Fundy two years after its initial detection via government monitoring in 2016. We also document the re-appearance of *D. listerianum* in the coastal waters of Îles-de-la-Madeleine, Quebec, after a six-year hiatus. These early patterns of detection suggest that Eastern Canada is at risk of further regional spread and re-introduction of these two invasive ascidians unless appropriate eradication measures are taken (*e.g.,* Deibel et al. 2014).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Confirmed records of *Ascidiella aspersa* and *Diplosoma listerianum* from eastern Canada by means of morphological methods.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_Ma_etal_Table_S1.xlsx

Article 5. Biogeographical patterns of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39º and 47º north latitude (New Jersey to Newfoundland)

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Research Article

Biogeographical patterns of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39° and 47° north latitude (New Jersey to Newfoundland)

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Abstract

Colonization of eelgrass (*Zostera marina* L.) by tunicates can lead to reduced plant growth and survival. Several of the tunicate species that are found on eelgrass in the northwest Atlantic are highly aggressive colonizers, and range expansions are predicted in association with climate-change induced increases in seawater temperature. In 2017, we surveyed tunicates within eelgrass meadows at 33 sites from New Jersey to Newfoundland. Eight tunicate species were identified colonizing eelgrass, of which four were non-native and one was cryptogenic. The most common species (*Botrylloides violaceus* and *Botryllus schlosseri*) occurred from New York to Atlantic Canada. Tunicate faunas attached to eelgrass were less diverse north of Cape Cod, Massachusetts. Artificial substrates in the vicinity of the eelgrass meadows generally supported more tunicate species than did the eelgrass, but fewer species co-occurred in northern sites than southern sites. The latitudinal gradient in tunicate diversity corresponded to gradients of summertime sea surface temperature and traditional biogeographical zones in the northwest Atlantic, where Cape Cod represents a transition between cold-water and warm-water invertebrate faunas. Tunicate density in the eelgrass meadows was low, ranging generally from 1–25% cover of eelgrass shoots, suggesting that space availability does not currently limit tunicate colonization of eelgrass. This survey, along with our 2013 survey, provide a baseline for identifying future changes in tunicate distribution and abundance in northwest Atlantic eelgrass meadows.

Key words: regional study, *Zostera marina*, fouling organisms, Ascidiacea, introduced species

Introduction

The prevalence and distribution of non-native tunicates on a wide variety of substrates in the northwest Atlantic has been increasing (Bullard et al. 2007; Locke et al. 2007; Sargent et al. 2013; Moore et al. 2014; Carman et al. 2016). In addition, expansion to new substrates, such as the submerged vascular plant *Zostera marina* Linneaus, 1753 (eelgrass) has been documented for a number of tunicate species (Carman and Grunden 2010; Carman et al. 2014). Colonization of eelgrass by tunicates can cause reduced growth, production, and even mortality of eelgrass shoots (Wong and Vercaemer 2012; Long and Grosholz 2015).

Eelgrass is considered a foundational species that serves many critical ecological functions. It is highly productive and is widely recognized for its importance in coastal food webs and as a nursery and refuge habitat for many fish and invertebrate species (Thayer et al. 1985; Heck et al. 1995; Lazzari et al. 2003). Eelgrass stabilizes sediments and provides some shoreline protection and erosion control (Thayer et al. 1985). In addition, by sequestering large quantities of carbon, eelgrass may buffer climate change (Fourqurean et al. 2012; Colarusso et al. 2016a). Distribution and abundance of eelgrass has declined substantially in the northwest Atlantic from direct physical disturbance by dredging, dock and pier construction, and damage from boats and moorings (Burdick and Short 1999; Orth et al. 2017) and indirect effects of watershed disturbance from development that leads to reduced water clarity (Hauxwell et al. 2003; Lee et al. 2007). Colonization of eelgrass by tunicates increases stress on eelgrass plants, which could hasten losses of this important habitat.

In 2013, the first tunicate survey of northwest Atlantic eelgrass meadows was conducted to determine the extent of colonization in the region (Carman et al. 2016). In that survey, twenty-one sites between Newfoundland and New Jersey were surveyed, and a total of 8 species of tunicates were found colonizing eelgrass. Four of those 8 colonizers are considered non-native. The most common tunicates present were *Botryllus schlosseri* (Pallas, 1766) and *Botrylloides violaceus* Oka, 1927. In general, tunicate coverage of eelgrass shoots was < 25%, though it approached 75–100% in some locations, and several of the tunicate species found on eelgrass at that time (*B. schlosseri, B. violaceus, Ciona intestinalis* (Linneaus, 1767), and *Didemnum vexillum* Kott, 2002) are considered highly aggressive colonizers (Carver et al. 2006; Bullard et al. 2007; Epelbaum et al. 2009).

Among marine fouling communities, seawater temperature governs species growth rates and competitive abilities (Lord 2016), and temperature increases are expected to strongly influence changes in community composition (Hansen et al. 2006; Lord and Whitlatch 2015). Water temperatures are expected to increase 2–4 °C in the northwest Atlantic by 2100 (IPCC 2007). The 2013 eelgrass tunicate survey showed fewer tunicate

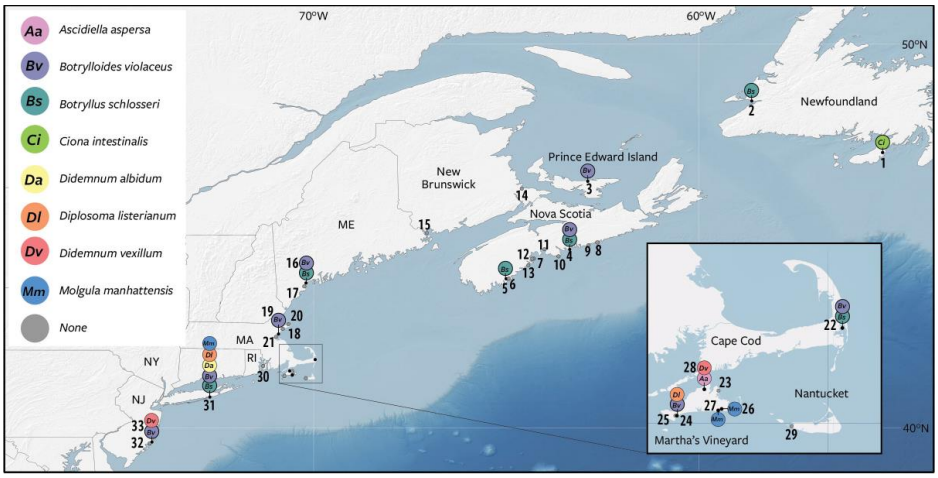


Figure 1. Map of study sites and tunicate species occurrences on eelgrass.

species in northern than in southern eelgrass meadows (Carman et al. 2016). Range expansions are predicted for non-native and native species in association with climate-induced changes in seawater temperature (Parmesan et al. 2005; Rius et al. 2014). Colder sites, often characterized by more open space available, are likely the most susceptible to future invasions. As climate change extends the ranges of potential invaders (Lord 2016), eelgrass may provide substrate for expansion of tunicate species. Thus, northern eelgrass meadows may see increases in the number of tunicate species and in tunicate overgrowth on eelgrass shoots. The purpose of this study was to improve understanding of the distribution, diversity, and abundance of tunicate species colonizing eelgrass along the northwest Atlantic coast. We revisited 11 sites that were surveyed in 2013, while adding more sites to the latitudinal gradient between New Jersey and Newfoundland. The result is an expanded baseline that can be used to predict and identify future changes in the utilization of eelgrass as substrate by tunicates.

