UNIVERSITÉ DU QUÉBEC À RIMOUSKI

INTRODUCTION DE DINOFLAGELLÉS NON INDIGÈNES DANS LES ÉCOSYSTÈMES AQUATIQUES CANADIENS VIA LES RÉSERVOIRS DE BALLAST DES NAVIRES

Thèse présentée

dans le cadre du programme de doctorat en Océanographie

en vue de l'obtention du grade de philosophiæ doctor, océanographie

PAR

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AVRIL 2012

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Dépôt initial le 17 Avril 2012 Dépôt final le 28 aout 2012

A mis padres y hermana por motivarme a superar mis límites. A mi familia: Paola, mi principal motor, Avril mi solecito que ha sabido quebrar la soledad, Mathias mi angel en la infinidad, ...y a mis dos futuras razones de seguir siendo (Isaac y Sofia).

In questions of sciences the authority of a thousands is not worth the humble reasoning of a single individual: Galileo Galilei.

Always listen to those that say they are searching for truth, never those that say they have found it: Albert Einstein.

We are all atheists about most of the gods that societies have ever believed in. Some of us just go one god further: Richard Dawkins.

REMERCIEMENTS

Je voudrais remercier les membres du jury de Thèse et mon comité, Dr. Philippe Archambault (président ISMER-UQAR), Dr. Lucie Maranda (examinatrice externe, URI), Dr. C.W. McKindsey (membre du comité de thèse) et mes directeurs, Dr. Suzanne Roy (directrice ISMER-UQAR) et Dr. André Rochon (codirecteur, ISMER-UQAR). Leurs commentaires et précisions constructifs apportés sur les questions examinées ont contribué grandement aux avancées de cette thèse. Plus particulièrement, je voudrais remercier mes directeurs de recherche, *Suzanne Roy* et *André Rochon* qui m'ont fait confiance en m'acceptant comme étudiant à ce projet. Merci beaucoup pour votre support tout le long de cette recherche. Je suis très reconnaissant pour votre disponibilité, pour répondre à mes questions ou pour corriger mes écrits. Merci pour cette formation dans les sciences de la mer, mais surtout pour me transmettre votre passion pour le phytoplancton, pour les dinoflagellés et leurs kystes. Merci de toute votre patience!!

Merci aux chercheurs partenaires de l'Institut Maurice Lamontagne à Pêches Océans Canada, Nathalie Simard, Andrea Weise et Chris W McKindsey pour vos commentaires et votre appui scientifique dans les différents volets de cette recherche. Merci aux biologistes François Roy et Jean Yves Couture pour les heures dans les réservoirs des bateaux, où chaque gramme de sédiment ou chaque millilitre d'eau faisait la différence. Merci aux différentes équipes d'échantillonnages à Vancouver, dans les Grands Lacs, ainsi que dans l'est du pays, notamment Sarah Bailey, Elizabeta Briski, Francis Choi, Jean-Yves Couture, Rémis Daigle, Donald Humphrey, Matthieu Huot, Olivia Lacasse, Gavin Leung, Petra Logic, Colin van Overdijk et Beth Piercey. Je voudrais remercier vivement la précieuse collaboration de Marie Parenteau. Au début avec les échantillons vivants et les cultures des dinoflagellés; ensuite avec son expertise dans l'identification des cellules végétatives. Merci à Sylvie Lessard de m'avoir ouvert les portes du laboratoire de taxonomie de phytoplancton à l'Institut Maurice Lamontagne et d'avoir partagé les références bibliographiques avec moi, *quels beaux cadeaux*! Merci à DomDom et Thomas, avec qui j'ai appris sur les kystes. Merci à tous les employés de l'ISMER qui m'ont aidé à résoudre les différents problèmes, que ce soit au plan administratif: Sylvie, Brigitte, Nycole, Martine, Marielle ou au niveau technique: Dominique, Mélanie, Pascal, Gilles, Sylvain et Bruno.

Je remercie aussi les étudiants de mon bureau, *quelle belle diversité culturelle*: Sahar, Chian, Waibba, Nico, Heicke, Sarah, Eva, Jessi, Philippe. Merci d'avoir supporté ces longues journées d'obscurité, en m'épargnant de travailler sous la lumière flegmatique des néons. Merci Nico de résoudre n'importe quel problème technique et de partager ton savoir et ta passion pour la . Merci aux étudiants hors bureau (présents et déjà partis) et hors campus, qui m'ont aidé, qui m'ont soutenu, ou qui ont su me changer les idées pendant les moments difficiles: Aurore, Magdalena, Andréane, Amandine, Erwann, André-Anne, Max, Rachel, Marjo, Nassim, Souad, Adriano, Marine, Firoz, Emmanuelle. J'ai aussi une pensée très chaleureuse pour les gens qui nous ont soutenues lors de l'angoisse et le désespoir après le décès de Mathias: Manie, MarieLili, Marjo, Erwann, Sarah, JoannieF, Rachel, Eva, Charline et Julien; je remercie énormément l'appui infini de Renée, Iften, Pato, Vincent, JB et Isa.

Paola: *tu amor, tu paciencia y confianza en mi han sido mi motor todos estos año. Gracias por haber compartido esta aventura. Gracias por el milagro de la vida encarnado en Avril, Mathias, Sophia y Naikoa.* Je tiens aussi à remercier mes parents, Armando y Carmen Luz, qui m'ont encouragé toujours à repousser mes limites. *Gracias por los esfuerzos y sacrificios, por el amor y ejemplo.* Merci à ma sœur Helga qui tout en étant loin me surveille et continue de croire en moi. *Gracias mi hermanita por esos años de infancia y juventud perfectos, te adoro.* Merci aux amis de la Colombie, avec qui j'ai partagé ces années inoubliables à Santa Marta. Merci à toute la famille et ma belle famille pour les encouragements, l'appui et leur accompagnement dans la distance.

RÉSUMÉ

L'introduction d'espèces non indigènes de dinoflagellés via le transport maritime représente un risque pour les écosystèmes marins et d'eaux douces du Canada, autant du point de vue de leur biodiversité que de l'économie du pays (e.g. la problématique de la moule zébrée dans les Grands Lacs). Ailleurs dans le monde, des études récentes ont mis en lumière l'influence de vecteurs tels que l'eau et les sédiments de ballast, les routes utilisées par les navires ainsi que les méthodes d'échange d'eau de ballast sur les introductions d'espèces non indigènes. Au Canada, le Réseau de recherche du CRNSG Canadian Aquatic Invasive Species Network (CAISN) en partenariat avec Pêches et Océans Canada et Transport Canada fut mis sur pied en 2006 pour explorer les principaux vecteurs de transport des organismes envahissants et les facteurs influençant leur succès d'établissement afin de développer des modèles de risques pour les eaux canadiennes. Dans le cadre de ce réseau, le but du présent travail est de mieux comprendre le rôle des vecteurs, les patrons d'invasion selon la région et l'influence des différentes méthodes d'échange de l'eau de ballast, dans l'introduction des espèces non indigènes incluant les espèces nuisibles ou toxiques de dinoflagellés au Canada. Cette thèse se concentre sur la présence d'espèces non indigènes de dinoflagellés dans l'eau (formes végétatives) et les sédiments (formes de résistance) de ballast des navires commerciaux qui visitent les côtes est et ouest du Canada et la région des Grands Lacs. De plus, elle fait le lien avec les facteurs antérieurement nommés ainsi que la durée des voyages et le volume d'eau et de sédiments dans les réservoirs à l'arrivée des navires. Le corps de la thèse comporte trois chapitres dont les objectifs, méthodes et principaux résultats sont soulignés dans les lignes qui suivent.

L'objectif du premier chapitre est d'examiner la présence et l'abondance des espèces non indigènes et nuisibles ou toxiques de dinoflagellés dans les sédiments des navires visitant la côte est du Canada. L'étude se concentre plus spécifiquement sur l'influence des échanges d'eau de ballast, le type de navire et l'âge de l'eau sur la présence de kystes de dinoflagellés non indigènes. Les échantillons de sédiment proviennent de 65 navires et les espèces de kystes de dinoflagellés contenues ont été dénombrées à l'aide d'un microscope

inversé. Les résultats confirment la présence de kystes de dinoflagellés non indigènes dans les sédiments de ballast. De plus, certains d'entre eux ont été observés avec du contenu cellulaire (donc potentiellement viables) et des abondances importantes, notamment dans les navires en provenance du nord de la côte est des États-Unis qui, selon la législation canadienne en cours, ne sont pas obligés de faire un échange d'eau de ballast. Ce chapitre a été publié dans la revue Aquatic Invasions.

L'objectif du deuxième chapitre est de comparer l'introduction de kystes de dinoflagellés d'espèces non indigènes et nuisibles dans les sédiments de ballast des navires qui visitent la côte est, la côte ouest et les Grands Lacs du Canada. L'étude examine plus précisément l'abondance et la diversité des kystes de dinoflagellés en relation avec les facteurs qui expliquent la variabilité des patrons d'invasion selon la région étudiée, tels que les routes utilisées par les navires, les types d'échanges d'eau de ballast, l'âge de l'eau et la quantité de sédiments contenue dans les réservoirs de ballast. Un total de 147 navires ont été visités pendant trois campagnes d'échantillonnage au cours des étés 2007-2008-2009. L'étude montre que le patron d'invasion change d'une région à l'autre. Sur la côte est, l'échange de l'eau de ballast diminue l'abondance des espèces de kystes de dinoflagellés non indigènes, tandis que sur la côte ouest et les Grands Lacs, les catégories de navires présentent le même risque d'invasion. Ce chapitre a été soumis à la revue Aquatic Conservation: Marine and Freshwater Ecosystems.

Alors que les deux premiers chapitres s'intéressent aux organismes présents dans les sédiments des réservoirs de ballast, le troisième chapitre examine la pression d'invasion par les espèces de dinoflagellés non indigènes présentes dans l'eau de ballast des navires visitant les côtes est et ouest du Canada. Le dénombrement et l'identification des formes végétatives de dinoflagellés ont été faits par microscopie inversée. Dans certains cas, des techniques de coloration de cellules ont été réalisées afin de confirmer l'identification des cellules. L'étude met en évidence la contribution des routes transocéaniques et côtières en terme de pression d'invasion (actuelle et effective) des cellules de dinoflagellés non indigènes pour les écosystèmes marins canadiens. L'étude montre que les cellules végétatives de dinoflagellés peuvent survivre aux voyages dans les navires, influençant la pression effective des dinoflagellés non indigènes, et met en perspective les abondances de dinoflagellés déchargés par réservoir et par navire selon le trafic maritime annuel. Ce chapitre sera bientôt soumis à la revue Marine Pollution Bulletin.

Mots clés : Dinoflagellés, introduction d'espèces non-indigènes, eaux de ballast, sédiments de ballast, pression de propagule

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ABSTRACT

Introduction of non-indigenous species of dinoflagellates via shipping transport involves a risk to marine and freshwater ecosystems of Canada, in terms of biodiversity and economy (e.g., invasion of the Zebra mussel in the Great Lakes). Elsewhere, recent studies have highlighted the influence of vectors such as ballast water and sediments, routes and methods of ballast water exchange in the introduction of non-indigenous species. In Canada, the NSERC-funded research network entitled Canadian Aquatic invasive Species Network (CAISN) was established in 2006 in collaboration with the Department of Fisheries and Oceans Canada (DFO) and Transport Canada, in order to examine the main vectors in the transport of invasive species as well as the factors influencing the success of establishment of non-indigenous species and to develop risk models for Canadian waters. As part of this Network, the purpose of the present study is to better understand the role of vectors, the invasion patterns according to the region and the influence of different methods of ballast water exchange, in the introduction of non-indigenous species in Canada, including harmful and toxic dinoflagellates.

This thesis focuses on the presence of non-indigenous species of dinoflagellates in the ballast water (motile forms) and sediments (resistance forms) of commercial ships visiting the East and the West coasts of Canada and the Great Lakes region. Additionally, this study links the factors previously cited with the duration of the voyages and the volume of ballast water and sediments present in the tanks when ships arrive at their destination port in Canada. The body of the thesis has three chapters with their objectives, methods, results and conclusions. The main results of the present work are outlined in the following paragraphs.

The objective of the first chapter is to examine the presence and abundance of non-indigenous and harmful or toxic species of dinoflagellates in the sediments of ships visiting the East coast of Canada. The study focuses specifically on the influence of the ballast

water exchange, ship type and ballast water age on the presence of non-indigenous dinoflagellate cysts. Sediment samples were collected from 65 ships and dinoflagellate cyst species present were counted using an inverted microscope. The results confirm the presence of non-indigenous dinoflagellate cysts in ballast sediments. In addition, some non-indigenous dinoflagellate cysts were observed with cell content (potentially viable) and high abundance, especially in ships from the northern part of the East coast of the United States, which under Canadian legislation, are not required to exchange their ballast water. This chapter was published in the journal Aquatic Invasions.

The objective of the second chapter is to compare the introduction of non-indigenous and harmful species of dinoflagellate cysts in ballast sediments of ships that visit the East and West coasts and the Great Lakes of Canada. The study examines more precisely the abundance and diversity of dinoflagellate cysts in relation to the factors that could explain the variability of invasion patterns according to the area studied, such as routes used by ships, types of ballast water exchange, age of ballast water and the amount of sediments contained in the ballast tanks. A total of 147 ships were sampled during three campaigns in the summers of 2007-2008-2009. The study shows that the invasion patterns change from one coast to the other. On the East coast, the ballast water exchange reduces the abundance of non-indigenous species of dinoflagellate cysts, while on the West coast and the Great Lakes, all the categories of ships pose the same risk of invasion. This chapter was submitted to the journal Aquatic conservation: Freshwater and Marine Ecosystems.

While the first two chapters focus on organisms contained in the ballast sediments, the third chapter examines the pressure of invasion by non-indigenous species of dinoflagellates present in the ballast water of ships visiting the East and the West coast of Canada. Motile cells of dinoflagellates were counted and identified with an inverted microscope. In some cases, staining techniques were performed on cells to confirm the species identification. The study highlights the contribution of coastal and transoceanic routes in terms of invasion pressure (actual and effective) of non-indigenous dinoflagellates in Canadian marine ecosystems. The study shows that motile cells of dinoflagellates can survive ship-borne voyages influencing the effective pressure of non-indigenous dinoflagellates. It also assesses the abundance of dinoflagellates discharged per tank and per ship, according to the annual ship traffic. This chapter will be submitted to the journal Marine Pollution Bulletin.

Keywords: Dinoflagellates, non-indigenous species introductions, Ballast water, Ballast sediments, propagule pressure

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AFT	after peak
ALT	alternative method
ANOVA	analysis of variance
APP	actual propagule pressure
BWE	ballast water exchange
BWOB	ballast water on board
BWD	ballast water to be discharged
BWT	ballast water in tank
CAISN	Canadian Aquatic Invasive Species Network
CBT	cargo ballast tank
CE	continental exchanged ships
CNE	continental non-exchanged ships
DBT	double bottom tank
DF	degrees of freedom
EC	East coast
EC	empty cysts
ECNIS	empty cysts of non-indigneous species

EEZ	Exclusive Economic Zone
EPP	effective propagule pressure
E-R	empty-refill method
FPT	forepeak tank
F-T	flow-Trhough method
GL	Great Lakes
HAB	harmful algal blooms
HA	harmful algae
IMO	International Maritime Organisation
MOE	mid-ocean exchange
MC	Monte Carlo test
NIL	not available
NIS	non-indigenous species
NM	nautical miles
NOBOB	no ballast on board ships
OCC	occurrence
PERM	PERMANOVA test
PP	propagule pressure
PSP	paralytic shelfish poisoning
SE	standard error

SS	sum of squares
TC	total cysts
TCNIS	total cysts of non-indigneous species
TST	top side tank
UBWE	unballast water exchange
VCNIS	viable cysts of non-indigeneous species
VC	viable cysts
WBT	wing ballast tank
WC	West coast

INTRODUCTION GÉNÉRALE

LES INVASIONS BIOLOGIQUES

Les invasions biologiques dans les écosystèmes aquatiques constituent l'un des éléments les plus importants du changement environnemental à l'échelle du globe (Duke et Moonley 1999; Davis et al. 2000). Ces changements peuvent être d'ordre écologique (déplacement des espèces indigènes, réduction de la biodiversité locale et même l'extinction des espèces locales) ou socio-économique (dispersion de maladies, coûts élevés destinés au monitorage, au contrôle, à l'éradication des espèces non indigènes et à la restauration des écosystèmes) (Davis 2004; Pimentel et al. 2000).

Ces invasions biologiques se présentent quand des organismes natifs d'une région sont transportés en dehors de leurs aires de distribution naturelles, par dispersion naturelle ou par des activités humaines, lesquelles peuvent être intentionnelles ou accidentelles (Kolar et Lodge 2001a). Un des principaux vecteurs mondiaux de ce transfert d'organismes est le transport de marchandises par bateau (Carlton 1985; Carlton et Geller 1993; Chu et al. 1997; Gollasch 2002; Occhipinti 2007). En effet, les navires modernes possèdent des réservoirs de ballast qui sont remplis d'eau de mer (et des sédiments associés), et ces réservoirs sont vidés lors du chargement des marchandises. Les navires commerciaux voyagent à travers tous les océans du monde, couvrant de nombreuses routes, transocéaniques ou continentales, et ils utilisent différentes méthodes pour échanger l'eau des réservoirs de ballast afin de réduire le transfert d'organismes. Cependant, des études scientifiques récentes continuent d'observer des organismes dans les réservoirs de ballast, incluant des espèces phytoplanctoniques telles que les dinoflagellés, dont plusieurs espèces sont considérées comme nuisibles ou toxiques (Doblin et Dobbs 2006; Hallegraeff 1998; Hamer et al. 2001; Kelly 1993). D'ailleurs, plusieurs études ont montré que des espèces de dinoflagellés introduites par les navires ont causé des dommages importants, notamment à l'industrie aquicole, par exemple sur les côtes de l'Australie (Hallegraeff et al. 1997; Hallegraeff et Bolch 1992). Dans ce pays, sur 80% des navires échantillonnés, 40% contenaient des kystes viables de dinoflagellés et 6% d'entre eux (contenant environ 300 millions de kystes par navire) étaient des kystes d'espèces toxiques telles que *Alexandrium catenella* et *A. tamarense* (Anderson 1992).

En Amérique du Nord, Fofonoff et al. (2003) ont estimé que 52% des espèces aquatiques envahissantes et d'autres introductions marines étaient attribuables aux eaux de ballast. Au Canada, ces introductions ont augmenté considérablement dans les dernières décennies sur les côtes Est (Harvey et al. 1999; Simard et Hardy 2004; Martin et LeGresley 2008), Ouest (Piercey et al. 2000; Waters et al. 2001) et dans les Grands Lacs (Grigorovich et al. 2003; Bailey et al. 2005). Ces introductions d'espèces et leur établissement dans les eaux de compétence canadienne ont entrainé des dépenses pour les gouvernements, calculées à près de 340 millions de dollars CAD (Colautti et al. 2006). Annuellement, plus de 52 millions de tonnes d'eau de ballast en provenance des différents ports du monde sont déchargées sur les côtes canadiennes (Claudi et Ravishankar 2006).

En résumé, les espèces non indigènes, particulièrement les dinoflagellés (formes végétatives et/ou enkystées) viables avec un potentiel d'établissement dans les eaux canadiennes, constituent ainsi un groupe à risque qui mérite une attention particulière dans le contexte des invasions biologiques via le trafic maritime. L'influence des deux principaux vecteurs, eaux et sédiments de ballast, ainsi que des facteurs clefs comme le type de voyage (transocéanique vs continental) ou l'efficacité des échanges d'eau de ballast effectués lors des voyages seront discutés dans les sections qui suivent.

LE TRANSPORT MARITIME

La section précédente a signalé comment le transport de marchandises par bateaux comporte un des plus importants risques pour le transfert d'espèces non indigènes dans les écosystèmes aquatiques marins et d'eaux douces (Carlton 1985; Mills 1993; Bailey et al.

2003; Ruiz et al. 1997). Depuis que l'homme utilise des bateaux pour voyager le long des côtes et à travers les océans, ce transfert d'espèces indigènes a augmenté (par exemple, les Vikings dans les années 1000 AD) (Petersen et al. 1992). Au début, ce transfert impliquait des espèces mélangées avec le sable ou collées aux roches qui servaient de lest (Carlton 2003). De nos jours, ces organismes voyagent dans l'eau et les sédiments des réservoirs de ballast que les navires utilisent afin d'assurer leur stabilité (Gauthier et Steel 1996).

D'autre part, l'ouverture du canal de Suez en 1869, de celui de Panama en 1914 et de la voie maritime du Saint-Laurent en 1959, ainsi que l'augmentation de la vitesse des cargos modernes ont réduit considérablement la durée des voyages (Gauthier et Steel 1996). De plus, les études ont démontré que la diversité et l'abondance des espèces contenues dans l'eau et les sédiments de ballast, sont négativement corrélées avec la durée du voyage (Dickman et Zang 1999; Verling et al. 2005; Simkanin et al. 2009). Comme le tonnage d'eau de ballast peut varier de quelques centaines de tonnes à plus de 100 000 tonnes, de grandes quantités d'eau contenant des organismes vivants sont rapidement et continuellement transportées et déchargées dans tous les coins du monde. Ce transfert d'organismes vivants en moins de temps contribue considérablement à l'établissement potentiel d'espèces non indigènes dans les écosystèmes aquatiques.

Au Canada, Locke et al. (1993), Mills et al. (1993) et Leach et al. (1995) ont identifié le transport maritime comme un des facteurs les plus probables expliquant l'introduction de plus de 150 espèces non indigènes au pays. Plus récemment, les études de Harvey et al. (1999), Simard et Hardy (2004), Simard et al. (2011) et Martin et LeGresley (2008), Villac et Kaczmarska (2011) sur la côte Est, Piercey et al. (2000), Waters et al. (2001) et Lo et al. (2011) sur la côte Ouest et de Grigorovich et al. (2003), Bailey et al. (2003 et 2005) et Briski et al. (2011) dans le système des Grands Lacs, ont mis en évidence le besoin de comprendre les vecteurs d'introduction des espèces non indigènes afin de diminuer les risques pour les écosystèmes aquatiques. Cela nous mène aux questions centrales de la présente thèse: Quel est l'impact du transport maritime sur l'introduction d'espèces de dinoflagellés via les eaux et les sédiments de ballast de navires qui visitent les côtes canadiennes, et ce, après la mise en place de la règlementation en cours? Et combien de ces espèces sont nuisibles ou encore toxiques pour les écosystèmes canadiens et pour la santé humaine?

Pour mieux comprendre les patrons d'invasions d'espèces non indigènes dans les écosystèmes canadiens, une description des différents facteurs qui peuvent contribuer à cette introduction d'espèces s'avère nécessaire. Tout d'abord, un des principaux facteurs implique les échanges d'eaux de ballast. Cette pratique a été établie au niveau international afin de réduire le transfert d'espèces côtières entre les différents ports. Cette prise et décharge des eaux de ballast sont aussi utilisées pour fournir l'équilibre et la stabilité nécessaire aux navires et pour maintenir et assurer des conditions sécuritaires lors du transit en mer (Niimi 2004). Quand un navire quitte un port, sans marchandise à bord, ses réservoirs de ballast sont remplis avec de l'eau côtière. Lors de son arrivée au port de destination, ses réservoirs sont vidés et la marchandise est montée à bord. Ainsi, les eaux contenues dans les réservoirs de ballast des navires constituent un mécanisme de propagation d'espèces indigènes, bien au-delà de leur milieu naturel (Hallegraef et Bolch 1991; Ricciardi et MacIsaac 2000; Niimi 2004).

Un autre facteur qui contribue au transfert d'organismes est le type de voyage. L'introduction d'environ deux tiers des espèces non indigènes dans les écosystèmes aquatiques canadiens serait vraisemblablement expliquée par la présence de trois catégories de navires. La première catégorie est composée par les navires transocéaniques (=TOE). Ils représentent le transport inter-côtes puisqu'ils voyagent sur des grandes distances à travers les continents. La loi canadienne oblige ces navires à faire un échange de leurs eaux de ballast en zone océanique, à l'extérieur de la zone économique exclusive du Canada. Cet échange d'eau de ballast est connu sous l'acronyme de MOE, de l'anglais «mid-ocean exchange». Cependant, dans la présente recherche nous utiliserons l'acronyme BWE de l'anglais «ballast water exchange» et ce pour toutes les catégories qui font un échange de l'eau de ballast, car les échanges n'ont pas toujours lieu au milieu de l'océan. La deuxième catégorie de navires comprend les navires côtiers qui transitent à l'intérieur des eaux continentales de l'Amérique du Nord et qui doivent faire un échange de leurs eaux de ballast. Ils sont représentés par l'acronyme CE «coastal exchange». La troisième catégorie de navires inclue les navires côtiers qui ne sont pas obligés de faire un échange de leurs eaux de ballast. Ces navires sont représentés par l'acronyme CNE «coastal non-exchange». Ces derniers navires sont exemptés de changer leurs eaux de lest parce qu'ils transportent du *fuel* ou parce qu'ils arrivent d'un port des États-Unis, situé au nord de Cape Cod sur la côte est-américaine ou au nord de Cape Blanco sur la côte ouest-américaine. Ces deux dernières catégories de navires représentent le transport intra-côtes (Duggan et al. 2005; Grigorovich et al. 2003; MacIsaac et al. 2004).

L'échange d'eau de ballast en pleine mer est un élément essentiel dans la réglementation du transport maritime. Historiquement, les navires devaient changer volontairement leurs eaux de ballast en dehors de la zone économique exclusive (ZEE) des différents pays ayant des côtes marines et à une profondeur supérieure à 2000 mètres (IMO 2004). Cependant après 2004, il a été convenu internationalement que cette pratique pouvait être effectuée dans des eaux à au moins 200 milles nautiques de distance des côtes et avec une profondeur d'au moins 2000 mètres (IMO 2004).

Malgré la mise en application de mesures volontaires (Canada, 1989) et de règlements (É.-U. 1993; Canada 2006) sur l'échange des eaux de ballast avant l'arrivée près des côtes, des espèces non indigènes ont été retrouvées à un taux plus élevé pendant les années 1990, que durant les trois décennies précédentes (Duggan et al. 2005; Grigorovich et al. 2003). C'est ainsi que ces règlements ont été renforcés à partir de juin 2006, particulièrement sur la voie maritime du Saint-Laurent où tous les navires océaniques, y compris ceux qui déclarent ne pas avoir d'eau de ballast à bord, sont soumis à des inspections visant la totalité de leurs réservoirs de ballast. L'inspection a pour but de garantir que les navires ont rincé tous leurs réservoirs avec de l'eau salée, et ce, alors qu'ils étaient encore à au moins 200 milles nautiques (~ 370 km) au large (http://www.greatlakes-seaway.com) afin de protéger les écosystèmes d'eau douce du Canada.

Ces échanges d'eau côtière pour de l'eau plus salée sont considérés comme un mécanisme visant à diminuer l'abondance et la diversité des organismes côtiers. Cependant, ce même mécanisme pourrait agir comme une source de mélange génétique d'espèces en voie de dispersion (Carlton 1985). Par le fait même, le transport inter-côtes pourrait favoriser une augmentation du potentiel de dispersion des espèces, surtout celles qui ont une vie planctonique courte ou qui sont restreintes à des écosystèmes estuariens (Carlton 1985). Par exemple, Roy et al. (2011) ont noté que la plupart des espèces non-indigènes de dinoflagellés identifiées dans leur étude avaient une distribution océanique, c'est à dire, elles ont pu être prises au cours du BWE en plein milieu de l'océan. De plus, leur plus forte abondance dans les navires avec BWE, contrastait avec les abondances des espèces potentiellement nuisibles/toxiques avec une distribution généralement côtière, où le BWE aurait montré une moindre efficacité. Carver et Mallet (2002) ont aussi observé que le fait de faire un échange d'eaux de ballast augmente le risque d'introduction de taxa phytoplanctoniques non indigènes dans l'est du Canada. D'ailleurs, dans leur étude les navires qui avaient échangé leurs eaux de ballast contenaient des abondances élevées d'organismes dont 80% étaient vivants. Bien que les échanges en pleine mer puissent éliminer de 80% à 95% des espèces côtières présentes, il peut arriver que l'abondance et la diversité des espèces augmentent significativement suivant ces échanges. De plus, les espèces côtières qui restent après le BWE et arrivent dans les ports canadiens sont celles qui ont survécu au remplacement des eaux côtières par les eaux marines dans les réservoirs de ballast (Wonham et al. 2001). Donc la méthode des échanges d'eaux de ballast n'est pas totalement efficace, et elle peut entraîner des apports d'espèces marines non indigènes vers les régions côtières. Compte tenu du problème, cette stratégie doit continuer à être évaluée, étant donné, a) les multiples endroits choisis par les navires pour faire leurs échanges, dont font souvent partie les zones côtières à cause des conditions plus sécuritaires pour les navires, et b) le fait que les échanges d'eaux de ballast peuvent s'avérer moins efficaces pour éliminer les organismes dans les sédiments des réservoirs de ballast. Donc le vecteur sédiment constitue un risque potentiel aussi important que l'eau de ballast pour les invasions d'espèces, notamment pour celles qui produisent des stades de dormance benthiques au cours de leur cycle de vie (Hallegraeff et al. 1997; Hallegraeff 1998). De plus, ce vecteur «sédiments de ballast» a été moins étudié que le vecteur «eaux de ballast».

La compréhension des différents facteurs impliqués dans le transfert d'espèces par bateau est d'une importance particulière pour l'écologie des communautés. Bien que la viabilité de ces espèces lors du déchargement et leurs interactions avec les communautés locales soient souvent complexes, toute étude de l'influence de ces espèces au niveau écologique bénéficierait de la connaissance sur leur dispersion et leur possibilité d'établissement dans les écosystèmes aquatiques et leurs impacts potentiels.

LES INTRODUCTIONS D'ESPÈCES NON INDIGÈNES AQUATIQUES AU CANADA

En Colombie-Britannique, les introductions involontaires d'espèces telles que le copépode parasite *Mytilicola orientalis*, plusieurs espèces de perceurs d'huîtres telles que *Limnoria tripunctata*, de macrophytes comme *Sargassum muticum*, *Zostera marina*, ou *Z. japonica* ont eu des effets écologiques et économiques importants (Gauthier et Steel 1996). Cette liste inclut également le tunicier *Styela clava*, envahisseur agressif dont la répartition naturelle se situe dans la partie sud de la mer d'Okhotsk au large de la Russie (Lambert et Lambert 1998) et qui s'est établi avec succès dans les eaux tempérées un peu partout dans le monde. Ce tunicier a été observé pour la première fois à l'extérieur de son aire de répartition d'origine en 1933, sur les côtes de la Californie (Clark et Therriault 2007). Les vecteurs d'introduction les plus probables comprennent les bateaux infestés et les transferts d'huîtres. Le tunicier *S. clava* est maintenant présent sur les côtes du Pacifique au Canada, particulièrement dans le sud de la Colombie-Britannique, et il est considéré comme une espèce nuisible importante dans le domaine de la conchyliculture puisque ce tunicier étouffe les espèces cibles et salit les engins et l'équipement (Clark et Therriault 2007). Dans les ports de la même région Jamieson (2002) a étudié l'arrivée du crabe vert (*Carcinus maenas*), prédateur potentiel des mollusques endémiques. Une autre espèce de bivalve exotique (*Nuttallia obscurata*) arrivée dans les réservoirs de ballast des bateaux, s'est largement répandue le long du détroit de Géorgie (Merilees and Gillispie 1995). Waters et al. (2001) ont mis en évidence la croissance des algues qui provenaient de l'eau et des sédiments des réservoirs des navires. Ces études confirment que les systèmes aquatiques de l'ouest du Canada continuent d'être vulnérables aux invasions des espèces non indigènes (Larson et al. 2003). Cependant, peu d'études ont examiné l'introduction d'espèces non indigènes de dinoflagellés pour cette région.

Depuis l'ouverture de la voie maritime du Saint-Laurent en 1959, au moins 43 espèces zooplanctoniques et de protistes non indigènes se sont établies dans les Grands Lacs (Grigorovich et al. 2003; Duggan et al. 2005). En particulier, le système des Grands Lacs a été impacté par l'introduction involontaire de la moule zébrée *Dreissena polymorpha*, la moule quagga *D. bugensis* et la lamproie marine *Petromyzon marbeus*. Les moules ont été introduites par les navires commerciaux et ont affecté les systèmes d'aqueducs des municipalités. Dans le cas de la lamproie marine, son introduction date des années 1830 et affecte principalement l'industrie de la pêche commerciale et sportive. Récemment, de grands efforts ont été déployés afin d'éviter l'invasion des carpes asiatiques *Hypophthalmichthys molitrix* et *H. nobilis* dans le réseau des Grands Lacs. Ces espèces proviennent d'Asie et ont été introduites en Amérique du Nord dans les années 1960 et 1970. Au début des années 1990, ces deux espèces se sont échappées d'installations d'aquaculture dans le sud du Mississippi. Depuis, elles migrent vers le nord en recherchant les eaux froides ou à température modérée. Étant donné leurs taux élevés d'ingestion (jusqu'à 40% de leur poids par jour), leur établissement pourrait entraîner le déclin des espèces indigènes par manque de nourriture, et affecter les pêches sportive et commerciale qui ont des retombées économiques en Ontario.

Au plan environnemental, les invasions biologiques ont été aussi responsables de l'altération des habitats benthiques d'eau douce et marine. C'est le cas du bryozoaire épiphyte *Membranipora membracea*, qui a envahi la côte atlantique du Canada et des États-Unis, provoquant la fragmentation et la perte des frondes des laminaires (Gendron 2010). L'est du pays a aussi souffert de nombreuses invasions, notamment à l'Île du Prince-Édouard où le crabe vert européen (*Carcinus maenas*) et également quatre espèces de tuniciers se sont établis. Une espèce en particulier de tunicier (*Styela clava*) a remplacé plus de 50% des moules cultivées dans les fermes d'aquaculture en 2004. De même, l'espèce de tunicier *Ciona intestinalis* a infesté les fermes d'aquaculture en Nouvelle-Écosse et elle commence à remplacer *Styela clava* dans certaines régions de l'Île du Prince-Édouard. Toutes ces introductions d'espèces et leur établissement ont généré des dépenses aux gouvernements, estimées à près de 34.5 millard de dollars CAD par année (Colautti et al. 2006).

INTRODUCTION DES DINOFLAGELLÉS VIA LE TRANSPORT MARITIME

Plusieurs études ont mis en évidence l'effet du transport maritime dans le transfert de dinoflagellés, dont plusieurs espèces sont considérées comme nuisibles ou encore toxiques (Hallegraeff 1998; Doblin et Dobbs 2006). De plus, des études dans plusieurs régions du monde ont montré les conséquences économiques négatives de ces introductions d'espèces non indigènes, notamment pour l'Australie (Hallegraeff and Bolch 1992), la Nouvelle-Zélande (Hay et al. 1997), l'Angleterre (Hamer et al. 2001) et les États-Unis (Kelly 1993). Au Canada, sur la côte ouest plus spécifiquement, dans le port de Vancouver et le détroit de Juan de Fuca, des monitorages effectués entre 1995 et 1997 (Levings et al. 1998; Piercey et al. 2000) ont montré que des espèces phytoplanctoniques non indigènes, dont plusieurs espèces de dinoflagellés, continuaient d'arriver dans les réservoirs de ballast des navires commerciaux (Larson et al. 2003). Dans la région des Grands Lacs, Fanhestiel et al. (2009) ont regardé l'introduction des kystes de dinoflagellés via le transport maritime. Cependant, les seuls navires échantillonnés dans cette étude correspondent à la catégorie de navires des «no ballast on board», NOBOB. Cette catégorie (sans eau de ballast à bord) constitue 90% du transport maritime à l'intérieur du réseau des Grands Lacs. Or elle est absente du trafic maritime visitant les côtes canadiennes, ce qui rendait les comparaisons difficiles et c'est la raison principale pour laquelle elle n'a pas été considérée dans cette recherche.

Dans l'estuaire et le golfe du Saint-Laurent, Harvey et al. (1999) ont observé des densités de kystes de dinoflagellés allant de 18 à 509 kystes cm^{-3} dans les eaux et les sédiments de ballast. Plusieurs de ces kystes font partie des espèces toxiques, lesquelles peuvent se développer en cellules végétatives, une fois déchargées dans un environnement propice. Ces espèces toxiques peuvent affecter l'industrie maricole à cause de leurs diverses toxines, dont la saxitoxine, induisant des intoxications paralysantes par les mollusques (*paralytic shellfish poisoning*, PSP, ou intoxications paralysantes des mollusques, IPM) ou d'autres toxines induisant divers problèmes de santé. Ces toxines provoquent des troubles nerveux, respiratoires et intestinaux graves chez les humains et peuvent entrainer la mort (dans 15% des cas) (Hallegraeff et al. 1997). Dans la baie de Fundy, Martin et LeGresley (2008) ont mis en évidence la présence de 8 nouvelles espèces de dinoflagellés depuis 1995. Cette étude suggère que certaines de ces espèces ont pu arriver par dispersion naturelle, apportées par les courants qui entrent dans le Golfe du Maine, mais aussi par des activités anthropiques, comme le transport de marchandises par bateau.

Ces dinoflagellés viables sous formes végétatives et/ou de kystes de résistance, ont un potentiel d'établissement dans les eaux canadiennes s'ils proviennent d'environnements tempérés et ils constituent ainsi un groupe à risque qui mérite une attention particulière dans le contexte des invasions aquatiques via les réservoirs de ballast. La grande résistance des formes enkystées de dinoflagellés leur confère un grand potentiel de survie même sous les conditions particulières des réservoirs de ballast (obscurité, diminution d'oxygène, etc.). De plus, au Canada il y a une connaissance très limitée de la pression d'invasion (voir troisième chapitre) des cellules végétatives et des kystes de dinoflagellés et plus particulièrement, combien de ces espèces arrivent via les eaux ou les sédiments de ballast des navires; quelles sont les routes qui comportent le plus grand risque d'introduction, s'il y a une relation entre la durée de ces voyages et la viabilité des cellules et combien de ces espèces sont nuisibles ou toxiques pour les écosystèmes aquatiques.

LE CYCLE DE VIE DES DINOFLAGELLÉS

Plus de 200 espèces de dinoflagellés marins sont connues comme ayant la capacité de produire des stades enkystés, ou phases de dormance, pendant une période de leur cycle vital (Head 1996; Taylor 1987). La plupart de ces espèces ont deux noms, dû au fait que les kystes et les cellules végétatives furent décrits et nommés indépendamment par des palynologistes et par des phycologues, respectivement (Ellegaard 2003).

Les stades de dormance, nommés kystes, ont généralement une taille inférieure à 100 μm et leur densité se situe entre 1,5 et 2,3, ce qui leur permet de sédimenter dans le milieu naturel, tout comme les particules fines telles que les silts (Dale 1979). Cette caractéristique permet de trouver des grandes concentrations de kystes dans les milieux de dépôt des particules fines, avec des concentrations maximales lorsque celles-ci constituent 50 à 60% des sédiments (Lewis 1988). Les kystes peuvent être concentrés par les processus sédimentaires ou être dispersés par les courants et laisser des grandes zones qui en sont totalement exemptes (Goodman 1987). Dans certaines zones ouvertes, les kystes peuvent être transportés hors du milieu naturel d'où ils sont originaires et être concentrés par des processus hydrographiques et sédimentaires (Dale et al. 1978). Donc dans un milieu naturel, lorsque la quantité de sédiments en suspension augmente, la probabilité qu'un bateau pompe des sédiments (incluant les kystes) dans ses réservoirs augmente également. Ainsi, les espèces de kystes de dinoflagellés peuvent former des lits de kystes au fond des réservoirs au fur et à mesure que les sédiments se déposent, après chaque remplissage des réservoirs avec l'eau des ports (Pertola et al. 2006). Une fois les navires arrivés à un nouveau port, ils déchargent l'eau de leurs réservoirs et une partie des sédiments peut également être déchargée, avec les kystes de dinoflagellés. Ces sédiments de ballast constituent donc un risque d'introduction d'espèces aussi important que les eaux. Cependant, pour les côtes canadiennes et les Grands Lacs, il y a peu de données détaillées, particulièrement pour les espèces non indigènes de dinoflagellés.

Durant leur cycle de vie (Figure 1), les espèces peuvent former un premier type de kyste connu comme temporaire pouvant survivre pour quelques jours dans des conditions d'obscurité et de basses températures (Pfiester et Anderson 1987). Cependant, Anderson et Wall (1978) ont constaté que ce type de kyste chez *Alexandrium tamarense* n'est pas en mesure de survivre pendant l'hiver.

Les dinoflagellés peuvent aussi produire des hypnozygotes, soit des kystes formés lors d'une reproduction sexuée et pouvant survivre pendant des périodes de temps prolongées (mois à années) sous des conditions extrêmes (Dale 1977; Anderson 1980). Ces kystes trouvés dans les sédiments marins (Pfiester et Anderson 1987; Wyatt et Jenkinson 1997) peuvent résister aux longs voyages et aux faibles concentrations d'oxygène comme celles rencontrées dans les sédiments des réservoirs de ballast des navires (voir deuxième chapitre).

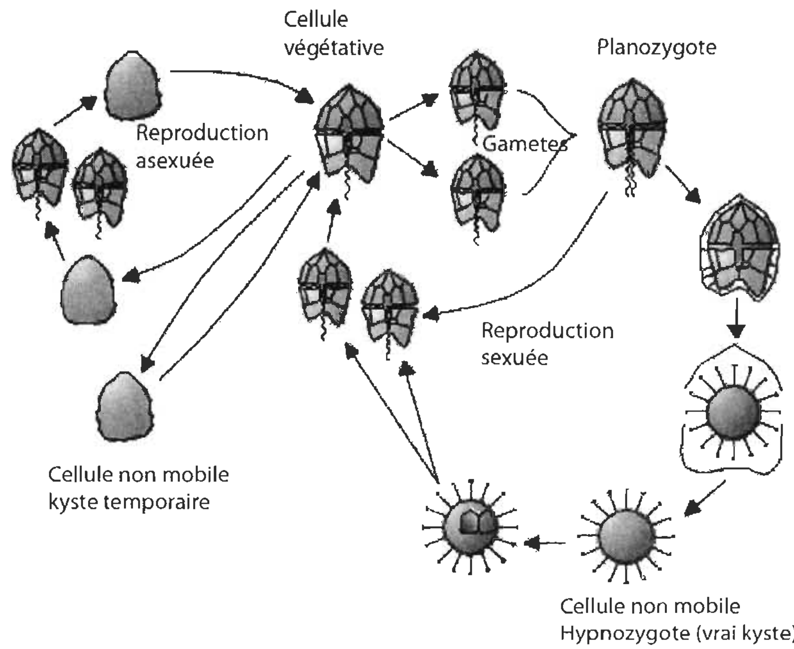

Figure 1. Cycle de vie des dinoflagellés produisant des kystes de dormance. Les cellules végétatives mobiles et haploïdes se reproduisent par mitose en produisant 2 cellules identiques (reproduction asexuée). Les cellules mobiles adultes haploïdes forment des gamètes via l'intermédiaire de la mitose, ayant pour résultat une cellule mobile de grande taille, nue ou ornementée. Quand les flagelles s'anastomosent les gamètes fusionnent pour former un planozygote diploïde (2N) (reproduction sexuée). Le zygote mobile s'agrandit formant une cellule non-mobile (kyste) diploïde (hypnozygote). L'hypnozygote après une période de dormance subit finalement la méiose, reconstituant la cellule mobile haploïde. (Tiré et modifié de Fensome 1993).

Dans un premier temps, le processus de développement et de maturation des kystes comprend une période pendant laquelle les cellules ne peuvent pas exkyster (vraie dormance) (Anderson et al. 1987; Kremp et Anderson 2000) et qui peut occuper plusieurs mois (jusqu'à 12 dans le cas de *A. tamarense* dans le Saint-Laurent) (Castell-Perez et al. 1998; Wyatt et Jenkinson 1997). Une fois la maturation complétée, la dormance est maintenue jusqu'à ce que les conditions du milieu soient les plus optimales pour germer (Anderson et al. 1987). À cet égard, des facteurs comme les températures extrêmes, les faibles taux d'oxygène et le manque de lumière peuvent inhiber ou retarder la germination de kystes (Anderson et al. 1987; Kremp et Anderson 2000).

