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Vessel Biofouling as a Vector for Nonindigenous Species Introductions in Canada

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Biofouling is the accumulation of organisms (such as algae, mussels, barnacles, and other taxa) on underwater surfaces. Biofouling on vessels is seen as undesirable, as it reduces vessel fuel efficiency through increased drag, and has potential to transfer organisms over long distances to locations outside their natural biogeographic region. Compared to other vectors that transfer aquatic organisms, such as ballast water, biofouling is relatively understudied despite being a major contributing vector of aquatic nonindigenous species (NIS) to coastal ecosystems globally. As a result, Transport Canada requested science advice from Fisheries and Oceans Canada, seeking an updated national assessment of the probability of NIS introduction and establishment via biofouling on vessels, to inform the development of biofouling management policies.

This study used a multistage mechanistic model (a multiple-step model describing the parts or stages of the invasion process) to assess the probability of introduction and establishment of NIS into Canada based on one year of data on first arrivals of foreign-flagged commercial vessels. The stages in the model included arrival, survival, and establishment of NIS, but throughout this document the term ‘establishment’ denotes the cumulative success through all three stages to result in a self-sustaining population in Canadian waters. Separate assessments were conducted for vessels’ main hull surfaces and combined niche areas (such as the sea-chest, propeller, and thruster tunnels, where biofouling may be more concentrated). Results were summarized for the four coastal regions of Canada based on the destination/arrival port of the vessels: Atlantic, Pacific, Great Lakes-St. Lawrence River, and Arctic regions. The model parameters were based on empirical vessel biofouling and environmental data, as well as estimates of biological processes with variability introduced.

Estimates of mean NIS primary establishments per year via vessel hulls ranged from <1 (Arctic region) to 2.2 (Pacific region). Similarly, the mean number of trips until at least one NIS establishment is successful via the hull ranged from 94 (Pacific region) to 174 (Great Lakes-St. Lawrence River region). Primary NIS establishments via vessel niche areas were generally higher than those associated with the hull, with the highest species establishments per year being 8.4, with 23 trips until establishment occurs (Pacific region). While there is uncertainty associated with these estimates, these results indicate a meaningful probability of NIS establishments by vessel biofouling in all regions of Canada. The Atlantic and Pacific coasts are expected to receive the greatest numbers of NIS establishments, driven by the higher number of vessel arrivals to these regions. NIS establishment rates via the main hull areas of vessels were lower compared to niche areas, with the niche areas (all combined) having higher abundance of biofouling but smaller wetted surface area. Vessel biofouling should be considered as a dominant, active vector for introduction of NIS to Canada.

INTRODUCTION

The introduction of aquatic nonindigenous species (NIS) is considered one of the major threats to global biodiversity and ecosystem health (Clavero et al. 2009; Havel et al. 2015). Nonindigenous species may be competitors, predators, or parasites directly impacting native species in the introduced range, altering population, community, and ecosystem structures, which in turn may lead to extirpation and/or extinction of some threatened native species (Roberts and Hawkins 1999; Gurevitch and Padilla 2004; Pacifici et al. 2015). International shipping transports most of the globally-traded goods and represents the single largest pathway for the introduction of coastal marine NIS (Hewitt et al. 2009; Bailey et al. 2020a). Globalization and population growth have increased the frequency of vessel movements and complexity of movement patterns, which, combined with changing climate, have contributed to widespread introduction and spread of NIS globally (Hopkins 2010; Chan et al. 2019). Primary mechanisms for NIS transport in or on vessels include (Hewitt et al. 2009; Hopkins 2010):

1. Ballast water and sediments,
2. Hull fouling, and
3. Fouling of niche areas such as sea chests, thruster tunnels, intake pipes, gratings, and internal seawater systems.

While the ballast water vector has been extensively studied and is regulated, biofouling is less studied and unregulated (in Canada) despite likely contributing to the transport and introduction of 55–69% of the ~1780 NIS detected in ports and harbors around the world (statistic based on the life-history characteristics of established aquatic NIS) (Hewitt et al. 2011; Bailey et al. 2020a).

Biofouling can be defined as the accumulation of microorganisms (such as fungi, algae, bacteria, diatoms), plants, and other marine life (e.g., bryozoans, mussels, barnacles, and polychaetes) on substrates immersed in sea water (Callow and Callow 2002; Yebra et al. 2004). Several environmental variables (such as salinity, temperature, conductivity, pH, organic material content, dissolved oxygen concentrations, currents, light, depth, and distance from the shore) influence the development of biofouling (Delauney et al. 2010). Biofouling can result in substantial negative impacts on aquatic life and the shipping industry through (Yebra et al. 2004; Hopkins 2010):

1. Biosecurity risks from NIS transfers,
2. Reducing vessel speed at a given engine power output due to reduction in hydrodynamic performance and maneuverability, and
3. Increasing fuel, maintenance and downtime costs to compensate for an increase in vessel hydrodynamic drag.

Historically, biofouling was managed by antifouling coating systems containing toxic paints such as tributyltin self-polishing copolymer paints (TBT-SPC paints) (Yebra et al. 2004). International regulations banned TBT in the 1990s due to adverse effects on the environment and marine life, triggering the development of non-toxic, environmentally friendly (and possibly less effective) antifouling methods (Yebra et al. 2004; Finnie and Williams 2010; Legg et al. 2015), however, copper-based coatings remain the primary type of antifouling coating system in use (Scianni et al. 2021). Despite widespread use of antifouling coatings, biofouling-associated transfer of NIS is still occurring due to several reasons, including (Ferreira et al. 2006; Coutts and Dodgshun 2007; Davidson et al. 2009; Hopkins 2010):

1. Antifouling coatings lose effectiveness with age,
2. Not all vessels undergo routine maintenance,
3. Inappropriate selection of antifouling coatings for vessel operations,
4. Efficacy of antifouling coating is compromised for some vessels that remain idle for extended periods (e.g., oil rigs),
5. Application of antifouling coatings on non-hull areas is often poor or nonexistent, and
6. Ability of some taxa to colonize even recently-antifouled surfaces (e.g., copper-resistant taxa).

The International Maritime Organization (IMO) has developed *Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species* (International Maritime Organization 2011). Introduced in July 2011, these IMO Guidelines recommend several steps for the prevention of biofouling, which include:

1. All vessels implement and maintain a biofouling management plan and a biofouling record book of management practices undertaken,
2. Using and maintaining antifouling coatings, biofouling resistant materials for uncoated surfaces, and marine growth prevention systems (MGPSs) in niche areas such as sea chests and internal seawater cooling systems,
3. In-water inspections of vessel surfaces and intelligent design of vessels to reduce the number and extent of niche areas that may support biofouling growth.

As the guidelines are voluntary in nature, their efficacy in preventing species introductions has not been demonstrated.

The Department of Fisheries and Oceans Canada (DFO) conducted a series of regional risk assessments between 2012 and 2017, which identified vessel biofouling as a vector for the introduction of NIS posing a threat to Canadian marine and freshwater ecosystems (Bailey et al. 2012; Chan et al. 2012; Adams et al. 2014; Linley et al. 2014; Simard et al. 2017). DFO is mandated under the *Fisheries Act* to protect fish and fish habitat, including the prevention and management of aquatic NIS (i.e., species not native to the receiving water body) and aquatic invasive species (i.e., those NIS likely to cause harm). Meanwhile, Transport Canada (TC) regulates shipping activities under the *Canada Shipping Act, 2001* and is responsible for preventing the introduction and spread of NIS through vessels' ballast water and biofouling. During the last 15 years, TC has worked collaboratively with DFO to develop science-based policies and regulations to effectively manage ballast water, and more recently, vessel biofouling. The research underlying these efforts often focuses on examining the probability of establishment of species that are nonindigenous to the receiving environment. While only a proportion of NIS may become invasive, the magnitude of impact may be unknown or difficult to predict for the hundreds to thousands of species that may be introduced by shipping. Assessments based on NIS are therefore the more precautionary and protective approach.

Transport Canada requested science advice from DFO to inform the development of biofouling management policies for vessels over 24 meters in length that will better protect Canadian marine and aquatic ecosystems against NIS. Specifically, DFO was asked to conduct an updated national assessment of the probability of species establishment through biofouling, incorporating methods advanced during the last decade for assessment of ballast water introductions. Following the precautionary approach described above, this assessment examines the probability of primary NIS establishment, where the term 'establishment' is used to describe the successful transition through the stages of arrival, survival, and establishment of a self-sustaining population in Canadian waters. Secondary NIS establishment (i.e., the spread of NIS by vessels moving between Canadian ports) was not assessed in this study.

OBJECTIVE

The objective of this study was to build on previous DFO regional assessments of vessel biofouling-mediated establishments of NIS (Bailey et al. 2012; Chan et al. 2012; Adams et al. 2014; Linley et al. 2014; Simard et al. 2017), in view of creating an up-to-date comprehensive national assessment using best-available science. This study incorporates 'new' data and modelling methods, to provide insight into the following questions:

1. What are the probabilities of arrival, survival, and establishment of biofouling NIS posed by domestic and international commercial vessels at freshwater and marine ports and anchorages, considering different operational and/or route characteristics and additional factors identified in the scientific literature that could be used to predict the probability of establishment of NIS by biofouling; and
2. What effect will forecasted changes in shipping activity and temperature (as predicted by climate change model(s)) have on the probability of establishment of NIS by biofouling to freshwater and marine ecosystems of Canada (in particular, to the Arctic and other waterways where greater changes are expected)?

METHODS

STUDY AREA

The study area included all regions with active international commercial shipping in Canada, including the Pacific coast (ports in British Columbia), Atlantic coast (ports in Quebec, east of Quebec City (in the St. Lawrence Estuary and Gulf of St. Lawrence), New Brunswick, Nova Scotia, Prince Edward Island, and the island of Newfoundland), Arctic region (ports in the Northwest Territories, Nunavut, Manitoba, northern Quebec and Labrador), and the Great Lakes-St. Lawrence River region (freshwater ports in Quebec and Ontario, west of and including Quebec City) (Table 1).

DATA SOURCES

Multiple data sets were obtained from a variety of sources as inputs to the multistage mechanistic model. Shipping data were obtained from Transport Canada Marine Security Operation Centres (East and West) for first arrivals to Canada by vessels that were greater than 100 tons of gross tonnage (other than a towing vessel), carrying greater than 12 passengers, or a towing vessel that is towing a barge astern or alongside or pushing ahead, if the barge is carrying certain dangerous cargoes. The shipping data included information for each vessel entry into Canadian waters in 2018, along with vessel identifiers and voyage history, including up to 10 last-ports-of-call, and the destination/arrival port in Canada. Transits with a Canadian last-port-of-call were removed, as domestic vessel movements were beyond the scope of this assessment (resulting in 8103 international arrivals to Canada during 2018). According to a review of data held in the Canadian Coast Guard Vessel Traffic Management Information System, 2018 was the year with the highest number of vessel transits entering Canadian waters between 2015–2020. As the annual average was 7956 (± 688), 2018 is considered as representative of a typical year of vessel traffic to Canada. However, it was noted that the number of transits to the Arctic region, and the distances they have travelled, have been steadily increasing in recent years and are projected to continue to increase (Dawson et al.

2017; Dawson et al. 2018). Therefore, the 2018 shipping dataset was adjusted to include data from 2019 (instead of 2018) for the port of Milne Inlet, to reflect the recent peak in operations during the early revenue phase at the Baffinland iron mine (74 international arrivals).

Hull wetted surface areas were calculated for each vessel according to its generic vessel type (Table 2) and gross tonnage, using the regression models in Ceballos-Osuna et al. (2021). For vessels where gross tonnage data were not available, wetted surface area was assigned based on a mean estimate of the wetted surface area of the global fleet based on vessel type by Moser et al. (2016). Specifically, this was necessary for vessels that were classified as 'Other' or 'Tugs/Supply vessels' (Table 2). Wetted surface areas specific to niche areas were calculated according to vessel type based on a study of proportion of niche area relative to hull wetted surface area of the global fleet (Moser et al. 2017). Table 2 describes the categories of vessels in the shipping dataset by vessel type, along with their frequency in each region. An additional figure showing the frequency of each vessel type per region is available in Appendix 1 (Figure A1).

Biological data, including percent cover, abundance and species richness of biofouling taxa, were obtained from underwater dive surveys conducted by the Canadian Aquatic Invasive Species Network (CAISN) for vessels sampled in the Atlantic (*n = 20*), Pacific (*n = 20*) (Sylvester et al. 2011) and Great Lakes-St. Lawrence River (*n = 19*) regions (Sylvester and MacIsaac 2010), as well as an additional 12 vessels subsequently sampled in the sub-Arctic (Churchill, MB) (Chan et al. 2015). These data are the best-available for biofouling on vessels entering Canada, and are considered representative of current vessel biofouling communities as no major changes in vessel routes or biofouling management practices have been reported. In addition, data from drydock inspections examining fouling inside vessel seachests were included from vessels sampled in the Pacific coast (*n* = 6) and the Atlantic coast (*n* = 2) (Frey et al. 2014). The biological data were pooled across regions (rather than kept as region-specific datasets) due to small sample sizes for each of the individual regions, although analysis using regional separation was performed as part of the sensitivity analysis. Each vessel was a single data point in this pooled data set. During the underwater dive surveys, fouling organisms were collected using 20×20cm quadrats sampled from the vessel hull or niche areas, which were later identified and counted (with a focus on invertebrates) if presumed to be alive at the time of collection. Estimates of percentage cover were made using video transects of the vessel. Individual counts scaled up to a per-meter-squared basis and percentage cover of the vessel area (hull or specific niche area) were used to calculate an estimate of the abundance of organisms per meter square (abundance = sample count * % cover). Using the percentage cover of the vessel surface accounts for potential bias in opportunistic sampling of the most fouled areas on the vessels, which are naturally patchy in coverage. Following the methods used by the authors of the underwater dive studies, data were adjusted to exclude species that were also found in background water samples taken from the port harbour. Full details on the methods and analysis for the underwater dive studies can be found in Sylvester and MacIsaac (2010), Sylvester et al. (2011) and Chan et al. (2015). Similarly, the internal seachest data were scaled up using counts of quadrats (10×10cm) scaled to one-meter-squared, and percentage cover of the seachest. Only samples where counts were recorded could be included from this dataset, as many measurements included a combination of presence-absence, counts, biomass, and percent cover, which could not be utilized in this model. Internal seachest data from navy vessels were excluded to keep vessel types consistent across studies. Full sampling details and analysis of the internal seachest study can be found in Frey et al. (2014). Information on last ports-of-call were available for vessel sampling data, and were used for the port history analysis (described later).

Across all vessels and regions, 59 distinct taxa were identified from the hull area, and 242 distinct taxa (both NIS and non-NIS) were identified from the combined niche areas, including representatives of the Anomopoda, Copepoda, Cirripedia, Ostracoda, Amphipoda, Acari, Bivalvia, Oligochaeta, Gastropoda, Polychaeta, Hydrozoa, Tardigrada, Trichoptera, Tanaidacea, Appendicularia, Decapoda, Isopoda, Nematoda, Chironomidae, and Echinodermata. Detailed taxonomic lists can be found in the original studies (see Sylvester and MacIsaac 2010 (Appendices S1 and S2); Sylvester et al. 2011 (Appendices S1 and S2); Frey et al. 2014 (Appendix 2); Chan et al. 2015 (Tables A1 and A2)). These taxa were identified to species level where possible, but were often more broadly classified as distinct taxa without identification to the species level. Both species-level and higher order distinct taxa data were used to create species abundance distributions, as species identity was not important for this analysis. Taxa were also classified as NIS, or non-NIS, in the original studies according to their population status in the specific regions where sampling occurred (Sylvester and MacIsaac 2010; Sylvester et al. 2011; Frey et al. 2014; Chan et al. 2015). If specimens were unable to be identified to species level, but the taxon was listed by the original authors as a 'non-established taxon', it was considered a NIS. Additionally, any taxa sampled in the Great Lakes that was noted to originate from a marine habitat was classified as NIS. All NIS data were included in this analysis regardless of population status in Canada (having already established populations, or not). All other population status categories (e.g., 'cryptogenic', 'unknown', or 'native' / 'indigenous') were considered non-NIS. Based on these classifications, proportions of NIS were calculated for each vessel (hull and niche areas), and for each seachest in the seachest dataset. The proportions were based on the total relative abundance of each species/distinct taxon for each sampled part of the vessel. The relative abundance of each distinct taxon that was identified as NIS on each vessel were used to create the species abundance distributions in the model. In total (combined across all vessels and regions), 37 distinct nonindigenous taxa were identified from the hull area, and 179 distinct nonindigenous taxa were identified from the combined niche areas. This list is likely non-exhaustive due to difficulties associated with sampling and identifying microscopic and/or non-invertebrate biofouling taxa.