Materials and methods

Eelgrass meadows were surveyed for tunicates at 33 sites between June and October 2017 (Figure 1, Supplementary material Table S1). In many cases, the tunicate surveys capitalized on existing eelgrass research and monitoring projects underway in the region. Methods for quantifying tunicates were comparable among sites but were not standardized. Estimates of tunicate cover on eelgrass were made by recording systematic underwater observations (snorkel, SCUBA, or wading) or evaluating replicate quadrats (0.25 m × 0.25 m) positioned randomly within meadows or along 50- to 100-m transects across meadows. Eelgrass and tunicate

measurements were made in situ or in the lab on harvested quadrat contents. Cover of eelgrass by each tunicate species present was estimated visually within 5 classes (0, 1–25, > 25–50, > 50–75, > 75–100 percent cover). Some investigators also documented eelgrass density (number of shoots/area) and canopy height (distance from the sediment to the top of the canopy, ignoring the tallest 20% of the leaves, following Duarte and Kirkman 2001). In addition, the tunicate faunas on the artificial substrates nearest to the surveyed eelgrass meadows were documented at 9 sites where both eelgrass and artificial substrate types were present (Table S1). General spatial patterns of average monthly sea-surface temperatures in 2017 were examined from satellite data collected at 1-km resolution by the NASA Earth Observing System Moderate Resolution Imaging Spectroradiometer (MODIS; NASA 2019).

Study site characteristics

Eelgrass meadows were surveyed for tunicates at 33 sites (Table S1). An additional 2 sites that formed part of the 2013 survey were visited in 2017 (intertidal zone off Town Neck Beach, Sandwich, MA; Lagoon Pond on Martha's Vineyard, MA; Carman et al. 2016) but eelgrass was no longer present, therefore these sites are not included in this compilation. Where measured, the average density of eelgrass ranged from 46 to 1030 shoots/m^2 and the average canopy height ranged from 18 to 84 cm.

Newfoundland

Little Bay (site #1)

This site is a small protected, enclosed embayment located within Mortier Bay, which is on the western side of Placentia Bay, with a tidal range between 1 and 1.5 m and a fetch of 0.40 km in the prevailing wind direction (SW). Surveys were conducted along a shallow transect (2 m deep), mid-depth transect (3 m deep), and deeper transect (3–4 m deep). In Little Bay, eelgrass is generally found within a narrow band (< 50 m wide) along the shoreline in water depths ranging from 0 to 6–7 m. It is also found throughout Mortier Bay in similar, shallow subtidal habitats.

Little Port Harmon (site #2)

This site is located in a channel that connects a tidal pond to St. George's Bay in southwestern Newfoundland. The channel remains connected to the ocean throughout the tidal cycle; the maximum spring tide is 1.6 m above absolute low. The sampled eelgrass meadow ranges from 0 to 5 m deep; there are additional patches of eelgrass throughout the intertidal/subtidal areas of Port Harmon.

Prince Edward Island

Savage Harbour (site #3)

This site is an enclosed embayment connected by a narrow channel to the Gulf of St. Lawrence, on the north shore of Prince Edward Island. The

embayment has a surface area of approximately 415 hectares (ha), a maximum depth of 3.65 m, and a tidal range of 0.9 m. There are patches of eelgrass throughout the embayment.

Nova Scotia

Lower Three Fathom Harbour (site #4)

This site is a shallow lagoon of approximately 39 ha, located on the eastern shore of Nova Scotia northeast of Halifax. The seagrass bed is located approximately 0.8 km from a narrow opening to the Atlantic Ocean. The 8 ha eelgrass bed is patchy, in soft sediment with a gravel underlayer. Water depth at low tide is approximately 0.5 m with a tidal range of 1 m.

Port l'Hebert (site #5)

This site is near the head of Port l'Hebert Bay on the south shore of Nova Scotia and is located in a migratory bird sanctuary. The 490 ha seagrass bed is in very shallow water, in muddy sediments. Eelgrass occurs intertidally to about 0.2 m at low tide, with a tidal range of 1.5 m.

Port Joli (site #6)

This site is near the head of Port Joli Bay on the south shore of Nova Scotia and is located in a migratory bird sanctuary. The eelgrass bed is about 164 ha and inhabits both sandy and muddy sediments. Eelgrass in both sediment types were surveyed for tunicates. The bed is exposed to about 0.2 m water at low tide and has a tidal range of about 1.5 m.

Sacrifice Island (site #7)

This site is located off of Back Harbor, Lunenburg, on the south shore of Nova Scotia. The eelgrass bed is approximately 3 ha and ranges in depth from 1.5 to 6 m at low tide. Both shallow (1.5 m) and deep (5 m) portions of the bed were surveyed.

Taylor's Head (site #8)

This site is located on the eastern shore of Nova Scotia and is in a provincial park. The eelgrass bed is extensive and ranges from 1.5 to 5 m deep at low tide. Both shallow (1.6 m) and deep (4.3 m) portions of the bed were surveyed.

Cable Island (site #9)

This site is located outside of Ship Harbor on the eastern shore of Nova Scotia. It is about 16 ha and ranges from 0.5 to 3.5 m deep. Both shallow (0.7 m) and deep (2.7 m) portions of the eelgrass bed were surveyed.

Inner Sambro Island (site #10)

This site is located at the mouth of Sambro Harbor and is influenced by oceanic conditions. It is subjected to a strong current and sediments are sandy. The eelgrass bed ranges from 2 to 6 m deep. Both shallow (2 m) and deep (5 m) portions of the bed were surveyed. The bed size is approximately 2.3 ha.

Croucher Island (site #11)

This site is located in St. Margaret's Bay, on the south shore of Nova Scotia. It has a small eelgrass bed of about 0.75 ha. Sediments are comprised of sandy-mud with some cobbles and small gravel. Depth ranges from 1.5 to 6 m deep. The narrow bed is located on a steep shoreline. Both shallow (2 m) and deep (5 m) portions of the bed were surveyed.

Mason's Island (site #12)

This site is located in Back Harbor, Lunenburg, on the south shore of Nova Scotia. The eelgrass bed is very shallow, close to intertidal at low tide and 2 m at high tide. The bed is quite extensive but total areal coverage is unknown. Surveying occurred at 1.5 m depth.

La Have Islands (site #13)

This site has an eelgrass bed that is located on the landward side of Cape La Have Island, in the La Have Island region on the south shore of Nova Scotia. The eelgrass bed is subjected to strong tidal currents. Depth ranges from 1 to 2 m, and the area surveyed was 1 m deep at low tide. The bed is extensive but total areal coverage is unknown.

New Brunswick

Shediac (site #14)

This site is in a sheltered part of an estuarine embayment of about 20 square km that is part of the Northumberland Strait. It is located on the eastern coast of New Brunswick, at Shediac. The sampled eelgrass meadow is about 25 ha with a depth of about 1 m at low tide and is relatively sheltered from the open sea. In the sampled area, eelgrass was common and extends throughout the meadow. The maximum tidal range in the bay is 1.4 m.

St. Andrews (site #15)

This site is located in southern New Brunswick, along an exposed shore in the northwestern region of Passamaquoddy Bay. The bay is connected to the Bay of Fundy and has a maximum tidal range of about 8 m. Eelgrass was absent outside of a relatively small patch (0.12 ha) at a water depth of < 1 m at low tide.

Maine

Broad Cove, Casco Bay (site #16)

This site is a shallow, 250 ha embayment along the western shore of Casco Bay near Cumberland Foreside and has a tidal range of about 4 m. Eelgrass extends continuously from the low intertidal zone to depths of about 3 m below mean low water and covers about 80 ha of the cove.

East End Beach, Portland Harbor (site #17)

This site extends from < 1 m to approximately 3 m (mean low water). The meadow is in a relatively sheltered harbor and encompasses approximately

12 ha. Tidal range in Portland Harbor is approximately 4 m. Bottom sediments are generally silty mud.