Certains des stades de dormance sont aussi reliés aux espèces qui forment des marées rouges le long des côtes et des estuaires (Anderson et Wall 1978). Ces stades de dormance ont plusieurs fonctions écologiques comme produire annuellement des évènements de proliférations dans des zones où les kystes hivernent dans les sédiments, synchroniser les floraisons printanières avec les changements saisonniers des conditions physiques des écosystèmes aquatiques et permettre aux espèces nuisibles ou toxiques d'élargir leurs aires de distribution géographique (Anderson et Wall 1978). Par exemple, pour les kystes d'*Alexandrium*, Cox et al. (2008) ont trouvé une corrélation positive entre la concentration des kystes dans les sédiments de la baie de Puget Sound et la magnitude du bloom dans la colonne d'eau. Ainsi en réponse à son cycle annuel, une fraction des kystes présents dans les sédiments peut germer quand les conditions de la colonne d'eau sont plus favorables (Wyatt et Jenkinson 1997). Les concentrations des cellules peuvent ainsi atteindre jusqu'à 10^6 cellules L^{-1} (Schrope 2008) et contaminer plusieurs kilomètres de côte en quelques semaines (Lorraine et al. 2006).

LA PRESSION DE PROPAGULE

La pression de propagule ou pression d'invasion est une composante de la mesure du nombre d'individus d'une espèce déchargés dans une région, où cette espèce est considérée comme non indigène (Figure 2). La pression de propagule inclut les estimations du nombre d'individus dans chaque évènement de décharge (*propagule size*) et le nombre d'évènements de décharge (*propagule number*). Une augmentation du nombre d'individus déchargés ou du nombre d'évènements de décharge fait en sorte que la pression de propagule augmente aussi (Lockwood 2005). La pression de propagule peut être définie aussi comme la quantité, la qualité et la fréquence d'introduction d'organismes envahissants (Groom 2006). La pression de propagule joue un rôle important dans la détermination du succès de l'établissement des espèces non indigènes (Ruiz et al. 2000; Lockwood et al. 2005; Colautti et al. 2006), bien que ce facteur ne soit pas toujours pris en considération dans les études sur les invasions biologiques. Cette pression relie aussi le nombre d'individus relâchés et la fréquence des évènements de relâche, avec le succès et les patrons d'établissement des espèces (Kolar et Lodge 2002). Plus les quantités d'espèces introduites sont élevées et constantes, plus les chances de survie de ces espèces non indigènes augmentent, tandis que les espèces introduites en petit nombre avec peu d'évènements de relâche seront plus susceptibles de mourir (Lockwood 2005).

La pression de propagule peut être expliquée par trois composantes: 1) la pression de propagule potentielle, représentée par la quantité d'eau de ballast déchargée; 2) la pression de propagule actuelle qui représente le nombre d'organismes contenus dans les réservoirs lors de l'arrivée des navires et 3) la pression de propagule effective qui détermine le nombre d'organismes *potentiellement vivants* déchargés par un vecteur (eau ou sédiments de ballast) (Lo et al. 2011). Cependant, le succès dans l'établissement des espèces non indigènes va dépendre aussi des caractéristiques d'invasibilité des espèces qui prolifèrent dans les habitats originaux (Lodge 1993; Kolar et Lodge 2001b; Davis et al. 2005) et/ou des caractéristiques des habitats susceptibles de subir la prolifération des espèces non indigènes (Occhipinti-Ambrosi 2007). Dans les écosystèmes aquatiques d'eaux douces et marines, le processus par lequel une espèce est considérée comme envahissante compte plusieurs étapes et filtres (Kolar et Lodge 2001; Colautti et MacIsaac 2004; Lockwood et al. 2005; Occhipinti-Ambrosi 2007) qui sont énumérés et décrits dans les lignes suivantes:

Étape 0 - Envahisseur potentiel : Correspond à la population source.

Étape 1 - La Prise: Commence avec les espèces établies dans la région d'origine ou la région source. Les propagules sont prises par les navires et transportées vers un nouvel environnement (NE).

Étape 2 - L'arrivée: Cette étape est atteinte quand les individus sont déchargés avec l'eau ou les sédiments des navires.

Filtre Survie: la survie des organismes dans leur nouveau milieu dépend de leurs interactions avec les communautés locales et de l'adaptation au nouvel environnement. Seuls les organismes les plus résistants pourront se rendre à l'étape suivante.

Étape 3 - L'établissement : elle est considérée uniquement pour les espèces qui ont été déchargées, qui ont survécu et qui se sont reproduites dans le nouvel environnement.

Étape 4a - La dispersion : Correspond à l'accroissement de la population.

Étape 4b - La dominance : Les espèces envahissantes deviennent dominantes par rapport aux espèces indigènes.

L'établissement est régi par deux filtres différents : un filtre qui relie la survie et la reproduction dans le nouvel environnement et un filtre qui détermine la dispersion locale. Le premier filtre (survie) est principalement lié à la pression de propagule. Ce filtre détermine, parmi toutes les espèces qui arrivent, lesquelles pourront s'établir, se disperser ou dominer. Le deuxième filtre, dit environnemental, agit en régulant le passage des espèces de l'étape 3 (établissement) à l'étape 4a (dispersion) et de l'étape 4b (dominance) à l'étape 5 (prédominance, cf. Figure 2).

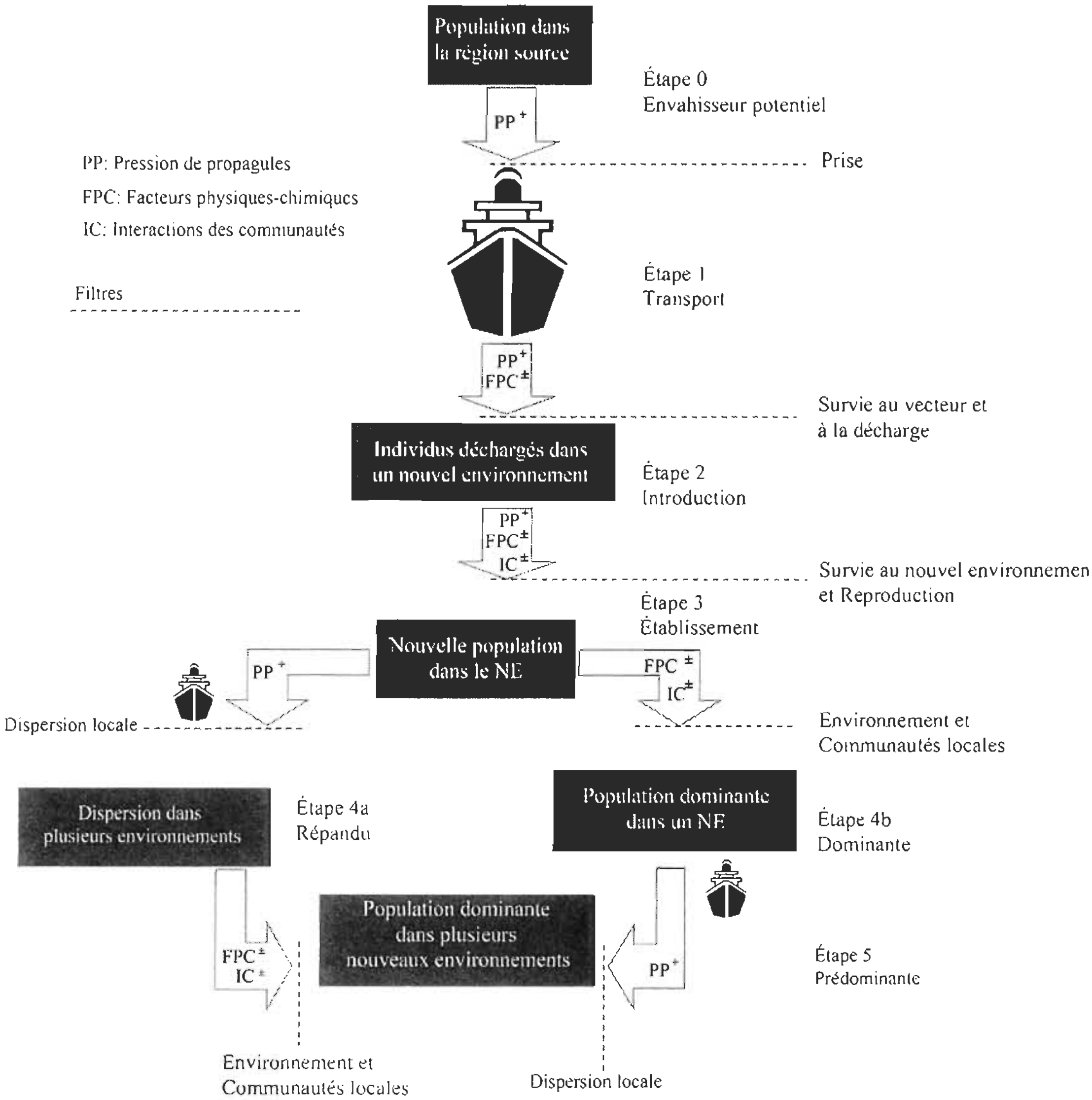

Figure 2. Le schéma montre les différentes étapes que doivent franchir les espèces envahissantes. Les propagules doivent passer la transition entre les étapes à travers différents filtres. Pour atteindre chaque étape, les espèces dépendent de deux grands facteurs : les facteurs physico-chimiques et la résistance biologique, lesquels peuvent agir d'une façon positive ou négative (modifié de Colautti et al. 2006; Occhipinti 2007).

Malgré cette connaissance, au Canada et plus particulièrement pour les espèces non indigènes de dinoflagellés, les pressions actuelle et effective n'ont pas été déterminées. Il n'y a pas non plus de données qui mettent en relation l'abondance des espèces de dinoflagellés présentes dans les réservoirs lors de l'arrivée des navires aux ports avec leur volume d'eau de ballast présent dans les réservoirs ainsi que leur volume déchargé. De plus, on connait mal l'influence de la durée du voyage, des routes empruntées par les navires et des méthodes d'échange des eaux de ballast sur les pressions actuelle et effective des espèces de dinoflagellés non indigènes pour les écosystèmes aquatiques marins et d'eau douce du Canada.

L'information présentée dans cette section, permet de voir l'importance et le risque que comporte l'introduction des dinoflagellés via les réservoirs de ballast, dans les écosystèmes aquatiques canadiens. D'abord, le cycle de vie des dinoflagellés leur permet de résister aux longs voyages et d'arriver encore vivants dans leurs nouveaux environnements. Puis, plusieurs espèces qui ont la capacité de former des kystes de résistance sont des espèces qui sont considérées comme des espèces nuisibles ou qui peuvent produire diverses toxines avec des conséquences négatives pour les environnements et pour les humains qui utilisent les ressources marines. La résistance et la viabilité des kystes contribuent de façon significative à la pression d'invasion. Toutefois, le succès d'établissement des espèces non indigènes va dépendre aussi des caractéristiques du milieu et des interactions écologiques avec les espèces qui prolifèrent dans les habitats originaux. La section suivante présentera les sujets qui ont été étudiés plus en détail dans le cadre de la présente recherche.

CHAPITRES ET OBJECTIFS DE LA THÈSE

Le but de cette thèse de doctorat est de déterminer la pression d'invasion par les dinoflagellés non indigènes (incluant les formes végétatives et enkystées) présents dans les réservoirs de ballast des navires fréquentant les ports des côtes est et ouest canadiennes, ainsi que les ports canadiens des Grands Lacs. Plus particulièrement, ce travail examine l'influence du type de voyage (transocéanique vs continental) et celle des échanges d'eau de ballast effectués lors des voyages côtiers selon la réglementation en cours pour les eaux canadiennes. Les deux premiers chapitres de la thèse considèrent l'impact du vecteur sédiments de ballast dans l'introduction des espèces de kystes non indigènes de dinoflagellés. Le troisième chapitre quant à lui examine la pression de propagule des cellules mobiles de dinoflagellés contenus dans les eaux de ballast pour les écosystèmes aquatiques canadiens.

L'objectif général de chaque chapitre est de:

Chapitre 1: Identifier les espèces non indigènes présentes dans les sédiments de ballast des navires fréquentant la côte est du Canada.

Chapitre 2: Comparer les patrons d'invasion des espèces non indigènes de dinoflagellés présentes dans les sédiments de ballast des navires fréquentant les côtes est et ouest du Canada, ainsi que la région canadienne des Grands Lacs.

Chapitre 3: Déterminer la pression d'invasion des espèces mobiles non indigènes de dinoflagellés contenues dans l'eau de ballast des navires fréquentant les côtes est et ouest du Canada.

La section suivante décrit les objectifs spécifiques de chacun des chapitres, mais le lecteur est invité à lire les chapitres appropriés pour plus de détails.

Chapitre 1: *Introduction d'espèces non indigènes de dinoflagellés par les sédiments de ballast sur la côte est du Canada*. Le transport de marchandises par bateau est un des vecteurs les plus importants dans le transfert d'espèces non indigènes. L'introduction d'espèces potentiellement viables de dinoflagellés non indigènes, nuisibles ou toxiques peut être liée à différents facteurs : (1) un risque différent associé à chacune des catégories de navires selon les routes utilisées, (2) une diminution de l'abondance des dinoflagellés non indigènes par l'effet de l'échange des eaux de ballast avant d'arriver aux ports de l'est du Canada, et (3) une différence dans la quantité de sédiments contenus dans les réservoirs qui peut jouer sur l'abondance des espèces de dinoflagellés non indigènes. L'objectif de ce premier chapitre est alors de déterminer si les navires visitant la côte est transportent des espèces non indigènes et la relation avec chacun des facteurs précédemment cités. L'hypothèse de ce chapitre est : *le transport maritime que ce soit transocéanique ou continental comporte le même risque d'introduction d'espèces non-indigènes de kystes de dinoflagellés*. Ce chapitre est la première étude détaillée sur l'introduction d'espèces non indigènes de kystes de dinoflagellés via les sédiments des réservoirs de ballast.

Chapitre 2: *Kystes de dinoflagellés dans les sédiments de ballast: différences entre la côte est, la côte ouest et les Grands Lacs*. Le risque potentiel d'invasion par des espèces non indigènes de kystes de dinoflagellés est-il le même pour les trois régions étudiées, soit deux régions marines et une d'eau douce? L'arrivée d'organismes provenant de milieux marins peut potentiellement mettre en danger les écosystèmes d'eau salée, mais peut-être pas les écosystèmes d'eau douce. L'objectif de ce chapitre est d'évaluer l'importance relative des facteurs tels que les routes, les échanges d'eau de ballast, la durée du voyage ainsi que les quantités de sédiments de ballast dans l'introduction d'espèces non indigènes de dinoflagellés pour les trois régions étudiées. L'hypothèse de ce chapitre est : *le patron d'invasion des espèces non-indigènes de kystes de dinoflagellés est le même entre les régions étudiées*.

Chapitre 3: *Pression de propagule des dinoflagellés non indigènes via les navires fréquentant les côtes est et ouest canadiennes*. La pression de propagule est en relation avec le succès de l'établissement des espèces non indigènes. Il est difficile de déterminer si pour les côtes canadiennes il y a une relation directe entre le nombre des espèces dans les réservoirs des navires et le nombre d'évènements de déchargement d'eaux de ballast, avec les chances d'établissement de ces espèces non indigènes. L'objectif de ce troisième chapitre est de déterminer la pression d'invasion des espèces mobiles non indigènes de dinoflagellés (formes mobiles) contenues dans les eaux de ballast des navires fréquentant les côtes est et ouest du Canada. Dans ce but, ce chapitre s'intéresse plus particulièrement à la pression de propagule actuelle et effective de toutes les espèces de dinoflagellés, ainsi que celles considérées comme non indigènes. Ces deux mesures sont mises en perspective en tenant compte du volume de trafic maritime pour les deux côtes. L'hypothèse de ce chapitre est : *pour les dinoflagellés contenus dans l'eau de ballast, la pression de propagule que ce soit actuelle ou effective ne diffère pas entre les régions étudiées*.

Effectuée dans le cadre du Réseau canadien sur les espèces aquatiques envahissantes (CAISN), le travail présenté ici tente de répondre aux questions les plus importantes pour la compréhension de l'introduction des dinoflagellés via les eaux et les sédiments de ballast au Canada. Plusieurs aspects n'ont pas pu être traités par cette recherche et plusieurs questions restent ouvertes. Cependant, nos résultats et nos conclusions suggèrent des pistes qui méritent être considérées dans de futures études sur l'introduction des espèces non indigènes phytoplanctoniques, et particulièrement sur les espèces non indigènes et nuisibles des dinoflagellés. Ces perspectives de recherche seront abordées plus en détail dans la section Conclusions générales.

CHAPITRE 1
TRANSPORT D'ESPÈCES NON-INDIGÈNES DE DINOFLAGELLÉS VIA LES SÉDIMENTS DE BALLAST SUR LA CÔTE EST DU CANADA

RÉSUMÉ

Dans le cadre du réseau canadien sur les espèces aquatiques envahissantes (CAISN), la présence et l'abondance des espèces de dinoflagellés non-indigènes, nuisibles ou toxiques sont examinées dans les sédiments de ballast de 65 navires ayant visité les ports de la côte est du Canada. Les navires faisant escale dans plusieurs ports des provinces du Québec, du Nouveau-Brunswick et de la Nouvelle-Écosse ont été échantillonnés pendant trois étés (2007, 2008, 2009). Ces navires incluent des navires de charge classiques, des vraquiers et des pétroliers. Ils représentent deux grandes catégories de navires: les navires faisant des voyages continentaux et transocéaniques. Nos résultats montrent que les kystes de dinoflagellés potentiellement viables sont présents dans les sédiments de ballast de toutes les catégories de navires qui arrivent à la côte est du Canada. Les concentrations de tous les types de kystes de dinoflagellés sont plus élevées dans les navires sans échange d'eau de ballast (BWE) que dans les navires avec BWE y compris les navires transocéaniques. Ces derniers présentent un risque plus faible d'introduction d'espèces non-indigènes (NIS) de dinoflagellés. Nous avons identifié 14 espèces non-indigènes de kystes de dinoflagellés qui n'ont pas encore été répertoriées dans les eaux des côtes canadiennes, y compris 4 espèces potentiellement nuisibles/toxiques, ce qui représente une possibilité de nouvelles introductions. Ces introductions de NIS toxiques pourraient représenter un problème pour les écosystèmes marins canadiens, avec des effets potentiellement désastreux sur les communautés halieutiques, l'aquaculture et la santé humaine. Ce risque potentiel peut être favorisé par les changements climatiques.

Ce premier article, intitulé « *Ballast sediment-mediated transport of non-indigenous species of dinoflagellates on the East coast of Canada* », fut corédigé par moi-même ainsi que par les professeurs Suzanne Roy et André Rochon. Il fut accepté pour publication dans sa version finale en 2010 par les éditeurs de la revue *Aquatic Invasions: 6(3): 231-248*. En tant que premier auteur, ma contribution à ce travail fut l'essentiel de la recherche sur l'état de la question, le développement de la méthode, l'identification et le dénombrement des espèces, l'exécution des tests statistiques et la rédaction de l'article. Le professeur Suzanne Roy, deuxième auteur ainsi que le professeur André Rochon troisième auteur ont grandement aidé à la recherche, au développement de la méthode ainsi qu'à la révision du manuscrit. Les principaux résultats de cette étude ont été présentés à la *16th International Conference on Aquatic Invasive Species* à Montréal, Canada, au printemps 2009.

BALLAST SEDIMENT-MEDIATED TRANSPORT OF NON-INDIGENOUS SPECIES OF DINOFLAGELLATES ON THE EAST COAST OF CANADA

ABSTRACT

The presence and abundance of non-indigenous, and/or harmful or toxic dinoflagellate species in ballast sediments is examined for 65 cargo ships visiting ports on the East coast of Canada, as part of the Canadian Aquatic Invasive Species Network (CAISN). Ships visiting several ports in the provinces of Quebec, New Brunswick and Nova Scotia were sampled during three summers (2007, 2008, 2009). These ships included general cargo, bulk carriers and oil tankers, and they represented two major categories: ships undergoing continental and trans-oceanic voyages. Our results show that potentially viable dinoflagellate cysts are present in ballast sediments of all the categories of ships arriving to the East coast of Canada. The concentrations of all types of dinoflagellate cysts are higher in continental ships without ballast water exchange (BWE) than in ships with BWE, including trans-oceanic ships, which presented lower risk of introduction of non-indigenous species (NIS) of dinoflagellates. We identified 14 non-indigenous dinoflagellate cyst species not yet reported from Canadian coasts, including 4 potentially harmful/toxic species, representing a possibility of new introductions. These introductions of toxic NIS could represent a problem for marine Canadian ecosystems, with potentially disastrous effects on fish communities, aquaculture and human health. This potential risk may be facilitated with climate change.

Introduction

Ship transport is a major vector for biological invasions, namely through the seawater carried in ballast tanks (Medcof 1975; Carlton 1985; Ruiz et al. 2000; Fofonoff et al. 2003). Ballast water is carried onboard ships to provide balance stability, and maintain safe transit conditions. Ballast tanks may also carry unpumpable residual water and sediments that can contain viable organisms (Williams et al. 1988; Bailey et al. 2005a). The invasion risk posed by live organisms associated with sediments or residual water from ships visiting the Great Lakes has been examined by Bailey et al. (2005a, b), but these studies did not look at the presence of phytoplankton species. Fahnenstiel et al. (2009) examined dinoflagellate cysts in ballast tank sediments of ships entering the Great Lakes but only for NOBOB (no ballast on board) ships. Ballast sediments from other regions have been shown to contain viable long-lived resting stages of phytoplankton (named cysts or dinocysts in the case of dinoflagellates), notably of non-indigenous and toxic species of dinoflagellates (Hallegraeff and Bolch 1992; Kelly 1993; Hamer et al. 2001; Persson 2002; Pertola et al. 2006). For the present study, we considered "non-indigenous" all species of dinoflagellates that had not been reported before in the scientific literature for the Gulf of St. Lawrence and Eastern Canada.

Approximately 2000 species of living dinoflagellates have been identified and less than 150 are known to produce cysts (Head 1996). At least 90 of these cysts producing species are known to be harmful (Sournia 1995) and a minimum of 45 species is considered as toxic (Sournia 1995; Smayda 1997; Hallegraeff 2003). Cysts are generally produced as part of the life cycle of dinoflagellates, and in some cases in response to adverse environmental conditions (low oxygen concentration, low temperature or light intensity) that may occur in ballast tanks. When conditions improve, dinoflagellate cysts can germinate (e.g. in a new environment), and resume the pelagic phase of their life cycle. Thus, encystment may help the cells survive the voyage, or cysts may be picked up with suspended sediments when ballast tanks are filled. Cases of non-indigenous dinoflagellate species introduction mediated by ballast sediments have been recorded in Australia

(Hallegraeff and Bolch 1992; Hay et al. 1997), North America (Kelly 1993; Harvey et al. 1999) and Northern Europe (Hamer et al. 2001; Pertola et al. 2006). The transfer of dinoflagellate species via ballast sediments contributes to the spread of harmful and toxic species and increases the frequency, intensity and geographic distribution of toxic poisoning such as paralytic shellfish poisoning (PSP) (Hallegraeff 1998), which can have a direct impact on human health, fisheries and aquaculture (Hallegraeff and Bolch 1991, 1992; Hallegraeff 2003).

The most commonly used method to reduce the risk of non-indigenous species introductions with current technologies is either open-ocean or mid-ocean ballast water exchange (BWE) (McCollin et al. 2007). The purpose of these exchanges is to replace almost all of the original ballast water from the coastal region of origin, and hopefully remove the sediments as well (IMO 2004). Under perfect conditions, approximately 95% of the ballast water from the original port should be replaced (Rigby and Hallegraeff 1994). In Canada, mandatory ballast water control and management regulations have been applied since June 2006 through the Canada Shipping Act (Office of the Auditor General of Canada 2008). This measure aims to prevent or reduce the release of foreign aquatic organisms and it is enforced for all ships arriving in Canada's coastal waters and particularly in the Great Lakes. There are three categories of ships traveling to Canadian coasts: 1) trans-oceanic ships (TOE) for which BWE is mandatory 2) continental ships traveling within North American waters that are required to do a ballast water exchange (CE) or continental ships for which BWE is not required (CNE) (includes most often oil tankers engaged in coastwise trade or vessels operating exclusively between ports on the East coast of the United States north of Cape Cod : 41°54'00''N – 70°17'0''W) (Canada Shipping Act 2001; United States Coast Guard 1993; Simard and Hardy 2004). There were no NOBOBs examined in this study.

In this study, we examine whether cargo ships visiting the East coast of Canada may be carrying non-indigenous, and/or harmful or toxic dinoflagellate species in their ballast sediments, which could inoculate local waters if discharged. The composition, abundance and percentage of occurrence of dinoflagellates present in ballast sediments from 65 ships is presented, along with pertinent ship information, details of ballast water exchange, including strategies used (empty-refill, flow-trough and no exchange) and age of ballast water. We identified both empty and live dinoflagellate cysts, since an empty cyst may represent a cell that germinated while inside the ballast tank. We also compared results among all categories of ships mentioned above (CE, CNE, TOE) and examined the efficiency of ballast water exchange to remove dinoflagellates from ballast tank sediments.

METHODS

Sediment ballast sampling and laboratory procedures

Sampling was carried out during three summers as part of the Canadian Aquatic Invasive Species Network (CAISN). Ships visiting the ports of Sept-Îles, Port Cartier and Baie-Comeau (Quebec) were sampled from June to August 2007. In 2008, ships visiting the ports of Hantsport and St. John (New Brunswick) and Canso, Halifax and Liverpool (Nova Scotia) were sampled from May to July. Finally, in 2009 several CNE ships were sampled in St. John (New Brunswick) (Figure 3).

A total of 65 ballast sediment samples were collected from general cargo, bulk carriers and oil tankers (see Annex 1) – this number of ships represents roughly 10% of the yearly number of foreign ships visiting the marine sector of the St. Lawrence (Bourgeois et al. 2001). The CAISN program aimed to sample at least 20 ships from each of the three ship categories (TOE, CE, CNE). Hence ships were selected on an opportunity basis until we had reached a maximum of about 20 ships for each category. Once the ships arrived in port, the sampling team met the captains and asked for the ballast reporting form (required by Transport Canada for all ships entering Canadian waters), they sampled the water column (data not presented here) and waited, when possible, until a tank was completely empty in order to go into the tank for sediment sampling. Sediment samples were taken from one tank per ship, mostly from wing tanks or top side and forepeak tanks according to the quantities of sediments found at the bottom of the tanks (Annex 1), and the accessibility of the tank. In either case, sediment samples were collected through direct access to the tanks, after complying with security conditions (e.g., prior tank oxygenation).

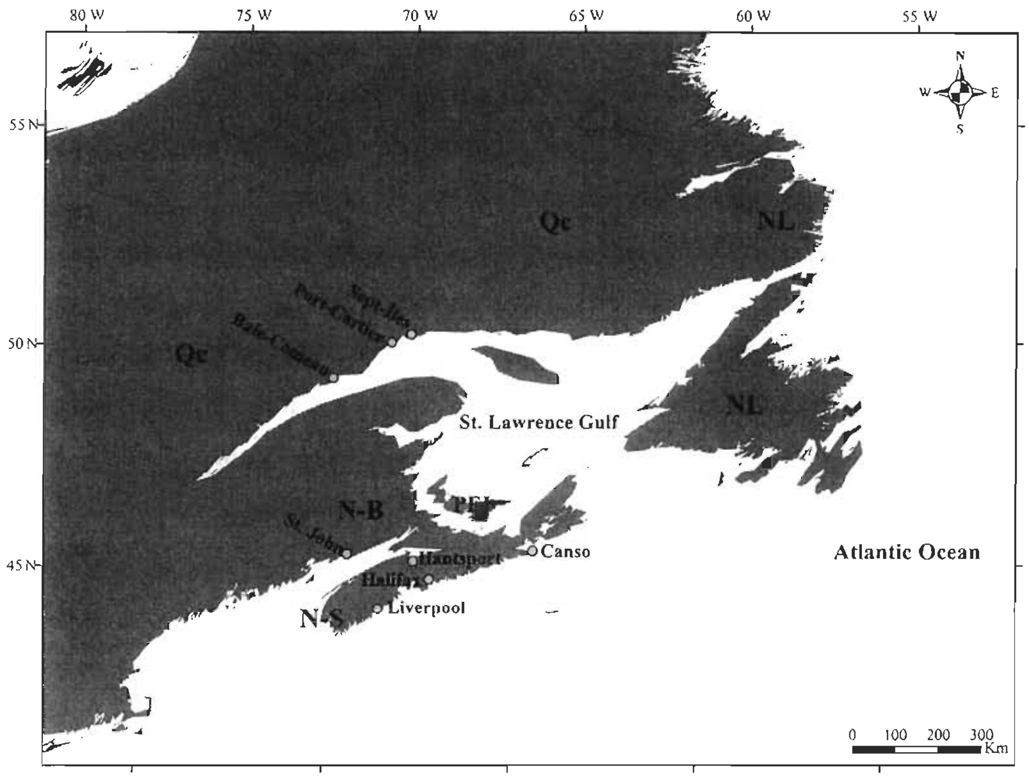

Figure 3. Location of ports where the ships were sampled on the East coast of Canada. QC = Quebec, NL = Newfoundland, N-B New Brunswick, N-S Nova Scotia, PEI = Prince Edward Island. The map was made using the World Robinson projection.

Approximately 500 g of the top 10 cm of the bottom sediments were collected with a scoop, from different areas within each tank, placed in a single plastic bag and stored in the dark at 4°C for a maximum of 12 months, prior to laboratory analysis. The quantities of ballast sediment were estimated by multiplying the surface area covered with sediment, with the average thickness of the sediment layer and correcting for the percent coverage of the sediment in the tank bottom.

All sediment samples were processed at the Institut des sciences de la mer de Rimouski (ISMER), of the University of Québec at Rimouski (UQAR). In the laboratory, each sample was mixed in the bag prior to the analysis and two sub-samples of 5 cm^3 of sediment were taken. The first one was for sediment dry weight determination and the second one for dinoflagellate cysts identification and counts. The first wet sub-sample was weighted and dried in an Imperial II radiant heat oven (Lab Line Instruments Inc.), at 55°C. Dry weight was taken after 48 h. The second wet sub-sample was transferred into a 250 mL beaker with 30 mL of filtered seawater originating from the Sept-Îles region. This seawater had a salinity of 26 and was pre-filtered on hydrophilic polysulfone 0.2 μm pore size membranes (Mini Capsule, Pall Corporation). Samples were manually homogenized and sonicated with a Fisherbrand FS-20® sonicator for 2 - 3 min to help detach the cysts from sand and detritus. These samples were then sieved on a 73 μm Nytex mesh to remove coarse sand followed by a 20 μm Nytex sieve. The fraction larger than 20 μm (which contains the cysts), was sieved twice on the 20 μm Nytex and washed with filtered sea water. Finally, the coarse particles were concentrated in the centre of a Petri dish by circular movements. Dinocysts were identified and counted (more than 200 cysts per sample) using an inverted microscope (Olympus) at 200X to 400X magnification. All specimens were mounted in a "micro slide chamber" following Horiguchi et al. (2000) and observed in a transmitted light microscope (Zeiss Axiovert) at 250X to 1000X in order to improve the identification. Dinocyst concentrations are given per gram of dry sediment.

Germination tests

Dinoflagellate cysts were separated into empty cysts (not viable) and full cysts, which were deemed viable (more precisely potentially viable, using a criterion of cellular content). As there were too many samples to do germination tests for all cysts, we randomly selected over 800 cysts that were tested for germination. To do this, viable dinocysts were isolated, cleaned and cultured in sterilized f/2 medium without silica prepared with filtered seawater from the Sept-Îles region (salinity = 26), sterilized in an autoclave (Guillard 1975). Dinocysts were washed through successive transfers through three Petri dishes with f/2 medium. They were then put in a 24-well Falcon® non-tissue culture treated plate, with each well filled with 2 ml of f/2 medium. These plates were placed in an environmental chamber at a temperature of 15°C, and a 14L: 10D photoperiod, provided by Phillips cool white fluorescent lamps with a light intensity between 100 and 120 μmol photons m^{-2} s^{-1}. Each Falcon® plate was observed daily during 10 days and then once a week during 4 weeks. Successful germination of cysts was used to verify their viability and to observe the archeopyle, which facilitates identification.

Nomenclature of dinoflagellate cysts

The taxonomical nomenclature used in this work follows Blanco (1989a, 1989b), Fensome et al. (1993) and Rochon et al. (1999), with help also from de Vernal et al. (2001), Head et al. (2001, 2005), Mudie and Rochon (2001), Louwye et al. (2004) and Head (2007). Some species in our samples are similar to those from Russian East coasts described by Orlova et al. (2004) and from Baja California, Mexico as illustrated by Peña-Manjarrez et al. (2005). Cysts of *Polykrikos kofoidii* (Chatton 1914) were compared with cysts described by Matsuoka and Fukuyo (2000) and with illustrations in Radi et al. (2001). Some taxa of e.g. *Brigantedinium cariacoense* (Wall 1967), *B. irregulare* (Matsuoka 1987) and *B. simplex* (Wall 1965) were identified from the shape of the archeopyle and compared with description and illustrations in Rochon et al. (1999) and McMinn et al. (2010). Finally, dinoflagellate cyst names (paleontological names) and their corresponding biological affinities (thecate names) were provided following Head (1996), Lewis et al. (1999), Pospelova et al. (2002, 2006) and Ellegaard et al. (2003) (see Table 1).

Table 1. Dinoflagellate cyst names found in this study (paleontological names) and their corresponding biological affinities (thecate names). In some cases, the same name is used for both nomenclatures.

Cyst species Paleontological name	Dinoflagellate motile cell Biological name
Brigantedinium cariacoense (Wall, 1967)	*Protoperidinium avellana* (Meunier, 1919) Balech, 1974
Brigantedinium simplex (Wall, 1965)	*Protoperidinium conicoides* (Paulsen, 1905) Balech, 1973
Brigantedinium irregulare (Matsuoka, 1987)	*Protoperidinium denticulatum* (Gran & Braarud, 1935) Balech, 1974
Bitectatodinium tepikiense (Wilson, 1973)	*Gonyaulax digitalis* (Pouchet, 1883) Kofoid, 1911
Cyst of *Alexandrium tamarense* (Hallegraeff & Bolch, 1992)	*Alexandrium tamarense* *
Cyst of *Cochlodinium polykrikoides* (Margalef, 1961)	*Cochlodinium polykrikoides* *
Cyst of *Gymnodinium catenatum* (Graham, 1943)	*Gymnodinium catenatum* *
Cyst of *Gymnodinium nolleri* (Ellegaard & Moestrup, 1998)	*Gymnodinium nolleri*
Cysts of *Protoperidinium americanum* (Gran & Braarud, 1935) Balech, 1974	*Protoperidinium americanum*
Cyst of *Pentapharsodinium dalei* Indelicato & Loeblich, 1986	*Pentapharsodinium dalei*
Cyst of *Polykrikos kofoidii* (Chatton, 1914)	*Polykrikos kofoidii*
Cyst of *Polykrikos schwartzii* (Bütschli, 1873)	*Polykrikos schwartzii*
Cyst of *Scrippsiella trochoidea* (Braarud, 1957)	*Scrippsiella trochoidea* *
Dubridinium caperatum (Reid, 1977)	*Preperidinium meunieri* (Pavillard, 1907) Elbrächter, 1993
Ensiculifera spp. (Balech, 1967)	Cf. *Pirumella quiltyi*
Impagidinium aculeatum (Wall, 1967) Lentin & Williams (1981)	*Gonyaulax* sp. indet.
Impagidinium pallidum (Bujak, 1984)	*Gonyaulax* sp. indet.
Impagidinium paradoxum (Wall, 1967) Stover & Evitt, 1978	*Gonyaulax* sp. indet.
Impagidinium sphaericum (Wall, 1967) Lentin & Williams (1981)	*Gonyaulax* sp. indet.
Islandinium minutum (Harland & Reid in Harland et al., 1981) Head et al., 2001	*Protoperidinium* sp. indet.
Lingulodinium machaerophorum (Deflandre & Cookson, 1955; Wall, 1967)	*Lingulodinium polyedrum* * (Stein, 1883) Dodge, 1989
Nematosphaeropsis labyrinthus (Ostenfeld, 1903) Reid (1974)	*Gonyaulax spinifera* complex
Operculodinium centrocarpum sensu (Wall & Dale, 1966)	*Protoceratium reticulatum** (Claparède & Lachmann, 1859) Bütschli, 1885
Operculodinium janduchenei (Wall & Dale, 1967)	
Polysphaeridium zoharyi (Rossignol, 1962) Bujak et al., 1980	*Pyrodinium bahamense* * Plate, 1906 var. *compressum* (Böhm, 1931)
Quinquecuspis concreta (Matsuoka, 1985)	*Protoperidinium leonis* (Pavillard, 1916) Balech, 1974
Selenopemphix quanta (Wall & Dale, 1968)	*Protoperidinium conicum* (Gran, 1900) Balech, 1974
Spiniferites belerius (Reid, 1974)	*Gonyaulax scrippsae* (Kofoid, 1911)
Spiniferites bentorii (Rossignol, 1964) Wall & Dale (1970)	*Gonyaulax digitalis*
Spiniferites delicatus (Reid, 1974)	*Gonyaulax* sp. indet.
Spiniferites elongatus (Reid, 1974) Ellegaard et al., 2003	*Gonyaulax elongata* (Reid, 1974)

	Ellegaard et al., 2003 *Gonyaulax spinifera-Spiniferites group*
Spiniferites membranaceus (Rossignol, 1964) Sarjeant (1970)	*Gonyaulax membranacea* (Rossignol, 1964) Ellegaard et al., 2003
Spiniferites mirabilis (Rossignol, 1967) Sarjeant (1970)	*Gonyaulax spinifera* complex
Spiniferites ramosus (Ehrenberg, 1838) Mantell (1854)	*Gonyaulax scrippsae* *Gonyaulax spinifera* complex
Spiniferites spp.	
Stelladinium reidii (Bradford, 1975)	*Protoperidinium* sp. cf. *P. compressum* (Abé, 1927) Balech, 1974
Trinovantedinium applanatum (Bradford, 1977) Bujak & Davies, 1983	*Protoperidinium pentagonum* (Gran, 1902) Balech, 1974)
Tuberculodinium vancampoae (Rossignol, 1962) Wall, 1967	*Pyrophacus steinii* (Schiller, 1935) Wall & Dale, 1971
Votadinium calvum (Wall & Dale, 1968)	*Protoperidinium oblongum* (Aurivillius, 1898) Parke & Dodge, 1976
Votadinium spinosum (Reid, 1977)	*Protoperidinium claudicans* (Paulsen, 1907) Balech, 1974

* Harmful or toxic species

Statistical analysis

Ships were separated into three categories depending on the type of voyage and ballast water exchange (see above). We verified whether the location of ballast exchange was indeed done in coastal or oceanic waters by comparing the location of the exchange with the Exclusive Economic Zone (EEZ) which defines coastal waters as those within ~370 km or 200 nautical miles from coastal regions (United Nations 1982), using ArcView version 9.3. Since this definition of coastal waters is more political than biological, we also plotted the location of ballast exchange sites with respect to Longhurst's biogeographical provinces or biomes (Longhurst 1998).

Cysts were separated into six categories: viable cysts (VC), empty cysts (EC) and total cysts (TC = VC + EC), as well as total cysts of NIS (TC NIS), empty cysts of NIS (EC NIS) and viable cysts of NIS (VC NIS). One-way analysis of variance was performed using PERMANOVA for PRIMER v.6 (Primer-E Ltd), with 9999 random permutations of appropriate units (McArdle and Anderson, 2001; Anderson et al. 2008). *P*-values were obtained by permutation, not requiring data normality. In addition, independence and homogeneity of dispersions are directly implied by the permutation procedure (Anderson, 2001; Anderson et al. 2008). Significant terms within the full models were analysed using appropriate pair-wise comparisons. Analyses were applied in order to 1) examine differences for each dependent cyst variable among ship categories, 2) characterize relationships among trans-oceanic (TOE), continental exchanged (CE) and continental non-exchanged (CNE) transport, as well as the influence of ballast water exchange strategies, including Flow-Through (FT), Empty-Refill (ER) or No-Exchange (NE). The ballast water age (BWA), defined as the residence time of water within ballast tanks, was calculated as the number of days after ballast water exchange (for TOE and CE ships) or after ballast water uptake at the port of origin (for CNE ships). Finally, dinoflagellate concentrations were compared with BWA and the volume of the sediment contained in the bottom of the tanks.

RESULTS

Vessels sampled

A total of 65 sediment samples were collected from 66 foreign ships visiting ports of Atlantic Canada (for one ship visited, there was not enough sediments). Ballast sediments were collected mostly from bulk carriers (58% of all ships), oil tankers (33%), general cargo (8%) and Ro/Ro type ships (1%). Sediment samples were collected mostly from wing tanks or topside tanks (65%), 28% from forepeak tanks (where we found the highest quantities of sediments), 6% from double bottom tanks and only 1% from cargo holds (Annex 1). These last tanks are the only ones used to transport merchandise, but once the merchandise is downloaded, they are filled up with water in a similar way to the other tanks, to provide stability during voyages.

Information from the Ballast Reporting Forms showed that, of the 65 ships sampled, 65% undertook ballast water exchange (BWE). About 54% of these ships exchanged their ballast waters in a coastal region and 46% in mid-ocean, according to the International Maritime Organization (IMO) forms provided by ship crews. To verify that BWE was done, we used salinity data from ballast water prior to emptying the tank before sediment sampling (whenever this was possible) or from another tank with the same ballast water source. These data showed that the TOE ships had a mean salinity of 34 ± 0.36 (n=12) while CE ships had a mean salinity of 32 ± 0.43 (n=9), which can be used to corroborate that BWE was done in waters of a salinity typical of truly marine environments.

Concerning the mode of BWE, about 33% of all ships exchanged ballast water using the empty/refill method, 32% used the flow-through method (ca 300% tank volume overflow, to achieve about 95% water replacement), 30% did not do any BWE, 2% used alternative methods such as a mix between flow-through and empty/refill methods, and for the remaining 3% of ships, ballast water management was undetermined. The method of ballast water exchange mostly used for TOE ships was FT while CE ships used mostly the ER method (with the exception of EC-28 that used an alternative method with only 20% of ballast volume replacement). We also examined where exactly BWE was done (whether it was in coastal or oceanic waters, according to the EEZ and to Longhurst's (1998) biogeographic provinces – see Annex 1). About 78% of TOE ships exchanged their ballast water in oceanic waters (at a distance between 417 and 998 km from any coast) and 13% of these ships did the exchange inside the EEZ (9% were undetermined).

Based on Longhurst's provinces, most of the TOE ships exchanged ballast in the NADR (North Atlantic Drift) province. For CE ships, 95% of these did the exchange in coastal waters according to EEZ limits, while 5% exchanged ballast outside of this area. Fourteen of 21 CE ships exchanged ballast in Longhurst's NWCS (Northwest Atlantic Shelves) province. The minimum and maximum distance from the coast where CE ships undertook ballast exchange was 30 and 378 km respectively, while the average distance was 203 km. These distances were calculated using geographic information systems analysis (ArcView, version 9.3, Figure 4).

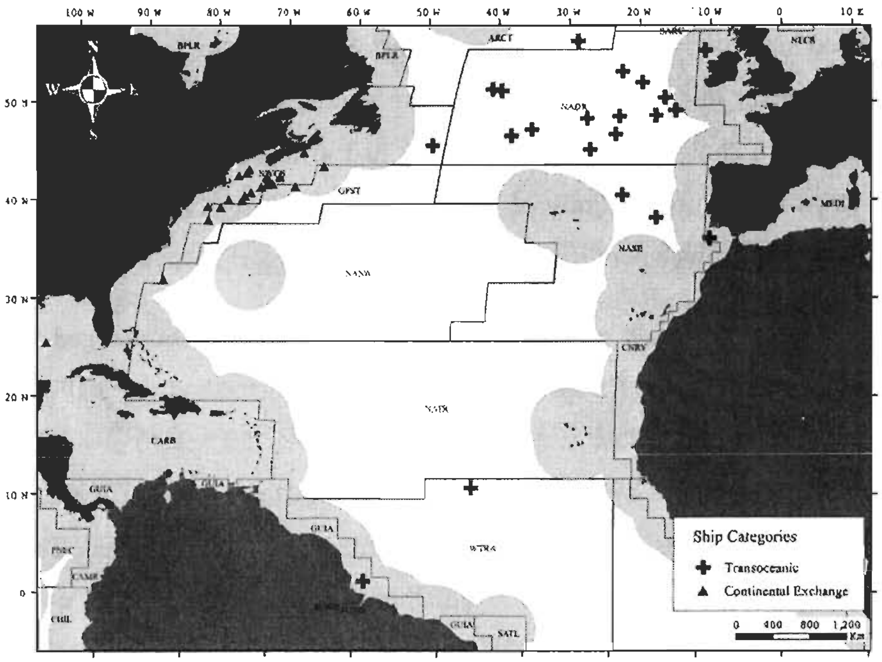

Figure 4. Location of ballast water exchange sites for ships arriving to the East coast of Canada. The light grey zone indicates the exclusive economic zone EEZ (370 km or 200 n.m.). The map was made using the World Robinson projection. Partitions correspond to the provinces defined by Longhurst (1998), ARCT Atlantic Arctic Province, SARC Atlantic Subarctic Province (Atlantic Polar Biome); NADR North Atlantic Drift Province, GFST Gulf Stream Province, NASW, NASE North Atlantic Subtropical Gyral Province West and East (Atlantic Westerly Winds Biome); WTRA Western Tropical Atlantic Province (Atlantic Trade Wind Biome); NECS Northeast Atlantic Shelves Province, NWCS Northwest Atlantic Shelves Province, CNRY Eastern Canary Coastal Province, GUIA Guinea Coastal Province (Atlantic Costal Biome); CARB Caribbean Province (Atlantic Trade Wind Biome).