All vessels that were sampled for biological data by underwater dive surveys had some type of antifouling system (AFS) in place (e.g. paint coatings of different compounds, or active cathodic protection). The original studies collected information including the specific AFS type and time since last dry-dock and coating application, where time varied from 1–57 months (Sylvester and MacIsaac 2010; Sylvester et al. 2011; Chan et al. 2015). In these studies, the effect of AFS on fouling levels was examined, but showed varying results. In the Great Lakes, the age of the AFS was found to be unrelated to vessel hull fouling (Sylvester and MacIsaac 2010). In the Atlantic and Pacific coasts, vessel hull fouling was found to increase in relation to the time-since-application of antifouling paint (Sylvester et al. 2011). In the Arctic study, the age of the antifouling coating was related to the fouling cover of the entire vessel, but not to the specific niche areas or to total abundance (Chan et al. 2015). Further, in a synthesis of Arctic biofouling data, the age of antifouling coating was determined to be related to the percent cover and abundance of biofouling invertebrates on vessels, while the number different biogeographic regions and duration of stay were associated with species richness (Chan et al. 2022). Information on specific AFS type and age was not available for the seachest dataset (Frey et al. 2014). Further, information on AFS was not available in the shipping dataset acquired from Transport Canada (the full year of vessel arrivals). As a result, the effects of vessel AFS could not be accounted for in this study.

Additional potential sources of biological data were examined, such as those from underwater surveys of Canadian naval vessels (A. Valenta, DND, pers. comm.) and CAISN fouling collector plate studies (Gartner et al. 2016). Navy vessel data were not included due to differences in methodology for total fouling counts (sampling only the most fouled areas without percent cover estimates). Collector plate data were not used due to very low abundance of NIS and limited species diversity overall.

Port environmental data (i.e., annual salinity, mean temperature during the warmest month, mean temperature during the coldest month and annual average temperature) were obtained from Keller et al. (2011) and World Ocean Atlas 2013 Vol. 2 (Locarnini et al. 2013, Zweng et al. 2013; sea surface level data at a one-degree resolution, from 2005-2012) with corrections to salinities for freshwater ports where errors were found (Bailey et al. 2020b; Drake et al. 2020). Future/predicted minimum, maximum, and mean values of surface temperature and salinity (2050, RCP 8.5) were obtained from Bio-ORACLE marine raster layers (Tyberghein et al. 2012; Assis et al. 2018). The 'extract multi values to points (Spatial Analyst)' tool was used in ArcMap 10.8.1 to extract the environmental data cell values for the specified locations of ports included in the shipping dataset.

MODELLING APPROACH

The modelling approach in this assessment built on existing peer-reviewed multistage mechanistic models used in Drake et al. (2020), Bradie et al. (2020), and DFO (2020) for assessments of NIS introductions via ballast water. This multistage model estimated the establishment of NIS via biofouling based on three components:

1. The probability of arrival (biofouling abundance and proportion of NIS);
2. The probability of survival based on the environmental similarity (temperature) between the Canadian destination/arrival port and each vessel's last two ports-of-call; and
3. The probability of establishment based on a theoretical equation for establishment (Leung et al. 2004) with an adjustment based on the salinity match between the Canadian destination/arrival port and each vessel's last two ports-of-call (Figure 1).

Although the survival and establishment stages are interrelated, with temperature and salinity influencing both initial survival after release from the vessel and longer term population establishment, they were implemented in the model as discrete steps. This approach was investigated in Bradie et al. (2020) where the use of salinity in the survival and establishment components of the model were compared, with similar outcomes. The hull and niche areas (all niche areas combined) were modelled separately, to allow for detailed understanding of NIS arrival and establishment metrics via biofouling associated with these parts of vessels (that are likely to be managed in different ways). Table A1 (Appendix 1) outlines the values for each of the parameters used in the model. In addition to the main model, the relative importance of previous ports-of-call in relation to biofouling NIS presence was examined, to inform the survival step of the model.

Port History Analysis

Unlike ballast water, where each tank is typically filled at a single known location, the history of port visits needs be taken into consideration when predicting the survival and establishment of biofouling organisms because biofouling accumulates over a longer time span and may incorporate species from multiple geographic regions. Feature selection, a machine learning technique, was used to evaluate the information gain achieved by including multiple last-ports-of-call into the analysis. The CAISN biological survey data, including the corresponding vessel-specific list of the last ten ports-of-call prior to arrival at the Canadian sampling location, were used for the feature selection analysis. The influence of previous ports-of-call was examined on the target variable (presence of NIS), with the assumption that the ports which contribute highest information gain for determining presence/absence of NIS at the time of arrival to Canadian waters will also have highest information gain for predicting survival.

The first step of the feature selection analysis was to extract or generate relevant features from the dataset. A linear historical combination approach was used, which generated features based on the environmental distance between each port-of-call and the prior port-of-call, in succession, across the last ten ports-of-call. Environmental distance was calculated as the Euclidean distance between four environmental variables (annual salinity, mean temperature during the warmest month, mean temperature during the coldest month and annual average temperature) for each set of port-pairs, following Bradie and Bailey (2021) and implemented in Python following Etemad et al. (2021).

In the second step, the CAISN biological data (abundance of NIS in biofouling on vessels) was categorized as two groups (presence/absence). This enabled the development of a classifier using the 10 extracted features (environmental distances calculated over the last ten ports-of-call) from step one and the labels from step two. The data were divided into training (70%) and testing sets (30%) to train and fine-tune the classifier. Next, a random forest model was developed as a feature selection method, using the Scikit-learn python library (Pedregosa et al. 2011), to find the importance of each feature in building the best configuration classifier.

Applying random forest as a feature selection method is possible in two ways:

1. The impurity-based feature importance (default approach in Scikit-learn), or
2. Permutation importance (Breiman 2001).

The first method has some weaknesses in providing the correct importance in cases with high cardinality of features or having numerical variables in the features. In this study, a permutation importance method was applied to overcome these limitations and provide more accurate importance results. In order to train a random forest with the highest possible accuracy, the model must be tuned on its parameters. As the biological dataset used here is small, (*n = 67* vessels with hull-only abundance data) high accuracy for this model is not expected, however, the model was configured so that the random forest gets the maximum possible accuracy through tuning. In this experiment, the minimum number of samples required to be at a leaf node was set to five, where the highest average for accuracy of our classifier can be achieved.

The results provided in Figure 2 show that the two most recent last ports-of-call are the most important features extracted from the empirical data in relation to the presence of NIS on arrival. The moving average trend of feature importance shows that the importance of these features decreases when older ports-of-call are applied (Figure 2); however, some past ports-of-call can be of importance. One explanation for the multi-modal pattern of importance of older ports-of-call is that some vessels may be repeating visits to the same set of ports-of-call in loops or circuits.

To examine this hypothesis, the last ten ports-of-call visited by sampled vessels were plotted on a map. As the environmental data (temperature, salinity) are available at a one-degree resolution, ports closer than 0.5 degrees were clustered for analysis. After that, the sequence for each vessel in the dataset was generated by assigning a number to each cluster, and cluster sequences were processed to identify cycles with the sampling port and the most recent two ports-of-call. From the total 67 samples, two samples had incomplete voyage history, where ports visited were unknown beyond their 4^{th} and 7^{th} last ports-of-call, respectively. In order to process these two samples, the unknown locations were substituted with the last known ports-of-call port (i.e., the location of the 4^{th} last port-of-call was repeated for the 5^{th} – 10^{th} last ports-of-call, and the 7^{th} last port-of-call was applied for 8^{th} – 10^{th} last ports-of-call, respectively). After cleaning data, 20 of the 67 sampled ships were determined to have at least one repeat visit to the sampling port (29.85%), 27 had at least one revisit of sampling port or the last port-of-call (40.29%), and 31 had at least one revisit of sampling port of call, first or second last ports-of-call (46.26%), supporting the selection of the last two ports-of-call as the most important features for predicting presence (and survival) of NIS on arrival to a Canadian port. A cord diagram was used to display these inter-relationships between the last ten ports-of-call across all sampled ships with arcs (segments of the circumference of the circle) representing the different ports-of-call and cords (connecting two arcs together) representing each vessel trip between a pair of ports (Bostock et al. 2011) (Figure 3A). By removing the trips between adjacent ports-of-call from the matrix of trips, the occurrence of repeat visits to individual ports by ships is magnified (Figure 3B).

ESTIMATING NIS ARRIVAL

Three main steps were conducted to build probability distributions associated with biofouling of vessels arriving to Canada, based on the available empirical data. These distributions described: the total abundance of all species, the proportion of species being NIS, and the species abundance distributions for NIS (Drake et al. 2014; Drake et al. 2020). A fourth step made random draws from each of the distributions from the first three steps to create vessel-specific estimates and applied a probability of organism release from the vessel (later described in more detail) into the destination port environment (as not all fouling organisms may be released during a vessel stay). It is important to note that these steps were informed by biological data collected from vessels upon arrival to Canada, such that any processes impacting biofouling organisms (organism attachment, drop-off, and survival) during or prior to the vessel transit are already captured in the empirical data. Thus, the steps in this model account only for processes that occur after vessel arrival to the destination port in Canada.

Biofouling abundance

The first arrival step was to build probability distributions describing the total abundance of all fouling organisms presumed to be alive at collection (whether NIS or not) associated with each vessel arrival (for hull and combined niche areas). Using the pooled CAISN and seachest data of biofouling organism abundances per vessel, probability density distributions were generated respectively for the hull and combined niche areas using a negative binomial distribution fit to the data (Figure 4). The Akaike Information Criterion (AIC) statistic showed that the variation among vessel samples was best described by the negative binomial statistical distribution. This is consistent with negative binomial distributions used for describing the abundances of organisms in ballast water samples (Casas-Monroy et al. 2014; Drake et al. 2020). These distributions made use of the available empirical data to more generally describe the pattern of biofouling for the broader population of vessels.

Proportion of nonindigenous species

The second arrival step was to estimate the proportion of total biofouling organisms which are NIS. This was informed by hull and niche fouling data from the combined regions (Sylvester and MacIsaac 2010; Sylvester et al. 2011; Chan et al. 2015), as well as seachest data included for the niche assessment (Frey et al. 2014). The proportion of NIS was calculated for each vessel, and beta distributions (with range 0 through 1) were fit to these proportions for both the hull and combined niche areas (Figure 5). These beta distributions were used to describe the general pattern of proportion of fouling NIS out of the total organism abundance on vessels across all regions.

Species abundance distributions

In the third arrival step, species abundance distributions based on NIS identified in the fouling abundance data were used to estimate how the total abundance of nonindigenous individuals was distributed among different NIS (described in Drake et al. 2014). Using the CAISN data only (Sylvester and MacIsaac 2010; Sylvester et al. 2011; Chan et al. 2015), the relative abundances of individual NIS (or higher level distinct taxa) were used to create a species abundance distribution for each vessel. This was done separately for the hull and combined niche areas. For the hull area, a total of 24 species abundance distributions representing 37 distinct taxa were created, and for the niche areas, a total of 58 species abundance distributions representing 179 distinct taxa were created. For each vessel arrival into Canada, a species abundance distribution was drawn at random which determined the number of NIS expected to be arriving, and their relative abundances. This was done for both the hull and combined niche areas analyses. The identities of individual species were not maintained throughout the remainder of the analysis (i.e., a single species could become established multiple times via different vessel arrivals).

To calculate the total amount of biofouling organisms arriving, for each vessel trip in the shipping dataset, a value of biofouling abundance was randomly drawn from the total abundance probability distribution. Next, a value was drawn from the distribution for the proportion of nonindigenous individuals out of total fouling individuals. Multiplying these two values (number of biofouling individuals × proportion of nonindigenous individuals) results in the total number of biofouling nonindigenous individuals per vessel. A random species abundance distribution was selected to determine the assemblage (number of NIS and associated individual counts) for each vessel arrival to Canada.

Probability of NIS release

Finally, a generic estimate of probability of release (0.5) was applied, following an earlier study by Drake et al. (2017) modelling the release of organisms associated with biofouling on recreational boats. A binomial distribution was generated with a mean value of 0.5 so that probability of release could vary across each vessel arrival. A random draw from this distribution was made and applied to the associated species abundance distribution for the vessel entry, so that a proportion of individuals from each species that were present in the assemblage was selected to be released into the port environment.

The probability of release may be influenced by the duration of stay of a vessel, as longer residence times in ports may lead to greater chance of organism release into a new environment (Minchin and Gollasch 2003; Ruiz et al. 2022). However, actual values for this relationship are unknown and the duration of stay was not available for many of the vessels in the arrivals dataset. The influence of probability of release was explored in the model sensitivity analysis using higher (0.75) and lower (0.25) estimates for probability of release.

ESTIMATING NIS SURVIVAL

Following the arrival estimate, the probability of survival of released individuals in the destination port was estimated based on environmental similarity between predictor/previous ports and destination ports. Following the approach of the most recent ballast water model (DFO 2020; Bradie et al. 2020), three temperature variables (minimum, maximum, and mean) were used to evaluate environmental similarity at the survival step in the model. Minimum and maximum temperatures are important variables for limiting species survival in a new environment. Mean temperature was also included, as Bradie et al. (2015) demonstrated that Euclidean distance calculated using mean and minimum temperatures was often the best performing distance metric. Salinity was taken into account at the establishment step to ensure that the effect of salinity mismatch between predictor/previous and destination ports, based on a single parameter, was not outpowered when there was a close match in the three temperature variables (based on Bradie et al. 2020).

Based on the results of the feature selection analysis (described earlier), the last two ports-of-call were used to estimate survival of released NIS in the multistage biofouling model. Note that the choice to use the last two ports-of-call does not indicate that all fouling individuals on arriving vessels originate from the last two ports, but that these ports are the best predictors of survival of the existing complement of biofouling organisms on arrival to Canada. Biofouling assemblages could have originated from any ports-of-call since the last dry-docking or in-water cleaning, with vessels having visited a greater number of biogeographic regions having a higher diversity of species (Chan et al. 2022).

A binomial distribution was used to select between the last two ports-of-call, such that the survival estimate for each released NIS for each vessel had a 50% chance of being predicted by either the last or second-last port-of-call. This allowed for variation in the survival estimates, where survival of some vessels' fouling communities are better predicted by the last port-of-call compared to the second-last port-of-call, and vice versa. The probability of survival for released NIS was then calculated based on the environmental distance in the three temperature variables between the arrival and predictor port (being either the last or second-last port-of-call). The relationship between environmental distance and probability of organism survival was established for aquatic organisms by Bradie et al. (2020), using a binomial generalized linear model (Figure 6). This resulted in a relationship where the probability of survival is high when port temperatures are similar, and probability of survival is low when port temperatures are very dissimilar. A random draw, based on the survival probability, was then made for each species in the vessel assemblage to determine whether the *n* individuals of each released NIS survived in the destination port.

ESTIMATING NIS ESTABLISHMENT

The probability that NIS that have been released and survive in the destination environment will then establish a viable population was estimated based on the following equation from Leung et al. (2004):

$$P_e = 1 - e^{-\alpha N^c}$$

Here, P_e represents the probability of establishment; alpha (α) is the probability that a single individual will establish a population, N is the initial population size of NIS that are released and survive in the destination environment (determined by the prior steps in the model), and c describes the existence of an Allee effect[1] (if $c > 1$). This equation relates the probability of establishment to the population density or propagule pressure of individuals. The upper limit for the alpha values used in this model are based on mesocosm trials with parthenogenetic zooplankton in the Great Lakes, which estimated their upper limit of P_e based on population density, where individuals per m^3 was the unit used for the initial population size N (Bailey et al. 2009). However in this study, total abundance (total individuals per vessel) was the unit of initial population size. As there were no units associated with the original equation developed in

[1] An Allee effect is defined as a positive association between individual fitness (and therefore population growth) and population size or density, with small or low density populations subject to Allee effects having lower probability to become established (Drake and Kramer 2011).