Massachusetts

Beverly (site #18)

This site is located on the northern shore of Salem Sound. The 97 ha patchy eelgrass meadow extends seaward between West Beach and Misery Island. The offshore edge of the meadow is blocked from direct exposure to the open ocean by Misery Island. The meadow is approximately 3 m deep (mean low water) with an approximate tidal range of 2.7 m. The bottom is sandy with areas of underlying clay and pockets of silty sand.

Nahant (site #19)

This site covers most of a cove on the southwestern side of the peninsula and is exposed to the ocean. The eelgrass meadow at Nahant is approximately 1.5 ha and occurs at water depths from 1 to 5 m (mean low water); tidal range is approximately 3.5 m.

Niles Beach, Gloucester (site #20)

This site parallels a sandy beach in Gloucester Harbor. The eelgrass meadow lies in a depth range of 1 to 5.5 m (mean low water) and is exposed to the sea to the southwest. This is a large meadow of approximately 21 ha and is very patchy throughout most of this area. Tidal range in Gloucester Harbor is approximately 3.5 m.

Long Island, Boston Harbor (site #21)

This site is on the south side of Long Island in Boston Harbor near the mouth of Boston Harbor. The eelgrass is growing on the lee side of the island. The meadow covers a narrow depth range from approximately 1.5 to 3 m (mean low tide) and covers approximately 4 ha. Tidal range is approximately 3.5 m.

Little Pleasant Bay, Cape Cod (site #22)

This site lies at the upper end of the Pleasant Bay estuary which connects to the Atlantic Ocean through a barrier beach. It is the largest coastal embayment on Cape Cod. Eelgrass occurs throughout most of the Little Pleasant Bay. The mean tidal range is approximately 1.5 m. Surveys were conducted along 3 transects parallel to the shoreline. Water depths (mean low water) were 1.35 m at the deepest transect, 0.84 m at the mid-depth transect, and 0.64 m at the shallow transect.

Farm Pond, Martha's Vineyard (site #23)

This site is a 13 ha pond at Oak Bluffs on the northeast coast of Martha's Vineyard. It has restricted access to the sea via a culvert. The eelgrass meadow (5.3 ha) lies at water depths up to 1.5 m in a tidal range of < 1 m. Maximum water depth is 1.5 m.

Stonewall Pond, Martha's Vineyard (site #24)

This site is a shallow coastal pond in the backwaters of the Menemsha Pond system located at Aquinnah on the southwest coast of Martha's Vineyard. It is connected to Vineyard Sound via Nashaquitsa Pond and Menemsha Pond and lies in an area of low anthropogenic impact. Water depths are < 3 m, and the tidal range is < 1 m. Eelgrass occurs throughout the pond.

Nashaquitsa Pond, Martha's Vineyard (site #25)

This site is a shallow, protected coastal pond between Stonewall Pond and Menemsha Pond located on the southwest coast of Martha's Vineyard. Water depth is approximately 1–2 m (mean low water) throughout the pond. Eelgrass occurs in small patches at numerous locations within the pond.

Edgartown Great Pond, Martha's Vineyard (sites #26, 27)

These 2 sites are located in Great Pond, a 360 ha brackish coastal pond located on the south shore of Martha's Vineyard. It is separated from the Atlantic Ocean by a barrier beach that is artificially breached 3–4 times per year. Water depth varies, with the deepest areas about 3 m deep. Slough Cove (site #26) is narrow and shallow, with freshwater input from groundwater at the head of the cove. Mid-Beach (site #27) is in a large open basin located just north of the barrier beach, thus it tends to be more saline than Slough Cove. Eelgrass occurs throughout most of the pond.

Lake Tashmoo (site # 28)

This site is a 109 ha elongate coastal pond on the north shore of Martha's Vineyard with access to Vineyard Sound via a narrow channel. Water depth is up to 3 m and the tidal range is < 1 m. Eelgrass occurs in patches throughout the pond.

Jackson Point, Nantucket (site #29)

This site is a shallow coastal area along the west coast of Nantucket that is open to Nantucket Sound on the northwest. Water depths are < 3 m, and the tidal range is < 1 m. Eelgrass occurs throughout the area.

Rhode Island

Prudence Island, Narragansett Bay (site #30)

This site is located on the southwest side of the island, which is mid-bay in Narragansett Bay. The eelgrass meadow is approximately 6 ha in size and occurs in shallow water to a depth of approximately 2.5 m. Tidal range in Narragansett Bay is 1.2 m. There is a pile-supported pier and large rock outcroppings on the north and western side of the meadow that supports extensive tunicate growth.

New York

Moriches Bay, Long Island (site #31)

This site is an embayment on Long Island's south shore and is located behind a barrier beach that separates it from the Atlantic Ocean. The

eelgrass meadow is located approximately 3 km east of Moriches Inlet in Westhampton Dunes. The meadow is shallow, at depths < 2 m, with a tidal range of < 1 m.

New Jersey

Middle Sedge and Ham Island, Barnegat Bay (sites #32, 33)

These sites are located in the southern part of Barnegat Bay, which is a long, narrow tidal basin that is separated from the Atlantic Ocean by a series of barrier beaches. It is shallow (1.5 m average depth) with a limited tidal range (~ 0.2–1 m). Middle Sedge (site #32) had a water depth of 1.1 m with the eelgrass bed approximately 1 ha in size, while Ham Island (site #33), located about 8 km northeast of Middle Sedge, had a similar water depth, but lies within an expansive eelgrass mosaic in this region of Barnegat Bay.

Results

Eelgrass meadows

A total of 8 tunicate species were identified colonizing eelgrass at the 33 sites sampled (Table S1). Four are non-native species, including the solitary *Ascidiella aspersa* (Müller, 1776) and colonials *Botrylloides violaceus, Didemnum vexillum,* and *Diplosoma listerianum* (Milne Edwards, 1841). Two are native species, colonial *Didemnum albidum* (Verrill, 1871) and solitary *Molgula manhattensis* (De Kay, 1843). One species is cryptogenic, the colonial *Botryllus schlosseri* and 1 species is considered native in the US and non-native in Canada, the solitary *Ciona intestinalis* (Figure 1). The species with the greatest latitudinal ranges were *B. schlosseri* (5 sites, from New York to Newfoundland) and *B. violaceus* (7 sites, from New Jersey to Prince Edward Island). The least common species were *D. albidum* and *A. aspersa* (1 site each) and *D. listerianum* (2 sites). Tunicate faunas attached to eelgrass were less diverse north of Cape Cod, Massachusetts, where most individual survey sites exhibited a single species and several sites exhibited 2 species. From Cape Cod south, most sites surveyed exhibited from 2 to 5 tunicate species. The average eelgrass density was greater north of Cape Cod (414 shoots/m^2) than south of Cape Cod (259 shoots/m^2). The average eelgrass canopy height was also greater north of Cape Cod (49 cm) than south of Cape Cod (31 cm; Table S1).

Where tunicates occurred on eelgrass, the average tunicate coverage was low; the average percent cover of tunicates on eelgrass was 1–25% (Table S1). At 19 sites, no tunicates were found attached to eelgrass. Within-site distribution of tunicates was patchy, and some eelgrass quadrats were observed with tunicate cover of 25–50% (Lower Three Fathom Harbour, NS; Moriches Bay, NY) and 50–75% (Slough Cove in Edgartown Great Pond, MA; Ham Island, NJ).

The depth distribution of tunicates attached to eelgrass ranged from the low intertidal zone to depths of about 4 m. The shallowest occurrence among the sites surveyed was at Broad Cove, in Casco Bay, Maine (Table S1, site #16), where *B. violaceus* and *B. schlosseri* were found at very low abundance in the low intertidal and shallow subtidal portion of the eelgrass bed. The deepest occurrence was at Nahant (Table S1, site #19), where *B. violaceus* was found on eelgrass growing at 4 m (MLW), which is close to the deepest edge of the eelgrass bed at that site.