Dinocyst diversity and identification

Dinoflagellate cyst assemblages were characterized by a relatively high diversity, with species belonging to the orders Gonyaulacales, Gymnodiniales and Peridiniales, including Calciodinelloides and Diplopsaloides. In terms of number of species, diversity varied from 2 to 24 species for TOE ships, with an average of 15; from 3 to 17 species for

CE ships, with an average of 16; and from 8 to 17 species for CNE ships with an average of 10. Annex 2 gives a detailed list of all species identified.

A total of 51 taxa belonging to 40 genera were identified in the ballast sediments among which, 9 that could not be identified to the species level. Eighteen different species of cysts were common to all categories of ships and *Brigantedinium simplex*, *B. cariacoense* and the round brown protoperidinioid cysts dominated dinoflagellate cyst assemblages. Those assemblages were accompanied by *Dubridinium caperatum*, *Quinquecuspis concreta*, and *Votadinium calvum*. Cysts of the Gonyaulacales were dominated by the potentially harmful species (indicated with an asterisk after the name) *Operculodinium centrocarpum** and *Scrippsiella trochoidea**. Cysts belonging to the order Gymnodiniales were dominated by *Polykrikos schwartzii*. Seven of these 51 taxa are considered as potentially harmful taxa (*Alexandrium tamarense**, *Cochlodinium polykrikoides**, *Gymnodinium catenatum**, *Lingulodinium machaerophorum**, *Operculodinium centrocarpum**, *Polysphaeridium zoharyi** and *Scrippsiella trochoidea**).

In addition, 14 of these 51 taxa are considered as non-indigenous species for the East coast of Canada. Dominant non-indigenous species included cf. *Polysphaeridinium zoharyi**, *Polykrikos kofoidii*, *Lingulodinium machaerophorum** and *Gymnodinium catenatum**. These species were recorded with empty and potentially viable cysts, posing a potential risk to marine ecosystems in case they germinate. In addition, 4 of these 14 non-indigenous species (29%) are potentially harmful or toxic. Six unidentified cyst species were also recorded (Annex 2).

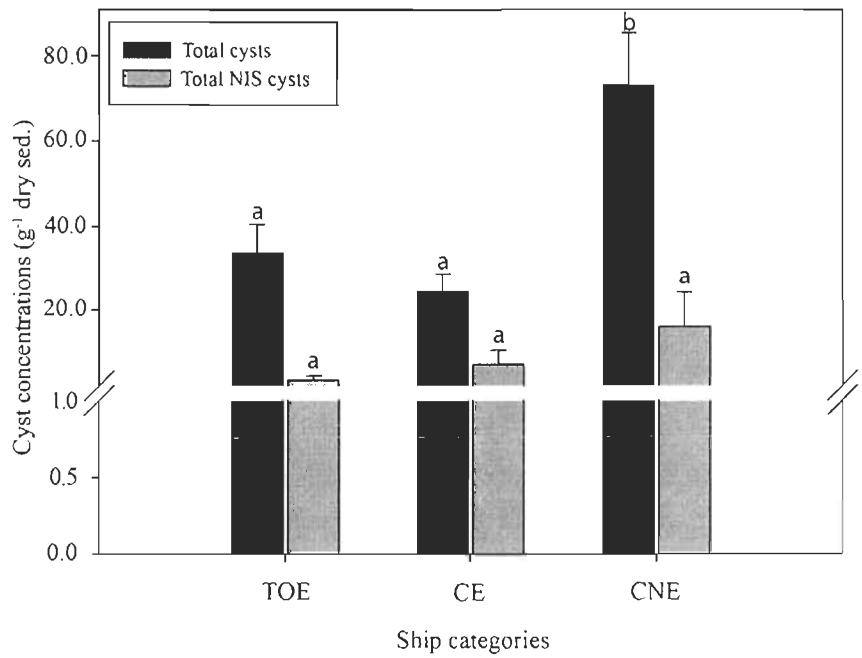

Figure 5. Mean concentration (± standard error) of total cysts (TC) and total NIS cysts (TC NIS) present in ballast sediment for each category of ships. Letters above columns indicate the results of pair-wise tests that showed significant differences between continental ships without ballast water exchange (CNE) and both transoceanic (TOE) (PERMANOVA test; $p = 0.001$) and continental ships with exchange (CE) (PERMANOVA test; $p = 0.0002$) in terms of TC. Categories of ships that differ significantly are indicated with different letters above data bars.

Abundance of dinoflagellate cysts in ballast sediments

The East coast ballast sediment samples revealed cyst concentrations that ranged from 1 to 220 total cysts g^{-1} dry sediment. Total cyst abundances were significantly different among ship categories (PERMANOVA, $p = 0.01$) (Figure 5). Ships that did not exchange ballast water (CNE), reached the East coast of Canada with the highest abundances of all types of cysts. Empty cyst concentrations were also significantly higher in CNE ships (mean = 46 ± 5 cysts g^{-1} dry sediment; median = 42 cysts g^{-1} dry sediment) than in TOE and CE ships (PERMANOVA, $p < 0.001$). For these two later ship categories, higher values of empty cysts were recorded in TOE ships (mean = 30 ± 6 cysts g^{-1} dry sediment; median = 18 cysts g^{-1} dry sediment) than in CE ships (mean = 22 ± 5 cysts g^{-1} dry sediment; median = 15 cysts g^{-1} dry sediment).

Cysts that were full were considered viable; random tests on over 480 full cysts from various species showed an average percentage of excystment of 38%, indicating that a significant proportion of all full cysts could germinate. Although not significant (PERMANOVA test, $p > 0.05$), the mean concentrations of viable dinocysts were almost the same between TOE ships (mean = 4 ± 1 cysts g^{-1} dry sediment; median = 1 cyst g^{-1} dry sediment) and CE ships (3 ± 1 cysts g^{-1} dry sediment; median = 1 cyst g^{-1} dry sediment), whereas it was higher in CNE ships (mean = 28 ± 10 cysts g^{-1} dry sediment; median = 9 cysts g^{-1} dry sediment) (Figure 6).

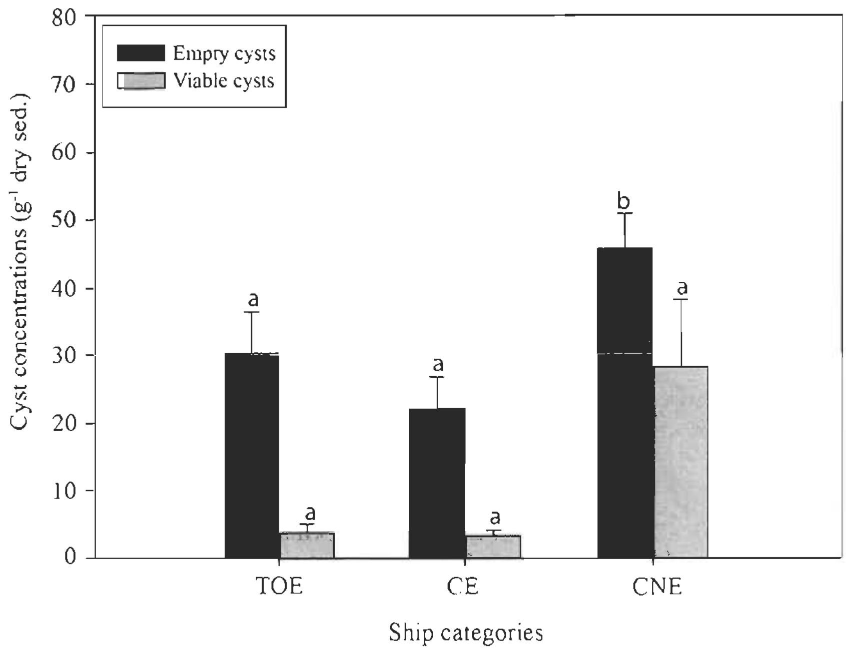

Figure 6. Mean concentration (± standard error) of empty and viable cysts of dinoflagellates present in ballast sediment for each category of ships. Categories of ships that differ significantly are indicated with different letters above data bars. Values for viable cysts were seemingly higher in CNE vessels compared with CE and TOE vessels (significantly higher for empty cysts: PERMANOVA test, $p < 0.05$)

For empty and viable NIS cysts, there were no significant differences among the three ship categories (PERMANOVA test, $p > 0.05$). Concentrations for empty NIS cysts were similar in CNE ships and CE (mean for both categories = 7 ± 3 cysts g^{-1} dry sediment; median = 3 cysts g^{-1} dry sediment) in contrast with TOE ships (mean = 2 ± 0 cysts g^{-1} dry sediment; median = 0 cysts g^{-1} dry sediment). Although not significant, values for viable NIS cysts were seemingly higher in CNE ships (mean = 9 ± 5 cysts g^{-1} dry sediment; median = 1 cysts g^{-1} dry sediment), compared with CE and TOE ships (mean for both categories = 1 ± 0.1 cysts g^{-1} dry sediment; median = 0 cysts g^{-1} dry sediment), respectively.

Finally, the concentrations of viable NIS cysts in CNE ships were also seemingly higher than that of empty NIS cysts, although the differences were not significant (Figure 7).

Percentage of occurrence

Table 1.2 presents the percentage of occurrence for the dominant dinocyst taxa. *Polykrikos schwartzii* was the most commonly observed species present in the ballast sediment samples (in 78.5% of all ships sampled), across all ship categories, followed by *Brigantedinium simplex* (70.8%), *B. cariacoense* (60.0%), *Quinquecuspis concreta* (55.4%) and *Votadinium calvum* (55.4%) (Table 2).

However there were some dinoflagellate cyst species more commonly observed in categories that performed ballast water exchange. For TOE ships, these were *Bitectatodinium tepikiense* (in 6 ships out of 24), *Operculodinium centrocarpum* (in 12 ships out of 24) and *Spiniferites elongatus* (in 7 ships out of 24), while for CE ships, there was only *Spiniferites ramosus* (in 11 ships out of 21). Finally, *Dubridinium caperatum* (in 10 ships out of 20), the cyst of *Protoperidinium americanum* (in 14 out of 20) and *Selenopemphix quanta* (in 11 ships out of 20) were among the most commonly-present dinoflagellate cyst species for CNE ships, apart from those listed above.

Table 2. Percentage of occurrence for the seven numerically-dominant taxa of dinoflagellate cyst species from sediment samples collected on ships arriving to Canada's East coast. Values in columns 2 to 5 are the number of samples containing the indicated species found in ships from the three categories and from all ship categories (column 5). The last column presents values (as totals for all ship categories) expressed as percentage of the total number of ships (i.e. 24 TOE + 21 CE + 20 CNE = 65 ships). Nd= no data.

	TOE	CE	CNE		
	n=24	n=21	n=20	All categories	All categories
Species	Number of ship samples			Total	% Occurrence
Bitectatodinium tepikiense	6	Nd	Nd	6	9.2
Brigantedinium cariacoense	15	12	12	39	60.0
B. simplex	15	13	18	46	70.8
Polykrikos schwartzii	16	17	18	51	78.5
Quinquecuspis concreta	14	11	11	36	55.4
Scrippsiella trochoidea	13	12	9	34	52.3
Votadinium calvum	11	13	12	36	55.4

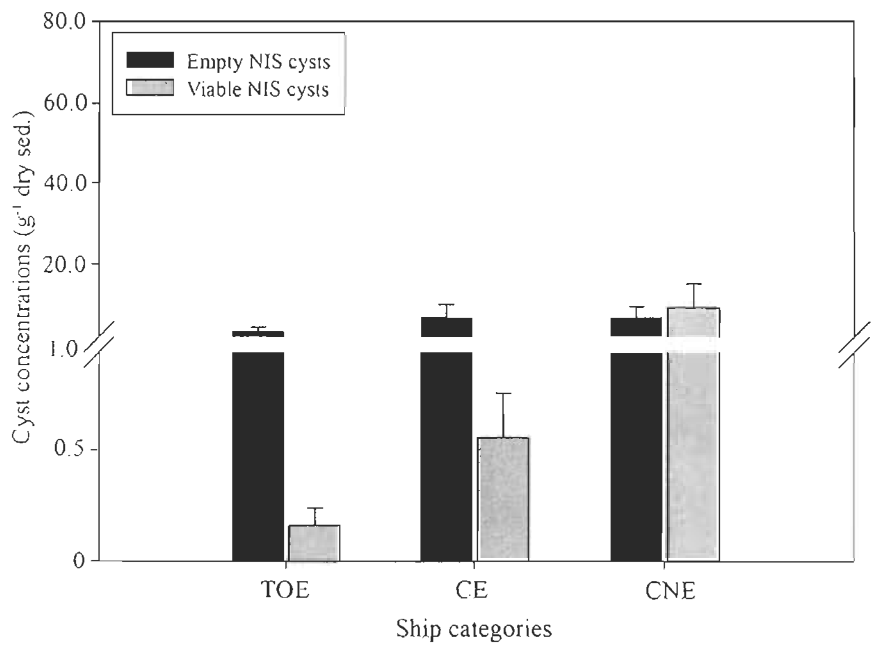

Figure 7. Mean concentration (± standard error) of empty and viable NIS cysts of dinoflagellates present in each category of ships. There are no significant differences among ship categories (PERMANOVA test; $p > 0.05$)

Percentage of occurrence of NIS cysts

Table 3 presents the percentage of occurrence for all non-indigenous dinocyst taxa. *Polysphaeridium zoharyi** (30.8%), *Lingulodinium machaerophorum** (26.2%) and *Polykrikos kofoidii* (20.0%) were the most frequently found non-indigenous species for all categories of ships (Table 3; Figure 8).

In addition, *Trinovantedinium applanatum* (in 5 ships out of 24) and *Votadinium spinosum* (in 4 ships out of 24) were the most frequently found species in TOE ships. The most commonly observed non-indigenous species for continental ships (with and without ballast water exchange) were *Brigantedinium irregulare* (in 8 ships out of 41) and *Cochlodinium polykrikoides** (in 10 ships out of 41).

Table 3. Percentage of occurrence for all non-indigenous dinoflagellate cyst taxa from sediment samples collected on ships arriving to Canada's East coast. Values in columns 2 to 5 are the number of samples containing the indicated species found in ships from each ship category and from all of them (column 5). The last column presents values (as totals for all ship categories) expressed as percentage of the total number of ships (65 ships).

	TOE	CE	CNE		
	n=24	n=21	n=20	All categories	All categories
Species	Number of ship samples			Total	% Occurrence
Brigantedinium irregulare	4	2	6	12	18.5
*Cochlodinium polykrikoides**	1	5	5	11	16.9
Cyst of *Polykrikos kofoidii*	7	3	3	13	20.0
Cyst of *Protoperidinium latissimum*	0	1	2	3	4.6
Fragilidium mexicanum	5	2	1	8	12.3
*Gymnodinium catenatum***	4	5	2	11	16.9
G. nolleri	1	1	0	2	3.1
*Lingulodinium machaerophorum***	8	7	2	17	26.2
*Polysphaeridium zoharyi***	2	13	5	20	30.8
Spiniferites bentorii	0	1	1	2	3.1
Stelladinium reidii	0	0	4	4	6.2
Trinovantedinium applanatum	5	4	1	10	15.4
Turbeculodinium vancampoae	0	1	0	1	1.5
Votadinium spinosum	4	2	0	6	9.2

* Harmful species

** Saxitoxin producers. *L. machaerophorum* is mostly associated with yessotoxin production

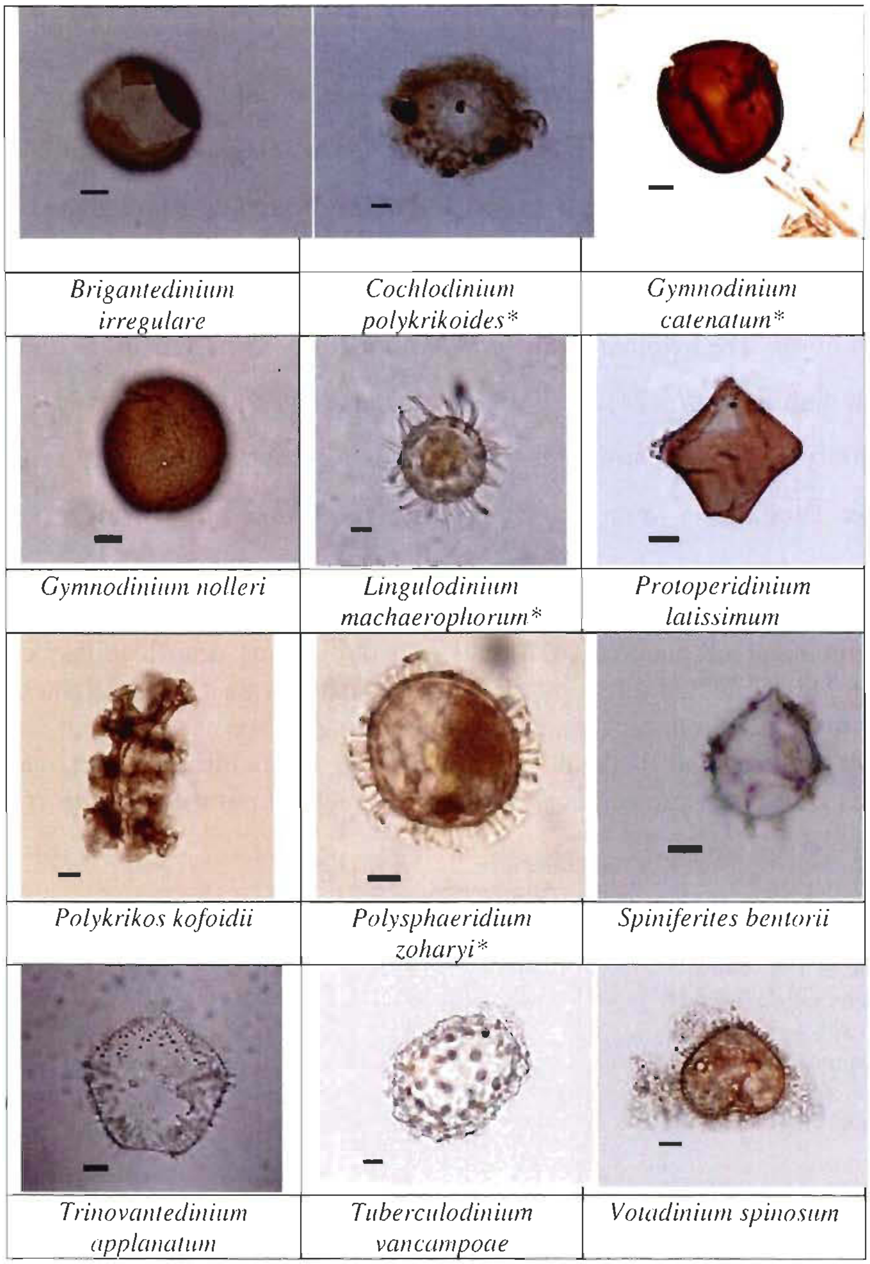

Figure 8. Non-indigenous species found in the ballast sediment of ships sampled on the East coast of Canada. *Fragilidinium mexicanum* and *Stelladinium reidii* are not. Harmful or toxic species are indicated with an asterisk. Scale bars represent 10 µm. Photographs by Oscar Casas-Monroy.

Ballast water exchange strategies

Figure 9 and the statistical analyses using Permanova show that viable and empty cyst abundances were significantly higher in ships with no BWE compared to ships with BWE. Viable cyst abundances were also significantly higher in non-exchanged ships ($p < 0.05$). In contrast, ballast water management practices (FT, ER) have no significant effect on empty or viable cysts of NIS (PERMANOVA, $p = 0.766$ and $p = 0.069$, respectively).

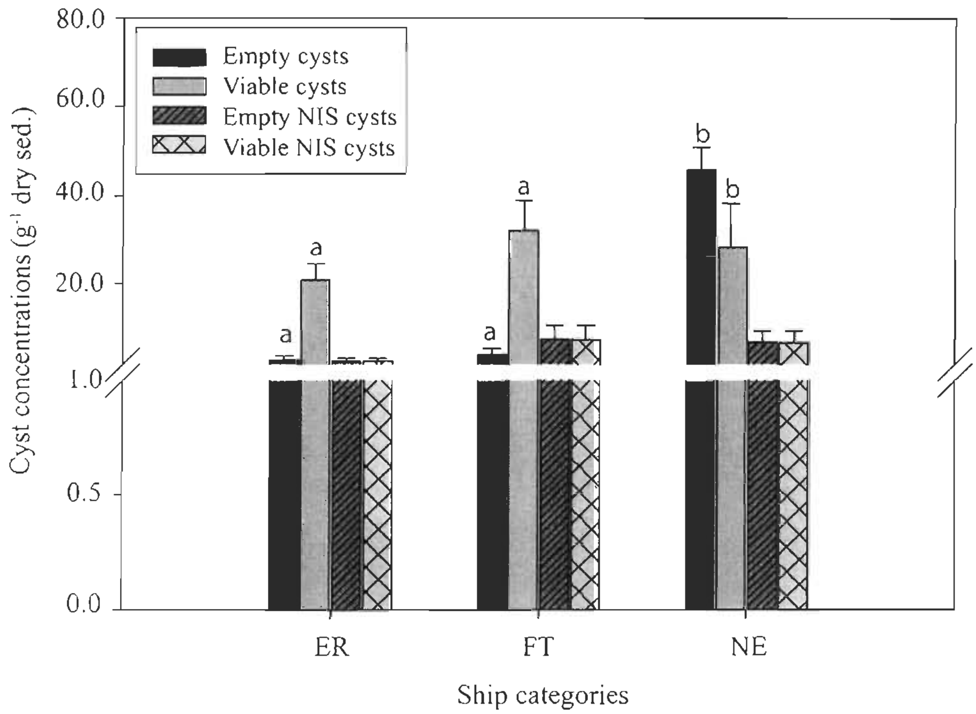

Figure 9. Mean concentration (± standard error) of empty cysts (EC), viable cysts (VC), empty NIS cysts (ECNIS), and viable NIS cysts (VC NIS) of dinoflagellates present in ballast sediments for each ballast water exchange strategy. Letters above columns indicate the results of pair-wise tests that showed significant differences among ships without ballast water exchange (NE) and ships with exchange (ER = empty-refill, FT = flow-through), in terms of VC and EC (PERMANOVA test; $p < 0.05$). Categories of ships that differ significantly are indicated with different letters above data bars.

Ballast water age

Ballast water age was significantly different among TOE and continental (CE and CNE) ships (PERMANOVA test, $p < 0.05$) (Figure 10), while there was no significant difference between the two continental ship categories. The average water age (number of days) was higher for TOE ships (mean = 6 ± 0.5 days; median = 6) than for CE (mean = 3 ± 0.5 days; median = 2) and CNE ships (mean = 4 ± 1 days; median = 2). Ballast water age shows no clear statistically-significant effect on dinoflagellate cyst abundances, even though the two continental categories, with generally shorter trip duration, have higher abundances of total and viable dinoflagellate cysts of NIS. Ships with "younger" ballast water, less than 4 days old, have the highest abundances of viable cysts of NIS.

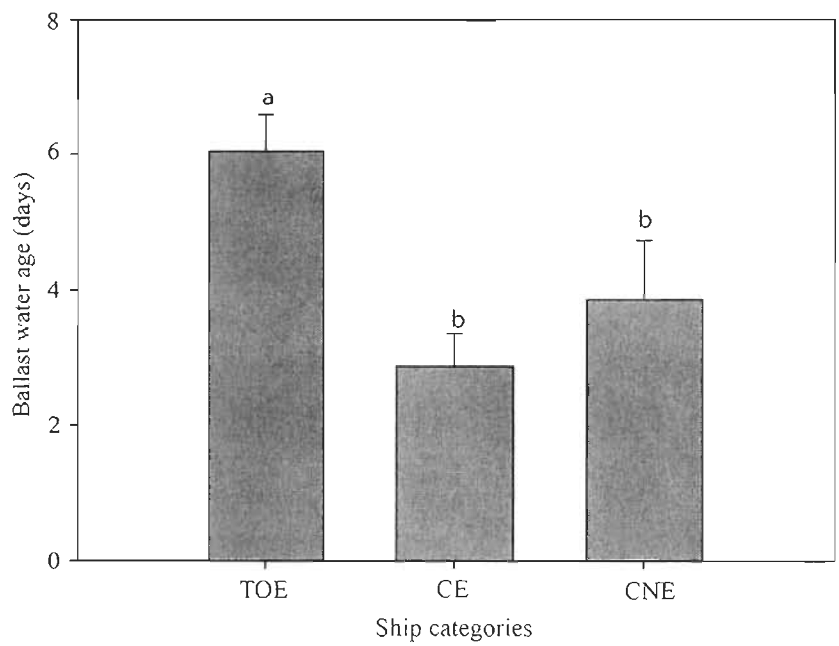

Figure 10. Mean ballast water age (± standard error) estimated for each category of ships. . Letters above columns indicate the results of a pair-wise test that showed significant differences among trans-oceanic and continental ships (CE and CNE) (PERMANOVA test; $p < 0.05$). Categories of ships that differ significantly are indicated with different letters above data bars.

Ballast sediments

The volume of sediments was significantly different between CNE ships and BWE categories (P< 0.001) (Figure 11A). The highest sediment volumes were recorded in CE ships (mean= 2.02 ± 0.8 m^3; median = 0.31 m^3) followed by TOE ships (mean = 1.3 ± 0.5 ± m^3; median = 0.68 m^3). The lowest quantities were recorded in CNE ships (mean = 0.1 ± 0.05 m^3; median = 0.067 m^3).

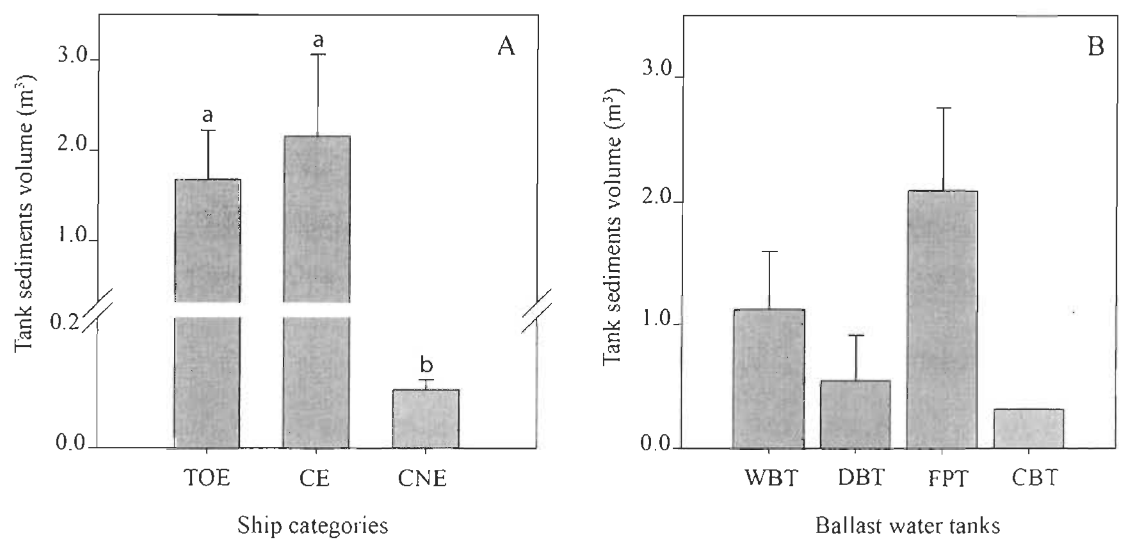

Figure 11. A) Volume of sediments (± standard error) estimated for all ship categories. Letters above columns indicate the results of a pair-wise test that showed significant differences between continental non-exchanged ships (CNE) and the two ship categories with ballast water exchange (PERMANOVA test; $p < 0.001$). B) Volume of sediments estimated in each type of tanks for all ship categories. WBT = wing tanks; DBT = Double bottom tanks; FPT = forepeak tanks; CBT = cargo ballast tank

The two categories performing BWE were not significantly different. Regardless of the ship category, in some ballast tanks, sediments were uniformly distributed on the bottom. In other tanks, sediments were mostly found in tank corners. The shapes and dimensions of tank compartments varied depending on the tank sampled, type of ship (e.g.

bulk carrier, oil tanker or RO/RO) (Annex 1) and/or gross tonnage of ships visited (data not shown), making it difficult to get an accurate estimate of the volume of sediment per tank or per ship. Since the shape and dimensions of the various types of tanks may affect the accumulation of bottom sediment, we plotted the sediment volume for the major types of tanks encountered (Figure 11B).

The highest mean sediment volumes were recorded in forepeak tanks ($2.1\ m^3 \pm 0.7\ m^3$) followed by wing tanks ($1.2\ m^3 \pm 0.5\ m^3$). The lowest mean quantities were recorded in double bottom and cargo tanks (less than $1\ m^3$). Sampling was done mostly in wing tanks in the two continental ship categories (15/21 ships for CE and 19/20 ships for CNE), while forepeak and wing tanks were used for trans-oceanic ships (22/24 ships). Although the same type of tanks was sampled for CE and CNE ships, the amount of sediments was clearly different between the two (Figure 11A). Part of the explanation may lie in the different types of ships sampled, with oil or chemical tankers dominating the CNE category while there are more bulk carriers in the CE category (Annex 1).

DISCUSSION

There are a few studies examining the composition and abundance of planktonic species in ballast water of ships visiting ports of the East coast of Canada, but this is the first extensive study dealing with phytoplankton in ballast sediments for this region. Our results show that cysts of dinoflagellates are present in ballast sediments and many of those are potentially viable (cyst not broken and with cell content, some that successfully excysted in the laboratory). Non-indigenous viable species are present (total abundance = 206 cysts g^{-1} dry sediment for all viable NIS cyst species over 65 ships), as well as some harmful/toxic species, some of which are also non-indigenous. Along with studies from other regions of the world, this confirms the importance of ballast sediment as a vector for the potential introduction of non-indigenous viable organisms between and within different biogeographic zones. Our results also highlight a significant transfer of dinoflagellate cysts, including non-indigenous, harmful/toxic species through continental transport, especially by non-exchanged ships traveling in coastal waters of the East coast of North America.

Previous studies in North America also reported viable phytoplankton contained in ballast sediments of bulk cargo ships using various ballasting practices (Kelly 1993; Harvey et al. 1999). Most studies, including ours, sampled mostly bulk carriers for two principal reasons: 1) these ships carry a large volume of ballast water because, in addition to the tanks generally used for ballast water (such as fore peak, after peak, double bottom and wing tanks), they also often fill empty cargo holds with ballast water to improve stability (Kelly 1993), and 2) they represent 64% of the ships arriving to the East coast of Canada (Bourgeois et al. 2001; Simard and Hardy 2004). In the present study 31% of ships sampled were oil or chemical tankers, and most of these were non-exchanged. Our results show that these ships reached the East coast of Canada with the highest abundances of total NIS cysts (144 cysts g^{-1} dry sediment) and of potentially viable NIS cysts (101 cysts g^{-1} dry sediment) (maximum values found per ship). A previous study carried out for ships arriving into Atlantic Canadian waters, also showed higher phytoplankton abundances in ballast water from oil tankers compared with bulk carriers, general cargo and container ships (Carver and Mallet 2002), however ballast sediments were not examined in that study. Simkanin et al. (2009) found that bulk carriers and oil tankers discharged a greater volume of water per ship than other ship types, (perhaps removing some of the ballast sediment at the same time, which would explain the smaller sediment volume in the CNE ship category in the present work), and oil tankers undertaking coastal voyages discharged significantly greater volumes compared to tankers arriving from overseas to ports of the United States. Moreover, the United States are considered one of the main export regions of ballast water by crude oil carriers (Endresen et al. 2004), with high numbers of ship arrivals (higher frequency of discharge events) on the East coast of Canada. Internationally, oil tankers account for some 37% of the ballast water transported annually, while dry bulk cargo carriers account for 39% (coal, iron ore, grains and other bulk commodities) (Endresen et al. 2004). During the present study, we only have data from 13 oil and chemical tankers that discharged about 2.5×10^5 MT (Metric Tonnes) of ballast water in total. The Canada Port State Control National Ballast Water Database from Transport Canada indicates that bulk carriers discharged more than 10.4×10^6 MT of ballast water during 2008 at all ports of the marine coastal region of the East coast of Canada, while oil and chemical tankers discharged the same volume during the same period. Hence, both categories of ship (carriers and tankers) can strongly contribute to the introduction of species into a new region.

Continental exchanged ships may have implications for the spread of non-indigenous or harmful/toxic species. Our results show that non-living and potentially viable cysts of non-indigenous species are present in 30% of ships arriving to the East coast of Canada. Just in 2008, more than 25 x 10^6 MT of ballast water were discharged in all ports on this coast (Transport Canada, unpublished data). This transport of water and sediments in ballast tanks of continental exchanged ships poses a risk of introduction and spread of NIS, in spite of the ballast water exchange practice. The role of ballast water exchange is to reduce to a large extent the abundances of non-indigenous species and to increase salinity to protect in-land freshwater ecosystems (Rigby and Hallegraeff 1994). If we compare the two continental categories CNE and CE, we see that the CNE ships presented significantly higher concentrations for all categories of dinoflagellate cysts compared to CE, for which water exchange relative to the ballast tank volume varied between 90% (E-R) and more than 300% (F-T, see Annex 1). Ballast exchange in mid-ocean should reduce the amount of sediment (and associated organisms) picked up during ballast intake. This would be consistent with the decresing sediment volume trend in the trans-oceanic ships (TOE) compared to the continental ships with a BWE (CE), although these are not significantly different. Indeed, a majority of the CE ships exchanged their ballast water within the EEZ or in areas under continental influence, according to the concept of coastal biomes proposed by Longhurst (1998) (cf. Annex 1). However, the very low sediment volumes observed in the continental non-exchanged ships (CNE) is counter-intuitive. We believe this may be due to the fact that ships of the CNE category were mostly tankers while ships of the CE category were mostly bulk carriers (Annex 1) hence differences between them cannot be attributed only to the presence or absence of BWE or the specific location to perform BWE. These results also indicate that 1) cyst abundances are highly variable and 2) continental ship traffic (with and without ballast water exchange) represents a risk of introduction of dinoflagellate species as cyst forms, including NIS. These observations, particularly the highest dinocyst abundance in CNE ships, are in accordance with those of Burkholder et al. (2007), who found phytoplankton abundances 4-fold higher in tanks with coastal water than in tanks with ballast water from the open-ocean.

In addition, the voyages are of shorter duration for continental ships (3-4 days) compared with trans-oceanic ships (6-12 days). The shorter trip duration within the coastal waters of North America can favor the survival of dinoflagellate cysts. Consequently, the shorter voyage duration for continental compared to trans-oceanic ships may also have contributed to the higher concentrations of potentially viable cysts in continental ships. In addition, continental ships (CE) seemingly carry more sediment, increasing the concentration of cysts per tank. When comparing the cysts concentrations and the ballast tank sediment volumes between continental ships, our results show that CNE presented the highest cyst concentrations but the lowest sediment volumes, while CE was the opposite. These results suggest that both categories of continental ships present a risk of NIS introduction. More potentially viable cysts may be found in the upper layers of the ballast sediments if they have been deposited recently, explaining the highest concentration in ships with shorter voyage duration. In that case, more viable cysts could be discharged with sediment slurries when ballast water is discharged in ports. Scientists from Fisheries and Oceans Canada are currently examining whether or not sediments can be discharged along with ballast water during loading operations in port. Preliminary results show that little suspended particulate matter is discharged with ballast water at the beginning of the deballasting procedures but it increases towards the end (Nathalie Simard, Maurice Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli QC, personal communication). These results indicate that ballast sediments can be flushed out of ballast tanks and thus could play an important role in the introduction of non-indigenous species. Methods used to estimate the ballast sediment quantities per ship were set-up by the CAISN program and have been used by others in this program (Briski et al. 2010). These results show estimations close to 5 tonnes of sediments per ship (averaged over 17 ships), while our data show an average close to 15 tonnes (over 65 ships). Nevertheless, only a single tank per ship was sampled and often spots and areas with higher sediment accumulation were observed in double bottom, topside or forepeak tanks. Forepeak tanks, due to their size, usually have the most sediment (personal observations). Here, the maximum sediment volumes recorded per tank were 16.8 m^3 or 12 tonnes in a topside tank and 12.1 m^3 or 10 tonnes in a forepeak tank.

This study is the first one describing in detail the dinoflagellate cysts present in ballast sediments from ships arriving to the East coast of Canada. The dinoflagellate cyst diversity was relatively higher in CNE ships than in TOE and CE. There were also differences in the dinoflagellate cyst assemblages, for example CE ships were characterized by a higher proportion of Gonyaulacales cysts (37%) while in TOE and CNE ships, this proportion was lower (24% and 19%, respectively). In addition, Gymnodiniales such as *Polykrikos schwartzii* and Peridiniales such as *Brigantedinium* spp., *Dubridinium* spp., *Quinquecuspis concreta* and *Votadinium calvum* were dominant species with highest percentage of occurrence in all categories of ships. Except for *Quinquecuspis concreta* that may be present in open ocean environments around the British Isles (Dodge and Harland 1991), these species occur mostly in neritic areas, in line with the origin of the water used for ballast water exchange or to fill the ballast tanks of CE and CNE ships. Moreover, these dominant species are often considered biological indicators of eutrophic water conditions in coastal systems (Pospeslova et al. 2006). Considering their usual distribution, it is not surprising to find more Gonyaulacales in CE ships instead of the TOE, even though certain genera such as *Impagidinium, Nematosphaeropsis* and *Spiniferites* are often present in open ocean environments (Mudie and Rochon 2001). Species such as *Spiniferites ramosus*, associated with both neritic and oceanic domains (Dodge and Harland 1991), were here more abundant in continental exchanged ships.

Of the 51 dinoflagellate species found in all ballast sediment samples (TOE, CE and CNE), more than 50% are cosmopolitan and distributed from coastal to oceanic environments, and from temperate to tropical regions. Of these, 14 have not yet been reported in Canada's East Coast waters (Annex 2). In addition, out of these 14 NIS, 4 are considered as potentially harmful/toxic and their concentrations ranged from 2 to 70 cysts g^{-1} dry sediment. Some of these NIS harmful/toxic species are restricted to tropical and subtropical regions and their invasion threat is low for temperate regions, although climate change may facilitate this. Given the non-indigenous species with empty and potentially viable NIS cyst concentrations found in continental exchanged ships our results suggested that continental traffic could contribute to future invasions. Once a non-native population is successfully established within a port, the port system itself may become a source for subsequent introductions (particularly if cyst resuspension occurs) through passive range expansion, and continental exchange ships may influence this secondary spread of NIS (Wasson et al. 2001; Ruiz et al. 2006).

The proliferation of non-indigenous species of dinoflagellates poses enormous risks, particularly when these are harmful or toxic. These proliferations have had major economic consequences for the Australian aquaculture industry (Hallegraeff 2002). Cysts of potentially toxic algal species found in this study such as PSP producers *Alexandrium* spp., *Gymnodinium catenatum* and *Polysphaeridium zoharyi* or yessotoxin producers such as *Lingulodinium machaerophorum* can remain dormant in sediments for a period up to 10-20 years (Hallegraeff and Bolch 1992). Cysts of species such as *Gymnodinium nolleri* and *Operculodinium centrocarpum** have even been found in viable form after about 37 years in the sediments of Koljö Fjord on the west coast of Sweden (McQuoid et al. 2002). Hence according to our results, coastal shipping represents a higher risk than trans-oceanic shipping in terms of these organisms despite lower abundances (9-541 cysts cm^{-3}, this work) than in previous studies for ships visiting Scottish ports (5-1450 cysts cm^{-3} in ballast water discharge, Macdonald 1995) and Australian ports (40-22 500 cysts cm^{-3}, Hallegraeff and Bolch 1992). For some species, such as *G. catenatum**, the chances of survival under typical Canadian winter conditions are low because this species is restricted to tropical/subtropical regions. It is the same case for species such as the cyst of *Fragillidium mexicanum* mostly found in coastal waters of Mexico, Japan, Russia and Europe (Blanco 1989a; Selina and Orlova 2009), or *Polysphaeridium zoharyi* which was abundant in our samples and is mostly distributed in the Caribbean Sea and Gulf of Mexico. In the case of

*L. machaerophorum**, it has been recorded occasionally in sites with mean winter temperature as low as -1.5°C, but this species is generally found mostly in coastal sediments from the subtropical/tropical Southern Hemisphere and from tropical/temperate regions of the Northern Hemisphere. This species is generally reported to be restricted to regions with summer temperatures between 10 to 12°C (Rochon et al. 1999), however, temperatures as low as 4°C do not prevent survival of the cysts (Lewis and Hallet 1997). The presence of cysts of *Cochlodinium polykrikoides** is also worth noting here, since this harmful species seems to be expanding its range and it is generally found in temperate environments (Kudela et al. 2008). Climate change may facilitate the introduction of these toxic species (Dukes and Mooney 1999; Vitousek et al. 1997), increasing the risk to Canada's coastal waters.

In summary, our data show that potentially viable dinoflagellate cysts are present in ballast sediments of three categories of ships arriving to the East coast of Canada. The concentrations of all types of dinoflagellate cysts are higher in continental ships without ballast water exchange than in ships with BWE, including trans-oceanic ships that presented the lowest risk of introduction of NIS for these ballast sediment organisms. Even though CE ships had a lower concentration of dinocysts than CNE ships, sediment volume was higher in CE ships, suggesting that they also present a significant risk to coastal environments. The presence of non-indigenous species in continental ships (particularly non-exchanged ones) may be related to the upsurge in aquatic invasions that have affected some ports on the US Eastern seaboard, such as Chesapeake Bay (Ruiz and Reid 2007).

We identified 14 non-indigenous dinoflagellate cyst species not yet reported from Canadian coasts, including 4 harmful/toxic species, representing a possibility of new introductions. In addition, non-indigenous species were present in close to 30% of the ships arriving to the East coast ports. The risk of new invasions increases with the volume of ballast water discharged in receiving ports and the frequency of ships' visits, but also with the number of NIS released (Carlton 1996; Verling et al. 2005). Some of these non-indigenous dinoflagellate species have a wide distribution (Dodge 1982). Some species found in TOE ships (e.g. *L. machaeorophorum**) live in marine environments with relatively similar temperature and salinity conditions as local coastal ecosystems, which could facilitate their successful establishment. Other species, particularly harmful/toxic species, have a more restricted distribution limited to the tropical and subtropical regions. A warming climate may facilitate the introduction, establishment and spread of these species. Further research on NIS and on ballast water and sediment discharge can help us to understand, prevent and predict new possible introductions of non-indigenous phytoplankton species. Ballast water treatment methods presently available or proposed seem to have limited efficacy for phytoplankton, depending mostly on cost, biological efficacy and environmental impact (Gregg et al. 2009; Tsolaki and Diamadopoulos 2010). Dinoflagellate cysts pose significant technological challenges because of their resistance to most treatments (except heat when applicable), and difficulties associated with high flow rates, and large volumes of ballast water and sediments. A combination of two or more methods may increase the efficacy when feasible but further research on IMO-approved treatment methods are needed.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC), Fisheries and Oceans Canada (DFO), the Canadian Aquatic Invasive Species Network (CAISN) and the Institut des Sciences de la Mer de Rimouski (ISMER) of the Université du Québec à Rimouski for financial support. We thank specially Nathalie Simard, Andrea Weise and Dr. Chris McKindsey (DFO), for help during sampling and for discussion. Also, we thank Transport Canada, shipping agents in different ports, captains, ship officers and crews of the vessels sampled. Finally, we thank all the persons involved in the East Coast Sampling Teams and Jean-Guy Nistad for help with the production of maps.

