Measuring and managing invasive species threats in the Arctic

by

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Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Statement of co-authorship

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Abstract

Several decades of invasive species research have yielded a broad understanding of the nature of species transfer mechanisms and associated threats globally. This is not true of the Arctic, however, a region where increasing human activity and ongoing climate change is expected to promote species invasion. This thesis examines the potential for both terrestrial and marine non-indigenous species (NIS) to be introduced to and establish in the Arctic under present and future climatic conditions. Throughout, the work uses the high-Arctic archipelago Svalbard as a model for the wider Arctic region. The research focuses on two of the most well-described pathways of species introduction globally, human visitation and shipping, both of which are increasing in intensity in Svalbard. Potential for species introduction and establishment is examined by quantifiying and identifying propagule loads transferred to the Arctic; developing and testing species identification methods; evaluating present and forecasting future habitat suitability for NIS; measuring the spread of established non-indigenous vascular plants; and testing the efficacy of management measures designed to prevent further species introduction.

Results demonstrate high plant propagule transport by people travelling to highly-visited Arctic regions is occurring. Furthermore, propagule pressure associated with ship hull fouling poses immediate risks, while if more stringent management related to ships' ballast water discharge is not enacted this vector will pose an increasing risk over the coming century. Improved vector screening methods were achieved through testing a molecular species identification approach for organisms transported with ships, but the approach was found to be inefficient in a biosecurity management context. Climate changes, and particularly temperature increases, over the coming century are expected to increase Svalbard habitat suitability for both terrestrial and marine species. Acknowledgment of the negative impacts NIS may have in Svalbard has led to the implementation of preventative management measures designed to reduce species transfer by visitors and ships; however, these were found to have limited effect. Scope for improved management is outlined.

Where species invasion risks are found to exist at the transport stage, the body of invasion ecology knowledge suggests a precautionary approach whereby NIS introduction should be prevented. The imperative to ensure this in polar regions has historically been lacking, owing largely to the strength of climatic barriers, and assumed weak propagule pressure. By quantifying propagule pressure across different pathways and vectors, and estimating changing habitat suitability under forecast climatic conditions, this research provides the basis upon which to develop more informed biosecurity management for Svalbard. Moreover, given the similarity in pathways of species introduction across the Arctic region, the work presented here suggests an Arctic-wide need to address management and policy gaps.

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Chapter 1: Measuring and managing biological invasions in the Arctic: general introduction

Increases in the intensity and scale of international travel and trade have escalated the frequency and extent of global species transfer (Mack et al. 2000, Tatem and Hay 2005, Hulme 2009, Pyšek et al. 2010). Most activities associated with travel and trade have the potential to mediate the introduction of non-indigenous species (NIS) (Hulme 2009), and as a result few regions are free of NIS (Catford et al. 2012). A proportion of introduced species – somewhere around 10 % (Strayer 2011, Vila et al. 2010) – establish, spread, and become invasive, often negatively impacting on indigenous biodiversity, ecosystem functioning and services, economies, or human health (Pyšek and Richardson 2010). Understanding the processes underlying species invasion is pivotal to forecasting where future risks of invasion and impact lie (Faacon et al. 2006). The research effort has been biased towards later stages of invasion (stages post NIS-introduction – see Fig 1) (Puth and Post 2005), and has neglected the important role of inadvertent species introduction (Hulme et al. 2008, Huiskes et al. 2014). We still have a poor understanding of the rate, type, and magnitude of threat posed by species transferred inadvertently, yet introduction is the stage in the invasion process where management interventions are most successful (Mack et al. 2000, Leung et al. 2002). Where socio-economic systems and climates are changing so too are rates of species transfer. Refocusing research effort to address this knowledge gap is vital.

Why measure and manage invasive species?

Critics of the fields of invasion ecology research and invasive species management often highlight the benefits NIS may provide in calling for reduced or no efforts to study or prevent species introduction (e.g. Davis et al. 2011, Schlaepfer et al. 2011). While this view acknowledges the important role some NIS play in modern societies (our dependence on crop species which are often NIS for example), it is ignorant of the wider picture. Several decades' worth of research and data collection enables meta-analyses of large and diverse data sets, making it possible to now empirically evaluate the benefit of preventing NIS introductions. Such analyses clearly demonstrate that the ratio of NIS that produce desirable as compared to undesirable impacts is low (Vitule et al. 2012).

Box 1 What are biological invasions?

Biological invasion is a process whereby an organism is transferred by human agency (purposefully or inadvertently) beyond the biogeographical range achieved naturally, and then subsequently establishes and spreads in the novel habitat (IUCN 2000, Blackburn et al. 2011). Human agency is a key aspect to the definition of biological invasion as it stipulates those range expansions that are not considered invasions – that is, species naturally occurring in a given region which for one reason or another spread rapidly, and species which are naturally transferred beyond the geographical range they otherwise achieve. This is important as it defines the scope of biological invasion management.

Discussion has centred on whether this definition should include a measure of impact (e.g. Ricciardi and Cohen 2007). Generally (but not strictly), impact is not implied in the ecological definition (though impact at some level might be assumed given the spread of a non-indigenous organism), but is assumed in policy papers and legislation (Ricciardi and Cohen 2007).

Box 2 Terminology

The variety of terminology and synonyms used in biological invasion research – and the often indiscriminate mixing of terminology – has long been a source of confusion that has hindered comparisons and processes in biological invasion, and has led to the reinvention of concepts and hypotheses (Blackburn et al. 2011).

Invasive species is a term that applies to any organism that fits the definition at the beginning of this section. **Non-native**, **non-indigenous**, **alien**, and **exotic** are all synonyms for species that have been moved beyond their natural geographic range through human agency, but these terms have no measure of impact associated with them. That is, an invasive species is always a non-native species, but the reverse is not always true. The term **pest** species is commonly used with reference to invasive vertebrates, while the term **weed** is often used with reference to invasive plants. Similarly, **harmful** algae are invasive algae. **Noxious** and **nuisance** are two further synonyms applied to non-indigenous species that cause negative impacts.

In this thesis, the term non-indigenous (NIS) or alien will be used and reserved for any introduced species that has not yet spread. Invasive species will be used for any NIS/alien that has spread, but will not imply impact.

Vectors are the physical means by which species are transported beyond their indigenous range.

Pathway refers to the processes that result in the introduction of alien species from one location to another.

Impacts on biological diversity vary from reducing genetic variation and eroding gene pools, through to extirpating populations of indigenous species, and irreversibly altering habitat and ecosystem functioning (Hulme 2008, Bergstrom et al. 2009, Moles et al. 2012). Impacts may be rendered directly (e.g. through predatory actions, Goldschmidt 1998), or indirectly, generally over longer time scales (e.g. through the use of limiting resources, Didham et al. 2005). Some of the most severe ecological impacts caused by invasive species include the extinction of over 150 indigenous species of fresh water fish through the introduction of one species of NIS (Nile perch, *Lates niloticus*) (Goldschmidt 1998); trophic cascades on remote islands associated with the introduction of Norway rats (*Rattus norvegicus*) (Kurle et al. 2008); and the destruction of tens of thousands of hectares of wetland vegetation following the invasion of a semiaquatic mammal (nutria, *Myocastor coypus*) (Pyke et al. 2008).

Biological invasions also cause economic and human health impacts that can be measured as financial costs or human morbidity (Leung et al. 2002, Pimentel et al. 2005, Keller et al. 2007, Vila et al. 2010). For example, costs incurred by biological invasions have been estimated to amount to 5% of the global gross domestic product (Pintennal et al. 2005). Regional estimates of costs are also substantial. For NIS established in the US across all taxonomic divisions, impacts were estimated to cost US$120 billion per year (Pimentel et al. 2005). Sinden et al. (2004) estimated yearly costs to government agencies associated with monitoring, control, management, and research on weeds were at least AUS$116.4 million. NIS impacts may affect a wide range of ecosystem services that underpin human well-being, including provisioning of food and fibre; regulating the spread of human diseases; and tourism benefits (Pejchar and Mooney. 2009, Pyšek and Richardson 2010). Thus, disruption of ecosystem services as a result of biological invasions may have adverse socioeconomic, and human health impacts, of which the financial and healthcare-associated burden can be high (Pyšek and Richardson 2010).

Further economic justification for preventing NIS introduction is evident in the relative costs of implementing preventative measures compared with the costs of species invasion. One of the most comprehensive studies of the relative costs of applying preventative management used a simple cost:benefit bioeconomic framework to quantify the net benefit of prescreening plant species prior to their introduction (Keller et al. 2007). Even when using low estimates of the damages caused by the small proportion of introduced plants that become invasive, prescreening produced net benefits (Keller et al. 2007).

Finally, social attachment to either invasive or indigenous species as a result of the cultural, recreational, or aesthetic benefits they confer must also be taken into account. For example, people living in and around Golden Gate Highlands in Table Mountain National Park in South Africa use non-indigenous woody *Acacia*, *Eucalyptus*, *Hakea*, and *Pinus* species for food, fuel, and building materials (Shackleton et al. 2007, Kueffer et al. 2014). The particular NIS used are highly invasive, and under other circumstances environmental management would mandate their removal (Kueffer et al. 2014). In contrast, protected areas are typically valued for their indigenous ecological integrity, and the introduction of NIS made illegal through legislative articles (e.g. Svalbard Environmental Protection Act 2002).

Therefore, invasion biology sits at a juncture where ecology, social science, public perception, and resource management meet. This positioning means any decision taken to manage an introduced species or not is done so not in isolation, but with regard to the potential for impact to a diverse range of values (immediate and forecast) including the public value ascribed to the particular species (Nuñez and Simberloff 2005). An organism may be deemed unwanted based on its non-indigenous status, but management not warranted based on a perceived lack of impact, lack of resources, or positive value attached to the species by the public. Measures of impact are inherently subjective, although several approaches to empirically measure impact have been suggested (e.g. Parker et al. 1999, Catford et al. 2012, among others). Any measure should go beyond the 'good versus bad' indigenous/non-indigenous dichotomy (Shackleford et al. 2013, Simberloff et al. 2012), and ideally account for the full range of ecological, economic, and sociological consequences of a species invasion.

Is it possible to predict which species will become invasive?

Given the need to identify those NIS that are likely to cause undesirable impacts, is it possible to predict which NIS will potentially become invasive? Above all, invasion biology as a field of research attempts to improve our understanding of how the addition of a single species to an environment can modify biodiversity and ecosystem functioning (Simberloff et al. 2012). Yet one of the enduring hurdles in invasion ecology is the difficulty of prediction. The task is particularly challenging given that species may respond differently in different habitats (Pyšek et al. 2012). In seminal work, Elton (1958) conceived and tested numerous theories related to biological invasions. Our understanding of invasion process remains less than perfect. How useful is information which has been assimilated over the last three decades of intensive research in advancing our predictive abilities?

One of the most important advances over recent decades has been the development of substantial bodies of data and literature that now permit meta-analyses of invasive species patterns and processes across spatial, temporal, and taxonomic scales. Meta-analyses paint a picture of increasing clarity at some scales and related to some taxa, while identifying conflicting patterns related to others (Moles et al. 2012). A few robust

generalisations have emerged: (1) the probability of a species becoming established increases with the magnitude of associated propagule pressure (the number of individuals of a species released into an environment multiplied by the number of release events) (Cassey et al. 2005, Lockwood et al. 2005, Simberloff 2009); (2) the probability of invasiveness increases if the species has a history of invasion (Kulhanek et al. 2011); (3) habitat disturbance does not unequivocally render a community more invasible (Facon et al. 2006, Moles et al. 2012); (5) insular ecosystems such as oceanic islands are more invasible than mainland (Simberloff 1995, Lonsdale 1999); (6) a relationship between lower community diversity and invasiveness is not consistent across spatial scales (Levine and D'Antonio 1999, Moles et al. 2012); (7) climate-matching between donor and recipient habitats is a consistent predictor of NIS establishment (Williamson 1996, Duncan et al. 2001, Richardson and Thuiller 2007); (8) despite the idiosyncrasy of results and generalisations at different spatial scales, satisfactory generalisations may be evident over limited domains (e.g. fire-prone grasslands, suspension feeding bivalves) (Strayer 2011, Moles et al. 2012).

Despite the poor understanding of processes underlying successful species invasion, a practical outcome is the general acknowledgement that at the initial stages of introduction (transport or newly established), decisions to prevent or exclude species from introduction or establishment based on their non-indigenous status are wise (Strayer 2011, Shackleford et al. 2013). This logic has carried over into biosecurity agencies which typically employ a 'guilty until proven innocent' approach to vectors of species introduction carrying unknown loads of putative NIS (e.g. Biosecurity NZ, http://www.biosecurity.govt.nz/enter/personal).

How to organise research around identifying and managing risks of species introduction?

Several decades of species invasion research have yielded a broad understanding of the nature of species transfer mechanisms and associated threats globally (Chown et al. 2012), and the formulation of frameworks for understanding biological invasions (Blackburn et al. 2011). Broadly, frameworks have developed along separate, but complementary, lines. These can be grouped as those focussing on the ecological and evolutionary processes underlying invasion (e.g. Elton 1958, Shea and Chesson 2002, Facon et al. 2006, Gurevitch et al. 2011), or those attempting to define the various stages of the invasion continuum (e.g. Williams 1996, Richardson et al. 2000, Hulme et al. 2008, Blackburn et al. 2011). Of the two approaches the latter is more relevant to the setting of research, policy, and management agendas (Gurevitch et al. 2011), assisting in aligning research and management to the various processes acting at different stages of species invasion. These frameworks typically conceptualise species invasion as a series of stages through which a species must pass to in order to become invasive (Fig 1).

Research tasked with identifying risk at the transport and introduction stages of invasion has been pursued along a number of avenues. Propagule load and composition is most robustly measured by sampling individual vectors associated with a pathway of introduction (e.g. Chown et al. 2012), while proximal means are used to derive cruder estimates (e.g. Verling et al. 2005). Proximal means include estimating introduction effort based on vector activity (McGee et al. 2006), or using dispersal models to estimate whether NIS transfer might be occurring (Bossenbroek et al. 2001). While these are often valid approaches certain assumptions must be accounted for. For example, the number of ships arriving to ports is commonly used as a proxy for the volume of ballast water discharged, yet no relationship between number of visiting ships and the number of invasive species has been identified (Verling et al. 2005). The explanation offered here is that not all ballast water is of equal risk (e.g. old ballast water versus newer ballast water; high versus low salinity ballast water), and

moreover, not all ships discharge ballast water. Thus, the use of ship arrivals as a proxy for introduction effort may grossly overestimate risk.
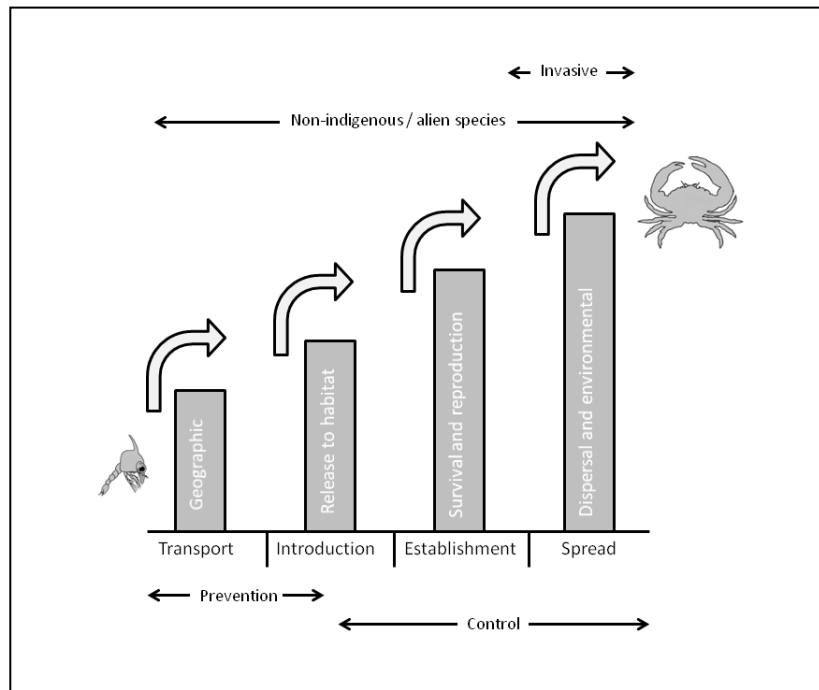
Figure 1 Simplified conceptualisation of the invasion process whereby organisms must overcome barriers to progress to the next stage of the invasion continuum (*sensu* Richardson and Pysek 2000, Blackburn et al. 2011). Terminology is shown at the top, while management options are shown at the bottom. The various stages are labelled, below the respective barriers operating on organisms.

The range of taxa spread via anthropogenic vectors is wide, including plants (Clifford 1956; Powell 1968; Falinski 1972; Higashino et al. 1983; Whinam et al. 2005; Lee and Chown 2009; Wichmann et al. 2009), arthropods (McCullough et al. 2006; Hughes et al. 2010), bacteria (Curry et al. 2002; Drake et al. 2007; Hughes et al. 2010; McNeill et al. 2010), terrestrial vertebrates (Gillespie 1985), and marine organisms (Carlton 1985; Gollasch 2002, Coutts and Taylor 2004). Screening all vectors is resource and time intensive, and frequently some form of extrapolation, proximal estimation, or a combination of both is used to characterise propagule loads. Focussing on exemplar taxa is an efficient way of reducing sampling effort, the results of which can then be extrapolated over wider taxonomic domains (e.g. Shaw et al. 2010).

Measures of the diversity and abundance of organisms associated with a vector permit analyses of risk. Risk is calculated separately across individual stages of the invasion continuum. In this way, risk of transport might be high, but the risk of dispersal lower, as in the case of ship hull fouling (Minchin and Gollasch 2003, Hewitt et al. 2009). Similarly, plant seeds might have specific adaptations for attachment to vectors, yet have a narrow climatic regeneration niche, and therefore risk of establishment may be low. Where the identities of organisms associated with a given vector remain unknown, qualitative estimates of risk may be ascribed based on measures such as abundance (as a proxy for propagule pressure), or climatic similarity between donor and recipient regions. Such risk assessments are known as vector-based risk assessments (Barry et al. 2008), and are readily translatable into management outcomes given an entire assemblage of putative NIS can be managed at the vector level (Ruiz and Carlton 2003, Chan et al. 2012).

More precise risk assessments, however, are based on the identification of individual organisms carried by a vector, with risk of establishment or invasion evaluated empirically. This approach, known as species-specific risk assessment (Barry et al. 2008), entails the capacity to identify risk species *a priori*. This may reduce the burden of needlessly managing entire vectors where risk has been overestimated. Considerable limitations to species-specific risk assessments associated with pathways of species introduction include the time and resources required to sample and identify associated organisms (Barry et al. 2008, Chan et al. 2012). Organism identification in particular can be a time consuming process often requiring the need for deep taxonomic expertise. For example, samples of ships' ballast water may contain thousands of juvenile meroplanktonic organisms, for which species recognition based on morphological characters alone is either challenging or impossible due to: 1) the size and immaturity of organisms; 2) a lack of published taxonomic keys; 3) the broad geographical range from which organisms may have been sourced; and 4) the possibly damaged physical condition in which the organisms are sampled. In these circumstances, organisms have often been identified only to phylum or family level (e.g, Choi et al. 2005, David et al. 2007, DiBacco et al. 2011) which provides little information on which to base an assessment of risk. Molecular tools to aid in the identification of early life stage organisms, in particular, present a promising avenue of research, the utility of which has been demonstrated in a number of pathway-assessment settings (e.g. Armstrong and Ball 2005, Ball and Armstrong 2006, Armstrong 2010, Collins et al. 2012). Nonetheless, such methods require context-specific testing to evaluate efficacy given a number of published technical hurdles (e.g. Hoareau and Boissin 2010, Siddal et al. 2009, Bhadury and Austen 2010).

Species-specific risk assessments typically use experimentally derived physiological measures of a species' capacity to survive and/or reproduce (Monahan 2009); modelling methods to characterise the environmental conditions under which successful establishment, survival (Elith et al. 2010), or spread may occur (Kearney et al. 2008, Elith et al. 2010); or combinations of both approaches (Elith et al. 2010, Kearney et al. 2010, Buckley et al. 2011). Methods based solely on one or two critical physiological determinants of reproduction, and certainly those based on critical limits to survival, will usually overestimate the geographical range of a species (Svenning 2004). Such physiological delimitations do not account for the many biotic and abiotic interactions that also shape a species' realised niche. Nonetheless, such methods provide suitable model complexity for estimating the response of species to changing environmental gradients (Monahan 2009), such as those forecast under climate change.

Invasive species in the Arctic

Like other biomes, the Arctic is exposed to the introduction of NIS. The potential for any NIS to establish has, however, been limited by a low level of opportunity for human-mediated species transfer, and severe climatic conditions (e.g. Ruiz and Hewitt 2009). The strength of these barriers is such that there are few invasive species in the Arctic. Throughout the entire Arctic (following the biogeographical definition of Elvin et al. 2011), no non-indigenous plants are considered to be producing negative impacts (though some are spreading) (Elvin et al. 2011), while in the marine environment just one NIS is considered invasive and to be causing negative impacts (the crab *Paralithodes camtschaticus*, Jørgensen and Primicerio 2007; but see also Alvsvåg et al. (2009) for reference to the expansion of the snow crab *Chionoecetes opilio*). Invasive vertebrates are few, and their persistence largely synanthropic (Coulson et al. 2012), while few data exist on the status of microorganisms (Lovejoy 2013). Low air and soil temperatures, large temperature variations and a short

growing season characteristic of the Arctic are major challenges for plant growth (Callaghan et al., 2004), while for marine zooplankton low temperatures directly limit larval development rate, restricting the capacity of many species to metamorphose (Thatje et al, 2005 deRivera et al, 2007). Though the status of NIS in the Arctic is presently favourable, the wider Arctic region – and particularly a number of Arctic locations – has become more steadily influenced and trafficked by humans, a trend which is set to continue (Hall et al. 2012, Chan et al. 2012). Temperature increases in the Arctic as a result of global climate change are predicted to be greater than any other region over the coming century (Steele et al. 2008, IPCC 2007, Serreze et al. 2011, Xu et al. 2013). Therefore, the very factors which have maintained Arctic isolation and ecosystem integrity may no longer continue to do so.

Unsurprisingly there is a paucity of research on species invasion in the Arctic compared to that from temperate and tropical regions. The few NIS known from the Arctic region, and the widely-held perception that the region is largely immune to the impacts caused by NIS, has presented little impetus for broad-scale research or management development. The present situation is at odds with that in the Antarctic, where, on some sub-Antarctic Islands, terrestrial NIS may be more numerous than native species (Frenot et al. 2005). Analyses of pathways of species introduction, and quantification of the potential risk NIS transferred on pathways pose, have both been performed for the Antarctic with a focus on marine crustaceans, terrestrial plants, and microorganisms (Lewis 2003, Whinam et al. 2005, Lee and Chown 2009, Chown et al. 2012). This collective research effort identified risk at a number of levels related to most pathways and taxa, and has resulted in the development of a number of preventative management initiatives (Huiskes et al. 2014). In contrast, few comprehensive pathway analyses have been performed in the Arctic where few preventative invasive species management measures are employed. Exceptions exist in the form of proximal analysis of ships' ballast water for the Canadian Arctic (Chan et al. 2012), sample-based analyses of ballast water organisms from sub-Arctic Alaska (Ruiz and Hines 1997), and isolated vector analyses focussed on invasive plants (Alaska – Carlson et al. 2007, Conn et al. 2008).

Given the limited knowledge of the NIS propagules arriving in Arctic regions, current estimations of invasion risk are compromised. Climate changes, and particularly temperature increases, are expected to be most pronounced in Arctic regions (IPCC 2007). Reducing sea ice extent is one of the most publicised consequences of climate changes to date (Wang and Overland 2009), and is increasingly permitting the use of northern sea routes for shipping further promoting the potential for NIS introduction in Arctic waters (Liu and Kronbak 2010). On land, changes in precipitation regimes and critical degree day sums will overlap for the first time with the climate niche thresholds of lower latitude plant species (Milbau et al. 2010, Walther et al. 2009). Arctic tourism, with the majority of opportunities being sea-borne and including frequent shore excursions, is also expanding and intensifying in terms of the number of sites visited and the number of tourists (Governor of Svalbard 2006). Polar tourists have been demonstrated to be effective vectors of vascular plant NIS (Lee and Chown 2009, Chown et al. 2012, Huiskes et al. 2014), and so the rate and spread of terrestrial NIS transfer to Arctic locations will likely increase in the absence of preventative management. Furthermore, the majority of NIS known to have been introduced to Arctic locations are plants (e.g. Elven et al. 2011, Ruiz and Hewitt 2009). These have historically been ephemeral in their presence, or stable but not spreading (Elven et al. 2011), and are often reported only in vegetative stages (Liška and Soldon 2004). Little monitoring of such species has been reported, but moderating climates will reduce abiotic stresses (Walther et al. 2009). Climate changes will also affect the distribution and abundance of indigenous species. Range extensions have already been observed in

both marine (Sorte et al. 2010, Canning-Clode et al. 2011) and terrestrial systems (Sturm et al. 2001, Walther et al. 2009), and the responses of indigenous species – particularly those presently existing at range margins – may affect the establishment success of NIS. Thus, while the positive effects of climate change on NIS in the Arctic may be generalised at larger scales, the implications at more regional scales are not clear given the uncertainty of changes in niche opportunity (Shea and Chesson 2002).

Measuring and managing invasive species threats in the Arctic

It is clear is that moderating climatic conditions in the Arctic, coupled with increasing introduction potential, will together increase the *potential* for the introduction and establishment of NIS in the Arctic. What impacts these introduced species might have on indigenous communities, ecosystem functioning, ecosystem services, human livelihoods or health will be primarily determined by the type of NIS, their effect on native ecosystems, and any managerial response by human populations. Identifying impacts might be possible in some cases by generalising those caused by individual invasive species outside the Arctic. Yet, this requires some *a priori* understanding of the species being introduced, their vectors, their viability, and their number. Other species being introduced may have no history of invasion elsewhere, but may still impact on Arctic ecosystems. For the majority of all introduced species however, their impact (negative or positive) will be unclear. Identifying vectors of species dispersal, quantifying propagule loads carried with these, and estimating the potential for species to establish and cause impacts are therefore major knowledge gaps that need addressing.

Therefore, in the present study, I evaluate current and future invasive species threats in the Arctic. I use the high-Arctic archipelago, Svalbard, as a model system throughout the work. Svalbard's utility as a model system is based on recent increases in tourists and other visitors, ongoing mining operations, and indications of temperature increases as a function of climate change (Førland et al. 2012) (see next section for location description). This research addresses substantial knowledge gaps in our understanding of NIS introduction processes in the Arctic region. Specifically, I aim to:

1. Quantify the non-indigenous plant propagule load transferred to Svalbard and test germination rates under set conditions;
2. Evaluate the status of non-indigenous plants present around Svalbard, and investigate factors that may be associated with their persistence;
3. Evaluate the efficacy of disinfection measures as a tool limiting the introduction of microorganisms to Svalbard;
4. Undertake a shipping pathway analysis to evaluate the potential for known invasive NIS to be transferred to, and survive in, Svalbard, currently and in the future;
5. Test the efficacy of molecular tools to assist in the identification of marine zooplankton sampled from ships' ballast water tanks; and
6. Evaluate the potential for non-indigenous zooplankton introduced to Svalbard in ships' ballast water to establish based on eco-physiological tolerances and species distribution models.

Thus, this research encompasses both terrestrial and marine threats, and consideration of the extent to which management measures reduce threat. The results are generalised where appropriate across the Arctic region such that a broader picture of invasive threats is presented.

Thesis outline

Chapter 2 presents results that address Aim 1. For this research, I used the footwear of visitors arriving in Svalbard and plant propagules as exemplars of wider human-mediated NIS introduction patterns to Svalbard. Vascular plant propagules were identified and germination rates tested under favourable conditions realistic of Svalbard summer ground temperatures. Chapter 3 addresses aim 2 describing vascular plant surveys I undertook together with project collaborators in Svalbard, and analyses performed with historical survey records. The study focuses on the persistence of NIS recorded in Svalbard, and their phenological state. Relationships between the phenological stage of individuals and both time and measures of climate were investigated. These data provide indications of spread, and potential of spread, of vascular plant NIS on Svalbard. Chapter 4 addresses aim 3, in evaluating the efficacy of a management measure designed to reduce NIS introduction. This work was accomplished in collaboration with the Association of Arctic Expedition Cruise Operators (AECO), and was designed to test the efficacy of recently trialled footwear disinfection methods to reduce microbial NIS introduction to Svalbard. The study was performed on an expedition ship during the Arctic summer, with the procedures evaluated by swabbing footwear both before and after disinfection treatment with contact plates. Contact plates were then incubated on board the ship to measure microorganism growth. Chapters 5-7 address marine research. In addressing aim 4, chapter 5 made use of data available characterising shipping patterns to Svalbard, and also data reporting the distributions of known invasive species, to evaluate the potential for transfer of NIS to Svalbard. I used a simple similarity metric to evaluate similarity between donor and recipient (Svalbard) locations, and thus the potential for NIS to survive in Svalbard waters. These calculations were repeated using forecast environmental data for the coming century. Importantly, I also developed and employed a qualitative model to characterise the many external factors that may affect the risk of NIS introduction to Svalbard. In this way, the use of ships as proxies for propagule pressure was justifiable. Chapters 6 and 7 build on the results of Chapter 5, and address aims 5 and 6. Chapter 6 is based on samples collected from the ballast water tanks of coal ships discharging ballast water at Svalbard ports. Owing to the substantial proportion of meroplankton, and particularly early life stage organisms, in the samples, I tested the utility of DNA barcoding methods to improve identification rates and efficiency. A range of universal primers were tested for their ability to amplify one of three different mitochondrial DNA markers. This work represented the first attempt to test such general methods on zooplankton transported in ballast water. Chapter 7 incorporates all data collected from the sampling of ships ballast water and those generated in Chapter 6, detailing the abundance and diversity of zooplankton discharged through ballast water into Svalbard waters. This research also provided the opportunity to qualitatively evaluate the effectiveness of ballast water exchange (a method of reducing the abundance of NIS in ballast water). A number of species were selected from those identified that had both a known invasive distribution and sufficient data characterising their eco-physiology and range, to model their potential to establish and reproduce in Svalbard. In this way, range maps based on the eco-physiological tolerances and correlative models were generated for eight NIS. Finally, Chapter 8 synthesises results from the individual studies outlined in Chapters 2-7, presenting an overview of the vulnerability of Svalbard, and by inference the wider Arctic, to species invasion.