Artificial substrates

Where surveyed, artificial substrates generally supported more tunicate species than eelgrass within the same site (Table S1). At some sites, the difference in tunicate diversity between substrate types was quite dramatic; for example, at Nashaquitsa Pond, MA (Table S1, site #25) 4 tunicate species were found attached to mooring lines, whereas none were found attached to eelgrass. The general latitudinal gradient in tunicate diversity on artificial substrates mirrored that on eelgrass, with fewer species co-occurring within northern sites than within southern sites.

Sea surface temperature patterns

Sea surface temperature increased predictably from the northern to southern limit of the survey during the warmer months of the year. From May through November, the coastal surface waters north of Cape Cod, MA were markedly cooler than the coastal waters south of Cape Cod (Figure 2).

Discussion

Most of the tunicate species found on eelgrass in this survey were non-native or cryptogenic species and the majority of these are very aggressive colonizers (*B. schlosseri, B. violaceus, D. vexillum, D. listerianum,* and *C. intestinalis*). The 2 native species occurred only in Edgartown Great Pond, MA (*M. manhattensis*) and Moriches Bay, NY (*D. albidum* and *M. manhattensis*). Although space often limits the settlement of tunicates in coastal habitats (Whitlatch and Osman 2009; Lord 2017) and tunicate cover on artificial substrates is often near or at 100% (Valentine et al. 2016; Lord 2016), we found cover on eelgrass shoots to be generally 1–25% throughout the northwest Atlantic meadows we surveyed. Tunicate spatial distribution in eelgrass was patchy and tunicates were not found in all eelgrass beds across the latitudinal gradient. This is consistent with earlier studies (Wong and Vercaemer 2012; Carman et al. 2016; Colarusso et al. 2016b) and suggests that space availability does not currently limit tunicate colonization in eelgrass habitat.

During spring and summer, when tunicate larvae are colonizing open space, eelgrass is routinely creating new substrate by defoliating and growing

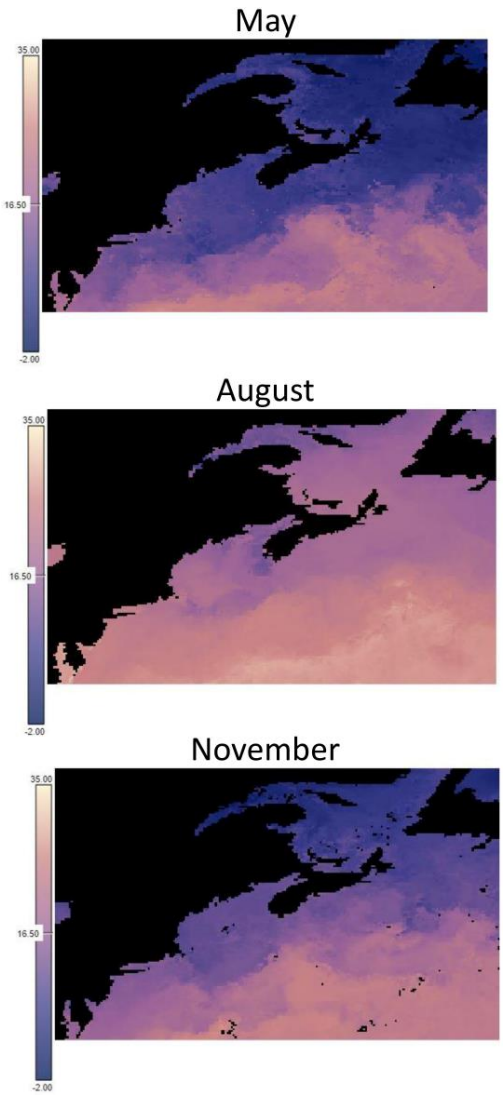


Figure 2. Average monthly sea surface temperatures in the study region during May, August, and November 2017. Scale is degrees C; data and images from NASA Earth Observations (NASA 2019).

new leaves. Defoliated eelgrass blades (with tunicate epifauna) either settle on the seafloor where the tunicates can continue to live and grow (Carman and Grunden 2010; Carman et al. 2014, 2016) or float on the water's surface providing a rafting vector for tunicate distribution to new locations (Worcester 1994). Thus, eelgrass may provide substrate for range expansion of tunicate species.

The latitudinal gradient in tunicate diversity we observed corresponds to traditional biogeographical zones of the northwest Atlantic, where Cape Cod represents a transition zone between northern, cold-water invertebrates of the Acadian Biogeographic Province and southern, warm-water invertebrates of the Virginian Biogeographic Province (Hale 2010). This pattern correlates with gradients of summertime sea surface temperature, with cooler temperatures north of Cape Cod (Figure 2). Temperature affects tunicate metabolism and growth, space acquisition, and reproductive output. For example, warmer regions of the temperate zone such as Japan and the Mediterranean experience continuous spawning by *C. intestinalis*, producing up to 4 generations annually, but the cooler temperate regions such as Atlantic Canada experience 2 spawning events per year (Harris et al. 2017). Ultimately, rapid growers that can overgrow neighbors (Osman 2015) and reproduce earlier or later than others in the community have a competitive advantage (Stachowicz et al. 2002), and there is indication that some non-native species may benefit from predicted increases in global seawater temperatures. During a 12-year study of tunicate recruitment, Stachowicz et al. (2002) found that *A. aspersa, B. violaceus,* and *D. listerianum* recruited earlier in warmer years, whereas native tunicates showed no such relationship between water temperature and onset of recruitment. Mesocosm experiments show that tunicate growth responses to elevated temperature are greater in the cooler parts of their range (Lord and Whitlatch 2015), suggesting that *B. violaceus* will likely increase in abundance in the northern portion of our survey as seawater temperatures rise.

Warming seawater temperatures are also expected to enable the arctic-ward range expansion of invasive, temperate tunicates. For example, *A. aspersa, D. listerianum, D. vexillum,* and *Styela clava* Herdman, 1881, have recently spread from northern New England to Atlantic Canada via shipping, boating, rafting, and fragmentation (Moore et al. 2014). *Ciona intestinalis* has similarly spread poleward, from Maine (Trott 2004) to Nova Scotia in 1997 (Carver et al. 2003) to Prince Edward Island in 2004 (Ramsay et al. 2009) to Newfoundland in 2012 (Sargent et al. 2013; McKenzie et al. 2016), a range expansion of about 1,000 km in about 15 years. *Didemnum vexillum* fragments on eelgrass have the ability to reattach and grow on new substrates in temperatures as low as 6 °C (Carman et al. 2014), implying that rafting fragments drifting northward in colder temperatures could survive, asexually reproduce, and colonize. Invasive species of tunicates in North Carolina, south of our study area, that are more abundant in the colder months, such as *Polyandrocarpa anguinea* (Sluiter, 1898) and *P. zorritensis* (Van Name, 1931), may spread northward into southern New England as temperatures warm (Villalobos et al. 2017). Interestingly, although tunicate assemblages similar to those reported in our survey occur on hard substrates of coastal North Carolina and Puget Sound, tunicates were not found attached to eelgrass in those regions

(Susanna Lopez-Legentil and Jeff Gaeckle *pers. comm.*), but have been recorded elsewhere on the US west coast in California (Worcester 1994; Williams 2007; Long and Grosholz 2015). Conditions have already favored tunicate expansion to eelgrass in the northwest Atlantic. Understanding the mechanisms of thermal adaptation and responses to stressors will help improve predictions of future distribution of these species (Woodin et al. 2013).