CHAPITRE 2
LES KYSTES DE DINOFLAGELLÉS DANS LES SÉDIMENTS DE BALLAST: DIFFÉRENCES ENTRE LES CÔTES EST ET OUEST DU CANADA ET LES GRANDS LACS

RÉSUMÉ

L'abondance et la diversité des kystes de dinoflagellés (en particulier pour les espèces non-indigènes ou nuisibles) dans les sédiments de ballast ont été examinées pour trois régions du Canada: les régions marines côtières de l'est et l'ouest et les Grands Lacs, afin d'évaluer les risques potentiels d'invasions de ces organismes et déterminer la similitude entre les régions. Un total de 147 navires ont été échantillonnés, répartis en trois catégories: (1) les navires transocéaniques, avec échange obligatoire de leurs eaux de ballast, (2) côtiers avec échange d'eaux de ballast, et (3) côtiers sans échange d'eau de ballast. Cette étude a examiné divers facteurs qui pourraient expliquer la variabilité des résultats, y compris les routes empruntées par les navires (transocéaniques ou côtières), l'échange de ballast, l'âge de l'eau et le volume des sédiments dans les réservoirs. Le patron du risque potentiel d'invasion change selon la région examinée. Sur la côte est, les navires côtiers transportent les plus fortes concentrations de tous les kystes viables incluant ceux d'espèces non-indigènes (par g de sédiment sec et par réservoir de ballast). Les navires côtiers sans échange avaient des concentrations maximales par g de sédiment sec, mais de plus petits volumes de sédiments, ce qui diminue l'abondance globale de kystes par réservoir. L'échange des eaux de ballast a eu une influence différente selon la région: sur la côte est, le risque d'invasion a été réduit par l'échange au milieu de l'océan, alors qu'il n'a eu aucun effet significatif dans les deux autres régions. L'âge de l'eau de ballast et le

volume de sédiments étaient également différents entre les régions, mais ils n'étaient pas clairement en relation avec les concentrations de kystes dans les réservoirs de ballast. La gestion future des sédiments de ballast devrait prendre en considération ces différences régionales.

Ce deuxième article, intitulé « *Dinoflagellates cysts in ballast sediments: Differences between Canada's East coast, West coast and the Great Lakes* », fut corédigé par moi-même ainsi que par le professeur Suzanne Roy et le professeur André Rochon. Il fut soumis et accepté pour publication, sous réserve de modifications, en mars 2012 à la revue *Aquatic Conservation: Freshwater and Marine Ecosystems*. En tant que premier auteur, ma contribution à ce travail fut l'essentiel de la recherche sur l'état de la question, le développement de la méthode, l'identification et le dénombrement des espèces, l'exécution des tests statistiques et la rédaction de l'article. Le professeur Suzanne Roy deuxième auteur, ainsi que le professeur André Rochon troisième auteur ont grandement contribué à la recherche sur l'état de la question, au développement de la méthode ainsi qu'à la révision du manuscrit. Les résultats préliminaires de cette étude ont été présentés lors du Forum Québécois des Sciences Marines, Rimouski, QC, Canada, à l'automne 2009. Par la suite les principaux résultats ont été présentés lors des Assemblées Générales et conférences du réseau canadien sur les espèces aquatiques envahissantes « CAISN », à Victoria, C.-B., au printemps 2010 et à Québec QC, au printemps 2011.

DINOFLAGELLATE CYSTS IN BALLAST SEDIMENTS: DIFFERENCES BETWEEN CANADA'S EAST COAST, WEST COAST AND THE GREAT LAKES

ABSTRACT

The abundances and diversity of dinoflagellate cysts (including non-indigenous or harmful species) in ballast sediments were examined for three regions of Canada: the marine coastal regions of the East and West coasts and the Great Lakes, to assess the potential invasion risk for these organisms and determine similarity across these regions. A total of 147 ships were sampled, distributed among three categories: (1) transoceanics, with mandatory ballast water exchange (BWE), (2) coastal with BWE, and (3) coastal without BWE. We examined various factors that could account for the variability in our results including ship routes (transoceanic or coastal), BWE, ballast water age and the volume of sediments in ballast tanks. The pattern of potential invasion risk differs according to the region examined. On the East coast, coastal ships carried the greatest concentrations of all viable cysts including those of non-indigenous species (per g of dry sediment and per ballast tank). Unexchanged coastal ships had maximum concentrations per g of dry sediment, but smaller volumes of sediments, decreasing the overall abundance of cysts per tank. Ballast water exchange had a different influence according to the region: on the East coast, the risk of invasion was reduced by BWE while it had no significant effect in the other two regions. Ballast water age and sediment volume were also different among regions, but they were not clearly related with cyst concentrations in ballast tanks. Future management of ballast sediments should take into consideration these regional differences.

INTRODUCTION

Worldwide trade by ship has contributed to the establishment of non-indigenous species (NIS) in marine and freshwater ecosystems (e.g., Carlton 1985; Mills et al. 1993; Ruiz et al. 2000). Research on ballast water and sediment has revealed the presence of several types of viable organisms (Bailey et al. 2005; Bailey et al. 2007; Williams et al. 1988) including dinoflagellates and their resting stages (Hallegraeff and Bolch 1992; Hamer et al. 2001; Kelly 1993; Pertola et al. 2006). Non-motile dinoflagellate cysts or hypnozygotes are produced during the sexual reproduction phase and they normally sink to the sea bottom; encystement can be triggered in response to unfavourable environmental conditions (Garcés et al. 2002; Taylor 1987). Similar unfavorable conditions may be present in ballast sediments (e.g. no light, low oxygen and low temperature). Thus, cysts can be introduced in ship tanks during ballast intake, or they may be produced inside these tanks during voyages. Their highly resistant character allows them to survive ship transport, even over several years (Lewis et al. 1999; Rigby and Hallegraeff 1994). Although sediments accumulate at the bottom of ballast tanks, a few studies have shown that a portion of this sediment can be resuspended (Villac et al. 2001) and discharged upon de-ballasting (Weise et al. in prep.). Increasing ship transport provides opportunities for dinoflagellate species, notably non-indigenous and toxic species, to expand their distribution ranges (Carlton 1999; Hallegraeff 1998). This could represent one cause for the increased frequency, intensity and geographic extent of harmful algal blooms often accompanied by toxic poisoning, such as paralytic shellfish poisoning (PSP) (Hallegraeff 1998), which can have a direct impact on human health, fisheries and aquaculture (Hallegraeff and Bolch 1991, 1992; Hallegraeff 2003).

The introduction of non-indigenous dinoflagellate species mediated by ballast sediments has been recorded in Australia (Hallegraeff and Bolch 1992; Hay et al. 1997), Northern Europe (Hamer et al. 2001; Pertola et al. 2006), and the United States (Kelly 1993). In Canada, Harvey et al. (1999) assessed the risk of introduction for non-indigenous dinoflagellate cyst species into the Estuary and the Gulf of St. Lawrence by ballast sediments from eight commercial ships. Among taxa observed in this study, dormant cysts were present in almost 50% of the samples. Fahnenstiel et al. (2009) recorded 35 marine dinoflagellate cyst taxa from ballast sediments of 25 no ballast on board (NOBOB) ships entering the Great Lakes. Recently, Casas-Monroy et al. (2011) also reported the presence of viable non-indigenous species of dinoflagellate cysts in ballast sediments of ships arriving to the East coast of Canada, in particular in coastal ships that do not undertake ballast water exchange (BWE), which thus represents a high risk. Coastal ships with BWE are also a concern because they often contain a large volume of sediments. In the study by Casas-Monroy et al. (2011), transoceanic ships presented the lowest risk of introduction of NIS of dinoflagellates, since most of these ships exchanged their ballast offshore, contrary to coastal ships with BWE, which perform their exchange closer to shore. These differences in ballast water management may favour the potential transfer of dinoflagellate species via ballast sediments and the eventual spread of harmful/toxic species into Canadian aquatic ecosystems.

To reduce potential transfers of non-indigenous species, voluntary (1989) followed by mandatory (1993) BWE regulations were enacted for ships carrying coastal waters entering the United States, especially the Great Lakes systems (US Coast Guard 1993). Under these regulations, ships are required to perform a ballast water exchange in open-ocean or outside of the 200 nautical miles limit from coast (IMO 2004; McCollin et al. 2007; Office of the Auditor General of Canada 2008). The purpose of these exchanges is to replace coastal waters and their organisms with oceanic waters reducing the abundance of coastal species, and hopefully removing the sediments as well (IMO 2004). During normal operations BWE can be highly effective at replacing coastal planktonic organisms (80-95% reduction in concentration), when conducted according to guidelines and regulations (Ruiz and Reid 2007).

Requirements to perform BWE vary among numerous governmental jurisdictions, at the international (International Maritime Organization or Europe), national (Canadian Coast Guard or US Coast Guard), or even state level (states of California, Oregon or

Washington). In Canada, BWE is mandatory for incoming transoceanic ships and for coastal ships not remaining in close proximity to Canadian coastal waters. Coastal ships that do not need to perform BWE include ships such as oil tankers or other ships which operate exclusively between ports in Canada and ports on the East coast of the United States north of Cape Cod (41°54'0" N – 70°17'0" W), or north of Cape Blanco (42°50'15" N - 124°33'50" W) on the West coast (Canada Shipping Act 2001; Simard and Hardy 2004; United States Coast Guard 1993). Mandatory ballast water control and management regulations have been applied in Canada since June 2006 through the Canada Shipping Act (Office of the Auditor General of Canada 2008). These measures aim to prevent or reduce the release of foreign aquatic organisms and they are enforced for all ships arriving in Canadian coastal waters, and particularly those entering the Great Lakes. But despite the implementation of BWE regulations, new potential invaders continue to be reported in Canada's coastal regions and the Great Lakes (Briski et al. 2011; Casas-Monroy et al. 2011; Ricciardi 2006).

Until now, coastal ships traveling short distances have been considered low risk for non-indigenous introductions, with the argument that ports located in the same biogeographic region have similar biological communities (Simkanin et al. 2009). In contrast, some studies have considered coastal traffic as an invasion pathway. Some coastal ships have been shown to transport large numbers of coastal non-indigenous organisms particularly when ports along domestic routes are highly invaded (secondary spread: Lawrence and Cordell 2010). Even if these coastal ships perform BWE, this exchange may be relatively inefficient (Cordell et al. 2009; Lawrence and Cordell 2010; McCollin et al. 2007), because of insufficient exchange, different strategies of BWE or inadequate zones to perform BWE (Simard and Hardy 2004). For example, ships on the West coast are allowed by law to perform BWE close to land, near the line of 50 nautical miles, where the influence of coastal waters may still be present and could contribute to the presence of coastal propagules on board. Furthermore, if BWE promotes the stirring and resuspension of sediments it may influence the abundance and composition of dinoflagellate cysts present in ballast sediments and discharged in water at destination (Villac et al. 2001).

Hence coastal traffic may not always be low risk.

In the present study, we compare the abundance and diversity of dinoflagellate cysts in ballast sediments from ships visiting Canada's East and West coast regions as well as the Great Lakes to assess the similarity in patterns of invasion risk for these regions. We also examine whether BWE has the same level of efficacy among these regions to reduce the introduction of non-indigenous species of dinoflagellates cysts. Comparison among these regions is facilitated by common sampling and cyst identification protocols (Casas-Monroy et al. 2011). For each region, we examined at least 20 ships from each one of the three categories of ships: (1) transoceanic (TOE), (2) coastal with BWE (CE) and (3) coastal without BWE (CNE). This number of ships was sufficiently large to get a good overall estimate of the abundance and diversity of organisms present in ballast sediments across categories and regions. The following hypotheses were examined: 1) transoceanic ships should have higher abundances of nonindigenous dinoflagellate cysts in ballast sediments relative to coastal ships, since coastal ships come from ports located in the same biogeographic region while transoceanic ships carry organisms from far away (Simkanin et al. 2009), 2) ships that undertake BWE (TOE and CE) should have lower abundances of risk organisms than coastal non-exchanged ships (CNE), if BWE helps to reduce the abundance of non-indigenous species (NIS) and/or harmful species (HA) dinoflagellates in tanks (Ruiz and Reid 2007), and 3) the invasion risk for NIS of dinoflagellates is similar across the three regions studied for a given ship category.

METHODS

Sediment ballast sampling and laboratory procedures

Sampling was carried out during three summers (2007 to 2009) as part of Phase I of the Canadian Aquatic Invasive Species Network (=CAISN, http://www.caisn.ca). Samples were collected in at least 20 ships from each of the three ship categories (TOE, CE, CNE) for the two marine regions. For the Great Lakes ships from only two categories were sampled : TOE (13 ships) and CE (5 ships). In this last region, ships with ballast exchange were chosen because they have been less studied, since so far most of the attention has been focused on "no ballast on board" ships, which represent ~90% of ships entering the Great Lakes (Holeck et al. 2004). For the East coast, sampling was done in the ports of Sept-Îles, Port Cartier and Baie-Comeau (Quebec), St. John (New Brunswick) and Canso, Halifax Hantsport and Liverpool (Nova Scotia). For the West coast, ships were sampled only in the port of Vancouver, while in the Great Lakes ships were sampled in Nanticoke, Hamilton (Ontario) and Toledo (OH, United States). A total of 147 ballast sediment samples were collected from general cargos, bulk carriers and oil tankers. Ships were selected on an opportunity basis. Once a ship arrived in port, the sampling team met the captain and asked for the ballast reporting forms (required by Transport Canada for all ships entering Canadian waters), then sampled the water column (data not presented here) and waited, when possible, until a tank was completely empty in order to go into the tank for sediment sampling. In many cases, different ships were sampled for ballast water and ballast sediments, because empty tanks (necessary for sampling ballast sediments) were not always available.

Sediment sampling and sample processing methods were similar to those described in Casas-Monroy et al. (2011) and are described here only briefly. Samples were taken from one tank per ship (mostly wing ballast tanks and forepeak tanks), according to accessibility and the quantities of sediment present in the bottom of the tanks. Sediment samples were collected through direct access to the tanks, after complying with security measures (e.g., prior tank aeration). Approximately 500 g of the bottom sediments were collected from different areas within each tank, placed in a single plastic bag and stored in the dark at 4°C, prior to laboratory analysis. The total quantities of ballast sediments in a tank were estimated by measuring the surface area covered, the average thickness and the percent of coverage.

In the laboratory, each sample was mixed in the bag prior to the analysis and two sub-samples of 5 cm^3 of sediment were taken. The first one was used for sediment dry weight determination (48 hours at 65°C). The second one was for dinoflagellate cysts identification and enumeration. Dinoflagellate cysts were classified as empty cysts (not viable) or full cysts, which were deemed viable, using a criterion of cellular content. As there were too many samples to do germination tests for all cysts, we randomly selected over 800 cysts that were tested for germination, as a verification of their viability. To do this, viable dinoflagellate cysts were isolated, cleaned and cultured in sterilized f/2 medium without silica (Guillard 1975) prepared with filtered seawater from the Sept-Îles region (salinity = 26) and sterilized in an autoclave (see Casas-Monroy et al. 2011). All specimens were mounted in a "micro slide chamber" according to Horiguchi et al. (2000) and observed in a transmitted light microscope (Zeiss Axiovert) at 250X to 1000X magnification. Micrographs of dinoflagellate specimens were taken with a digital camera Nikon CoolPix P5000 mounted fitted with a Nikon MDC CoolPix lens in order to help identification.

Ship information

Ships were classified into the three categories mentioned above depending on the type of voyage and strategy of ballast water exchange. We verified whether the location of ballast exchange was indeed done in coastal or oceanic waters by comparing the location of the exchange with the Exclusive Economic Zone (=EEZ) which defines coastal waters as those within ~370 km or 200 nautical miles (NM) from coastal regions (United Nations 1982), using ArcView version 9.3. This 200 NM limit is also the one imposed by Canadian Law for BWE (see introduction). Since this definition of coastal waters is more political than biological, we also plotted the location of ballast exchange sites with respect to Longhurst's biogeographical provinces or biomes (Longhurst 1998). The age of ballast water was calculated using information from ballast reporting forms. For TOE and CE ships it was calculated as the number of days after ballast water exchange and for CNE ships, after ballast water uptake at the port of origin. Dissolved oxygen concentrations in ballast water were examined as a factor that might influence the dinoflagellate cyst concentrations in ballast sediments. These concentrations were obtained from CAISN colleagues who had examined changes in various groups of organisms during two transoceanic voyages (Klein et al. 2009 ; Seiden et al. 2011).

Diversity and composition of dinoflagellate cysts

The diversity of dinoflagellate cyst species was assessed using the total number of cyst taxa identified in a sample (S). The relative abundance (%) of cyst taxa in a sample was calculated by dividing the number of cysts of a given taxon by the total number of cysts present in the samples. Cysts were separated into four categories: viable and empty cysts for all dinoflagellate species and viable and empty cysts of non-indigenous dinoflagellate species. Viable or full cysts represented cysts that may be able to germinate after discharge, representing a potential risk for Canadian ecosystems. Empty cysts were considered here also as they could have germinated while inside the ballast tanks, increasing the risk associated with ballast water discharge. Taxa were considered non-indigenous in the present study when no previous reports of their occurrence could be found in local floristic data or based on information from well-known taxonomic works such as Taylor and Waters (1988), Bérard-Therriault et al. (1999) and Taylor et al. (2007), as well as consultation with specialists from Fisheries and Oceans Canada (Sylvie Lessard, Maurice-Lamontagne Institute, Mont-Joli, Québec) and the GEOTOP (Anne de Vernal, Université du Québec à Montréal).

Nomenclature of dinoflagellate cysts

The paleontological taxonomy of dinoflagellate cysts used here conforms to that presented in previous publications such as Blanco (1989a, 1989b), Fensome et al. (1993), Rochon et al. (1999), de Vernal et al. (2001), Head et al. (2001, 2005), Mudie and Rochon (2001), Louwye et al. (2004), Head (2007) and Radi et al. (2001, 2007). Some species present in our samples (including those of genera *Brigantedinium* and *Polykrikos*) were identified using microphotography to observe the shape of the archeopyle (Casas-Monroy et al. 2011, and references therein). The taxonomic list of dinoflagellate cysts recovered in this study is presented in Table 4, which also gives the nomenclature for vegetative (thecal) forms.

Statistical analysis

Statistical analyses were used to 1) examine differences for each dependent cyst variable among categories of ships for each region, and 2) examine differences for each dependent cyst variable among BWE strategies for each region. One-way ANOVA was performed using PERMANOVA with PRIMER v.6 (Primer-E Ltd). To analyze differences among categories of ships for each region and differences among regions for each category of ship, a similarity matrix was calculated using Canberra distance, which takes into account double zeros. To analyze differences among regions by each BWE method the similarity matrix was calculated using Euclidean distance, which enables the analysis with factors combined in only one column (ship categories and exchange methods). *P*-values were obtained with 9999 random permutations (Perms) (Anderson 2001). When there were too few possible permutations to obtain a reasonable test, a *P*-value was confirmed by the Monte Carlo (MC) random draws from the asymptotic permutation distribution (Terlizzi et al. 2005). Significant terms within the full models were analysed using appropriate pair-wise comparisons. Data normality was not required with this type of analysis. In addition, independence and homogeneity of dispersions were directly implicit in the permutation procedure (Anderson et al. 2008).

Table 4. Names of the dinoflagellate cysts found in this study (paleontological names) and their corresponding biological affinities (thecate names). In some cases, the same name is used for both nomenclatures.

Cyst species **Paleontological name**	**Dinoflagellate motile cell** **Biological name**
Autotrophic Gymnodiniaceae	
Cochlodinium polykrikoides Cyst (Margalef, 1961)	*Cochlodinium polykrikoides* *
Gymnodinium catenatum Cyst (Graham, 1943)	*Gymnodinium catenatum* *
Gymnodinium nolleri Cyst (Ellegaard & Moestrup, 1998)	*Gymnodinium nolleri*
Gymnodinium impudicum Cyst (Fraga & Bravo, 1995)	*Gymnodinium impudicum* (Fraga *et* Bravo 1995) G. Hansen *et* Moestrup 2000 [Fraga *et al.* (1995), Daugbjerg *et al.* (2000)].
Gonyaulacaceae	
Alexandrium tamarense Cyst (Lebour, 1925) Balech, 1985	*Alexandrium tamarense* *
Bitectatodinium tepikiense (Wilson, 1973)	*Gonyaulax digitalis* (Pouchet, 1883) Kofoid, 1911
Fragilidium mexicanum (Balech, 1988)	
Impagidinium aculeatum (Wall, 1967) Lentin & Williams, 1981	*Gonyaulax* sp. indet.
Impagidinium pallidum (Bujak, 1984)	*Gonyaulax* sp. indet.
Impagidinium paradoxum (Wall, 1967) Stover & Evitt, 1978	*Gonyaulax* sp. indet.
Impagidinium sphaericum (Wall, 1967) Lentin & Williams, 1981	*Gonyaulax* sp. indet.
Lingulodinium machaerophorum (Deflandre & Cookson, 1955) Wall, 1967	*Lingulodinium polyedrum** (Stein, 1883) Dodge, 1989
Nematosphaeropsis labyrinthus (Ostenfeld, 1903) Reid, 1974	*Gonyaulax spinifera* complex
Operculodinium centrocarpum sensu (Wall & Dale, 1966)	*Protoceratium reticulatum** (Claparède & Lachmann, 1859) Bütschli, 1885
Operculodinium janduchenei (Wall & Dale, 1967)	
Operculodinium israelianum (Rossignol, 1962) Wall 1967	? *Protoceratium reticulatum*
Polysphaeridium zoharyi (Rossignol, 1962) Bujak et al., 1980	*Pyrodinium bahamense** Plate, 1906 var. *compressum* (Böhm, 1931)
Spiniferites belerius (Reid, 1974)	*Gonyaulax scrippsae* (Kofoid, 1911)
Spiniferites bentorii (Rossignol, 1964) Wall & Dale, 1970	*Gonyaulax digitalis*
Spiniferites delicatus (Reid, 1974)	*Gonyaulax* sp. indet.
Spiniferites elongatus (Reid, 1974) Ellegaard et al., 2003	*Gonyaulax elongata* (Reid, 1974) Ellegaard et al., 2003 *Gonyaulax spinifera-Spiniferites* group
Spiniferites hyperacanthus (Deflandre & cookson, 1955) Cookson & Eisenack, 1974	*Gonyaulax spinifera* complex
Spiniferites lazus (Reid, 1974)	*Gonyaulax* sp. indet.
Spiniferites membranaceus (Rossignol, 1964) Sarjeant, 1970	*Gonyaulax membranacea* (Rossignol, 1964) Ellegaard et al., 2003
Spiniferites mirabilis (Rossignol, 1967) Sarjeant, 1970	*Gonyaulax spinifera* complex

Spiniferites ramosus (Ehrenberg, 1838) Mantell, 1854	*Gonyaulax scrippsae* *Gonyaulax spinifera* complex
Spiniferites spp.	
Tectatodinium sp. (Wall, 1967)	*Gonyaulax spinifera* complex
Tuberculodinium vancampoae (Rossignol, 1962) Wall, 1967	*Pyrophacus steinii* (Schiller, 1935) Wall & Dale, 1971
Peridiniaceae	
Ensiculifera spp. (Balech, 1967)	Cf. *Pirumella quiltyi*
Pentapharsodinium dalei Cyst (Indelicato & Loeblich, 1986)	*Pentapharsodinium dalei*
Scrippsiella lachrymosa/trifida Cyst	*Scrippsiella lachrymosa/trifida* Sp. indet.
Scrippsiella trochoidea Cyst (Braarud, 1957)	*Scrippsiella trochoidea* *
Heterotrophic	
Diplopsalidaceae	
Diplopsalis orbicularis Cyst Steidinger & Williams, 1970)	*Diplopsalis orbicularis*
Dubridinium caperatum (Reid, 1977)	*Preperidinium meunieri* (Pavillard, 1907) Elbrächter, 1993
Polykrikaceae	
Polykrikos sp. Cyst	
Polykrikos kofoidii Cyst (Chatton, 1914)	*Polykrikos kofoidii*
Polykrikos schwartzii Cyst (Bütschli, 1873)	*Polykrikos schwartzii*
Protoperidiniaceae	
Brigantedinium cariacoense (Wall, 1967)	*Protoperidinium avellana* (Meunier, 1919) Balech, 1974
Brigantedinium simplex (Wall, 1965)	*Protoperidinium conicoides* (Paulsen, 1905) Balech, 1973
Brigantedinium irregulare (Matsuoka, 1987)	*Protoperidinium denticulatum* (Gran & Braarud, 1935) Balech, 1974
Brigantedinium sp.	*Protoperidinium thorianum* (Paulsen, 1905) Balech, 1973
Echinidinium delicatum (Zonneveld, 1997)	*Protoperidinium* sp. indet.
Echinidinium granulatum (Zonneveld, 1997)	*Protoperidinium* sp. indet.
Echinidinium spp. (Zonneveld, 1997)	*Protoperidinium* sp. indet.
Lejeunecysta oliva (Reid, 1977) Turon & Londeix, 1988	*Protoperidinium* sp. indet.
Lejeunecysta sabrina (Reid, 1977) Bujak, 1984	*Protoperidinium leonis*
Protoperidinium americanum Cyst (Gran & Braarud, 1935) Balech, 1974	*Protoperidinium americanum*
Protoperidinium latissimun Cyst (Kofoid) Balech, 1974	*Protoperidinium latissimun*
Protoperidinium minutum Cyst (Kofoid 1907) (Loeblich, 1970)	*Protoperidinium minutum*
Protoperidinium nudum Cyst (Meunier, 1919) Balech, 1974	*Protoperidinium nudum*
Protoperidinium obtusum Cyst (Matsuoka et al., 2004)	*Protoperidinium obtusum*
Protoperidinium stellatum Cyst (Wall in Wall & Dale, 1968) Head, 1996	*Protoperidinium stellatum* (Wall, 1968) Balech, 1994
Islandinium minutum (Harland & Reid in Harland et al., 1981) Head et al., 2001	*Protoperidinium* sp. indet.
Quinquecuspis concreta (Matsuoka, 1985)	*Protoperidinium leonis* (Pavillard, 1916) Balech, 1974
Selenopemphix quanta (Wall & Dale, 1968)	*Protoperidinium conicum* (Gran, 1900) Balech, 1974
Stelladinium reidii (Bradford, 1975)	*Protoperidinium* sp. cf. *P. compressum* (Abé, 1927) Balech, 1974
Trinovantedinium applanatum (Bradford, 1977) Bujak & Davies,	*Protoperidinium pentagonum* (Gran,

1983	1902) Balech, 1974
Votadinium calvum (Wall & Dale, 1968)	*Protoperidinium oblongum* (Aurivillius, 1898) Parke & Dodge, 1976
Votadinium spinosum (Reid, 1977)	*Protoperidinium claudicans* (Paulsen, 1907) Balech, 1974

* Harmful or toxic species

RESULTS

Ship information

A summary of pertinent information from the 147 ships sampled, including ballast water age, dominant ballast source and exchange method is presented in Table 5. For the East coast of Canada, sediment samples were collected from 65 foreign ships: 23 TOE, 22 CE and 20 CNE visiting the 8 ports included in this study. Ballast sediments were collected mostly from bulk carriers (38 ships out of 65) and oil tankers (20 out of 65). Sediment samples were collected mostly from wing tanks or topside tanks (in 42 ships out of 65) and forepeak tanks (in 18 ships). For East coast TOE ships, the source of ballast water was mostly from European ports (20 ships out of 24). Most of these TOE ships performed ballast water exchange using a flow-through method (12 ships out of 24), while 9 ships used empty-refill. About 78% of TOE ships exchanged their ballast water in oceanic waters (at a distance between 417 and 998 km from any coast) and 13% of these ships did the exchange inside the EEZ (9% were undetermined). Based on Longhurst's biogeographic provinces, most of the TOE ships exchanged ballast water in the North Atlantic Drift province (NADR) (see Casas-Monroy et al. 2011 for a map showing BWE sites for the East coast). The source of ballast water for CE ships was predominantly from eastern coastal ports of the United States (18 ships) and 3 ships filled up their ballast in the Gulf of Mexico. The method most commonly used to exchange ballast water in CE ships was empty-refill (11 ships) followed by flow-through (9) (Table 5). Most CE ships (95%) did the exchange in coastal waters according to EEZ limits. Fourteen of 21 CE ships exchanged ballast in Longhurst's Northwest Atlantic Shelves province (NWCS). The minimum and maximum distances from the coast where CE ships undertook ballast exchange were 30 and 378 km respectively, while the average distance was 203 km. Finally, for all CNE ships, samples were collected mostly from wing ballast tanks and the dominant ballast water source was also from the eastern coastal ports of the United States.

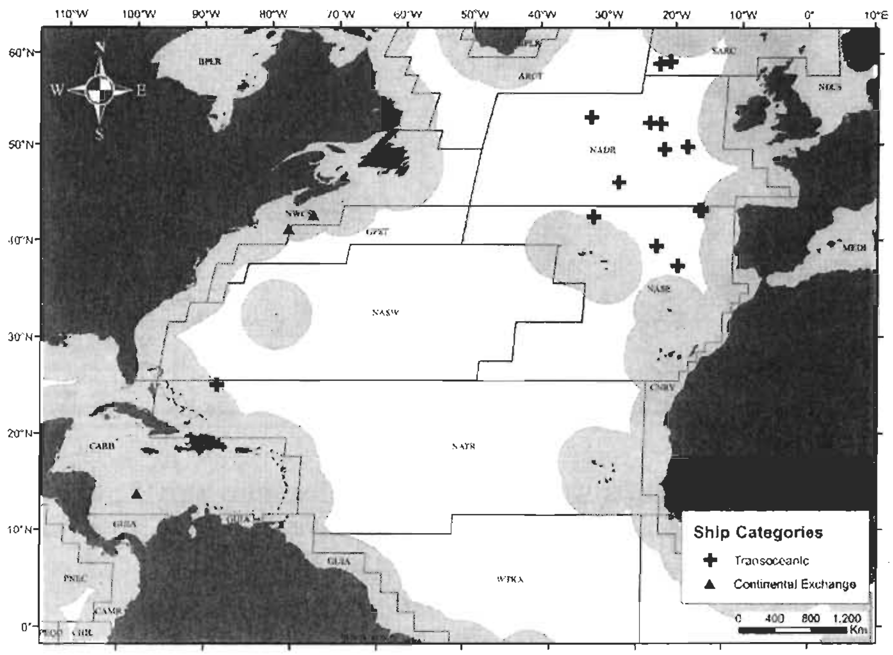

Figure 12. Location of ballast water exchange sites for ships visiting the Great Lakes of Canada. The light grey zone indicates the exclusive economic zone EEZ (370 km or 200 n.m.). The map was made using the World Robinson projection. Partitions correspond to the biogeographic provinces defined by Longhurst (1998): ARCT, Atlantic Arctic Province; SARC, Atlantic Subarctic Province (Atlantic Polar Biome); NADR, North Atlantic Drift Province; GFST, Gulf Stream Province; NASW, NASE North Atlantic Subtropical Gyral Province West and East (Atlantic Westerly Winds Biome); WTRA, Western Tropical Atlantic Province (Atlantic Trade Wind Biome); NECS, Northeast Atlantic Shelves Province; NWCS, Northwest Atlantic Shelves Province; CNRY, Eastern Canary Coastal Province; GUIA, Guinea Coastal Province (Atlantic Costal Biome) and CARB, Caribbean Province (Atlantic Trade Wind Biome).

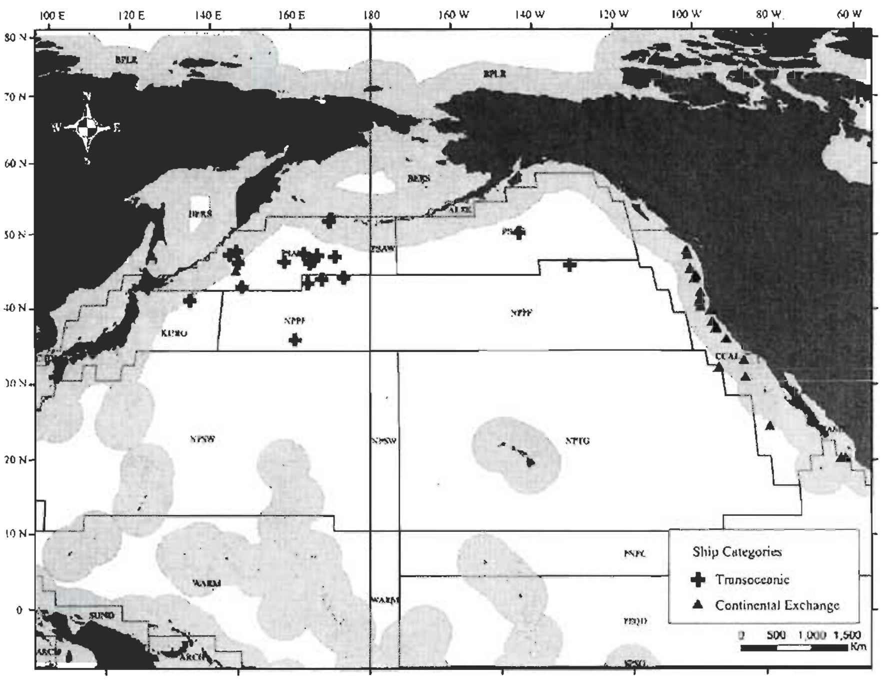

Figure 13. Location of ballast water exchange sites for ships arriving to the West coast of Canada. The light grey zone indicates the exclusive economic zone EEZ (370 km or 200 n.m. from coasts). The map was made using the World Robinson projection. Partitions correspond to the biogeographic provinces defined by Longhurst (1998): BERS, North Pacific Epicontinental Sea Province (Pacific Polar Biome); ALSK, Alaska Downwelling Coastal Province; CCAL, California Current Province; CAMR, Central American Coastal Province; CHIN, China Sea Coastal Province; SUND, Sunda-Arafura Shelves Province (Pacific Coastal Biome); ARCH, Archipelagic Deep Basins Province; NPTG, North Pacific Tropical Gyre Province; PNEC, North Pacific Equatorial Countercurrent Province; SPSG, South Pacific Subtropical Gyre Province (Pacific Trade Winds Biome); KURO, Kuroshio Current Province; NPPE, North Pacific Transition Zone Province and PSAG, Pacific Subarctic Gyres (East and West) Province (Pacific Westerly Winds Biome).

In the Great Lakes, ballast sediment samples were collected from 18 ships: 13 TOE and 5 CE, that had undertaken BWE, mostly bulk carriers (78%), oil tankers (11%) and general cargo (11%). Sediment samples were collected mostly from wing tanks (83%). For TOE ships ballast water originated mostly from Europe (Belgium, Netherlands, Italy, Germany and United Kingdom). Most of these ships used the empty-refill method (9 ships). About 92% of the TOE ships exchanged their ballast waters in oceanic waters (between 377 and 1122 km away from any coast). Based on Longhurst's biogeographic provinces, 6 TOE ships performed BWE in the NADR province while 4 ships used the North Atlantic Subtropical Gyral province (NASE). For CE ships, ballast water originated from coastal ports along the eastern coast of the United States. All these ships conducted BWE, one of them used the empty-refill method, two others used an alternative method (combination of empty refill and flow-through methods in the same voyage) and for the other two ships information was not available. Two of these CE ships performed BWE inside the EEZ, in Longhurst's NWCS province. The minimum and maximum distance from the coast where CE ships undertook ballast exchange was 172 and 291 km respectively (Figure 12).

For the West coast, ballast sediment samples were collected from 64 ships: 22 TOE, 22 CE and 20 CNE. Most of the ships sampled were bulk carriers (91%). Tanks used to collect sediment samples were mostly wing tanks or topside tanks (91%). For TOE ships arriving to Canada's West coast the largest source of ballast water was Japan (7 ships out of 22), followed by China (6 ships out of 22) and Taiwan (3 out of 22). Most of these TOE ships used the flow-through method (16 ships out of 22), while 5 ships used the empty-refill method for ballast water exchange. About 73% of the TOE ships exchanged their ballast in oceanic waters (at a distance between 554 and 2488 km away from any coast). Twelve TOE ships used Longhurst's Pacific Subarctic Gyre West (PSAG) province to perform BWE and at least 4 ships did their BWE in the North Pacific Transition Zone province (NPPE, Figure 13). For CE ships, the largest ballast source was somewhere in the North Pacific Ocean (14 ships out of 22) and from the coastal ports of western United States (5 ships). For ballast exchange, CE ships used mostly the empty-refill method (14 ships out of 22) while the 8 remaining ships performed their exchange using the flow-through method. All CE ships exchanged their ballast water inside the EEZ. Most of these ships (20 ships out of 22) performed BWE in Longhurst's California Current province (CCAL). For CNE ships ballast tanks sampled were mostly wing tanks (18 ships out of 20). Most of these CNE ships came to Canada with ballast water from ports of the United States

West coast (17 ships out of 20), and only 3 ships indicated that their ballast source was the North Pacific Ocean.

Table 5. Ship categories and types, length of voyage, dominant ballast source, tank sampled and exchange method used for ships arriving in each Canadian region. Numbers in parentheses correspond to number of ships for each variable. E-R= empty-refill; F-T= flow-through; ALT= alternative; NIL= without exchange; CBT= cargo ballast tank; DBT= double bottom tank; FPT= forepeak tank; WBT= wing ballast tank. * : represents ships for which information about ballast water exchange was not available. Table should be read by columns.

Region	Ship category	Ship type	Ballast tank sampled	Mean length of voyage (days)	Dominant ballast source	Exchange method used			
						E-R	F-T	ALT	NIL
East coast	TOE (23)	Bulk carrier (19)	DBT (2)	6	Europe (20)	7	10		3
		General cargo (3)	FPT (13)		South America (2)	1	2		
		Ro/Ro (1)	WBT (8)		Undetermined (1)	1			
	CE (22)	Bulk carrier (16)	CBT (1)	3	USA (18)	10	5	1	
		Oil tanker (3)	DBT (2)		Gulf of Mexico (2)		3		
		General Cargo (1)	FPT (4)		Caribbean (2)		1		
		Ro-Ro (1)	WBT (15)			1			
	CNE (20)	Bulk carrier (3)	FTP (1)	4	USA (20)				3
		Oil tanker (17)	WBT (19)						17
Great Lakes	TOE (13)	Bulk carrier (11)	CBT (1)	14	Europe (12)	8		3	
		General cargo (2)	FPT (2)		Undetermined (1)	1		1	
			WBT (10)						
	CE (5)	Bulk carrier (3)	WBT (5)	15	USA (4)	1			2*
		Oil tanker (2)			Caribbean 1			2	
West coast	TOE (22)	Bulk carrier (22)	WBT (18)	11	Asia (16)	5	16		1
	CE (22)	Bulk carrier (19)	WBT (21)	6	Pacific Ocean (14)	11	8		
		General cargo (2)			Central America (2)	2			
		Oil tanker (1)			USA (6)	1			
	CNE (20)	Bulk carrier (17)	WBT (18)	6	USA (17)				17
		Container ship (1)							1
		General cargo (2)							2

Relative abundance, diversity and composition of dinoflagellate cysts in ballast sediments

Well-preserved organic-walled dinoflagellate cysts were recorded from ballast sediment samples with 60 taxa identified including 33 autotrophic, 27 heterotrophic species and 9 unidentified taxa. Dinoflagellate cyst taxa in an individual ship sample varied from undetectable to 25, with an average of 10 taxa.

For all cysts of dinoflagellates, the highest concentrations (g^{-1} dry sediment) were recorded in CNE ships, followed by TOE ships arriving to the East coast (respectively 1779 and 887 cysts g^{-1} dry sediment, Table 6), and the lowest cyst concentrations for all dinoflagellates were found in CE ships from the Great Lakes region. Viable non-indigenous dinoflagellate cysts were present in concentrations up to 194, 122 and 32 cysts g^{-1} dry sediment in samples collected in ships from the East coast, West coast and the Great Lakes, respectively (data not shown). These results are in the range of previous studies in other regions of the world (Hallegraeff and Bolch 1992; Macdonald 1995). Figure 14 shows the relative abundances of the different species of dinoflagellate cysts present in the ballast sediment samples for species with more than 5% of relative abundance. The genus *Brigantedinium* was the most abundant in all categories of ships, including viable and empty cysts. *Alexandrium tamarense* was a potentially toxic native species that recorded high relative abundances of viable cysts, mostly in coastal ships (CE and CNE) from the United States.

Information on species richness and cyst concentrations are summarized in Table 6. The mean number of taxa was slightly greater in CE ships visiting the West coast (17), followed by CE and TOE ships visiting the East coast (16 and 15, respectively). TOE ships visiting the Great Lakes showed a mean number of taxa slightly greater (13) than CE ships (11). When comparing ballast water exchange strategies, ships performing an empty-refill exchange showed a greater mean number of taxa on the East coast, while the mean number of taxa in flow-through ships and empty-refill ships was the same on the West coast (Table 6).

Table 6. Total number of species identified in a ship sample (S), mean, minimum and maximum number of taxa and concentration of cysts (g^{-1} dry sediment) for all dinoflagellates recorded in the three regions for each category of ships and ballast water exchange strategy. * : represents ships for which infromation about ballast water exchange was not available.

Region	Category	S	Mean	Min	Max	Concentration
East coast	TOE	51	15	3	24	887
	CE	52	16	3	18	535
	CNE	47	10	8	20	1779
Great Lakes	TOE	31	13	3	12	136
	CE	28	11	0	16	67
West coast	TOE	42	13	0	17	586
	CE	51	17	4	24	480
	CNE	42	13	3	20	654

Region	Strategy	S	Mean	Min	Max	Concentration
East coast	FT	44	12	3	20	756
	ER	54	16	3	25	666
	NE	47	10	8	20	1779
Great Lakes	ER	33	13	3	10	122
	ALT	24	9	3	6	69
	NE*	9	5	0	2	12
West coast	FT	47	15	0	25	583
	ER	47	15	5	18	483
	NE	42	13	3	20	654

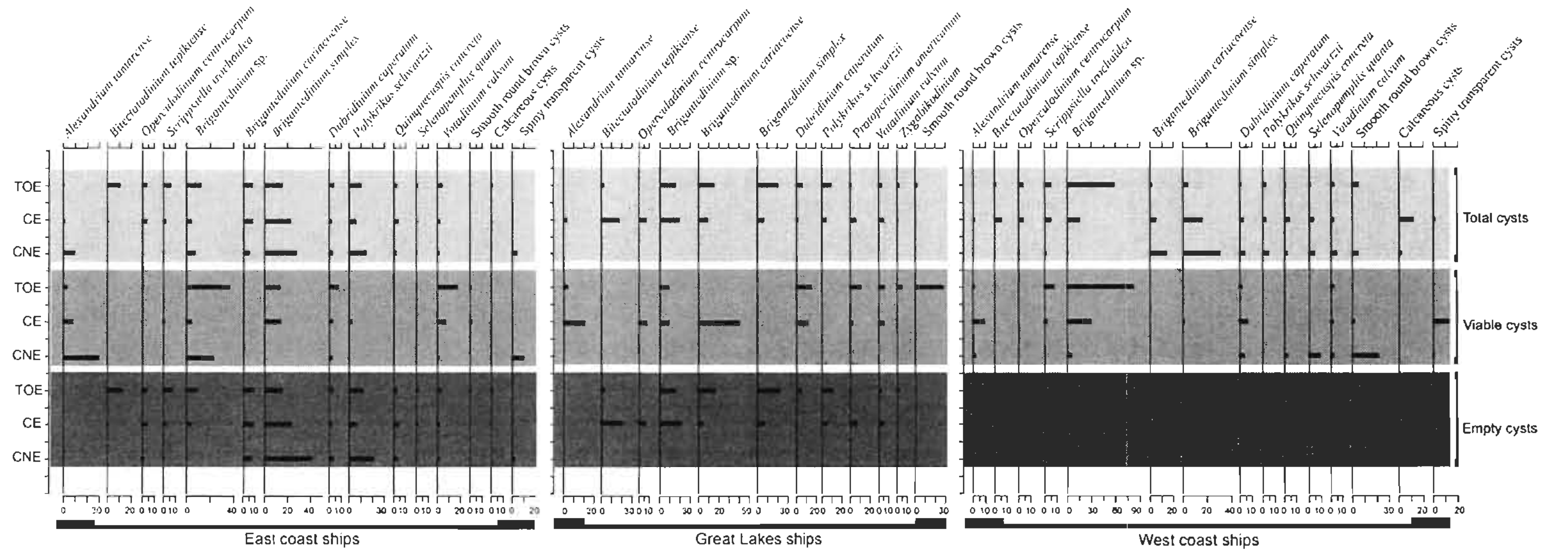

Figure 14. Relative abundance of cysts found in ballast sediments from ships arriving to the West coast, the Great Lakes and East coast of Canada. Taxa shown have more than 5% of relative abundance. Relative values were calculated independently for Total (viable + empty cysts), Viable and Empty cysts (hence the total abundances for each one of these categories of cysts was set at 100% for each region).