Leung et al. (2004), it is unclear if one of these metrics should be preferred. This inconsistency is important to note as an uncertainty in this study, and is later explored in sensitivity analysis.

Following Drake et al. (2020) and Bradie et al. (2013), it was assumed no Allee effect was present (c = 1) allowing for establishment to occur when initial population sizes are small. The alpha values for each species were estimated using a beta distribution. True alpha values are not known and will be species-specific (Wonham et al. 2013). With no information on alpha values specific to biofouling species, the parameters used in Drake et al. (2020) for ballast water species were also used for this study (beta distribution with parameters α = 0.005, β = 5) since they were designed to describe a wide range of aquatic species under a variety of conditions, with the upper limit bound by the empirical data for parthenogenetic species (Bailey et al. 2009).

For each vessel arrival, an alpha value was randomly selected from the alpha distribution for each species that was released and survived. The alpha values were then adjusted based on environmental salinity match between the predictor and destination ports. This restricted the chance of establishment if salinities were highly mismatched, as establishment is constrained when a species is introduced to a new environment with low potential of survival (Bradie et al. 2020). Port environments were categorized as either marine (salinity >18.1 g/kg), brackish (salinity between 5.1–18.0 g/kg), or freshwater (salinity <5.0 g/kg) based on a three-tier scale (Por 1972; Bald et al. 2005) using average annual salinity data (Bailey et al. 2020b). Following Bradie et al. (2020), alpha values were halved when the destination-predictor pair salinity difference was marine-brackish or brackish-freshwater, or vice-versa (one tier difference in salinity). The alpha values were divided by 10 if the destination-predictor pair salinity difference was marine-freshwater or vice versa (two tier difference in salinity). The alpha values remained unchanged for port-pairs within the same salinity category (Table 3). Additionally, any destination ports that were located in freshwater (i.e., all Great Lakes-St. Lawrence River region ports) were assigned to the marine-freshwater salinity difference category, since any transits into these ports from outside Canada required travel through marine environments. Thus all alpha values for these ports were divided by 10, as high salinity during transit is expected to impact the arrival, survival and establishment probabilities of most freshwater organisms.

Using the alpha values and equation described above, estimated probabilities of establishment were calculated for each species associated with each vessel arrival, as predicted by both the first and second last-ports-of-call. These values were then combined to have probabilities of establishment for the full assemblage associated with each vessel. Then, comparing the establishment probabilities to a uniform distribution, a draw was made for each species to determine if it establishes (1) or becomes extinct (0) in the destination port based on the calculated establishment probabilities. This resulted in a final outcome of whether or not a given NIS on a given vessel entry into Canada establishes a population in the destination port.

FINAL PROBABILITY OF ESTABLISHMENT

The final probability of establishment was calculated using the above steps, for n vessel entries to each region (Atlantic, Pacific, Great Lakes-St. Lawrence River, and Arctic; Table 1) per year. This process was repeated 1,000 times to achieve variation in resampling distributions and randomization. Metrics of interest were calculated separately for hull and combined niche areas, to allow for comparisons of relative likelihood of establishment associated with the different underwater vessel surfaces.

Values were obtained as number of unique NIS establishments per year (SpPY). Mean values of NIS establishments per year across all regions were also examined by vessel type. Finally, estimates were also summarized as the mean number of trips until at least one unique NIS establishment occurs (calculated as the yearly number of trips ÷ unique species establishments). Note that the number of trips until establishment is calculated as an estimate of the mean number of trips where an establishment is predicted to occur over time; if the annual number of trips falls below this value, it does not indicate that there is no risk of NIS establishment.

For the remainder of this document, reported species establishments per year refer to unique NIS establishments per region and by vessel part (hull or combined niche areas), acknowledging that a single species could establish multiple times associated with different vessel arrivals and in multiple locations. When examining species establishments associated with the different two last ports-of-call, species were unique per port but may be repeated across the different ports. When examining species establishments associated with different vessel types, the total number of species establishments, rather than unique species, was used to account for all trips for each vessel type into the region. The unique species metric was calculated per region and per vessel part (either hull or niche areas), where multiple of the same species will not be counted in the same region or vessel part, but will be counted across regions. Therefore, if the model predicts a species to establish once, but then later predicts the same species to establish again in another event (within the same region and vessel part), only one NIS establishment will be recorded. Bootstrapped 95% confidence intervals were calculated for each metric, sampling full results with replacement 5,000 times.

FUTURE SCENARIO

To examine how the probability of establishment of NIS by biofouling into Canada could change in the future, it was planned to rerun the model with forecasted changes in shipping activity and environmental conditions (as predicted by climate change models) as data inputs, keeping all steps in the model the same as described in the methods above. Projected shipping activity data were obtained for the Canadian Arctic (e.g., 2015-2050 forecasts available from Environment and Climate Change Canada, Marine Emissions Inventory Tool), although these are limited to number of transits and voyage length without any assessment of potential changes in the geography of port connections (i.e., if the identity or frequency of different predictor and destination ports would change). Future shipping activity projections were not available/could not be obtained for the other Canadian regions during the timeframe of this analysis. It was therefore explored whether a future scenario could be modelled for only the Arctic region, by extrapolating the current port connections to the expected levels of shipping activity.

Future environmental temperature (used in probability of survival component) and salinity (used in probability of establishment component) variable values were extracted from Bio-ORACLE marine layers (Tyberghein et al. 2012; Assis et al. 2018) for the year 2050 (RCP8.5). However, projected climate variables were not available across all Arctic ports and their connected last two ports-of-call, resulting in data gaps for 66% of (current) Arctic transits. These missing data may be the result of several factors such as mismatch of the port locations to the marine climate layers (i.e., ports are in the Great Lakes, or are inland on major rivers).

Due to uncertainty in the future geographic shipping patterns and gaps in projected environmental data, the future scenario analysis was not completed as planned. Instead, two additional scenarios were conducted using the same methodology as the ‘current’ scenario model, specifically examining the impacts of increased vessel transits in the Arctic region using hull data only. The number of transits to Milne Inlet were increased as predicted under phase 2 of the Baffinland iron mine expansion (176 international arrivals, instead of 74) and additional transits into Churchill were added to reflect its peak year of operations between 2008-2020 (at 22 international arrivals in 2010, based on data obtained from the Canadian Ballast Water Information System; Etemad et al. 2021). Although the port of Churchill closed in 2016, it is expected to resume operations in 2023. These additions resulted in a future scenario of 221 annual vessel arrivals to the Arctic (an increase of 124 arrivals from the current scenario). A second scenario included an increase in overall vessel wetted surface area for vessel arrivals in the Arctic corresponding with a proposal to increase both vessel size and frequency to support higher rates of ore production in the Arctic (Baffinland Iron Mines Corporation 2020). Data were unavailable to precisely project this increase, so 10 vessel arrivals into Milne Inlet were randomly selected to increase gross tonnage to 100,000 for calculating associated wetted surface areas, under the expectation that Cape size vessels will be added to the fleet in the future. In the original dataset, the fleet is mainly Panamax size vessels, with the largest having gross tonnage values of up to 44,218. As there was insufficient information to determine how many Cape size vessels should be expected, the effect of increased wetted surface area was examined under a realistic scenario based on current vessel population. This scenario also had the increased vessel traffic as described above. Final species establishments in unique species per year were calculated in the same way as prior model runs, and values were compared across the two future scenarios and original baseline species establishments for the Arctic region. In addition, a brief literature review was conducted to establish the current state of knowledge with respect to future distributions of NIS in Canadian waters.

SENSITIVITY ANALYSIS

Sensitivity analyses were conducted to explore how the results of the model changed when different variables were increased, decreased, or otherwise altered to assess how sensitive the model was to any of the selected parameters (i.e., to determine if there are any variables that have a larger effect on the output of the model). These parameter alterations were also used to explore uncertainties in parts of the model, such as combining biological data across regions or using total abundance to estimate P_e. The parameters altered in the sensitivity analysis were: the number of vessel entries into Canada (+/- 25%), separation of biological data by region (for fouling abundance and proportion of NIS distributions), fouling abundance (+/- 25%), the proportion of NIS out of total fouling organisms (+/- 25%), the probability of release factor (set to 0.25 or 0.75), the environmental distance between ports (+/- 25%), the alpha establishment value (set to 0.005 for all species), salinity factor change to alpha (increased division to /20 and /100, or decreased division to /1 and /2), and changing the units of N in the P_e equation to reflect population density (individuals/m^2) instead of total abundance. Each parameter was changed individually to examine the response of the model (the number of unique species establishments per year (SpPY)) to that parameter in comparison to the original results, and not on an interacting basis. Percent change was calculated based on the final species per year metric of the model for each sensitivity analysis parameter change compared to the original baseline model. Note that these sensitivity analyses were performed only on the main hull fouling estimates, as the same model was used for both hull and niche analyses and differences are not expected.

RESULTS

At the arrival step (prior to release into the destination port), vessel niche areas tended to carry a greater number of individuals of NIS compared to the main hull area, except for the Arctic region (Figure 7). The estimated number of NIS individuals arriving in association with vessel hulls and combined niche areas was highest in the Pacific region (mean 948,978,861 individuals on vessels' hulls/yr and 1,329,916,371 individuals in niche areas/yr) and lowest in the Arctic region (mean 22,358,217 individuals on vessels' hulls/yr and 21,890,065 individuals in niche areas/yr). After accounting for the probability of release and survival at the destination port, the estimated number of NIS individuals decreased 78–88% across all regions and both underwater surface areas (greatest decrease in the Arctic region; Figure 7).

After factoring in the probability of establishment, the Atlantic and Pacific regions had the highest estimates for the mean number of NIS establishments per year (SpPY), with 2.136 (Atlantic) and 2.231 (Pacific) SpPY associated with vessels' hulls, and 7.622 (Atlantic) and 8.391 (Pacific) SpPY associated with vessels' combined niche areas (Figure 8). The estimated number of mean NIS establishments for the Great Lakes-St. Lawrence River region is 1.544 SpPY (by vessels' hulls) and 4.664 SpPY (combined niche areas). The Arctic region had the lowest estimates for mean number of NIS establishments, with 0.588 SpPY associated with vessels' hulls and 1.741 SpPY associated with the combined niche areas.

In all regions, NIS establishments associated with biofouling of the combined niche areas are much greater than those coming from the main hull, with a percent difference ranging from approximately 99–116%, with the greatest difference observed in the Pacific region. The number of NIS establishments followed a similar pattern to the number of vessel entries into each region, with the highest number of vessels destined for ports in the Atlantic and Pacific regions, and the lowest number of vessels entering via the Arctic region (Table 1).

Large differences were observed in the number of NIS establishments per year by vessel type (Figure 9). The highest rates of NIS establishments were associated with container vessel niche areas (at 36.8 SpPY). Bulkers, passenger vessels, and tanker niche areas had the next highest numbers of NIS establishments per decade, with values over 14 SpPY. The top vessel types for NIS establishments into Canada also had the largest number of entries into Canada per year, and largest wetted surface areas.

Similar regional patterns were observed for the estimated number of trips for at least one NIS establishment to occur. The lowest number of trips was estimated for the Pacific (94 trips via vessels' hulls, 23 trips via the combined niche areas) and Atlantic (101 trips via vessels' hulls, and 26 trips via niche areas) regions (Figure 10). The Great Lakes-St. Lawrence River region had the greatest number of trips until at least one NIS establishment occurs - with 174 (hull) and 49 (niche) trips, while the Arctic region had 134 (hull) and 35 (niche) trips until at least one NIS establishment occurs.

Examination of establishment patterns predicted by the last two ports-of-call revealed few differences in the estimated number of NIS establishments per year by region (Figure 11). Most regions had similar mean values of SpPY predicted by each last-port-of-call, although the Arctic region appeared to have a greater number of SpPY NIS establishments predicted by the second last port-of-call (POC2; hull: 0.51; niche: 1.49) compared to the more recent last port-of-call (POC1; hull: 0.11; niche: 0.48).

For the Arctic future scenarios, the estimated number of NIS establishments per year on vessel hulls increased by approximately 50% for both scenarios (increased traffic and increased traffic with increased gross tonnage) (Figure 12). Both future scenarios had similar values for mean NIS establishments per year, with 0.895 SpPY establishing in the increased trips scenario, and 0.891 SpPY establishing in the increased trips with increased gross tonnage scenario.

DISCUSSION

PROBABILITY OF NIS ESTABLISHMENT

The extent of NIS establishment via vessel biofouling in Canada is relatively unknown, although biofouling on commercial vessels has been previously identified as a vector of introduction for potentially harmful NIS into Canadian marine ecosystems (Adams et al. 2014; Linley et al. 2014) and there has been research on NIS introduction and spread by biofouling on recreational boats (e.g., Clarke Murray et al. 2011; Simard et al. 2017). In this study, the first quantitative estimate of the magnitude of the biofouling vector for the establishment of NIS via commercial vessels across Canada's four coastal regions is assessed.

The establishment of NIS by vessel biofouling was consistently highest for Atlantic and Pacific ports, each with approximately two unique SpPY established via vessels' main hull and eight unique SpPY established via the combined niche areas in each of the regions. Note that species being transported in association with vessel hulls and niche areas may overlap, so these estimates are not mutually exclusive. The higher rates of NIS establishment for these two regions is likely driven by the greater number of vessel arrivals relative to the other two regions. Differences between hull and niche areas are likely due to the high concentration of organisms, and high likelihood of NIS, in the niche areas. Although the hull wetted surface areas are much larger than for niche areas, the abundance of organisms in niche areas can be high, as reflected in the abundance distribution (Figure 4). Niche areas are more susceptible to biofouling than main hulls due to their complex surface types (which provide protection from turbulence and higher water velocities while underway) and lower efficacy of (or ability to apply) antifouling coatings (Coutts and Taylor 2004; Davidson et al. 2009; Moser et al. 2017). This is clearly important for the final estimate of NIS establishments in a region, with the combined niche areas having approximately three to four times as many NIS establishments compared to the hull region. Higher organism accumulation in niche areas has also been observed in other studies (e.g., Davidson et al. 2009).

The results of this study indicate that probability of establishment is not completely driven by greater shipping traffic as there are differences in the per trip estimates, resulting from the different abundances of NIS and the influence of environmental match between the last two ports-of-call and the destination port. The fewer trips until NIS establishments via the niche areas highlights that they are much higher risk areas on the vessel relative to the main hull. Note that the number of trips until NIS establishment does not indicate a minimum required number of voyages until a NIS can establish in a region, but rather a measure of the frequency that NIS may be expected to establish over the course of a year-long period (i.e., if a region has fewer trips than the trips until NIS establishment occurs, there is still risk of NIS establishment).

Various other factors may be influencing the risk of establishment of NIS via biofouling on vessels entering Canada. The age of the antifouling coating on a vessel may impact the extent of biofouling (Sylvester et al. 2011), which was unable to be assessed in this study due to limited data, though it is noted that the biological sampling informing this model was performed on vessels with a variety of antifouling systems of different ages. The duration of stay of a vessel at prior ports-of-call may also influence the amount of biofouling organisms accumulated, as greater time in a port allows for greater uptake of individuals (Davidson et al. 2009; Chan et al. 2022). Other factors that have been identified as having influence on the diversity and abundance of biofouling are: time since last dry-dock (Davidson et al. 2009), environmental factors resulting in synchronized spawning of organisms (Minchin and Gollasch 2003), vessel sailing speed (Coutts et al. 2010) and location of previous port visits (Sylvester et al. 2011; Chan et al. 2022).