Notes:

1. Chapters 2 and 5 are published in journals, while the remaining chapters are either being submitted to journals or are currently in review. Accordingly, there is an unavoidable repetition of methods and discussion in some chapters.
2. I am lead author on all manuscripts with the exception of Chapter 3, but combinations of collaborators appear as co-authors, and as a result 'we' is often used within these chapters. Details of author contributions are outlined in the authorship statement.

Study site

Svalbard is an Arctic archipelago located in the high north (74° - 81°N and 10° - 30°E) administered by Norway. Two main Norwegian settlements exist on the main island of Spitsbergen, together with a Russian settlement (Fig 1). The three settlements service operational coal mines and harbours, while a fourth settlement, Ny Alesund, houses an international scientific research community.

Around 60 % of the islands are covered in ice (Jónsdóttir 2005), leaving coastal pockets suitable for vegetation. Mean air temperatures of the warmest month throughout the archipelago are between 1-6°C (Elvebakk 2005), while annual mean sea surface temperatures are around 3°C (Ware et al. 2013).

Environmental management in Svalbard is administered in accordance with the 2002 Svalbard Environmental Protection Act (amended in 2012). The purpose of the Act is to preserve a virtually untouched environment in Svalbard, and section 26 and 27 explicitly prohibits the introduction of flora and fauna that do not naturally occur in Svalbard (Government of Norway 2014).
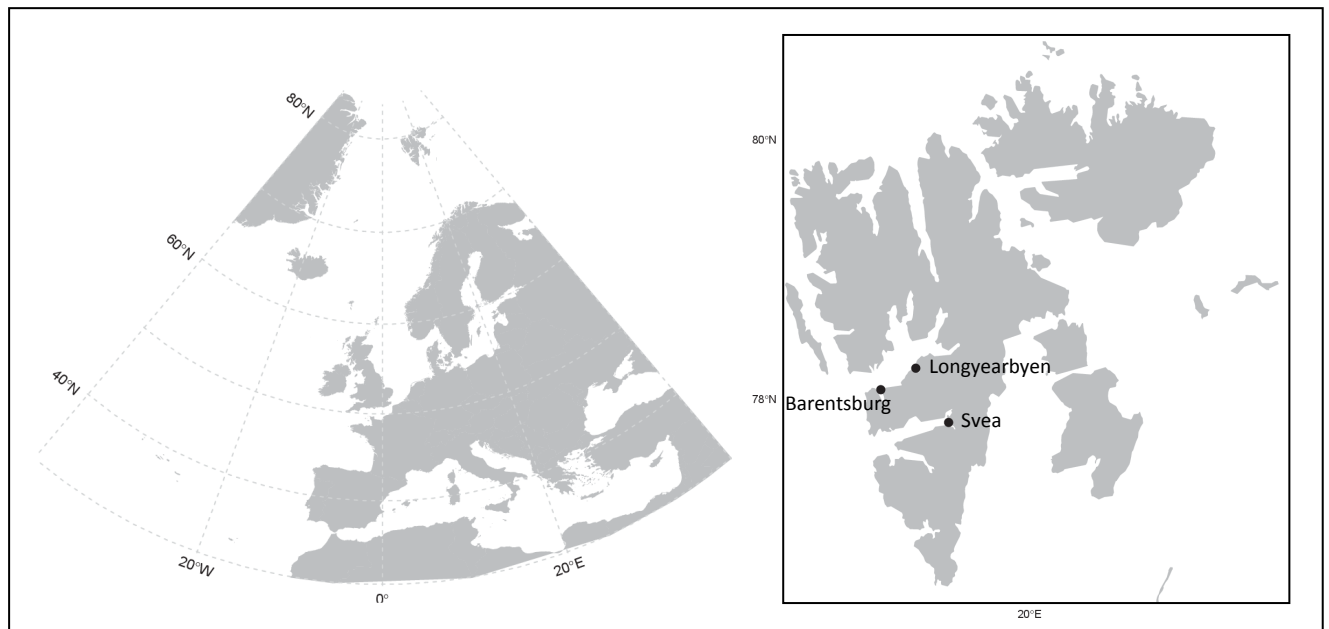
Figure 1. Map showing the location of Svalbard, and inset the location of the three largest coal mining settlements on Svalbard.

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Chapter 2: Humans introduce viable seeds to the Arctic on footwear

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Abstract

Expanding visitation to Polar regions combined with climate warming increases the potential for alien species introduction and establishment. We quantified vascular plant propagule pressure associated with different groups of travelers to the high-Arctic archipelago of Svalbard, and evaluated the potential of introduced seeds to germinate under the most favorable average Svalbard soil temperature (10°C). We sampled the footwear of 259 travelers arriving by air to Svalbard during the summer of 2008, recording 1019 seeds: a mean of 3.9 (± 0.8) seeds per traveler. Assuming the seed influx is representative for the whole year, we estimate a yearly seed load of around 270,000 by this vector alone. Seeds of 53 species were identified from 17 families, with Poaceae having both highest diversity and number of seeds. Eight of the families identified are among those most invasive worldwide, while 88.2% of the species identified were non-native to Svalbard. The number of seeds was highest on footwear that had been used in forested and alpine areas in the three months prior to traveling to Svalbard, and increased with the amount of soil affixed to footwear. In total, 26% of the collected seeds germinated under simulated Svalbard conditions. Our results demonstrate high propagule transport through aviation to highly visited cold-climate regions and isolated islands is occurring. Alien species establishment is expected to increase with climate change, particularly in high latitude regions, making the need for regional management considerations a priority.

Introduction

Until recently in the high-Arctic and Antarctic, two processes have maintained ecological integrity: low frequency of human-mediated dispersal, and the prevailing climate, both of which are rapidly changing (Convey et al. 2006; Elven et al. 2011). Seed dispersal by humans and cargo is to some degree documented for the Antarctic (Whinam et al. 2005; Frenot et al. 2005; Lee and Chown 2009a, b; SCAR 2010), and has been the subject of management development by the Antarctic Treaty Parties (Australia and SCAR 2007). In contrast, no such quantification exists for the Arctic, where few biosecurity measures are currently employed.

The total number of archaeophytic, persistent, and transient alien plants in the Arctic is low, constituting a very low to zero proportion of the regional Arctic floras (Elven et al. 2011). Exceptions exist in some of the millennium-old Viking settlements and more recent Russian settlements where non-indigenous plants are more prevalent. In some Arctic settlements, sometimes very far to the north, casual introductions are quite frequent (Liška and Soldán 2004; Elven et al. 2011). For other taxa, records of established alien species exist, although these are also few. A vole is known to be established on Svalbard (Fredga et al. 1990) while the first records of a non-native crustacean and species of kelp have been made at lower Arctic latitudes (Ashton et al. 2008).

Elsewhere in the world, humans and their associated activities have been demonstrated to be effective vectors of unintentional species transfer, providing carriage for plants (Clifford 1956; Powell 1968; Falinski 1972; Higashino et al. 1983; Whinam et al. 2005; Lee and Chown 2009a; Wichmann et al. 2009), arthropods (McCullough et al. 2006; Hughes et al. 2010), bacteria (Curry et al. 2002; Drake et al. 2007; Hughes et al. 2010; McNeill et al. 2010), terrestrial vertebrates (Gillespie 1985), and marine organisms (Carlton 1985; Gollasch 2002). Generally, the little amount of research concerning invasion processes in the Arctic is biased towards post-invasion – a trend identified globally (Puth and Post 2005). To date, the only attempt to quantify the significance of a pathway of species introduction to the Arctic focused on ship-mediated introductions to Alaska (Hines and Ruiz 2000).

Human activity in the Arctic has rapidly increased over the past 40 years (Kaltenborn 2000; Forbes et al. 2004). Between 1995 and 2004 there was a 255% increase in the number of tourists visiting Svalbard (Governor of Svalbard 2006), while Greenland recorded a 500% increase over the same time period (Statistics Greenland 2009). While the tourism sector is increasing rapidly, so too are other travel sectors such as that associated with science. Polar scientists often visit and work in several alpine or high latitude environments, and may move frequently between them (e.g. Whinam et al. 2005), increasing the chances of introducing organisms pre-adapted to Arctic environmental conditions.

While dispersal is a critical step in species invasions, the Arctic climate also presents a significant barrier to species colonization (Alsos et al. 2007). The effect of climate on new species colonization is complex, and varies at different stages of colonization (Shevtsova et al. 2009). For plants, the initial bottleneck of colonization may be germination. Low temperatures have been shown to limit germination in Arctic plant species (Sørensen 1941, Müller et al. 2011) suggesting also that germination of introduced alien plant species would be similarly impaired. Despite this, many temperate grassland, shrub and herbaceous species have been shown experimentally to be capable of germination at surface temperatures commonly recorded in the Arctic today (Baskin and Baskin 1998; Trudgill et al. 2000). Indeed, Arctic summer surface temperatures can be

several degrees warmer than those reported from meteorological stations recorded at two meters above the surface (Scherrer and Körner 2010). Furthermore, it is possible that seeds introduced today, capable of lying dormant in soil for many years (Thompson et al.1997), may be capable of germination under future climates.

Alien plants are widely documented at higher latitudes (Alaska, sub-Antarctic), and are relatively easy to monitor and identify (compared to e.g. bacteria, arthropods, fungi). For these reasons, and due to the incomplete knowledge of other taxonomic groups in the Arctic (Coulson et al. 2004; Elvebakk and Prestrud 1996; Alsos et al. 2009), plant seeds make appropriate exemplars to investigate the extent to which new species could be transported to, and survive in, Arctic regions. Humans can carry a high plant propagule load on footwear (Clifford 1956; Powell 1968; Falinski 1972; Higashino 1983; Whinam et al. 2005; Lee and Chown 2009a; Wichmann et al. 2009; McNeill et al. 2011), from which seeds can disperse (Lee and Chown 2009a; Wichmann et al. 2009; Pickering and Mount 2010; Ware and Bergstrom, unpublished data), and as such, humans are likely to introduce alien seeds while traveling. Here, by using footwear as a pathway of introduction, we investigated the threat of species transfer to Svalbard. We asked the following questions: (1) What is the size and composition of the seed load being carried to Svalbard on travelers' footwear? (2) What factors explain the number of seeds on footwear? (3) Could seeds transported to Svalbard on footwear germinate under current Svalbard conditions? Based on the results of these investigations, implications for management are discussed.

Methods

Location

Around 60% of the Svalbard archipelago (74° - 81°N and 10° - 30°E) is covered in ice, leaving coastal pockets suitable for vegetation (Jónsdóttir 2005). Three of the five Arctic bioclimatic subzones identified by Walker et al. (2005) are present in Svalbard: polar desert (subzone A), northern Arctic tundra (subzone B) and middle Arctic tundra (subzone C). Mean air temperatures of the warmest month in each zone respectively are between 1-2.5ºC, 2.5-4ºC, and 4-6ºC (Elvebakk 2005). There are 165 native plant species (Alsos et al. 2011). Around 60 non-indigenous plant species have been recorded around the main settlements (Liška and Soldán 2004), of which around 28-37 are established or are frequently re-introduced (Elven and Elvebakk 1996; Elven et al. 2011).

Most travelers to Svalbard travel by plane, arriving at the major airport located in Longyearbyen, on the island of Spitsbergen. During 2008, 68,901 travelers flew into Longyearbyen (Governor of Svalbard, personal communication) with more than 90 % of these typically arriving over the tourist season (March-September) (Governor of Svalbard 2006). Many travelers join expedition ships at the local port, exploring the archipelago by ship.

Footwear sampling

We sampled the footwear of 259 travelers arriving at Svalbard Airport between 20 June and 28 September 2008. Around 75% of travelers arriving in Svalbard were wearing footwear with soles capable of carrying substantive quantities of soil, such as those typical of hiking/running shoes (Ware, unpublished data); only these travelers were asked to participate in the survey. We scraped off any soil attached to participants'

footwear using a stiff-bristled brush and forceps, scrubbed the shoe sole, and inspected the shoe lacing and tongue for biological material. Footwear was cleaned until all visible material was removed, and material was collected in plastic bags. A sampling unit was considered as a pair of shoes and in instances where travelers arrived with two pairs of shoes (i.e. one pair in their luggage) the second pair was considered a separate sample. In between samples, sampling equipment was cleaned thoroughly and visually inspected for dirt and propagules so as to avoid sample contamination. Samples were tagged with unique identifiers. We sorted samples into the following categories with the aid of a dissecting microscope (3×): seeds; plant and invertebrate fragments (bryophyte fragments, leaves, macroscopic invertebrate parts); soil (organic material); and non-organic material (highly variable, but commonly including metal and plastic fragments, chewing gum, and feathers). Total seed and bryophyte fragment numbers were tallied. Seeds were identified to the lowest taxonomic group possible using an identification guide (Cappers et al. 2006) and online resources (Kirkbride et al. 2006). Families were crosschecked against the most invasive families listed within the Global Invasive Species Database (ISSG 2010). While bryophyte fragments collected may have been capable of vegetative growth, we excluded these from substantive analysis due to the difficulties associated with their identification (e.g. Rowntree et al. 2010). We considered a focus on vascular plants to be a priority, owing to their significance in the global invasive flora (e.g. ISSG 2010). Soil samples were stored at − 20° prior to sample sorting, and weighed following sorting using Metter Toledo scales.

Germination

We placed collected seeds on filter paper (grade 1, Whatman, Maidstone, UK) moistened with distilled water via a wick attached to a reservoir. Seeds were then kept in a phytotron chamber at 10°C, under 24-hour light (approximately 40 μmol m^{-2} s^{-1} at seed surface, 35 W fluorescent tube, 840 HE (Osram, Munich, Germany)) to simulate ambient average summer soil conditions in Svalbard. A temperature of 10°C was selected as this best reflects average soil surface records from a number of favorable Svalbard sites; the temperature was fixed as this reflects the relatively low standard temperature deviation recorded from these sites (Müller, unpublished data). Seeds were monitored for germination (protrusion of a radical) for 48 days.

Traveler statistics

Participants also completed a questionnaire (linked to their footwear sample using a unique identifier), categorizing themselves as a tourist, scientist, businessperson, resident, or student. Furthermore, they indicated whether, and when, they had last cleaned their footwear; whether they had used their footwear in the three months prior to traveling; and in what type of habitat they had used their footwear (forested, alpine, rural, or urban areas) over the three previous months.

Statistical analysis

To determine the relationship between the weight of soil collected from footwear and the number of seeds found, we fitted a generalized linear model (GLM) with a quasipoisson error distribution and logarithmic link function, and an overdispersion characteristic. From this, we also calculated seeds per 1g of soil. To test for correlation between seed load and the two explanatory variables, traveler categories and previous footwear use, we fitted GLMs separately. Again, we fitted the GLMs using a quasipoisson error distribution with a logarithmic link function and an overdispersion characteristic. We began by fitting maximum models

containing all predictor variables and interaction terms. Model simplification was then achieved by removing variables and interaction terms stepwise. Model fit was assessed using analysis of variance (ANOVA) tests to determine whether simplified models significantly increased deviance; the significance of difference was assessed using *F*-tests. Where deviance was not increased (i.e. p < 0.05), variables or interaction terms were omitted from further modeling. To determine the effectiveness of traveler footwear cleaning prior to travel, we used the Wilcoxon rank sum test with continuity correction. For all mean values calculated in our analyses, standard errors were also calculated (± SE). We used the statistical and programming package R (version 2.10.0, R Development Core Team 2008) to carry out all analyses.

Results

Footwear samples

Overall, 40 % of the 259 footwear samples examined contained seeds. A total of 1,019 seeds were collected representing a mean of 3.9 (±0.8) seeds per traveler, or 9.9 (±1.1) seeds per traveler that had seeds attached to their footwear. The maximum number of seeds found in a single sample was 117 (Table 1), and 26 samples (10%) contained 10 or more seeds. In addition, we also found 465 bryophyte fragments in the samples representing a mean of 1.8 (±0.6) fragments per traveler. A mean of 0.27 g (± 0.06) of soil was found on a pair of footwear, with a range: 0 – 9.9 g (35 % of footwear did not contain any soil). The amount of soil present on footwear was significantly correlated with the number of seeds present (F = 165, *p* < 0.05, 166 *df*). Where soil was present in a sample, there was an average of 2.9 ± 1.2 seeds per gram of soil. We did not find any live invertebrates, eggs, or larvae in the samples.

Table 1 Summary of footwear samples, and survey information collected from people arriving to Svalbard. CI: confidence interval

Traveler category	n	% Contaminated	% With soil	Total seeds	Mean seeds	Bootstrapped 95% CI	Max. seeds per sample	% Cleaned
Tourist	170	41	69	631	3.71	2.3, 6.6	117	21
Scientist	37	57	65	212	5.73	2.8, 12.0	62	19
Student	28	36	46	98	3.50	1.1, 9.1	39	21
Business	19	47	63	59	3.11	1.0, 7.7	25	10
Resident	5	20	40	19	3.80	0.0, 7.6	19	0
Total	259	48	65	1,019	3.93	2.8, 6.1	-	20

The majority of the identified seeds collected were grasses (60 %), with 17 Poaceae species identified (Table 2). Other seeds present were tree, herb and sedge seeds, with a proportion (13 %) unable to be identified (Fig. 1). Four of the herb species and three grass species identified from our samples have already established as alien species in Svalbard. Only two possible native species were found (Table 2).

Germination

Of the total 1,019 seeds tested for germination, 266 (26%) germinated under the test conditions. *Taraxacum* sp. (n = 9)*, Cerastium brachypetalum* (n = 8), *C. glomeratum* (n = 12), and *Dactylis glomerate* (n = 2) all recorded 100% germination, while *Deschampsia flexuosa, Poa annua* and *P. trivialis* all recorded germination > 40% (Table 2).

Table 2 Number of seeds of native or alien species found on people's footwear arriving in Svalbard, and seed germination percentages Numbers in bold indicate those alien species that have already established on Svalbard. Life form: perennial (P), annual (A) or both (A/P) according to www.plants.usda.gov. [a]Families that are those identified as the most invasive worldwide (as per Pysek 1998) or families with a high number of invasive species (ISSG 2010).

Group and family	Taxa	Native	Alien	Unidentified species	Life form	% Germination
Gymnosperm						
Cupressaceae	*Thuja plicata*		1		P	
Angiosperms - dicotyledons						
Apiaceae	*Torilis japonica*		1		A	
Asteraceae[a]	Unidentified			1		
	Taraxacum sp.		**9**		P	100
Betulaceae	*Betula pubescens*		143		P	1
Brassicaceae[a]	Unidentified			1		
	Erucrastrum sp.		1			
	Isatis sp.		7			
	Nasturtium microphyllum		23		P	9
Caryophyllaceae	Unidentified					
	Cerastium brachypetalum		8		A	100
	Cerastium glomeratum		12		A	100
Ericaceae	*Vaccinium* sp.			5		
Fabaceae[a]	*Astragalus glycyphyllos*		1		P	
	Medicago falcata		1		A/P	
Papavaceae[a]	Unidentified			1		
	Papaver sp.			3		
Plataginaceae	*Plantago major*		**27**		P	7
Polygonaceae	Unidentified			4		100
	Polygonum aviculare		**3**		A/P	
	Rumex sp.			**1**		
	Rumex crispus		1		P	
Ranunculaceae[a]	*Ranunculus* sp.			1		
	Ranunculus acris		**5**		P	
Rosaceae[a]	*Geum macrophyllum*		1		P	
	Geum rivale		1		P	
Angiosperms - monocotyledons						
Cyperaceae	*Carex* sp.			14		

	Carex acutiformis		1		P	
Juncaceae[a]	Unidentified			3		
	Juncus sp.			6		
	Juncus effuses		10		P	60
	Juncus pygmaeus		22		A	
Juncaginaceae	*Triglochin maritima*		20		P	
Poaceae[a]	Unidentified			73		4
	Agrostis sp.			**1**		100
	Agrostis stolonifera		24		P	38
	Alopecurus pratensis		**6**		P	50
	Ammophila arenaria		2		P	50
	Bromopsis sp.		1			
	Bromus sp.		2			
	Bromus hordeaceus		4		A	75
	Calamagrostis pseudophragmites		2		P	
	Dactylis glomerata		2		P	100
	Deschampsia sp.			1		
	Deschampisa caespitosa		**3**		P	
	Avenella flexuosa		89		P	45
	Festuca sp.			21		5
	Festuca lemanii		1		P	100
	Festuca rubra	1			P	
	Holcus lanatus		1		P	100
	Hordeum sp.			**2**		50
	Lolium perenne		2		A/P	50
	Phleum pratense ssp. *pratense*		2		P	
	Phleum pratense ssp. *serotinum*		1		P	
	Poa sp.			41		39
	Poa annua		**36**		A	58
	Poa trivialis		180		P	57
	Poa pratensis	62			P	10
	Trisetum flavescens		3		P	
Unidentified seeds				118		7
Totals		63	659	293		26
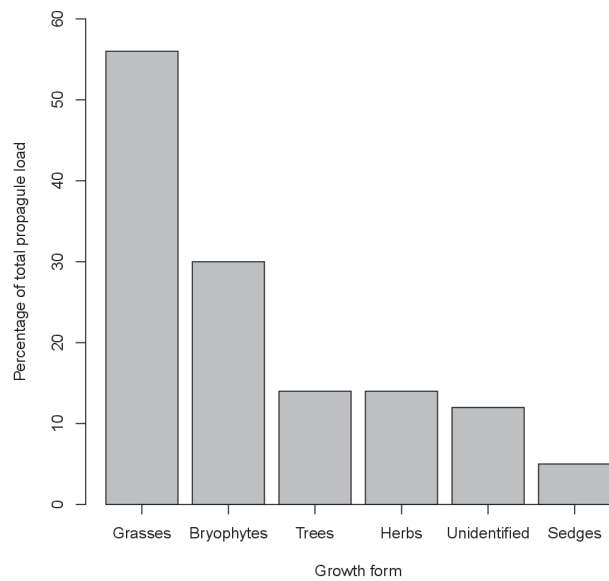
Figure 1 Percentage of total seeds by previous use collected from the footwear of travelers to Svalbard
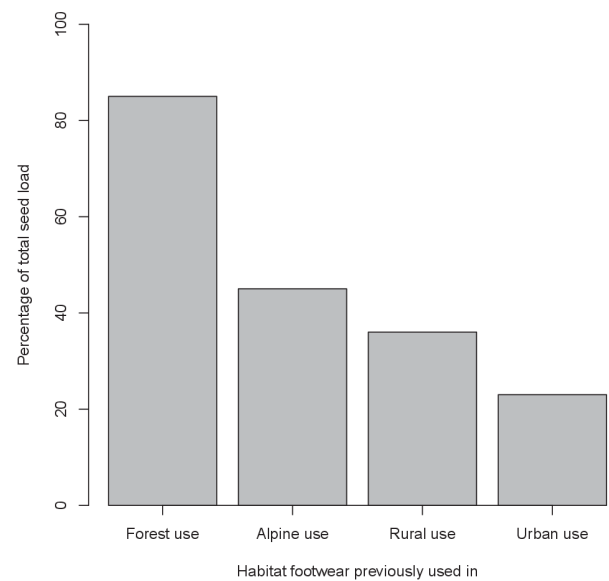
Figure 2 Percentage of total seeds by previous use collected from the footwear of travelers to Svalbard

Traveler statistics

Category of traveler arriving to Svalbard had no significant effect on the number of seeds imported on footwear (Table 1). Twenty percent of participants reported cleaning their footwear prior to travel: of this percentage, 49% contained seeds. There was no evidence that footwear cleaning by participants lowered the number of seeds transported on shoes (W = 5,615.5, *p* > 0.05).

Fifty seven per cent of the participants had used their footwear in forests, while 30% had used their footwear in alpine regions (Fig. 2). A GLM that included use of footwear in both forest and alpine areas provided the best fit to the data (Table 3), demonstrating that footwear previously used in these two habitats contained a significantly higher seed load (F = 11.06, *p* = 0.001, 257 *df*).

Table 3 The results of ANOVA tests comparing different generalized linear models investigating the effects of where footwear had been previously used (forest, alpine, rural or urban habitats) on the number of seeds affixed to footwear

Model variables	Deviance residuals (max – min)	Deviance on *df*	Δ Deviance	*p*
Seeds = forest × alpine × rural × other	(-5.0398 to 2.8748)	2,932.4 on 244	-	-
Seeds = forest + alpine + rural + other	(-4.249 to 2.876)	3,294.4 on 254	-362.00	0.1325
Seeds = forest + alpine + rural	(-4.076 to 2.892)	3,314.6 on 255	-20.2	0.4263
Seeds = forest + alpine	(-3.8360 to 3.1327)	3,331.0 on 256	-16.4	0.4785
Seeds = forest	(-3.423 to 3.423)	3,370.6 on 257	-39.6	0.272

Discussion

This study demonstrates that people arriving in Svalbard pose an identifiable hazard to the local environment through the introduction of alien plant seeds that are capable of germination even under current climatic conditions. Travelers are providing the means to increase the plant species pool capable of reaching the Arctic, a trend identified already in the sub-Arctic (Carlson and Shephard 2007). The seed load per person transferred to the Arctic is similar to that being introduced by expeditioners to the Antarctic, and the same types of species are being transported (Lee and Chown 2009a). Our findings support those of others demonstrating that humans are capable of translocating many of the world's widespread alien plant species (Pickering and Mount 2010).

Our analysis demonstrated that footwear previously used in forested or alpine areas carried significantly higher numbers of seed than that used in rural or urban areas. Few studies have attempted to investigate previous use as a factor predisposing an item to contamination. McNeill et al. (2010) found golfing footwear to be the most highly contaminated item in a study of the footwear of arriving airplane passengers in New Zealand, while Whinam et al. (2005) found many Antarctic expeditioners had recently used their clothing in natural environments. The positive relationship between outdoor use and clothing and equipment contamination is logical, and our study reaffirms the notion that these items provide the greatest biosecurity hazard.

The strong association between the presence of soil and incidence of seeds and bryophyte fragments is consistent with other studies (Hughes et al. 2010; McNeill et al. 2011), and highlights the potential for any clothing and equipment capable of carrying soil to mediate alien organism introduction. The mean number of seeds found per gram of soil reported here (2.9 ± 1.2) is comparable to that found in soil attached to the footwear of arriving aircraft passengers to New Zealand (2.5 ± 0.37 per 1g soil - McNeill et al. 2011). As with McNeill at al. (2011), our seed counts may be underestimated owing to imperfect visual searches in our samples, while counts would be slightly inflated by the few occasions where seeds were found in the absence of soil (e.g. on footwear lacing or tongue).

The sampled seed load contained a number of cosmopolitan species, and eight of the 17 families identified belonged to those families ranked as most invasive at a global scale (Pyšek 1998). Considering the dominance of Poaceae seeds found in connection with other human-mediated seed dispersal studies (e.g. Schmidt 1989; Hodkinson and Thompson 1997; Lee and Chown 2009a), and the wide geographic range of establishment that some Poaceae species have achieved (i.e. *Poa annua* and *Poa trivialis* have both established in the Antarctic – Frenot et al. 2005; Hughes et al. 2010), the finding that Poaceae seeds dominated our samples was not unexpected.

Our relatively high germination rates indicate that germination may not be a barrier to establishment in Svalbard for many non-indigenous species. Germination occurred rapidly under the test conditions, with 87 % of those that germinated doing so within 14 days, and the remainder within 48 days – well within the growing season. These germination results are based on present climatic means; however, temperature increases of 0.61ºC per decade are expected for the period 1961 – 2050 (Hanssen-Bauer 2002) which would likely favour the germination of more northerly plants if introduced to Svalbard (e.g. Trudgill et al. 2000; Milbau et al. 2009).

Further improving the chances of successful establishment are the few samples that contained many propagules. One sample contained over 100 seeds, and 26 samples had 10 or more seeds (10 %). If these were dispersed into suitable habitat, the effects of propagule pressure would increase their likelihood of successful establishment (i.e. Williamson 1996; Lockwood et al. 2005, 2007; Colautti et al. 2006). Similarly, where seeds are pre-adapted to the Svalbard climate, the potential for establishment is greater. Many participants had used their footwear in either northern boreal forested regions or alpine regions, and many of the species identified from shoe samples are found in these habitats. While we would expect these species to be better adapted to the challenges of establishing in Svalbard, establishment of other more generalist species cannot be precluded. Indeed, *Barbarea vulgaris* ssp. *arcuata* and species of the *Tarxacum ruderalia* aggregate – both generalist European natives – have established on Svalbard and are spreading locally (Alsos, pers. obs.).

As our data were collected over a summer period, they were not suited to testing the effects of seasonality on seed load (no data were collected during winter or spring). There was however, a large variation in seed loading (see CIs – Table 1), and our models failed to account for parts of this variation (Table 2). Factors such as footwear use at times of seed production and dispersal may then be important considerations in more precisely modeling human-mediated seed influx to Svalbard.