For the East coast region, a total of 51 taxa (S in Table 6) pertaining to 40 genera were identified throughout all ballast sediment samples, among which 9 could not be identified to the species level. Eighteen different heterotrophic taxa, including cysts of Peridiniaceae such as *Brigantedinium simplex,* round brown protoperidinioid cysts, as well as Gymnodiniaceae cysts such as *Polykrikos schwartzii*, were common to all categories of ships. All these cysts included viable and empty cysts (Figure 14). Micrographs of several of these species can be found in Casas-Monroy et al. (2011). Autotrophic taxa of Gonyaulacaceae cysts were dominated by *Operculodinium centrocarpum* and *Scrippsiella trochoidea*, each representing less than 10% of all taxa. In addition, the round brown protoperidiniaceae cysts, *Alexandrium tamarense* and *Votadinium calvum* were the most abundant species with viable cysts, followed by spiny transparent cysts. Finally, out of the 51 taxa, 14 were considered non-indigenous for this region of Canada and seven were considered potentially harmful taxa or bloom forming (indicated in the rest of the text with an asterisk following the name): *Alexandrium tamarense**, *Cochlodinium polykrikoides**, *Gymnodinium catenatum**, *Lingulodinium machaerophorum**, *Operculodinium centrocarpum**, *Polysphaeridium zoharyi** and *Scrippsiella trochoidea**.

All taxa recorded in ballast sediment samples from the ships arriving to the Great Lakes were considered non-indigenous species, including harmful species, as all of them are marine species not present in freshwater environments. A total of 37 taxa were identified, representing 24 genera. One taxon could not be identified to the species level. The heterotrophic Peridiniaceae cysts were dominated by *Brigantedinium* sp., followed by *B. simplex* and *B. cariacoense*. Autotrophic Gonyaulacaceae cysts were dominated by *Operculodinium centrocarpum*, *Bitectatodinium tepikiense* and *Alexandrium tamarense*, this last species being present as viable cysts, especially in CE ships (Figure 14). A total of six dinoflagellate species were considered as potentially harmful/toxic or bloom forming: *Alexandrium tamarense**, *Cochlodinium polykrikoides**, *Lingulodinium machaerophorum**, *Operculodinium centrocarpum**, *Polysphaeridinium zoharyi** and *Scrippsiella trochoidea**.

A total of 60 cyst taxa belonging to 31 genera were identified from ballast sediment samples of ships visiting the West coast. Five taxa could not be identified to the species level. In general, heterotrophic-related taxa were dominant in all categories of ships (more than 50% of the total number of taxa). They included cysts of Peridiniaceae such as *Brigantedinium* sp., *B. simplex* and *B. cariacoense* as well as cysts of Gymnodiniaceae such as *Polykrikos schwartzii*, especially for empty cysts (Figure 14). Autotrophic taxa included cysts of Gonyaulacaceae such as *Scrippsiella trochoidea** and *Operculodinium centrocarpum**, each representing less than 15% of all taxa. In addition, most of the unidentified taxa were composed of smooth round brown and calcareous and spiny transparent cysts, present mostly in CE and CNE ships. These cysts are very difficult to identify hence they are generally described by their appearance in palaeoceanographic studies. Sediment samples from the West coast also revealed the presence of 14 non-indigenous species dominated by species such as *Lingulodinium machaerophorum**, *Polysphaeridinium zoharyi**, *Gymnodinium catenatum** and *Cochlodinium polykrikoides**. All of these species are considered potentially harmful/toxic. They were accompanied by native harmful/toxic or bloom forming species such as *Alexandrium tamarense**, *Operculodinium centrocarpum**, and *Scrippsiella trochoidea**. This last species was observed mostly as viable cysts, and showed greatest abundance in coastal ships (CNE and CE).

When considering the major groups of dinoflagellates, we observe that the Peridiniaceae were dominant in all regions and categories of ships, with maximum values in the Great Lakes (79% in TOE ships) and on the West coast (78% for TOE and CNE ships; Table 7). The Gonyaulacaceae were dominant in CE ships arriving to the Great Lakes (43%) and were generally greater in ships from the East coast compared to the West coast (Table 7).

Table 7. Dominance of major groups of dinoflagellates identified in the ballast sediment samples from ships in the three regions examined in this study. Dominance was calculated as the sum of relative abundance for each species in a given group.

	East coast			Great Lakes		West coast		
	TOE	CE	CNE	TOE	CE	TOE	CE	CNE
Gonyaulacaceae	24	36	19	7	43	12	15	7
Gymnodiniaceae	15	8	16	12	4	5	3	8
Peridiniaceae	60	55	60	79	53	78	68	78
Unidentified Taxa	1	1	5	2	0	5	14	7

Differences among ship categories and types of dinoflagellate cysts

The only significant difference among ship categories was found for the East coast, where viable and empty cysts abundances were significantly greater in CNE ships (respectively, 28 viable cysts g^{-1} dry sediment; PERMANOVA, $p < 0.05$ and 46 empty cysts g^{-1} dry sediment; PERMANOVA, $p = 0.0001$) than in TOE and CE ships (Figure 15 a ; Table 8 a). Our results also revealed significant regional differences in concentrations of cysts within a particular category of ships. For example, pair-wise comparison tests indicate that TOE ships from the East coast contained significantly higher concentrations of empty cysts (30 ± 6 cysts g^{-1} dry sediment) than TOE ships from the West coast (10 ± 2 cysts g^{-1} dry sediment) (PERMANOVA, $p < 0.05$) and the Great Lakes (10 ± 3 cysts g^{-1} dry sediment) (PERMANOVA, $p < 0.05$) (Figure 15 b). This was accompanied by lower concentrations of viable cysts in TOE ships from the East (4 ± 1 cysts g^{-1} dry sediment) compared to the West coast (12 ± 5 cysts g^{-1} dry sediment) (PERMANOVA, $p < 0.05$). When considering NIS species of dinoflagellates specifically, a pair-wise test indicate that TOE ships from the Great Lakes region had higher concentrations of empty cysts of NIS (10 ± 2 cysts g^{-1} dry sediment) compared to the East coast (3 ± 1 cysts g^{-1} dry sediment) (PERMANOVA, $p = 0.00014$) (Figure 15 c). No statistical differences were found for viable cysts of NIS between East and West coasts for TOE ships. For CE ships, significantly lower concentrations of empty cysts (18 ± 6 cysts g^{-1} dry sediment) were found on the West coast compared to the East coast (22 ± 4 cysts g^{-1}

$^{-1}$ dry sediment) (PERMANOVA, $p < 0.05$), when pair-wise tests were performed. No statistical differences were found for other comparisons among regions for empty cysts. For NIS of dinoflagellates, pair-wise tests indicated that CE ships from the Great Lakes showed significantly greater concentrations of empty cysts of NIS (12 ± 3 cysts g^{-1} dry sediment) than in the West coast (2 ± 1 cysts g^{-1} dry sediment) (PERMANOVA, $p < 0.05$). No statistical differences were found for viable cysts and viable cysts of NIS among regions for CE ships. No statistical differences were found for viable cysts of NIS and empty cysts of NIS between the East and West coasts for CNE ships.

Influence of the ballast water exchange methods

There were no significant differences between the flow-through and the empty-refill mode of ballast exchange in all three regions considered in this study (PERMANOVA, $p > 0.05$). There were also no significant differences between ships without BWE (CNE) compared with exchanged ships (TOE or CE) in two of the three regions (Great Lakes and West coast) (PERMANOVA, $p > 0.05$). However for the East coast, CNE ships had significantly greater concentrations of viable and empty cysts g^{-1} of dry sediment than TOE ships, and these ships also had significantly greater concentrations of empty cysts than CE ships (Table 8 a). Hence, ships having performed ballast exchange showed lower cyst concentrations only in one of the three regions examined.

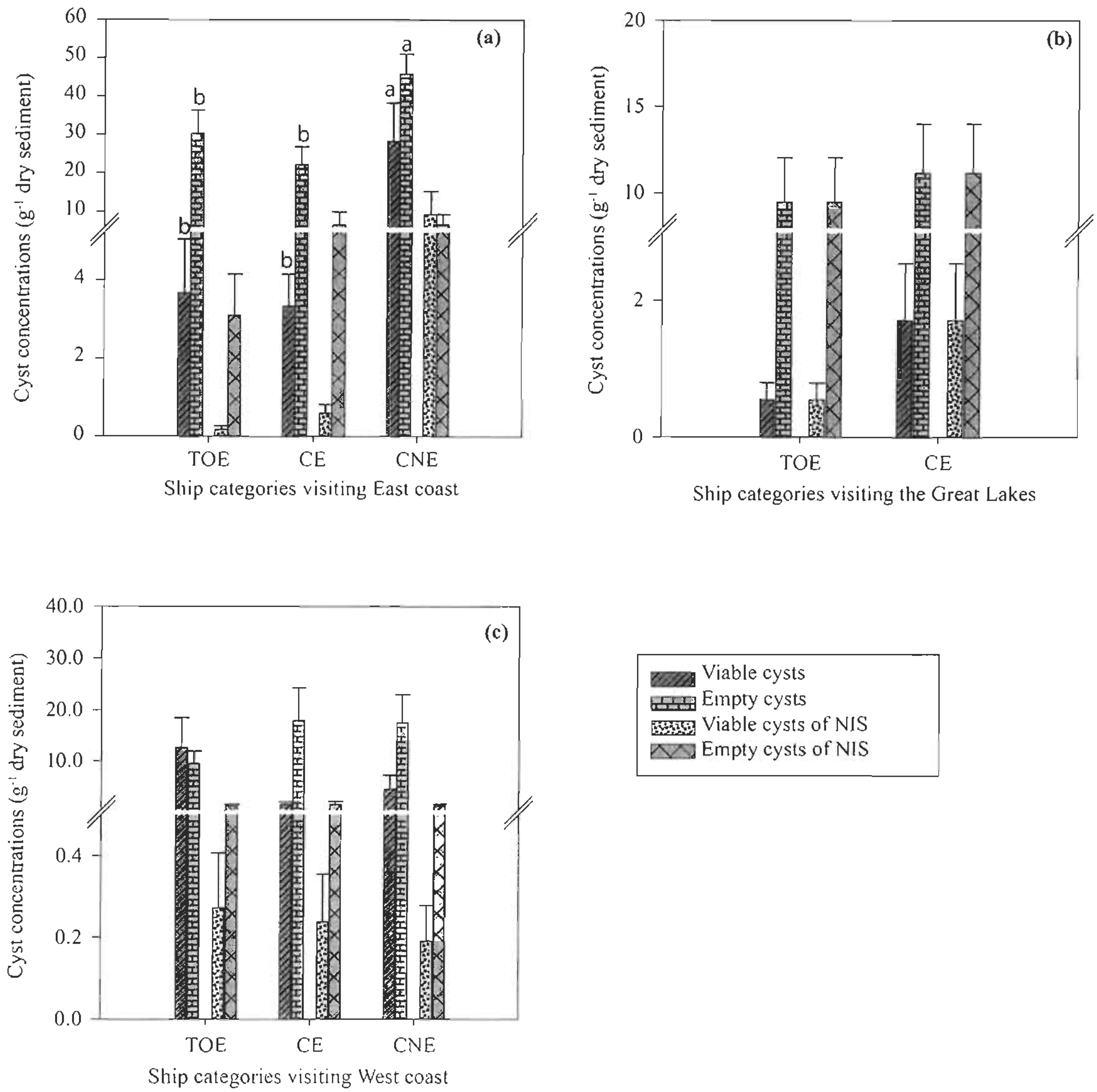

Figure 15. Mean concentrations (± standard error) of viable and empty cysts for all dinoflagellate cyst species and for viable and empty cysts of non-indigenous species for each category of ships arriving to a) the East coast, b) the Great Lakes and c) the West coast. Letters above columns indicate the results of pair-wise tests that showed significant differences between continental ships without ballast water exchange (CNE) and both transoceanic (TOE) (PERMANOVA test; $p < 0.05$) and continental ships with exchange (CE) (PERMANOVA test; $p < 0.05$) in terms of viable and empty cysts. Categories of ships that differ significantly are indicated with different letters above data bars. See statistic tests Table 8.

Table 8. a) Results of PERMANOVA tests (only cases with significant differences are shown). Values in bold indicate significant differences ($p \leq 0.05$) between groups of main factors for each dependent variable (viable cysts, empty cysts, viable cysts of NIS and empty cysts of NIS per g. dry sed.) for each region and among regions for each category of ships. b) Results of PERMANOVA tests for variables other than cysts. DF = degrees of freedom ; SS = sum of squares ; P(Perm) = permutation P-value; P(MC) = Monte Carlo asymptotic P-value (see Anderson et al. 2001; Anderson et al.2008).

a) Ship category comparisons							
East coast							
Viable cysts	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Category	2	1.3448	2.457	0.0304	9938	**0.0347**
	Residual	62	16.9670				
	Total	64	18.312				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	TOE, CNE	1.9155	0.0144	**0.0161**			
	TOE, CE	1.0521	0.3013	0.3282			
	CNE, CE	1.6188	0.0527	0.0553			
Empty cysts	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Category	2	1.6875	6.698	0.0003	9948	**0.0001**
	Residual	62	7.8101				
	Total	64	9.4976				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	TOE, CNE	2.6721	0.0006	**0.0009**			
	CE, TOE	1.4652	0.1159	0.1087			
	CNE, CE	3.9897	0.0001	**0.0001**			
Comparisons among regions							
TOE ships							
Viable cysts	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Regions	2	1.48	2.5275	0.049	9952	**0.0438**
	Residual	55	16.103				
	Total	57	17.583				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	EC, GL	1.5831	0.0975	0.0919			
	EC, WC	2.0223	0.0192	**0.0175**			
	GL, WC	0.79731	0.5381	0.5334			
Empty cysts	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Regions	2	1.2132	3.1106	0.007	9939	**0.008**
	Residual	55	10.726				
	Total	57	11.939				

Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	EC, GL	2.0436	0.0173	**0.0173**			
	EC, WC	1.9886	0.0088	**0.0101**			
	GL, WC	0.8961	0.5101	0.4836			
Empty cysts of NIS	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Regions	2	2.5409	5.0413	0.0014	9950	**0.0022**
	Residual	55	13.86				
	Total	57	16.401				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	EC, GL	2.6641	0.0025	**0.0014**			
	EC, WC	1.0614	0.3071	0.2986			
	GL, WC	3.0639	0.0001	**0.0004**			
CE ships							
Empty cysts							
	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Coast	2	0.7552	2.586	0.0026	9946	**0.0259**
	Residual	45	6.5706				
	Total	47	7.3258				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	EC, GL	0.9917	0.4015	0.399			
	EC, WC	2.0625	0.0141	**0.0141**			
	GL, WC	1.2907	0.1549	0.1727			
Empty cysts of NIS	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Regions	2	1.3119	2.3653	0.056	9930	**0.050**
	Residual	45	12.479				
	Total	47	13.791				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	EC, GL	1.5655	0.0781	0.0846			
	EC, WC	1.1296	0.2583	0.2646			
	GL, WC	2.0361	0.0169	**0.0242**			
CNE ships							
Viable cysts	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Regions	1	1.0225	3.4929	0.0227	9936	**0.0219**
	Residual	39	11.4170				
	Total	40	12.440				
Empty cysts	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Regions	1	2.7750	29.4760	0.0001	9953	**0.0001**
	Residual	39	3.6716				
	Total	40	6.4466				

b) Ship category comparisons							
East coast							
Length of voyage	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Category	2	1.9005	10.604	0.0001	9945	**0.0001**
	Residual	61	5.48				
	Total	63	7.3853				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	TOE, CNE	3.4247	0.0008	**0.0003**			

	TOE, CE	4.8048	0.0001	**0.0001**			
	CNE, CE	1.0109	0.3334	0.3319			
Sediment volume	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Category	2	2.4490	4.8237	0.0002	9938	**0.0003**
	Residual	63	15.9930				
	Total	65	18.4420				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	TOE, CNE	3.1516	0.0001	**0.0001**			
	TOE, CE	0.76799	0.6475	0.6369			
	CNE, CE	2.3675	0.0008	**0.0014**			
West coast							
Length of voyage	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Category	2	0.9570	4.6567	0.0017	9945	**0.0022**
	Residual	66	6.782				
	Total	68	7.739				
Pair wise tests							
	Groups	t	P(perm)	P(MC)			
	TOE, CNE	2.3996	0.0043	**0.0051**			
	TOE, CE	2.7422	0.0021	**0.0021**			
	CNE, CE	1.1835	0.2306	0.24			

Percentage of occurrence of non-indigenous cysts

Non-indigenous species were present in up to 32% of all ships from the three categories (Table 9). For the East coast, the most common non-indigenous species were *Polysphaeridium zoharyi** (32%), *Lingulodinium machaerophorum** (28%) and *Scrippsiella lachrymosa/trifida* (26%). *Polysphaeridium zoharyi**, *Lingulodinium machaerophorum** and *Cochlodinium polykrikoides** (17%) were the most common harmful/toxic species in both coastal ship categories (with and without ballast water exchange), accompanied by the indigenous species *Alexandrium tamarense** (32%). In addition, *Gymnodinium catenatum** (17%) was more common in ship categories doing ballast water exchange (TOE and CE).

For the Great Lakes, all of the species recorded were marine or estuarine and can thus be considered non-indigenous. Table 9 shows only species common to the other regions. The most frequently encountered potentially harmful or non-indigenous species found in this region were *Lingulodinium machaerophorum** (28%), *Alexandrium tamarense** (17%) and *Scrippsiella lachrymosa/trifida* (17%).

For the West coast, the percent occurrence of non-indigenous species in ballast sediment ranged between 2 to 27% of the ships sampled. Non-indigenous species such as *Brigantedinium irregulare* and *Trinovantedinium applanatum* were present in 13% and 20% of ships, respectively. The most commonly observed potentially harmful species were *Alexandrium tamarense**, *Cochlodinium polykrikoides** and *Lingulodinium machaerophorum** with a percent occurrence up to 27%.

Table 9. Percentages of occurrence (% Occ.) of the most frequently observed non-indigenous species found in the three regions. The first 3 columns for each region are the number of samples containing the indicated species. The last column for each region presents values expressed as percentage of the total ships (% Occ.). The % Occ. for the indigenous *A. tamarense** is also added on the last line, for comparison purposes.

	East coast				Great Lakes			West coast			
Species	TOE	CE	CNE	% Occ.	TOE	CE	% Occ.	TOE	CE	CNE	% Occ.
Brigantedinium irregulare	4	2	6	19	0	0	0	2	2	4	13
*Cochlodinium polykrikoides**	1	5	5	17	2	0	11	5	2	2	14
Fragilidinium mexicanum	5	2	1	12	0	1	6	0	0	0	0
*Gymnodinium catenatum***	4	5	2	17	0	0	0	2	3	1	10
Gymnodinium nolleri	1	1	0	3	1	0	6	0	0	0	0
Gyrodinium impudicum	0	0	0	0	0	0	0	1	0	0	2
*Lingulodinium machaerophorum**	8	7	3	28	3	2	28	6	8	3	27
Polykrikos kofoidii	7	3	3	20	2	0	11	2	2	4	13
*Polysphaeridium zoharyi***	2	13	6	32	1	0	6	0	1	4	8
Cyst of *Protoperidinium latissimum*	0	1	2	5	0	0	0	3	1	0	6
Cyst of *Scrippsiella lachrymosa/trifida*	1	8	8	26	3	0	17	0	4	3	11
Spiniferites bentorii	0	1	1	3	0	0	0	1	1	0	3
Trinovantedinium applanatum	5	4	1	15	0	1	11	3	7	3	20
Tuberculodinium vancampoae	0	1	0	2	0	0	0	0	1	0	2
Votadinium spinosum	4	2	0	9	0	1	6	0	0	0	0
*Alexandrium tamarense***	5	6	10	32	1	2	17	5	5	2	19

* Harmful species. *L. machaerophorum* is mostly associated with yessotoxin production

** Saxitoxin producers. *Alexandrium tamarense* is not considered as a non-indigenous species, however it is a PSP producer and has been recorded in 25% of the samples, on average.

Potential viability of dinoflagellate cysts

Potential viability of cysts, especially those of non-indigenous species was tested randomly by examining if they could germinate. After the transfer into multi-well plates, successful cyst germination was often observed after 1 or 2 days. Motile cells of dinoflagellates were sometimes observed during the microscopic counts. In some cases, the motile cells died one or two weeks after the transfer to the culture media. For autotrophic dinoflagellates, motile cells lived up to 3 or 4 weeks, being able to undergo cell division. Several heterotrophic species also showed successful germination. However, these species require preys (especially diatoms and ciliates), which were not added to the media, hence they died a few days after excystment.

The percentage of germination of the 480 isolated full cysts from East coast samples was close to 38%, indicating that a significant proportion of all full cysts could germinate. Higher percentages of germination were observed for potentially harmful native species such as *Operculodium centrocarpum** and *Scrippsiella trochoidea** (up to 97 and 45%, respectively). Potentially toxic native species such as *Alexandrium tamarense** also showed high percentages of germination (close to 45%). Additionally, non-indigenous species such as *Votadinium calvum* showed a percentage of germination of 90% and the non-indigenous toxic species *Polysphaeridium zoharyi** also showed a high germination rate (62%).

The potential viability of cysts from the West coast samples was also tested randomly for various species present in sediment samples. A smaller fraction of these cysts germinated successfully (15% of the 122 cysts isolated) compared with the East coast. Heterotrophic native species such as *Votadinium spinosum* recorded a percentage of germination close to 90%, followed by *Protoperidinium nudum* (67%), *Brigantedinium simplex* and *B. cariacoense* (40% each). Among the potentially harmful native species, 25% of the cysts tested germinated for *Scrippsiella trochoidea**. In contrast, non-indigenous potentially toxic species such as *Polysphaeridium zoharyi** presented the lowest percentage of germination (less than 5%) for this region. Cysts (~200) from ships sampled in the Great Lakes showed no excystment when the sediment slurry was incubated in freshwater.

Ballast conditions

Ballast tank conditions such as the age of ballast water and the volume of sediments contained inside the tanks differed among groups of ships, and varied with the region where they were sampled. Associated with the greater duration of transoceanic travel compared to coastal shipping, pair-wise tests indicated that the ballast water age was significantly greater in TOE ships than for the two other categories of ships, in the marine regions (PERMANOVA, $p = 0.0022$; $p = 0.0001$, respectively) (Figure 16; Table 8). TOE ships from the East coast had the youngest average water age (3 to 6 days) compared with the other regions. In contrast, TOE and CE ships from the Great Lakes showed the oldest average water age (14 ± 2 days and 15 ± 3 days, respectively). For the West coast, ballast water age was greatest for TOE ships (11 ± 2 days) and CNE ships (11 ± 5 days) compared to CE (6 ±1 days). Although the age of ballast water had no statistically significant effect on dinoflagellate cyst abundances, we observed that ships with ballast water less than ten days old showed more viable dinoflagellate cysts of non-indigenous species.

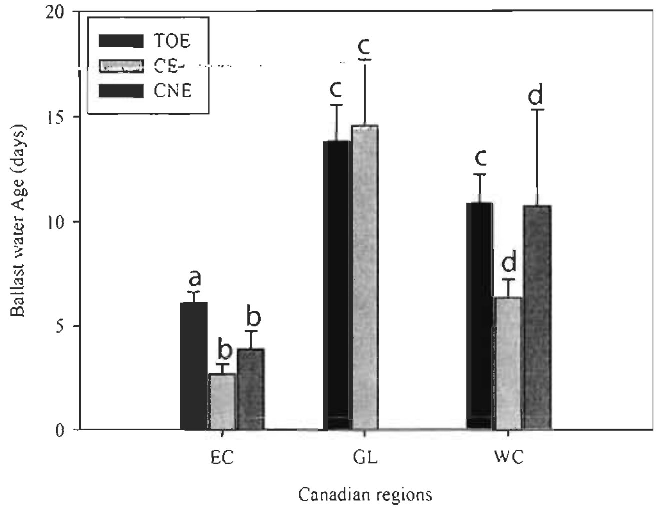

Figure 16. Mean of ballast water age (± standard error) for each category of ships in the three regions of Canada examined in this study (EC = East coast; GL = the Great Lakes; WC = West coast). Letters above columns indicate the results of pair-wise tests that showed significant differences between transoceanic (TOE) and both continental ships without ballast water exchange (CNE) and continental ships with exchange (CE) (see table 2.6). Categories of ships that differ significantly are indicated with different letters above data bars.

The volume of ballast sediments in ships that undertook ballast water exchange was much greater for East coast ships (CE and TOE) compared to the other regions and it was least for TOE ships in the Great Lakes (Figure 2.6a). For coastal non-exchanged ships, ballast sediment volume was greater for West coast CNE ships compared to the East coast. Sediment volume also differed among categories of ships, within each region. For the East coast, the volume of ballast sediments was significantly greater (PERMANOVA; $p = 0.0001$; $p = 0.0014$, respectively) in TOE and CE ships (1.8 ± 0.6 m^3 and 2.1 ± 0.9 m^3, respectively) compared with CNE ships (Figure 17a). In contrast, there were no significant differences for sediment volume among categoies of ships for the Great Lakes and the West coast regions. These quantities of ballast sediments become relevant when an estimate is made of the abundance of dinoflagellate cysts of NIS per ballast tank (cyst concentration per m^3 multiplied by sediment volume in m^3 per tank) (Figure 17b). In fact, the large volume of sediments in coastal ships from the East coast with BWE (CE) resulted in high

NIS cyst concentrations per tank and, along with ships without BWE (CNE), these two categories of ships recorded significantly higher cyst concentrations of non-indigenous species per tank than TOE ships (PERMANOVA; $p < 0.05$). For the other two regions, sediment volumes also influenced cyst concentrations per tank, with exchanged coastal ships arriving to Canada with a greater number of NIS cysts per tank (PERMANOVA; $p < 0.05$) compared with TOE and CNE ships (West coast) and TOE ships (Great Lakes).

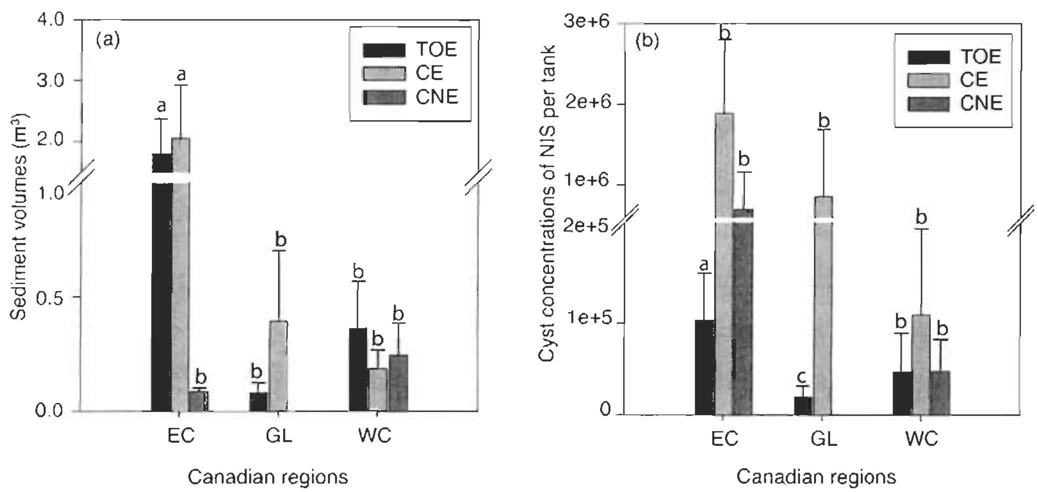

Figure 17. Mean ballast sediment volumes (m^3) per tank (± standard error) a) recorded in all ship categories for the East coast, the Great Lakes and the West coast of Canada; b) Mean concentration of viable dinoflagellate cysts of NIS per tank recorded in each category by regions (EC= East coast; GL= the Great Lakes; WC=West coast).). Letters above columns indicate the results of pair-wise tests that showed significant differences. (see table 8). Categories of ships that differ significantly are indicated with different letters above data bars.

DISCUSSION

This is the first study examining in detail the presence of dinoflagellate cysts including harmful and non-indigenous species, in ballast sediments from ships visiting three regions in Canada: the East coast, West coast and the Great Lakes. We found that non-indigenous dinoflagellate species (some of them potentially harmful or toxic) were present in ballast sediment from 21%, 22% and 14% of ships arriving to the East coast, the Great Lakes and West coast, respectively. Maximum concentrations of viable non-indigenous dinoflagellate cysts were 194, 122 and 32 cysts g^{-1} dry sediment in samples collected in ships from the East coast, West coast and the Great Lakes, respectively.

Previous studies in North America also reported the introduction of phytoplankton species in ballast sediments, including cysts of dinoflagellates, even for viable toxic species (Kelly, 1993; Harvey et al. 1999). The proliferation of harmful or toxic species of dinoflagellates poses enormous risks, particularly when these are non-indigenous. These non-indigenous proliferations have had major economic consequences (e.g., the Australian aquaculture industry) (Hallegraeff 2002). If we compare with earlier ballast sediments studies, abundances are in the same order of magnitude (9-1779 cysts cm^{-3}, in this work) compared to previous studies for ships visiting Scottish ports (5-1450 cysts cm^{-3} in ballast water discharge, Macdonald 1995) but lower if we compare with ships visiting Australian ports (40-22 500 cysts cm^{-3}, Hallegraeff and Bolch 1992).

If we extrapolate NIS dinoflagellate cyst concentrations per tank to whole ships, we obtain mean values of 75 x 10^6 cysts of NIS per ship for the East coast, 12 x 10^6 for the West coast and 22 x 10^6 for the Great Lakes. However, not all sediments from the bottom of ballast tanks are not all discharged: a recent study from Weise et al. (in prep.) estimated that about 0.4% of the sediment volume at the bottom of ballast tanks are discharged during de-ballasting. Hence more realistic estimates of cysts that could be discharged into the environment would be respectively 3.0 x 10^5, 4.8 x 10^4, 8.6 x 10^4 cysts of NIS per ship for the East and West coasts and for the Great Lakes. Considering total ship arrivals per year in Canada (more than 4000) (Lo et al. 2011), and the percentage of occurrence of non-indigenous species or harmful species, the amount of cysts introduced annually by ballast sediments could be equivalent to more than 5.6×10^{10}, 6.9×10^{8} and 1.5×10^{10} cysts of NIS for the above three regions respectively.

Composition of dinoflagellate cyst assemblages

Dinoflagellate cyst assemblages found during this study showed similarities between categories of ships and regions when considering all the species. Cyst taxa such as *Alexandrium tamarense*, *Operculodinium centrocarpum*, *Scrippsiella trochoidea*, *Dubridinium caperatum*, *Polykrikos schwartzii* and species of the genus *Brigantedinium* were common in all categories of ships arriving in the three Canadian regions. These species are considered cosmopolitan and they are distributed from coastal to oceanic environments and from temperate to tropical regions (Dodge and Harland 1991).

Cyst assemblages from ships on the East coast were differentiated by the high occurrence of the Gonyaulacales *Polysphaeridinium zoharyi** and *Lingulodinium machaerophorum**. *Polysphaeridinium zoharyi** was abundant in our samples from CE ships which visited ports of southern United States, corresponding with the natural distribution of this species (the Caribbean Sea and Gulf of Mexico). *Lingulodinium machaerophorum** was found mostly in ballast sediments of ships arriving to the East coast of Canada from northern Europe (Netherlands, Belgium and United Kingdom). The cyst form of this species is generally found in coastal sediments from the subtropical/tropical Southern Hemisphere and from tropical/temperate regions of the Northern Hemisphere. This species is generally reported to be restricted to regions with summer temperatures between 10 to 12°C (Rochon et al. 1999), however, temperatures as low as 4°C do not prevent survival of the cysts (Lewis and Hallet 1997), hence it is not surprising to find it in ships arriving from Europe. On the West coast, *L. machaerophorum** was found in CE ships arriving from Asian ports. Its lower abundance in West coast samples could be due to relatively lower abundances of this species in Western Pacific marine sediments than on the European shelves, even if this species can occasionally form blooms in these regions (Smayda and Trainer, 2010). Hence, presence of this species associated with ballast sediments of coastal route ships could support a secondary spread (Simkanin et al 2009). Interestingly, in recent studies this species has not been reported (Radi et al. 2007), or has rarely been reported (Mudie et al. 2002) or with low concentrations (3 cysts over three years of research), in sediments from Canada's West coast, suggesting it is not well established there (Taylor and Waters 1982; Pospelova et al. 2010). The highest percentages of occurrence of *L. machaerophorum** were recorded in ships with BWE from the two marine coasts, which could indicate the relative inefficiency of BWE at removing organisms contained in the ballast sediments. In the Great Lakes samples, cyst assemblages were different from those found in West coast ship samples. For example, CE ships visiting the Great Lakes were characterized by the presence of *Bitectatodinium tepikiense* (up to 25%). Since this species is common in temperate and sub-Arctic environments of the North Atlantic Ocean and is more abundant in coastal regions than the open ocean (Rochon et al. 1999), it may have been picked up during BWE in these CE ships. Considering their usual coastal distribution, it is not surprising to find more Gonyaulacales in CE ships instead of the TOE, even though some species are present in open ocean environments (Mudie and Rochon 2001).

Several species identified in this study are non-indigenous-harmful/toxic species of global concern (IOC-UNESCO 2009). Interestingly, they were present in all categories of ships throughout all regions. These species included autotrophic NIS such as *Cochlodinium polykrikoides**, *Lingulodinium machaerophorum** and *Scrippsiella lachrymosa/trifida*. *Cochlodinium polykrikoides** was found in all regions examined in this study (Table 9). The cyst form of this species has been reported from Puerto Rico, the Atlantic coast of North America, the Mediterranean Sea, the Indian Sea, the Western Pacific Ocean and the Eastern Pacific Ocean (Matsuoka et al. 2008). It is one of the most harmful species for the fish aquaculture industry in western Japan and southern Korea (Matsuoka et al. 2008). We note that this species was mostly found in CE ships arriving to the East coast, suggesting that it was picked up in ports from the Atlantic coast or during BWE in coastal waters from this region (Gobler et al. 2008). For the West coast samples, this species was not only found in ships involved in coastal traffic but also in several TOE ships from Asia, which matches with its distribution in both Western and Eastern Pacific Ocean (and could also suggest that BWE was not very efficient at removing these coastal organisms). Although we considered this species as non-indigenous, it has been previously recorded from the East coast (Kudela et al. 2008) and the West coast of the United States and Canada (Whyte et al. 2001). In fact, a harmful bloom has been reported along the coast of British Columbia in 1999 (Whyte et al. 2001). However, recent palaeoceanographic studies (see Radi and de Vernal 2004; Radi et al. 2007) did not observe any cysts of this species for this region of Canada, suggesting it is not a regular member of the local flora. *Scrippsiella lachrymosa/trifida* was found mostly in coastal ships (with and without BWE). This taxon is dominant in the North Sea and the Baltic Sea (Nehring 1994). However, we found no match between the origin of ships and the occurrence of this species in ballast sediments, but we note that this species was rarely present in our samples (observed in only 4% of all ships examined on the East coast).

The dominance of heterotrophic dinoflagellate taxa over the autotrophic ones in ballast sediments could be an indication of the resilience and survival potential of these species that do not require light or photosynthesis. Examples of heterotrophic non-indigenous species include *Polykrikos kofoidii* and *Trinovantedinium applanatum*. The former species is present in coastal waters of Japan (Matsuoka et al. 2003) and the Mediterranean Sea (Montresor et al. 1998). It is also thought that this species was introduced from Asia into Australian and Tasmanian port waters by ballast water (Hallegraeff and Bolch 1992). In our study, it was mostly found in East coast TOE ships from Europe and in West coast ships with BWE (TOE and CE ships originating from Asia), matching with its known geographic distribution (but also suggesting that BWE was unable to remove this species from ballast sediments). The latter species is present in coastal and oceanic environments of southern and northern hemispheres but mostly in central North Atlantic (Rochon et al. 1999). The presence of these two species in most of the TOE ships visiting the East coast and in CE ships visiting the West coast is likely related with their wide distributions and ability to resist different environmental conditions. They have been observed in a broad range of environments, from coastal waters to open oceans and from oligotrophic to eutrophic regions (Marret and Zonneveld 2003).

Other non-indigenous species were common only in sediment samples from ships in the marine regions and were recorded with low abundance values (e.g. *Gymnodinium nolleri*, *Protoperidinium latissimum*, *Spiniferites bentorii*) including toxic NIS such as *Gymnodinium catenatum**. This last species is considered a PSP producer and has major economic consequences for the Australian aquaculture industry (Hallegraeff 2002). All these non-indigenous species occur mostly in coastal regions, in line with the origin of the water used for ballast water exchange or to fill the ballast tanks including CE and in some cases CNE ships. Despite their low relative abundance, the majority of the potentially harmful/toxic NIS were found in all categories of ships, which could also indicate the widespread distribution of these species. Cyst assemblages were differentiated by the presence of NIS *Votadinium spinosum* for East coast samples and *Gyrodinium impudicum* and *Tuberculodinium vancampoae* for West coast samples. The presence of these last two species in West coast samples is not surprising as they are naturally present in the Western Pacific. The presence of *V. spinosum* in East coast samples could indicate that it was picked up during previous voyages in other regions of the world since it is less commonly observed in the Atlantic.

Some of the species found in ships from the Great Lakes have been recorded in NOBOB ships (no ballast on board) (Fahnenstiel et al. 2009). However, the following non-indigenous and/or harmful species have not been reported before: *Cochlodinium polykrikoides**, *Diplopsalis orbicularis*, *Gymnodinium nolleri*, *Operculodinium centrocarpum**, *Polysphaeridium zoharyi**, *Trinovantedinium applanatum* and *Votadinium spinosum* which are associated with both neritic and oceanic domains and were most abundant in transoceanic ships. These marine species are not expected to be able to survive in freshwater environments.

Even though the match between the presence of non-indigenous dinoflagellate cysts in ships from various regions of the world and their natural distribution was found to be relatively good in this study, such a match should not always be expected since ballast sediments may represent the accumulation of several voyages from different ports around the world.

Comparisons among regions and ship categories

When comparing the three regions examined in this study, the East coast stands out with greater cyst concentrations per tank for total dinoflagellate as well as viable cysts of NIS. The reasons why cyst concentrations, particularly for viable cysts, were greater on the East coast could include a shorter duration of voyages and more sediment per ballast tank (for ballast exchanged ships). When comparing viable cyst concentrations and the ballast tank sediment volumes between TOE ships and the two coastal ship categories for the East coast, both coastal categories showed higher concentrations of viable cysts than TOE ships, associated with shorter voyages. Thus, recently deposited viable cysts may be found in the upper layers of the ballast sediments, and these cysts could be discharged with sediment slurries when de-ballasting takes place in ports (Casas-Monroy et al. 2011). We also examined whether oxygen concentrations in ballast tanks could provide some clues to explain the relative abundance of empty cysts, since oxygen is an absolute requirement for cyst germination (Anderson et al. 1987) and the bottom stirring and oxygen replenishment associated with BWE could possibly trigger cyst germination, increasing the numbers of empty cysts. Results from co-researchers in the CAISN programme showed that oxygen concentrations decreased over the first week of a trans-Pacific voyage and were strongly replenished following ballast exchange (Klein et al. 2009). However, changes in oxygen concentrations were much less in a trans-Atlantic voyage (Seiden et al. 2011), barely showing any changes following BWE. Biological oxygen demand combined with a shorter voyage duration of trans-Atlantic ships contributed to the slow decline in dissolved oxygen (Seiden et al. 2011). If these results can be generalized then oxygen increase following ballast water exchange might provide an explanation for the abundance of empty cysts.

Comparison of the three categories of ships for each region showed no significant differences among them on the West coast or the Great Lakes, indicating that ballast water exchange, conducted for two of the three ship categories had no influence on dinoflagellate cyst concentrations. In contrast, on the East coast, ships with ballast water exchange had significantly lower cyst concentrations than unexchanged coastal ships, suggesting that ballast water exchange contributed also to lower the concentrations of undesirable viable NIS even in ballast sediments (possibly through resuspension and flushing of sediment and cysts during BWE). It is unclear why this was not observed on the West coast, although one possible reason is that unexchanged coastal ships on the East coast were mostly oil tankers, while they were bulk carriers on the West coast. Hence the type of ships as well as the absence of ballast exchange (unexchanged bulk carriers were not present on the East coast at the time of sampling) could influence the higher cyst concentrations observed in East coast CNE ships.

Sediment volume per tank varied greatly among regions, with a lot more sediment on the East coast for ships that carry out ballast exchange (CE and TOE). This has a clear influence on the abundance of cysts per tank, increasing values for the East coast. We have no explanation why there was more sediments in ships from the East coast – the age of this sediment was recently assessed at several years (Rochon et al. in prep.), which would suggest infrequent ballast tank cleaning. Interestingly, we see the reverse trend for unexchanged coastal ships, which carry more sediment per tank on the West coast. This is possibly related to the type of CNE ships (oil tankers on the East coast, bulk carriers on the West coast). Oil tankers from coastal ports have been shown to discharge significantly greater ballast volumes compared to tankers arriving from overseas in ports of the United States (Simkanin et al. 2009). This may contribute to clean the bottom of ballast tanks and reduce the amount of sediment. Furthermore, the sediments present may be more recent than the sediments accumulating in other types of ships, which may contribute to greater concentrations of viable cysts even though there is less sediment. It would be interesting to test this hypothesis in future studies. However, it does not explain why the concentration of *non-indigenous* species of viable dinoflagellate cysts was so high in these CNE ships from the East coast if their routes are local. Part of the answer may reside in inadequate published lists of local organisms, resulting in incorrect attribution of the non-indigenous character. Another possibility is secondary spreading from "hotspots" of non-indigenous species in the United States, such as Chesapeake Bay on the East coast or San Francisco on the West coast (Wasson et al. 2001; Ruiz et al. 2006). Some of these "hotspots" sites may be located within the zone considered "coastal" for Canadian waters (which is north of the two hotspot zones just mentioned), hence not requiring a ballast water exchange according to local regulations (Office of the Auditor General of Canada 2008), but nevertheless contributing to the transport of non-indigenous species.

The routes used for coastal non-exchanged transport from the United States are also different between the two marine coasts. On the East coast, coastal non-exchanged ships traveled only between the United States and Canada, whereas on the West coast, coastal non-exchanged ships arriving to Canada transited through the United States from ports in Asia or Oceania (information from ballast reporting forms submitted to Transport Canada). Thus, sediments accumulated in ballast tanks on the West coast could originate from prior visits to Asian ports – this could explain the similarity among all three ship categories on the West coast. Similar results were found for diatoms in ballast tank sediments in a subset of the ships examined here (Villac and Kaczmarska 2011). These authors suggested that all ship categories that reach the West coast should receive the same level of concern because there are no statistical differences among them, in contrast with the East coast of Canada. Our results on dinoflagellate cysts in ballast sediments concur with this.

The mode of ballast water exchange (flow-through or empty-refill) had no significant influence on cyst concentrations, for all three regions. While BWE contributed to decrease total cyst concentrations on the East coast, there were no differences between ships with and without BWE on the West coast. Although BWE may sometimes be inefficient (McCollin et al. 2007; Cordell et al. 2009), the different patterns on the two coasts are surprising. For the East coast, the invasion risk could be reduced by BWE, while for the West coast and the Great Lakes the three categories of ships present the same level of invasion risk (no significant differences) suggesting that BWE is inefficient at removing dinoflagellate cysts from ballast sediments. As mentioned above, shipping routes may account for the similarity among all three categories of ships on the West coast, several ships arriving in Vancouver as "coastal" having in fact originated from across the Pacific Ocean. While ballast water may have been exchanged along the way, ballast sediments may still reflect the port of origin, and thus would show some similarity with transoceanic ships arriving directly to Vancouver.