MODEL GROUND-TRUTHING

There are no data available for the discovery rate of NIS in Canadian waters by biofouling, preventing the calibration of this model. While it is desirable to ground-truth any model, this is a difficult task when modelling establishments of NIS due to the lack of data concerning failed invasions and the many uncertainties surrounding observed species discovery data (e.g., inconsistent search effort, detection bias, etc.). As a result, this study relied on a mechanistic approach to estimate the probability of establishment of NIS based on well-known processes (stages of biological introduction) using a multistage model similar to previous work assessing the introduction and establishment of NIS in Canada via vessels' ballast water (Drake et al. 2020; Bradie et al. 2020).

While it would be inappropriate to compare the biofouling model results with that of the prior ballast water models due to differences in units of measurement for initial population sizes of NIS (density vs. total abundance), it is important to note that the foundational ballast water model was calibrated using discovery data for ballast-mediated species in the Great Lakes (Drake et al. 2020). The main steps of organism arrival, survival, and establishment in this biofouling model followed the prior calibrated method, with the addition of a probability of release factor, to account for partial release of organisms into the destination port, and the inclusion of prior ports-of-call to predict the survival and establishment of biofouling NIS.

It is surprising that the estimate for number of NIS establishments by biofouling to the Great Lakes-St. Lawrence River is about half that of the Pacific and Atlantic coasts, since biofouling has previously been considered lower risk for this freshwater area (Sylvester and MacIsaac 2010). Although this model included an adjustment to lower the establishment of organisms arriving in the Great Lakes-St. Lawrence River region, further work to fine-tune the model to better reflect survival and establishment probabilities for (mostly marine) biofouling species in freshwater recipient ports is warranted. Dedicated surveillance monitoring of biofouling on vessels and NIS in port areas would be beneficial for the calibration of such models in the future (e.g., Ojaveer et al. 2014).

SENSITIVITY ANALYSIS

Sensitivity analyses were conducted on model parameters including the number of trips per year, the abundance of fouling organisms and the proportion which are NIS, the probability of release, the environmental distance, and the establishment alpha values. The change in model output (unique species establishments per year) during sensitivity analysis was relatively small in most cases (changed by less than 10%), however, larger changes in the Arctic region and in parameters such as altered alpha values and population units were observed (Table 4). This sensitivity analysis indicates that many of the parameters alone have a relatively low effect on model outcome, but that a few parameters may be larger drivers in the model. The change in output when alpha values were set to 0.005 for all species was disproportionately large (i.e., 2000% change in estimate for approximately 90% change in alpha). This is not surprising as the 0.005 value is at the tail-end of the alpha distribution and setting alpha this high for all species is expected to markedly increase the probability of establishment irrespective of any mismatch in salinity. This is an unrealistic scenario – as many biofouling invertebrates are not parthenogenetic species, and establishment success is known to be affected by salinity mismatch (e.g., Ricciardi and MacIsaac, 2000; Paiva et al. 2018), though the magnitude of that effect is often unknown.

The alteration of the salinity factor adjustment to alpha resulted in a fairly large change in output for the Great Lakes-St. Lawrence River region compared to the other Canadian regions (changes >18% vs. <3 %). This is logical as all of the ports in the Great Lakes-St. Lawrence River region are freshwater, and therefore will be highly influenced by the salinity factor adjustment. Additional research to parameterize alpha and the salinity adjustment factor are recommended to fine-tune the output of this model to reflect survival rates of biofouling taxa in fresh waters.

Another large driver in the model is the unit choice for the population of NIS used in the probability of establishment (P_e) equation. In the main model, total abundance (individuals per total vessel area) was used, which was noted to be different from the units used to develop the upper limits of the model in Bailey et al. (2009), where population density (individuals per m^3) was used. Switching the abundance from a vessel-wide scale to a per-square-m scale had large effects on the model output, and all regions had decreases between 72–93% in species establishments per year. The choice of unit is somewhat philosophical – with total abundance reflecting the risk of all biofouling propagules released from a vessel as a single inoculum while the population density metric is more reflective of individual patches of biofouling having independent rates of establishment success. As the unit used in the model has a large influence on the model output, it should be carefully considered in future analyses.

The separation of regions in the construction of distributions describing the abundance and proportion of NIS on vessels was explored via the sensitivity analysis as well. The distributions used in this portion of the sensitivity analysis are provided in Figure A2 and A3 (Appendix 1). Additional distributions of niche areas are also available for comparison in Figure A4 and A5 (Appendix 1), but were not used in any analyses. Due to small sample sizes of vessels used to build these distributions, it is unknown if the differences in shapes (particularly for A2 with increases in the right tails for the Pacific and GLSLR regions) are due to actual regional differences or an artefact of small sample sizes. As expected, there were large shifts in some regions, with the Atlantic region experiencing a decline of 0.523 SpPY (-24.5%) and the Arctic region experiencing an increase of 0.153 SpPY (+26.0%). The Pacific and Great Lakes-St. Lawrence River regions experienced smaller declines as well. This highlights that there likely are regional differences in species abundances and the proportion of NIS associated with vessels arriving to each region, which are lost when regional data are combined. However, due to small sample sizes for each of the regions, it is difficult to determine the magnitude of differences in abundances and proportion of NIS by region. Additional biological sampling is recommended to increase knowledge of regional differences.

The Arctic region also had greater changes in output compared to the other regions when altering the number of trips per year, fouling abundance, and probability of release factor. This may be due to the limited number of vessel trips into the Arctic region in the shipping dataset, making it more sensitive to the randomization process in the model. However, it is a realistic scenario that the probability of NIS establishment will markedly increase in the future if there are large increases in vessel traffic and/or abundance of biofouling organisms, indicating that this region may be more sensitive to smaller changes that influence risk of invasion via biofouling compared to other regions.

Changes in the sensitivity analysis can also be used to understand how the model output would change due to variability in the underlying data and population processes. For example, the probability of survival will be highly variable across biofouling taxa, with some being better able to survive during voyages and after release into new destination ports. For example, some bivalves can tightly close their shells to survive short-term exposure to harsh salinity conditions, which may increase their likelihood of establishment, particularly at lower temperatures (Riley et al. 2022). This species-specific phenomenon is difficult to capture in a pathway-level model, but may be similar in effect to a shift in the relationship between environmental distance and survival (Figure 6), where the 25% decrease in environmental distances between ports results in higher survival. In the sensitivity analysis, this change resulted in a 2.39–7.31% increase in species establishments per year. Similarly, the probability of release was adjusted to 0.25 and 0.75, which may represent shorter and longer durations of stay, respectively. These adjusted values showed increases of up to 1.61% (probability of release set to 0.75) and decreases of up to -22.28% (probability of release set to 0.25) in NIS establishment, which may be indicative of the influence of duration of stay on biofouling risk.

MODEL LIMITATIONS AND UNCERTAINTIES

Although the outputs of this model provide insight on the probability of NIS establishments by biofouling to Canadian ports, they should be considered more as relative relationships rather than precise numerical estimates. Additional fine-tuning (especially regarding species release rates and establishment potential) is required as more data become available to more realistically capture the survival and establishment probabilities of biofouling NIS released into the freshwater Great Lakes-St. Lawrence River region. Conversely, this study used shipping data only for first arrivals of vessels to Canada, so it will underestimate NIS establishments by vessels making multiple port-calls per trip. In addition, there was no analysis of secondary establishments (i.e., spread) of NIS across Canadian ecosystems by vessels operating within Canada. Secondary establishments by domestic movements of recreational boats and ballast water are known to rapidly spread established NIS across Canadian ecosystems, hindering management efforts and amplifying economic and ecological costs (e.g., Simkanin et al. 2009: Drake, 2017). Previous research has reported that coastal domestic vessels undertake more than 1,000 trips annually in the Atlantic coast (Adams et al. 2014) while shipping traffic in the Arctic region is rapidly growing (Dawson et al. 2018). The secondary spread of NIS within Canada by biofouling remains a core issue for future research and management.

This biofouling model made use of best-available biological and shipping data, however, sample sizes were limited (biological biofouling data from 78 vessels and seachests across the four regions, and one year of shipping data). Due to the low sample size, it was necessary to combine the biological data across all regions to create a single distribution of biofouling abundance, thereby restricting examination of differences in NIS establishment owing to regional differences due to distinct patterns in vessel histories (e.g., voyage lengths and connected bioregions; Chan et al. 2014). Additionally, there were limited species-level identifications in the biological data for all regions except the Arctic, which reduced sample size even further for creating the distribution for the proportion of organisms being NIS. Regional estimates of NIS establishments may be further refined through collection of additional biofouling data for both main hull and niche areas. Further, the biological data that were used were collected more than 10 years ago and re-sampling may be warranted to assess the influence of any recent changes in biofouling management practices.

Uncertainties exist around various parameters and assumptions made with the model. The probability of release factor was set as $p = 0.5$ (on average) following the Drake et al. (2017) study modelling the release of organisms from biofouling on recreational boats although there are no data to inform the probability for biofouling release and the factors that influence release rates. Additionally, in the survival step, temperature was the only factor used for calculating environmental distance and thus for assessing organism survival in the destination port. Salinity was incorporated into the establishment portion of the analysis, using a categorical salinity match which was then used to decrease alpha values for population establishment. Other factors such as substrate type are likely important for survival and establishment of certain NIS, but the ‘right’ conditions will be species-specific, temporally variable, and the ability to include them in any model will be limited by available data. Similarly, alpha values for establishment probability are species-specific and largely unknown.

Finally, this analysis considered the last two ports-of-call in the survival assessment, based on their relatively higher importance for predicting the presence/absence of biofouling NIS compared to prior ports-of-call. The duration since last drydock can be a predictor of biofouling extent and diversity (Frey et al. 2014), with the combination of ports and vessel routes taken influencing the biofouling assemblage. Incorporating higher-order patterns of vessel traffic may improve the accuracy of predicting NIS establishments (Saebi et al. 2020).

NIS ESTABLISHMENTS BY VESSEL BIOFOULING UNDER FUTURE CLIMATE SCENARIOS

Summer sea ice extent in the Canadian Arctic has decreased over the past five decades (by 5–20% per decade, depending on the location), facilitating an increasing volume of shipping traffic across the region (Tivy et al. 2011; Dawson et al. 2018). Climate-induced reductions in sea ice are projected to increase navigability across the Arctic through Northern and Transpolar sea routes and the Northwest Passage (Stephenson et al. 2011). For instance, modelling results project 100% navigation probability along the Northwest Passage and Arctic Bridge trade routes for part of the year, above 2°C of global warming, placing the Canadian Arctic as a key region for future trans-Arctic shipping (Mudryk et al. 2021). In fact, several studies have reported the probability of a sea ice-free Arctic in summer months under different climate scenarios (Screen and Williamson 2017; Jahn 2018; Sigmond et al. 2018), with significant implications for the introduction and establishment of NIS in the Hudson Bay complex and other Canadian Arctic regions (Goldsmit et al. 2021).

Understanding the observed and projected changes in climate change indices is important to learn the nature and extent of likely impacts on marine and freshwater ecosystems in Canada (Steiner et al. 2015; Beaugrand et al. 2019; Arrigo et al. 2020). Most predictions of future NIS establishments by shipping have been conducted for Arctic regions, where changes in climate are expected to increase the level of shipping activity and influence the survival and establishment of new species (e.g., Miller and Ruiz 2014; Ware et al. 2014; Chan et al. 2019). Habitat suitability in the Canadian Arctic has been predicted to increase for at least some NIS under future climate scenarios (Goldsmit et al. 2018; Goldsmit et al. 2019; Goldsmit et al. 2020). For instance, Goldsmit et al. (2020) modelled spatial distribution of 23 ‘high risk’ species at pan-Arctic and global scales, revealing that all taxa studied will gain suitable habitat under future conditions at pan-Arctic scale while loss of habitat or no change was predicted at the temperate/southern ends of distributions for some of the same species.

Lyons et al. (2020) used more than 12 years of occurrence data and modelled the distribution of marine invertebrates and algae already established across the northwest Atlantic and northeast Pacific. They found that existing hotspots of aquatic invasive species are predicted to expand both in the Atlantic and the Pacific, and new hotspots are likely to appear in the Pacific. Additional assessments of predicted species distributions for biofouling taxa at a pan-Canadian scale may provide useful information on habitat suitability under future climate scenarios and could be tailored for directing ecosystem management and conservation planning for freshwater and marine ecosystems in Canada.

This analysis included theoretical scenarios to reflect the increased and projected changes in shipping transits to the Arctic (Dawson et al. 2017; Dawson et al. 2018). These scenarios may be used to examine how sensitive the Arctic is to changes in shipping patterns, in terms of how the number of NIS establishments per year may be expected to increase in the future. Under both scenarios, more than 50% increase was seen with relatively small increases of vessel arrivals to Churchill and Milne Inlet ports. The increase in gross tonnage (and thus vessel wetted surface area) did not appear to have strong influence on the results as the value for NIS establishments was similar to the scenario that only included increased trips. However, as there is limited information for projecting the proportion of vessels with increased size, it could be useful to explore this factor in more detail.

CONCLUSIONS

The main objective of this science advisory process was to build on previous DFO regional assessments of aquatic NIS establishments via vessel biofouling, to create an up-to-date national assessment incorporating ‘new’ data and modelling methods. A multistage mechanistic model was developed to assess the biofouling risk associated with vessels entering Canada across the Atlantic, Pacific, Arctic, and Great Lakes-St. Lawrence River regions over a one-year timespan. Available Canadian biofouling data and global estimates of underwater wetted surface areas by vessel type were used to develop estimates of the number of NIS arriving on vessel hulls and niche areas. Additional parameters, such as probability of release, survival and establishment in the destination port (predicted by environmental similarity in temperature and salinity to the last two ports-of-call) were incorporated into the model following the known stages of the biological invasion process.

The results of this analysis show that biofouling is a dominant vector for the establishment of NIS into Canadian coastal regions via shipping. A relatively large number of NIS per year are estimated to establish (arrive, survive, and establish) across all regions in Canada. Niche areas are identified as being greater risk for the establishment of NIS compared to the main hull of the vessels, likely due to higher abundance of biofouling organisms in these areas. The number of NIS establishments varies across regions, being highest on the Atlantic and Pacific coasts where vessel traffic is higher. The Arctic region had relatively lower rates of NIS establishment, however, this region may be more sensitive to changes in factors that influence NIS establishment via biofouling, such as increased vessel traffic, fouling abundance, or duration of stay of vessels, and is vulnerable to greater rates of establishment with climate change. Additional biological sampling of biofouling on vessels (both international and domestic voyages) and of rates of establishment of biofouling NIS in Canadian port areas would facilitate better calibration and fine-tuning of such multistage models and help to elucidate regional differences in biofouling risk.

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Zweng, M.M, Reagan, J.R., Antonov, J.I., Locarnini, R.A., Mishonov, A.V., Boyer, T.P., Garcia, H.E., Baranova, O.K., Johnson, D.R., Seidov, D., and Biddle, M.M. 2013. World Ocean Atlas 2013, Volume 2: Salinity. Edited by S. Levitus and A. Mishonov. NOAA Atlas NESDIS 74: 39 p. doi:10.7289/V5251G4D

TABLES

Table 1. Annual number of entries into Canadian waters by foreign-flagged vessels, by region (with associated provinces for each region), and number of destination ports within each region, in the shipping dataset used for this assessment. Data were obtained from Transport Canada Marine Security Operation Centres (East and West) for 2018 (with transits to Milne Inlet in the Arctic region from 2019 substituted for 2018).

Region	Provinces	Number of entries	Number of ports
Pacific	BC	3447	24
Atlantic	PE, NB, NL**, NS, QC*	3138	54
Great Lakes-St. Lawrence River	ON, QC*	1421	22
Arctic	MB, NL**, NT, NU, QC*	97	10

*Atlantic Coast region includes ports to the east of Quebec City, while Great Lakes-St. Lawrence River region includes ports to the west of and including Quebec City. Northern Quebec ports are included in the Arctic region.

**The island of Newfoundland is included in the Atlantic Coast region, while Labrador (mainland) is included in the Arctic region.

Table 2. Types of vessels and underwater wetted surface areas based on Moser et al. (2016) and associated vessel types and frequencies by region (PC = Pacific, AC = Atlantic, GL = Great Lakes-St. Lawrence River, AR = Arctic) in the shipping dataset used for this assessment.