While recognizing the above caveat, it is possible to project an estimate of a yearly seed load introduced on footwear based on visitor numbers alone. For the year of the study (2008) 68,901 people arrived at the Svalbard airport, equating to an estimated yearly seed load of 270,000 from travelers' footwear alone. In addition, approximately 30,000 cruise ship passengers land on Svalbard. In a separate preliminary study we sampled the footwear of three hundred cruise ship passengers landing on Svalbard and found just 21 seeds and bryophyte fragments (Ware, unpublished data), suggesting that cruise ship passengers may contribute a smaller propagule load to the region. Many other pathways of species introduction to Svalbard exist, including cargo, planes, scientific equipment and the clothing and personal equipment of travelers (e.g. Whinam et al. 2005; Barnes et al. 2006; Lee and Chown 2009a, b). From these, organisms of other taxa may be transferred. Thus, the total propagule load being introduced to Svalbard would be considerably higher than estimated here.

Management implications

Our study suggests that modern aviation, as the means by which tourism has achieved its rapid increase, has the potential to increase the pressure of plant species introduction to highly visited cold-climate regions and isolated islands globally. Studies elsewhere demonstrate that footwear and the soil attached to it are furthermore capable of carrying a variety of other taxa (e.g. McNeill et al. 2011). While we found no evidence that footwear could transport live invertebrates, eggs, or larva, we did not analyze collected samples for the presence of bacteria or fungi which may have been present. The question of whether alien plants can establish on Svalbard requires further investigation (being dependent on a variety of factors including soil moisture, aspect, and season); however, our germination results, and the artificial ranges achieved by other introduced plants at high latitudes, suggest that a more conservative approach to regional biosecurity need be considered if the ecological and genetic integrity of the local flora is to be maintained. As many other organisms can be transported in association with soil, measures taken to reduce the seed load imported to Svalbard will also reduce the hazard of other organisms being introduced.

Measures to address the introduction of seeds via footwear exist. The ineffectiveness of footwear cleaning by travelers participating in this study suggests that educating travelers of the need to clean footwear prior to arrival may not be effective alone. While the effectiveness of educating Antarctic expeditioners to clean footwear and personal equipment of seeds and contaminants was apparent (Bergstrom, pers. obs.), this may have been due to the combination of follow-up inspections. Beyond education, more stringent management measures could include the adoption of a biosecurity policy at entry points to Svalbard, such as those in place in New Zealand (Biosecurity New Zealand 2010), and for Antarctic tour operators (IAATO 2010). Any management interventions in Svalbard would ideally be positioned within a more comprehensive framework, incorporating all pathways and vectors of introduction, and all organisms, especially pathogens.

Our study makes a case for a more precautionary approach to the management of alien species in Svalbard. The pathway analysis described here suggests that more non-indigenous plant species can be expected in Svalbard if measures to prevent their introduction are not considered. Moreover, our study implies that isolated islands or regions worldwide, which are experiencing similar increases in human traffic as is occurring in Svalbard, may be exposed to similar hazards or even greater hazards if climate is less of a limiting factor.

Acknowledgments

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Chapter 3: Past Arctic aliens have passed away, current ones may stay

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Abstract

Increased human activity and climate change are expected to increase the numbers and impact of alien species in the Arctic, but knowledge of alien species is fragmentary for most Arctic regions. Through field investigations over the last ten years, and review of alien vascular plant records for the high Arctic Archipelago Svalbard over the past 130 years, we explored long term trends in phenology and persistence. In total, 447 observations (299 when accounting for possible re-sightings) of 105 taxa had been recorded at 27 sites. Recent surveys at 18 of these sites revealed that alien species had disappeared at half of them. Investigations at a further 25 sites characterised by former settlements and/or current high visitation rates, but where no older records of flora were available, revealed no alien species. Alien species in fertile phenological stages were associated with positive mean July temperatures more frequently than were those in vegetative stages. This demonstrates a clear effect of temperature on the reproductive potential of recorded alien plants, and thus the potential for spread in Svalbard. Given that human activity and temperatures are expected to continue increasing into the future, there is a need to respond in policy and action to the heightening potential for further alien species introduction and spread in the Arctic.

Introduction

The proportion of alien species is lower in polar regions than in other regions (Frenot et al. 2005; Elven et al. 2011; Ellis et al. 2012). Until recently, two processes have maintained the ecological integrity of these cold regions: low frequency of human-mediated dispersal, and the prevailing climate, both of which are rapidly changing (Thuiller et al. 2007; Walther et al. 2009). Propagule pressure, a primary determinant of alien species occurrence and spread (Lockwood et al. 2005; Carboni et al. 2011), is increasing in polar regions due to escalating human activity (Chown et al. 2012; Ware et al. 2012; Lassuy and Lewis 2013; Ware et al. 2013). Likewise, ongoing global warming has enabled alien species to expand into regions in which they previously could not survive and reproduce (Walther et al. 2009), and has increased invasion rates independent of propagule pressure in China, the United Kingdom and the United States (Huang et al. 2011). Ongoing climate change affects biodiversity most immediately through poleward and uphill range shifts (Kelly and Goulden 2008; Lenoir et al. 2008) and changes in phenology (Bates et al. 2012). The Arctic has so far experienced the highest rates of temperature increase globally, and is expected to continue to do so (Xu et al. 2013), with changes in phenology and range shifts observed already in Arctic species (Sturm et al. 2001; Callaghan et al. 2011; Myers-Smith et al. 2011). Thus, the risk of alien species establishment and spread in the Arctic has already increased and is expected to escalate dramatically in the near future.

The Arctic represent a steep climatic gradient from the low Arctic with a mean July temperature of 10-12°C, to the polar desert zone with mean July temperatures of 1-3°C (Walker et al. 2005). While no alien species have been recorded in the northernmost zones of the Arctic, the numbers of both casual and naturalized alien species increase towards southern zones (Elven et al. 2011; Daniëls et al. 2013). The majority of Arctic aliens are confined to settlements and their close surroundings, trading posts, mining areas, airstrips, harbours, and the few roads and railways, and are not considered a threat to the native flora (Elven et al. 2011; Gederaas et al. 2012; Daniëls et al. 2013; Lassuy and Lewis 2013). However, in the low Arctic, plants introduced through agriculture have been a significant part of local and regional floras for more than a millennium, and have caused substantial impacts (Elven et al. 2011). Furthermore, an increasing number of alien species, among them some ranked as highly invasive, have been recorded in the low Arctic and in all Arctic bordering zones (Alaska (Lassuy and Lewis 2013; AKEPIC 2014) cf. (Carlson and Shepard 2007), Canada (http://www.wildspecies.ca), Greenland (http://nobanis.org/), Iceland (Wasowicz et al. 2013), Norway (Gederaas et al. 2012), and Russia (Elven et al. 2011)). These represent a large pool of alien species potentially able to invade the Arctic in the near future either by human-mediated or natural dispersal (Alsos et al. 2007; Ware et al. 2012).

Future change is best understood when measured against a credible baseline (Lassuy and Lewis 2013). However, with the exception of Iceland (Wasowicz et al. 2013), no comprehensive overview of alien species distribution or status exists for any Arctic region. The high Arctic archipelago Svalbard is among the best studied Arctic regions in terms of biodiversity (Elvebakk and Prestrud 1996; Prestrud et al. 2004). Accordingly, the higher number of casual alien species known from Svalbard compared to other Arctic regions (Elven et al. 2011) may be a result of survey bias. Svalbard was uninhabited until the first whaling stations were established in the early seventeenth century. The first record of vascular plants in Svalbard dates back to 1675 (Malmgren 1862), whereas the first records of alien species were documented more than 200 years later in 1883 (Gyllencreutz 1884), 1897 (Ekstam 1899) and 1898 (Andersson and Hesselman 1900). The flora of the

archipelago was extensively investigated during the 20th century (e.g. (Hadač 1944; Rønning 1972; Elvebakk 1989). However, with some exceptions (Høeg and Lid 1929; Hadač 1941; Sunding 1961), alien species were only sporadically recorded. In 1988, Liška and Soldán (2004) surveyed the surrounds of the two largest Russian settlements, Barentsburg and Pyramiden. However, no attempts have been made to summarize all alien vascular plant species in Svalbard since 1941 (Hadač 1941). Here we: 1) present a complete record of all alien vascular plant species recorded in Svalbard based on field investigations and review of previous records; 2) evaluate if phenological stage is related to temperature; and 3) based on the results of 1-2, discuss the risk of alien species becoming naturalized or invasive in the near future.

Methods

Records of alien species

Records of alien species were compiled from the literature, the Norwegian herbaria, nobanis.org, and GBIF.org (access date 9th November 2012). Field investigations were undertaken in 1) Barentsburg in 2007, 2008, and 2011; 2) Pyramiden in 1998 and 2011; 3) Advent City and Hiorthhamn in 2013; 4) Sverdrupbyen, Nybyen, Hotellneset and Longyearbyen airport in 2013; and brief visits at 18 stations in NW Svalbard in 2013. Surveys at all sites ranged between 2-6 hours, and were undertaken with the help of a number of individuals (see acknowledgements). In addition, alien species have been recorded occasionally in Longyearbyen in 2006-2013, and all three authors have also done fieldwork at many other sites during recent years. This included mainly undisturbed sites, but also trapper huts (Kapp Berg, Hyttevika at Kvartsittodden, Fredheim at Sassendalen, Kvalhovddalen, Bohemanflya), a German weather station from World War II (Biskayahuken), a former whaling site (Magdalenafjorden), former mining sites (Skansebukta, Ny-London, Vårsolbukta), and a former research station (Svenskhuset at Kapp Thordsen).

Place names of collection sites are given with their modern equivalent and spelling according to http://miljo.npolar.no/placenames/pages/searchE.asp (e.g. Moskushamn = Hiorthhamn, Longyear City = Longyearbyen, Hotelneset = Hotellneset) (Figure 1). "Tempelfjorden: Nøis' hut" (Hadač 1941) is assumed to be Fredheim. The largest settlement, Longyearbyen, has had around 2000 inhabitants in recent years, the highest ever recorded (Statistics Norway, http://ssb.no). At the time of the investigation by Liška and Soldán in 1988, Pyramiden had about 1000 inhabitants (abandoned in 1998), whereas Barentsburg had 1200 inhabitants (about 500 in 2012). We follow the most recent taxonomy for the Arctic, the Panarctic Flora checklist (PAF, Elven et al. 2011). For species not mentioned there, we follow the United States Department of Agriculture/Natural Resources Conservation Service Plant Database (http://plants.usda.gov/java/).

Phenology and climate

Phenological stages were classified where possible into 'vegetative', 'with bud', 'in flower', and 'with fruit' based on herbarium specimens or information given in the literature. Date of investigation or herbarium voucher collection was noted. When dates were given as a period, the latest date of this period was used in the calculations. Dates were transformed to July date starting from the 1st of July. Mean July temperature data were downloaded from eKlima (http://met.no).

Statistics

For analysis, phenological stages were first transformed into the categories 'vegetative' (= vegetative) and 'fertile' (= any of the three latter phenological stages). We then analysed the effect of year and temperature on phenological stage using generalised linear models with mixed effects (GLMM) and a binomial error distribution. Models were fitted with the binary phenology categories as a response variable and three predictor variables: July date, the deviation of mean July temperatures, and the deviation of mean July temperatures of the previous year. A separate model was fitted with phenology as a response and the year of record as a predictor variable. Species was included as a random effect in both models. Model fit was assessed by simplifying models and removing interactions between effects and assessing the impact of simplification. This was done in two ways. The first was based on changes in Akaike's information criterion (AIC), whereby ΔAIC <2 were considered to demonstrate equally adequate models. The second considered changes in deviance, where fixed effects or interactions were dropped if they did not significantly increase deviance. The latter was achieved by comparing models of reducing complexity using analysis of variance tests (ANOVA), and assessing the significance of difference between models using Chi Square tests. Constant variance of the residuals, presence of outliers, and approximate normality of the random effects were checked graphically for final models. We also investigated possible relationships between the number of alien species records collected and time using generalised linear models (GLM). Initial models were fitted with a poisson distribution, but resulted in substantially greater residual deviance than degrees of freedom. We subsequently fitted models with a quasipoisson error distribution to account for the overdispersion. All analyses were done in R (version 2.12.0, R Core Team 2013), and GLMM models fitted using functions in the R package lme4 (Bates et al. 2012).

Results

Frequency and stability of introductions

370 records of alien species were found in the literature and herbarium records, and an additional 77 records were made during our field investigations (Appendix 1). In total, 447 observations of 105 taxa (including 7 taxa not determined to species or subspecies level) had been reported from 27 sites (35 if sub-sites within settlements were counted). Accounting for potential re-sighting of the species within sites (Appendix 1), this represents a minimum of 299 independent introductions of alien species. Among the 27 sites where alien species had been recorded during the last 130 years, the same species were not found at nine sites and were still present at nine during our field investigations over the last ten years. For nine sites no recent observations were available. Recent examination of 25 sites characterised by former settlements and/or current high visitation rates, but where no previous records were available, revealed no alien species (Fig. 1). For the majority of taxa fewer than ten records had been made; for eight taxa 10-20 records were made; and only *Deschampsia caespitosa*, *Festuca rubra* ssp. *rubra*, *Poa pratensis* ssp. *pratensis* and *Stellaria media* were recorded more than twenty times (Appendix 1). All except two species that had been observed 10 times or more were observed in flower or fruit stage. In the two settlements Pyramiden and Barentsburg, where records are most comprehensive and where 40 alien species were observed in 1988, 28 taxa had disappeared, 12 had persisted and 17 new species had been introduced (Appendix 1).

Shift in phenology

Data on phenology, mean July temperature, and July date were obtained for 448, 442, and 379 records respectively, and data on all three variables were obtained for 348 records. For each degree of warmer temperature, the proportion of fertile plant records was 0.71 times greater (CI: 0.33-1.08) when July date was included in the model.
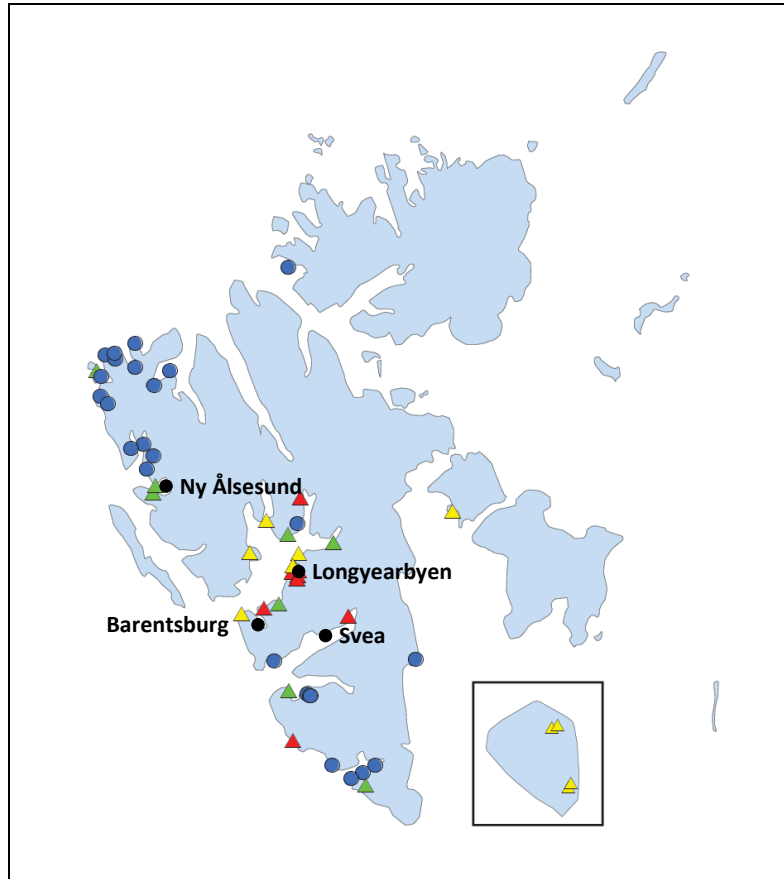
Figure 1. Maps of a) the high Arctic archipelago Svalbard showing major settlements. Triangles show sites where alien species have been recorded. Green triangles: earlier years only. Red triangles: both earlier and recent years. Yellow triangles: no recent investigations performed. Blue circles show sites with former settlements and/or current high number of visitors, where no older records of flora are available, and no alien species were found during recent investigations. The island of Bjørnoya is inset.

(Table 1, Fig. 2). A model including deviation from mean July temperature of the previous year was not strongly supported. The difference between the AIC of this model and the reduced model were small (2.2), and the removal of this variable did not significantly increase deviance ($\chi2 = 38.14$, *p* = <0.001). A further reduced model not including July date increased AIC (33.4) and deviance ($\chi2 = 0.71$, *p* = 0.39). Similarly, proportions of fertile records were 0.13 times greater in more recent years of observation (CI: 0.013-0.014) (Table 1, Fig. 2). The random effect of species was not strong in either model (Table 1). The number of alien species records did not increase over the 130 years of observations (*p* = 0.21, df = 12, *F* = 1.84).

Discussion

Alien species in Svalbard compared to other regions

Through this thorough exploration of records of alien plant species in Svalbard, we recorded 105 taxa, about twice as many as recorded for the same region previously (Elven et al. 2011; Gederaas et al. 2012). The percentage of alien species recorded in Svalbard (57 %, 105 alien compared to 185 native; Alsos et al. 2014)

Table 1. Parameter estimates for minimum adequate generalized linear mixed models with restricted maximum likelihood explaining the phenological stage of alien vascular plants in Svalbard compared to a) deviation from mean July temperature (n = 348 observations of 88 taxa), and b) year of record (n = 392 observations of 100 taxa). Species was included as a random effect and variance ± standard deviation is given.

a)	Fixed effect	Estimate	CI 95 %	SE	z-value
Fertility ~ July temp + July date	(Intercept)	-2.489	-	0.715	-3.479
Species 4.556 ± 2.135	July temp	0.706	0.333-1.079	0.910	3.716
	July date	0.045	0.021-0.068	0.011	3.732
b)	Fixed effect	Estimate	CI 95 %	SE	z-value
Fertility ~ Year + July date	(Intercept)	-26.289	-	0.001	-23511
Species 4.001 ± 2.000	Year	0.134	0.013-0.014	0.000	96
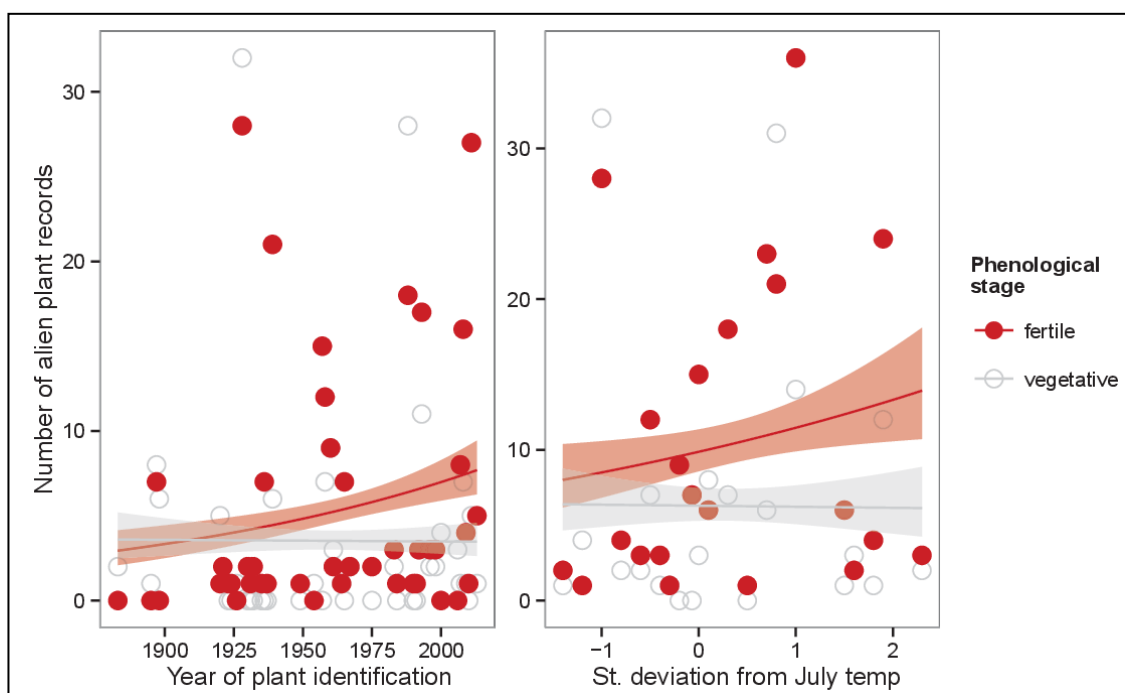
Figure 2. Phenology of alien species recorded in Svalbard in relation to (left panel) year of record and (right panel) deviation from mean July temperature. Lines of best fit are plotted using a generalised linear model with a quasipoisson error distribution to account for overdispersion. Grey shaded areas represent the 95% confidence intervals.

was higher that recorded in any other Arctic region (Elven et al. 2011), and considerably higher than that found in the mainly temperate-boreal Alaska (13 %, 283 alien compared to 2100 native; Carlson and Shepard 2007)

and Canada (24 %, 1252 alien compared to 5111 native; http://www.wildspecies.ca). It approaches the percentage found in boreal areas, e.g. Norway (61 %, 1719 aliens, 2802 natives; Gederaas et al. 2012) and Iceland (78 %, 336 aliens recorded 1840-2012, 429 natives; Wasowicz et al. 2013). While the exact numbers may depend on factors such as the inclusion of casuals and degree of exploration, it is nevertheless clear that the proportion of alien species in Svalbard is as high as in some non-Arctic regions. Further, eleven species (10 %) were assumed to have established permanently and one species (1 %) is potentially invasive (classified as "high impact") (Appendix 1) (Gederaas et al. 2012). These are broadly similar percentages to those observed in other biomes (Vila et al. 2010), indicating that the risk of alien species becoming invasive in the Arctic is similar to that of other places.

Effect of climate

As the phenological stage of the plants advanced with summer temperature, the likelihood of species spreading will increase with ongoing warming. Also, increased seed germination in native species indicates an effect of climate change (Müller et al. 2011; Alsos et al. 2013). In addition, we may expect that extreme weather events will increase natural dispersal from neighbouring regions as observed for moth introductions to Svalbard (Coulson et al. 2002). Although climate change will likely have complex effects on alien species (Bellard et al. 2013), our data support the expectation that it will mainly favour establishment and spread of alien species in the Arctic. With the 3-4°C increase in summer temperature expected by 2100 (Førland et al. 2011), and assuming that climate requirements of the introduced range are similar to the native range (Petitpierre et al. 2012), the future climate will meet the requirement of the majority of species recorded as casuals (Appendix 1), and also the majority of alien species currently found in the low Arctic and Arctic neighbouring territories (e.g. Gederaas et al. 2012).

Managing risk

If impacts by invasive species common in temperate and low Arctic regions are to be avoided in Svalbard, our study indicates protective policy and proactive management should be implemented. Livestock have been present at all places where most species were recorded, (Barentsburg, Longyearbyen, Pyramiden, Ny-Ålesund, and Hjorthhamn). Thus, unintended introduction through fodder is the most likely cause of past introductions. While fodder imports have decreased in terms of both total amount and number of sites used, the number of visitors to Svalbard is increasing. Seeds attached to visitors' footwear, clothing and equipment may currently be the most important pathway of introduction (Chown et al. 2012; Ware et al. 2012; Huiskes et al. 2014). Travellers (e.g. tourists, scientists) to the Arctic typically visit natural settings including some of the most pristine areas. Whereas propagules introduced with fodder may disperse within settlements only, introductions from travellers are more likely to pose greater ecosystem risks. While strict biosecurity measures are executed in the Antarctic, few biosecurity measures exist in any Arctic region. Burning animal manure is a simple measure that could be employed to limit potential impacts from fodder imports, while adopting a biosecurity framework modelled on existing Antarctic measures (Hughes and Convey 2010; Huiskes et al. 2014). Other common sources of aliens such as the ornamental plant trade and introduction through agriculture are currently not relevant to the high Arctic, but adopting measures to ensure these activities do not present species invasion risks in the future seems prudent.

Records of introduced species reported here, were, with few exceptions, made by professional botanists. The status of our knowledge of alien species could be greatly increased by engaging local residents as well as visitors to the Arctic in contributing to survey efforts. To facilitate identification, alien species should be included in regional flora guides as has been done for Svalbard (Alsos et al. 2014). This layer of additional survey effort could provide for cost-effective early detection monitoring.

Conclusions

Our comprehensive evaluation of survey records demonstrating trends of alien species persistence, abundance, diversity, and phenology over time was made possible through a long history of botanical exploration in Svalbard. By collating and exploring these data we have been able to demonstrate that alien species turnover has been substantial over the past century. More recent records are of fertile plants compared to earlier records, constituting a phenological shift we expect will promote the persistence, establishment, and spread of current alien plants. The positive effect temperature has on alien plants also suggests that increasingly higher proportions of introduced species may persist. Simple management measures may greatly limit alien species introduction and spread, and should therefore be implemented.

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Chapter 4: The efficacy of footwear disinfection to prevent microbial species introduction to the Arctic

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Abstract

Biosecurity measures are commonly used to prevent the introduction of non-indigenous species to natural environments globally, yet the efficacy of practices is rarely monitored. A voluntary biosecurity measure was trialed in the Norwegian Arctic following concern that non-indigenous species might be transferred to the region on the footwear of travelers. Passengers aboard an expedition cruise ship disinfected their footwear prior to and in-between landing at sites around the remote Svalbard archipelago. The efficacy of this measure was evaluated by measuring the number of colony forming units on footwear both before and after disinfection under different conditions. Disinfection reduced the microbial burden on only 28 % of footwear when sampled within one minute of disinfection, and 66 % when footwear was permitted to dry before sampling. Thus, the procedures used on board the study ship were ineffective at removing microbial burden, and were effective at reducing microbial burden only when footwear was given more time to dry than that granted under operational conditions. Monitoring of this measure suggests that empirical research underpinning the practice of footwear cleaning and disinfection needs to be communicated more effectively. We make suggestions to this end, with relevance to all tourism operators undertaking footwear cleaning and disinfection for biosecurity purposes globally.

Introduction

Increases in human travel, tourism, and trade have facilitated the spread of non-indigenous species (NIS) across the earth (Keller et al. 2011). Acknowledgement of the serious impacts caused by a proportion of these species, and the difficulties associated with their eradication, has spurred the implementation of management interventions designed to prevent biological introductions. Monitoring the efficacy of such interventions is fundamental to ensuring the ongoing effectiveness of biosecurity management.

Footwear has been demonstrated to be contaminated by a range of NIS (McNeil et al. 2011, Ware et al. 2012). Soil-borne organisms found on footwear have caused substantial impacts to wildlife (Hernandez et al. 2007), and native vegetation (Cahill et al. 2008), while footwear has been directly identified as the likely vector leading to the establishment and spread of non-indigenous plants (Lloyd et al. 2006) and plant pathogens (Cahill et al. 2008), and the transmission of diseases (Phillot et al. 2010).

Strategies used to reduce the risk of footwear-mediated NIS introductions are typically inexpensive and rapid, and are designed to both clean and disinfect. Empirical evaluations have been undertaken in controlled settings to determine processes under which efficacious outcomes can be achieved (Amass et al. 2001, 2005, Curry et al. 2005, Ware, unpublished data). As a result, best-practice or evidence-based footwear cleaning strategies have been incorporated into public (PAWS 2013) or industry-based guidelines (IAATO 2013), and state-based regulations (USDA 2013) in efforts to minimize NIS transmission.

One industry that has adopted guidelines to reduce NIS transmission via footwear is the polar tourism industry. While there are generally fewer invasive NIS in the Arctic and Antarctic than in more temperate regions (Elven et al. 2011, Coulson et al. 2012, Frenot et al. 2005), some sub-Arctic and sub-Antarctic environments are heavily invaded (Frenot et al. 2005, Carlson and Shephard 2007). Moreover, increasing human activity in the polar regions combined with the effects of ongoing climate change stands to promote the possibility of high-latitude invasion (Cowan et al. 2011, Gederaas et al. 2012, Ware et al. 2012). Concern exists that disease transmission to, and between, wildlife populations might occur at high latitudes (Curry et al. 2005, Kerry and Riddle 2009), as might the introduction of pathogens (Cowan et al. 2011, Hughes et al. 2011), invertebrates (Hughes et al. 2011), and invasive plants (Chown et al. 2012, Ware et al. 2012). The consequences of such introductions are as yet, largely unknown, but are likely to impact on existing community structure and functioning (Litchman 2010), and may cause disease to both fauna and flora (Kerry and Riddle 2009, Hughes et al. 2011).

Here, we evaluate the efficacy of footwear disinfection practiced by expedition companies operating ship-based tourism ventures in the Arctic. Expedition ship cruising constitutes a large proportion of tourism opportunities in polar regions. In the Arctic, most expedition ship operators are members of the industry-based Association of Arctic Expedition Cruise Operators (AECO). Among other objectives, AECO is dedicated to managing respectable, environmentally-friendly, and safe expeditions in the Arctic (AECO 2013). In 2012, AECO trialed voluntary biosecurity measures aimed at reducing the risk of NIS introduction mediated by tourists and ship-crews. One measure aims to prevent the transmission of microorganisms to the natural environment through footwear disinfection. We tested whether procedures reduced microbial loads on footwear under

operational practices, and in doing so, monitored the efficacy of the new measure. As the practice of footwear decontamination is undertaken by many expedition and tourism companies in a similar manner, our study has wide relevance.

Methods

Svalbard and expedition tourism

The biosecurity measure was undertaken by ships operating around the Svalbard archipelago (74-81°N, 10-35°E), approximately 700 km north of mainland Norway. Around one hundred non-indigenous plants have been observed in Svalbard, about 40 of them in recent years (Elven and Elvebakk 1996, Gederaas et al. 2012, Alsos et al. unpublished data). Also, a number of non-indigenous invertebrates have been observed (Coulson et al. 2012), while ecto- and intestinal parasites are known to be associated with the introduced sibling vole *Microtus levis* (the vole's survival in Svalbard is likely synanthropic). Microorganism biogeography is poorly understood in the Arctic, and consequently it is not known whether non-indigenous microbes have been introduced to the region (Prestrud et al. 2004, Lovejoy 2013).