Coastal traffic is increasingly recognized as a significant invasion pathway (Cordell et al. 2009; Simkanin et al. 2009; Casas-Monroy et al. 2011) and the present study concurs with this. Our results show greater ballast tank concentrations of viable cysts for non-indigenous dinoflagellate species in coastal ships. Hence, we suggest that coastal ship traffic in Canada poses at least as great a risk for dinoflagellates as transoceanic shipping. Our results also suggest that secondary spread from highly invaded regions contributes to bring risk organisms into Canadian coastal waters via coastal traffic. This may be favoured by the fact that most of the coastal exchanged ships perform ballast exchange close to shore (often within the EEZ zone) or in areas under a continental influence, where the abundance of phytoplankton taxa is more important (Cebrian and Valiela 1999). Furthermore, we see no evidence that coastal ships with BWE contain significantly fewer taxa of non-indigenous species, suggesting that undertaking a ballast water exchange in these zones may increase the risk of introduction of NIS phytoplankton taxa.

Due to the magnitude of international shipping, international regulations are particularly important for the effective prevention of marine bio-invasions. Regulatory frameworks need to be established at different levels (global, regional, and national) in order to prevent the introduction of undesirable species such as NIS and harmful dinoflagellates. Unlike terrestrial systems, the majority of demonstrable marine bio-invasion impacts appear to be primarily on native biodiversity and ecosystem health (Carlton and Ruiz 2004) with direct impacts on the economy (Hallegraeff 2002). Ballast management is important to prevent new introductions which could threaten ecosystems and which could have negative impacts on biological diversity or human health. The present study suggests that ballast management will need to take into account differences observed among coastal regions in Canada and the importance of coastal traffic for the introduction of non-indigenous species.

ACKNOWLEDGMENTS

This research was made possible by the financial support from the Canadian Aquatic Invasive Species Network (CAISN), the Natural Sciences and Engineering Research Council of Canada (NSERC), Fisheries and Oceans Canada (DFO), the Institut des Sciences de la Mer de Rimouski (ISMER, Université du Québec à Rimouski) and Québec-Océan. We thank all CAISN sampling teams on the West and East coasts and the Great Lakes. Special thanks to Transport Canada, shipping agents in ports and shipping companies sampled. Finally, we also thank Jean-Guy Nistad for help with the production of maps.

CHAPITRE 3

PRESSION DE PROPAGULES DES DINOFLAGELLÉS (INCLUANT LES ESPÈCES NON-INDIGÈNES) À PARTIR DES NAVIRES VISITANT LES CÔTES EST ET OUEST DU CANADA

RÉSUMÉ

La présente étude a quantifié et identifié les espèces de dinoflagellés qui sont introduits par des navires commerciaux (pression de propagules, PP, actuelle) et le nombre d'organismes capables de survivre au transport et croître après la décharge dans les ports récepteurs (PP effective) pour les côtes est et ouest du Canada. Un total de 134 navires a été échantillonné et séparé en trois catégories : (1) les navires transocéaniques avec échange d'eau de ballast obligatoire (BWE), (2) les navires côtiers avec échange de leurs eaux de ballast, et (3) les navires côtiers sans échange. Le patron d'invasion diffère selon la région examinée. Ceci est illustré par une interaction significative entre la région (côte est vs côte ouest) et les catégories de navires. Pour la côte ouest, il n'y a pas de différence significative entre les trois catégories de navires pour les pressions de propagules actuelle et effective, que l'on considère tous les dinoflagellés ou bien seulement les non-indigènes. Cependant, la PP effective y est corrélée négativement à l'âge de l'eau de ballast. Par conséquent, les navires dont la durée des voyages était plus courte après l'échange avaient plus de cellules végétatives et d'espèces non-indigènes viables par navire (jusqu'à un maximum de 2,0 x 10^9). Pour la côte est, les concentrations de dinoflagellés par navire sont significativement plus élevées dans les navires transocéaniques comparativement aux navires côtiers n'effectuant pas de BWE. Ces concentrations sont aussi plus élevées sur la côte est comparativement à la côte ouest, sauf pour les navires côtiers sans échange où c'est

l'inverse. Nos résultats ne montrent pas d'évidence que les échanges d'eau de ballast soient efficaces pour réduire l'introduction d'espèces de dinoflagellés non-indigènes : il n'y a pas de différence significative entre les trois catégories de navires pour la côte ouest, et pour la côte est, les navires ayant effectué un échange de leurs eaux de ballast ont des concentrations de dinoflagellés significativement plus élevées, suggérant un apport lié aux échanges. Les expériences de croissance ont confirmé que plusieurs espèces de dinoflagellés étaient encore viables lorsque les navires sont arrivés aux ports et qu'elles sont capables de croître et se reproduire. Par conséquent, si les conditions environnementales sont favorables, ces espèces pourraient éventuellement se reproduire et/ou s'établir dans les milieux marins côtiers de l'est et l'ouest du Canada.

Ce troisième article intitulé « *Non-indigenous dinoflagellate propagule pressure from ships arriving to the East and West coast of Canada* », fut corédigé par moi-même ainsi que par le professeur Suzanne Roy, le professeur André Rochon et ma collègue Marie Parenteau. Il sera soumis pour publication en 2012 à la revue *Marine Pollution Bulletin*. En tant que premier auteur, ma contribution à ce travail fut l'essentiel de la recherche sur l'état de la question, le développement de la méthode, l'exécution des tests statistiques et la rédaction de l'article. Ma collègue Marie Parenteau, deuxième auteur, a identifié et dénombré les espèces par microscopie. Le professeur Suzanne Roy troisième auteur, le professeur André Rochon quatrième auteur et le chercheur Chris McKindsey ont grandement contribué à la recherche sur l'état de la question, au développement de la méthode ainsi qu'à la révision du manuscrit. Les résultats de cette étude ont été présentés lors de la Conférence Zones Côtières du Canada 2012, à Rimouski, QC, Canada, en juin 2012.

PROPAGULE PRESSURE OF DINOFLAGELLATES (INCUDING NON-INDIGENOUS SPECIES) FROM SHIPS ARRIVING TO THE EAST AND WEST COASTS OF CANADA

ABSTRACT

In order to determine the actual and effective propagule pressure (PP) of non-indigenous dinoflagellate species, we identified and quantified the organisms being introduced (actual PP) and the number of organisms that could survive ship transport and be able to grow after discharge (effective PP) in the receiving ports from commercial ships arriving to the East and West coasts of Canada. A total of 134 ships were sampled and separated into three categories: (1) transoceanic, with mandatory ballast water exchange (BWE), (2) coastal with BWE, and (3) coastal without BWE. The pattern of invasion pressure differed between the regions examined. This is illustrated by a significant interaction between the region (East vs West coast) and ship categories. For the West coast, there is no significant difference among ship categories for actual and effective PP (all dinoflagellates and NIS). However, effective PP was negatively correlated with ballast water age in that region. Hence, ships with shorter trip duration after ballast water exchange had more non-indigenous motile cells per tank (up to 2.0×10^9). For the East coast, concentrations of dinoflagellates per ship were significantly greater in transoceanic ships compared to coastal ships without BWE. East coast concentrations were greater than on the West coast, except for coastal ships without BWE, where it was the reverse. Our results show no evidence that ballast water exchange is efficient at reducing the introduction of non-indigenous dinoflagellates, since there is no significant difference among ship categories on the West coast, and on the East coast, ships which performed BWE had significantly greater concentrations of dinoflagellates, suggesting an input associated with the ballast water exchange. Growth experiments confirmed that several dinoflagellate species were still alive when ships arrived in ports and that they were capable of growth and reproduction. Hence, if environmental conditions are favourable, these species could eventually reproduce and/or get established in coastal marine environments of the East and

West coasts of Canada.

INTRODUCTION

Shipping transport promotes the inoculation of species across their natural dispersal barriers, resulting in biological invasions (Kolar and Lodge 2001; Ruiz et al. 2000). In recent times, the observed rate of new invasions has increased dramatically across global regions, habitat types and taxonomic groups (Cohen and Carlton 1998), particularly in North America (Mills et al. 1993). Commercial ships use ballast water to provide balance and stability and to maintain safe and efficient transit conditions, but uptake of ballast water at the departure ports and its subsequent discharge at destination is also a recognised mechanism to transfer non-indigenous species (NIS) to new regions (Carlton 1985; Niimi 2004; Wonham et al. 2001). Potentially serious environmental problems (e.g., increasing harmful algal blooms) could arise when this ballast water contains harmful phytoplankton species, such as dinoflagellates (Hallegraeff 1993). Several of these species can produce temporary or long-lived resting cysts, facilitating survival during transport. These harmful and/or toxic species can result in human diseases, massive kills of marine fish or mammals, and even impact fisheries and aquaculture (Hallegraeff 2002).

Propagule pressure is defined as the total number of individuals introduced at a given location (Williamson 1996; Lockwood et al. 2009). Propagule pressure may be viewed as having two components: the number of introduction events (propagule number) and the number of individuals per introduction event (propagule size) (Carlton 1996; Lockwood et al. 2005). Thus, the propagule pressure is the sum over all introduction events of the number of individuals liberated (Lockwood et al. 2009). It may be increased either through an increased number of ship arrival events (increased frequency of inoculation) or an increased intensity of exposure during any one event (increased abundance of organisms released into the recipient water body from any one vector) (Johnston et al. 2009). Empirical and theoretical models predict a higher likelihood of establishment with higher propagule pressure (Lockwood et al. 2007; Johnston et al. 2009). Evidence indicates that reducing propagule pressure, specifically the quantity, quality and frequency of introduced individuals, can decrease the invasion risk (Drake and Lodge 2004; Wonham et al. 2005).

However, there is limited information on propagule pressure, in part due to the difficulty in quantifying the number of non-indigenous organisms arriving in a new environment (Colautti and MacIsaac 2004); and the relationship between propagule pressure and establishment success remains poorly defined. "Propagule pressure", as it is usually defined, represents a "potential" for introduction rather than a realized introduction and may be considered at three levels (Lo et al. 2012): 1) *potential* propagule pressure –vector or pathway characteristics (e.g., number of ships or volume of ballast water discharged over time), 2) *actual*: propagule pressure –the abundances of organisms being introduced into a new area that can be quantified and identified (density of entrained taxa), and 3) *effective* propagule pressure –the number of organisms that survived ship transport and may potentially survive and grow after discharge in a receiving area. These latter two classes of propagule pressure are a function of the ability of NIS to survive several "filter barriers" between a source region and a receiving area (survival through ballast uptake, transport, deballasting, establishment, dispersion and dominance) (Coulatti and MacIsaac 2004).

To limit the propagule supply (actual and effective), the only mitigation measure currently widely applied is the ballast water exchange (BWE), which takes place either as a mid-ocean exchange for transoceanic ships or outside of the Exclusive Economic Zones for continental ships (Transport Canada 2006). These exchanges aim to replace water of coastal origin with oceanic water (with 95% volumetric efficiency or having replaced 95% of the tank volume) removing most of the coastal organisms in the process (Simard et al. 2011). These measures are enforced for all ships arriving in Canada's coastal waters and particularly in the Great Lakes region. Mandatory ballast water control and management regulations have been applied since June 2006 through the Canada Shipping Act (Office of the Auditor General of Canada 2008). Three major categories of ships arriving in Canadian ports were considered in this study: 1) transoceanic ships (= TOE), 2) coastal exchanged ships (= CE) and 3) coastal ships with no BWE (= CNE). This last category of ships includes most often oil tankers engaged in coastwise trade or other vessels operating exclusively between ports in Eastern Canada and ports on the East coast of the United States north of Cape Cod, or between ports on the West coast of Canada and ports on the

West coast of the United States north of Cape Blanco (United States Coast Guard 1993; Transport Canada 2006).

As part of the Canadian Aquatic Invasive Species Network (= CAISN 2006-2011, http://www.caisn.ca), the major goal of the present study was to determine the actual and effective propagule pressure of dinoflagellates in ballast water from each category of ships (TOE, CE, CNE) visiting the East and West coast ports of Canada. Both the abundance and composition of dinoflagellate communities were evaluated. We also examined the presence of non-indigenous species and whether there was any influence of ship categories, age of water and ballast water exchange. We hypothesized that ships that undertake BWE show a low actual propagule pressure of dinoflagellates (i.e. they have low concentrations of dinoflagellates being introduced). In addition, if effective propagule pressure represents the organisms that are potentially alive at discharge, it is assumed that effective propagule pressure would be greater for ships completing coastal voyages (with and without BWE) than those completing transoceanic voyages, for which trip duration is longer than for coastal ships (Smith et al 1999; Lawrence and Cordell 2010; Casas-Monroy et al. 2011). Finally, we expect that actual an effective propagule pressure is similar for both coasts.

METHODS

Sampling was conducted during three summers (2007 to 2009). For the East coast, sampling was done from the ports of Sept-Îles, Port Cartier and Baie-Comeau (Quebec), St. John (New Brunswick) and Canso, Halifax, Hantsport and Liverpool (Nova Scotia). For the West coast, ships were sampled only in the port of Vancouver. A total of 137 ballast water samples were collected from general cargo, bulk carriers and oil tankers; these ships represent the largest contribution to ship traffic and volume of ballast transport across both regions (Lo et al. 2012). The objective of the CAISN sampling program was to sample at least 20 ships from each of the three ship categories (TOE, CE, CNE). Ships were selected on an opportunistic basis until we had reached a maximum of about 20. Once the ships arrived in port, the sampling team met the captains and asked for the ballast reporting form, which is required by Transport Canada for all ships entering Canadian waters. We obtained the following information from each ship's ballast reporting form: ship type, last port of call, ballast water source, volume of the ballast tank sampled, BWE location, BWE method used, total ballast water capacity, total ballast volume on board, volume of BW discharged, and dates when tanks were filled with water in the source region, exchanged and when they were discharged at ports.

Ballast water samples were collected from a single ballast tank per ship. The selection of ballast tanks was done based on: 1) tanks with BWE (transoceanic or coastal); 2) tanks without ballast BWE; 3) tanks had to be full or with a minimum of 4 meters deep of ballast water; 4) the ease of sampling tanks via manholes (mostly wing or topside tanks, but cargo hold tanks were also sampled); 5) any tanks assigned by captains or chief officers of the ship.

Prior to the ballast water sampling, the in-tank water depth was measured with a 35 m weighted measuring tape, from tank bottom to the top of the water column to define 4 equidistant sampling depths. Temperature and salinity were also measured at these depths using a YSI model 30 salinity, conductivity and temperature meter (YSI environmental monitoring, Yellow Springs, Ohio, USA). A single phytoplankton sample per tank was collected with a 5 L Niskin bottle: water at each of the four pre-described depths was mixed in a 20 L carboy and divided into subsamples to send to all CAISN researchers involved in the program (for analysis of diatoms, virus and bacteria). Both fresh and fixed samples containing dinoflagellates were collected. Fresh subsamples were used to test viability and to assess the concentration of viable dinoflagellates (extrapolated per ship). A volume of 500 mL of unfiltered and unpreserved ballast water from the 20 L carboy was shipped immediately in a cooler to be processed fresh in the laboratory 1 or 2 days after collection. For preserved samples, the remaining volume of carboy water (minimum of 13 L) was filtered through 73 μm and 20 μm mesh sieves. All the material collected on the 20 μm mesh was concentrated in a 250 mL glass bottle using filtered seawater (0.2 μm), to which was added 0.5 mL of acid Lugol. Preserved samples were sent to Rimouski and kept at 4°C in the dark until they were examined within 12 months of collection.

To evaluate viability, fresh samples were filtered through 73 µm and 20 µm mesh sieves and the 20-73 µm fraction was transferred to a beaker using 25 mL of filtered (0.2 µm) seawater. The 25 mL was divided into 5 subsamples, transferred into Petri dishes with 10 mL of sterilized f/2 culture medium without silica. The culture medium was prepared using filtered seawater from the Sept-Îles region (salinity = 26) and sterilized in an autoclave (Guillard 1975). The five Petri dishes were placed in an environmental chamber at a temperature of 15°C, and a 14L:10D photoperiod, provided by Phillips cool white fluorescent lamps with a light intensity between 100 and 120 µmol photons m^{-2} s^{-1}. Petri dishes were observed every second day and viable dinoflagellate cells (swimming cells) were isolated, cleaned with sterile culture medium, and transferred into Pyrex tubes filled with the same culture medium. The survival and growth experiments were carried out in triplicate from cultures with at least 1000 cells·L^{-1}, and left to grow under the same chamber conditions. Most of the dinoflagellates that grew in these cultures were autotrophic species (see Annex 3).

For microscopic counts and identification, all preserved samples were mixed by overturning at least 20 times prior to subsampling. An aliquot of 50 mL was put in a sedimentation chamber for 24 hours following Uthermöhl's method (Uthermöhl, 1958). The settled phytoplankton cells were identified and enumerated using an inverted microscope. Only cells with intact protoplasm and cell contents were considered, assuming they were viable. Taxonomic nomenclature used in this work follows Dodge (1982), Steidinger and Tangen (1996), Bérard-Therriault et al. (1999), Taylor and Waters (1982), and Taylor et al. (2003). To improve identification, specimens were mounted in a "micro slide chamber" following Horiguchi et al. (2000) and observed using a transmitted light microscope (Zeiss Axiovert) at 250X to 1000X or at times, Calcofluor was added (Fritz and Triemer 1985) to visualise dinoflagellate thecal plates under fluorescence using an ultraviolet mercury lamp at 350-380 nm of excitation wavelength. Transmitted light and fluorescence microphotographs of dinoflagellate specimens were taken with a digital camera Nikon CoolPix P5000 fitted with a Nikon MDC CoolPix lens.

Estimation of the ballast water volume per tank and ballast water discharged by ship was based on information from the ballast reporting forms. The ranking of ship categories changed according to whether data were presented as cells L^{-1}, cells per tank or cells per ship. Cell concentrations estimated from fresh, live samples were lower than the estimates from lugol-fixed samples, because of the difference in sample volume (13 L for fixed samples compared with 500 mL for fresh samples).

We evaluated two measures of propagule pressure: the actual propagule pressure (= the concentration of cells per tank for each category of ship, from the fixed samples) and the effective propagule pressure (= the number of organisms that survived ship transport and may potentially survive and grow after discharge in a receiving area, estimated as the concentration of cells per Liter from the fixed samples times the volume of ballast water discharged). To examine differences for both measures within each coast and for the various ship categories, multivariate analyses were based on the Canberra similarity matrices performed on untransformed data (Clarke and Warwick 1994). Variations in both measures of propagule pressure were studied using a Permutational Based Multivariate Analysis of Variance PERMANOVA (PRIMER-E v.6, ltd) performed with 9999 random permutations of appropriate units (McArdle and Anderson 2001; Anderson et al. 2008). When there were too few possible permutations to obtain a reasonable test, a p-value was calculated using Monte Carlo random draws from the asymptotic permutation distribution (Terlizzi et al. 2005). Significant terms and interactions (between coast and categories) within the full models were analysed using appropriate pair-wise comparisons. Spearman rank correlations were used to explore the relationship between each measures of propagule pressure and the number of days after exchange, salinity, and the percentage of ballast water exchange for ships with BWE.

RESULTS

Ship traffic information and characteristics

A total of 134 ships were sampled in both coasts. A summary of pertinent ship information such as ship types, ballast tank sampled, dominant source of ballast water, exchange method used by ships and ballast water age for all ships sampled is presented in Table 10.

Pacific voyages

For the West coast, ballast water samples were collected from 71 ships divided into TOE (28), CE (20) and CNE (23) categories. Most of these were bulk carriers (52% of ships). Most ballast water for TOE ships was from Japan (11 of 28 ships), followed by Korea (5 of 28 ships) and China (3 of 28 ships) (Table 10). For CE ships, the ballast source was mostly the West coast of USA (16 of 20 ships) and coastal ports of Mexico (4 ships), whereas ballast water for all CNE ships originated from West coast USA ports. Most of the ships that did BWE used the empty-refill method (30 of 48 ships), and 37.5% of them used the flow-through method. About 86% of the TOE ships did BWE in oceanic waters while 90% of CE ships did BWE inside the EEZ and only 10% did it outside of the EEZ.

Atlantic voyages

For the East coast of Canada, ballast water samples were collected from 63 ships divided into TOE (22), CE (22) and CNE (19) categories. Ballast water samples were collected mostly from bulk carriers (56% of the ships) and oil tankers (37% of the ships). Europe was the largest source of ballast water for TOE ships (21 of 22 ships), and the East coast of the USA was for coastal ships (with and without BWE). All the TOE ships did ballast water exchange, and 15 of 22 of these used a flow-through method and 7 the empty-refill method. About 86% of TOE ships exchanged their ballast water in oceanic waters while the remainder did so within the EEZ. For coastal ships, the empty-refill method was the most common method used to exchange ballast water (13 ships) followed by the flow-through (9 ships) method (Table 10). Of these ships, 91% did BWE in coastal waters according to EEZ limits.

Table 10. Information on the ships sampled on the West and the East coasts of Canada. Ship categories are transoceanic (TOE), coastal with BWE (CE) and coastal without BWE (CNE) ships. n.a. = data not available. AFT = After Peak; CBT = cargo ballast tank; DBT = Double ballast tank; FPT = Forepeak tank; WBT = Wing ballast tank. ER = Empty/refill; FT = Flow-through; NE = non-exchanged. BW age = ballast water age; Max days = maximum days of trip; Days after exch. = Days after exchange. SE = standard error.

	West coast			East coast		
	TOE	CE	CNE	TOE	CE	CNE
Ship	28	20	23	22	22	19
Bulk carrier	21	5	11	19	15	1
Container		1	2			
General cargo/container	1	1	2			
General cargo	4	8	7	2	1	
Oil tanker	2	4			5	18
Ro-Ro			1	1	1	
n.a.		1				
Tank sampled						
AFT					1	
CBT	1			7		
DBT	1	2		7	1	
FPT		2	1		3	1
WBT	26	16	22	8	17	18
Last port						
China	6					
Canada	1					8
Ecuador	1					
Europe				21		
Guatemala	1					
Hawaii	1					
Japan	6					
Korea	3					
Mexico	2	2				
USA	7	17	23	1	22	11
n.a.		1				
Ballast origin						
China	3					
Canada						
Ecuador	1					
Europe				21		
Guatemala	1					

Hawaii	1					
Japan	11					
Korea	5					
Mexico	1	4				
Mid-ocean	3					
USA		16	23	1	22	19
n.a.	2					
Method of BWE						
ER	15	15		7	13	
FT	13	5		15	9	
NE			23			19
BW age						
Max days	22±2	12±2	8±4	16±1	5±1	3±1
Days after exchange	11±1	7±1	0	8±1	2±1	3±1
Salinity						
Mean	33	31	12	35	32	29
SE	0.5	0.6	2.7	0.5	0.4	0 4
Temperature						
Mean	15	16	15	12	17	16
SE	0.6	0.6	0.6	0.9	1.2	0.4

Ballast water volume.

On the East coast, TOE ships had the greatest volumes of ballast water on board (BWOB), ballast water to be discharged (BWD), ballast water per tank at the time of sampling (BWT), and maximum volume discharged in ports (Table 11). Similarly, CE ships had greater volumes of ballast water (variables mentioned above) than CNE ships. For the West coast, TOE ships had the greatest volume of BWOB and BWT and they discharged the largest volumes of ballast water. With respect to the coastal ship categories, CNE ships had greater volumes of BWD (more than 6.0×10^6 L) than did CE (Table 11). CNE ships also discharged similar or greater concentrations of viable dinoflagellates during deballasting operations than did CE ships (Tableau 11).

Table 11. Mean volumes of ballast water on board (BWOB), ballast water to be discharged (BWD), ballast water in sampled tanks (BWT), and maximum discharged volume by ships arriving to the marine coasts of Canada.

Ship type	BWOB (L ship^{-1})	BWD (L ship^{-1})	BWT (L ship^{-1})	Maximum discharge (L ship^{-1})
East coast				
TOE	4.06 E+07	3.93 E+07	8.58 E+06	7.51 E±07
CE	1.91 E+07	1.82 E+07	3.03 E+06	3.92 E±07
CNE	1.63 E+07	8.94 E+06	1.40 E+06	3.47 E±07
West coast				
TOE	2.23 E+07	1.30 E+07	2.47 E+06	5.11 E±07
CE	1.28 E+07	6.59 E+06	9.96 E+05	2.80 E±07
CNE	1.90 E+07	6.96 E+06	7.83 E+05	3.00 E±07

Characteristics of ballast water

Ballast water characteristics such as salinity, temperature, and ballast water age were differed among ship categories, depending on the region from where they were sampled. Ballast water salinities ranged between 24 and 37 for ships visiting the East coast. In contrast, salinities were lower for ships arriving to the West coast, ranging between 0 and 36. Ships performing BWE had the greatest salinity on both coasts (over 31 ± 0.5) (Figure 18 A and B). CNE ships had the lowest salinity values (12 ± 3) on the West coast in contrast with ships of the same category on the East coast (29 ± 0.5) (Figure 18 A).

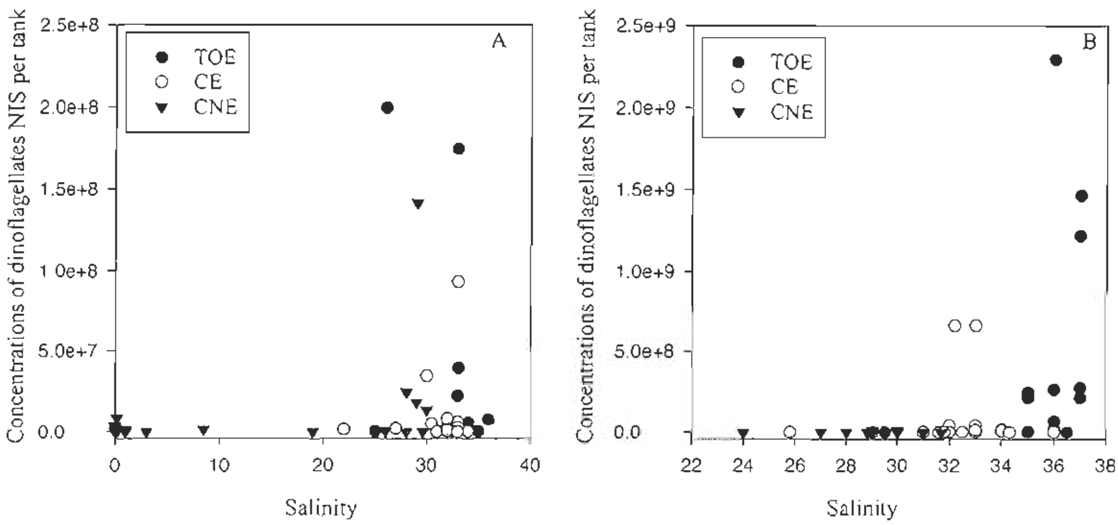

Figure 18. Concentrations of non-indigenous dinoflagellate species per tank as a function of salinity of ballast water for ships arriving to (A) the West coast and (B) the East coast of Canada.

Water temperature in ballast tanks on the East coast varied from 12°C± 1°C (TOE ships) to 17°C± 1°C (CE ships) and from 15°± 1°C (TOE ships) to 16°C± 0.5°C (CE ships) on the West coast (Table 10). We used similar temperatures for our culture conditions during the dinoflagellate motile cell growth experiments.

The ballast water age represents the number of days after BWE for TOE and CE ships, and the number of days after ballasting at port source for CNE ships. TOE ships from both coasts contained the oldest ballast water, averaging 22 ± 2 (SE) days for West coast and 16 ± 2 (SE) days for East coast ships. Coastal ships contained younger ballast water, averaging respectively 8 ± 4 (SE) and 3 ± 1 days after filling ballast tanks for CNE ships for West coast and East coast ships, and respectively 12 ± 2 (SE) and 5 ± 1 days after BWE for CE ships from the same regions. Ships with shorter trip duration after ballast water exchange (less than 15 days) had more non-indigenous motile cells per tank up to 2.0 x 10^9 (Figure 19 B).

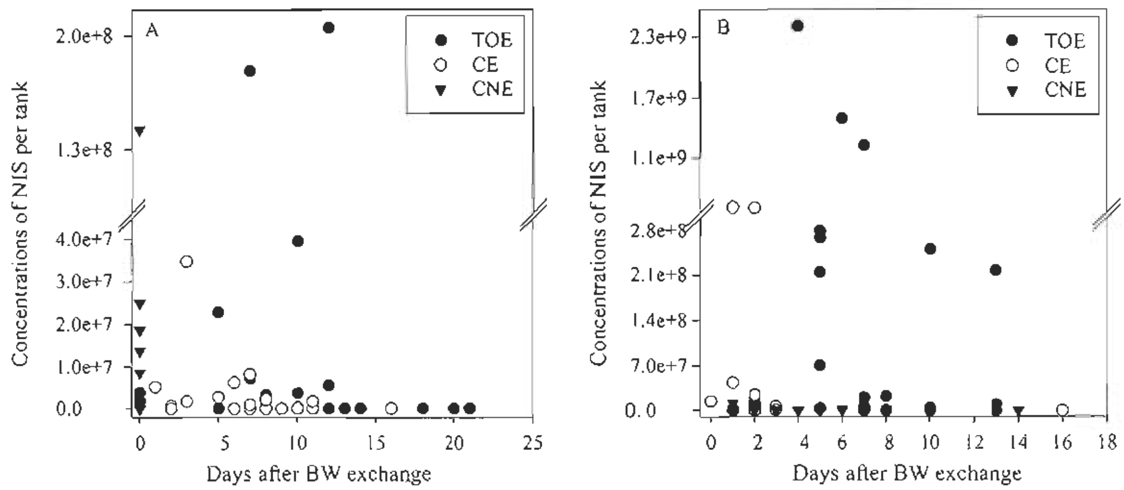

Figure 19. Concentration of non-indigenous dinoflagellates species per tank as a function of days after ballast water exchange for ships arriving to (A) the West coast and (B) the East coast.

Most of the ships arriving to the West coast performed BWE using the empty-refill method, in contrast with ships on the East coast, which performed BWE mostly using the flow-through method (Table 10). Pair-wise tests, from a two-way crossed design PERMANOVA revealed that ships using flow-through on the East coast had significantly higher concentrations for all dinoflagellates than ships using the same method on the West coast ($p = 0.002$) (Figure 20 A). No other significant differences were found.

When non-indigenous species were considered, a two-way crossed design PERMANOVA showed a significant interaction between the two factors, coast and ballast water exchange method ($p = 0.004$). Indeed, pair-wise tests showed that ships without BWE (NE in fig 20 B) had significantly lower concentrations per tank of non-indigenous species of dinoflagellates than ships using the ER method ($p < 0.05$) and FT method ($p = 0.0004$), on the East coast. These tests also showed that ships using the FT method on the East coast had significantly higher concentrations of NIS than ships using the FT method on the West coast ($p = 0.0014$) (Figure 20 B).

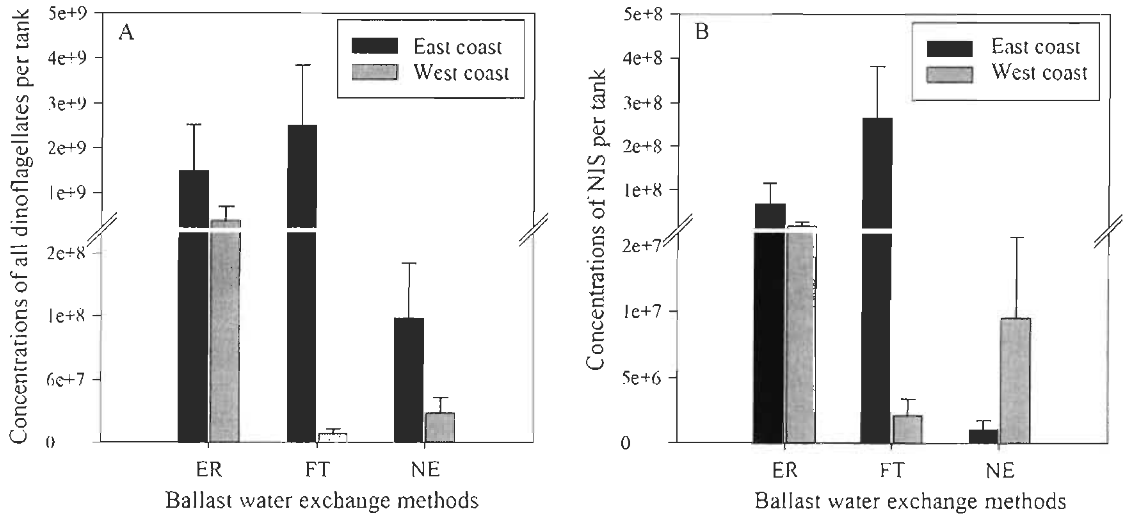

Figure 20. Mean concentrations of (A) all dinoflagellates and (B) non-indigenous species per tank according to ballast water exchange methods for ships visiting Canada's East and West coasts. ER = empty-refill; FT = flow-through; NE = non-exchanged. Bars represent the standard error.

Dinoflagellate diversity

The number of taxa per tank varied from 0 to 39 (mean of 10 ± 1 taxa) (Figure 22 A). For the West coast, transoceanic (TOE) ships recorded the lowest average number of taxa (3 ± 1) for all dinoflagellates compared to coastal ships with and without BWE (average number = 8 ± 2 and 7 ± 2 taxa, respectively). In contrast, for the East coast, ships with BWE (TOE and CE) showed the highest average number of taxa (17 ± 3; 16 ± 2, respectively). Coastal ships without BWE (CNE) showed the lowest average (7 ± 1) for this region. For non-indigenous species (NIS), the number of taxa in the West coast ships was greater in the CNE category (3 ± 1) compared with ships with BWE (TOE and CE) (2 ± 1 NIS taxa). For the East coast, the highest diversity values were recorded in TOE and CE ships (5 ± 1 and 3 ± 1 NIS taxa, respectively), while CNE ships presented the lowest average number of taxa (1 ± 0).

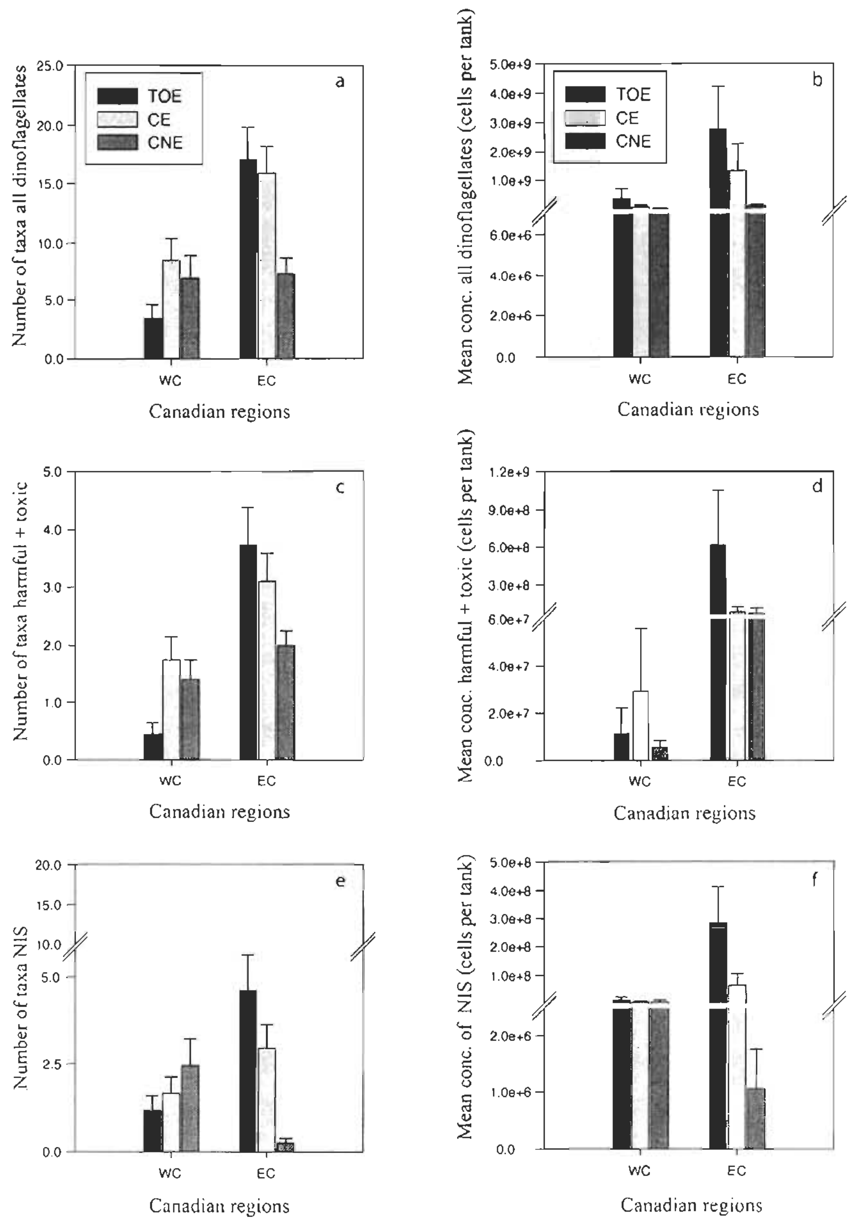

Figure 21. Number of taxa (left-side panel) and mean concentrations in cells per tank (right-side panel) of (a, b) all dinoflagellate species, (c, d) harmful + toxic, and (e, f) non-indigenous species of dinoflagellates present in the three ship categories: transoceanic (TOE), coastal with (CE) and without ballast water exchange (CNE). WC = West coast; EC = East coast. Bars represent the standard error.

Annex 3 summarizes the details of the 190 different dinoflagellate taxa found during the present study, including 33 non-indigenous species for the West coast of Canada and 39 non-indigenous taxa for the East coast. Annex 3 also shows that 20 of the identified dinoflagellate species may be considered as potentially harmful or toxic, regardless of whether they are indigenous or not. Some of these are recognized as toxin producers such as *Alexandrium*, *Dinophysis*, or *Protoceratium*. Their concentrations were often close to a million cells per tank and considering the large volumes of ballast water discharged per ships, these concentrations represent potential abundances of several million cells per ship. Furthermore, some of these potentially harmful/toxic species have not been reported yet from local waters of eastern Canada (*Dinophysis caudata*, *D. fortii*, *D. tripos*, *Gonyaulax polygramma*, *G. scrippsae*, *Neoceratium furca* and *Phalacroma mitra*) or from western Canada (*G. scrippsae*).

Actual propagule pressure

Concentrations of dinoflagellates in the 134 preserved samples varied from 0 to 4935 cells L^{-1} (mean ± SE, 188 ± 56 cells L^{-1}) (Table 12). With respect to harmful and/or toxic species, the concentrations varied from 0 to 3536 cells L^{-1} (mean ± SE, 56 ± 27 cells L^{-1}) and for non-indigenous species from 0 to 216 cells L^{-1} (mean ± SE, 11 ± 3 cells L^{-1}). When concentrations are extrapolated to the entire ballast water volume of each tank as an estimate of actual propagule pressure, dinoflagellate cell concentrations varied from 0 to $3 \times 10^{10} \pm 6 \times 10^{9}$ per tank (mean ± SE, $8 \times 10^{8} \pm 3\times10^{8}$) (Figure 21 B).

Table 12. Statistics on the concentration of all dinoflagellate species (cells L^{-1}) from ships sampled in the West and East coasts of Canada. Ship categories are transoceanic (TOE), coastal ships with BWE (CE) and coastal ships without BWE (CNE). SE = standard error.

	West coast			East coast		
	TOE	CE	CNE	TOE	CE	CNE
Number of ships	28	20	23	22	22	19
Cells L^{-1}						
Minimum	0	0	23	0	0	0
Maximum	2279	508	464	1409	4935	3897
Median	0	7	7	30	41	15
Mean	87	48	54	222	406	365
SE	81	26	21	78	232	239

For all dinoflagellates per tank a two-way crossed design PERMANOVA revealed significant differences between the two marine coasts ($p = 0.0001$). There were also significant differences among the three ship categories ($p < 0.05$), but no significant interaction between coasts and ship categories (Table 13). A pair-wise test revealed that TOE ships were significantly different between the two coasts ($p = 0.0014$). They also indicated significant differences between TOE ships and CNE ships on the East coast ($p < 0.05$).

When harmful/toxic species were considered, the two-way crossed design PERMANOVA showed that there was no significant interaction between coasts and ship categories, a significant difference according to the coast and to ship categories. For the West coast, pair-wise tests indicated that TOE ships were significantly different from coastal ships (with and without BWE) for harmful and toxic species ($p = 0.0071$; $p < 0.05$; respectively). Results also indicated that CNE ships and TOE ships were significantly different between coasts ($p = < 0.05$; $p = 0.0001$) (Table 13).

Non-indigenous species were present in all categories of ships visiting both coasts. The two-way crossed design PERMANOVA revealed a significant interaction between coasts and ship categories ($p < 0.05$), as well as a significant difference among categories of ships (PERMANOVA $p < 0.05$, Table 13). When pair-wise tests were performed, results indicated that TOE ships were significantly different between the two coasts ($p < 0.05$). They also indicated that ships with ballast water exchange (TOE and CE) on the East coast had significantly greater concentrations of non-indigenous species of dinoflagellates than CNE ships ($p = 0.0004$; $p = 0.0037$; respectively).

In this region, BWE seems to increase the number of taxa of all dinoflagellates irrespective of whether they are harmful or non-indigenous. The concentrations and taxonomic richness of non-indigenous dinoflagellates did not vary among ship categories on the West coast. However CNE ships showed a slightly greater (although not significant) number of taxa than the other two categories (Figure 21 e). For the East coast, the actual propagule pressure was correlated positively with the percent of ballast water exchanged (relative to total tank volume) (Spearman $r = 0.52$, $p = 0.0001$). For the West coast, the actual propagule pressure was correlated negatively with ballast water age (Spearman $r = -0.25$, $p < 0.05$).

Effective propagule pressure

The concentration of potentially viable dinoflagellates in the ballast water to be discharged per ship was used to estimate the effective propagule pressure of ships visiting both coasts.

When all dinoflagellates per ship were considered, the two-way crossed design PERMANOVA revealed significant differences between coasts ($p = 0.0001$). There was also a significant interaction between coast and categories ($p < 0.05$). However, statistical results did not reveal differences among categories of ships. Pair-wise tests revealed that the two marine coasts were significantly different for TOE ships and for CE ships ($p = 0.0002$; $p = < 0.05$) (Table 13). These tests also revealed significant differences between TOE ships and CNE ships for both coasts ($p = <0.05$).

For harmful/toxic species per ship, the two-way crossed design PERMANOVA indicated significant differences between coasts ($p = 0.0001$) and also a significant interaction between coasts and categories of ships ($p < 0.05$). Pair-wise tests revealed that the two marine coasts were significantly different for TOE ships and CE ships ($p = 0.0001$; $p = < 0.05$). For the West coast, TOE ships were significantly different compared to continental ships irrespective of whether or not they performed BWE ($p < 0.05$ and $p = 0.005$ for TOE compared with respectively CE and CNE). On the East coast statistical analyses did not show differences among categories of ships.

For NIS of dinoflagellates per ship, the two-way crossed design PERMANOVA indicated significant differences between coasts ($p < 0.05$) and also a significant interaction between coasts and categories ($p = 0.0004$). Pair-wise tests revealed that the two marine coasts were significantly different for TOE ships and for CNE ships ($p = 0.0008$; $p = < 0.05$). They showed no significant difference among categories of ships on the West coast whereas on the East coast, ships with ballast water exchange (TOE and CE) had significantly greater concentrations of non-indigenous species of dinoflagellates than CNE ships ($p = 0.0007$; $p = 0.0015$; respectively).

Finally, on the East coast the effective propagule pressure was positively correlated with the percent of ballast water exchanged (relative to total tank volume) (Spearman $r = 0.54$, $p = 0.0001$). On the West coast, the effective propagule pressure showed a significant positive correlation with ballast water salinity (Spearman $r = 0.22$, $p = 0.01$ and a negative correlation with ballast water age (Spearman $r = -0.30$, $p = 0.001$).