General Vessel Type (Moser et al. 2016)	**Specific Vessel Type (Canadian shipping data)**	**Frequency**			
		PC	**AC**	**GL**	**AR**
Bulkers	Bulk carrier, Bulk/Oil carrier, Cement carrier, Ore carrier, Wood chips carrier	1444	726	454	80
Container Vessels	Container vessel, Container/ro-ro cargo vessel, Passenger/ro-ro vessel (vehicles), vehicles carrier	1039	1092	376	0
LNG/LPG Carriers	LNG tanker, LPG tanker	1	11	0	0
Tankers	Asphalt/bitumen tanker, Chemical/Products tanker, Crude oil/Oil products tanker, Shuttle tanker, Tanker	266	753	423	2
Other	Buoy tender, Cable layer, Diving support vessel, Icebreaker, Offshore support vessel, Platform supply vessel, Research survey vessel, Sail training vessel, Trailing suction hopper dredger	9	28	4	3
General Cargo	General cargo vessel, Heavy load carrier, Open hatch cargo vessel, Refrigerated cargo vessel, Replenishment dry cargo vessel, Ro-ro cargo vessel	188	352	154	3
Passenger Vessels	Passenger vessel, Cruise, Yacht	461	175	4	8
Tugs/Supply Vessels	Anchor handling tug supply, Articulated pusher tug, Offshore tug/supply vessel, Tug	39	1	6	1

Table 3. Matrix showing salinity factor adjustments to alpha values based on the salinity match between the last port-of-call (either first or second) and the destination port. Salinity categories for each port are marine (salinity >18.1 g/kg), brackish (salinity 5.1–18.0 g/kg), or freshwater (salinity <5.1 g/kg), where alpha values are divided based on the magnitude of difference.

		Last port-of-call (first or second)		
		Marine	**Brackish**	**Freshwater**
Destination port	**Marine**	*α*	*α/2*	*α/10*
	Brackish	*α/2*	*α*	*α/2*
	Freshwater	*α/10*	*α/2*	*α*

Table 4. Sensitivity analysis results, as % change in species per year from baseline value, for model parameters: number of trips per year, separation of regions, initial fouling abundance, proportion of NIS, probability of release, environmental distance between ports, alpha values for all species, salinity factor change, using density for establishment equation. Values directly below each parameter header indicate the change applied: increase/decrease by 25% or set to certain values (separate distributions of abundance and proportion of NIS by region, probability of release set to 0.75 and 0.25, alpha values for all species set to 0.005, salinity factor adjustment increased by a multiple of 10, or decreased to no division for medium difference and halved for large difference, probability establishment using density of organisms rather than abundance).

Region	**Baseline species per year**	**Trips per year**		**Separate regions**	**Fouling abundance**		**Proportion of NIS**		**Probability of release**		**Environmental distance**		**Alpha values**	**Salinity Factor**		**Density**
		+25%	**-25%**	**-**	**+25%**	**-25%**	**+25%**	**-25%**	**=0.75**	**=0.25**	**+25%**	**-25%**	**=0.005**	**/20 or /100**	**/1 or /2**	**-**
Atlantic	2.136	+5.38%	-3.23%	-24.49%	+7.72%	-5.20%	+2.20%	-2.15%	+0.37%	-8.71%	-1.92%	+2.39%	+1632.02 %	-2.11%	+2.11%	-72.57%
Pacific	2.231	+4.57%	-3.50%	-5.38%	+7.08%	-5.29%	+2.24%	-2.06%	+1.61%	-7.17%	-2.33%	+3.36%	+1558.45 %	-0.54%	+1.12%	-72.88%
Great Lakes-St. Lawrence River	1.544	+5.31%	-9.00%	-12.63%	+9.26%	-7.84%	+2.07%	-4.08%	+1.41%	-10.30%	-7.38%	+6.22%	+2187.76 %	-27.14%	+18.26%	-88.92%
Arctic	0.588	+13.10%	-27.04%	+26.02%	+4.59%	-16.84%	+1.70%	-3.57%	-8.16%	-22.28%	-3.57%	+7.31%	+2111.73 %	-2.04%	+2.21%	-90.82%

FIGURES

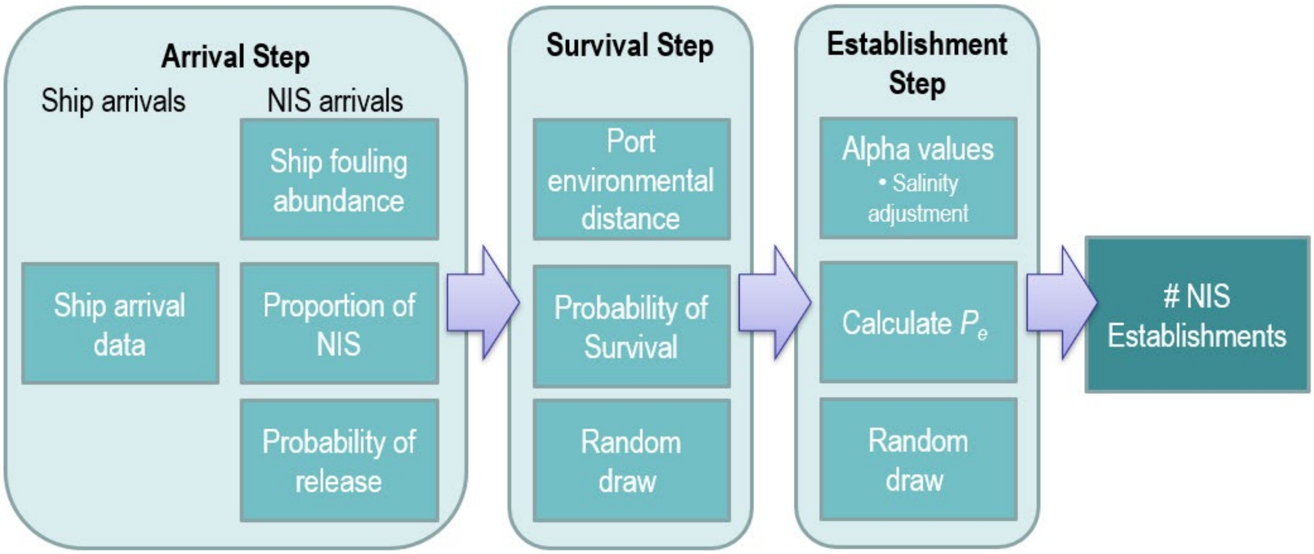


Figure 1. Flow chart outlining the main steps in the model in the arrival, survival, and establishment steps (large bubbles) each with specific steps (smaller squares) to obtain the final number of nonindigenous species (NIS) establishments.

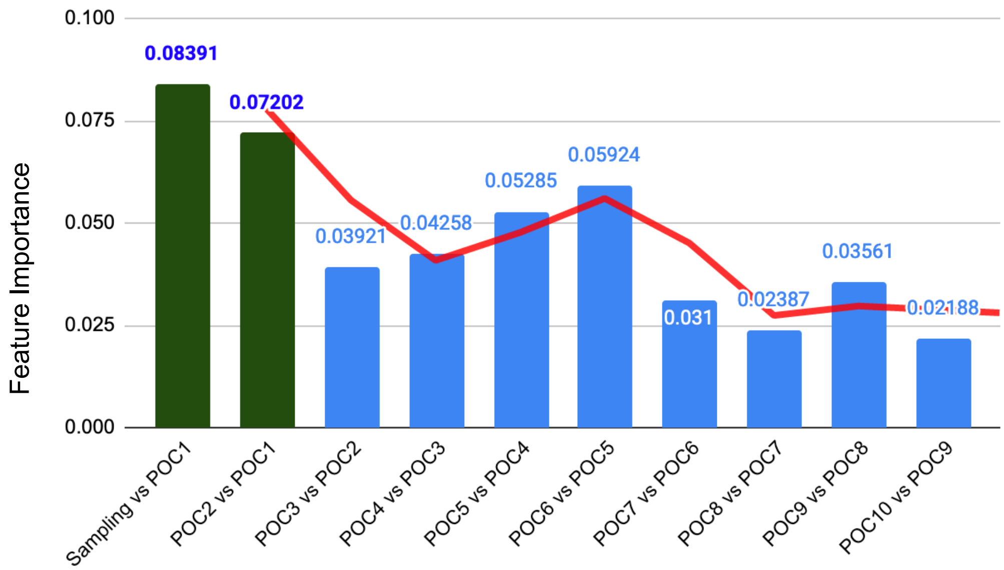
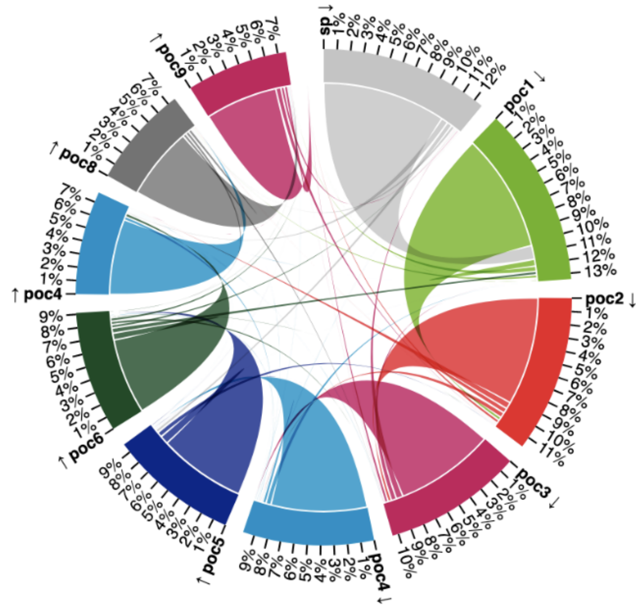


Figure 2. Results of the port history analysis examining the importance of each of the last 10 ports-of-call for predicting presence of NIS on arrival to the Canadian sampling/destination port.-Each bar represents the relative importance of each port-pairing, while the line represents the moving average trend across all port pairs.

A.



B.

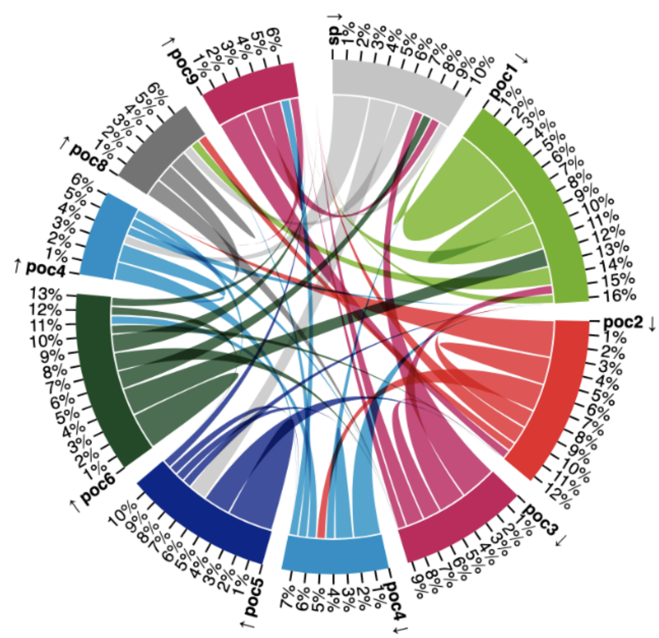


Figure 3. A) Cord diagram representing the trips between sampling port (SP) and previous ports-of-call (POC), in sequence from most recent (poc1) to 10th last POC. B) Magnified cord diagram showing repeated visits at different POC by removing adjacent connections.

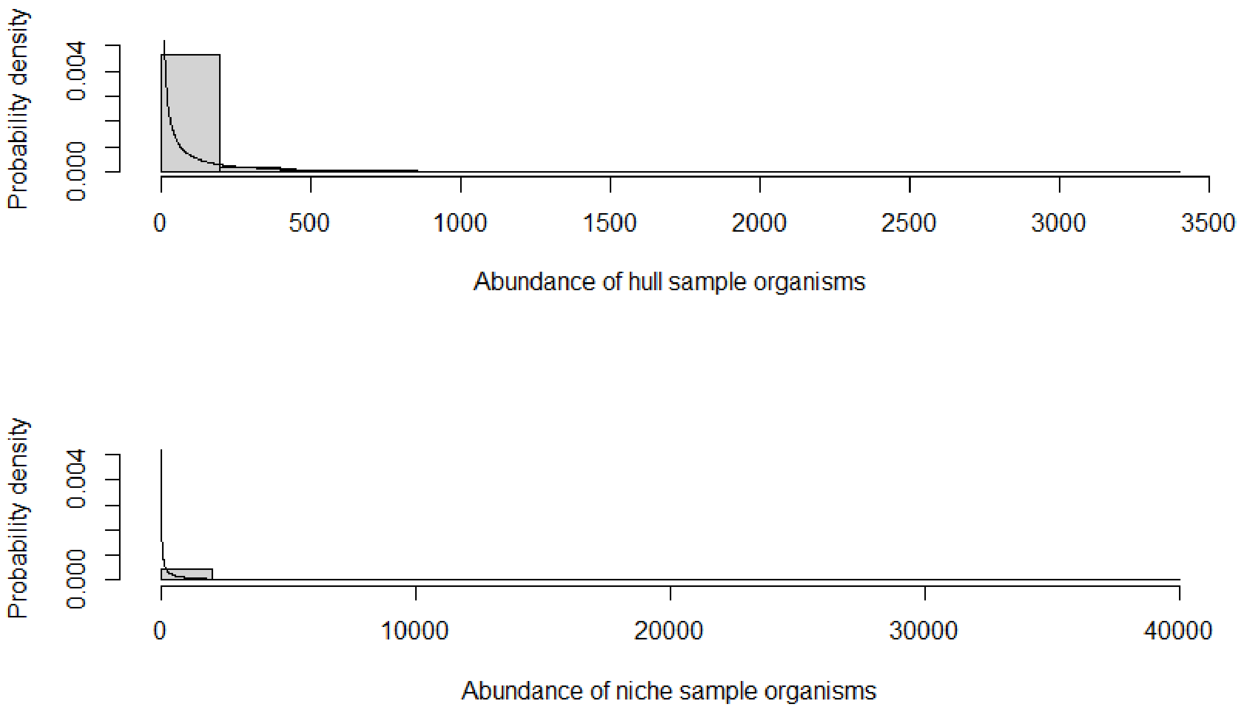


Figure 4. Probability distributions describing the abundance of biofouling organisms on individual vessels based on biological fouling data (combined across all regions). The top distribution describes abundance of biofouling organisms on main hulls, while the bottom distribution describes abundance of biofouling organisms in combined niche areas. The black line indicates the probability function.