Annually up to 20 expedition ships operate around Svalbard between the months of June and September. These ships take between 5-220 passengers and carry nearly 10,000 passengers collectively during a season (Governor of Svalbard 2012). Landings are carried out multiple times per cruise at nearly 180 different sites (Governor of Svalbard 2012). Tourists undertaking an expedition cruise typically first fly to Svalbard and board expedition ships at the local port in Longyearbyen. Opportunities for NIS dispersal via footwear may occur upon landing in Svalbard, through the introduction of NIS following a landing around the archipelago, or through the translocation of NIS between Svalbard locations.

Disinfection methods

Participating expedition cruise ships used baths of Virkon S® (DuPont, America) to disinfect footwear. Virkon S® is a broad spectrum virucidal disinfectant, commonly used in farm biosecurity settings. Used as a 1% solution, the agent is active for around five days, after which a loss of pink color indicates the need to replace the solution. Disinfectant baths were typically placed at the gangway such that passengers would step through the bath prior to entering tender boats before a landing. Some ships used an additional bath containing water and scrubbing brushes in which passengers would first clean their footwear before disinfection. Alternatively, a few ships reported that they conduct footwear disinfection following boarding by passengers. In this manner, footwear is left in a room near the gangway immediately after boarding the ship where crew would spray footwear with disinfectant, leaving this to dry until the time of the next landing.

Tests were carried out on board a single ship during the shipping season. Owing to variation in procedures used as reported by ships, we evaluated the effect of disinfection under two scenarios. The first (Test 1) tested the immediate effect of footwear disinfection on microbial removal without the complete drying of disinfectant. Tender boat trips to shore vary in length between landings and are dependent on the weather (typically 3-10 minutes). Given this, little time is afforded to allow the disinfectant to dry which may be further compromised

by water pooled on the floor of a tender boat. Due to the operational procedures on board the ship, we were unable to test the effect of disinfection following a tender boat trip to shore. Therefore, instead, our first test evaluated microbial removal immediately following footwear disinfection. The second procedure evaluated (Test 2) tested the effect of microbial reduction following disinfectant drying.

The study ship used a new solution of Virkon S® for each voyage (four days' duration) with which to disinfect footwear. Contact plates (55mm with Columbia 5% sheep blood agar base, Oxoid) were used to sample the sole of footwear prior to disinfection, and following disinfection. Time constraints imposed by the expedition-ship setting indicated that this would be the most effective method to sample the footwear of a large number of passengers. For Test 1, samples were collected from 60 passengers while they waited to board tender boats prior to a landing. A subsequent paired sample was then taken within 1 minute of a passenger disinfecting their footwear. This time period was the maximum afforded between passengers waiting to take a tender boat to shore. Disinfectants designed for footbaths are required to be fast acting on microorganisms, and Virkon S® is advertised as being able to achieve disinfection following footwear being scrubbed for 30 seconds in a footbath (DuPont 2013). Test 2 was performed on 30 passengers returning to the ship following a landing. Here, samples were taken from footwear prior to passengers disinfecting their footwear. The footwear was then allowed to dry for one hour before a subsequent paired sample was taken. In all cases contact plates were pressed lightly on a flat area of the sole, preferentially in an area containing visible soil. It is important to note that our tests were not aimed at testing the effectiveness of the disinfectant product which has been done elsewhere (e.g. Amass et al. 2001, DuPont 2013), but to determine whether footwear disinfection as practiced aboard expeditions ships was effective.

Contact plates were stored in a drying oven at 37° C for 48 hours following sampling. Growth on the contact plates was scored at 24 and 48 hours, following the method of Curry et al. (2005) using the categories in Table 1. Differences in growth on pre- and post-treatment contact plates were calculated using the Wilcoxen signed rank test for paired samples. As we were focused on evaluating the effect of treatment, we did not attempt to identify any organisms collected from footwear.

Table 1. Descriptions used to score growths on sample contact plates. CFUs = colony forming units

Growth score	Growth descriptor
1	No growth
2	Scanty growth (5-10 CFUs visible)
3	Moderate growth (> 10 CFUs but none extending beyond a single grid square)
4	Heavy growth (CFUs extending beyond a single grid square)
5	Profuse growth (CFUs extending beyond two grid squares)

Results

Pre-disinfection samples produced microbial growth on all contact plates, generally with heavy-to-profuse growth (growth score 4-5: 75 % for Test 1; 80 % for Test 2). In Test 1, only 17 of 60 samples (28%) exhibited a reduction in microbial growth compared to pre-disinfection samples after 48 hours (Fig. 1), demonstrating a

non-significant effect of disinfection (Wilcox test: *p* = 0.69). Microbial colonies often 'carpeted' the contact plate, and appeared morphologically similar to that on pre-disinfection plates. Thus, there was no significant effect of disinfection on reducing microbial load on footwear immediately following disinfection.

The effect of disinfection was more pronounced in Test 2. In this test, 23 of the 35 post-disinfection samples (66%) showed reduced growth compared to pre-disinfection samples, demonstrating a significant effect of reducing (but not completely removing) microbial load (Wilcox test: *p* = < 0.001) (Fig. 1). Considering only the instances where growth was reduced, growth was either scanty or moderate in 87 % of the samples after 48 hours.
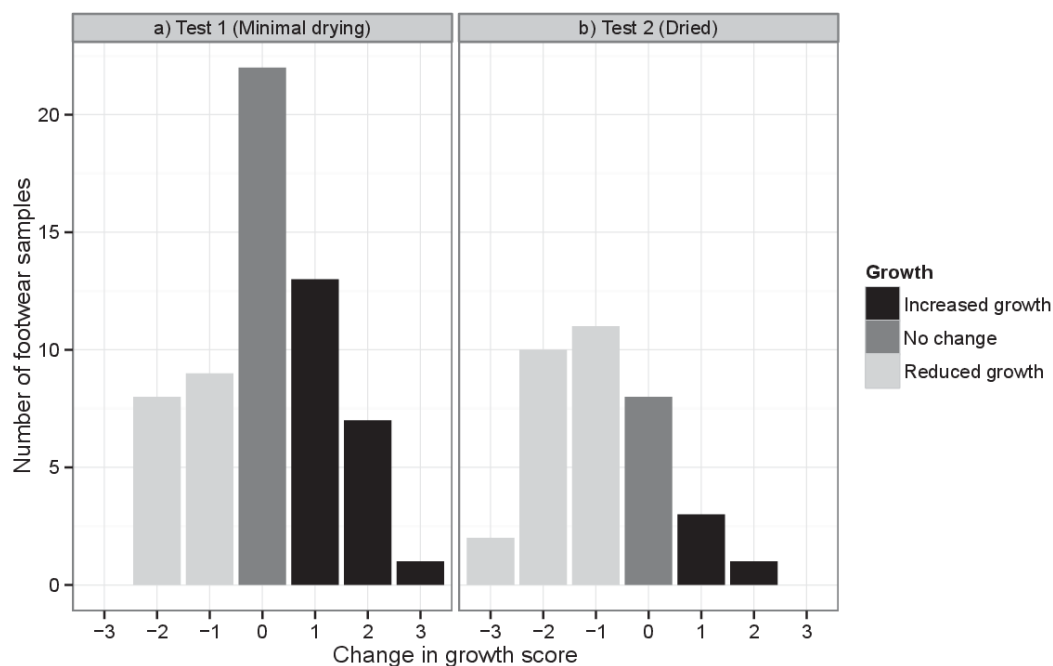
Figure 1. Change in growth on contact plates used to sample the microbial content on disinfected footwear of expedition ship passengers. Panel a) samples collected prior to a landing (at the gangway permitting only minimal drying time) and in panel b) following a landing (whereby footwear was allowed to dry completely before contact plate sampling). Change in growth score indicates the number of categories that growth reduced (-) or increase d (+) on samples according to the categories in Table 1. In Test 1, reduced growth was evident in 28% of the samples (panel a); in Test 2, reduced growth was evident in 66% of the samples (panel b)

Discussion

Footwear disinfection is performed by tourism operators in the Arctic as a precautionary measure. We show that the practice as undertaken by one expedition ship operator is ineffective at reducing the microbial load on footwear. Considering the method assessed is similar to that of most ships, the practice of footwear disinfection is likely ineffective across a wide section of the tourism sector. In contrast, operators who disinfect footwear upon passenger boarding, permitting disinfectant to dry completely in between landings, likely

substantially reduce microbial loads transferred to, and in-between, landing sites. Nonetheless, permitting disinfectant to dry on footwear may reduce, but not completely remove microbial loading. Our findings corroborate those of others indicating where footwear is not thoroughly cleaned with brushes and water or scrubbed while in a disinfectant bath, disinfection is unlikely to be achieved (Amass et al. 2001, 2005, Curry et al. 2001, 2005). Most importantly, our study demonstrates the need to monitor biosecurity interventions to determine their efficacy.

While footwear disinfection was focused on reducing associated microbial load, a biosecurity intervention would ideally also reduce the risk of introducing plant propagules and invertebrates. A range of plant (Alsos et al. 2012) and invertebrate NIS (Coulson et al. 2013) are already established around the archipelago, yet footwear disinfection alone is unlikely to prevent the further introduction of plant or invertebrate NIS. While disinfectants are effective against bacteria, viruses, and yeasts, they are not designed to render plant propagules or invertebrates non-viable, and the act of stepping through a footbath does not reliably remove propagules (Curry et al. 2005). Requesting that passengers scrub footwear with brushes and water prior to stepping through a disinfection bath would reduce the transmission risk of a greater range of taxa.

Disinfection outcomes would also likely improve following the prior cleaning of footwear. When footwear was thoroughly cleaned of any organic material prior to disinfection in a study in the Antarctic, disinfection rates were significantly improved (Curry et al. 2005). Amaas et al. (2005) showed that by the additional practice of wiping the cleaned and disinfected soles of footwear with paper towels, associated bacterial levels were significantly reduced. Such a modification to the existing protocol would be relatively easy to implement on board expedition ships, and would improve the efficacy of disinfection.

Potential impacts caused by introduced microbial NIS are not well indicated in Svalbard, though are likely to be similar to those indicated elsewhere (e.g. Litchman 2010, Cowan et al. 2011). Impacts could include the transmission of disease to, or between, wildlife populations (particularly when visitors encounter landings where there is fecal material), genetic homogenization and disruptions to ecosystem functions, or impacts on indigenous flora through the introduction of plant pathogens. Impacts from established plant and invertebrate NIS on Svalbard are presently highly localized (Gederaas et al. 2012, Coulson et al. 2013), though if they should colonize the floristically diverse and nutrient rich bird cliff environments characteristic of the high Arctic (Coulson et al. 2013) more substantial impacts to Svalbard's natural ecology would likely follow. Moreover, while the prevailing high-Arctic climate of Svalbard prevents the establishment of many NIS, the establishment of new microbial NIS will likely be favored under future moderating climatic conditions (Cowan et al. 2011, Ware et al. 2012).

The present study was limited to one ship, and to the testing of disinfection procedures under restricted conditions. We did not test the range of disinfection practices, nor did we test the potential for microbial growth under different temperatures. These aspects are avenues for future research. The focus of the present study was evaluating the efficacy of a biosecurity measure to reduce microbial footwear burden as practiced by most operators, for which our data demonstrate improvements should be made. It is also important to note that other means of microbe introduction are likely active in transporting organisms to Svalbard, including both natural and anthropogenic means. Natural vectors of dispersal, such as sea-ice, birds, or wind, may be effective

transporters of microbes (Alsos et al. 2007, Pearce et al. 2009). Anthropogenic transport and dissemination of microorganisms is an inevitable consequence of almost all forms of human presence: food, cargo, planes, vehicles, and the human body itself may all carry and disseminate large numbers of microorganisms (Cowan et al. 2011). Given this, effective footwear disinfection can only prevent a fraction of the transferred microbial propagule load. Nonetheless, when considering the capacity of footwear to collect soil, guano, and biological material that likely harbors microorganisms (McNeil et al. 2011), the pervasiveness of footwear as a species transport vector in Svalbard (Governor of Svalbard 2012), and the relative ease of managing footwear as a species transport vector (Amass et al. 2005), properly practiced footwear disinfection presents as an efficacious means to reduce NIS threats to Svalbard.

Conclusion

Our study underscores the need to monitor the efficacy of management interventions. Footwear cleaning and disinfection protocols are underpinned by empirical research, yet, as evidenced through this study, details of best-practice had not filtered through to ships operators carrying out the intervention. Monitoring can uncover such deficiencies. Through this study we highlight ways in which this practice can be improved, consistent with other published research. While our focus was on expedition ships operating around Arctic Svalbard, the findings have relevance for ship and tour operators using similar footwear cleaning practices globally.

Acknowledgements

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Chapter 5: Climate change, non-indigenous species, and shipping: assessing the risk of species introduction to a high-Arctic archipelago

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Abstract

Aim Anticipated changes in the global ocean climate will affect the vulnerability of marine ecosystems to the negative effects of non-indigenous species (NIS). In the Arctic there is a need to better characterise present and future marine biological introduction patterns and processes. We use a vector-based assessment to estimate changes in the vulnerability of a high-Arctic archipelago to marine NIS introduction and establishment.

Location Global, with a case study of Svalbard, Norway.

Methods We base our assessment on the level of connectedness to global NIS pools through the regional shipping network, and predicted changes in ocean climates. Environmental match of ports connected to Svalbard was evaluated under present and future environmental conditions (2050 and 2100 predicted under the RCP8.5 emissions scenario). Risk of NIS introduction was then estimated based on the potential for known NIS to be transported (in ballast water or as biofouling), environmental match, and a qualitative estimate of propagule pressure.

Results We show that Svalbard will become increasingly vulnerable to marine NIS introduction and establishment. Over the coming century sea surface warming at high latitudes is estimated to increase the level of environmental match to nearly one third of ports previously visited by vessels travelling to Svalbard in 2011 (n = 136). The shipping network will then likely connect Svalbard to a much greater pool of known NIS, under conditions more favourable for their establishment. Research and fishing vessels were estimated to pose the highest risk of NIS introduction through biofouling, while ballast water discharge is estimated to pose an increased risk by the end of the century.

Main conclusions In the absence of focused preventative management, the risk of NIS introduction and establishment in Svalbard, and the wider Arctic, will increase over coming decades, prompting a need to respond in policy and action.

Introduction

Many marine non-indigenous species (NIS) have been introduced into tropical and temperate zones in or on ships (Minton *et al.*, 2005; Molnar *et al.*, 2008). These have included economically and environmentally harmful species, difficult or impossible to eradicate (Bax *et al.*, 2003). Management approaches to help prevent the introduction of marine NIS target regional vectors (i.e. ships) (Hewitt & Campbell, 2007), although the magnitude and type of risk is unknown for many regions, particularly where changing patterns of shipping or climate change are likely to occur. Global changes in climate, and patterns of trade and travel, may promote or inhibit the introduction and establishment of new NIS by altering port-environment conditions and regional shipping intensity. Given that preventing the introduction of NIS remains the most effective course of management (Sylvester *et al.*, 2011), identifying existing and potential biological introduction risks is a priority for environmental managers.

Marine vector-based risk assessment methodology is well established in the scientific literature (Campbell and Hewitt, 2011; Keller *et al.*, 2011; Chan *et al.*, 2012; Floerl *et al.*, 2013) and in management arenas (Clarke *et al.*, 2003; Gollasch *et al.*, 2006). This approach commonly uses environmental matching to quantify vulnerability to the negative impacts of NIS, whereby a high degree of environmental match is taken to mean high risk (Floerl *et al.*, 2013). Risk is also a function of the number and rate at which NIS are introduced to a region (i.e. propagule pressure – Lockwood *et al.*, 2009). By coupling environmental matching data to ship arrivals as a proxy for propagule pressure, vector-based assessments can identify potential high-risk introduction pathways. In this way, recent studies have estimated current invasion risk associated with global (Keller *et al.*, 2011; Seebens *et al.*, 2013), and regional shipping networks (Chan *et al.*, 2012; Floerl *et al.*, 2013).

While these methods are able to assign meaningful risk ratings in the absence of direct measures of ship-associated biota, they are not without limitation. Principally, a number of studies have demonstrated that vessel arrival details are a poor proxy of propagule pressure, usually leading to overestimates (Verling *et al.*, 2005; Lawrence & Cordell, 2010; Ruiz *et al.*, 2013). The alternative of directly measuring ship-associated propagule pressure is logistically challenging and resource intensive. Ships predominately transfer marine species in ballast water tanks (in ballast water, attached to tank walls, or within tank sediment), or on the wetted surface of hulls as biofouling. The task of representatively sampling ship biota is difficult because of the number and variety of ships, the number of shipping routes, and the number of connected potential source NIS that exist within even the simplest network. For example, Keller *et al.*, (2011) demonstrated that Laurentian Great Lakes Ports were indirectly connected to over 2000 global ports by 716 ships during 2005-2006. Adding to this complexity is the need to adequately account for the myriad influences on propagule loads, such as the potential for inoculation (e.g. port layover period, antifouling paint age: Coutts, 1999; Coutts & Taylor, 2004; Davidson *et al.*, 2009; Sylvester *et al.*, 2011), *en route* survivorship (Gollasch *et al.*, 2000; Coutts *et al.*, 2010), and management measures intended to mitigate propagule pressure (e.g. ballast water exchange: McCollin *et al.*, 2008; Bailey *et al.*, 2011; Briski *et al.*, 2012). In the face of uncertainty surrounding the exact conditions under which potentially invasive species are introduced however, decisions must be made about how and when to limit risk (Keller *et al.*, 2011). Qualitatively characterising the processes affecting propagule pressure may guide these decisions.

Here, we develop a temporal framework for estimating change in vulnerability to NIS introduction based on relative estimates of propagule pressure and climate matching. As a case study, we analyse the shipping

network linked to the high-Arctic Svalbard archipelago to evaluate whether this region will become increasingly vulnerable to NIS establishment under future predicted environmental conditions. The archipelago remains one of the most pristine marine environments in the world with no known NIS (though sampling effort in port environments is low). Svalbard extends from 74° to 81°N and 10° to 35°E, with a mean annual sea surface temperature of 6°C (mean range: -2°-8°) reflecting warm inflow of Atlantic water towards the Arctic and, thus, salinities approaching 35psu. To the north of the islands, temperatures are low and salinity affected by the fresher polar mixed layer. Consistent with other polar regions, shipping to the archipelago has increased markedly over the past 40 years (Governor of Svalbard, 2012), and evidence of sea surface warming is apparent (Berge *et al.*, 2005; Bjørklund *et al.*, 2012). We expect that, as with much of the wider Arctic, long-term barriers to species introduction and establishment may be breached (de Rivera *et al.*, 2011), and that the region will become vulnerable to impacts caused by NIS.

The present study builds upon the approach of Floerl *et al.*, (2013) who predicted effects of climate change on potential sources of NIS. Our method involves three major steps. First, we identify shipping connections that present higher risks of NIS introduction based on environmental matching and relative estimations of propagule pressure. Second, we determine how climate change will affect the vulnerability of regions to NIS introduction using environmental data projected for 2050 and 2100. Third, we consider the potential effect of regional management interventions. Our aim is to evaluate the potential change in vulnerability of a region to NIS introduction as a means to direct further research and the development of targeted preventative management.

Methods

Shipping network characteristics

Details of ship visits and ballast water discharges were obtained from port authorities and individual vessels respectively for the year 2011. To identify potential biofouling donor pools that may contribute to ship biofouling, the last three ports visited by vessels prior to visiting Svalbard were identified from the FleetMon database (www.fleetmon.com). FleetMon provides information on present and historical vessel itineraries through coverage of 5531 of the world's ports together with technical information for most of the world's ships. These data were not available for recreational vessels. Since biofouling organisms can be acquired at any port, and may persist on a vessel for several ports (or years) thereafter, we also include secondary and tertiary potential source ports visited by vessels in our analysis.

Only bulk carriers transporting coal from Svalbard discharge ballast water in the region (Port Master, Longyearbyen pers. comm.). Ships travelling to Norway carrying ballast water sourced from an area outside of the Norwegian Exclusive Economic Zone, or Norwegian territorial waters including Svalbard, are required to manage ballast water under the Norwegian Ballast Water Regulation (Norwegian Ministry of the Environment, 2009). The primary management option currently employed under the regulation is ballast water exchange (BWE). This requires that vessels replace port-sourced ballast water with open ocean water as a means to limit the number of coastal organisms discharged at the destination which are assumed to be of greater invasion risk. The following data were collected from eight of these vessels: last port of ballasting, date of most recent ballasting, whether or not BWE was undertaken and if so where, and the date and volume of ballast water discharge in Svalbard. For the remaining bulk carriers discharging ballast water in Svalbard, we estimated

discharge based on discharge from a known vessel of the same size and class (e.g. sister ships) (Rup *et al.*, 2010, Chan *et al.*, 2012). For these vessels, we assumed that ballast water was sourced from the last port of call.

Environmental matching

We examined present-day and future (2050 and 2100) environmental match between Svalbard and potential NIS ports connected by the shipping network. We restricted our analysis to the northern hemisphere as we consider it unlikely that biofouling organisms sourced in the southern hemisphere and transported to the Arctic would survive (Sylvester *et al.*, 2011). Environmental match was based on sea surface temperature (SST) and sea surface salinity (SSS) for each port, evaluated for the upper 10 metre surface layer. This depth is characteristic of coastal ports, and other shallow water environments associated with marine NIS (Floerl *et al.*, 2013). We base our analyses solely on SST and SSS as both variables have been shown to substantially restrict species distributions (Van den Hoek, 1982) and have been identified as the most appropriate for marine environmental match assessments (Barry *et al.*, 2008; Floerl *et al.*, 2013). We incorporated maximum and minimum values for each variable in addition to mean values to better characterise variability of port environments.

Environmental data were modelled using the EC-Earth climate model participating in CMIP5. Present day SST and SSS values were obtained, as were predictions for the years 2050 and 2100 based on the RCP8.5 emissions scenario (see Appendix 2.1). From this coarse resolution model archive we extracted minimum, maximum and mean annual values for the years 2011, 2050, and 2100 for the nearest model grid-point of all ports in the study. We examined changes for a more managerially meaningful time period (2050), and a date at which predicted environmental change for higher latitudes relative to temperate regions is maximal (2100). Data were extracted for all coastal regions and inland waterways for which data were available (n = 3189 global ports; 60% of all study ports) (see Appendix 2.1).

Following Floerl *et al.*, (2013), data were processed prior to calculations to remove correlation and scaling errors (see Appendix 2.1). Environmental match was estimated by calculating the Euclidean distance (*d*) between data points (network ports) over the three time periods. To determine the relative importance of each environmental variable in environmental distance calculations we conducted a sensitivity analysis (Keller *et al.*, 2011; Chan *et al.*, 2012). In addition we also compared environmental distances when based on environmental data predicted under a different emissions scenario (see Appendix 2.1).

Potential donor pool

For ports within the Svalbard shipping network, we compiled lists of known NIS for ecoregions within which ports were located. Lists were extracted from the Nature Conservancy's Marine Invasive Database (Molnar *et al.*, 2008), which reports NIS occurrences by marine coastal ecoregions (Spalding *et al.*, 2007; Molnar *et al.*, 2008). As current, port-specific lists of NIS are typically not available this database is the most current and comprehensive compilation of marine NIS.

Evaluating risk

Environmental match between ports visited by vessels within the 2011 Svalbard shipping network was filtered to ports with an environmental match of $d < 1.0$ for the time periods present and 2050, and $d < 2.2$ for 2100. Minimum SST and SSS of ports separated by less than these distances fell within the range of values characterising Svalbard. When cross-checked with the environmental tolerances of a number of NIS established in port-ecoregions separated by greater environmental distances, NIS were found to be filtered-out appropriately (data not shown). The appropriate cut-off increased over time as predicted Svalbard SSTs overlapped with the tolerances of NIS found in port-regions separated by greater distances. This method of filtering environmental distances gives the distance metric an increased biological relevance suggested to be necessary by several authors (e.g. Barry *et al*., 2008; Campbell & Hewitt, 2011; Floerl *et al*., 2013). To evaluate whether secondary or tertiary source ports could also act as potential biofouling source pools, we filtered secondary and tertiary potential donor ports according to whether they were environmentally matched to Svalbard (as per the above values of d), and between steps (e.g. between a tertiary port and a secondary port) as a measure of *en route* survivorship.

Lists of known NIS were matched to those ports which exhibited high environmental match ($d < 1.0$ for the present and 2050; $d < 2.2$ for 2100). We then applied a qualitative model to derive relative estimates of low, medium, or high propagule pressure associated with each vector. Our model makes assumptions about: 1) the probability of a vessel entraining or providing habitat for an NIS; 2) the probability of an organism surviving transport; 3) the effect of ballast water management practices; and 4) the probability of repeat inoculations based on data published in the scientific literature. Current understanding of processes affecting propagule pressure does not permit the formal modelling of propagule pressure for an 'unknown' vessel along a particular pathway of potential introduction in the absence of biological sample data; therefore we do not attempt to predict propagule pressure, but characterise the process of propagule inoculation, transport, and introduction to estimate relative levels of propagule pressure (see Appendix 2.2 in Supporting Information).

Results

Shipping network characteristics

We identified 90 ships making 155 visits to Svalbard. Twenty-two ships visited Svalbard more than once during 2011. Including the previous three ports vessels had visited, Svalbard was connected to 136 global ports, 46 of which were primary ports. Ports visited by vessels were concentrated in Western Europe (Fig. 1a) while primary ports of departure were concentrated in Scandinavia (34%). The majority of ships visited the largest settlement on the archipelago, Longyearbyen (Fig. 2a), and the tourism sector accounted for the majority of ship visits (Fig. 2b). The composition of vessel types at any port was strongly spatially dependent: the port of Longyearbyen received the full range of vessel types visiting Svalbard, while no cruise or tourist ships visited the port of Svea. There was a strong seasonality in ship arrivals, with 77 % between June and September. Vessels' mean duration in ports prior to visiting Svalbard was 12.6±2 days (mean±SE), though substantial differences existed between vessel classes. For example, bulk carriers and cruise ships spent a mean of 2.3±0.9 and 3±1 days in port respectively, whereas fishing and research vessels spent a mean of 19.3±9.5 and 20.4±2.3 days in port respectively.
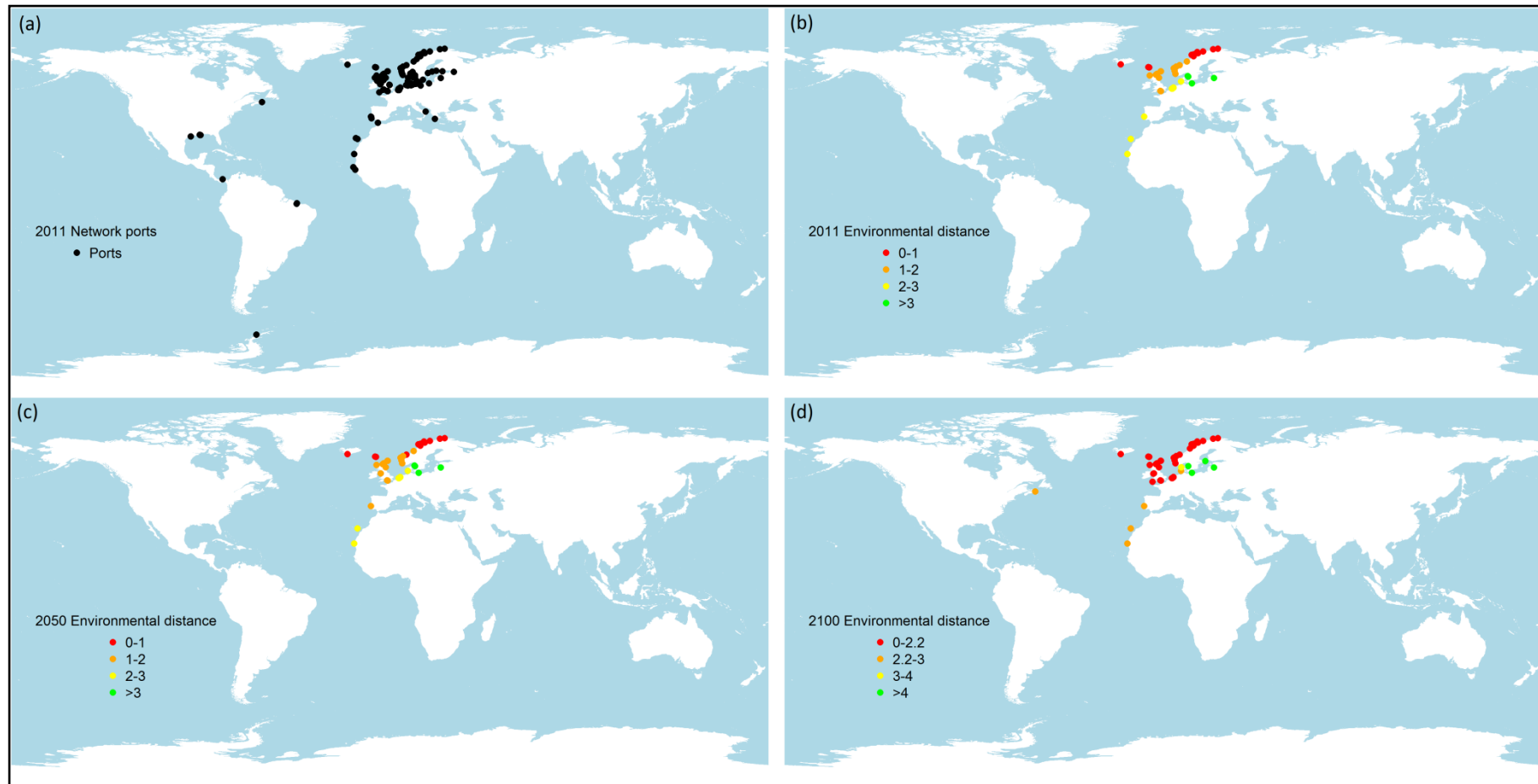
Figure 1 Figure 1. Ports connected to Svalbard through the 2011 shipping network, and environmental distances from Svalbard. Environmental distance (d) is based on temperature and salinity with lower values of d indicate higher environmental match. (Panel a) All primary, secondary, and tertiary ports connected to Svalbard during 2011. (Panels b-d) Environmental distances from primary ports of call for the year 2011, and also environmentally matched (d < 1 for b-c; d < 2.2. for d) secondary and tertiary ports.