To best conserve the critical ecosystem services that eelgrass meadows provide, natural resource managers need information on the magnitude of existing and potential threats to this species. The data reported in our survey provide a baseline for identifying changes in tunicate cover of eelgrass. Continued systematic monitoring has provided for an expanded and important understanding of tunicate populations within eelgrass beds, a valuable and severely threatened coastal habitat. Rapidly warming ocean temperatures may enhance tunicate abundance on eelgrass substrate in northern latitudes. Because of the negative impacts of tunicates on seagrasses (Wong and Vercaemer 2012; Long and Grosholz 2015; Colarusso et al. 2016b), consistent monitoring of sentinel eelgrass sites would allow increased threats to eelgrass from tunicate cover to be detected.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Locations of study sites, dates of observations of eelgrass meadow properties, and tunicate occurrences.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2019/Supplements/MBI_2019_Carman_etal_SupplementaryTable.xlsx

Article 6. Reconstructing the distribution of the non-native sea anemone, *Diadumene lineata* (Actiniaria), in the Canadian Maritimes: Local extinction in New Brunswick and no regional range expansion in Nova Scotia since its initial detection

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Reconstructing the distribution of the non-native sea anemone, *Diadumene lineata* (Actiniaria), in the Canadian Maritimes: Local extinction in New Brunswick and no regional range expansion in Nova Scotia since its initial detection

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ABSTRACT

Since its first observations in New Brunswick (Sam Orr's Pond) and Nova Scotia (Bedford Institute of Oceanography in Dartmouth) in 2009 and 2013, respectively, *Diadumene lineata* has been observed spreading to new locations in the Canadian Maritimes. In 2017 and 2018, regional surveys for non-native species did not detect *D. lineata* in New Brunswick, suggesting local extinction. Recent surveys also did not detect *D. lineata* in Prince Edward Island. However, between 2013 and 2018, *D. lineata* was detected in Nova Scotia (in chronological order) from Lunenburg Harbour (Lunenburg), Hermans Island (Lunenburg), Black Rock Beach (Halifax), Oak Island Marina (Chester), and Dartmouth Yacht Club (Dartmouth). These new observations combine to reconstruct the known recent distribution of this non-native sea anemone in the Canadian Maritimes. Further, after five years since its first detection in Nova Scotia, the regional distribution of *D. lineata* remained unchanged, restricted to a region of approximately 100 km between Halifax and Lunenburg. Although the primary focus is on *D. lineata*, additional records of the non-native macroalga, *Codium fragile*, from Sam Orr's Pond and the native sea anemone, *Metridium senile*, fouling dock structures throughout the Canadian Maritimes are included in this report as *M. senile* commonly overlaps in habitat with *D. lineata*.

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1. Introduction

The orange-striped green sea anemone, *Diadumene lineata* (Verrill, 1869), is an infralittoral species considered to be native to China and Japan, where it was first described (Verrill, 1869; Uchida, 1932). It is now known as one of the most widely distributed sea anemones in the world, as it has been introduced via a variety of ways, including (a) the translocation of fouled boat hulls, bivalve aquaculture products, and aquaculture equipment, (b) ballast water exchange, and (c) long-distance rafting on anthropogenic debris (Verrill, 1898; Shick, 1976; Shick and Lamb, 1977; Gollasch and Riemann-Zürneck, 1996; Carlton et al., 2017).

Diadumene lineata is a relatively small, solitary sea anemone with a maximum diameter of up to 22 mm at the base, although bodies ranging from 5 to 10 mm in diameter are typically encountered (Uchida, 1932; Hand, 1955; Fautin and Hand, 2007; Fautin et al., 2009). The smooth columnar body is greyish-green in colour with thin, orange or white longitudinal stripes (Uchida, 1932; Hand, 1955). In its native range, up to four colour morphs have been described, which vary in stripe size, colour, and arrangement (Uchida, 1936; Ryan and Kubota, 2016). Tentacles are slender and typically number about 25 but can be as many as 100 (Carlgren and Hedgepeth, 1952; Fautin and Hand, 2007). Individuals expel defensive acontia when disturbed (Uchida, 1932). A native congener, *Diadumene leucolena* (Verrill, 1866) is known from the Canadian Maritimes (Thomas et al., 1983); it can be distinguished from *D. lineata* by its transparent or pinkish body, large cinclides, and the absence of coloured stripes on the body column.

Diadumene lineata inhabits marine and estuarine environments tolerating a wide range of salinities (5 to 35 psu) and water temperatures (0 to 27.5 °C), which contributes to its status as a global invader (Shick, 1976; Seaton, 1985; Konecny and Harley,

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2019). Outside its native range, *D. lineata* mainly reproduces asexually either by longitudinal fission or pedal laceration (Shick, 1976; Ting and Geller, 2000). However, mixed sex populations containing eggs and sperm have been observed along the east coast and west coast of the United States (Newcomer et al., 2019; Ryan and Miller, 2019). The planula larvae have a disposition to attach to boat hulls that they encounter, suggesting that this is one of the main introduction vectors (Gollasch and Riemann-Zürneck, 1996).

Throughout the world, *D. lineata* and the sea anemone, *Metridium senile* (Linnæus, 1761) are often found as part of the fouling community on artificial structures in marinas and harbours (Griffiths et al., 1996; Hancock et al., 2017). Both species have a cosmopolitan distribution but, at present, *D. lineata* has not been detected from southern Africa, Australia, the Arctic Ocean, and the Southern Ocean and *M. senile* has not been detected from Oceania, and the Southern Ocean (Hancock et al., 2017; OBIS, 2019). *Diadumene lineata* can be easily distinguished from *M. senile* based on external features: *M. senile* has a long, smooth columnar body with numerous slender tentacles, giving a feathery appearance in larger specimens. Its body colour is uniform and ranges from orange to pink to white (Fox and Pantin, 1941). The parapet, in the distal region of the column, typically forms a prominent collar-like ring (Batham and Pantin, 1951; Fautin and Hand, 2007).

In the Canadian Maritimes, *D. lineata* was first detected in October 2009 as a non-native species at Sam Orr's Pond, New Brunswick (Saunders et al., 2013). In November 2013, *D. lineata* was documented for the second time in the Canadian Maritimes off a dock at the Bedford Institute of Oceanography in Dartmouth, Nova Scotia (Moore et al., 2014).

This study consolidates several regional surveys that detected *D. lineata* since the records of Saunders et al. (2013) and Moore et al. (2014) to better understand its distribution in the Canadian Maritimes (New Brunswick, Nova Scotia, and Prince Edward Island). Here, observations of *D. lineata* from five sources are documented: (a) 2013 benthic community surveys in Lunenburg, Nova Scotia, (b) 2017 surveys throughout New Brunswick targeting *D. lineata*, (c) a 2017 survey of Sam Orr's Pond, New Brunswick targeting *D. lineata* and another non-native species, *Codium fragile* (Suringar) Hariot, 1889, (d) a 2017 observation from Halifax, Nova Scotia, contributed by a citizen scientist, and (e) 2018 surveys throughout the Canadian Maritimes targeting *D. lineata* and *M. senile*.

2. Methods

2.1. 2013

2.1.1. Artificial structures

In Nova Scotia, sampling of benthic species was done at the Lunenburg Yacht Club on Hermans Island (44.4109°N; 64.3218°W), and along two wooden wharves in Lunenburg Harbour (44.3755°N; 64.3095°W). Settlement plates were deployed at random locations within each site, suspended from floating docks (N = 50 plates in Hermans Island and N = 6 plates in Lunenburg Harbour) on 20 July and retrieved on 19 September. Between 18 and 19 September, the same number of quadrats per site were scraped by SCUBA divers from the undersides of floating docks and the vertical sides of wooden pilings. The dimensions of the quadrats and settlement plates both measured 20 × 20 cm in size and, upon collection, benthic samples were transported to a laboratory where they were maintained in flowing seawater holding tanks until taxonomic examination (see Section 2.4 below). Only observations of *D. lineata* and *M. senile* from these settlement plates and benthic quadrats were used for this report.

2.2. 2017

2.2.1. Artificial structures

At each of the 20 sites throughout New Brunswick (Table 1), a systematic survey of the undersides of floating docks as viewed from the water surface at the edge of floating docks was done between 1 and 9 October. This site-level survey was followed by a detailed examination of 20 different 1-m wide dock sections for the presence and absence of *D. lineata*. Only the presence of *M. senile* was noted from these sites in New Brunswick (i.e., no data on their absences).