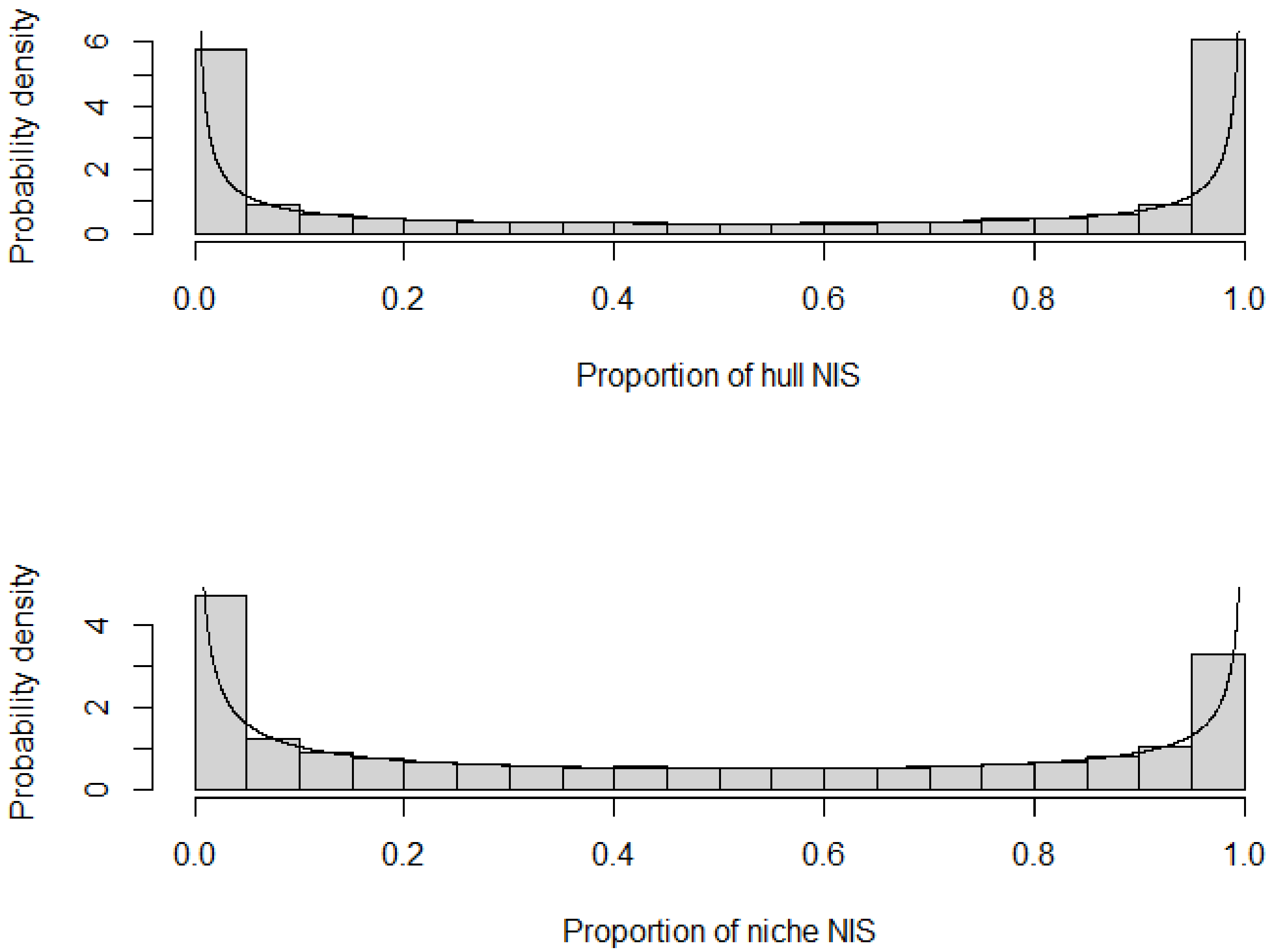


Figure 5. Probability distributions describing the proportion of nonindigenous individuals based on biological fouling data (combined across all regions), for both main hull (upper distribution) and combined niche (lower distribution, including seachest data) areas of vessels. The black line indicates the probability function.

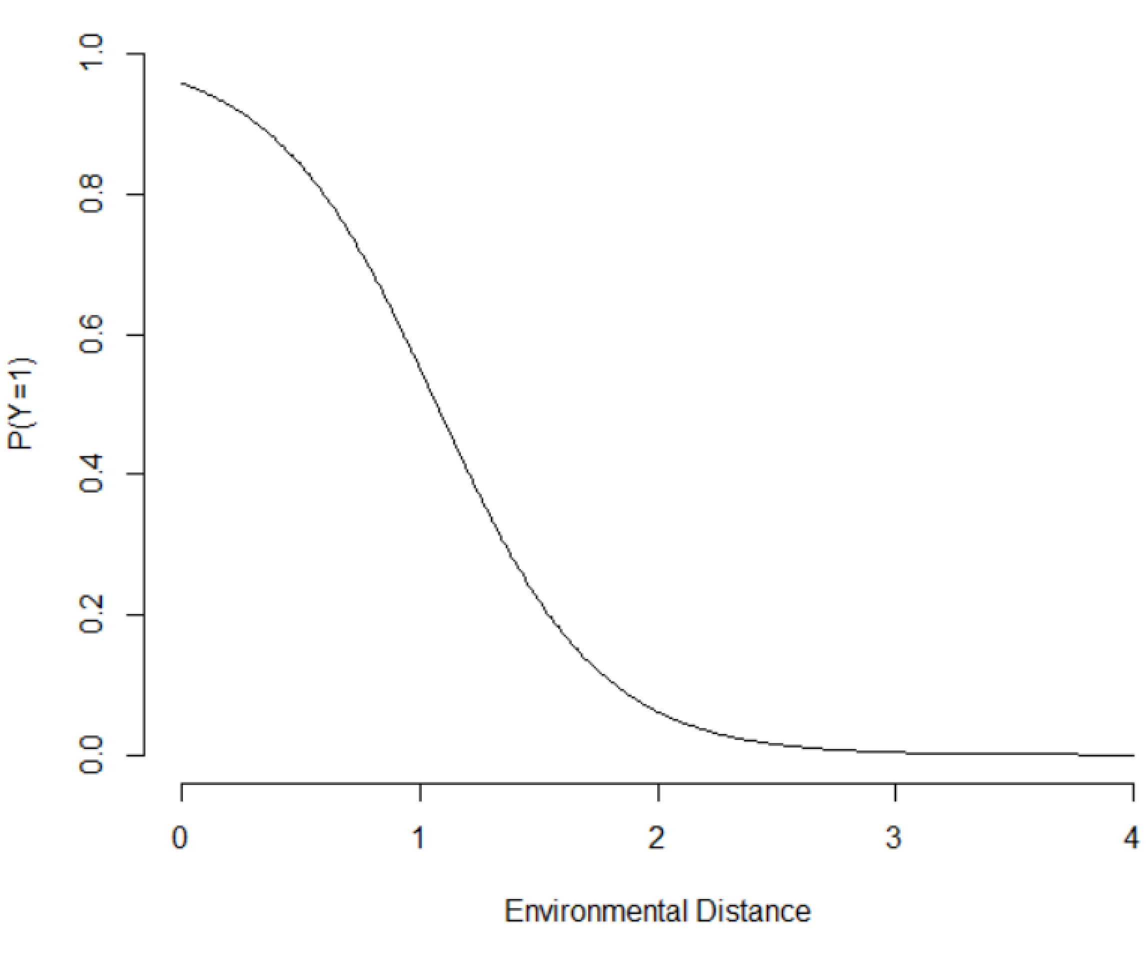


Figure 6. Survival curve relating environmental distance (Euclidean distance of mean temperature during the warmest month, mean temperature during the coldest month and annual average temperature) to the probability of survival based on presence-presence vs. presence-background data for 603 aquatic organisms obtained from the Global Invasive Species Information Network. A binomial generalized-linear model was fit to the data to produce the curve. Adopted from Bradie et al. (2020).

A.

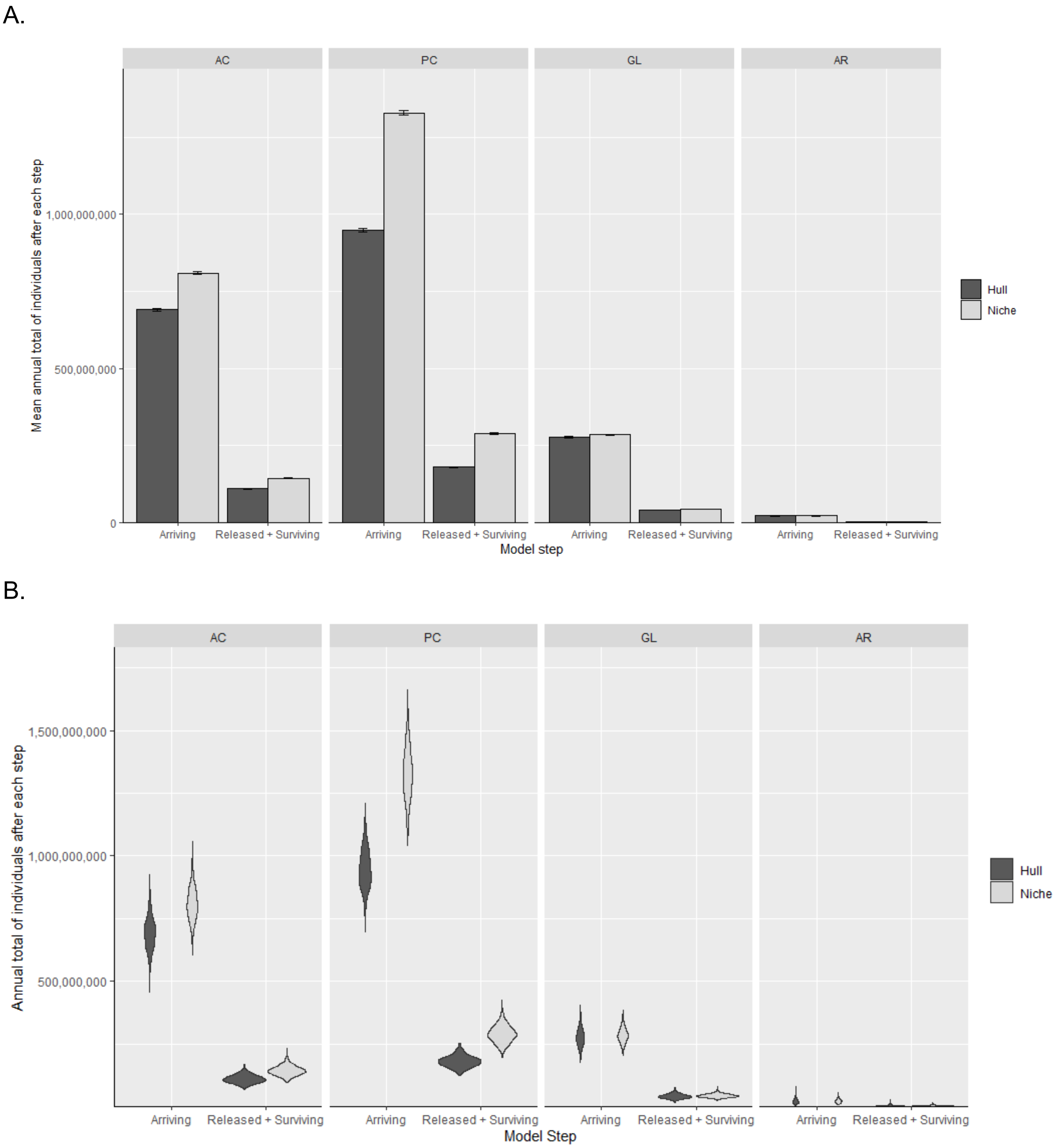


Figure 7. A) Mean annual total number of NIS individuals arriving on vessels (left bars of each panel) and mean annual total number of NIS individuals that are released and survive in the destination port (right bars of each panel), by region (AC = Atlantic, PC = Pacific, GL = Great Lakes-St. Lawrence River, AR = Arctic), associated with main hull (dark bars) and combined niche areas (light bars). Mean annual total is the mean value of the summed individuals of NIS per year across all model iterations. Error bars represent the 95% bootstrapped confidence intervals on the mean for 1,000 simulations. B) Violin plots displaying the same results described above, but representing the entire spread of results across all runs of the model (probability density of the data).

A.

B.

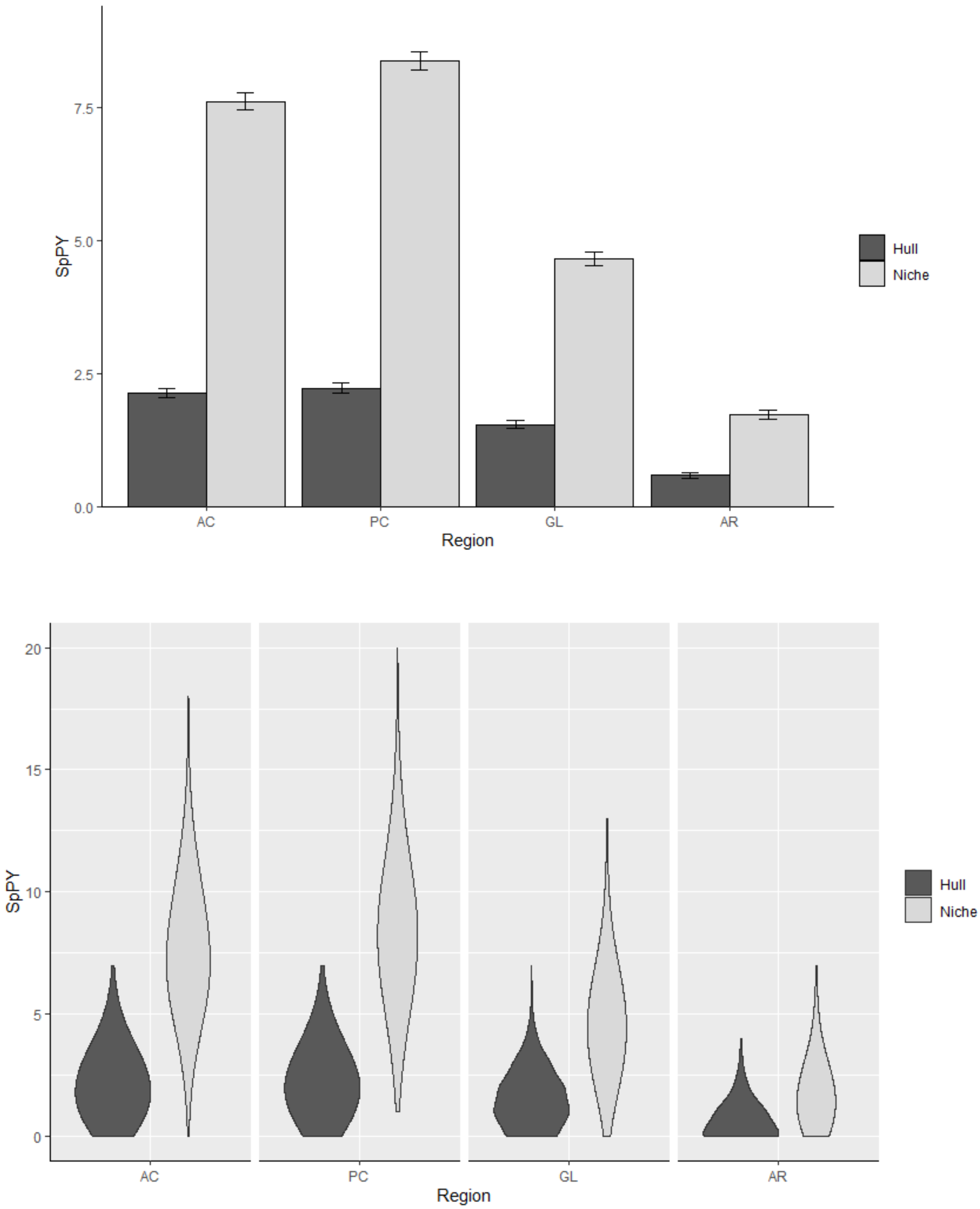


Figure 8. A) Mean number of unique NIS establishments per year (SpPY) for each region (AC = Atlantic, PC = Pacific, GL = Great Lakes-St. Lawrence River, AR = Arctic) by main hull (dark bars) and combined niche areas (light bars). Error bars represent the 95% bootstrapped confidence intervals on the mean for 1,000 simulations. B) Violin plots displaying the same results described above, but representing the entire spread of results across all runs of the model (probability density of the data). An adjustment was made to smooth out the plots due to discrete values for species establishments.

A.

B.