During 2011, 13 ships made 31 fully ballasted trips collectively to Svalbard, discharging ballast water upon each arrival. We estimate the volume of ballast water discharged by the entire fleet to be 653,000m^3 (mean = 21,060m±2070m^3). Vessels all sourced ballast water from one of 16 European ports (Fig. 3). Five of the eight ships for which we have data reported having exchanged ballast water mid-ocean, while three reported no form of exchange. The age of ballast on these ships upon discharge varied (range: 1-22 days). From all vessels, ballast water discharged in Svalbard was mostly sourced from marine waters (92%), with the remainder sourced from brackish ports (14-19psu). Both Longyearbyen and Barentsburg ports received modest quantities of un-exchanged ballast water, while the port of Svea received substantial quantities of exchanged ballast water. Thus, coastal organisms are being transferred to two ports, whereas predominately oceanic organisms are likely being transferred to a third (Svea).
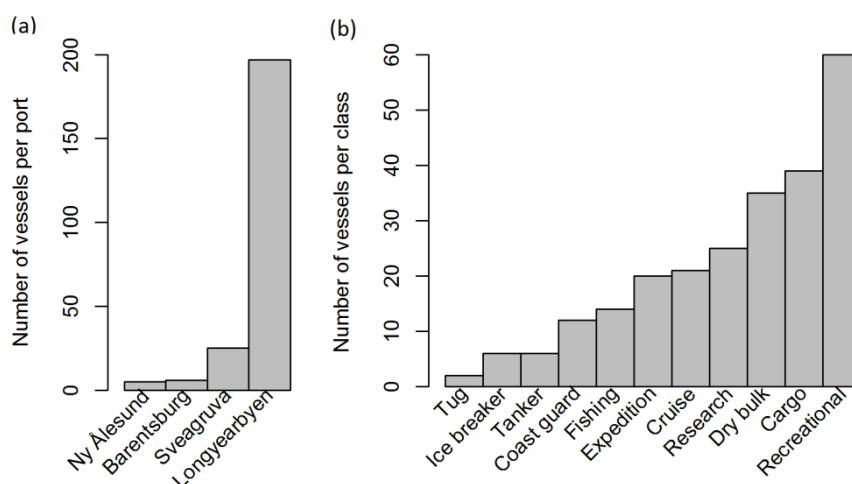
Figure 2. (a) Vessel arrival by Svalbard port during 2011. (b) Vessel arrivals by class across all Svalbard ports during 2011.

Environmental similarity

Sensitivity analysis revealed that temperature variables explained the majority of variation in environmental distance about the mean (linear regression with only temperature variables: R^2 = 0.64). Both temperature variables (see Appendix 2.1) were independently important, reflecting the higher proportion of global ports that are more saline (e.g. similar to Svalbard: more than one half of global ports have salinities > 30psu) compared with the overall low number of global ports with similar temperature characteristics to Svalbard. Nonetheless, removing salinity from the calculations increased deviance significantly between the full and reduced linear models (ANOVA: F = 22352, p < 0.001, 12 df). Based on this result, salinity data were retained in environmental distance calculations.

The current environmental distance between ports where ballast water was sourced and Svalbard ranges from 2.1 to 2.6 (Fig. 1b). Environmental distances between all primary ports of departure and Svalbard ranges from 0.8 to 5.0 under present conditions (Fig. 1b). Twenty-eight vessels connect Svalbard to primary ports of departure (six different ports) with d < 1.0 presently (range: 0.8-1.0). These same vessels connect Svalbard to a further five secondary and tertiary ports of high environmental match (d < 1) to Svalbard.
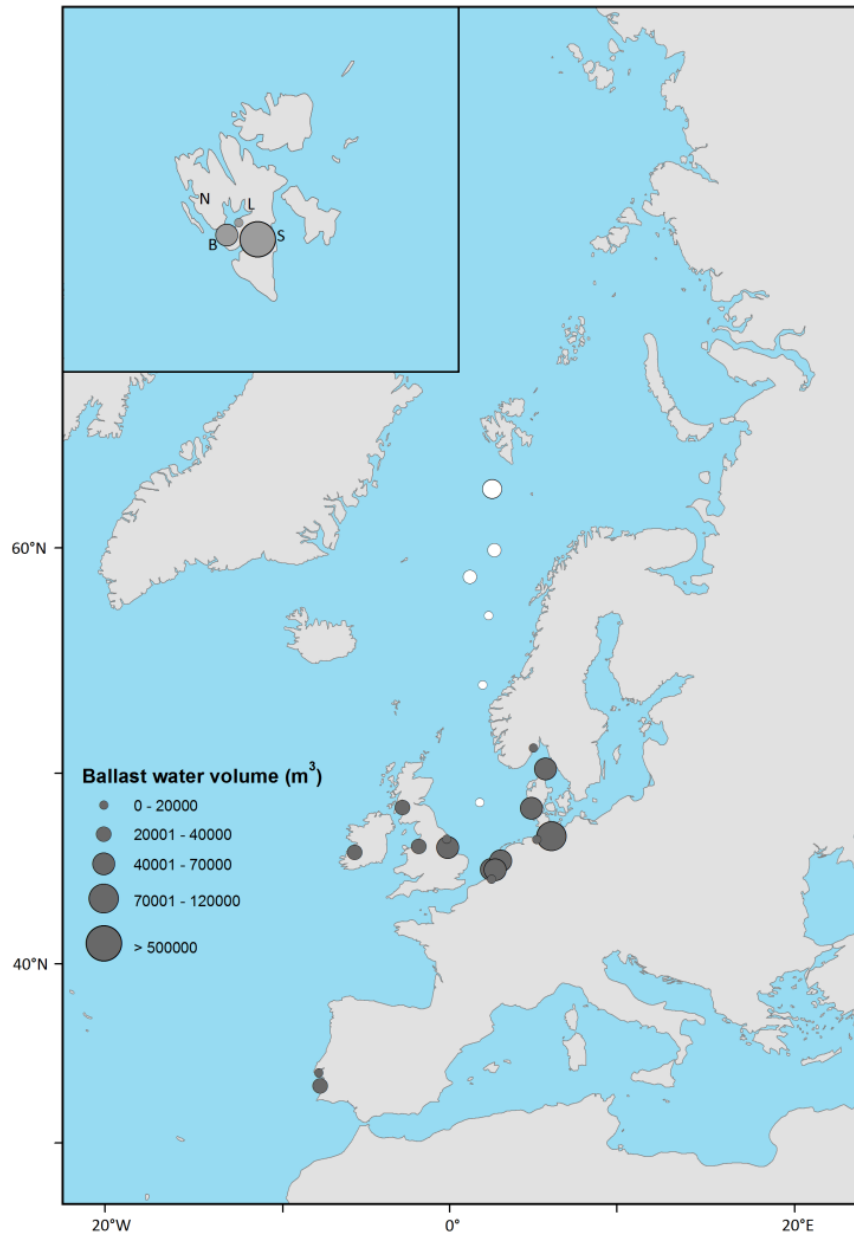
Figure 3. Regions from which ballast water was sourced by vessels prior to discharge in Svalbard in 2011: grey circles – original ballast water source estimated for all vessels; open circles – mid-ocean exchanged ballast water reported by eight vessels. Inset: ballast water discharged in Svalbard. S – Svea; B – Barentsburg; L – Longyearbyen; N – Ny Ålesund: no ballast water was discharged in Ny Ålesund.

Considering present shipping network connections, Svalbard would be connected to seven ports with $d < 1.0$ by 2050, and 16 ports with $d < 2.2$ by the end of the century (Fig. 1c-d). Considering secondary and tertiary ports, five and 22 further ports of high environmental match would be connected to Svalbard under the same shipping network by 2050 and 2100 respectively.

No ballast water source ports are predicted to be environmentally matched ($d < 1.0$) to Svalbard by 2050, yet two current ballast water source ports will become matched ($d < 2.2$) to Svalbard by 2100.

By 2100 predicted environmental distances < 2.2 to Svalbard are characterised by maximum temperatures in the range 7.9 – 20.9°C and salinity levels greater than 32psu.

Environmental match using data modelled under the A1B scenario (see Appendix 2.1) estimated only marginally smaller degrees of environmental match between ports (mean = 0.2±0.2).

Potential donor pool

Under present conditions, the shipping network connects Svalbard to four ecoregions with similar environmental conditions (d < 1.0). Sixteen NIS are known from these regions (Molnar *et al.*, 2008), including one species indigenous to Svalbard (the soft-shelled clam *Mya arenaria*) (see Appendix 2.3). Of the remaining 15 species, 14 are suited to transport as biofouling on ships (see Appendix 2.3) (Molnar *et al.*, 2008).

Assuming climate change predictions and the same shipping network, by 2050 Svalbard will remain connected to the three same highly environmentally matched port-ecoregions. By 2100, the number of highly matched port-ecoregions is estimated to increase to nine. The pool of current NIS in these 9 regions is 640% greater (see Appendix 2.3) (Molnar *et al*., 2008) than that in the four regions currently connected to Svalbard. Therefore, while it is impossible to know the number of NIS that will be present in these regions in coming decades, it is likely that an increase in connected regions of high environmental match will expose Svalbard to a larger number of NIS.

Evaluating risk

Ballast water discharged in Svalbard waters was not estimated to pose a risk currently, or by 2050. By the end of the century two ballast water sourced ports will be environmentally matched to Svalbard (d < 2.2). Propagule pressure associated with ships currently sourcing ballast water from these ports is estimated to be low for those vessels currently performing BWE, and high for those not.

Risk associated with biofouling is estimated presently to be limited to the 28 ships connecting Svalbard to six highly environmentally matched ports. Of these, 11 were estimated to pose high propagule pressure, and six low. All cruise ships are estimated to pose low propagule pressure, while those posing high propagule pressure include vessels from all other classes with the exception of bulk carriers which are estimated to pose low or medium propagule pressure.

Discussion

Regulatory mechanisms, ship operations, trading patterns, the distributions of NIS, and ecological values need to be taken into account when assessing the potential risks for NIS transfers. In the first such assessment for the European Arctic, we have demonstrated an efficient means to do this. Our assessment of the Svalbard shipping network indicates an increasing vulnerability to NIS introduction and establishment over coming decades. Risk is differentiated by vector, shipping routes, recipient location, and time. All Svalbard ports are estimated to be at high risk of biofouling introductions mediated by a small number of vessels; yet the NIS donor pool is small (15 species) owing to the small number of ports environmentally matched to Svalbard. Vulnerability to biofouling introductions are likely to increase towards the end of the century however, due to

the increasing diversity of the potential NIS donor pool and moderating SSTs. Ballast water introductions are not estimated to pose a risk presently, or by 2050. By the end of the century however, two ports will be matched to current ballast water source ports. These results suggest that the values for which Svalbard is managed will come under threat as the region becomes increasingly vulnerable to the effects of NIS.

Densities of organisms in ballast water sourced from the same ecoregions have been reported to be high (though varied) in other studies ($5 \times 10^3 - 8 \times 10^5$ organisms m/^{3} – McCollin *et al.*, 2008; Simard et *al.*, 2011). While mortality is known to increase with time, the short voyages in our analysis are likely to maintain some level of survivorship (Simard *et al.*, 2011). BWE heavily reduces the number of coastal NIS transferred in ballast water (McCollin *et al.*, 2007; McColling *et al.*, 2008; Simard *et al.*, 2011), and was undertaken by the majority of ships discharging ballast water in Svalbard. Nevertheless, BWE efficacy varies according to the method of BWE, source port, and taxa (McCollin *et al.*, 2007; McCollin *et al.*, 2008; Simard *et al.*, 2011), and has been shown to increase propagule diversity (McCollin *et al.*, 2008) and even survivorship of ballast water organisms (Briski *et al.*, 2011).

Ballast discharge in Svalbard is restricted to bulk carriers which travel to Svalbard from European ports to collect coal. One of the two coal mining companies on Svalbard has recently expanded (Store Norske, 2013), while the other has access to considerable reserves of coal on Svalbard (Arktikugol, 2013). Therefore, ballast water sourced from European ports is likely to continue to be discharged in Svalbard in the foreseeable future. Subject to the ratification and phasing in of the International Ballast Water Convention in 2016, and modifications to the Norwegian Ballast Water Regulation to mandate ballast water treatment (currently optional), ships will be required to install ballast water treatment systems with strict discharge limits (IMO, 2004; Norwegian Ministry of the Environment, 2009). These systems would substantially reduce any risk of NIS introduction associated with ballast water transfer to Svalbard. Yet, there is some non-compliance with the current Norwegian Ballast Water Regulation among Svalbard shipping operators; our results press the need to improve this over coming years.

Vessel traffic in 2011 included movement that could be expected to differ little from year to year (e.g. cargo and local tourism associated shipping), and movement which may change from year to year (e.g. cruise and bulk shipping, and recreational vessel traffic). Due to the seasonality of shipping, the geographical range of ports vessels visited prior to arrival in Svalbard is wide (Fig 1a). Durations spent in ports visited by vessels prior to Svalbard were related to vessel class: cruise ships typically spent less than one day in port, while research and cargo ships routinely spent periods between one week and one month in port. No cruise ships connected ports with a high environmental match to Svalbard, whereas all research and cargo ships repeatedly visited ports (primary, secondary, and tertiary) of high environmental match to Svalbard. Propagule pressure associated with these vessels was therefore estimated to be high. Under present conditions, fishing, research, expedition, and cruise ships connected Svalbard to the most distant ports with a high environmental match (Torshavn and Vestmanna – Faroe Islands; Vestmannaeyjar – Iceland), with the former two estimated to pose low-medium propagule pressure. The size of the potential NIS donor pool from these ports, however, is low (six species).

Some of the increase in estimated vulnerability to NIS introduction and establishment by the end of the century is attributable to our increase of the environmental distance cut-off beyond which assumed risk is considered to be low (i.e. to $d < 2.2$). The rationale behind this increase lies in the thermal reproductive

requirements of a number of NIS. Conditions under which species can reproduce are more relevant in estimating establishment potential than physiological tolerances. By the end of the century maximum SSTs in Svalbard are predicted to rise beyond 10°C (12.5°C). A number of NIS (e.g. the European shore crab *Carcinus maenas,* the edible crab *Cancer pagrus*, and the green algae *Codium fragile* ssp. *tomentosoides*) have been shown to be able to reproduce at temperatures between 10° and 12°C. As the maximum SST in Svalbard poses a clear barrier to species invasion, we accordingly align estimates of risk to corresponding values of environmental distance (i.e. $d < 2.2$ by 2100). This cut-off also eliminated low salinity (< 15psu) ports from our analysis.

Over coming decades, our analysis indicates that vessel biofouling is likely to pose a greater risk of NIS transfer than ballast water discharge. While transfer suggests the potential for introduction, there are distinct differences in the way introduction is mediated. Whereas most organisms transported in ballast water are actively discharged at the recipient port, biofouling dispersal is a passive process that occurs when organisms reproduce in port, when an environmental cue triggers an organism to leave a ship hull in port (especially for mobile fouling organisms such as amphipods or isopods), or through dislodgement (for example during ship berthing) (Minchin & Gollasch, 2003). Despite the stochastic nature of the process, several studies have indicated that biofouling likely accounts for more NIS introductions than ballast water (Fofonoff et al., 2003; Davidson *et al*., 2009b, Hewitt & Campbell 2010). Thus, this vector should be included in a marine NIS risk assessment regardless of an inability to predict inoculation rates. Whereas ballast water discharge in Svalbard is regulated under the Norwegian Ballast Water Regulation (Norwegian Ministry of the Environment, 2009), no comparable regulation exists for the management of biofouling.

The importance of biofouling in the spread of NIS is further exemplified by the recent adoption of the '2011 Guidelines for the Control and Management of Ship's Biofouling to Minimize the Transfer of Invasive Aquatic Species' by the International Maritime Organisation (IMO 2011). The degree to which the voluntary guidelines will affect levels of propagule pressure associated with ships identified as high risk in this study presently remains unknown. Our study, however, underscores the need for high standards of biofouling management practices. We expect similar vulnerability to NIS introduction and establishment to evolve in other Arctic destinations, and in destinations receiving increasing vessel traffic. Increasing shipping traffic along the Northern Sea Route, for example, provides more rapid connections between Western Europe and East Asian ports (compared with travelling via the Suez Canal) and subjects potential biofouling to a range of different environmental conditions. These factors may promote or inhibit survivorship of biofouling, the extent of which will likely alter with climate change. Substantial increases in marine vessel traffic are expected in the wider Arctic region associated with tourism (Eger, 2011) and resource exploitation (Arctic Council, 2009). Vessels are likely to travel frequently to, from, and between Arctic regions, and operate under a range of different profiles. These movements will entail diverse and dynamic risk profiles. While the focus of this analysis has been on risks posed by vessels travelling to an Arctic location, vessels travelling from Arctic locations may also acquire biofouling and pose a return risk. The type of analysis used in our present study can be readily adapted and applied to increasing and evolving shipping networks to estimate changes in vulnerability to NIS introduction, and indicate vessel-related risk.

Limitations of our approach should be noted. Our analyses necessarily excluded recreational vessels as voyage histories are not readily available for these craft. However, the potential for recreational vessels to mediate

species transfer is high (Floerl & Inglis, 2005; Davidson *et al.*, 2010; Clarke Murray *et al.*, 2011). Our use of environmental distance assumes that the ranges of organisms will be limited to their current realised niche. A more full evaluation of the biological relevance of environmental distance metrics would be welcomed and would aid and improve risk assessment. Furthermore, while we identified NIS in potential source regions connected to Svalbard, our analysis does not identify species indigenous to source regions that may also pose a threat of impact if introduced.

Finally, assumptions we made in our qualitative model of propagule pressure were necessarily basic, and would benefit from better characterisations of the different influences on propagule pressure. In particular, determining the relative contributions of each factor to overall propagule pressure would improve accuracy, as would the incorporation of the age of antifouling paints on vessels which is positively related to the diversity and abundance of biofouling (but see Sylvester & MacIsaac, 2010). Preliminary data collected from ships in this study (n = 20) indicates that the age of antifouling paint varies greatly between ships (range: 1 – 36 months). Despite these limitations, we believe our estimates of propagule pressure, while heuristic, provide meaningful indications of broad levels of risk associated with individual vectors. Our objective for characterising propagule pressure was to improve the sensitivity of the risk assessment process, reducing overestimates of risk. However, we emphasise that our focus was on estimating relative, as opposed to absolute, risks.

Conclusion

By spatially and temporally characterising a regional shipping network, and by examining present and future environmental conditions and NIS pools in both donor and recipient regions, we have been able to identify biological introduction risks warranting management attention. These data present the first forecast of changing biological introduction risks associated with a regional shipping network. Similar increased vulnerability to species invasion can be expected in other Arctic locations.

IMO regulations provide a vital international context within which to position regional management. The recent adoption of biofouling guidelines signals an important move towards improving the management of this vector. Despite these layers of international governance, biological introduction risks are likely to persist and increase as a result of climate change. The Svalbard shipping network does not constitute a large shipping network: the number of connected ports is one order of magnitude lower than for the Laurentian Great Lakes (Keller *et al.*, 2011). In the absence of more comprehensive data, the method we used provides an efficient means of combining shipping, environmental, and biological data to identify current and future risks, prioritise further research, and identify management gaps in Svalbard, the wider Arctic, and for ports connected by regional shipping networks.

Acknowledgments

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Chapter 6: Applicability of universal primers for identifying zooplankton in ship ballast water

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Abstract

Biosecurity management relies on efficient and accurate means of identifying invasive species. We tested the capacity of universal primers to efficiently and accurately identify early life stages of Crustaceans and Molluscs transported in the ballast water tanks of ships. Amplification of CO1 sequences using four primer combinations provided poor-to-moderate success (12-49 %, n=120 PCR trials), while primers amplifying 12S rDNA and 16S rDNA proved more successful overall (40 % and 69 %, respectively, n=84 PCR trials combined). All markers considered, species identifications were made for 38 % of study organisms, whereas genus identification was possible for 55 % of organisms (n=112). Organisms were resolved into 22 higher taxa, and 13 species, including non-indigenous and known invasive species such as the crabs *Carcinus maenas* and *Hemigrapsus penicillatus*, and the barnacles *Astrominius modestus* and *Amphibalanus improvisus*. Species identification success varied among groups of taxa: Cirripeds - 58 % (n=19); Decapods – 57 % (n=28); Gastropods – 18 % (n=23); Cumacea – 0 % (n=7). PCRs failed for 29 organisms (26 %), and amplified non-target DNA prevented the correct identification of 17 organisms (15 %). We conclude that universal barcoding primers can be used to substantially improve identification rates of ballast water zooplankton, but that the efficiency and coverage required for biosecurity management is currently lacking. Metabarcoding and/or NGS approaches would allow direct analysis of unsorted samples reducing analysis time, while increasing coverage through the use of multiple primer pairs. Thus, there is a need to develop primers for such approaches to improve invasive species management.

Introduction

Biosecurity research and management requires efficient screening of species introduction vectors and accurate identification of associated biota (Armstrong and Ball 2005). There is often a need to identify early life stages of species that are typically poorly represented in morphological keys (David et al. 2007, Tang et al. 2010). Marine macroinvertebrates are usually abundant in the ballast water tanks of ships (Ruiz et al. 2000, Gollasch et al. 2002), and their introduction to new habitats has caused substantial impacts (e.g. the zebra mussel *Dreissena polymorpha*, Carlton 1992; the Pacific seastar *Asterias amurensis*, Byrne et al. 1997; and the toxic dinoflagellate *Alexandrium "tamarensis"* complex, Bolch and Salas 2007). As a result, the transport of potentially invasive species has become a focus of marine conservation research and management. Of all invasive marine taxa, Crustacea and Mollusca constitute the largest proportion (Ruiz et al. 2000, Molnar et al. 2008), and often dominate zooplankton communities in ballast water tanks. However, the identification of all meroplankton such as small Crustacea and Mollusca cannot be achieved by microscopy (see for example Williams et al. 1998, David et al. 2007, Choi et al. 2005, DiBacco et al. 2011). Thus, the invasion risk associated with discharging ballast water may be severely underestimated. There is a need to develop improved methods of species identification for marine biosecurity research and management.

DNA barcoding (Hebert et al. 2003, Valentini et al. 2009) presents a promising method of resolving early life stage taxonomy, and has been demonstrated to successfully discriminate and identify organisms such as meroplanktonic bivalve larvae, the resting stages of organisms, and diapausing eggs (e.g. Briski et al. 2012). The approach relies on the use of universal primers to amplify DNA over a wide range of taxa and match generated sequences to a reference database, while removing the need to trial primer combinations, isolate DNA fragments, or use internal primers (Hoareau and Boissin 2010). Here we use the term "DNA barcoding" in the less restrictive sense, where the method of matching gene sequences to identify species may be performed with any DNA fragment (i.e. DNA barcoding *sensu lato*; see Valentini et al. 2009 for discussion). DNA barcoding has been used in a small number of studies surveying ballast water organisms (Briski et al. 2011 – 64% species identification success rate; 2012 – 72% species identification success rate, Kreiser et al. 2004 – success not quantified), and more broadly on meroplankton from mixed marine plankton samples (Webb et al. 2006 – 22% PCR product success rate, Heimeier et al. 2010 – 35% PCR product success rate). DNA barcoding has a clear application in biosecurity given these success rates almost certainly represent marked improvements over what could be achieved relying on microscopy techniques alone, yet several of these, and other studies (Hoareau and Boissin 2010, Siddal et al. 2009, Bhadury and Austen 2010), have demonstrated the need for substantial methodological testing following variable success. Limitations include the inefficiency of the universal primers used to amplify the standard barcoding gene, cytochrome *c* oxidase 1 (CO1) (Hebert et al. 2003, CBOL (Consortium for the Barcode of Life), http://barcodeoflife.org) of certain taxa (Geller et al. 2013, Prosser et al. 2013).

Some limitations are difficult to circumvent (e.g. small size of organisms, or sampling method). For others, such as a reliance on one marker and universal primer set, alternatives exist. Studies commonly make use of mitochondrial or nuclear markers in addition to the standard CO1 barcoding gene (Webb et al. 2006 – 16S, Heimeier et al. 2010 – 16S and 18S, Machida and Tsuda 2010 – 12S and 28S, Briski et al. 2011 – 16S). A focus on CO1 is generally favoured given its endorsement as the standard barcoding gene, its range of phylogenetic signal (Hebert et al. 2003), and the number of CO1 references in online databases (Kwong et al. 2012).

Nonetheless, other markers may provide greater genetic resolution for some taxa (e.g. Dove et al. 2013), and be more appropriate in resolving the complex genetic patterns that can emerge in invasive species (Zhan et al. 2013).

Furthermore, in circumstances where sample organisms are mixed, such as ballast water samples, universal primers are liable to amplify sequences of non-target DNA. This can occur when non-target DNA molecules are collected on the target, or when another organism is present on the target organism (e.g. bacteria) (Siddall et al. 2009, Heimer et al. 2010). The method of sampling ballast water (typically with a plankton net or pump) may also degrade organisms, while the small size of many ballast water organisms means DNA yield is likely to be low potentially affecting PCR success and limiting the number of trials possible. Combined, these factors suggest that despite DNA barcoding presenting a promising method for improving ballast water taxonomy compared to traditional means, its efficacy requires testing.

In the present study we test the utility of barcoding methods to identify species of Crustacea and Mollusca in their early life stages found in ballast water tanks of ships. Our study focussed on the Arctic archipelago Svalbard (Fig 1), owing to the elevated risk of species invasion in this region due to ocean warming, and increasing shipping activity as a result of opening of polar sea routes (Ware et al. 2014). As this is a region which has not experienced significant levels of biological invasion, the accurate identification of all introduced species is fundamental in evaluating the magnitude of invasion threat. We test the success rate of six primer pairs targeting different genes in an effort to overcome previously reported PCR failure with some markers. In doing so, we determine the efficacy of standard published barcoding methods in resolving the challenge of ballast water biota taxonomy, and consider the application of these to biosecurity management.

Methods

Ballast water samples (n=16) were collected from ships making eight visits collectively to three ports around high-Arctic Svalbard (study area described in Ware et al. 2014). Ships originally sourced ballast water from a total of seven different European ports (Lisbon, Hull, Rotterdam, Amsterdam, Ternuezen, Esbjerg, and Aughinish). On five separate occasions, ships exchanged ballast water with oceanic water *en route*, resulting in mixed coastal and marine ballast water biota. Samples were collected from two ballast water tanks on board ships as they arrived to Svalbard, using either a plankton net (60µm) or by sieving ballast water drawn up from the tank using a hand pump. A total of 250 L of ballast water was sampled through the net by drawing the net through the water column or pumping the water out through the net, and organisms collected in a 0.5 L receptacle. Samples were transferred to 95% ethanol, and returned to the laboratory where the ethanol was replaced (within 24hr) according to published protocols (Bucklin 1999). Samples were then filtered and sorted to retain only Crustacea and Mollusca, and further sorted into lower taxonomic units under a dissecting microscope. We excluded copepods from the analyses reported here as they are generally identifiable based on morphological characters.

Over 300 Crustacea and Mollusca early life stages (mostly larvae, cyprids, and veligers) were recovered from the 16 samples of ballast water. Of these, 112 individuals representing duplicates of different morphotypes were selected for barcoding. We selected early life stage forms present, and additional small holoplankton for which identification was deemed to be difficult based on morphological characters alone. Organisms were

photographed using a stereomicroscope camera. DNA was then extracted from whole organisms according to the manufacturer's protocol (DNeasy Blood and Tissue Kit, Qiagen, Hilden, Germany), with the addition of an initial step whereby organisms were macerated using a pestle in a microtube (Halos et al. 2004). DNA concentration was assessed using a NanoDrop 2000c (Thermo Scientific). Different sets of primer combinations were then used to amplify a region of the CO1, 12S, or 16S genes (Table 1). For both groups of organisms, the barcoding metazoan primers (Folmer 1994) were initially used to amplify the barcoding (mtDNA CO1) gene (Hebert et al. 2003). The reaction success rate with these primers proved to be low, and non-target DNA was commonly amplified. *Taq* polymerase, and other chemicals, were trialled and exchanged, and negative controls were always run beside target DNA to determine whether any contamination stemmed from a laboratory source. We then trialled alternative CO1 primers, before using primers designed to amplify the 12S and 16S rDNA genes. The choices of 12S and 16S rDNA genes as alternatives were made based on the availability of universal primers and the high number of reference sequences in GenBank (12S – 894, 16S – 3954, 26 January 2014). The point biserial correlation coefficient was used to evaluate whether low DNA yield or quality was associated with PCR success.