2.2.2. Re-survey of Sam Orr's Pond

As the original site of the initial reports of *D. lineata* in the Canadian Maritimes and *C. fragile* in the southwest New Brunswick region of the Bay of Fundy, the shallow waters of Sam Orr's Pond (45.1583°N; 67.0442°W) were re-surveyed for both non-native species during low tide on 19 October 2017.

2.2.3. iNaturalist species record

On 6 October 2017, an observation of a sea anemone (later identified as *D. lineata*) attached to the chela of *Cancer borealis* Stimpson, 1859 was made by a citizen scientist at Black Rock Beach, Point Pleasant Park in Halifax, Nova Scotia (44.6247°N; 63.5640°W). The observation, accompanied by a photograph of the specimen, was uploaded to iNaturalist (https://www.inaturalist.org/), which is an online database of species records and social network to crowdsource taxonomic identifications of these records. The iNaturalist network and other similar citizen science databases were further searched for potential photographic observations of the species in the Canadian Maritimes.

2.3. 2018

2.3.1. Artificial structures

Surveys of the undersides of floating docks as viewed from the water surface were done at six sites in New Brunswick, 21 sites in Nova Scotia, and eight sites in Prince Edward Island for the occurrence and abundance of two species of sea anemones, *D. lineata* and *M. senile*, between 28 September and 7 October 2018 (Table 1). Sea anemone abundance was classified using the semi-quantitative SACFOR scale, which consist of six categories ranging from super-abundant to rare (Connor and Hiscock, 1996).

2.4. Morphological identification

The identities of *D. lineata* and *M. senile* specimens collected live (2013 survey) and those observed and photographed *in situ* (2017 and 2018 survey) were confirmed by examination of external morphological features, the method also used by Griffiths et al. (1996), Saunders et al. (2013), Moore et al. (2014), and Hancock et al. (2017). Similarly, the identities of specimens of *M. senile* observed *in situ* were based on external features. The macroalga *C. fragile* was identified morphologically by its spongy, cylindrical, finger-like, green thalli that branches dichotomously (Hubbard and Garbary, 2002).

3. Results

3.1. New Brunswick

No new records of *D. lineata* were made as a result of the 2017 and 2018 surveys of submerged portions of dock structures (Table 1). In Sam Orr's Pond, no new observations of *D. lineata* were made (Table 1); however, *C. fragile* continued to be present at this site as of October of 2017 (Fig. 1).

Table 1
Presence and absence of the non-native sea anemone, *Diadumene lineata*, and the native sea anemone, *Metridium senile*, from the Canadian Maritimes.

Site	Survey date(s)	Latitude (°)	Longitude (°)	Occurrence of *D. lineata*	Occurrence of *M. senile*	Observer[a]
New Brunswick						
Sam Orr's Pond	14 October 2009	45.1620	−67.0450	Present	No data	Saunders et al. (2013)
Shediac	01 October 2017	46.2274	−64.5448	Not detected	No data	KCKM
L'Etete	04 October 2017	45.0513	−66.8954	Not detected	No data	KCKM
St. Andrews Harbour	04 October 2017	45.0714	−67.0545	Not detected	Present	KCKM
Blacks Harbour	05 October 2017	45.0558	−66.7943	Not detected	Present	KCKM
Ingalls Head	05 October 2017	44.6626	−66.7563	Not detected	No data	KCKM
North Head	05 October 2017	44.7630	−66.7494	Not detected	No data	KCKM
Back Bay	06 October 2017	45.0559	−66.8632	Not detected	No data	KCKM
Beaver Harbour	06 October 2017	45.0688	−66.7387	Not detected	No data	KCKM
Boynes Cove	06 October 2017	45.1309	−66.4979	Not detected	No data	KCKM
Chance Harbour	06 October 2017	45.1226	−66.3514	Not detected	No data	KCKM
Fairhaven	06 October 2017	44.9640	−67.0080	Not detected	No data	KCKM
Lorneville	06 October 2017	45.1923	−66.1486	Not detected	No data	KCKM
Leonardville	07 October 2017	44.9717	−66.9527	Not detected	No data	KCKM
Lords Cove	07 October 2017	45.0060	−66.9466	Not detected	No data	KCKM
Seal Cove	07 October 2017	44.6491	−66.8385	Not detected	No data	KCKM
Stuart Town	07 October 2017	45.0190	−66.9377	Not detected	No data	KCKM
Dipper Harbour	08 October 2017	45.0943	−66.4180	Not detected	No data	KCKM
Five Fathom Hole	08 October 2017	45.1867	−66.2575	Not detected	No data	KCKM
Saint John	08 October 2017	45.2723	−66.0657	Not detected	No data	KCKM
St. Andrews Biological Station	09 October 2017	45.0825	−67.0848	Not detected	No data	KCKM
Sam Orr's Pond	19 October 2017	45.1583	−67.0442	Not detected	No data	KCKM
Richibucto	29 September 2018	46.6791	−64.8617	Not detected	Not detected	HEG
St. Andrews Harbour	30 September 2018	45.0715	−67.0544	Not detected	Present (abundant)	HEG
Beaver Harbour	01 October 2018	45.0684	−66.7385	Not detected	Present (abundant)	HEG
Dipper Harbour	01 October 2018	45.0938	−66.4180	Not detected	Present (abundant)	HEG
Seeleys Cove	01 October 2018	45.0938	−66.6355	Not detected	Not detected	HEG
Stuart Town	01 October 2018	45.0189	−66.9374	Not detected	Present (abundant)	HEG
Nova Scotia						
Hermans Island (quadrats)	18 September 2013	44.4109	−64.3218	Present (28% of quadrats)	Present (16% of quadrats)	HLH
Hermans Island (settlement plates)	20 July – 19 September 2013	44.4109	−64.3218	Not detected	Not detected	HLH
Lunenburg Harbour (quadrat)	19 September 2013	44.3755	−64.3095	Present (17% of quadrats)	Not detected	HLH
Lunenburg Harbour (settlement plates)	20 July – 19 September 2013	44.3755	−64.3095	Present (17% of plates)	Not detected	HLH
Bedford Institute of Oceanography	19 November 2013	44.6810	−63.6101	Present	No data	Moore et al. (2014)
Black Rock Beach	06 October 2017	44.6247	−63.5640	Present	No data	CNC
Brooklyn	02 October 2018	44.0505	−64.6898	Not detected	Not detected	HEG
Dennis Point	02 October 2018	43.6181	−65.7888	Not detected	Present (occasional)	HEG
Digby	02 October 2018	44.6267	−65.7539	Not detected	Present (rare)	HEG
Meteghan	02 October 2018	44.1939	−66.1668	Not detected	Not detected	HEG
Yarmouth	02 October 2018	43.8315	−66.1230	Not detected	Not detected	HEG
Armdale Yacht Club	03 October 2018	44.6350	−63.6137	Not detected	Present (abundant)	HEG
Gold River	03 October 2018	44.5470	−64.3166	Not detected	Not detected	HEG
Hubbards	03 October 2018	44.6379	−64.0592	Not detected	Not detected	HEG
Oak Island Marina	03 October 2018	44.5156	−64.2919	Present (super-abundant)	Present (rare)	HEG
Purcell's Cove	03 October 2018	44.6108	−63.5736	Not detected	Not detected	HEG
Shearwater	03 October 2018	44.6280	−63.5201	Not detected	Not detected	HEG
St. Margaret's Bay	03 October 2018	44.6354	−63.9191	Not detected	Present (occasional)	HEG
Bedford	04 October 2018	44.7257	−63.6637	Not detected	Not detected	HEG
Dartmouth Yacht Club	04 October 2018	44.7007	−63.6113	Present (abundant)	Present (abundant)	HEG
Guysbourough	04 October 2018	45.3920	−61.4971	Not detected	Present (abundant)	HEG
North Sydney	04 October 2018	46.1918	−60.2684	Not detected	Present (abundant)	HEG
Baddeck	05 October 2018	46.0995	−60.7483	Not detected	Present (abundant)	HEG
Inverness	05 October 2018	46.2302	−61.3166	Not detected	Not detected	HEG
Pictou	05 October 2018	45.6745	−62.7117	Not detected	Not detected	HEG
Port Hawkesbury	05 October 2018	45.6138	−61.3651	Not detected	Present (abundant)	HEG
Pugwash	05 October 2018	45.8465	−63.6632	Not detected	Present (common)	HEG
Prince Edward Island						
Borden-Carleton	06 October 2018	46.2494	−63.7012	Not detected	Not detected	HEG
Charlottetown	06 October 2018	46.2312	−63.1213	Not detected	Present (abundant)	HEG
French River	06 October 2018	46.5089	−63.5006	Not detected	Not detected	HEG
Georgetown	06 October 2018	46.1789	−62.5305	Not detected	Present (common)	HEG
Malpeque-Darnley	06 October 2018	46.5344	−63.6645	Not detected	Not detected	HEG
Stanley	06 October 2018	46.4640	−63.4591	Not detected	Not detected	HEG
Summerside	06 October 2018	46.3885	−63.7857	Not detected	Not detected	HEG
Tracadie Bay	06 October 2018	46.4029	−63.0296	Not detected	Not detected	HEG