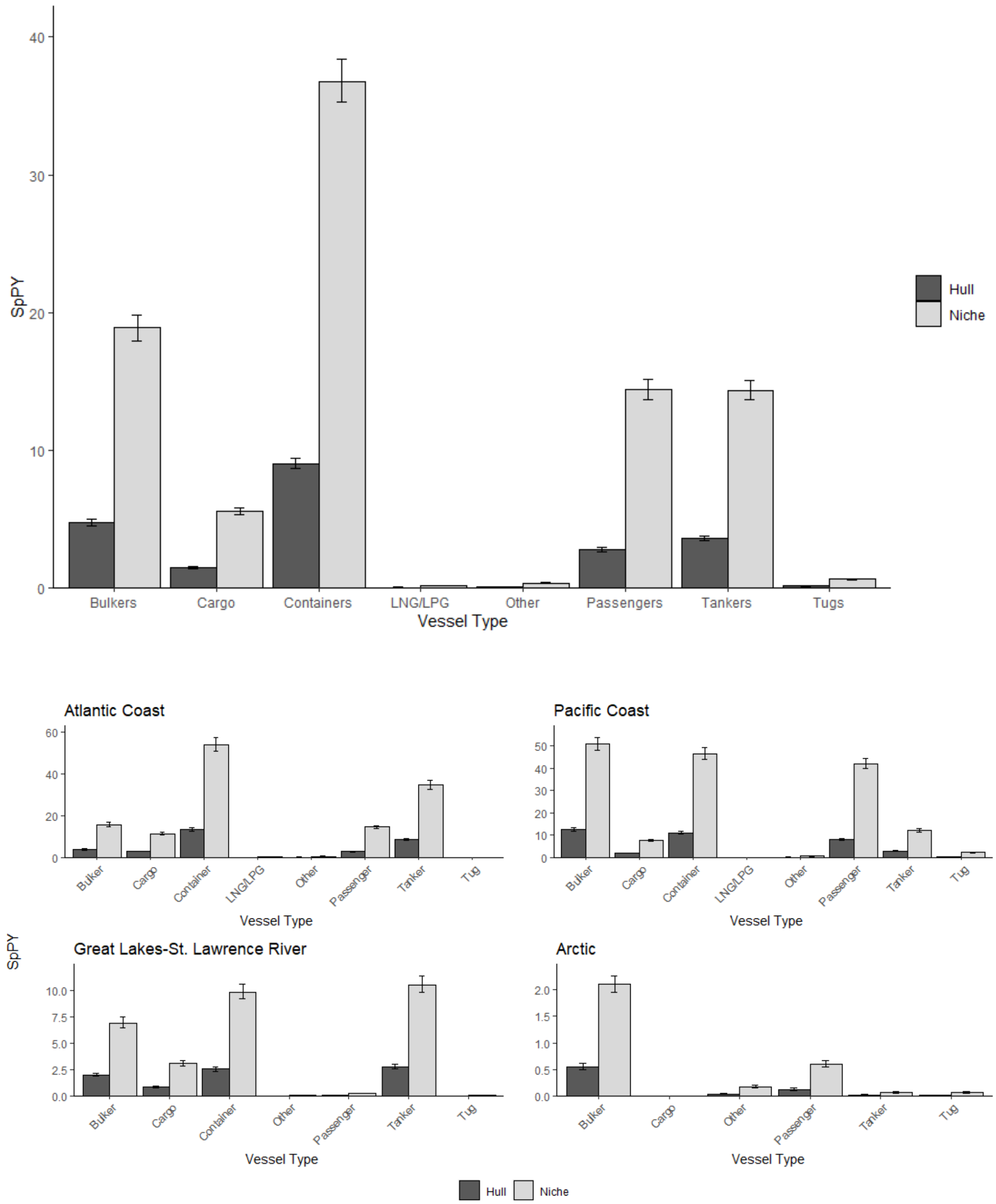


Figure 9. A) Mean number of NIS establishments per year (SpPY) attributed to each vessel type across all regions, by main hull (dark bars) and combined niche areas (light bars). Error bars represent the 95% bootstrapped confidence intervals on the mean for 1,000 simulations. Note that species may not be unique across each vessel type, and that container vessels were not present in the Arctic region, and LNG/LPG vessels were not present in the Arctic or Great Lakes-St. Lawrence River regions. B) Mean number of NIS establishments per year (SpPY) attributed to each vessel type separated by region. Error bars represent the 95% bootstrapped confidence intervals on the mean for 1,000 simulations.

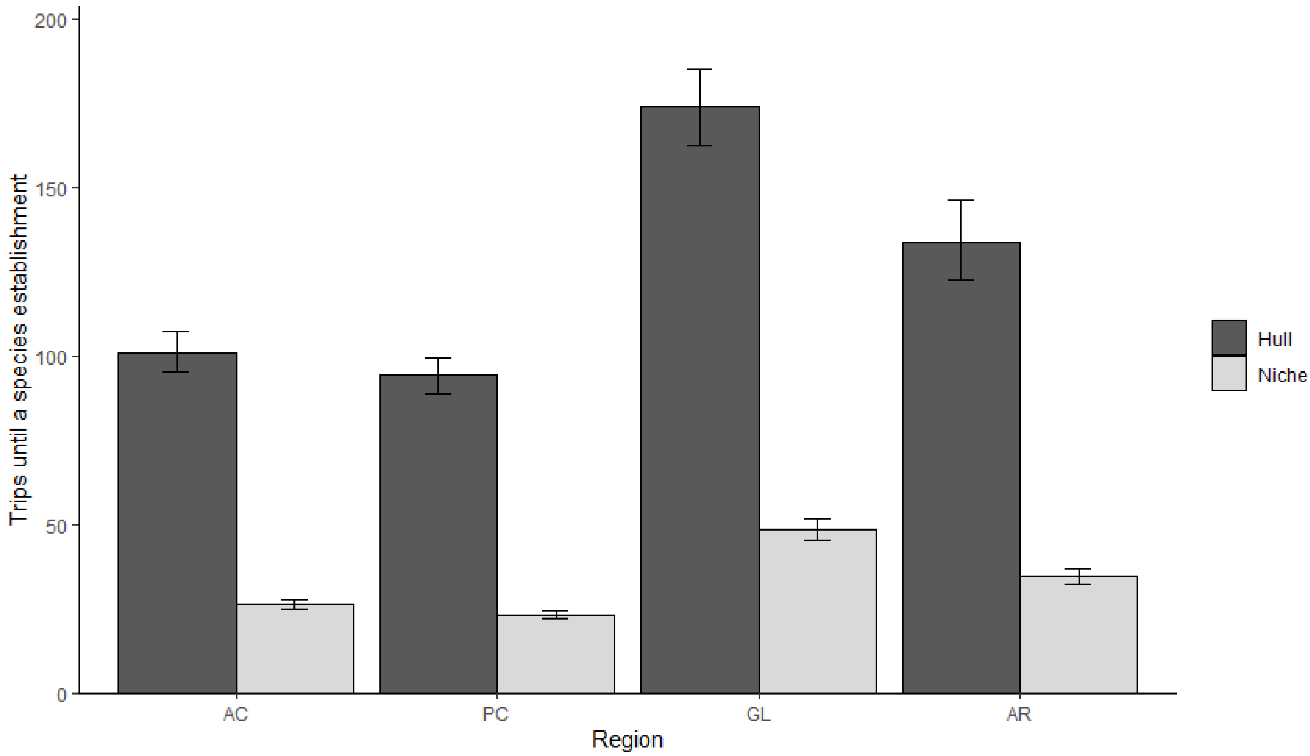


Figure 10. Mean number of trips until at least one NIS establishment occurs for each region (AC = Atlantic, PC = Pacific, GL = Great Lakes-St. Lawrence River, AR = Arctic) for both main hull (dark bars) and combined niche areas (light bars). Error bars represent the 95% bootstrapped confidence intervals on the mean for 1,000 simulations.

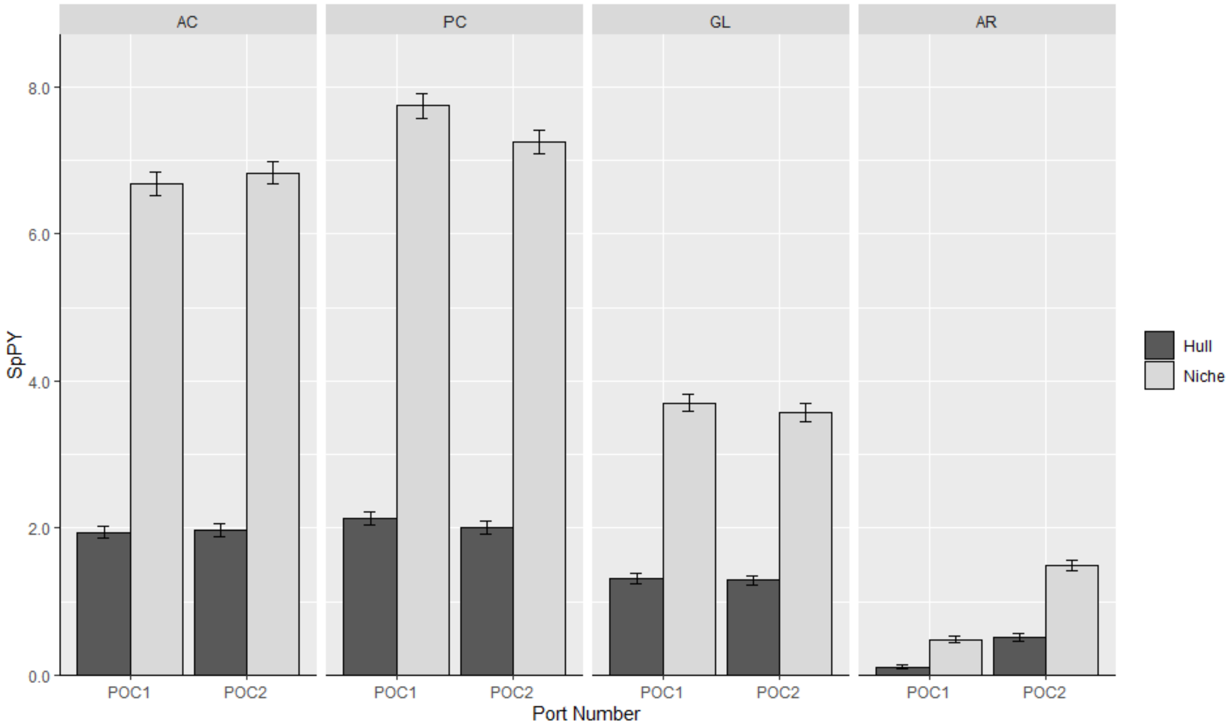
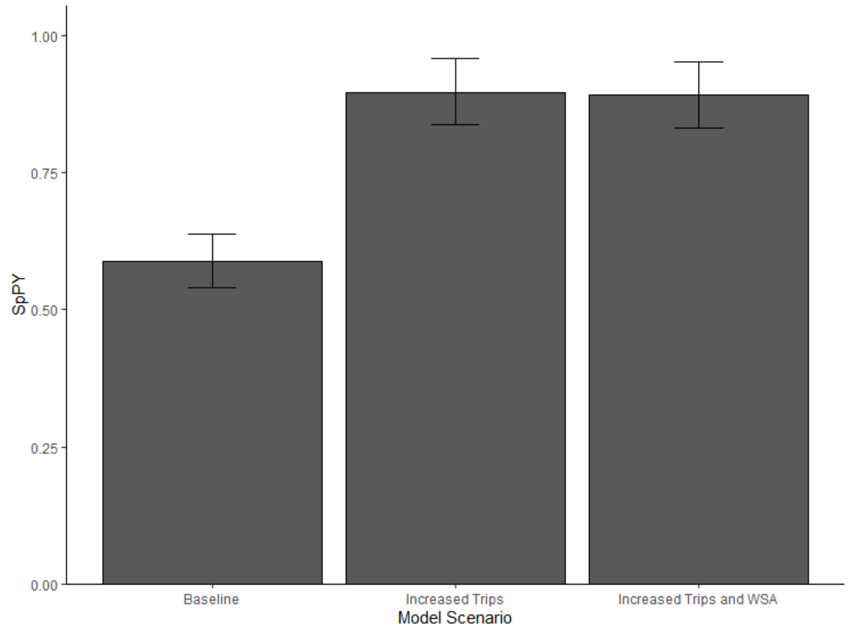


Figure 11. Mean number of NIS establishments per year (SpPY) as predicted by the most recent last port-of-call (POC1) and second-last port-of-call (POC2) on combined scales across regions. Main hull (dark bars) and combined niche (light bars) areas are represented. Error bars represent the 95% bootstrapped confidence intervals on the mean for 1,000 simulations.

A.



B.

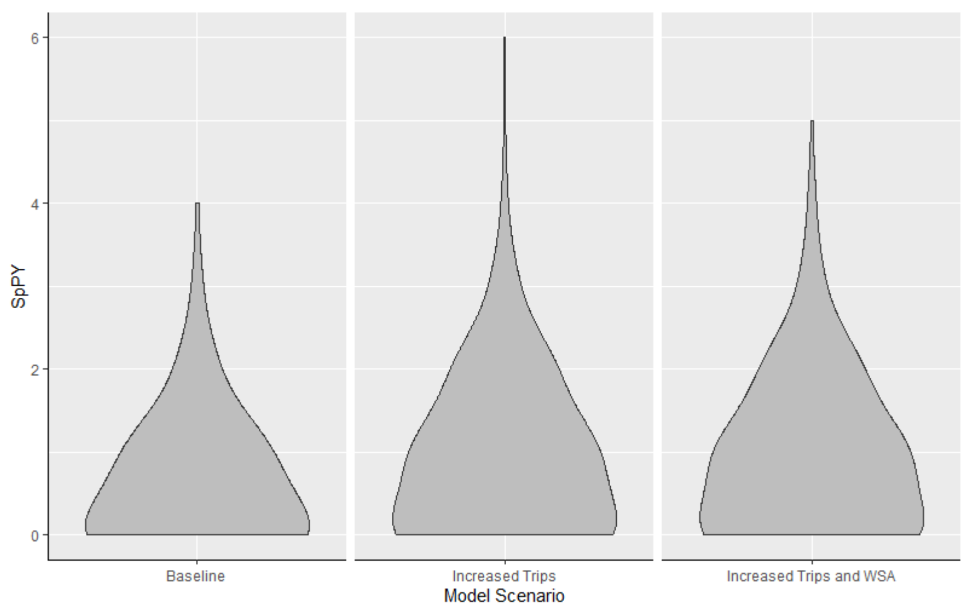


Figure 12. A) Mean number of unique NIS establishments per year (SpPY) under future scenarios in the Arctic region for main hulls only. "Baseline" scenario represents the current scenario with no changes, "Increased Trips" has an increase of 124 vessel (for a total of 221) arriving in the Arctic region, and "Increased Trips and WSA" has an increase of 124 vessels along with 10 vessels randomly selected with increased wetted surface areas (WSA) (GRT set to 100,000). Error bars represent the 95% bootstrapped confidence intervals on the mean for 1,000 simulations. B) Violin plots displaying the same results described above, but representing the entire spread of results across all runs of the model (probability density of the data). An adjustment was made to smooth out the plots due to discrete values for species establishments.

APPENDIX 1

Table A1. Model parameters that were used to quantify the estimated number of NIS establishing in Canada via biofouling per region.