PCRs were performed in a 12 µl volume containing 6 µl of multiplex PCR kit (3 mM $MgCl_2$; 1 U HotStar Taq; 1 × PCR buffer, dNTP mix, Qiagen, Germany), 10 pmol of each primer, 2-10 ng of template, and water. Thermal cycling conditions for each primer combination are shown in Table 1. PCR products were visualized on 1.5% aragose gels, before being purified with 1 U of Alkaline Phosphatase and Exonuclease I (Illustra). Single or bidirectional sequencing was performed using BigDye Termination chemistry on either an Applied Biosystems® 3130 (www.unn.no, Norway) or 3170 (www.macrogen.com, Netherlands) Genetic Analyzer. Sequences were manually inspected and edited using Geneious v. 6.1.6 (Biomatters), and deposited in GenBank (accession numbers: pending). Bi-directional sequences were aligned using the Geneious software package (v. 6.1.7) (70% similarity cost matrix; default parameters). Sequences were matched to the NCBI GenBank nucleotide collection database (http://blast.ncbi.nlm.nih.gov) using the BLAST algorithm 'megablast' with default parameters (Zhang et al. 2000), and CO1 sequences were matched to the Barcode of Life Database (www.boldsystems.org) using the identification engine BOLD-IDS with the option 'All Barcode Records on BOLD' to determine their taxonomic identities. For organisms identified to species (i.e. BLAST or BOLD maximum identity and similarity scores ≥ 98%, Ward 2009), genetic divergences both within and among taxa were calculated using the Kimura-2-parameter (K2P). These parameters were available on the BOLD distance summary for available CO1 sequences. For all other sequences, the K2P distance was calculated using the software MEGA v.4 (Tamura et al. 2007) as the divergence between the study sequence and the two closest matching conspecific or congeneric sequences respectively, provided in the BLASTn summary. Where definitive species identifications could not be made based on identity and similarity scores (i.e. < 98%), we followed the liberal tree-based assignment criteria of Wilson et al. (2011). Using these criteria sequences were assigned to a taxon if it was a sister to a single member of a taxon, or to a clade of members of a single taxon (Wilson et al. 2011).

Table 1 Primers used in this study to amplify either the CO1, 12S or 16S genes of Crustaceans and Molluscs. Nucleotides in grey represent the M13 tail portion of primers. PCR cycling conditions: 1 [15 min at 95 °C; 5 x (30 s at 94 °C, 45 s at 45 °C, 1 min at 72 °C); 35 x (30 s at 94 °C, 45 s at 52 °C, 1 min at 72 °C); 10 min at 72 °C]; 2 [15 min at 95 °C; 35 x (1 min at 94 °C, 1 min at 51 °C, 1.5 min at 72 °C); 10 min at 72 °C]

ID	Primer name	Sequence 5'-3'	Ref.	PCR protocol
1	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994	1
2	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994	1
3	LCO1490_M13	TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994	1
4	HCO2198_M13	CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994	1
5	dgLCO	TAAACTTCAGGGTGACCAAARAAYCA	Meyer 2003	1
6	dgHCO	GGTCAACAAATCATAAAGAYATYGG	Meyer 2003	1
7	ZplankF1_t1_M13	TGTAAAACGACGGCCAGTTCTASWAATCATAARGATATTGG	Prosser et al. 2010	1
8	ZplankR1_t1_M13	CAGGAAACAGCTATGACTTCAGGRTGRCCRAARAATCA	Prosser et al. 2010	1
9	12sf	GTGCCAGCHNHHGCGGTYA	Machida et al. 2012	2
10	12sr	RRRDYGACGGGCRRTDTGT	Machida et al. 2012	2
11	16sar	CGCCTGTTTATCAAAAACAT	Palumbi et al. 1991	2
12	16sbr	CCGGTCTGAACTCAGATCACGT	Palumbi et al. 1991	2

Results

Sequences were successfully amplified from 83 individuals using the range of primer combinations listed in Table 1. However, 17 of these sequences corresponded to non-target DNA. Thus, putative target sequences were generated for 66 (59%) of the 112 individuals. These were resolved into 13 different species and nine higher taxa (Table 2) using all primer combinations (Table 1). Sixty-two individuals (55%) were assigned to genus, while 42 individuals were resolved into species (38 % of all individuals barcoded; 64% of all individuals for which target sequences were produced). Where species identifications were made, they were generally supported well K2P distances (K2P > 2 %). Maximum K2P distances from study organisms to conspecific matches were <1.83% (i.e. intraspecific distance, mean: 0.84), whereas the minimum distance to the nearest congeneric match was 1.16% (i.e. interspecific distance, mean: 5.54).

Table 2 Crustaceans and Molluscs identified from ballast water samples using DNA barcoding methods. * - non-native status in Svalbard. No. – number of organisms. Max. ident./sim. – mean maximum identity or similarity as determined by BLASTn or BOLD matches. Primer numbers in bold indicate they generated successful PCR product from which sequences were successfully matched to putative target taxa. See Table 1 for details of the primers trialled.

Taxa	No.	Max. ident./sim.	Primers trialled
Crustacea			
Sessilia			
Amphibalanus improvises*	7	99	**9/10**
Elminius modestus*	3	100	**9/10**
Semibalanus balanoides	1	100	**9/10**
Semibalanus sp.	3	89	**1/2**
Sacculina sp.	1	88	**5/6**
Failed PCR	4	-	1/2 , 9/10
Euphausiacea			
Nematoscelis megalops*	1	99	**1/2**
Nyctiphanes sp.	5	92	**1/2, 7/8**, 11/12
Thysanoessa sp.	8	96	**1/2, 11/12**
Mysidae			
Mysidae	1	86	**11/12**
Mesopodopsis slabberi*	6	98	1/2, **11/12**
Failed PCR	1	-	1/2, 11/12
Cumacea			
Non-target (*Homo sapiens*)	2	100	1/2, 11/12
Non-target (*Risa tridactyla*)	1	99	11/12
Failed PCR	4	-	1/2, 11/12
Decapoda			
Cancer pagarus*	1	100	**1/2**
Carcinus maenas*	7	99	1/2, **7/8**, **11/12**
Hemigrapsus penicillatus*	1	99	**1/2**
Crangon crangon*	7	99	**7/8**, **11/12**

Paguridae	1	88	**11/12**
Portunidae	1	91	**11/12**
Non-target (*Homo sapiens*)	8	100	1/2, 7/8, 11/12
Failed PCR	2	-	1/2, 3/4, 7/8, 11/12
Isopoda			
Failed PCR	1	-	1/2, 11/12
Cladocera			
Evadne nordmanni	3	99	**7/8**, **11/12**
Podon leuckarti*	2	100	**7/8**
Non-target (*Homo sapiens*)	3	100	1/2, 7/8, 11/12
Failed PCR	2	-	1/2, 7/8, 11/12
Mollusca			
Bivalvia			
Non-target (*Meleagris gallopavo*)	1	99	3/4, 11/12
Failed PCR	1	-	11/12
Gymnosomata			
Clione limacine	2	99	**5/6**
Thecosomata			
Limacina sp.	3	83	**1/2, 5/6**
Anaspidea			
Aplysia punctata	1	98	**1/2**
Caenogastropoda			
Caenogastropoda	1	85	**5/6**
Gastropoda			
Non-target (*Homo sapiens*)	2	100	1/2, 5/6
Failed PCR	14	-	1/2, 5/6, 9/10

Species both indigenous and non-indigenous to the study region were identified (Table 2). Among the non-indigenous species a number of well-known invaders from other parts of the world were identified, including the Asian shore crab (*Hemigrapsus takanoi*), the European shore crab (*Carcinus maenas*), and the Australasian barnacle (*Elminius modestus*).

DNA yield from most organisms was sufficient for only a few PCR trials, so it was not possible to test all primer combinations on all organisms. Extracted DNA concentration varied greatly (1.8ng – >500 ng), as did quality of extracted DNA (A260/280: 1.2-2.9); however neither measure correlated with target amplification success (biserial correlation: r=0.14, p = 0.17, df=89; r=0.1, p=0.43, df=89 respectively – non-target amplification sequences removed). Read lengths varied between 400-658, 388-537, and 294-325 base pairs for CO1, 16S, and 12S amplicons, respectively.

Only three organisms were identified to species using sequences generated by the original Folmer primer pair (pair 1/2) (Folmer et al. 1994) when tried on a subset of the study organisms (n=57; 5 % success). As a result, these primers were abandoned in favour of those which proved more successful. In a total of 217 trials including all primer pairs, primers designed to amplify markers other than CO1 were more successful (45 %

success rate, versus 20 %). Primer pair 7/8 delivered the best CO1 success rate (49 %), while 12S delivered the greatest overall success rate (69 %) (though was tested in a much smaller number of trials: 133 CO1 trails versus 16 12S trials,) (Fig 2). These numbers do not account for the possible effect of species group on amplification success as a comprehensive evaluation of primer efficiency between taxonomic groups was not possible given the limited yield of DNA extracted from organisms. Species identification success varied among taxa: Cirripeds - 74 % (*n*=19); Decapods – 57 % (*n*=14); Gastropods – 18 % (*n*=23); while all Cumacea PCR reactions failed (*n*=7) (Fig 2). Within taxonomic groups, there existed small differences in primer pair success. For example, for the most abundant group, Decapoda (n=40), primer pair 7/8 (CO1) produced species identifications in 58 % of trials, whereas primer pair 11/12 produced species identifications in 50 % of trials. More specifically, for Euphausiaceae organisms, primer pair 7/8 was capable of producing sequences for *Nyctiphanes* species, whereas primer pair 11/12 failed. The latter pair did, however, produce sequences for *Thysanoessa* species (family: Euphausiaceae) (Table 2).
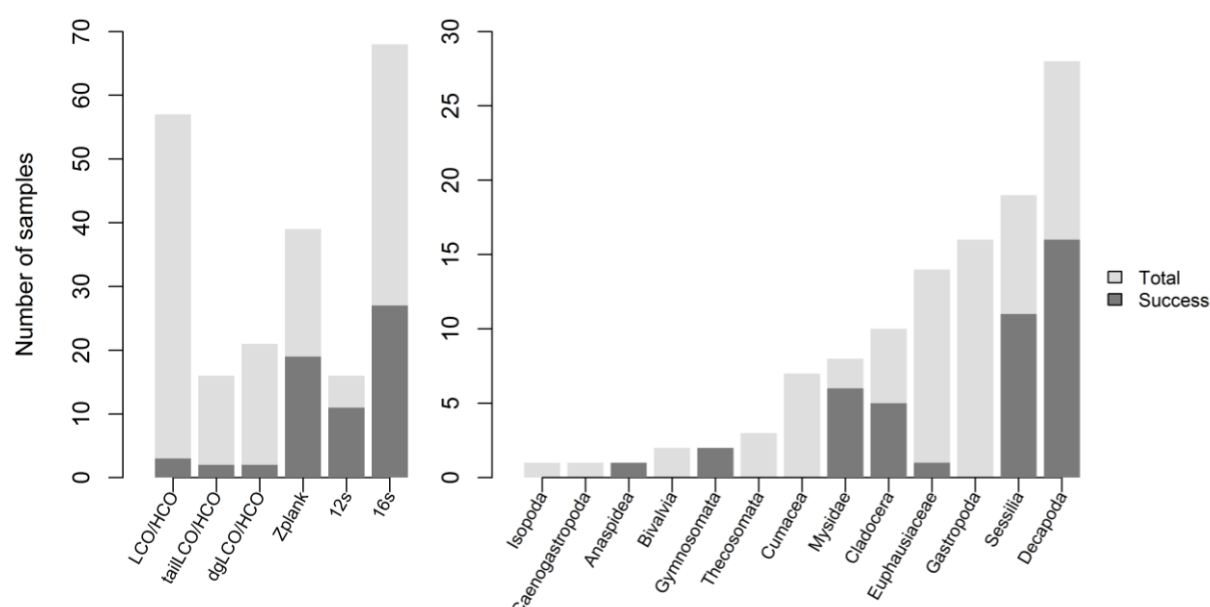
Figure 2 (left panel) Success of each primer pair against the total number of trials for all study organisms. Details of primer pairs are in Table 1. (right panel) Successful species identifications made for each class/order of Crustacean and Molluscs organisms, against the total number of organisms tested for each division.

Clear differences in primer effectiveness emerged at the phylum level. Crustaceans were amplified most successfully with the CO1 primer pair 7/8 (Prosser et al. 2013), and the 16S primer pair 11/12 (Palumbi et al. 1991), and for the case of barnacles, the 12S primer pair 9/10 (Machida et al. 2012) produced a high success rate (69 %). The degenerate Folmer primer pair 5/6 (Folmer et al. 1994, Meyer et al. 2003) was most successful for Mollusca (Table 2).

Multiple bands in gel electrophoresis images were frequently present, suggesting co-amplification of non-target DNA. Non-target DNA was sequenced routinely. Human DNA representing 16 genotypes was amplified (seabird (*Rissa tridactyla*) and turkey (*Meleagris gallopavo*) DNA was also amplified. These sequence matches were 99 % or 100 % (Table 2). All primer combinations used, with the exception of those amplifying the 12S region, amplified non-target DNA. The 12S and 16S primers successfully amplified putative target taxa DNA

from one sample each, where CO1 primers amplified human DNA. Negative controls did not produce amplified DNA. No turkey DNA entered the lab, although seabird work has been undertaken in it previously. We questioned whether DNA concentration might be related to the amplification of non-target sequences, assuming that, in lower concentrations of extracted total DNA, non-target DNA would comprise a greater proportion if present. However, the proportion of non-target versus template sequences was not correlated with quantity of DNA extracted (biserial correlation: r=0.03, p=0.78, df=89).

Discussion

We demonstrated that by using DNA barcoding methods, a wide diversity of Crustacea and Mollusca early life stages sourced from ballast water can be identified to species level. All three genetic markers tested in this study were required to satisfactorily identify the study organisms, which suggests a flexible, though presently complex, approach to barcoding is necessary. A number of non-indigenous and known invasive species were identified, including the European shore crab (*Carcinus maenas*), and the Australasian barnacle (*Elminius modestus*). These examples demonstrate the potential for biological invasion to be mediated via ballast water discharge in the Arctic, and also the utility of barcoding in identifying early life stages. Crab individuals were in both zoea and megalopae stages, while barnacle individuals were in both naupli and cyprid stages, all of which are particularly difficult to confidently assign to a species based on their morphology alone. Elsewhere, these species have caused extensive impacts through predation (Walton et al. 2002) habitat transformation (Floyd and Williams 2004), and competition (O'Riordan et al. 2009). The possibility for species such as these to establish in the Arctic is likely to increase with predicted climate changes (Ware et al. 2013).

Amplification and identification success

The success rate of species identifications (38%) is modestly higher than comparable barcoding studies of meroplankton (< 22% - Webb et al. 2006; < 35% - Heimeir et al. 2010), and demonstrates a clear advantage over traditional means of taxonomy, which would not have permitted the identification of early life stages to species level. Species identifications were well supported by measures of genetic divergence. Generated sequences enabled a large proportion of the samples to be identified at the genus level, but species level assignments were less common. Occasional high intra- and low-interspecific divergences both suggest that we might have sequenced members of species complexes – a finding not surprising given the geographical range from which organisms may have been sourced. Our experience, however, corroborates the findings of others (e.g. Hoareau and Boissin 2010, Heimeier et al. 2010) in that universal barcoding metazoan primers were not alone proficient in successfully amplifying target species. As a result, substantial methodological testing was required as was the use of multiple molecular markers.

A number of factors may explain PCR failure, including primer mismatches, organism preservation techniques, and the quality of DNA extracted from organisms, with the latter two factors potentially related. Mismatches of Folmer primers to some organism DNAs, and consequently lack of PCR success, have been reported (e.g. Hoareau & Boissin 2010, Geller et al. 2013, Prosser et al. 2013), and PCR success using these primers on aquatic organisms is variable (22 % Webb et al. 2006, 82 % Prosser et al. 2013; note that these studies focus largely on organism groups not trialled in the present study). Given that the Folmer primers were designed using a far smaller pool of full length metazoan CO1 sequences than is available today, it is not surprising that

it is nowadays relatively easy to find sequences of metazoan taxa for which these primers do not work (Geller et al. 2013, Table 3). Other CO1 primers used in this study are based on the Folmer primers, and while permitting improved PCR for some taxa, are also unlikely to serve as truly universal primers. Indeed, we found greater success using primer combinations designed to amplify other loci.

Our preservation and handling of organisms followed standard best practice protocols for marine organisms (e.g. Bucklin 1999, Aarbakke et al. 2011). Physical damage to the organisms cannot be avoided owing to the sampling method. A requirement for prior handling of organisms also exists, as some higher order morphological identification is required to guide primer selection. However, preservation and handling may be an area in which improvements could be made. Heimer et al. (2010) found improved amplification rates when organism handling was limited, while Prosser et al. (2013) found, subsequent to the present study, significant improvements in amplification rates (>25% improvement) when organisms were immediately stored on ice following fixation in ethanol. As the latter study showed improved PCR success rate from 57 % to 81 % with this preservation technique, it should be adopted in further studies. Protein contamination in the extracted DNA from some specimens was evident in spectrophotometer readings. There was, however, no pattern that might suggest an effect on PCR failure.

Non-target amplification

While failed PCRs were the greater source of failure in this study (29 samples), a substantial number of spurious results were a result of the amplification of non-target DNA (17 samples). We relied on universal primers, which by their nature may amplify non-target DNA. This amplification may occur if non-target DNA is present in a sample and primers are more closely matched to it than the target molecule, especially, if multiple homologous targets are present and DNA concentration of the non-target homolog is greater. The latter outcome may occur when DNA quantity of the template is low, particularly when organisms are small. Organisms in this study were small (30 – 100 μm), though there was no evidence to suggest that DNA extraction yield was correlated with template PCR success.

Despite efforts made to ensure any contaminating material was removed from the target organism, the means of sampling ballast water meant that the content of a large volume of sea water is filtered through a small receptacle, affording ample opportunity for individual organisms or exogenous DNA molecules to become entangled (Heimeier et al. 2010). We further believe that a similar explanation for the routine amplification of human DNA is plausible, whereby the source of contamination is the ballast water itself. We cannot rule out the possibility that human genotypes amplified in our study were a result of contamination by laboratory personnel, but the greater number of human genotypes amplified than the number of individuals working in the laboratory suggests other sources. All primers used in this study anneal well to human DNA and that of other species (Table 3) (Siddall et al. 2009). Sources of non-target DNA may be within the ship itself (e.g. drains to the ballast water tanks), or ports from which ballast water is sourced. Ship transit times were typically <10 days (mean 7 days) – a duration over which DNA might easily persist in a tank, particularly considering the cool temperatures the ballast water tanks were exposed to during transport from NW Europe to Arctic Norway.

Table 3. Alignment showing the exact match between the forward Folmer primer (LCO1490) and several common ballast water organisms and also two non-target sequences amplified (*Homo sapiens* and *Meleagris gallopavo*) (left panel), and the exact match between the 16S regions of the same species and the 16Sar forward primer. All sequences were obtained from Genbank (accession numbers shown in parentheses). Mismatching nucleotides are highlighted

CO1		16S	
LCO1490	**GGTCAACAAATCATAAAGATATTGG**	16Sar	**CGCCTGTTTATCAAAAACAT**
Mytilus edulis (AY484747.1)	**GGTCAACAAATCATAAAGATATTGG**	*Mytilus edulis* (AF023549.1)	**CGCAATTTCTCCCGAAAGAT**
Amphibalanus improvis (FJ845840.1)	**TATCAAGTAATATTGCACATTCTGG**	*Amphibalanus improvis* (FJ862079.1)	**AAACTCTTTATTTTAAAAAT**
Carcinus maenas (AY616444.1)	**TTATAACAACTATTATCAATATGCG**	*Carcinus maenas* (AJ130811.1)	**GGCCTGCTCACTGATAAAAT**
Clione limacine (AY227377.1)	**GGAAATTGAATGCTACCTCTATTGG**	*Clione limacine* (AJ223406.1)	**CTTGTTGTGATAAAAAGAAT**
Evadne nordmanni (EU675892.1)	**GCTCATGCTTTTATTATGATTTTCT**	*Evadne nordmanni* (GQ343305.1)	**CGCCTGTGCATCAAATGTTA**
Mesopodopsis slabberi (AJ966978.1)	**GTTCTGCAGTGGATATGGGGATTTT**	*Mesopodopsis slabberi* (AJ966898.1)	**CGATGTTGAATTAAAAAAAT**
Meleagris gallopavo (JX160013)	**GACGACCAAATCTATAACGTAATCG**	*Meleagris gallopavo* (JX160013)	**CGACTGTTTACCAAAAACAT**
Homo sapiens (JF682349.1)	**TCTCTACAAACCACAAAGACATTGG**	*Homo sapiens* (JF682349.1)	**CGCCTGTTTACCAAAAACAT**

Developments and application

Approaches to overcome these challenges exist. Rinsing organisms prior to DNA extraction (E. Briski pers. comm.), and minimising handling (Heimer et al. 2010), may lead to lower contamination rates, while cooling or freezing samples immediately following fixation may improve PCR success (Prosser et al. 2013). Alternative means to avoid sequencing non-target DNA include the development and use of blocking primers (e.g. Boessenkool et al. 2012), or RNA sequencing. Given that unsuccessful PCR was the greater source of failure in this study, improvements here stand to provide the largest gains. The development of improved universal or group specific primers would likely be particularly advantageous. This may prove challenging for some groups, although high levels of success have been reported for similar initiatives (e.g. Echinoderms – Hoareau and Boisson 2010; freshwater microzooplankton – Prosser et al. 2013; marine benthic invertebrates – Geller et al. 2013).

While Table 2 provides a useful resource to guide primer selection for future ballast water barcoding studies, success will depend very much on the diversity of organisms sampled from a ballast water tank, the region from which they were originally sourced, and the conditions experienced in the ballast tank. Thus, additional protocol development may be required. In a biosecurity context, quick turnaround time of sample identifications is vital. Therefore, for barcoding to be a viable resource for environmental managers, improvements are needed to meet taxonomic coverage and efficiency requirements.

Management practices employed to limit the transfer of non-indigenous organisms in ballast water are expected to change over the coming decade, with technological treatments of ballast water superseding the current practice of exchanging port-sourced ballast water mid-ocean (IMO 2004). The need to taxonomically screen ballast water will not a requirement of ballast water treatment measures as discharge will be regulated by organism density discharge limits; nonetheless, improved means to screen ballast water will be required into the foreseeable future given: a) the lag in adoption of ballast water treatment technologies by ships; and b) the regional need to evaluate invasion risks associated with ballast water accurately, as individual States determine the imperative for in-bound ships to adopt ballast water management technology; and c) to permit assessment of the effectiveness of current ballast water management procedures. We believe the most efficacious avenue to pursue in this regard is the development and testing of universal primers for use in metabarcoding studies (e.g. Wu et al. 2012, Ji et al. 2013, Leray et al. 2013), or combining multiple primers targeting different taxonomic groups and using next- generation sequencing (NGS) technologies (Parducci et al. 2012, Pochon et al. 2013). Metabarcoding primers have been developed and tested on a range of coral fish species using internal sections of the Folmer primers (Leray et al. 2013), while multiple primer sets and NGS approaches have been tested for use in marine invasive species surveillance (Pochon et al. 2013, see also). Such developments are particularly suited to the efficient identification of organisms in bulk samples such as those comprising ballast water samples, removing the time-consuming process of prior sample sorting into higher order taxa, and the reliance on a single set of quasi-universal primers.

Conclusion

By using barcoding protocols and published primer sets, we have been able to improve the identification rate of early developmental stages of zooplankton sampled from ballast water substantially over what would have

been achievable using traditional microscopy, and to a lesser extent also over previous marine barcoding studies. Our methods enabled us to document the transport and discharge of non-indigenous and known invasive species to the Arctic, a consequence that has potential ecological implications. There were difficulties in amplifying target DNA, and unwanted amplification of non-target DNA. The techniques we tested are not efficient for biosecurity purposes. Future ballast water studies may be better served by developing appropriate metabarcoding methods, or making use of multiple primer sets in NGS approaches. This will remove the need for laborious sample sorting and performing multiple PCRs with different primer sets, while extending taxonomic coverage. The outcomes of the present study therefore provide the means for guiding marine biosecurity improvement.

Acknowledgments

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Is marine species invasion a threat to a warming Arctic?

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Abstract

1. Several decades of marine species invasion research have yielded a broad understanding of the nature of species transfer mechanisms and associated threats globally. This is not true of the Arctic however, a region where ongoing climate change is expected to promote species invasion.

2. Here we present a comprehensive evaluation of risk associated with the non-indigenous propagule load discharged in ships' ballast water to the high-Arctic archipelago, Svalbard, as a model for the wider Arctic region. We do so by sampling and identifying transferred propagules, and assessing habitat suitability under present and forecast future climates (RCP 8.5 warming scenario) based on critical temperature and salinity reproductive thresholds.

3. Ships discharging ballast water in Svalbard carried high densities of living organisms (range: 10 – 4500 individuals m^{-3}; mean 1522 ± 335 SE individuals m^{-3}), predominately comprised of indigenous species. Nonetheless, non-indigenous species (NIS) were present in all except one ballast water sample (n = 16; eight ships; mean 2.7 ± 0.4 SE NIS individuals per sample), a trend that was not prevented through the practice of ballast water exchange.

4. Of a total of 79 unique taxa, 41 species including 23 NIS were identified. Of the 23 NIS, we evaluated habitat suitability for eight widely-known invaders (Copepoda – *Acartia tonsa, Eurytemora affinis*; Decapoda – *Carcinus maenas*, *Hemigrapsus takanoi*, *Crangon crangon*; Cladocera – *Podon leuckartii*; Balanidae – *Amphibalanus improvisus*). Conditions were estimated to permit northward expansion for all species, with only reproductive thresholds for *C. crangon* and *H. takanoi* not overlapping with Svalbard environmental conditions by the end of the century.

5. *Synthesis and applications.* Ballast water management did not prevent NIS introduction, and a number of NIS that survived transport were estimated to be capable of reproduction under forecast Svalbard climatic conditions. Together, we consider these results indicative of an increasing vulnerability of Svalbard to species invasion. Similarity in Svalbard bulk shipping patterns to those of other Arctic regions (e.g. Canadian Arctic) highlights the increasing potential for ballast water-mediated NIS colonisation throughout the wider Arctic. While measures to reduce the introduction of NIS through ballast water are in a period of transition globally, our results press the need to further evaluate ballast water management on board Arctic-bound ships, both in policy and in practice.

Introduction

Globally, few marine ecosystems remain immune from the potential impacts of NIS introduction (Catford *et al*. 2012). With the exception of commercial shellfish species, most marine invasive species have been introduced to their invasive habitats unintentionally, largely as a result of shipping activity (Carlton 1985, Gollasch 2002). Shipping connects distant global regions (Keller *et al*. 2011, Seebens *et al*. 2013), and even remote Antarctic and Arctic port-regions are vulnerable to species introduction through active shipping networks (Chan *et al*. 2013, Ware *et al*. 2014). Numerous ports worldwide have become heavily invaded by NIS and now serve as hubs for the further spread of invasive NIS (Adebayo *et al*. 2014, Briski *et al*. 2012). As a result, a major prerogative of environmental managers is developing an understanding of if, and where, marine species invasion threats lie, and to implement measures to reduce identified threats.

Ships may transfer species via biofouling (through organism attachment to vessel hulls) or through ballast water discharge (being collected during ballast water up-take). To reduce the transmission of NIS, international and domestic efforts have been made to regulate both the standard to which ship hulls are maintained (IMO 2011) and also the way ballast water is managed (IMO 2004). These approaches are currently in transition around the world (Frazier *et al*. 2013). With respect to ballast water management, the primary method of regulation has been to require ballast water exchange or saltwater flushing (collectively referred to as BWE – Frazier *et al*. 2013) to reduce invasion threat. In theory, these practices should reduce species abundance and richness of ballasted organisms by either purging individuals, or killing taxa through osmotic shock. In practice, it appears BWE can effectively reduce invasion risk for freshwater ecosystems, though efficacy is less apparent in marine ecosystems (Wonham *et al*. 2005, Briski *et al*. 2013). Requirements to install ballast water treatment systems in ships to limit (or even eliminate) NIS transfer will likely be realised in the coming years under the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004, Gollasch *et al*. 2007, Norwegian Ministry of Environment 2009, Frazier *et al*. 2103). Technological and logistical hurdles are expected to delay the immediate impact this requirement will have (Gregg *et al*. 2009, Balaji *et al*. 2014), and until such time that systems are installed on all vessels discharging ballast water for a given shipping network, some level of species introduction threat will likely remain.

One broad region for which few marine biological invasion data exist is the Arctic. The number of established marine NIS across the region is low (Ruiz and Hewitt 2009, Miller and Ruiz 2014), and invasive species are rare (Jørgensen and Primicerio 2007, Falk-Petersen *et al*. 2011) (though detection effort is substantially lower than other regions, Ruiz and Hewitt 2009). Given the rapid changes in regional climates forecasted for the coming century (Trenberth *et al*. 2007), the pronounced effect of changes in the Arctic region (Comiso 2003, 2006; Steele *et al*. 2008, McPhee *et al*. 2009, Serreze *et al*. 2011), and the positive effect they are estimated to have on the establishment of NIS (Stachowicz *et al*. 2002, Hoegh-Guldberg and Bruno 2010), recent efforts have been made to quantify the vulnerability of the Arctic to marine species introduction and invasion (Chan *et al*. 2013, Ware *et al*. 2014). The latter indicate some level of threat exists presently and is set to increase as climate changes progress; however, conclusions have largely been drawn in the absence of biological samples. While these remain worthy approximations of threat, the strength of conclusions are necessarily limited within the constraints imposed by the types of analysis.

Vector sampling provides the most powerful type of assessment in order to gain data from which risk at the transport stage of species introduction can be evaluated. From sample data, direct measures of biotic

composition and propagule pressure can be obtained, these providing information directly related to establishment and invasion processes (Lockwood *et al.* 2007). While such information may afford qualitative assessments of risk, more robust assessments are derivable by modelling the recipient habitat suitability for candidate species (e.g. Ficetola *et al.* 2007, Elith *et al.* 2010, Verbruggen *et al.* 2013).