[a]CNC = C.N. Chapman; HEG = H.E. Glon; HLH = H.L. Hawk; KCKM = K.C.K. Ma.

3.2. Nova Scotia

In Lunenburg, new occurrences of *D. lineata* were observed in 2013 in Lunenburg Harbour and Hermans Island (Table 1; Fig. 2). In September of 2013, a total of 37 individuals of *D. lineata* were collected in quadrats scraped from the undersides of floating docks at Hermans Island (18.5 individuals m^{-2}), one individual in a quadrat scraped from a wooden piling in Lunenburg Harbour (4.2 individuals m^{-2}), and one individual on a settlement plate in Lunenburg Harbour (4.2 individuals m^{-2}; Fig. 1). *Diadumene lineata* was not observed on settlement plates deployed at Hermans Island. Twenty-two individuals of *M. senile* were observed in benthic quadrats at Hermans Island (11.0 individuals m^{-2}). Out of the 50 quadrats at Hermans Island, three had both *D. lineata* and *M. senile*. *Metridium senile* was not detected on settlement plates at Hermans Island, nor was it detected by either sampling method in Lunenburg Harbour.

In October of 2017, a new observation of an individual of a sea anemone was made at Black Rock Beach in Halifax (Fig. 2). This observation (and accompanying photograph; Fig. 1) was uploaded to iNaturalist and later identified as *D. lineata* (Table 1). Further database enquiries did not yield additional observations of the species in the Canadian Maritimes.

As part of the survey of floating docks in October of 2018, two new occurrences of *D. lineata* (Fig. 1) were reported from Oak Island Marina near Chester (abundance: super-abundant; Fig. 2) and Dartmouth Yacht Club (abundant with two colour morphs present; Fig. 2) where populations of *D. lineata* co-occurred with *M. senile* (Table 1 and Fig. 3).

3.3. Prince Edward Island

Diadumene lineata was not detected from Prince Edward Island during the 2018 survey.

4. Discussion

4.1. Overall distribution in the Canadian Maritimes

The lack of observations of *D. lineata* in New Brunswick from the combined surveys of 2017 and 2018 suggest that this species went extinct (permanently or temporarily) in New Brunswick at some time between 2009 and 2017. The systematic surveys in 2017 and 2018 show that its distribution in the Canadian Maritimes is restricted to a region of approximately 100 km from Halifax to Lunenburg, Nova Scotia (Fig. 2). At some of the sites (i.e., Dartmouth Yacht Club, Hermans Island, and Oak Island Marina), *D. lineata* co-occurred in close proximity with *M. senile*.

4.2. Local extinction in New Brunswick

The local extinction of *D. lineata* from southwest New Brunswick is not unique and follows a similar extinction pattern in the Gulf of Mexico and in Germany. In the Gulf of Mexico, it was first observed from Port Aransas in 1952 but it was not recorded again from this site despite regular sampling in the 1980s (Carlgren and Hedgepeth, 1952; Hancock et al., 2017). In Germany, it was detected once in the Büsum (on the North Sea) between 1920 and 1924 (Gollasch and Riemann-Zürneck, 1996). Such patterns of local extinctions of *D. lineata* are known from the literature and could be caused by an environmental catastrophe exerted on a small, non-native population consisting of a single clone (Shick et al., 1979; Gollasch and Riemann-Zürneck, 1996; Hancock et al., 2017).

The likelihood of future re-introduction and establishment of an introduced species in a region can be difficult to determine, even in the context of climate change. For example, in the Gulf of Mexico, *D. lineata* was detected for the second time on a jetty on Galveston Island, Texas, in November of 2016, about 280 km north of Port Aransas and sixty-four years after its initial observations (Hancock et al., 2017), which suggests a separate introduction event. Correspondingly, the local re-appearance of *D. lineata* in New Brunswick is also possible. Reporting local extinctions of non-native populations is an important endeavour (e.g., see Robinson et al., 2005), which should help focus finite resources on monitoring existing biological invasions while working to predict future re-introduction events.

In contrast with the extinction of *D. lineata* from southwest New Brunswick, the non-native macroalga *C. fragile* was still present as of October 2017 in Sam Orr's Pond, which was the site of its first observation from the Bay of Fundy made on 23 July 2008 (Saunders et al., 2013). Since it was first documented in Atlantic Canada in December 1991, *C. fragile* is now a well-established and widely distributed throughout the Atlantic coast of Nova Scotia and the Gulf of St. Lawrence, with northernmost records from substantially colder waters of Newfoundland (Bird et al., 1993; Garbary et al., 1997; Schmidt and Scheibling, 2007; Matheson et al., 2014). However, the geographic extent of *C. fragile* in the Bay of Fundy remains unknown, since all observations of this species were reported from the one site in Sam Orr's Pond. Therefore, systematic surveys for *C. fragile* of the Bay of Fundy should continue to be carried out to determine its distribution and to monitor its spread and impact.

4.3. No regional range expansion in Nova Scotia

The seasonal timing of the *D. lineata* observations in Lunenburg (September 2013) was approximately two months earlier than the previously documented observation in Dartmouth (November 2013; Moore et al., 2014). By considering a recruitment and growth period of as little as three weeks and up to two months to obtain the sizes observed (at least 7 mm in basal diameter), these new records from Lunenburg suggest that this species was distributed from Halifax to Lunenburg at least as early as the summer of 2013.

The first report of *D. lineata* from Nova Scotia was based on an individual collected from a settlement plate. However, this study found that detection of *D. lineata* in the region was more effective using surveys of established communities on floating docks and wharves than by using settlement plates. The low recruitment of *D. lineata* on plates despite its abundance on anthropogenic structures at Lunenburg Harbour and Hermans Island sites indicated that either (a) the timing of reproduction was not during late summer or (b) the species does not often reproduce sexually in the region, as has been documented in other regions of its introduced range (Shick, 1976; Shick and Lamb, 1977; Dunn, 1982; Fukui, 1991; Ting and Geller, 2000; Ryan and Miller, 2019; Newcomer et al., 2019).