<table>
<tr><th colspan="2" rowspan="2">Parameter</th><th colspan="4">Region</th></tr>
<tr><th>Pacific</th><th>Atlantic</th><th>Great Lakes-St. Lawrence River</th><th>Arctic</th></tr>
<tr><td colspan="2">Number of arrivals (2018)</td><td>3447</td><td>3138</td><td>1421</td><td>97</td></tr>
<tr><td rowspan="2">Abundance of sample organisms</td><td>size</td><td colspan="4">Hull: 0.0861
Niche: 0.1483</td></tr>
<tr><td>μ</td><td colspan="4">Hull: 43.2985
Niche: 618.6712</td></tr>
<tr><td rowspan="2">Proportion NIS</td><td>α</td><td colspan="4">Hull: 0.1972
Niche: 0.3296</td></tr>
<tr><td>β</td><td colspan="4">Hull: 0.1899
Niche: 0.3938</td></tr>
<tr><td rowspan="2">Probability of release (binomial)</td><td>size</td><td colspan="4">100</td></tr>
<tr><td>prob</td><td colspan="4">0.5</td></tr>
<tr><td rowspan="2">Survival probability curve (logistic)</td><td>Intercept</td><td colspan="4">3.132</td></tr>
<tr><td>Slope</td><td colspan="4">-2.913</td></tr>
<tr><td rowspan="2">Probability of single propagule establishment (the alpha parameter in Leung et al. 2004, modelled as beta distribution)</td><td>α</td><td colspan="4">0.005</td></tr>
<tr><td>β</td><td colspan="4">5</td></tr>
<tr><td>Allee Effect</td><td>c</td><td colspan="4">1</td></tr>
</table>

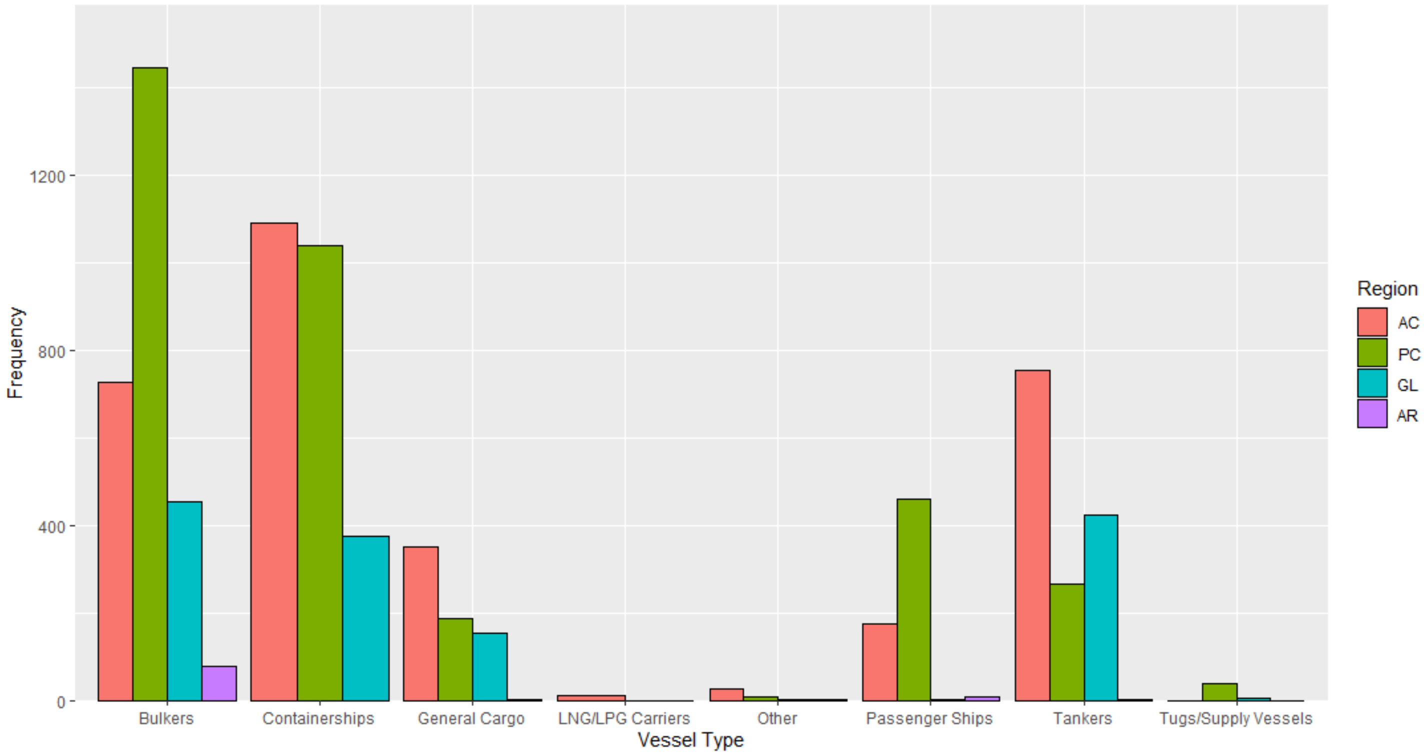


Figure A1. Frequency of vessel types in each category (x-axis) by region (colours; AC = Atlantic, PC = Pacific, GL = Great Lakes-St. Lawrence River, AR = Arctic).

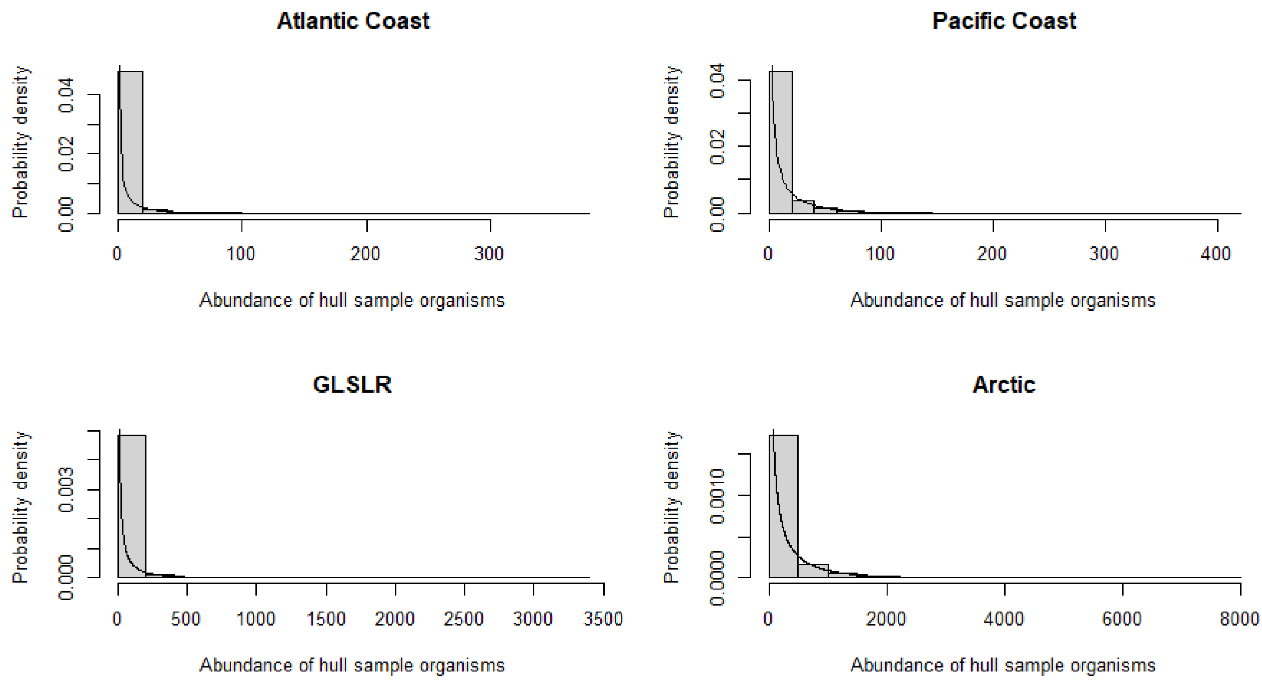


Figure A2. Probability distributions describing the abundance of biofouling organisms on vessel hulls for each region. These distributions were used in the sensitivity analysis but combined for the main analysis. Note that these distributions show hull-only abundances without niche abundances, as these were the only ones included in the sensitivity analysis. The black lines for each distribution describe the probability functions. Note differences in scale for each panel to visualize the pattern in each region.

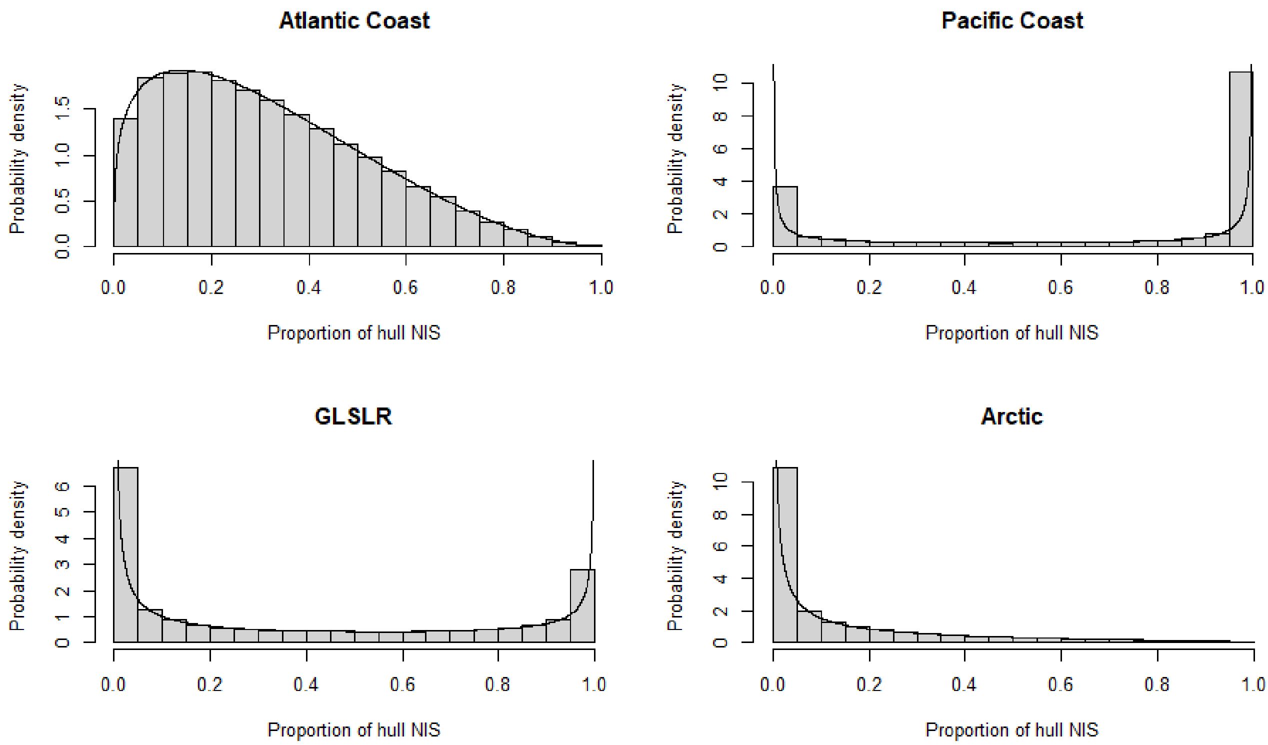


Figure A3. Probability distributions describing the proportion of nonindigenous individuals based on sample data from each region, for the main hull only. These distributions were used in the sensitivity analysis where regions were separated, but combined in the main analysis. The black lines indicate the probability functions. Note differences in scale for each panel to visualize the pattern in each region.

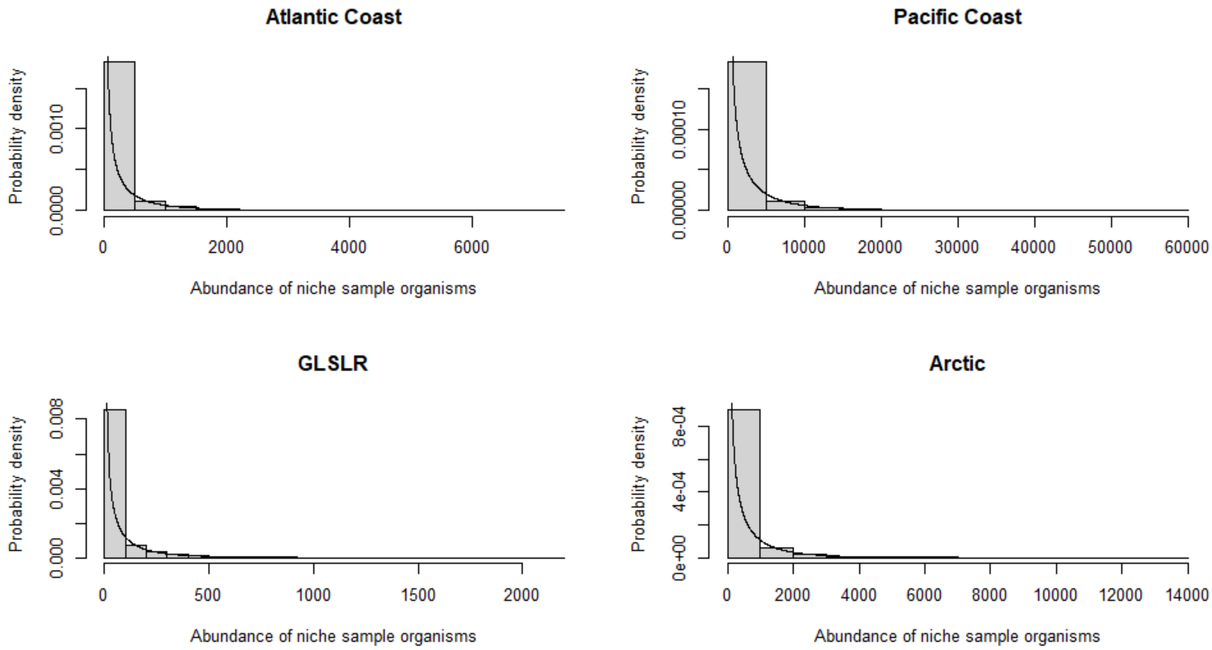


Figure A4. Probability distributions describing the abundance of biofouling organisms on vessel niche areas for each region. Note that these distributions show niche-only abundances which were not included in the sensitivity analysis, and these distributions were combined in the main analysis. The black lines for each distribution describe the probability functions. Note differences in scale for each panel to visualize the pattern in each region.

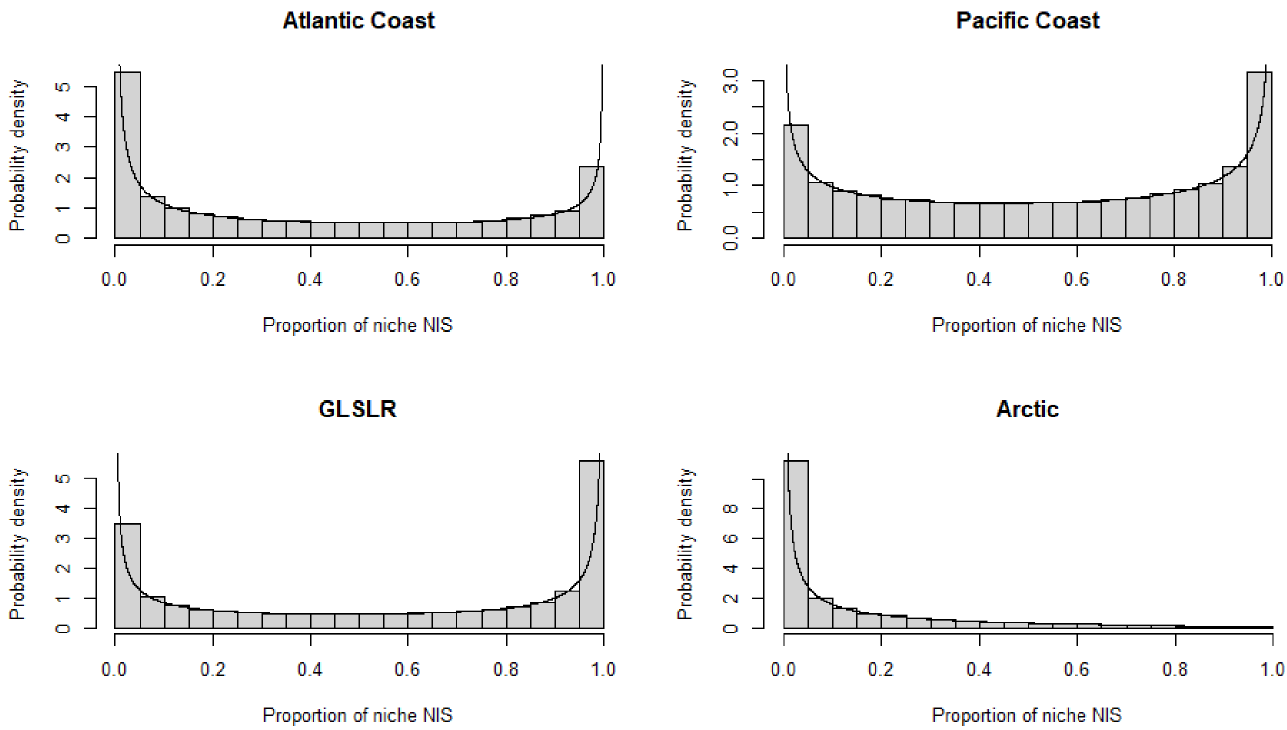


Figure A5. Probability distributions describing the proportion of nonindigenous individuals based on sample data from each region, for the niche areas only. Note that these distributions show niche-only abundances which were not included in the sensitivity analysis, and these distributions were combined in the main analysis. The black lines indicate the probability functions. Note differences in scale for each panel to visualize the pattern in each region.