At coarse scales, climate is considered to be a major determinant of species distributions (Pearson and Dawson 2003, Wisz *et al.* 2013), and a number of modelling approaches have been used to estimate habitat suitability for invasive species based on climate. Correlative approaches, whereby environmental conditions characterising the locations of known species occurrences are statistically modelled (e.g. species distribution models), are commonly used to infer habitat suitability in other spatial or temporal domains. These, however, rest on numerous theoretical assumptions, and the temporal and spatial transfer of such models may not always be appropriate (Pearson and Dawson 2003, Jeschke *et al.* 2008, Maiorano *et al.* 2013, Woodin *et al.* 2013). Conversely, processed-based (syn. mechanistic) approaches use physical processes underlying species' physiology to map habitat suitability and are thus not reliant on species distributional data or on the same assumptions such as niche conservatism or model transferability (see e.g. Kearney *et al.* 2009, Elith and Leathwick 2009 for reviews). For marine zooplankton, the two physical factors most important to population maintenance are temperature and salinity (Hoek 1982, Summerson *et al.* 2007; Barry *et al.* 2008, Sunday *et al.* 2012), as both influence survival and successful progression through life stages. Zooplankton have been shown to occupy large portions of the extent of latitudes tolerable within their fundamental thermal tolerance limits (Sunday *et al.* 2012), suggesting these limits are a useful predictor of habitat suitability. Experimentally derived data characterising marine species' threshold tolerances to both temperature and salinity are commonly available, and mapping these in joint environmental-geographical space thus presents a biological meaningful way of estimating a species' fundamental climatic niche (*sensu* Monahan *et al.* 2011, Rodda *et al.* 2011). More accurate estimates of colonisation potential should be obtainable by mapping the narrower range of tolerance thresholds that are required to be met for successful reproduction (i.e. the regeneration niche – Jackson et al. 2009).

In this study, we investigated zooplankton quantity, composition, and survivorship in the ballast water tanks of ships travelling to the Arctic. We then evaluated the potential for ballast-transported NIS to establish in the Arctic by mapping ecophysiological reproduction thresholds for individual species onto projections of oceanic climates for the remainder of this century. Macroinvertebrates constitute a large proportion of all marine organisms demonstrated to cause negative impacts on natural systems. As such, our overall aim was to evaluate vulnerability to zooplankton NIS introduction and establishment. We did so based on an assessment of the following three factors: 1) the composition and survivorship of zooplankton communities in ballast water tanks of ships travelling through polar waters; 2) the effectiveness of BWE at removing coastal zooplankton from ships travelling to the Arctic from European ports; and 3) the suitability of recipient habitats for population establishment of transported NIS, under present and future projected climatic conditions. We use the bulk shipping network to the Norwegian archipelago, Svalbard, as a case study for this assessment. Our results are generalisable to other Arctic shipping networks, providing the first sample-based assessment of ballast water-mediated biological introduction threats to the Arctic region.

Methods

Svalbard and the bulk shipping network

Svalbard is a Norwegian administered archipelago extending from 74° to 81°N and 10° to 35°E (Fig 1). There are four active ports around the archipelago, with three recieving bulk shipping servicing the three operational coal mines. Of the range of vessel classes visiting Svalbard, bulk carriers are the only class to discharge ballast water (Port of Longyearbyen, pers comm.). Ships travelling to Norway carrying ballast water sourced from an area outside of the Norwegian Exclusive Economic Zone, or Norwegian territorial waters including Svalbard, are required to manage ballast water under the Norwegian Ballast Water Regulation (Norwegian Ministry of the Environment, 2009). Bulk carriers visiting Svalbard typically visit from a non-Norwegian European port where they ballast (Ware *et al*. 2014), and are therefore required to manage ballast water under this regulation.

The port marine environments of Svalbard are characterised by a mean annual sea surface temperature of 3°C (range: -2° – 8°) reflecting warm inflow of Atlantic water towards the Arctic and, thus, salinities approaching 35 psu (Ware *et al*. 2014). To the north of the islands, temperatures are low and salinity is affected by the fresher polar mixed layer. Mean sea surface temperatures are expected to increase by as much as 1.7° and 5.2°C by 2050 and 2100 respectively (Ware *et al*. 2014), and evidence of sea surface warming is apparent around the archipelago (Berge *et al*. 2005, Bjørklund *et al*. 2012).

Ship operations and sample collection

Seventeen ballast water samples were collected from eight ships (two samples per ship plus one control sample: see below) arriving to Svalbard in 2011, the ballasting operations of which have been reported in a complementary study (Ware *et al*. 2014). All eight ships arrived in Svalbard from European ports where ballast water was originally sourced (Fig 1). Vessels were sampled between July and October 2011, the period encompassing the majority of coal shipments from Svalbard (and thus ballast water discharged into Svalbard waters). Five vessels for which we obtained data exchanged ballast water in accordance with the Ballast Water Regulation (Norway 2009), while the remaining three did not perform any form of ballast water management. Thus, ballast water discharged in Svalbard was mostly sourced from marine waters (92%), with the remainder sourced from brackish ports (14-19 ppt) (Ware *et al*. 2014). Total ballast water discharged by all eight vessels was 148,000m^3; total ballast water estimated to have been discharged by the entire 2011 fleet of 31 ships was 653,000 m^3 (Ware *et al*. 2014).

Exchange locations varied greatly (Fig 1) as did the age of exchanged ballast upon discharge (range: <1 – 12 days). The age of ballast water aboard the three vessels that did not perform any BWE was seven, 12, and 14 days old upon discharge. Voyage times ranged from seven to 22 days (mean 10.2, SE ± 1.7, median 8, n = 8 days) (unseasonal sea ice prevented one ship from berthing for several days, heavily inflating the mean) (Ware *et al*. 2014).

Samples were collected from wing tanks (n = 11) and double bottom tanks (n = 6) of ships. Where possible (75 % of samples), samples were taken by lowering and raising a 30 cm Ø, 50 μm mesh conical plankton net into the opened access hatch of a ballast tank (Gollasch *et al*. 2003). These hatches are located on the deck level of

ships, and vary in depth between tanks and ships. The plankton net was lowered to the bottom of the tank and then raised at a speed of approximately 1 ms^{-1} (Briski *et al.* 2013). Sampled volumes depended on the type of tank sampled, the volume of ballast water within the tank, and the sampling method (see below). Thus, sampled volumes for wing tanks were between 210 and 280 L, while samples from double bottom tanks were between 450 and 560 L.
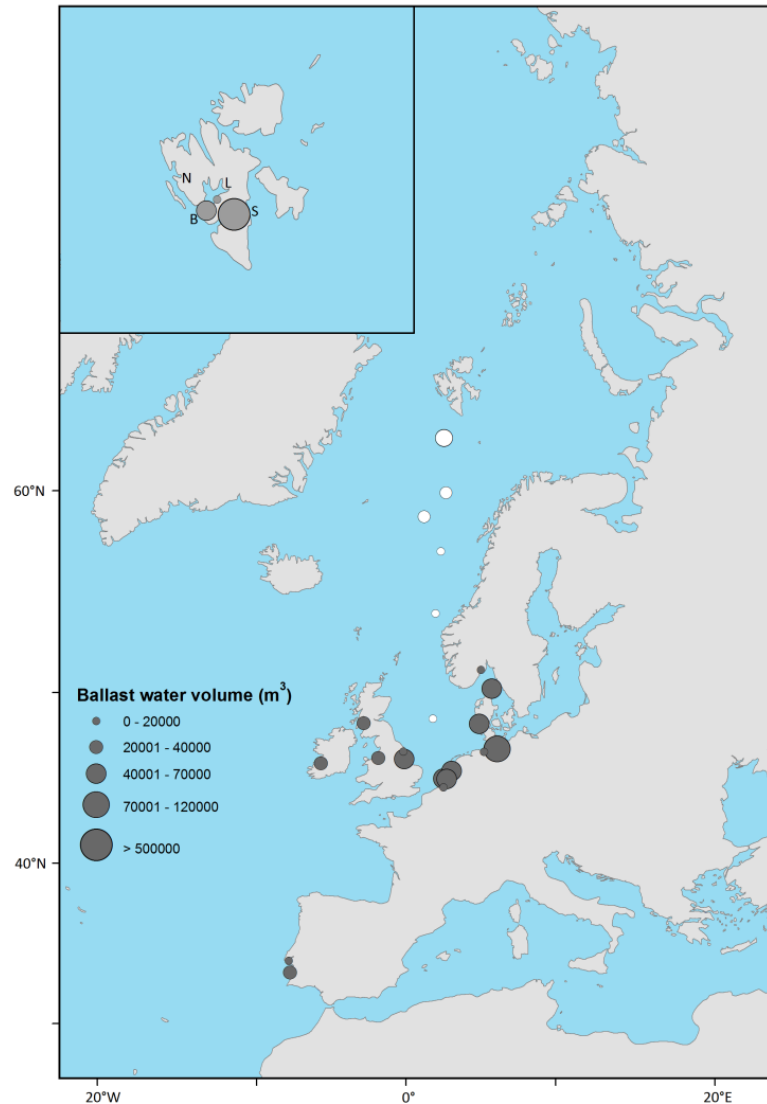
Figure 1. Regions from which ballast water was sourced by vessels prior to discharge in Svalbard in 2011: grey circles – original ballast water source estimated for all vessels; open circles – mid-ocean exchanged ballast water reported by eight vessels. Inset: ballast water discharged in Svalbard. S – Svea; B – Barentsburg; L – Longyearbyen; N – Ny Ålesund: no ballast water was discharged in Ny Ålesund.

Where ships did not have access hatches to ballast water tanks, samples were drawn through sounding pipes using a hand pump (David and Perkovič 2004). Sounding pipes are present on all ships and are used to gauge the level of water in ballast tanks. For sampling, a hose was inserted into the sounding pipe until it reached the tank base. Approximately 100 L of water was then pumped up to deck level and filtered through 50μm mesh into collection containers. An additional pump sample was taken from one ship to help evaluate species differences sampled by the two methods. Tank selection on all sampling occasions was made by ship's officers

according to their preference regarding ballasting and loading operations. All samples were inspected for organism motility and then preserved in 95 % ethanol according to published protocols (Bucklin *et al*. 2000).

Zooplankton enumeration

Samples were sorted under a dissecting microscope into operational taxonomic units (OTUs). The number of individuals per sample was counted, and densities based on the volume of ballast water sampled calculated. Where an individual OTU was highly abundant in a sample (> 500) the number of individuals was estimated. To do this, five × 1 ml subsamples of the OTU were taken from a well-mixed 50 ml of solution. The resulting number of individuals in each subsample was tallied and multiplied by ten to obtain a total abundance estimate.

Organisms were then identified based on morphological characters where possible, and/or analysed using molecular methods. Larval organisms commonly form a large proportion of zooplankton organisms present in ballast water tanks, and present particular difficulties in species identification based on morphology. Typically, studies of organisms collected from ballast water tanks fail to identify a large proportion of meroplanktonic larval forms, which compromises subsequent assessments of risk (see for example David *et al*. 2007, Choi *et al*. 2005, DiBacco *et al*. 2011). Sequencing of genes permits the discrimination of species based on recognition of unique DNA sequences, overcoming the challenges of larval identification where differences between organisms cannot be determined through morphological analysis alone. We used DNA barcoding to resolve species identity primarily in larval organisms, but also to confirm or refine identifications based on morphological characters of more mature organisms. Morphological species identifications were performed under a dissecting microscope by the authors, and several taxonomic experts (see acknowledgments). The DNA barcoding methods used for species identification are described in Chapter six of this thesis.

Zooplankton abundances were standardised to numbers per cubic metre of water sampled. Associations between voyage time and time since last ballast up-take on total zooplankton abundance in ballast water tanks were then explored using linear regression. The effect of BWE on zooplankton abundance and the number of NIS was assessed using generalised linear models and the chi-squared test. Analyses were performed in R (version 3.0.1, R Core Team 2013).

Habitat suitability

From the list of species identified in ballast water samples, we selected species to model Svalbard habitat suitability for based on the availability of experimentally derived ecophysiological data. Given that we wished to evaluate changing habitat suitability for NIS colonisation, we sought data on the critical minimum thermal and salinity thresholds for reproduction, and the requisite period of time required at these levels. Values were obtained for the number of threshold days required to complete all juvenile life stages (including egg hatching where available) for each selected species (see Appendix 1 in supporting information). Only minimum thresholds were sought as these were the parameters of relevance to the study. We acknowledge that numerous other factors may affect whether a NIS colonises a novel habitat (both abiotic and biotic), but restrict our analysis to these fundamental thresholds as they provide a framework for understanding how species may respond to changing climatic gradients.

Mapping of climatically suitable regions for reproduction was then achieved using a series of 'if-then-else' statements for each point in climatic space (i.e. each raster cell) to determine whether threshold criteria were met. For example, if the two conditions (requisite number of days at temperature x and salinity x) were met for a cell, the cell was classified as suitable for reproduction; if both conditions were not met, the cell was classified as unsuitable. This procedure was then repeated for conditions projected under future climates. We used modelled environmental data for 2011 and model forecasts for the years 2050 and 2100 (RCP 8.5 emissions scenario – see Moss *et al*. 2010) with a 0.5° resolution (approx. 55km2 at the equator) (see Ware *et al*. 2014 Appendix S1 for a detailed description of the future climate data). Monthly mean data for sea surface temperature and salinity (upper 10m) were used, which were interpolated to daily values using splines so that degree days could be calculated. The resulting maps indicated areas of climatic suitability for reproduction, and areas that were outside of these fundamental thresholds.

For all species, occurrence data were downloaded from the GBIF (gbif.org) database. These were mapped onto current threshold ranges to inspect the present level of regeneration climatic niche filling. All spatial analyses were performed in R [version 3.0.1, libraries (raster, ncdf, SDMTools); http://www.r-project.org].

Results

Zooplankton composition

Ballast water samples represented 20 % of the total shipping fleet discharging ballast water in Svalbard during 2011 (n ships = 31). A marked difference was evident in the sampling efficiency of the different methods. Pumping ballast water to the deck surface recovered about 30 % fewer organisms than a comparative net sample. Pump ballast water samples were similar in species richness to net samples, and recovered similar species (65 % species similarity). Mean species richness across all samples was 12.2 taxa (± 2.2). Zooplankton abundance per sample ranged from 10 – 4500 m^{-3} (mean 1522 ± 335 SE individuals m^{-3}) with pump samples accounting for the three smallest sampled abundances. Increasing time since last ballast up-take (original port water or BWE) was also associated with lower zooplankton abundances ($p < 0.05$, df = 14, F = 0.5), though longer voyage durations overall were not ($p = 0.7$, df = 14, F = 0.1). There was a weak association between exchanged ballast water and higher zooplankton abundances ($p = 0.05$, df = 14, F = 4.3), while significantly more NIS were present in exchanged ballast water than un-exchanged ballast water ($\chi^2 = 27$, df = 1, $p = <0.01$). We acknowledge that these analyses are based on a low number of samples.

We found 79 unique zooplankton taxa in all ballast water samples. Of these, 37 different genera and 41 different species were identified from 5 phyla (Table 1). Twenty three species were considered non-indigenous to Svalbard. The copepod, *Calanus finmarchicus*, dominated samples in terms of abundance (mean = 235 ± 70 SE), and presence among samples (62 % of samples, 62 % of ships), while Copepoda species dominated samples overall (31 % of all taxa). The most abundant NIS present was the green crab, *Carcinus maenas* (mean = 2.75 ± 1.3 SE, present in 25 % of samples, 20 % of ships), and NIS were present in all but one sample (94 %; mean = 2.71 ± 0.4 SE).

Habitat suitability

Data were available to explore Svalbard habitat suitability under present and future projected environmental conditions for eight NIS (Copepoda – *Acartia tonsa, Eurytemora affinis*; Decapoda – *Carcinus maenas, Hemigrapsus takanoi, Crangon crangon*; Cladocera – *Podon leuckartii*; Balanidae – *Amphibalanus improvisus*; see Appendix 1 in supporting information for species descriptions).

Table 1. Zooplankton identified from ballast water samples collected from eight vessel arrivals in Svalbard (16 ballast water tanks). Taxa in bold are not considered indigenous to Svalbard. ID refers to the method of identification: M=morphological; BC=molecular (barcoding).

Taxa	No. Ships	No. BW tanks	Prevalence	Mean (m^3) (±SE)	ID
Copepoda	1	1	3	<1 (±NA)	M
Calanoida	6	11	877	54.8 (±45.1)	M
Acartia sp.	3	5	385	24.0 (±33.8)	BC/M
Acartia clausii	1	1	1	<1 (±NA)	M
Acartia tonsa	1	1	1	<1 (±NA)	BC
Anomalocera patersoni	2	3	9	<1 (±2)	BC/M
Calanoida nauplii	4	8	301	18.8 (±13.9)	M
Calanus sp.	3	3	132	8.2 (±31.6)	M
Calanus finmarchicus	5	10	2354	147.1 (±69.2)	M
Calanus helgolandicus	2	2	3	<1 (±0.5)	M
Centropages hamatus	3	4	10	<1 (±1.5)	M
Centropages sp.	4	6	592	37.0 (±95.6)	M
Centropages typicus	3	7	316	19.7 (±31.9)	BC/M
Eurytemora affinis	1	1	1	<1 (±NA)	M
Isias clavipes	1	2	2	<1 (±0)	M
Metridia lucens	1	1	5	<1 (±NA)	M
Paracalanus sp.	1	1	1	<1 (±NA)	M
Paracalaus parvus	1	1	1	<1 (±NA)	M
Parapontella brevicornis	1	2	2	<1 (±0)	M
cf. *Pseudocalanus* sp.	3	5	210	13.1 (±16.5)	M
Pseudocalanus minutus	2	2	3	<1 (±0.5)	M
cf. *Sinocalanus sp.*	1	1	1	<1 (±NA)	M
Temora sp.	2	2	5	<1 (±1.5)	M
Temora longicornis	7	10	67	4.1 (±2.6)	M
Cyclopoida	1	1	3	<1 (±NA)	M
Oithona sp.	1	1	1	<1 (±NA)	M
Oithona similis	5	8	83	5.1 (±4.2)	BC/M
Harpacticoida	3	3	7	<1 (±1.3)	M
Microsetella norvegica	2	2	3	<1 (±0.5)	M
Cirripedia	4	5	10	<1 (±0.5)	M
Balanidae	1	2	18	1.1 (±0.0)	BC
Amphibalanus improvisus	2	3	7	<1 (±0.6)	BC
Balanus balanus	1	1	4	<1 (±NA)	BC
Austrominius modestus	2	3	3	<1 (±0.0)	BC

Euphausiacea	1	1	6	<1 (±NA)	BC/M
Nematoscelis megalops	1	1	1	<1 (±NA)	BC/M
Nyctiphanes simplex	3	3	3	<1 (±0.0)	BC/M
Thysanoessa cf. *longicaudata*	1	1	4	<1 (±NA)	BC/M
Thysanoessa inermis	1	1	1	<1 (±NA)	BC/M
Thysanoessa longicaudata	1	1	1	<1 (±NA)	BC/M
Thysanoessa longipes	1	1	1	<1 (±NA)	BC/M
Thysanoessa raschii	1	1	1	<1 (±NA)	BC/M
Amphipoda	-	-	0	0.0 (±NA)	M
Gammarus* cf. *tigrinus	1	1	1	<1 (±NA)	M
Gammarus* cf. *zaddachi	1	1	1	<1 (±NA)	M
Mysida	2	2	2	<1 (±0.0)	BC/M
Mesopodopsis slabberi	1	2	5	<1 (±1.5)	BC/M
Cumacea	3	3	5	<1 (±0.6)	M
Decapoda	3	3	4	<1 (±0.3)	BC/M
Brachyura	2	2	4	<1 (±1)	BC/M
Cancer pagarus	1	1	1	<1 (±NA)	BC
Carcinus maenas	3	4	10	<1 (±1.5)	BC
Hemigrapsus takanoi	1	1	1	<1 (±NA)	BC
Caridea	-	-	0	<1 (±NA)	BC/M
Crangon crangon	2	3	6	<1 (±0.5)	BC
Anomura	-	-	0	<1 (±NA)	BC
Paguridae	1	1	1	<1 (±NA)	BC
Isopoda	-	-	0	<1 (±NA)	M
Eurydice pulchra	3	2	2	<1 (±0.0)	M
Idotea linearis	1	1	1	<1 (±NA)	M
Cladocera	2	2	8	<1 (±1.0)	BC
Evadne nordmanni	2	2	4	<1 (±1.0)	BC
Podon leuckarti	2	2	5	<1 (±1.5)	BC
Polychaeta	-	-	0	<1 (±NA)	M
Spionidae	2	3	10	<1 (±2.3)	M
Eteone sp.	1	1	1	<1 (±NA)	M
Polydora sp.	1	1	1	<1 (±NA)	M
Pygospio elegans	1	1	7	<1 (±NA)	M
cf. *Spio* sp.	1	2	2	<1 (±0.0)	M
***Scolelepis* sp.**	1	2	2	<1 (±0.0)	M
Spiophanes kroeyeri	1	1	1	<1 (±NA)	M
Chaetognatha	3	4	11	<1 (±0.8)	BC
Mollusca	-	-	0	<1 (±NA)	BC/M
Gastropoda	7	10	190	11.8 (±10.9)	BC/M
Caenogastropoda	1	1	1	<1 (±NA)	BC
Clione limacine	1	1	3	<1 (±NA)	BC/M
Limacina cf. *helicina*	3	3	3	<1 (±0.0)	BC/M
Bivalvia	1	2	2	<1 (±0.0)	M
Rotifer	1	1	1	<1 (±NA)	M

P. leuckartii was the only one of the eight species ecophysiologically suited to present Svalbard port conditions (Fig 2b bottom row). The known distributions of all species were within their respective ecophysiological reproductive thresholds (i.e. sensitivity = 1), with the exception of one occurrence location for *P. leuckartii*, that lies north of the threshold margin (i.e. sensitivity < 1) (Fig 3). Suitable habitat was also estimated to be unoccupied (i.e. specificity < 1), suggesting partial under-filling of the fundamental climatic niche (Fig 3). Conditions permitting reproduction were estimated to shift poleward for all species over the coming century, and overlap with Svalbard port environments by 2100, with the exception of thresholds for *C. crangon*, and *H. takanoi* (Fig 2a-b).

a)

b)
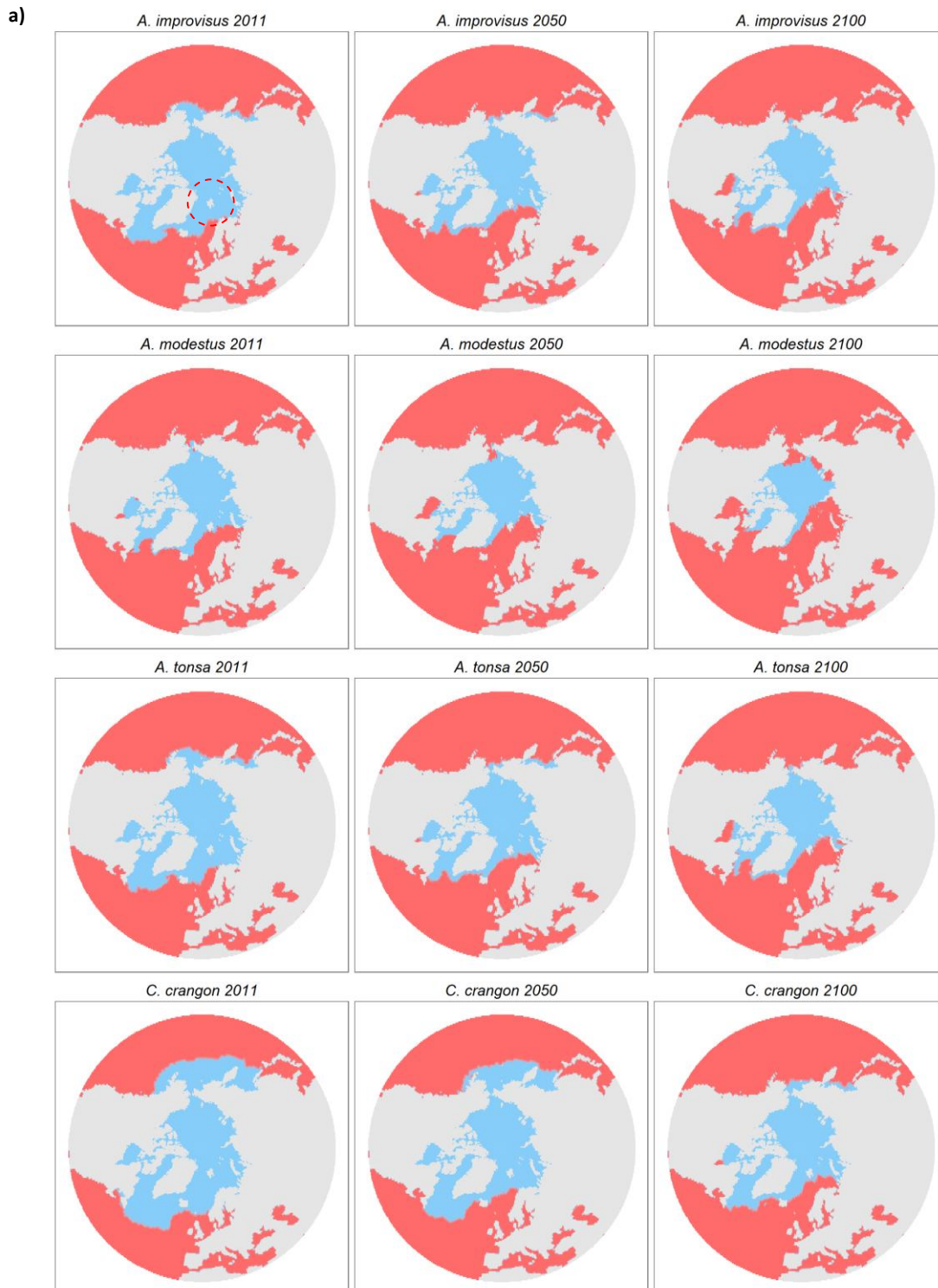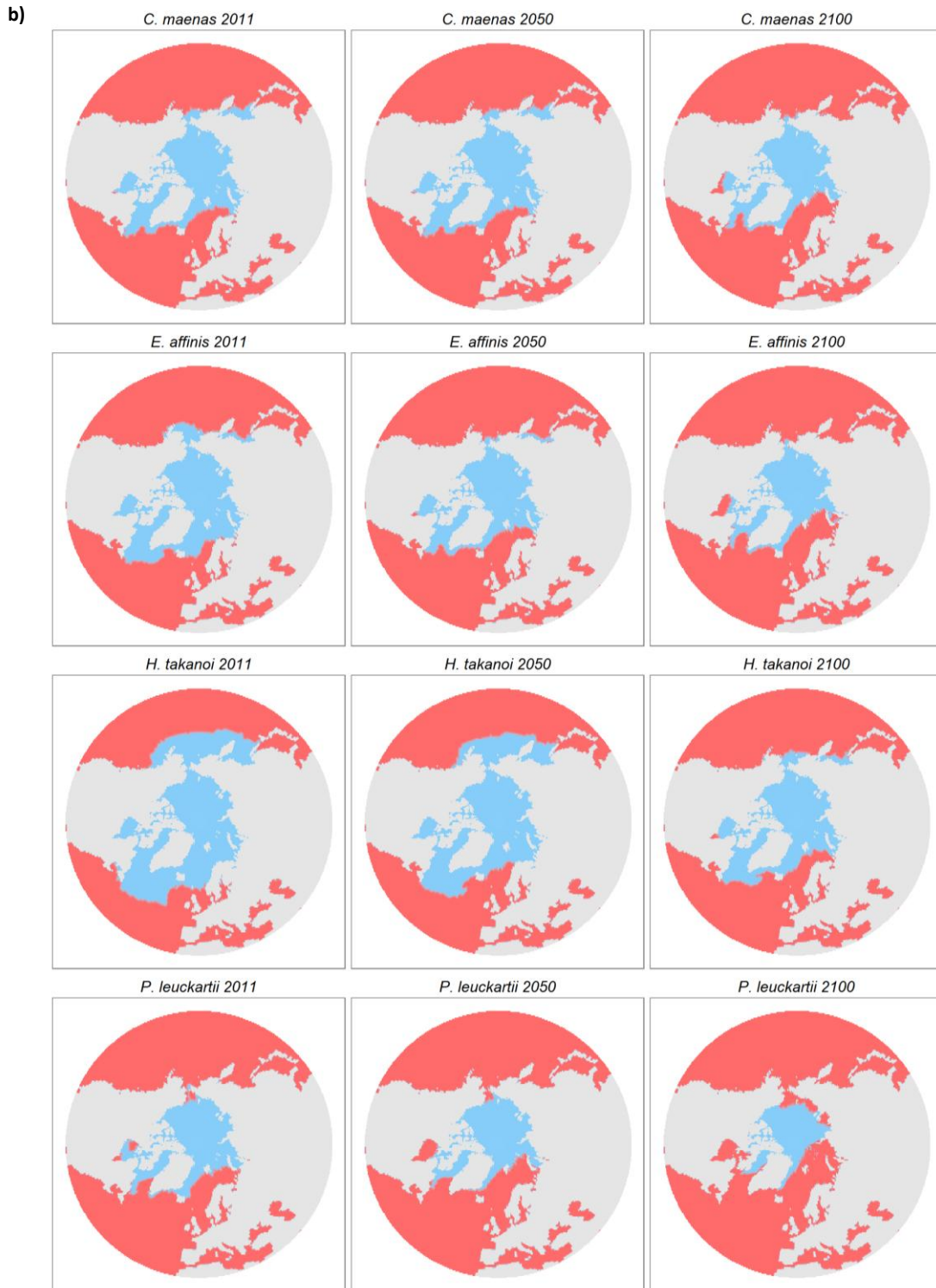
Figure 2a-b Projected ecophysiological thresholds for eight NIS sampled from the ballast water tanks of ships arriving to Svalbard during 2011. Thresholds were based on critical minimum temperature and salinity values, and the number of days required at these values for successful reproduction. Thresholds were projected into the future based on ocean climates forecast under the RCP 8.5 emissions scenario. Red indicates suitable habitat (i.e. temperature and salinity values above the minimum thresholds), while blue indicates unsuitable habitat (i.e. temperature and salinity values below the critical thresholds). Svalbard is highlighted in the upper left panel. Maps are North Pole Lambert Azimuthal Equal Area Projected.
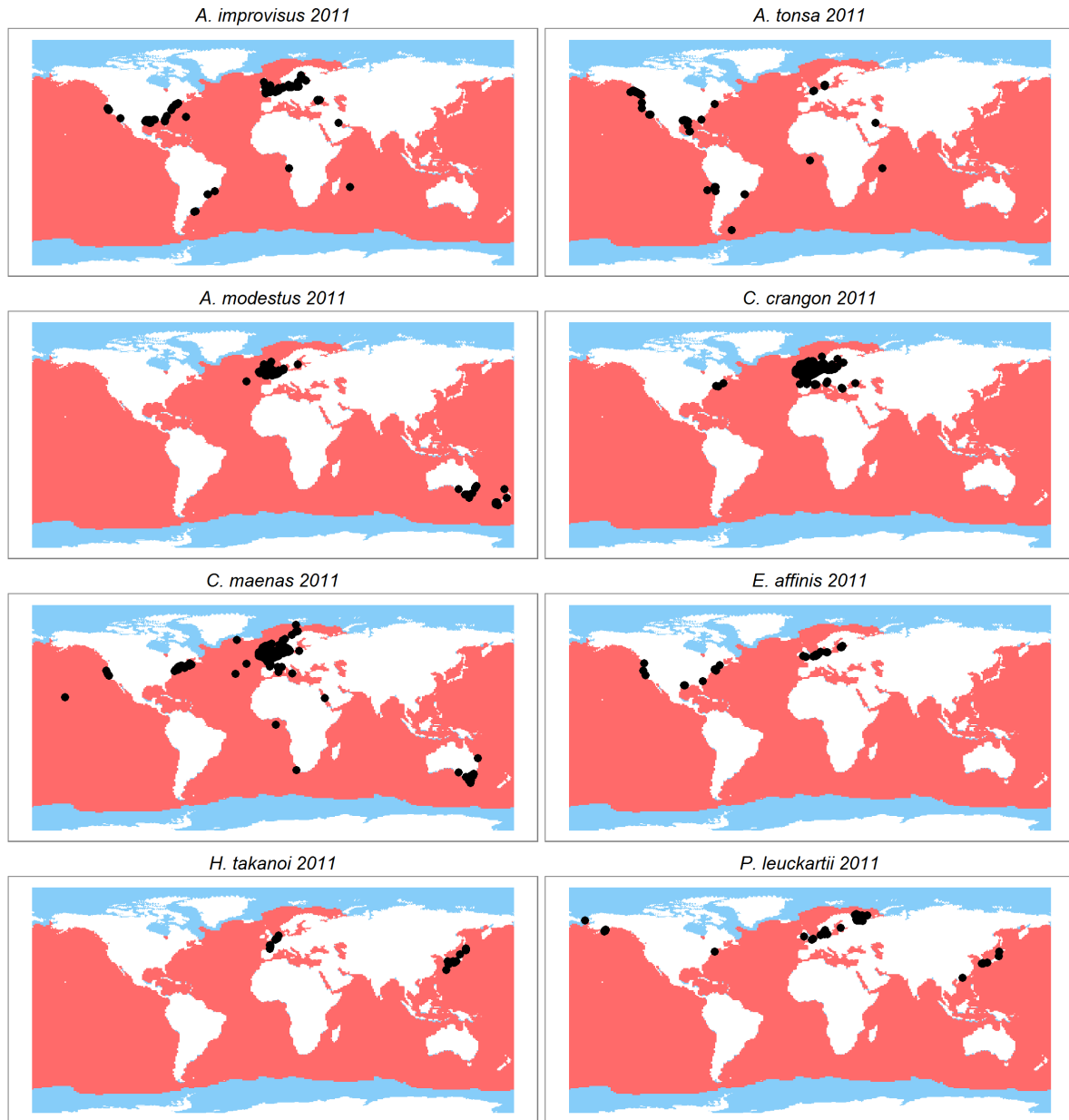
Figure 3 Known distributions of each of the eight NIS for which habitat suitability was projected. Occurrences points are plotted onto the projection of suitable habitat based on minimum ecophysiological tolerances under current oceanic conditions. Thus, these plots give an indication of present day range filling. Red indicates suitable habitat based on thresholds, while blue indicates unsuitable habitat. Thresholds are based on minimum requirements for reproduction and are not sensitive to maximum thermal thresholds (grid cells falling in warmer low latitude regions) which were not the focus of this study; therefore red cells only depict habitat with parameters exceeding minimum thresholds.