Table 13. Results of PERMANOVA crossed tests and pair-wise tests for a) actual propagule pressure for non-indigenous species of dinoflagellates and b) effective propagule pressure for all dinoflagellates, harmful and toxic species and non-indigenous species of dinoflagellates. Values in bold indicate significant differences ($p < 0.05$), only cases with significant differences are shown. Co x Ca = Interaction term for the factors coast (Co) and ship category (Ca). P(Perm) = permutation P-value ; P(MC) = Monte Carlo asymptotic P-value (see Anderson et al. 2008).

a) Actual propagule pressure							
All dinoflagellates per tank	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Coast	1	2.4828	7.311	0.0001	9930	**0.0001**
	Category	2	1.5523	2.2857	0.0242	9933	**0.0234**
	Co x Ca	2	0.5082	0.7483	0.6448	9917	0.6456
	Residual	128	43.465				
	Total	133	48.008				
Pair-wise tests							
	Groups	t	P(perm)	Perms	P(MC)		
Within level TOE	WC, EC	2.371	1.2694	9945	**0.0014**		
CE	WC, EC	1.1929	0.2106	9954	0.2178		
CNE	WC, EC	1.5758	0.1636	9943	0.0852		
Pair-wise tests							
Within level EC	Groups	t	P(perm)	Perms	P(MC)		
	TOE, CNE	1.7333	0.0133	9937	**0.0164**		
	CE, TOE	1.0467	0.3556	9947	0.3559		
	CNE, CE	0.8250	0.0133	9938	0.6233		
HA dinoflagellates per tank	Souree	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Coast	1	3.7309	11.901	0.0001	9953	**0.0001**
	Category	2	1.7140	2.7338	0.0125	9927	**0.0143**
	Co x Ca	2	0.6203	0.9893	0.4179	9949	0.4202
	Residual	128	40.126				
	Total	133	46.620				
Pair-wise tests							
	Groups	t	P(perm)	Perms	P(MC)		
Within level TOE	WC, EC	3.3055	0.0002	9938	**0.0001**		
CE	WC, EC	1.4063	0.098	9944	0.1021		
CNE	WC, EC	1.6901	0.0435	9939	**0.0429**		
Pair-wise tests							
Within level WC	Groups	t	P(perm)	Perms	P(MC)		
	TOE, CNE	2.0390	0.0191	9691	**0.0174**		
	CE, TOE	2.3153	0.0065	9633	**0.0071**		
	CNE, CE	0.5854	0.8007	9939	0.7839		
NIS dinoflagellates per tank	Souree	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Coast	1	0.4288	1.4153	0.2143	9948	0.2214
	Category	2	1.6353	2.6981	0.0226	9948	**0.0222**
	Co x Ca	2	1.7582	2.901	0.0179	9940	**0.0175**
	Residual	128	38.789				
	Total	133	42,611				
Pair-wise tests							
	Groups	t	P(perm)	Perms	P(MC)		
Within level TOE	WC, EC	2.0829	0.0094	4988	**0.0102**		
CE	WC, EC	0.75046	0.6306	4981	0.6148		
CNE	WC, EC	1.5758	0.0796	4560	0.0852		

Pair-wise tests							
Within level EC	Groups	t	P(perm)	Perms	P(MC)		
	TOE, CNE	2.4876	0.0039	9829	**0.0037**		
	CE, TOE	1.0864	0.3169	9951	0.3042		
	CNE, CE	2.7926	0.0002	9883	**0.0004**		
b) Effective propagule pressure							
All dinoflagellates per ship	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Coast	1	2.7584	8.3235	0.0001	9947	**0.0001**
	Category	2	1.0795	1.6286	0.1211	9931	0.1205
	Co x Ca	2	1.5653	2.3617	0.0233	9943	**0.0246**
	Residual	128	42.42				
	Total	133	47.823				
Pair-wise tests							
	Groups	t	P(perm)	Perms	P(MC)		
Within level TOE	WC, EC	2.8297	0.0004	9951	**0.0002**		
CE	WC, EC	1.7616	0.0213	9944	**0.0214**		
CNE	WC, EC	1.3792	0.1078	9927	0.1177		
Pair-wise tests							
Within level EC	Groups	t	P(perm)	Perms	P(MC)		
	TOE, CNE	1.5531	0.0806	9932	**0.0500**		
	CE, TOE	1.0208	0.371	9951	0.3822		
	CNE, CE	1.458	0.0806	9952	0.0753		
Within level WC	Groups	t	P(perm)	Perms	P(MC)		
	TOE, CNE	1.7333	0.0503	9951	**0.049**		
	CE, TOE	1.5672	0.0748	9931	0.0701		
	CNE, CE	0.9552	0.3972	9968	0.4165		
HA dinoflagellates per ship	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Coast	1	3.8561	12.536	0.0001	9945	**0.0001**
	Category	2	1.1462	1.8632	0.0802	9925	0.0807
	Co x Ca	2	1.3975	2.2717	0.0336	9943	**0.0333**
	Residual	128	39.371				
	Total	133	45.771				
Pair-wise tests							
	Groups	t	P(perm)	Perms	P(MC)		
Within level TOE	WC, EC	3.5629	0.0001	9924	**0.0001**		
CE	WC, EC	1.9215	0.0138	9951	**0.0169**		
CNE	WC, EC	1.3535	0.1311	9955	0.1291		
Pair-wise tests							
Within level EC	Groups	t	P(perm)	Perms	P(MC)		
	TOE, CNE	1.1008	0.2913	9947	0.2982		
	CE, TOE	0.9048	0.4992	9952	0.4966		
	CNE, CE	1.1896	0.2205	9936	0.2250		
Within level WC	Groups	t	P(perm)	Perms	P(MC)		
	TOE, CNE	2.3627	0.0067	9545	**0.0054**		
	CE, TOE	2.0045	0.0235	8073	**0.0259**		
	CNE, CE	0.89337	0.46	9942	0.4596		
NIS dinoflagellates per ship	Source	DF	SS	PseudoF	P(perm)	perms	P(MC)
	Coast	1	0.7930	2.7866	0.0467	9952	**0.0492**
	Category	2	1.2098	2.1256	0.0601	9948	0.0631
	Co x Ca	2	2.8548	5.0158	0.0005	9931	**0.0004**
	Residual	128	36.426				
	Total	133	45.284				
Pair-wise tests							
	Groups	t	P(perm)	Perms	P(MC)		

Within level TOE	WC, EC	2.8257	0.0002	9947	**0.0008**
CE	WC, EC	1.3746	0.1289	9948	0.1395
CNE	WC, EC	1.7504	0.0438	7746	**0.0468**
Pair-wise tests					
Within level EC	Groups	t	P(perm)	Perms	P(MC)
	TOE, CNE	2.8419	0.0013	9878	**0.0007**
	CE, TOE	1.3976	0.1036	9942	0.1081
	CNE, CE	2.7334	0.0011	9799	**0.0015**
Within level WC	Groups	t	P(perm)	Perms	P(MC)
	TOE, CNE	1.4649	0.1230	9593	0.1145
	CE, TOE	1.2709	0.1970	8922	0.1873
	CNE, CE	0.8391	0.5015	9847	0.5084

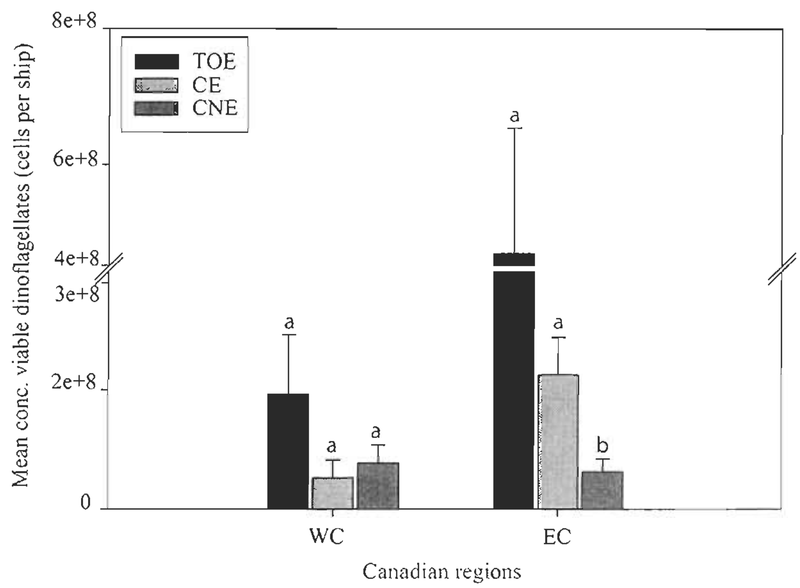

Figure 22. Mean concentration of viable dinoflagellates per ship arriving to the West coast (WC) and the East coast (EC) of Canada. These results are from ship samples containing live dinoflagellates that were incubated in culture media. Dinoflagellate concentrations were multiplied by the volume of the ballast water to be discharged per ship, at receiving ports. Bars represent the standard error (as mentioned in methods the survival and growth experiments were carried out in triplicate from cultures with at least 1000 cells·L^{-1}, and left to grow under the same chamber conditions). Categories of ships that differ significantly are indicated with different letters above data bars.

Estimates of viable cell concentrations are conservative because the live samples were only 500 mL (while preserved samples came from 13 L sieved on board ship and concentrated in 250 mL) and because the growth conditions (culture medium, salinity, temperature, irradiance) were perhaps not ideal for all cells found in ballast tanks. To confirm that cells were still viable when ships arrived at ports, we did growth experiments with nine indigenous autotrophic species (only one species was considered as non-indigenous) (Figure 24). Within seven days, these species reached concentrations ranging between 8.34×10^4 cells L^{-1} for *Gyrodinium resplendens* and 5.92×10^7 cells L^{-1} for *Gyrodinium estuariale* (Figure 23). Species that grew under laboratory conditions are noted in Table 1. These growth experiments confirmed that some dinoflagellates species were not only still alive when ships arrived in ports but they were also capable of growth and reproduction.

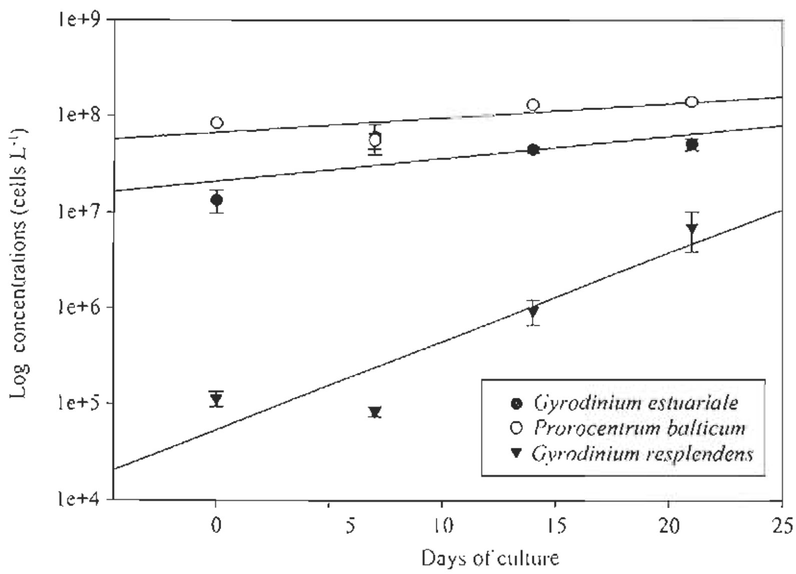

Figure 23. Survival growth curves (Log concentrations (cells·L^{-1}) of two indigenous species (*Gyrodinium estuariale* and *Prorocentrum balticum*) and one non-indigenous species (*Gyrodinium resplendens*), from unfiltered and live ballast water samples. Bars represent the standard error and lines indicate linear regressions between Log of cell concentrations and days of culture.

When cell concentrations of non-indigenous dinoflagellates per Liter were multiplied by the volume of water to be discharged per ship, the number of potentially viable cells that may have been discharged was calculated to range between 0.0 to 6.75 x 10^9 cells per ship (Table 14). For the East coast, concentrations of the non-indigenous species were significantly higher in ships performing BWE (TOE and CE) than in CNE ships (Table 8b) (PERMANOVA $p = 0.0007$ and $p = 0.0015$). Although the concentrations of non-indigenous species did not differ significantly among ship categories on the West coast, there was a trend for increased concentrations of non-indigenous species in CNE ships (mean ± SE, 7.25 x10^7 ± 3.5 x 10^7) compared to ships performing BWE (Table 14).

Table 14. Statistics for the cells per ship concentrations of non-indigenous species of dinoflagellates arriving to the West coast (WC) and the East coast (EC) of Canada. Asuming that the tanks of ships had viable species in their tanks during discharge operations we calculated concentations of non-indigneous dinoflagellates per ship by the volume of ballast water to be discharged at receiving ports.

Statistic	TOE	CE	CNE
West coast			
n	28	20	23
Minimum	0.00 E+00	0.00 E+00	0.00 E+00
Maximum	5.85 E+08	5.46 E+08	7.31 E+08
Mean	3.21 E+07	4.23 E+07	7.25 E+07
SD	1.13 E+08	1.27 E+08	1.68 E+08
SE of mean	2.14 E+07	2.83 E+07	3.50 E+07
East coast			
n	22	22	19
Minimum	0.00 E+00	0.00 E+00	0.00 E+00
Maximum	6.75 E+09	2.02 E+09	1.06 E+08
Mean	1.26 E+09	1.47 E+08	6.18 E+06
SD	2.18 E+09	4.26 E+08	2.43 E+07
SE of mean	4.66 E+08	9.09 E+07	5.57 E+06

Discussion

This study evaluated the actual and effective propagule pressures for dinoflagellate cells delivered to Canadian coastal ecosystems from two regions in Canada, Vancouver on the West coast, and several ports on the Eastern seaside, including in the Gulf of St. Lawrence. Given the large volume of ballast water discharged by ships visiting ports located in the marine coasts of Canada, it is evident that the practice of deballasting frequently introduces massive quantities of harmful and non-indigenous species to Canadian marine ecosystems. However, the relative contribution from transoceanic and coastal pathways to this propagule pressure varies between coasts, largely as a function of routes, BWE, and voyage duration.

For the West coast, there is no significant difference among ship categories for actual and effective PP (all dinoflagellates and NIS). However, effective PP was negatively correlated with ballast water age in that region. Hence, ships with shorter trip duration after ballast water exchange had more non-indigenous motile cells per tank. For the East coast, concentrations of dinoflagellates per ship were significantly greater in transoceanic ships compared to coastal ships without BWE. Our results also show no evidence that ballast water exchange is efficient at reducing the introduction of non-indigenous dinoflagellates, since there is no significant difference among ship categories on the West coast, and on the East coast, ships which performed BWE had significantly greater concentrations of dinoflagellates, suggesting an input associated with the ballast water exchange. In North America, several other studies have also assessed the invasion pressure risk associated with ballast water discharge. The main findings of these works are the importance of ships arrival rate (Drake and Lodge 2004), discharge volumes (McGee et al. 2006) and short transit times (Smith et al. 1999). Others have concluded that large numbers of arrivals does not necessarily mean large ballast discharge volumes, and the sole use of ship discharge volume is not a good predictor of propagule pressure to assess invasion risk (Lawrence and Cordell 2010). Thus, the major factors that could explain the variability in propagule pressurein these studies are the route types (Lo et al. 2012; Villac and Kaczmarska 2011), with size and frequency of NIS inoculations (Lawrence and Cordell 2010).

Actual propagule pressure

Motile dinoflagellate cells were identified and enumerated in 80% of ships sampled (20% of ships showed no dinoflagellates). TOE ships arriving to the East coasts of Canada contained greater volumes of ballast water per tank relative to CE and CNE ships, increasing the actual propagule pressure. However, there were no significant differences in the concentrations of all dinoflagellates among ship categories on the West coast. Hence all ship categories examined represent an invasion risk for this type of organisms, for this region. For non-indigenous dinoflagellate species in ballast water, the patterns of introduction differed between the two coasts, as evidenced by the significant interaction between coasts and ship categories in the PERMANOVA tests. Whereas the concentration of dinoflagellates in ballast water did not differ among ship categories on the West coast, it did on the East coast such that ships that undertook ballast water exchange (TOE and CE) had significantly greater concentrations of dinoflagellates than did CNE ships. These BWE ships also carried the greatest number of non-indigenous taxa. Hence in this particular case, the exchanged waters were apparently the source of NIS. These results show some similarity with those of Cordell et al. (2009), where BWE had no significant influence on coastal zooplankton species but increased the abundance of oceanic zooplankton species found in trans-Pacific ships. Several non-indigenous species for the East coast found in this work are from the genus *Dinophysis*, which has an oceanic distribution and thus may be taken up during the ballast water exchange (Roy et al. 2012). Carver and Mallet (2002) also indicated that BWE increases the risk of introducing non-indigenous phytoplankton taxa to Eastern Canada.

The two coasts of Canada are continuously being inoculated by a diverse assemblage of dinoflagellates, including harmful or toxic and non-indigenous species, transported from around the world. These inoculations occur frequently and represent considerable concentrations of dinoflagellates. For example, during the 12-month period between 2006 and 2007 (when samples were collected for the present study) more than two thousand bulk carriers, oil tankers and general cargo ships arrived in ballast to the East and West coast ports (Lo et al. 2012). Based on our data, a single ship could contain and discharge more than a million litres of foreign ballast water (from overseas or from USA ports), greatly increasing actual propagule pressure.

Effective propagule pressure

Live motile dinoflagellate cells were recorded in at least 25% of the ships sampled during the present study, ships which discharged their ballast water on Canada's East and West coasts. On the East coast, Burkholder et al (2007) showed that the relative abundance of viable dinoflagellates was significantly greater in ship categories with BWE, relative to coastal ships without BWE. For diatoms, Villac and Kaczmarska (2011) showed that propagule pressure differed significantly between ship categories with BWE and coastal ships without BWE on the East coast, but not among ship categories on the West coast. Their results also showed that ships without BWE represented a potential risk for invasion to the East coast based on their great frequency of arrival even though they had relatively low diatom cell concentrations, whereas TOE and CE ships were less frequent but had greater cell concentrations. In contrast, our results showed that ships with BWE (irrespective of routes, transoceanic or coastal) also present an invasion risk with significantly greater concentrations of non-indigenous dinoflagellates per ship than do CNE ships. One reason for this could be that BWE was incompletely conducted. In this case, laws should be enforced and BWE systematically verified. Another reason could be that ships sampled in our study belonged to different types of ships, which had different cargo capacities (gross tonnage) and which can discharge larger volumes of ballast water (i.e. some bulk carriers relative to some tankers) (Verling et al. 2005). The number of individuals introduced by the discharge of ballast water is affected not only by the volumetric concentration of organisms per tank but also by the total volume of ballast water which is discharged. There is also a positive relationship between the number of individuals introduced and the number of species of dinoflagellates (Briski et al. 2012), so the risk of introducing unwanted species grows with the discharge volume.

Ship type, ballast water capacity and the volume routinely carried on board show different trends between the East and West coasts of Canada and may also differ among trade routes within ship type (Verling et al. 2005). For example, on the West coast, all CNE ships were bulk carriers whereas they were mostly oil tankers on the East coast.

Additionally, East coast CNE tankers had low concentrations and numbers of non-indigenous dinoflagellate taxa per ballast tank. This may be because this type of ships transports less ballast water on board than TOE ships (mostly bulk carriers), which typically discharge four-times more ballast water than other categories of ships (Verling et al. 2005). In our study coastal ships discharged half the ballast volume of TOE ships. Lower concentrations of non-indigenous dinoflagellate taxa in CNE tankers may also be due to the proximity of ballast water sources and destinations such that few taxa would be considered as being non-indigenous species.

This study found no evidence that undertaking BWE reduced the concentration and number of non-indigenous dinoflagellate species that may be introduced with deballasting. On the East coast, ships that undertook BWE had significantly greater concentrations of non-indigenous dinoflagellate species as well as a larger number of taxa than non-exchanged ships. Our results concur with those of Carver and Mallet (2002) who observed an increased risk of non-indigenous phytoplankton taxa introduction in Eastern Canada when ships had undertaken BWE. On the West coast, the number of non-indigenous taxa did not vary among ship categories although there was a trend for increased numbers within CNE ships. Cordell et al. (2009) and Lawrence and Cordell (2010) found that coastal ships on the USA's West coast had greater numbers of high-risk taxa than did transoceanic ships. Several of the ships visiting the port of Vancouver come from overseas but first do a stopover in one of the West coast ports of the USA, hence these ships are then considered intra-coastal when they arrive to Canada from US West coast ports, increasing the risk of invasion through secondary spread. Indeed, these coastal routes should be a high priority for management of ballast water to reduce the risks of secondary invasions of non-indigenous species to Canadian West coast ports, as also noted by Humphrey (2008).

To reduce the potential introduction of NIS, most ships exchanged their ballast water using one of two methods: empty-refill or flow-through. For the first method, tanks are emptied and then refilled with oceanic water, to replace approximately 100% of the ballast tank volume. In the second method, water is pumped with a continuous overflow, replacing the original water by about 300% of the tank volume and thus removing greater than 90% of the organisms from the port source (Rigby and Hallegraeff 1994; Ruiz and Reid 2007; Simard et al. 2011). Although the present study did not detect significant differences between these BWE methods for either coast, the percentage of BWE seemed to affect actual and effective propagule pressure differently in the two regions. Surprisingly, for the East coast, we found a significant positive correlation between actual propagule pressure and the percentage of ballast water volume exchanged. These results contrast with the findings of Rigby and Hallegraeff (1994), Wonham et al. (2001) and Levings et al. (2004) who have shown the efficacy of BWE at reducing the abundance of NIS. For the West coast, both actual and effective propagule pressure were negatively correlated with the ballast water age. For West coast ships, the flow-through method seemed to be more efficient for reducing all and non-indigenous dinoflagellate species from ballast tanks (low abundances and number of taxa). Hence, while some studies have pointed out the relative inefficacy of BWE, especially for phytoplankton species (Ruiz and Reid 2007), it remains the best method to replace or remove original coastal water and reduce coastal non-indigenous species. However, it should be recognized that it may also contribute to the introduction of oceanic taxa, as reported previously by Carver and Mallet (2002), Simard et al. (2011), and more recently by Roy et al. (2012), particularly for eastern Canada.

In the present study significant relationship between the ballast water age and both measures of propagule pressure (actual and effective) was negative on the West coast, indicating that ballast water age has a negative effect on cell viability. In fact, all ship categories in this region undertook longer voyage duration and had lower concentrations of viable dinoflagellates than did East coast ships. Thus, shorter voyage duration could increase the abundances of viable cells. Verling et al. (2005) and Cordell et al. (2009) also found that domestic ships, which had much shorter voyages (2–3 days) than transoceanic ships (12–14 days), thus reducing mortality. Moreover, they observed greater reductions in plankton abundances during transoceanic voyages than during either Pacific or Atlantic coastal voyages. In fact, short transit time (<15 days) is probably associated with the presence of substantial numbers of live planktonic organisms, thus increasing invasion pressure, as previously reported by Smith et al. (1999). In the present study, for the East coast, although ships underwent BWE some still contained maximum values of viable dinoflagellates of non-indigenous species up to 2.0 x 10^9 cells per ship. Hence, shipping remains an important vector for transporting great quantities of harmful and non-indigenous dinoflagellates to Canada.

Although our results show that salinity was significant and positive correlated with actual propagule pressure on the East coast and with the effective propagule pressure on the West coast, patterns are not clear. Salinity shocks have been used to reduce the concentrations of viable organisms delivered by ballast waters in freshwater environments because it creates as a mismatch between donor and receiving systems (Smith et al. 1999; Ruiz and Reid 2007). This approach has been useful in reducing the number of species, particularly freshwater ones, transported by ships (e.g., for the Great Lakes region) (US Coast Guard 1993; Transport Canada 2006). Our results showed that these changes in salinity had no significant effect in most cases except for coastal ships without BWE, which had the lowest salinity values on the West coast (12 ± 3), potentially accounting for the lowest concentrations of all dinoflagellates per volume in this ship category and region (data not shown). This effect was less evident when concentrations of dinoflagellates (for all, harmful, and NIS) were considered per tank or per ship.

In summary, the present study shows that commercial shipping is an important vector and source of actual and effective propagule pressure for dinoflagellates. For both coasts, transoceanic ships contribute most to actual propagule pressure, introducing more species of dinoflagellates per tank, even if they have performed BWE. As hypothesized, our results also show that CE ships on the East coast and CNE ships on the West coast contained and may discharge non-indigenous species of dinoflagellates, influencing both actual and effective propagule pressure. The present study examined the potential release of viable individuals into a non-native environment as a measure of effective propagule pressure. Although testing the viability of all motile cells observed microscopically or if they may survive in the new environment was beyond the scope of the present work, it did show that a large number of motile dinoflagellate cells are viable when ships arrive in coastal Canadian ports and even possibly during deballasting operations. Factors such as the length of voyage and ballast water conditions (e.g., salinity or temperature) or even location of ballast water exchange should also be taken into account in the management of ballast water, in order to minimize new introductions of non-indigenous species of dinoflagellates. Other studies have also demonstrated the viability of dinoflagellates in ballast tanks using incubation experiments (Sutherland et al. 2001; Burkholder et al. 2007). In fact, the successful cultivation of motile cells of dinoflagellates in our laboratory experiments suggests that if environmental conditions are favourable, these species could eventually reproduce and/or establish in the coastal marine environments of the East and West coasts of Canada. For some non-indigenous species however, temperature could be a limiting factor, influencing their survival in temperate systems, particularly during winter.

ACKNOWLEDGEMENTS

We thank shipping companies, crews and port authorities to have allowed sampling of the ballast tanks. This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Aquatic Invasive Species Network, Fisheries and Oceans Canada, Université du Québec à Rimouski, Institut des Sciences de la Mer de Rimouski and Québec-Océan. We are grateful to the sampling teams on both coasts and for taxonomic assistance from Sylvie Lessard.

CONCLUSION GÉNÉRALE

Cette recherche fut principalement motivée par l'augmentation du transfert d'espèces non-indigènes entre différentes régions du monde (Ruiz et al. 2000; Carlton 2007) et la méconnaissance du nombre d'espèces et de l'abondance de dinoflagellés (formes enkystées et cellules végétatives) introduites par les navires commerciaux qui visitent les côtes est et ouest canadiennes ainsi que le réseau des Grands Lacs. Ainsi, le but du présent travail est de *mieux comprendre le rôle des vecteurs, les patrons d'invasion selon la région et l'influence des différentes méthodes d'échange de l'eau de ballast, dans l'introduction des espèces non indigènes incluant les espèces nuisibles ou toxiques de dinoflagellés au Canada*. Plusieurs des résultats nouveaux obtenus dans le cadre de la présente étude contribuent à ces objectifs et les résultats les plus significatifs sont soulignés dans les paragraphes qui suivent.

Le premier chapitre constitue la première étude au Canada à regarder en détail les espèces de kystes de dinoflagellés présentes dans les sédiments de ballast. Ce chapitre montre que des kystes potentiellement viables sont présents et peuvent être introduits via les sédiments des réservoirs de ballast de navires qui visitent la côte est du Canada. Les concentrations (par g de sediment sec) de tous les types de kystes de dinoflagellés incluant les kystes viables des espèces non-indigènes étaient plus élevées dans les navires côtiers qui ne font pas d'échange de leurs eaux de ballast que dans les sédiments des navires avec échange, y compris les navires transocéaniques. Ces derniers présentent le plus faible risque d'introduction d'espèces non-indigènes par les sédiments de ballast. La présente étude a permis de constater que les quantités de sédiments dans les réservoirs de ballast ne sont pas proportionnelles aux concentrations de kystes. Par exemple, les navires continentaux avec échange ont des volumes de sédiments plus importants que les navires des deux autres catégories, mêmes s'ils ont des concentrations plus faibles de kystes viables de dinoflagellés non-indigènes, ce qui suggère qu'ils représentent un risque aussi important que les autres catégories, pour les environnements côtiers. Une contribution majeure de l'étude est d'avoir identifié 14 espèces de kystes de dinoflagellés non-indigènes dans les sédiments des navires qui visitent la côte est du Canada. Ces espèces n'ont pas encore été répertoriées pour l'est du Canada. Parmi ces 14 espèces, quatre sont considérées comme toxiques ou nuisibles, et comportent donc un risque pour les écosystèmes canadiens et la santé humaine.

Le deuxième chapitre constitue une étude pionnière au Canada portant sur la comparaison des patrons d'introduction d'espèces de kystes de dinoflagellés contenus dans les sédiments de ballast des navires qui visitent les côtes est et ouest, ainsi que les Grands Lacs. Sur la côte est, ce sont les navires qui ne font pas d'échange qui comportent le plus grand risque d'introduction d'espèces non-indigènes de kystes de dinoflagellés. Pour la côte ouest et les Grands Lacs, les trois catégories représentent le même risque d'introduction d'espèces non-indigènes. Ainsi des mesures de contrôle devraient être prises afin de limiter leur introduction, plus spécialement si ces espèces sont également nuisibles ou toxiques. De plus, pour la côte ouest les navires avec échange des eaux de ballast ne montrent pas des concentrations de kystes d'espèces non-indigènes de dinoflagellés significativement moins élevées que ceux qui ne font pas d'échange. Ces résultats suggèrent que les échanges des eaux de ballast s'avèrent inefficaces pour réduire les concentrations d'espèces indésirables dans les sédiments de ballast pour cette région.

Le troisième chapitre montre que les routes transocéaniques et côtières agissent comme des vecteurs de la pression actuelle et effective des cellules végétatives de dinoflagellés sur les écosystèmes marins des côtes est et ouest canadiennes. Pour la côte est, les voyages transocéaniques influencent la pression actuelle et la pression effective, et ce, même si tous les navires font un échange de leurs eaux de ballast. Ces navires montrent une augmentation du nombre d'espèces des dinoflagellés ainsi que de celui des espèces non-indigènes, comparativement aux autres catégories de navires. Pour la côte ouest, il n'y pas de différences entre les voyages transocéaniques et les voyages côtiers. Les deux types de routes influencent la pression actuelle ainsi que la pression effective de propagules. Cependant, les voyages transocéaniques contiennent plus d'espèces viables de dinoflagellés que les navires côtiers. Ces résultats peuvent être reliés à un plus grand volume d'eau transporté dans leurs réservoirs de ballast. Pour la côte est, les concentrations élevées d'espèces viables pourraient être expliquées par une durée plus courte des voyages. Par exemple, les navires transocéaniques présentent en moyenne une durée de voyage de ≈ 11 jours entre un port de l'Europe et un port sur la côte est de l'Amérique du Nord, dû possiblement à une meilleure performance des navires modernes. De plus, il a été constaté que sur la côte est, les navires qui font des échanges d'eau de ballast contribuent davantage à la pression de propagule effective que les navires qui font des échanges sur la côte ouest. Pour cette dernière région, la catégorie des navires CNE présente un risque plus élevé d'introduction de dinoflagellés non-indigènes viables que la même catégorie de navires CNE sur la côte est, possiblement relié à une différence dans le type de navires échantillonnés (vraquiers sur la côte ouest, navires-citernes sur la côte est).

Ces résultats novateurs contribuent substantiellement à la compréhension des patrons d'introduction et de la pression d'invasion d'espèces non-indigènes de dinoflagellés via le transport maritime dans les écosystèmes aquatiques canadiens. Les implications, limitations et perspectives qui découlent de nos résultats sont discutées dans les paragraphes qui suivent.

Portée des résultats de la thèse

Plusieurs impacts possibles des introductions d'espèces non-indigènes pour les écosystèmes aquatiques canadiens furent présentés dans la section Introduction Générale. Un des plus importants impacts de ces introductions d'espèces non-indigènes au pays est celui relatif au rôle potentiel joué par l'utilisation des différentes routes par les navires. Tel que noté par Carlton (1995), le transport transocéanique a toujours été considéré comme l'une des plus importantes voies d'introduction d'espèces non-indigènes. D'ailleurs, afin de réduire ce transfert d'espèces, des méthodes d'échange d'eau de ballast au milieu de l'océan ont été mises en place suite à l'adoption de la Convention internationale maritime sur le contrôle et la gestion des eaux et des sédiments de ballast (IMO 2004). Cependant, des études récentes ont mis en évidence que les routes côtières peuvent être considérées comme des voies aussi importantes que les routes transocéaniques pour le transfert d'espèces non-indigènes (Cordell et al. 2009; Simkanin et al. 2009). Les résultats de la présente étude montrent que les concentrations les plus élevées de kystes non-indigènes viables, par gramme de sédiment sec ou par réservoir, ont été observées dans les sédiments des navires côtiers, qui visitaient la côte est (chapitres 1 et 2). Pour la côte ouest et les Grands Lacs, malgré qu'il n'y ait pas de différence significative entre les catégories de navires, les navires côtiers introduisent des concentrations de kystes viables d'espèces non-indigènes aussi élevées que les navires transocéaniques. Néanmoins, une différence importante entre les routes de chacune des régions étudiées a été remarquée. Dans l'est du pays, les navires côtiers font régulièrement des voyages le long de la côte est entre les ports des États-Unis et du Canada. Par contre, sur la côte ouest, dans la plupart des cas les navires côtiers ont visité un port asiatique avant d'arriver aux États-Unis, pour ensuite transiter vers les ports canadiens. Cette différence de routes entre les régions pourrait être à l'origine d'un transfert plus important d'espèces indésirables sur la côte est et elle pourrait expliquer la similarité entre les catégories de navires sur la côte ouest.

D'autres résultats de la thèse semblent pertinents à ce sujet. Étant donné l'influence du transport côtier dans l'introduction d'espèces non-indigènes, ce trafic contribue largement à la propagation secondaire, ou le transfert d'espèces non-indigènes depuis des régions aux États-Unis déjà envahies comme Chesapeake Bay sur la côte est ou la baie de San Francisco sur la côte ouest, ce qui semble être le cas pour l'espèce *Cochlodinium polykrikoides*. Cette espèce qui est originaire des mers asiatiques a causé des dommages économiques et écologiques importants à l'industrie aquicole au Japon. Elle a été identifiée dans au moins 14% des navires arrivant à la côte ouest et 17% des navires arrivant à la côte est (indépendamment des routes utilisées par les navires). Cet exemple pourrait être considéré comme un indice de l'étendue de la distribution et de la propagation de cette espèce (Matsuoka et al. 2008; Kudela et al. 2008).

Une autre avancée importante de la thèse correspond à l'effet des échanges d'eaux de ballast sur les organismes contenus dans les sédiments. Bien qu'actuellement ce soit le seul moyen accepté et suggéré (même parfois requis) afin de réduire et de prévenir l'introduction d'espèces non-indigènes, des études récentes montrent qu'il s'avère inefficace dans bien des cas (McCollin et al. 2007; Cordell et al. 2009) et ce, même pour les organismes contenus dans l'eau des réservoirs (chapitre 3). La présente étude montre que pour les sédiments, les échanges d'eau ont possiblement contribué aux faibles concentrations de kystes d'espèces non-indigènes retrouvées dans les navires TOE et CE qui sont arrivés dans l'est du pays. Pour leur part, les navires sans échange d'eau (CNE) présentent plus d'espèces non-indigènes avec des kystes potentiellement viables. Sur la côte ouest, les navires avec et sans échange présentaient des concentrations similaires de kystes d'espèces non-indigènes viables, ce qui suggère non seulement que les échanges d'eau de ballast ont été inefficaces, mais que des mesures de contrôle plus serrées devraient être considérées afin de réduire ce transfert d'espèces.

Ces observations sur l'efficacité des échanges d'eau de ballast ont aussi été analysées lors de notre étude sur la pression de propagules. Sur la côte est, les navires qui font un échange présentent des valeurs de pression de propagule actuelle et effective plus élevées que les navires côtiers qui n'échangent pas leurs eaux de ballast. Carver et Mallet (2002), et plus récemment Roy et al. (2012), ont également observé que les échanges d'eau de ballast contribuent davantage à l'introduction d'espèces non-indigènes dans cette région. Sur la côte ouest, malgré qu'il n'y ait pas de différence significative dans les valeurs de pression de propagules actuelle ou effective entre les catégories de navires, les navires côtiers sans échange présentent le plus grand nombre d'espèces non-indigènes de dinoflagellés. À la lumière de ces résultats, sur la côte ouest les trois catégories de navires agissent comme des voies d'introduction d'espèces non-indigènes mais aussi d'espèces nuisibles ou toxiques dans les écosystèmes aquatiques canadiens tant pour le vecteur eau que pour le vecteur sédiment.

Cette recherche a montré qu'au moins 25% de tous les navires contenaient des espèces potentiellement viables dans les eaux de ballast lors de l'échantillonnage et possiblement lors de la décharge des eaux de ballast dans les ports canadiens (voir chapitre 3). Ceci équivaudrait à au moins $1{,}6 \times 10^6$ cellules d'espèces non-indigènes viables par navire qui seraient introduites via les navires côtiers sans échange d'eau de ballast (CNE). Cette catégorie a été décrite précédemment comme celle où les concentrations des cellules de dinoflagellés par navire taient les moins élevées par navires. Ces valeurs restent toutefois sous-estimées, compte tenu du volume annuel du trafic maritime (Lo et al. 2011).

Cette recherche a identifié 14 espèces de kystes de dinoflagellés non-indigènes, contenues dans les sédiments de ballast des navires visitant les côtes canadiennes. Pour les Grands Lacs les 33 espèces de kystes de dinoflagellés identifiées ont été considérées comme non-indigènes et d'origine marine, donc leurs chances de survie dans un milieu d'eau douce sont faibles. Concernant l'eau de ballast, la présente recherche a identifié 33 espèces de dinoflagellés non-indigènes (cellules végétatives) pour la côte ouest et 39 espèces non-indigènes pour la côte est. Plusieurs de ces espèces non-indigènes appartiennent à des groupes taxonomiques reconnus pour être producteurs de toxines, tels que les genres *Alexandrium*, *Dinophysis*, *Gonyaulax*, *Gymnodinium* et *Prorocentrum*, ce qui a des répercussions importantes au niveau de la santé des écosystèmes, mais aussi de la santé humaine (Backer and McGillicuddy 2006).

Recommandations de recherche à poursuivre

Les résultats obtenus dans cette étude ont permis d'approfondir les connaissances concernant les vecteurs et les patrons d'invasions d'espèces non-indigènes de dinoflagellés. Ils ont aussi permis de soulever plusieurs questions concernant l'introduction d'espèces non-indigènes, leur viabilité et dispersion dans les écosystèmes canadiens. Certains aspects énoncés ci-dessous n'ont pas pu être traités dans le corps de cette thèse bien qu'ils auraient mérité une attention particulière.

Le dernier chapitre porte sur la pression de propagule actuelle et effective des formes végétatives de dinoflagellés sur les écosystèmes marins du Canada. De plus, ce chapitre montre une corrélation significative entre chaque mesure de pression de propagules avec des variables telles que l'échange des eaux de ballast, la salinité ou la durée du voyage. Ces corrélations illustrent le fait que le volume d'eau contenu dans les réservoirs et l'échange des eaux de ballast peuvent influencer les abondances des espèces, notamment celles potentiellement viables et considérées non-indigènes. Ces résultats sont en accord avec les résultats de l'étude de Briski et al. (2012) dans laquelle il a été démontré que le nombre de cellules de dinoflagellés introduites est positivement corrélé avec le nombre d'espèces de dinoflagellés introduites. Ceci souligne l'importance de mieux connaître les patrons de pression d'invasion des groupes phytoplanctoniques considérés à risque notamment les espèces nuisibles ou toxiques. Parmi les autres variables considérées dans cette thèse, la salinité et la durée des voyages peuvent aussi jouer un rôle important sur la pression d'invasion d'espèces non indigènes particulièrement sur leur viabilité. Tel que noté par Smith et al. (1999), le fait de changer la salinité peut influencer la survie des espèces marines, dulcicoles ou estuariennes étant donné que peu d'espèces supportent bien des changements drastiques de salinité. En effet, ces arguments ont été utilisés pour recommander des changements d'eaux de ballast pour réduire la présence des espèces non-indigènes dans les réservoirs de ballast, avant que cette eau ne soit déchargée dans des écosystèmes d'eau douce, par exemple le réseau des Grands Lacs (Dunggan et al. 2003). Dans la présente recherche, ces changements de salinité n'ont pas eu d'effets significatifs dans la majorité des cas, sauf pour les navires côtiers sans échange d'eau de ballast qui sont arrivés sur la côte ouest du Canada, pour lesquels la salinité moyenne montrait des valeurs de 12. Ces faibles valeurs de salinité expliqueraient les faibles concentrations de dinoflagellés (cellules par litre) pour l'ensemble des espèces de dinoflagellés, pour cette catégorie de navires.

Pour le vecteur sédiments de ballast, la présente recherche n'a pu faire ressortir de corrélation significative entre la durée des voyages et les concentrations de kystes viables de dinoflagellés. La formation de kystes de résistance dépend de plusieurs facteurs, notamment des conditions environnementales défavorables (lumière et/ou nutriments insuffisants). Dans bien des cas, ces kystes pourront demeurer viables pendant plus de 80 ans (Lundholm et al. 2011). L'oxygène joue un rôle important dans la germination des kystes (Anderson et al. 1987). Il peut être le facteur déclencheur de cette germination ou la prévenir en raison de teneurs réduites, comme c'est le cas pour les réservoirs de ballast. Plusieurs questions à ce sujet découlent de nos résultats par exemple, 1) est-ce que les kystes sont formés pendant les voyages? 2) est que les voyages plus longs (> 15 jours) contribuent davantage à une formation importante de kystes? Pour les voyages transocéaniques ou côtiers qui subissent un échange d'eau de ballast, est-ce que ces échanges peuvent favoriser une augmentation des teneurs en oxygène et par conséquent une augmentation dans le taux de germination des kystes? Puisqu'il y a souvent un déclin des teneurs en oxygène en raison de la consommation par différentes communautés biologiques dans les réservoirs de ballast lors des voyages transocéaniques (Klein et al. 2009; Seiden et al. 2011) est-ce que ce manque d'oxygène pourrait favoriser une formation des kystes de dinoflagellés? D'après nos résultats, les voyages plus courts peuvent favoriser la présence des kystes viables qui peuvent se déposer dans les couches de surface des sédiments au fond des réservoirs de ballast (Rochon et al. en prép.). La décharge de l'eau de ballast entraînant avec elle la décharge de 0.4 % des sédiments (Weise et al. en prép.) pourrait favoriser la décharge de ces kystes viables, augmentant ainsi l'introduction et le risque d'établissement d'espèces non-indigènes (incluant les espèces nuisibles ou toxiques) dans les écosystèmes aquatiques canadiens.

Une durée de voyage plus longue se traduit par une eau de ballast plus vieille. Généralement une eau plus vieille influence négativement la survie des espèces phytoplanctoniques qui dépendent notamment de la lumière (Rhee and Gotham 1981). La présente recherche a permis d'établir des corrélations significatives négatives entre la durée des voyages et les concentrations des formes végétatives de dinoflagellés. Ces résultats pourraient contribuer à expliquer pourquoi nos concentrations des dinoflagellés ont été plus faibles dans les navires arrivant à la côte ouest (voyages plus longs sur l'ensemble des catégories) que sur la côte est (voyages plus courts sur l'ensemble des catégories). Plusieurs études ont montré que la viabilité des cellules diminue avec une durée des voyages plus longue (Verling et al. 2005). D'ailleurs, après 33 jours de voyage, Burkholder et al. (2007) n'ont pas réussi à établir de cultures à partir de dinoflagellés récoltés dans l'eau de ballast des navires militaires aux États-Unis.

Les invasions biologiques sont un processus multi-étapes, lequel comprend la prise, le voyage, la décharge, la dispersion, l'établissement et la propagation des espèces (Elton, 1958; Colautti et al. 2006). Au cours de chaque étape, des pressions d'invasions sélectives agissent sur la survie des organismes diminuant progressivement le nombre total d'espèces et leur probabilité de succès d'invasion (Williamson 1996). Bien que les recherches et les ressources financières aient souvent été dirigées vers la caractérisation et l'identification d'espèces potentiellement envahissantes ou les étapes finales, ou les conséquences des propagations de ces espèces envahissantes, de nombreuses autres avenues de recherche reçoivent peu d'attention (Puth and Post 2005; Occhipinti et al. 2007) : la vulnérabilité ou l'invasibilité des écosystèmes aquatiques marins du Canada (Davis et al. 2005), le succès de survie des espèces non-indigènes après leur arrivée, les routes à partir desquelles les organismes pouvant bénéficier des changements climatiques peuvent se disperser, etc. Ces travaux pourraient s'avérer utiles pour mieux encadrer la gestion des eaux de ballast et potentiellement prévenir certaines invasions d'espèces.

Jusqu'à présent la plupart des études réalisées au pays se sont concentrées uniquement sur l'introduction d'espèces non-indigènes. Cependant, des questions relatives à l'introduction de différentes souches d'une même espèce restent encore sans réponse. C'est le cas de l'espèce *Alexandrium tamarense*, qui comporte différentes souches avec différents degrés de toxicité. Par exemple, la souche provenant de l'estuaire de la Tamar (Grande-Bretagne) ne s'est jamais révélée toxique (Balech 1995), tout comme les souches de l'Australie et du golfe de la Thaïlande, alors que la majorité des souches nordaméricaines et japonaises actuelles sont exclusivement toxiques (Hallegraeff et al. 1991 et références citées; Lilly et al. 2007). D'autant plus, que plusieurs des navires qui visitent la côte ouest du Canada arrivent en provenance des pays asiatiques (entre autres du Japon) ou en provenance des États-Unis mais après avoir visité un port asiatique auparavant.