The reconstructed range (from Halifax to Lunenburg) suggests that the regional distribution of *D. lineata* in Nova Scotia has remained relatively unchanged in the five years after its initial report. This lack of range expansion along the south shore Nova Scotia after five years is surprising given that its status as a globally successful invader (Seaton, 1985) and that the region is highly connected by commercial ships and recreational boats (Simard et al., 2017). Remarkably, extensive government monitoring for aquatic invasive species from 2012 to 2015 throughout New Brunswick and Nova Scotia did not mention any observations

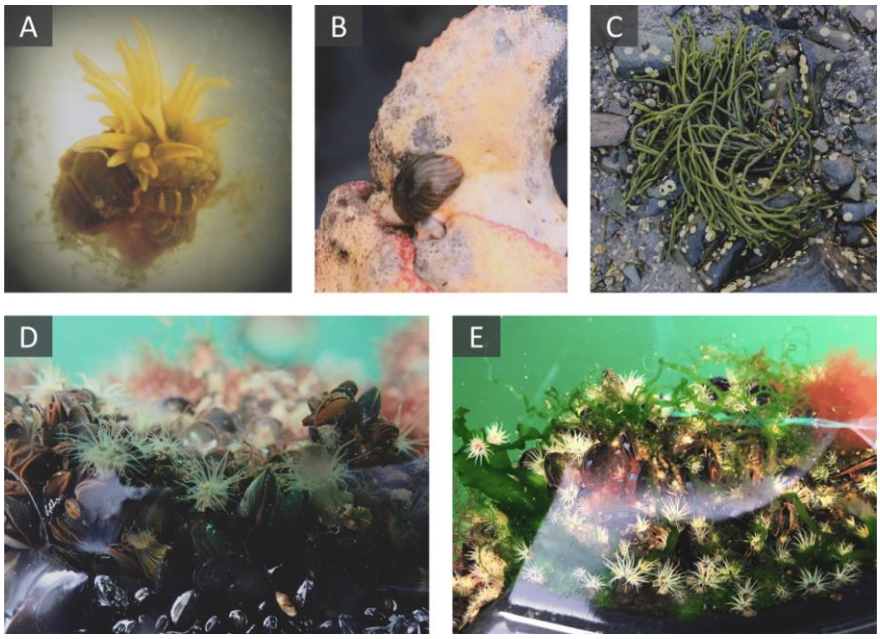


Fig. 1. Observations of *Diadumene lineata* in the Canadian Maritimes and *Codium fragile* from the Bay of Fundy. (A) A slightly contracted specimen of *D. lineata* collected from Hermans Island, Nova Scotia, in 2013 (basal diameter = 7 mm); (B) a completely contracted specimen of *D. lineata* observed from Black Rock Beach, Nova Scotia, in 2017; (C) *C. fragile* from Sam Orr's Pond, New Brunswick, in 2017; (D) extended specimens of *D. lineata* from Dartmouth Yacht Club, Nova Scotia, in 2018; (E) extended specimens *D. lineata* from Oak Island Marina, Nova Scotia, in 2018. Scale bars not available. Photo credit: H.L. Hawk (A), C.N. Chapman (B), K.C.K. Ma (C), H.E. Glon (D, E).

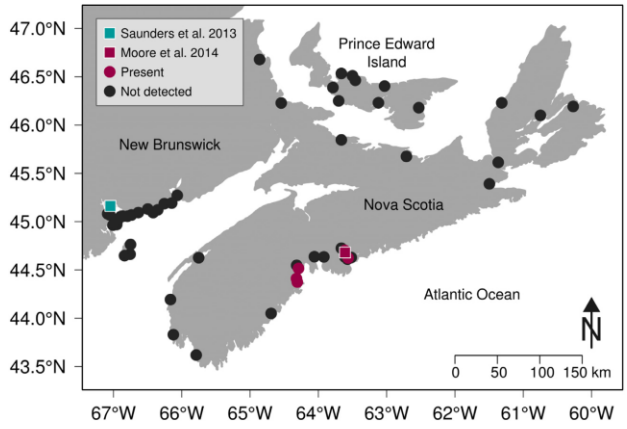


Fig. 2. Distribution *Diadumene lineata* in the Canadian Maritimes based on occurrence records collated in the present study and records documented by Saunders et al. (2013) in New Brunswick and by Moore et al. (2014) in Nova Scotia.

of *D. lineata* (DFO, 2015; Sephton et al., 2017). The probability of detection also depends on factors such as species density, sampling effort, method, and season. The high abundances of *D. lineata* observed at some sites that are also regularly surveyed by the government (e.g., Lunenburg) indicate that this non-native sea anemone is not targeted for monitoring. The inclusion

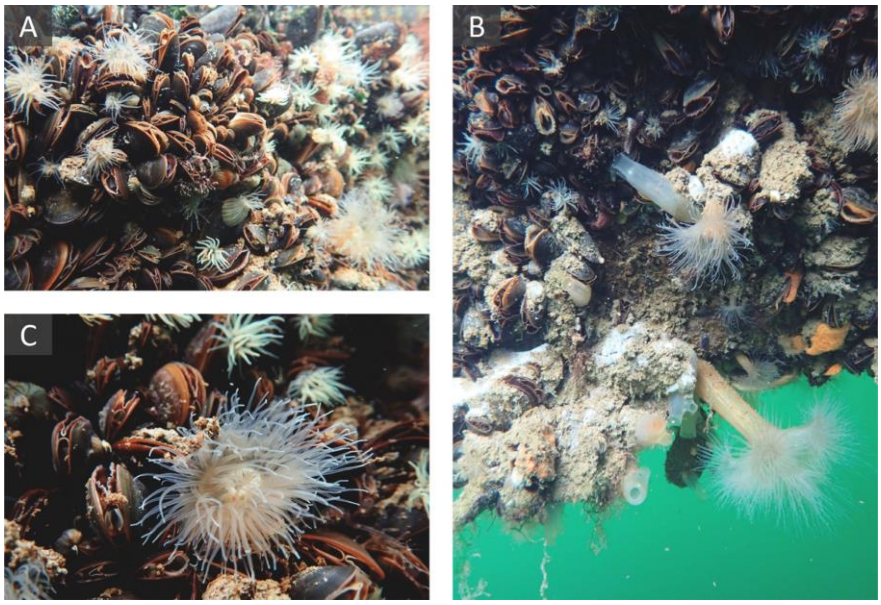


Fig. 3. Two sea anemones, the non-native *Diadumene lineata* and the native *Metridium senile*, co-occurring on fouling dock structures in Dartmouth Yacht Club, Nova Scotia. (A) Oblique view of the fouling community; (B) lateral view of the body of *M. senile*; (C) oral disc view of *M. senile*. Scale bars not available. Photo credit: H.E. Glon.

of this species in regular monitoring can substantially improve uncertainties associated with any absence records. The collection and identification of the iNaturalist observation of *D. lineata* from Black Rock Beach in Halifax highlights the potential role of citizen-science networks for detecting non-native species and monitoring their distribution in the Canadian Maritimes.

4.4. Conclusions

In this report, the known distribution of *D. lineata* in the shallow waters of the Canadian Maritimes is described. In Nova Scotia, its occurrence is closely associated with harbours and marinas. This association suggest that human activities likely facilitate the transport and introduction of *D. lineata* along the south shore of Nova Scotia. The regional and international connectivity of the Canadian Maritimes via human activities increases the risk of re-introduction and further geographic spread in the future. Therefore, systematic monitoring across the region (e.g., Sephton et al., 2017) should be carried out regularly to track the progression of its geographic spread over time.

Documenting the occurrence and circumstances (e.g., the various methods leading to a confirmed observation) of detecting non-native species throughout their invasion process (from introduction to establishment and to regional spread) is important to reconstruct their invasion history. In particular, this report provides evidence that *D. lineata* have gone locally extinct in New Brunswick and that its distribution in Nova Scotia have remained unchanged after five years since 2013, when it was first detected in Nova Scotia.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Kevin C.K. Ma: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Heather E. Glon:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - review & editing, Funding acquisition. **Heather L. Hawk:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - review & editing, Funding acquisition. **Cody N. Chapman:** Investigation.

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