Finalement, des questions sur l'efficacité des méthodes de traitements des eaux et de sédiments de ballast sont aussi pertinentes. Bien que l'échange au milieu de l'océan soit la méthode suggérée par l'Organisation Maritime Internationale (même si la nouvelle convention sur le contrôle et la gestion des eaux de ballast n'a pas encore été ratifiée), il y a eu parallèlement d'importantes recherches sur le développement de nouvelles technologies de traitement des eaux et des sédiments de ballast (Gregg et al. 2009). Cette convention suggère aux navires d'établir des procédures de gestion de ballast pour 2016, selon la date de construction des navires. Après 2016 les navires devront se munir d'un système capable de traiter les eaux et les sédiments à bord, avant de les décharger dans le milieu marin. L'implémentation de ces traitements pourrait atteindre une efficacité supérieure à 95% (efficacité des échanges d'eau de ballast) et devenir une meilleure alternative d'un point de vue environnemental, particulièrement pour éliminer les kystes de dinoflagellés contenus dans les sédiments. Une diminution de la viabilité des kystes pourrait ainsi mieux protéger les écosystèmes côtiers canadiens contre l'établissement potentiel d'espèces non-indigènes toxiques ou d'éventuelles formations de floraisons phytoplanctoniques nuisibles. Compte tenu du nombre élevé d'espèces qui produisent des floraisons nuisibles le long des côtes des États-Unis (Backer and McGillicuddy 2006) et les concentrations plus élevées de dinoflagellés potentiellement nuisibles ou toxiques dans les navires sans échange, observés dans la présente recherche, le transport côtier constitue une voie propice pouvant contribuer à augmenter le nombre, la fréquence et l'intensité des floraisons phytoplanctoniques nuisibles dans les eaux côtières du Canada (Roy et al. 2012).

D'autres alternatives envisageables seraient l'utilisation de méthodes combinées pour le traitement des eaux de ballast. L'efficacité d'une combinaison de systèmes de traitement d'eau de ballast pourrait s'avérer utile pour tous les nouveaux navires qui doivent être constuit à partir de 2012. Toutes les méthodes possibles de traitement des eaux de ballast impliquent un coût opérationnel qui normalement est une préoccupation majeure pour les armateurs et bien souvent le paramètre le plus important pour le choix d'une technologie de traitement des eaux de ballast qui doit être installé à bord. À partir des traitements mécaniques, les unités de filtration et hydrocyclone peuvent être une solution à être considéré dans un premier temps, pendant la prise de d'eau de ballast. Ces systèmes présentent l'avantage de laisser les organismes locaux dans leur milieu. Les deux sont aussi efficaces pour les plus grosses particules. L'application des UV et la désoxygénation constituent aussi des méthodes physiques à considérer. Les traitements UV sont bien connus des technologies des traitements des eaux usées et très efficaces contre un large éventail de micro-organismes. La combinaison de ces traitements UV avec l'addition d'agents oxydants, tels que l'ozone ou le peroxyde d'hydrogène, en général, améliore la performance des systèmes UV (Gregg et al. 2009). Finalement, l'utilisation des traitements chimiques comprend la chloration, l'ozonisation, ainsi que l'addition de dioxyde de chlore, d'acide peracétique, du peroxyde d'hydrogène et d'autres biocides. Cependant, les conditions de l'eau de ballast telles que le pH, la température et le type d'organisme sont des paramètres pouvant influencer l'efficacité de ces traitements. Enfin, il convient souligner que l'application de chaque méthode doit être sérieusement considérée selon l'impact que le système de traitement des eaux de ballast pourrait avoir sur l'environnement où l'eau de ballast sera déchargée. Il est important de ne pas transférer le problème à un niveau différent. À cet égard, la toxicité est le paramètre le plus important à être évalué, car le rejet de substances dans les milieux marins ne doit pas produire des effets de toxicité sur l'ensemble de la vie marine. Bien que plusieurs technologies de traitement des eaux de ballast ont été certifiées selon les directives de l'OMI, une évaluation plus approfondie est nécessaire en ce qui concerne de nouveaux organismes marins en mettant l'accent sur les kystes de dinoflagellés, le développement de nouveaux procédés à l'échelle de laboratoire et dans les navires, ainsi que d'étudier les incidences de ces technologies sur l'environnement.

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Annexe/Annex 1. Summary of ships sampled, ballast water exchange practice and location.

Ship Label	Ship Type[a]	Cat.[b]	Tank Sampled	Sampling Date	Ballast Water Source Origin	Ballast Water Source Date	Ballast Water Exchange Lat/Long	Ballast Water Exchange Date	BWE Location[c]	EM	Biome[d]	BWA Days
EC-01*	GC	TOE	WBT	27-Apr-07	Liverpool, UK	16-Apr	55 25 48 N - 10 37 18 W	17-Apr	North Atlantic Ocean 121 km NW of Ireland	E/R (100%)	NECS	5
EC-03	B	CE	WBT	29-Apr-07	Gulf of Mexico	6-Apr	31 52 36 N - 75 11 18 W	17-Apr	Atlantic Ocean 289 km NE of Charleston NC	F-T (300%)	NASW	5
EC-06	B	TOE	WBT	1-May-07	Genova, IT	8-Apr	40 25 00 N - 19 59 90 W	17-Apr	Atlantic Ocean, 548 km NW of Lisbon, Portugal	F-T (300%)	NASE	5
EC-07*	B	TOE	WBT	1-May-07	PortBury, UK	18-Apr	51 15 N – 38 48 W	23-Apr	Atlantic Ocean 872 km NE of St. John's NF, Canada	F-T (342%)	NADR	8
EC-10	B	TOE	WBT	4-May-07	-	-	-	-	-	-	-	-
EC-12	B	CE	WBT	5-May-07	Baltimore, MD	25-Apr	41 15 36 N - 61 30 48 W	2-May	Atlantic Ocean 240 km SE of Halifax, NS Canada	F-T (300%)	GFST	2
EC-14 [illegible]	B	TOE	WBT	7-May-07	Gijon, ES	24-Apr	45 04 N – 24 43 W	27-Apr	Atlantic Ocean 721 km NW of Lisbon Portugal	F-T (100%)	NADR	1
EC-15 [illegible]	B	CE	WBT	8-May-07	Atlantic Ocean, 210 km SE of Dover, MD	3-May	37 52 N – 71 21 W	3-May	Atlantic Ocean 271 km NE of Atlantic City NJ	E/R (100%)	GFST	1
EC-17*	B	CE	WBT	9-May-07	Eddystone, PA	4-May	39 55 N – 69 30 00 W	6-May	Atlantic Ocean 140 km SE of New York, NY	F-T (95%)	NWCS	3
EC-18*	B	TOE	WBT	23-May-07	Immingham, UK	10-May	48 26 N – 21 22 W	14-May	North Atlantic Ocean 989 km W of Brest, FR	F-T (300%)	NADR	6
EC-	GC	TOE	WBT	23-May-07	Rotterdam,	12-May	51 59 42 N -	14-May	North Atlantic	F-T	NADR	10

19*					NL		18 42 42 W		Ocean 657 km W of Ireland	(432%)		
EC-21*	B	TOE	DBT	24-May-07	Rotterdam, NL	6-May	47 02 24 N - 32 29 48 W	15-May	North Atlantic Ocean 792 km from any coast	F-T (522%)	NADR	5
EC-22	B	TOE	FPT	24-May-07	Trombetas, BZ	-	-	-	-	-	-	-
EC-23	B	TOE	FPT	24-May-07	Alicante, ES	20-Apr	10 34 N – 36 06 W	28-Apr	Atlantic Ocean 1168 km from any coast	E/R (500%)	WTRA	5
EC-28	B	CE	FPT	30-May-07	Gulf of Mexico	15-May	25 25 18 N - 87 58 48 W	22-May	Gulf of Mexico 378 km from any coast	(20%)	CARB	8
EC-29	B	TOE	FPT	30-May-07	Port Talbot, UK	20-May	51 06 N – 37 29 18 W	25-May	North Atlantic Ocean 913 km NE of St John's, Canada	F-T (338%)	NADR	4
EC-31	B	TOE	FPT	12-Jun-07	Liverpool, UK	1-Jun	53 06 N – 21 35 W	5-Jun	North Atlantic Ocean 880 km W of Ireland	(300%)	NADR	7
EC-33	B	CE	FPT	13-Jun-07	Salem, USA	8-Jun	42 06 N – 65 17 W	10-Jun	Atlantic Ocean 139 km NE of Boston MA	E/R (100%)	NWCS	3
EC-35	B	TOE	FPT	14-Jun-07	Ashkelon, IL	26-May	35 57 30 N - 8 52 18 W	3-Jun	Atlantic Ocean 114 km SW of Cadiz Spain	F-T (300%)	CNRY	9
EC-37*	GC	TOE	WBT	19-Jun-07	Zeebrugge, BE	8-Jun	50 22 48 N - 15 33 30 W	10-Jun	North Atlantic Ocean 452 km W of Ireland	F-T (351%)	NADR	12
EC-40	B	TOE	FPT	21-Jun-07	Rotterdam, NL	11-Jun	49 03 N – 14 01 W	14-Jun	North Atlantic Ocean 417 km W of Ireland	F-T (315%)	NADR	6
EC-42	B	TOE	FPT	22-Jun-07	Belem, BR	2-Jun	01 06 24 N - 48 30 18 W	2-Jun	Atlantic Ocean 132 km NE of Belem Brazil	E/R (100%)	GUIA	1
EC-43	B	TOE	FPT	23-Jun-07	Redcar, UK	10-Jun	45 27 N – 45 11 W	18-Jun	North Atlantic Ocean 610 km SE of St. John's NF	E/R (100%)	NWCS	4
EC-45	B	TOE	FPT	23-Jun-07	Ghent, BE	10-Jun	48 31 N	14-Jun	North Atlantic	F-T	NADR	9

							16 32 W		Ocean 625 km NE of Brest FR	(332%)		
EC-47	B	TOE	FPT	25-Jun-07	Immingham, UK	11-Jun	46 26 N – 35 08 W	17-Jun	Mid North Atlantic Ocean 763 km from any coast	F-T (336%)	NADR	5
EC-48	GC	CE	FPT	24-Jul-07	Baltimore, MD	17-Jul	39 15 06 N - 71 53 48 W	20-Jun	Atlantic Ocean 162 km E of Atlantic City, NJ	F-T (300%)	NWCS	3
EC-51	B	TOE	FPT	14-Aug-07	Port Talbot, UK	1-Aug	48 12 N – 25 31 W	5-Aug	North Atlantic Ocean, 1014 km from any coast	E/R (100%)	NADR	6
EC-52	B	TOE	FPT	14-Aug-07	English Chanel	7-Aug	-	-	North Atlantic Ocean	-	NADR	7
EC-53* I	B	TOE	FPT	15-Aug-07	Montoir, FR	26-Jul	46 35 N – 21 34 W	2-Aug	North Atlantic Ocean 998 km SW of Brest FR	E/R (100%)	NADR	6
EC-54	B	TOE	FPT	16-Aug-07	Liverpool, UK	3-Aug	56 27 N – 28 24 W	10-Aug	Mid North Atlantic Ocean 912 km from any coast	E/R (100%)	ARCT	6
EC-55	RR	CE	WBT	17-Aug-07	Philadelphia, PA	10-Aug	39 47 N – 70 14 W		Atlantic Ocean 222 km NE of Atlantic city NJ	E/R (100%)	GFST	9
EC-56	B	CE	DBT	5-Jun-07	Ravena, NY	1-Jun	42 41 N – 68 10 W	3-Jun	Atlantic Ocean 140 km NE of Boston MA	E/R (100%)	NWCS	2
EC-59*	T	CNE	WBT	7-Jun-08	Boston, MA	6-Jun	-	-	-	-	-	1
EC-61* I	B	CE	WBT	9-Jun-08	Newington, NH	6-Jun	42 24 06 N - 69 05 48 W	8-Jun	Atlantic Ocean 102 km NE of Boston	F-T (300%)	NWCS	1
EC-65 I	T	CNE	WBT	20-Jun-08	Portland, ME	18-Jun	-	-	-	-	-	1
EC-69 I	B	CE	WBT	24-Jun-08	New York, NY	19-Jun	41 32 12 N - 64 31 06 W	21-Jun	Atlantic Ocean 201 km SW of Halifax NS	E/R (300%)	NWCS	2
EC-71*	T	CE	WBT	25-Jun-08	Philadelphia, PA	21-Jun	40 17 48 N - 67 35 54 W	22-Jun	North Atlantic Ocean 205 km SE of Providence RI	F-T (300%)	NWCS	2

EC-74	RR	TOE	DBT	26-Jun-08	Gdynia, PL	7-Jun	58 53 N – 14 10 W	13-Jun	North Atlantic Ocean 493 km SE of Reykjavik Iceland	E/R (100%)	SARC	6
EC-75	B	CE	WBT	28-Jun-08	Portsmouth, NH	25-Jun	43 03 36 N - 67 58 36 W	26-Jun	Atlantic Ocean, 104 km N of Boston, mid Gulf of Maine	F-T (400%)	NWCS	2
EC-77	B	TOE	FTP	30-Jun-08	Yarimca, TR	10-Jun	38 05 N – 15 34 W	21-Jun	North Atlantic Ocean 540 km SW of Lisbon, Portugal	E/R (99%)	NASE	7
EC-78*	T	CNE	WBT	2-Jul-08	Searsport, ME	30-Jun	-	-	-	-	-	1
EC-80*	T	CNE	WBT	3-Jul-08	Boston, MA	1-Jul	-	-	-	-	-	1
EC-81*	T	CNE	WBT	3-Jul-08	Glouces, NJ	16-Jun	-	-	-	-	-	3
EC-83*	T	CE	WBT	7-Jul-08	Philadelphia, PA	3-Jul	39 52 36 N - 67 47 06 W	3-Jul	North Atlantic Ocean 208 km NE of Providence RI	F-T (300%)	NWCS	3
EC-85*	B	CE	WBT	9-Jul-08	Rosetown, NY	3-Jul	40 40 N – 66 56 W	7-Jul	North Atlantic Ocean 254 km SE of Providence RI	E/R (100%)	NWCS	2
EC-86	T	CE	DBT	10-Jul-08	New York, NY	6-Jul	43 20 36 N - 58 31 18 W	8-Jul	North Atlantic Ocean 108 km SE of Halifax NS	F-T (300%)	GFST	2
EC-89	B	CE	WBT	11-Jul-08	Brayton Point, MA	8-Jul	41 13 18 N - 65 48 42 W	9-Jul	North Atlantic Ocean 246 km SE of Halifax NS	E/R (100%)	NWCS	1
EC-92	B	CE	WBT	20-Jul-08	South Amboy, NJ	17-Jul	42 15 N – 65 28 W	19-Jul	North Atlantic Ocean 128 km NE of Boston MA	E/R (100%)	NWCS	1
EC-95	B	CE	WBT	5-Aug-08	Salem, NJ	3-Aug	44 46 06 N - 61 37 12 W	4-Aug	North Atlantic Ocean 30 km NE of Sheet Harbour NS	E/R (100%)	NWCS	1
EC-100	B	CE	CBT	9-Aug-08	Sparrows Point, MD	30-Jul	41 29 18 N - 65 03 30 W	8-Aug	North Atlantic Ocean 204 km SE of Boston MA	E/R (100%)	GFST	1

EC-101	B	CE	WBT	14-Aug-08	Brayton Point, MA	11-Aug	42 16 42 N - 63 48 48 W	12-Aug	North Atlantic Ocean 160 km S of Halifax, NS	E/R (100%)	NWCS	2
EC-102 ⚑	B	CNE	WBT	8-Jul-09	Sydney, NS	5-Jul	-	-	-	-		3
EC-105	T	CNE	WBT	19-Jul-09	Portland, ME	17-Jul	-	-	-	-		1
EC-106	T	CNE	WBT	19-Jul-09	Boston, MA	17-Jul	-	-	-	-		1
EC-107	T	CNE	WBT	20-Jul-09	Boston, MA	18-Jul	-	-	-	-		1
EC-108*	T	CNE	WBT	20-Jul-09	Belledune, NB	16-Jul	-	-	-	-		4
EC-109	T	CNE	WBT	21-Jul-09	Bootwood, NF	15-Jul	-	-	-	-		6
EC-110 ⚑	T	CNE	FPT	21-Jul-09	Stevenville, NF	7-Jul	-	-	-	-		14
EC-111	T	CNE	WBT	29-Jul-09	Portland, ME	26-Jul	-	-	-	-		1
EC-112	T	CNE	WBT	30-Jul-09	Boston, MA	26-Jul	-	-	-	-		2
EC-113	T	CNE	WBT	30-Jul-09	Charlottetown, PEI	24-Jul	-	-	-	-		6
EC-114 ⚑	T	CNE	WBT	30-Jul-09	St. John's, NL	24-Jul	-	-	-	-		6
EC-115	T	CNE	WBT	3-Aug-09	Boston, MA	1-Aug	-	-	-	-		1
EC-116 ⚑	T	CNE	WBT	3-Aug-09	Bucksport, ME	31-Jul	-	-	-	-		2
EC-117 * ⚑	B	CNE	WBT	6-Aug-09	Corner Brook, NL	25-Jul	-	-	-	-		11
EC-118*	B	CNE	WBT	7-Aug-09	Sydney, NS	27-Jul	-	-	-	-		11

* The asterisk after the ships codes indicates ships with harmful/toxic species. The order of ship code numbers follows the sampling order. Numbers are not consecutive because some ships were sampled for ballast water but not for sediment (no

empty tank available), hence these are not presented here

Ɪ The flag symbol after the ship codes indicates ships with non-indigenous species.

[a] Ship types: B = Bulk carrier; T = oil tanker or Chemical tanker; GC = General cargo; RR = Ro/Ro.

[b] Cat. = Categories utilized in statistical analyses; TOE = trans-oceanic ships with ballast exchange, CE = continental ships with ballast exchange and CNE = continental ships with no ballast exchange.

Tank sampled: CBT = cargo ballast tank; DBT = double bottom tank; FPT = forepeak tank; WBT = wing ballast tank

[c] Location of exchange = distance of the exchange site from coast, calculated with ArcView (Version 9.3). Some ships are named trans-oceanic, however their ballast exchange was in continental waters.

EM = exchange method. Practice used for ships to exchange ballast water; E/R = empty refill, F-T = flow-through, Alt = alternative. Percentages in parenthesis correspond to percentage exchanged relative to the total tank volume (information contained in the ballast reporting forms) to achieve a theoretical water replacement of ~95%.

[d] Biomes or provinces as defined by Longhurst (1998). ARCT Atlantic Arctic Province, SARC Atlantic Subarctic Province (Atlantic Polar Biome); NADR North Atlantic Drift Province, GFST Gulf Stream Province, NASW, NASE North Atlantic Subtropical Gyral Province West and East (Atlantic Westerly Winds Biome); WTRA Western Tropical Atlantic Province (Atlantic Trade Wind Biome); NECS Northeast Atlantic Shelves Province, NWCS Northwest Atlantic Shelves Province, CNRY Eastern Canary Coastal Province, GUIA Guinea Coastal Province (Atlantic Costal Biome); CARB Caribbean Province (Atlantic Trade Wind Biome)

BWA = ballast water age. Calculated as the number of days after ballast water exchange (for TOE and CE ships) or after ballast water uptake at the port of origin (for CNE ships).

Annexe/Annex 2. Summary of dinoflagellate cyst species found in the sediment samples.

	Trans-oceanic		Continental Exchange		Continental Non-exchange				
Species	Abund.[a]	Occ.[b]	Abund.	Occ.	Abund.	Occ.	NIS[c]	**Distribution**	**References**
	g^{-1} dry sed.		g^{-1} dry sed.		g^{-1} dry sed.				
Gonyaulacales									
*Alexandrium tamarense**	1	5	2	6	96	9		Widely distributed, coastal/estuarine sediments of temperate/subtropical regions	3; 22
Bitectatodinium tepikiense (*Gonyaulax digitalis*)	81	6	1	3	0	0		Temperate to sub-arctic environment North Atlantic Ocean	21
Fragilidium mexicanum	1	5	2	2	5	1	x	Sea of Japan, Sea of Okhotsk, Seas of Russia and European waters	1; 2
Impagidinium aculeatum (*Gonyaulax* sp. indet.)	1	1	0	0	0	0		Cosmopolitan species, neritic to oceanic and cool temperate to tropical regions	10
Impagidinium sphaericum (*Gonyaulax* sp. indet.)	1	2	0	0	0	0		Restricted to open oceanic environments; Cold temperate/tropical regions	10
Impagidinium paradoxum (*Gonyaulax* sp. indet.)	1	1	0	0	0	0		Oceanic to temperate/tropical marine environments	24

*Lingulodinium machaerophorum** (*Lingulodinium polyedrum*)*	9	8	3	7	1	2	x ‡	Coastal sediments from tropical/temperate regions throughout the world	13
*Operculodinium centrocarpum** (*Protoceratium reticulatum*)*	6	12	10	8	5	8		Widely distributed, from temperate/subtropical to tropical regions; from coastal to open waters	15; 18; 21
Operculodinium cf. *janduchenei*	1	1	1	1	1	4		Widely distributed, from temperate/subtropical to tropical regions; from coastal to open waters	15; 18; 21
Operculodinium cf. *israelianium*	1	1	0	0	3	1		Widely distributed, from temperate/subtropical to tropical regions; from coastal to open waters	18
Pentapharsodinium dalei	4	4	8	5	11	1		Widely distributed with respect to temperature, salinity and sea-ice cover	21
*Polysphaeridium zoharyi** (*Pyrodinium bahamense*)*	1	2	70	13	48	5	x ‡	Tropical/subtropical waters; mostly in Gulf of Mexico and Pacific Ocean in Central America	17
Spiniferites sp.	4	5	2	9	1	1			
Spiniferites belerius (*Gonyaulax*	1	3	1	1	1	1		Subtropical to temperate waters	3

scrippsae)									
Spiniferites bentorii (*Gonyaulax digitalis*)	0	0	1	1	1	1	x	Oceanic species; Temperate/tropical waters, North Amcrica, North Europe, East of Africa, Asia and South Australia	17
Spiniferites delicatus (*Gonyaulax* sp. indet.)	2	3	1	3	1	4		Coastal temperate/tropical species. Atlantic (North Sea) coasts of Europe, Africa, and Tasmania and near Japan.	17
Spiniferites elongatus (*Gonyaulax elongata*)	2	7	1	2	2	1		Cold to temperate regions, presumably endemic for the Northern hemisphere	17
Spiniferites hyperacanthus (*Gonyaulax* sp. indet.)	1	0	0	0	0	0		Temperate/tropical waters, oceanic/coastal regions	21
Spiniferites lazus (*Gonyaulax* sp. indet.)	2	2	1	1	0	0		Restricted to fully marine coastal regions of western Europe, Arctic to temperate regions	17
Spiniferites membranaceus (*Gonyaulax membranacea*)	2	4	1	2	2	2		From cold temperate/tropical regions.	17
Spiniferites mirabilis (*Gonyaulax spinifera complex)*	2	5	3	6	2	1		Temperate/tropical waters, coastal to oceanic regions	21

Spiniferites ramosus (*Gonyaulax scrippsae*)	4	6	1	10	3	3		Widely distributed, from coastal Arctic to the north of Antarctic subtropical front.	17
Spiniferites sp.1	1	1	0	0	1	1			
Tectatodinium sp.	3	3	1	1	0	1			
Tuberculodinium vancampoae (*Pyrophacus steinii*)	0	0	1	1	0	0	x	Tropical/subtropical coastal (estuarine, lagoonal) regions	
Gymnodiniales									
*Cochlodinium polykrikoides**	1	1	1	5	1	5	x ‡	Tropical/subtropical coastal regions of USA, Philippines, South China, and the Gulf of Thailand in Southwest Asia	12
*Gymnodinium catenatum**	2	4	2	5	2	2	x ‡	Tropical/subtropical coastal regions. Pacific/Atlantic coasts of North and South America, Europe, Japan and Tasmania	7; 8
Gymnodinium nolleri	1	1	1	1	0	0	x	Northern European waters and French North Sea	6
Polykrikos sp.	2	4	1	1	0	0	x		
Polykrikos kofoidii	17	7	1	3	20	3	x	Tropical/subtropical coastal regions, near upwelling zones	16
Polykrikos schwartzii	24	17	8	16	72	18		Temperate to tropical sediments; from coastal to open oceanic waters	21
Peridiniales									

Brigantedinium sp.	38	8	13	6	50	12		Widely distributed, coastal/oceanic sediments, cold to tropical regions	
Brigantedinium cariacoense (*Protoperidinium avellana*)	22	15	9	16	53	12		Widely distributed, coastal/oceanic sediments, cold to tropical regions	3
Brigantedinium irregulare (*Protoperidinium denticulatum*)	1	4	2	3	1	6	x	Widely distributed, coastal/oceanic sediments, cold to tropical regions	
Brigantedinium simplex (*Protoperidinium conicoides*)	27	16	42	13	104	18		Widely distributed, coastal/oceanic sediments, cold to tropical regions	3
Dubridinium sp.	5	6	2	6	8	14		Worldwide	22
Dubridinium caperatum (*Preperidinium meunieri*)	8	6	1	6	11	10		Worldwide	3; 22
Ensiculifera sp.	1	2	1	1	0	0	x	Caribbean Sea, Western Indian Ocean,	14
Echinidinium delicatum *Protoperidinium* sp. indet.	0	0	0	0	1	1		Coastal and upwelling regions, Arabian Sea, Oman upwelling region	23
Echinidinium sp.	1	7	4	7	3	9			
Islandinium minutum *Protoperidinium* sp. indet.	4	11	10	3	2	8		High latitudes; Arctic Ocean and adjacent seas and temperate North Atlantic Ocean.	21; 11

Lejeunecysta oliva	1	2	1	4	3	7			21
Lejeunecysta sabrina	1	1	1	1	1	3		Oceanic/neritic waters distribution in low and high latitude environments	4; 20
Leiosphaeridia sp.	0	0	1	1	0	0		Acritarch	
Protoperidinium americanum	7	3	1	5	16	14		Widely with broad temperature range	
Protoperidinium latissimum	0	0	1	1	1	2	x	Gulf of Mexico	19
Protoperidinium minutum	1	1	0	0	0	0		Widely distributed Atlantic and Pacific Subtropical/temperate,	3
Protoperidinium nudum	0	0	1	1	0	0		Widely distributed tropical to temperate waters, Mediterranean, Baltic, S. Atlantic, Amazon Estuary	3
Protoperidinium stellatum	0	0	1	3	1	4		Coastal from temperate/tropical regions, brackish to salted waters	
Quinquecuspis concreta (*Protoperidinium leonis*)	6	14	16	10	27	11		Oceanic/neritic waters distribution in low and high latitude environments	4; 20
*Scrippsiella trochoidea**	14	13	4	11	5	9		Widely distributed Atlantic and Pacific Subtropical/temperate	3
Scrippsiella lachrymosa/trifida?	4	1	3	8	122	8			
Selenopemphix nephroides (*Protoperidinium subinerme*)	2	4	2	8	1	4		Widely, restricted to marine regions, from temperate/tropical regions.	

Selenopemphix quanta (*Protoperidinium conicum*)	2	9	2	6	3	11		Coastal, from tropical to polar regions, absent in sediments south of 45°S	5; 9
Stelladinium reidii (*Protoperidinium* sp. cf. *P. compressum*)	0	0	0	0	1	3	x	Persian Gulf, Californian and western Australian coast, high abundances around Japanese Isles	
Trinovantedinium applanatum (*Protoperidinium pentagonum*)	1	5	1	4	1	1	x	Estuarine/neritic regions, and upwelling areas off N.W. Africa	4
Votadinium calvum (*Protoperidinium oblongum*)	13	11	3	13	13	12		Coastal distribution, Atlantic Ocean, Arabian Sea	3
Votadinium spinosum (*Protoperidinium claudicans*)	7	4	1	2	0	0	x	Temperate/subtropical, Northwestern Africa, around Japan, South Western Pacific.	3; 4
Zygabikodinium sp.	1	1	2	3	1	2		Present in the Baltic Sea, Caribbean Sea, Australia, Brazil	3
Unidentified cyst species									
Brown round smooth cysts	3	2	1	1	1	1		Widely	
Calcareous cysts	8	1	2	1	3	1			
Transparent cysts	2	3	0	0	1	1			
Spiny transparent cysts	1	2	1	2	33	12			

Species: Paleontological name and vegetative stage name in parentheses. In some cases, the same name is used for both nomenclatures.

[a] Abundance – Maximum abundances (cysts g^{-1} of dry sediment) per species in each ship category.
[b] Occurrence - number of times that species was found in ships of this category
[c] NIS – x: Non-indigenous species for the East coast of Canada
‡: Harmful and/or toxic
Code for references:

1. Blanco 1989a
2. Blanco 1989b
3. Dodge 1982
4. Dodge and Harland 1991
5. de Vernal et al. 2001
6. Ellegaard et al. 1998
7. Hallegraeff 2002
8. Hallegraeff and Fraga 1998
9. Harland 1983
10. Harland et al. 1998
11. Head et al. 2001
12. Iwataki et al. 2008
13. Lewis and Hallet 1997
14. Matsuoka et al. 1990
15. Matsuoka and Fukuyo 2000
16. Matsuoka and Cho 2000
17. Marret and Zonneveld 2003
18. McMinn and Sun 1994
19. Okolodkov 2008
20. Radi and de Vernal 2004
21. Rochon et al. 1999
22. Steidinger and Tangen 1996
23. Zonneveld 1997
24. Zonneveld and Brummer 2000

Annexe/Annex 3. Dinoflagellates found in the ballast water of ships arriving to West (WC) and East (EC) coasts of Canada. Harm = potentially harmful including toxic species. NIS = non-indigenous species for regions. Cultured = species put in culture media (f/2) to confirm viability of cells. Asterisks (*) indicate that the included species produced dense cultures (more than 1000 cells L^{-1}).

Species name	Comment
Akashiwo sanguinea (Hirasaka 1924) G. Hansen & Moestrup 2000	WC Harm Cultured
Alexandrium sp. 1	EC
Alexandrium sp. 2	EC
Alexandrium sp. 3	EC
Alexandrium sp. 4	EC
Alexandrium sp. 5	EC
Alexandrium sp. 6	EC
Alexandrium sp. 7	EC
Alexandrium sp. 8	EC
Alexandrium sp. 9	EC
Alexandrium sp. 10	EC
Alexandrium sp. 11	EC
Alexandrium cf. *minutum* Halin 1960	WC Harm
Alexandrium cf. *ostenfeldii* (Paulsen 1904) Balech & Tangen 1985	WC Harm
Alexandrium cf. *pseudogonyaulax* (Biecheler 1952) Horiguchi 1983 ex Kita & Fukuyo 1992	WC EC Harm
Alexandrium cf. *tamarense* (Lebour 1925) Balech 1985	WC EC Harm Cultured*
Amphidinium cf. *sphenoides* Wülff 1916	WC EC
Amylax cf. *triacantha* (Jorgensen 1899) Sournia 1984	WC EC
Ceratium hirundinella (O.F. Müller 1773) Bergh 1882	WC-NIS
Corynthodinium sp.	
cf. *Corynthodinium curvicaudatum* (Kofoid 1907) F.J.R. Taylor 1976	EC-NIS
cf. *Corynthodinium diploconus* (Stein 1883) F.J.R. Taylor 1976	EC-NIS
Dinophysis sp.	
Dinophysis cf. *acuminata* Claparède & Lachmann 1859	WC EC Harm Cultured
Dinophysis cf. *acuta* Ehrenberg 1839	WC EC Harm

Dinophysis caudata Saville-Kent 1881	EC-NIS Harm Cultured
Dinophysis dens Pavillard 1915	EC-NIS Cultured
Dinophysis fortii Pavillard 1923	WC EC Harm
Dinophysis norvegica Claparède & Lachmann 1859	WC EC Harm Cultured
Dinophysis odiosa (Pavillard 1930) Tai & Skogsberg 1934	WC EC NIS
Dinophysis cf. *ovum* Schütt 1895	EC-NIS
Dinophysis parva Schiller 1928	WC
Dinophysis rotundata Claparède and Lachmann, 1858-1859	EC Cultured
Dinophysis cf. *simplex* Böhm 1933	EC
Dinophysis tripos Gourret 1883	WC EC Harm Cultured
Diplopelta sp.	EC
Diplopsalis sp.	EC
cf. *Diplopsalis lenticula* Bergh 1881	WC-NIS, EC-NIS Cultured
Diplopsalopsis sp.	EC
Diplopsalopsis bomba (Stein 1883) Dodge & Toriumi 1993	EC
Dissodinium pseudolunula Swift 1973 ex Elbrächter & Drebes 1978	WC
Glenodinium sp.	WC
Goniodoma cf. *polyedricum* (Pouchet 1883) Jorgensen 1899	EC-NIS
Goniodoma sphaericum Murray & Whitting 1899	EC-NIS
Gonyaulax sp. 1	WC EC
Gonyaulax sp. 2	WC EC
Gonyaulax sp. 3	WC EC
Gonyaulax cf. *alaskensis* Kofoid 1911	EC
Gonyaulax diegensis Kofoid 1911	EC-NIS
Gonyaulax cf. *digitalis* (Pouchet 1883) Kofoid 1911	EC
Gonyaulax polygramma Stein 1883	EC-NIS Harm
Gonyaulax scrippsae Kofoid 1911	WC-NIS EC-NIS Cultured
Gonyaulax cf. *spinifera* (Claparède & Lachmann 1859) Diesing 1866	WC EC
Gonyaulax cf. *verior* Sournia 1973	WC EC
Gymnodinium sp. 1	WC EC
Gymnodinium sp. 2	WC EC
Gymnodinium sp. 3	WC EC
Gymnodinium sp. 4	WC EC
Gymnodinium sp. 5	WC EC

Gymnodinium sp. 6	WC EC
Gymnodinium sp. 7	WC EC
Gymnodinium sp. 8	WC EC
Gymnodinium sp. 9	EC
Gymnodinium sp. 10	EC
Gymnodinium cf. *aureolum* (Hulburt 1957) G. Hansen 2000	EC
Gymnodinium cf. *galeatum* J. Larsen 1994	WC-NIS EC
Gymnodinium cf. *variabile* Herdman 1924	EC-NIS
Gyrodinium sp.	EC
Gyrodinium cf. *crassum* (Pouchet 1885) Kofoid & Swezy 1921	EC-NIS
Gyrodinium cf. *estuariale* Hulburt 1957	WC Cultured
Gyrodinium cf. *fusus* (Meunier 1910) Akselman 1985	EC
cf. *Gyrodinium pepo* (Schütt 1895) Kofoid & Swezy 1921	WC-NIS EC
Gyrodinium cf. *pingue* (Schütt 1895) Kofoid & Swezy 1921	WC
Gyrodinium cf. *spirale* (Bergh 1881) Kofoid & Swezy 1921	WC EC
Gyrodinium cf. *resplendens* Hulburt 1957	WC Cultured*
Heterocapsa cf. *triquetra* (Ehrenberg 1840) Stein 1883	WC EC Cultured*
cf. *Heterodinium*	EC
cf. *Katodinium glaucum* (Lebour 1917) Loeblich III 1965	WC
Kofoidinium velelloides Pavillard 1928	NIS
cf. *Lingulodinium polyedrum* (Stein 1883) Dodge 1989	WC-Harm
cf. *Lophodinium polylophum* (Daday) Lemmermann 1910	WC-NIS
Neoceratium sp. 1	WC EC
Neoceratium sp. 2	WC EC
Neoceratium cf. *arcticum* (Ehrenberg 1854) Cleve 1901 Gómez et al 2010	EC
Neoceratium arietinum Cleve 1900 Gómez et al 2010	EC-NIS
Neoceratium azoricum Cleve 1900 Gómez et al 2010	WC-NIS EC-NIS
Neoceratium candelabrum (Ehrenberg 1860) Stein 1883 Gómez et al 2010	EC-NIS
Neoceratium compressum Gran 1912 Gómez et al 2010	EC-NIS
Neoceratium divaricatum var. *balechii* Hernandez-Becerril & Alonso-Rodríguez 2004 Gómez et al 2010	WC-NIS EC-NIS
Neoceratium furca (Ehrenberg 1834) Claparède & Lachmann 1859 Gómez et al 2010	WC EC-NIS Harm Cultured
Neoceratium cf. *fusus* (Ehrenberg 1834) Dujardin 1841 Gómez et al 2010	WC EC Cultured
Neoceratium cf. *gibberum* Gourret 1883 Gómez et al 2010	EC Cultured

Neoceratium cf. *horridum* (Cleve 1897) Gran 1902 Gómez et al 2010	WC EC
Neoceratium inflatum (Kofoid 1907) Jorgensen 1911 Gómez et al 2010	WC-NIS EC-NIS
Neoceratium kofoidii Jorgensen 1911 Gómez et al 2010	
Neoceratium cf. *lineatum* (Ehrenberg) Cleve Gómez et al 2010	WC EC
Neoceratium cf. *longipes* (Bailey 1850) Gran 1902 Gómez et al 2010	WC EC Cultured
Neoceratium longirostrum Gourret 1883 Gómez et al 2010	WC EC-NIS
Neoceratium cf. *macroceros* (Ehrenberg 1840) Vanhhöffen 1897 Gómez et al 2010	EC-NIS
Neoceratium cf. *minutum* Jorgensen 1920 Gómez et al 2010	WC EC-NIS
Neoceratium pentagonum Gourret 1883 Gómez et al 2010	WC EC-NIS
Neoceratium cf. *platycorne* Daday 1888 Gómez et al 2010	EC-NIS
Neoceratium pulchellum Schröder 1906 Gómez et al 2010	EC-NIS
Neoceratium teres Kofoid 1907 Gómez et al 2010	EC-NIS
Neoceratium tripos (O.F. Müller 1777) Nitzsch 1817	WC EC Cultured
cf. *Oblea*	WC EC
Oblea sp.	WC EC
cf. *Oblea rotunda* (Lebour 1922) Balech 1964 *ex* Sournia 1973	WC-NIS EC
Oxyphysis cf. *oxytoxoides* Kofoid 1926	WC
Oxytoxum sp.	EC
Oxytoxum cf. *frenguellii* Rampi 1943	WC-NIS
Oxytoxum cf. *laticeps* Schiller 1937	EC
Oxytoxum michaelsarsii Gaarder 1954	EC-NIS
Oxytoxum scolopax Stein 1883	EC-NIS
Peridinium cf. *quinquecorne* Abé 1927	WC-NIS
Peridinium cf. *volzii* Lemmermann 1905	WC-NIS
cf. *Peridinium willey/volzii*	WC
Phalacroma sp.	EC
Phalacroma cf. *contractum* Kofoid & Skogsberg 1928	EC
Phalacroma mitra Schütt 1895	EC-NIS Harm
Phalacroma pulchellum Lebour 1922	WC-NIS EC-NIS
Phalacroma cf. *rotundatum* (Claparède & Lachmann 1859) Kofoid & Michener 1911	WC EC Harm
Podolampas palmipes Stein 1883	WC EC-NIS
Podolampas spinifera Okamura 1912	EC-NIS
Polykrikos kofoidii Chatton 1914	WC-NIS

Polykrikos kofoidii Chatton 1914 (cyst)	EC-NIS
Polykrikos schwartzii Bütschli 1873 (cyst)	EC Cultured
cf. *Preperidinium meunieri* (Pavillard 1907) Elbrächter 1993	WC EC
Prorocentrum cf. *compressum* (Bailey 1850) Abé *ex* Dodge 1975	WC EC
Prorocentrum cf. *gracile* Schütt 1895	EC Cultured*
Prorocentrum cf. *lima* (Ehrenberg 1860) Dodge 1975	WC EC Harm
Prorocentrum cf. *mexicanum* Osorio-Tafall 1942	EC
Prorocentrum cf. *micans* Ehrenberg 1834	WC EC Cultured*
Prorocentrum sigmoides Böhm 1933	WC Harm
cf. *Protoceratium reticulatum* (Claparède & Lachmann 1859) Bütschli 1885	WC EC Harm Cultured
Cyst of *P. reticulatum* (cf. *Operculodinium centrocarpum*)	EC Harm
Protoperidinium sp. 1	WC
Protoperidinium sp. 2	WC
Protoperidinium sp.	
Protoperidinium cf. *americanum* (Gran & Baarud 1935) Balech 1974	EC
Protoperidinium cf. *bipes* (Paulsen 1904) Balech 1974	WC EC
Protoperidinium cf. *brevipes* (Paulsen 1908) Balech 1974	WC EC
Protoperidinium cf. *cerasus* (Paulsen 1907) Balech 1973	WC-NIS EC
Protoperidinium cerasus/quamerense/globulus	WC
Protoperidinium claudicans (Paulsen 1907) Balech 1974	WC-NIS EC
Protoperidinium cf. *conicoides* (Paulsen 1905) Balech 1973	WC-NIS EC
Protoperidinium cf. *conicum* (Gran 1900) Balech 1974	WC EC
Protoperidinium cf. *crassipes* (Kofoid 1907) Balech 1974	WC EC
Protoperidinium cf. *curtipes* (Jorgensen 1912) Balech 1974	WC-NIS EC-NIS
Protoperidinium cf. *curvipes* / pyriforme	
Protoperidinium cf. *decipiens* (Jorgensen 1899) Parke & Dodge 1976	NIS
Protoperidinium denticulatum (Gran & Baarud 1935) Balech 1974	WC-NIS EC
Protoperidinium depressum (Bailey 1850) Balech 1974	WC EC
Protoperidinium cf. *diabolus* (Cleve 1900) Balech 1974	EC-NIS
Protoperidinium cf. *divergens* (Ehrenberg 1841) Balech 1974	WC-NIS EC-NIS
Protoperidinium cf. *excentricum* (Paulsen 1907) Balech 1974	WC
Protoperidinium cf. *globulus* (Stein 1883) Balech 1974	WC-NIS EC
Protoperidinium cf. *granii* (Ostenfeld 1906) Balech 1974	WC-NIS EC
Protoperidinium laticeps (Grontved et Seidenfaden) Balech 1974	EC

Protoperidinium leonis (Pavillard 1916) Balech 1974	WC EC-NIS
Protoperidinium cf. *marielebouriae* (Paulsen 1931) Balech 1974	EC-NIS
Protoperidinium cf. *minutum* (Kofoid 1907) Loeblich III 1970	WC-NIS EC
Protoperidinium cf. *mite* (Pavillard 1916) Balech 1974	WC-NIS EC
Protoperidinium cf. *nudum* (Meunier 1910) Balech 1974	WC-NIS
Protoperidinium cf. *oblongum* (Aurivillius 1898) Parke & Dodge 1976	WC-NIS EC
Protoperidinium cf. *obtusum* (Karsten 1906) Parke & Dodge 1976	WC-NIS EC
Protoperidinium cf. *oceanicum* (Vanhöffen 1897) Balech 1974	WC-NIS EC-NIS
Protoperidinium cf. *ovatum* Pouchet 1883	WC-NIS EC
Protoperidinium cf. *pallidum* (Ostenfeld 1899) Balech 1973	WC EC
Protoperidinium cf. *pellucidum* Bergh 1881 *ex* Loeblich Jr. & Loeblich III 1966	WC-NIS EC
Protoperidinium cf. *pentagonum* (Gran 1902) Balech 1974	WC-NIS
Protoperidinium cf. *punctulatum / subinerme*	WC
Protoperidinium pyriforme (Paulsen 1907) Balech 1974	WC EC
Protoperidinium cf. *quarnerense* (Schröder 1900) Balech 1974	EC-NIS
Protoperidinium soltans (Meunier 1910) Balech 1973	EC
Protoperidinium cf. *sphaeroideum* (Mangin 1922) Balech 1974	EC-NIS
Protoperidinium cf. *steinii* (Jorgensen 1899) Balech 1974	WC EC Cultured
Protoperidinium cf. *subinerme* (Paulsen 1904) Loeblich III 1970	WC-NIS EC
Protoperidinium cf. *thorianum* (Paulsen 1905) Balech 1973	WC-NIS EC
Protoperidinium thulesense (Balech 1958) Balech 1973	WC-NIS EC-NIS
Protoperidinium cf. *tristylum* (Stein 1883) Balech 1974	EC-NIS
Pyrophacus horologium Stein 1833 emend. Wall & Dale 1971	WC EC
Scrippsiella sp. 1	WC EC
Scrippsiella sp. 2	WC EC
Scrippsiella sp. 3	WC EC
Scrippsiella sp. 4	WC EC
Scrippsiella sp. 5	WC
Scrippsiella cf. *trochoidea* (Stein 1883) Balech *ex* Loeblich III 1965	WC EC Cultured*
Spiraulax kofoidii Graham 1942	EC-NIS
cf. *Torodinium robustum* Kofoid & Swezy 1921	WC