Discussion

Zooplankton composition

Our sampling demonstrated that high abundances of zooplankton, including many NIS, are discharged through ballast water discharge to Svalbard. Most notably several well-known marine invaders (e.g. the barnacles *A. improvisus, A. modestus,* and the crab *C. maenas*) are introduced to Svalbard in this way. Zooplankton abundances found in this study (10 – 4500 m^{-3}, mean 1522 ± 335 individuals per m^3) were within the ranges of samples collected from ships arriving to other high latitude regions. Hines *et al*. (2000) sampled ballast water tanks of ships arriving to Port Valdez in Alaska and reported a range of zooplankton densities (14-29876 individuals m^{-3}) grouped by different ports of origin (mostly United States ports, mean across groups = 780 ± 596 SE individuals m-^{3}). Mean species richness (12 ± 2.2 taxa) sampled from ships visiting Svalbard was lower than in the Alaskan study (~20-28 taxa per ship), though the number of NIS was higher (14 NIS, cf. 23 NIS in the present study). Chan *et al*. (2014) reported lower mean abundances (942 individuals m^{-3}) and species richness (4.7 taxa per ship) of zooplankton of undocumented biogeographic origin sampled from the ballast water tanks of ships arriving to the Canadian Arctic from European ports. Samples of zooplankton abundances observed at more southerly latitudes (Chesapeake Bay, United States), but including samples from ships travelling from the same ports as ships in the present study, were lower (200 individuals m^{-3}), though diversity was much higher (168 taxa) (Smith *et al*. 1999). Therefore, mean zooplankton abundances in this study were marginally higher than those reported from other high-latitude and some lower latitude studies, but diversity similar to lower.

In terms of abundances, our samples were comprised heavily of species indigenous to Svalbard. These were predominately calanoid copepod species, the ranges of which extend much further south, but not to ports of origin (gbif.org). These species were probably collected during BWE. This accounts for the higher abundances of organisms found in samples from ballast water tanks that had been exchanged compared to un-exchanged, and the higher abundances in ships that had more recently collected ballast water. A trend of decreasing zooplankton abundances and richness with longer voyages was apparent in our data (but only significant when considering time since last ballast water up-take), a pattern which is further corroborated by survivorship studies carried out over the duration of voyages elsewhere (Gollasch *et al*. 2003, Verling *et al*. 2005). Zooplankton samples collected in the present study likely represent a conservative estimate of the overall diversity and abundance of zooplankton discharged in Svalbard waters, owing to a variety of factors influencing sampled abundance and composition [e.g. ships arriving from different ports, sampling intensity with relation to the peak spring abundances of zooplankton species (Fromentin and Planque 1996), and sampling techniques (Gollasch *et al*. 2003), among others].

Our finding of greater numbers of NIS in exchanged ballast water samples compared to un-exchanged ballast water samples highlights the possibility that survivorship within ballast water tanks that undergo BWE may be promoted. We sampled a smaller ballast water volume from two of the three vessels which did not perform BWE, but even when correcting for densities of NIS per cubic metre of sampled water, the differences remained significant. Therefore, it is plausible that the proportion of NIS entrained in ballast water that do not get flushed out during BWE, benefit from the addition of oxygen and nutrients introduced through BWE (Carver and Mallet 2004, Klein *et al*. 2010, Briski *et al*. 2012). Our data do not permit examination of the alternative hypothesis that the numbers of NIS initially entrained in ballast water tanks were different between ships performing BWE or not, though this may account for some of the difference. We cannot

therefore draw a robust conclusion on the overall merits of BWE for Svalbard-bound ships. What our data do highlight is that BWE does not prevent the introduction of NIS. Such organisms that would be most likely to survive transport and BWE should include those that are tolerant of oceanic salinities and are likely sourced from marine ports. One ship sampled in this study initially took on ballast water from the port of Esbjerg, Denmark (coastal salinity), performed BWE mid-voyage, and we recovered from the ships' ballast water seven *C. maenas* megalopae, a widespread coastal invasive species (Carlton and Cohen 2003). Briski *et al.* (2012) also highlighted the potential for BWE to promote survivorship, also finding several *C. maenas* individuals (adults) in ballast water that had recently been exchanged.

Further promoting the persistence of taxa otherwise tolerant to tank conditions, decreasing sea surface temperatures encountered by ships on the northward voyage would cool ballast water and slow declines in dissolved oxygen that are otherwise more rapid under warmer conditions (Reid *et al.* 2007, Briski *et al.* 2012). The reduction of this stressor, in combination with the processes outlined above which may promote NIS survival during transport, suggests the requirement of BWE for ships travelling to Svalbard and other Arctic destinations requires further evaluation. Specifically, the impacts of stress-reducing processes on NIS survival, and the interactions among processes, needs to be characterised for given voyage durations. Voyage duration clearly has an important impact on survivorship, but until this trend is quantified at regional scales, it remains uncertain whether voyage length and BWE may promote or limit zooplankton survival.

Implications of the quasi-effectiveness of BWE are also of relevance in the context of trans-Arctic shipping. Increasingly, trans-Arctic shipping routes (i.e. the Northern Sea Routes and the North East Passage) are becoming viable alternatives to established Asian-European routes via either the Suez or Panama canals (Stephenson *et al.* 2011; Miller and Ruiz 2014). The associated potential for the introduction of largely novel species assemblages to Asian or European ports with this change in shipping pattern has been recently highlighted (Miller and Ruiz 2014). Trans-Arctic shipping routes are longer than those considered in this study, but, as for the case for BWE on board ships travelling to Svalbard discussed previously, risk of NIS survivorship in ballast water tanks during trans-Arctic passages may be modulated by the same processes.

Interpreting habitat suitability

In estimating habitat suitability under forecast ocean climates we provide the first projection of marine NIS establishment potential in the Arctic linked to quantitative measures of propagule pressure. Changing conditions, driven largely by temperature increases, are likely to permit the successful recruitment in Svalbard of six of the eight NIS studied by the end of the century. Our projections also extended to other Arctic waters, and while not coupled to measures of propagule pressure in these regions, clearly demonstrate Arctic-wide increases in habitat suitability for the NIS considered. Collectively, the invasive species for which habitat suitability was mapped have caused wide-ranging impacts elsewhere including fouling (Gollasch 2002), parasite introduction (Stentiford *et al.* 2012), reducing indigenous diversity and abundance (Grosholz *et al.* 2000, Bracewell *et al.* 2012), and in the cases of *A. improvisus* and *C. maenas,* causing trophic cascades (Trussel *et al.* 2004, Kotta *et al.* 2006).

Potential habitat suitability for *C. meanas* and *A. improvisus* has been modelled previously (de Rivera *et al.* 2011). For both species, future estimates of habitat suitability were generated using correlative species distribution models based on a wider range of environmental variables than considered here. Modelled

present-day distributions were projected under an analogous oceanic warming scenario, and suitable habitat was similarly forecast to extend northwards for both species (de Rivera *et al*. 2011). Range margins were more conservative than those mapped in this study (ca 5-10° of latitude further south for *C. maenas* and *A. improvisus* respectively), though models were projected only until the year 2080. Nonetheless, suitable habitat was estimated to exist around Svalbard by 2080 for *C. maenas* (de Rivera *et al*. 2011).

Limitations of our habitat suitability analyses require consideration. We assume in our analyses that propagule pressure and climate are the major determinants of colonisation potential (Lockwood et al. 2007), but acknowledge that discounting factors such as competitive biotic interactions, optimal performance thresholds of the study species, and the use of coarse spatial (0.5°) and temporal (monthly) environmental data, may all serve to overestimate habitat suitability (Monahan 2009, Wisz *et al*. 2013, Woodin *et al*. 2013). Monthly sampling of future environmental data, in particular, may bias estimates of habitat suitability for coastal regions such as Svalbard that are influenced greatly by river or glacial outflow. Near-shore fjord environments in Svalbard, typical of port locations, are characterised by a brackish surface layer for much of the summer period, with extents varying daily (Zajączkowski *et al*. 2010). Our analyses were not sensitive to this fine resolution effect, but daily variations in salinity could preclude species survivorship. The importance of this caveat is minimized in the context of this study given the generalist salinity tolerances of species considered.

Furthermore, while minimum temperature and salinity levels for recruitment are critical for population establishment, these do not necessarily relate to optimal performance thresholds (Woodin *et al*. 2013). For example, *C. maenas* may survive in waters around 0° C (Cameron *et al*. 2005) and reproduce successfully in waters around 10° C, yet studies have shown that foraging activity is 15 to 20 times greater in waters at least 5° C warmer still (Aagaard *et al*. 1995). In such cases, biotic interactions can be expected to play a larger role in successful population establishment than climatic tolerances alone. If biotic interactions or other limiting factors presently restrict potential niche filling to optimal performance limits, this should be apparent in maps of fundamental climatic niche filling (Fig. 3). Yet, the distributions of species considered in this study, to a large degree, indicate that they are not limited within their fundamental niches, characteristic of many marine zooplankton (Sunday *et al*. 2012) (see Fig 3). Other techniques for estimating habitat suitability, such as correlational models, may implicitly model biotic processes in addition to a wider range of abiotic processes considered here. However, the accuracy with which such models transfer to different climate states is contingent to a large extent on how well sampled current species distributions are, and as a result how accurately the model is trained. In our case spatially explicit observations of several of the study species were few. The approach we used to estimating habitat suitability is not restricted by limited observational data, and estimates of the fundamental climatic niche over time should be robust as they are based on underlying physiological processes and not distributional data.

Projected climates are based on the RCP 8.5 emissions scenario, which assumes high population growth, relatively slow income growth, and modest rates of technological change and energy intensity improvements. Under this scenario, these processes lead to high energy demand and greenhouse gas emissions in the absence of climate change policies (Moss *et al*. 2010). They therefore constitute more extreme projections of climatic changes, though more suited to the generation of policy protective of the environment (Ware *et al*. 2014). Differences at higher latitudes between estimates based on the RCP 8.5 emission scenario, and those

derived from more conservative emissions scenarios, have, however, been shown to be minimal (Ware *et al*. 2014).

Conclusion

By presenting a comprehensive evaluation of a major vector of marine species discharge and associated risks thereof, our study offers an effective basis for developing more informed measures to limit marine species invasion in Arctic waters. It highlights that NIS are routinely introduced into Svalbard waters through ballast water discharge, and that the requirement of BWE warrants critical evaluation for ships travelling to the Arctic given the possibility that it promotes in-transit survival of NIS. The risk of a number of known invasive species establishing in Arctic waters is low presently but will increase rapidly over the coming decades. Our assessment thus grants environmental managers a rare buffer of time in which to implement preventative measures proactively. Importantly, planned international requirements for ships to treat ballast water should be implemented without delay. Bulk carrying ships travel from the same geographic port regions as did ships in this study to other Arctic destinations, and therefore similar species assemblages are likely transferred more widely across the Arctic. Our assessment thus provides proxies of risk posed at other Arctic ports, and offers guidance for early detection surveys at more southerly Arctic latitudes. Therefore, our risk assessment provides the understanding to guide more protective management of marine environments in a warming Arctic.

Acknowledgements

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Chapter 8: Thesis synthesis

In this research, I have evaluated current and future invasive species threats posed to the high-Arctic archipelago, Svalbard. In the following synthesis I address the specific aims outlined in the introductory chapter and summarise how each of these has been met. I then consider how this research as a whole has contributed to the field of invasion ecology, and highlight knowledge gaps for this region deserving future work.

Aim 1. Quantify the non-indigenous plant propagule load transferred to Svalbard and test germination rates under set conditions

Using visitors' footwear as an exemplar pathway of species introduction, Chapter 1 demonstrated that a large vascular plant seed load is transferred to Svalbard yearly. An estimated 270,000 seeds were transported to Svalbard in the year of this study, with nearly 90 % of these estimated to be non-indigenous to Svalbard. The sampling process did not favour any visitation type and a wide spread of visitor categories were included in the pool of samples (259 in total from five visitor categories); thus, the results are representative of the wider population of Svalbard visitors, and likely also of visitors to other Arctic regions. Among the seeds identified from samples, eight of the most invasive families of plants worldwide were represented. Just over a quarter of the seed load collected from footwear germinated at 10° C, a temperature which is representative of ambient Svalbard summer soil surface temperatures. Temperature increases over the remainder of this century for Svalbard are forecast to be greater than 2° C which will promote the germination potential for a greater range of plant taxa.

Management implications of these results were discussed in terms of the need to develop a more precautionary approach to non-indigenous species (NIS) introduction. Means to limit the introduction of NIS via people's footwear, clothing, and personal equipment have been developed elsewhere (e.g. Antarctica, Australia), and measures are often simple. For example, educating people about the need to undertake basic biosecurity measures has been shown to be effective, and such a campaign could be implemented in Svalbard. Results of this study demonstrated that footwear with soil attached routinely harboured seeds, as did footwear that had been used in forested or mountainous areas. Such information could be used to increase visitors' awareness and encourage self-assessments of risk. Beyond education, more stringent measures might include adopting biosecurity measures at entry points to Svalbard. Moves to address this need were made subsequently by members of the Association of Arctic Expedition Tour Operators (see Chapter 3). Most importantly, this study indicated a need to address the prevention of NIS to Svalbard through a region-wide framework, a move that is yet to be made.

Aim 2. Evaluate the status of non-indigenous plants present around Svalbard, and investigate factors that may be associated with their persistence

Chapter 2 investigated the status of NIS plants already present around the Svalbard archipelago. All records of vascular plant NIS occurrences for Svalbard were collated (survey years spanning 1893 – 2012) inclusive of survey records made as part of this research. Four hundred and forty-seven records of 105 taxa have been recorded in Svalbard over the 130 years of survey effort at 27 sites. Recent surveys at 18 of these sites revealed that NIS had disappeared at half of the sites, suggesting that NIS turnover was reasonably high with

reference to the total number of NIS. Surveys at a further 25 sites characterised by former settlements and/or current high visitation rates, but where no older records of flora were available, revealed no NIS.

Temporal and thermal correlates of phenological stage and record numbers were assessed using generalised linear models with mixed effects (GLMM), and generalised linear models (GLM). There was a weak association between the number of plants recorded in fertile phenological stages and increasing years of survey, while there was no relationship between the number of NIS records and years of survey. There was, however, a strong temperature signal in the number of fertile phenological stages recorded. Higher numbers of fertile NIS records were significantly associated with positive deviations from mean July temperatures (those at the start of the summer growing season). As discussed above, warming is forecast to continue for Svalbard over the remainder of this century. These results therefore indicate that there is a greater capacity for NIS spread in Svalbard given their increasing reproductive potential.

Aim 3. Evaluate the efficacy of disinfection as a tool to limit the introduction of microorganisms to Svalbard

Biosecurity measures are commonly used to prevent the introduction of non-indigenous species to natural environments globally, yet the efficacy of practices is rarely monitored. In 2012, Chapter 3 evaluated the efficacy of the Association for Arctic Expedition Cruise Operators (AECO) initiated biosecurity trial. Trialled measures included the disinfection of footwear prior to, and in between, shore excursions around Svalbard to limit the transfer of microorganisms. The efficacy of disinfection as assessed aboard one ship was found to be of limited effect. Soil remained attached to footwear following the procedure and the microbial burden was not removed.

This study did not provide new information on efficacious methods to perform disinfection, but instead tested disinfection performances under operation conditions. The practices of the study ship were representative of a number of ships operating around Svalbard, but not all. Our evaluation demonstrated that when disinfectant is not given time to dry, as was the case aboard the study ship, the microbial burden is not killed. In contrast, those ships that ensure disinfectant is given time to dry prior to or in between landings likely achieve improved disinfection rates as evidenced in our evaluation. This conclusion is further corroborated by research undertaken elsewhere. Disinfection is a simple and rapid measure easily employable aboard expedition or cruise ships. By drawing together the existing body of knowledge on footwear disinfection, and evaluating onboard performance of practices, both deficiencies and opportunities for improvement were identified. As footwear disinfection is widely practiced in ecotourism settings, this study serves as a call to better align operational biosecurity practices with the tested theory underpinning them.

Aim 4. Undertake a shipping pathway analysis to evaluate the potential for known invasive NIS to be transferred to, and survive in, Svalbard, currently and in the future

Ships as species introduction vectors are inherently challenging to sample, and as a result research addressing the potential risk posed by shipping vectors routinely uses proximal measures of propagule load. Chapter 4 described such a study whereby the Svalbard shipping network is reduced into proximal components that are known to increase or decrease the risk of a particular ship mediating NIS transfer and successful introduction.

Environmental match between a ship's port of origin and Svalbard was used as a proxy for habitat suitability, and therefore as a measure of the potential for a NIS to establish if transported. The environmental match of global ports connected to Svalbard through the 2011 shipping network was evaluated under present and future environmental conditions (2050 and 2100 predicted under the RCP8.5 emissions scenario). Match was based on sea surface salinity and temperature and was measured as the Euclidean distance. Risk of NIS introduction was then estimated based on the potential for known NIS to be transported (in ballast water or as biofouling), environmental match, and a qualitative estimate of propagule pressure. The latter measure overcomes general criticism of using shipping numbers, ship size, or ballast water volume as crude proxies for propagule pressure. Such measures have been shown to correlate poorly with propagule pressure as they are not sensitive to the many factors that increase or decrease the likelihood of successful NIS transfer. Factors including the age of antifouling paint, whether ballast water exchange was performed, vessel speed, and port layover times, were therefore incorporated into the qualitative model and used to downwardly constrain measures of risk where factors were known to inhibit species transport.

This study showed that Svalbard will become increasingly vulnerable to marine NIS introduction and establishment. Over the coming century, sea surface warming at high latitudes was estimated to increase the level of environmental match to nearly one-third of ports previously visited by vessels travelling to Svalbard in 2011 (n = 136). The shipping network would then likely connect Svalbard to a much greater pool of known NIS, under conditions more favourable for their establishment. Research and fishing vessels were estimated to pose the highest risk of NIS introduction through biofouling, while ballast water discharge was estimated to pose an increased risk by the end of the century.

The management of biofouling has only recently been addressed internationally, with the adoption of guidelines by the International Maritime Organisation. Domestic legislation giving effect to these has not yet been enacted for Svalbard. In contrast, the Norwegian Ballast Water Regulation requires all ships travelling to Svalbard from extra-territorial waters and intending to discharge ballast water to first exchange or treat ballast as a means to limit the introduction of NIS. Only 62 % of vessels from which data was collected as part of this research conducted any form of ballast water management, namely ballast water exchange. The extent to which these management practices reduce introduction risk is untested for ships transiting Arctic waters, and warrants evaluating.

Aim 5. Test the efficacy of molecular tools to assist in the identification of marine zooplankton sampled from ships' ballast water

The research reported in Chapter 4 was augmented by sampling the ballast water tanks of ships discharging ballast in Svalbard waters to determine the abundance and composition of zooplankton transported in managed and unmanaged ballast water. Traditional means to identify ballast water zooplankton are unsuited to the identification of the typically abundant meroplanktonic larval forms present in ballast water samples. In order to improve taxonomic resolution and thus generate better measures of risk, universal primers in a DNA barcoding approach were trialled to identify Crustacean and Molluscan larval forms. The performance of universal primers was inconsistent across taxonomic groups, with only a few generalisations emerging. Amplification of CO1 sequences using four primer combinations achieved poor-to-moderate success (12-49 %, n=120 PCR trials combined), while primers amplifying 12S rDNA and 16S rDNA proved more successful overall (40 % and 69 %, respectively, n=84 PCR trials combined). Considering all markers together, species

identifications were made for 38 % of study organisms, and genus identification was possible for 55 % of organisms (n=112). While substantially improving successful species identification rates of ballast water larval forms over those attainable relying on microscopy alone, our results suggest this approach to genetic sequence-based identification is inefficient in a biosecurity context. Sorting samples into individual higher taxonomic units prior to PCR is time-consuming, while inefficient priming reduced subsequent sequence generation. Next generation sequencing (NGS) and metabarcoding approaches may overcome these limitations by enabling the use of multiple primer pairs on pooled samples, providing the potential to mass-amplify DNA barcodes, and potentially eliminating the need for an amplification step. The development of these approaches is therefore recommended for future work.

Aim 6. Evaluate the efficacy of ballast water exchange to limit the introduction of NIS to Svalbard, and estimate habitat suitability for non-indigenous zooplankton introduced to Svalbard in ships' ballast based on eco-physiological tolerances

Chapter 6 demonstrated qualitatively that ballast water exchanged (BWE) was an inefficient means of preventing NIS introduction to Svalbard. While it may serve to limit NIS introduction, sampling ballast water tanks indicated that high numbers of known coastal invasive species survived both the voyage to Svalbard and BWE. Furthermore, BWE increased abundances of zooplankton discharged in Svalbard. Twenty- three NIS were present in discharged ballast water across all samples (exchanged and unexchanged), the majority of which were most likely sourced from ports ships had travelled from. NIS were present in all but one sample, though numbers were low (2.7 ± 0.4 SE individuals per sample).

Abundances of organisms discharged by ships in Svalbard were similar to or higher than those reported elsewhere. It is likely that the comparatively shorter voyage durations, decreasing sea surface temperatures, and act of BWE, promote zooplankton survivorship within the ballast water tanks of ships travelling from European ports to Svalbard.

Availability of eco-physiological data permitted habitat suitability modelling for eight of the recorded 23 NIS (Copepoda – *Acartia tonsa*, *Eurytemora affinis*; Decapoda – *Carcinus maenas*, *Hemigrapsus takanoi*, *Crangon crangon*; Cladocera – *Podon leuckartii*; Balanidae – *Amphibalanus improvises*, *Austrominius modestus*). All species' reproductive thresholds, based on the number of days required at minimum temperature and salinity levels, were estimated to permit northward expansion, with only *C. crangon* and *H. penicillatus*' thresholds not overlapping with forecast Svalbard environmental conditions by the end of the century. Increases in habitat suitability are driven primarily by temperature increases estimated over the coming decades (+ 8° C by 2100).

Habitat suitability estimates based solely on critical reproductive thresholds likely overestimate potential ranges given they do not account for factors such as biotic interactions or additional abiotic stressors that may limit species' ranges. Similarly, optimal performance thresholds for species may be higher than those required for population maintenance, and so invasion potential may be limited at range margins. In spite of these caveats, habitat suitability estimates based on critical eco-physiological thresholds provide an informative framework for understanding how species might respond to changing climate gradients. Temporal and spatial transferability of habitat suitability projections based on alternative methods (such as correlative species distribution modelling techniques) are typically based on a greater number of assumptions.

Chapter 6 therefore provided a risk assessment for the establishment potential of marine NIS transported in ballast water to Svalbard based on measures of propagule pressure. The effect of BWE is limited in reducing NIS numbers, and its suitability to ships travelling shorter distances across Arctic waters requires empirical testing. Habitat suitability for introduced NIS is presently low, but will increase rapidly under forecast conditions. Taken together, high propagule pressure posed by ballast water discharge in Svalbard measured in this study, and an increasing potential for NIS establishment forecast across the Arctic, suggest an increasing Arctic-wide vulnerability to species invasion.

Perspectives

Across the Arctic, biosecurity has a low profile in comparison to other regions. Few studies have investigated propagule transport to Arctic locations, or empirically estimated changing vulnerability to species establishment under forecast climates. Here, the primary pathways of species introduction, human visitation and shipping, were examined by quantifying transferred propagule loads, evaluating transport network risk profiles, and assessing habitat suitability and viability under forecast future climatic conditions. Existing measures to limit the introduction of species were tested to determine their efficacy. This research therefore addresses important knowledge gaps in our understanding of the nature and extent of invasive species risks in Svalbard, and by inference the wider Arctic. Importantly, a number of areas requiring further research have been identified through the course of this work. These include:

1. the effectiveness of educating visitors to Svalbard in the need to undertake basic biosecurity measures prior to their travel;
2. the potential for cargo and food imports to Svalbard to mediate species introduction;
3. quantification of the propagule load transported to Svalbard as biofouling;
4. measures of biofouling survivorship on all vessel types while in transit to Svalbard; and
5. quantification of the performance of BWE in eliminating NIS.

Shortcomings of biosecurity measures employed in Svalbard presently – or the absence of any preventative management measures – highlight a need to address a number of policy and management gaps. Primarily, measures to prevent species introduction are piecemeal across vectors and pathways. For example, few biosecurity measures exist to prevent plant propagule introduction, while ballast water but not biofouling requires explicit management. Moreover, this research demonstrated that current management efforts may not target those vectors and pathways posing the greater immediate risk.

Moves to improve biosecurity management in Svalbard are supported by the Svalbard Environmental Protection Act which explicitly prevents the introduction of NIS, and efforts to address shortcomings are in development (Governor of Svalbard, pers. comm.). Improvements should ideally constitute a holistic approach encompassing all vectors and pathways, a framework for which can be based on the results presented in this thesis.

Appendix 1

Table 1 List of all alien vascular plant species records collected on Svalbard over the past 130 years. Source references are given in Chapter 2. Phenology refers to the phenological stage plants were observed in

Taxon	Year	Phenology
Achillea millefolium L.	1897	Vegetative
Achillea millefolium L.	1928	Vegetative
Achillea millefolium L.	1936	In bud
Achillea millefolium L.	1939	-
Achillea millefolium L.	1939	In bud
Achillea millefolium L.	1960	Vegetative
Achillea millefolium L.	1961	Vegetative
Achillea millefolium L.	1988	In bud
Achillea millefolium L.	1993	Flowering
Achillea millefolium L.	1993	Flowering
Achillea millefolium L.	1996	Flowering
Achillea millefolium L.	1998	In bud
Achillea millefolium L.	2006	Vegetative
Achillea millefolium L.	2007	In bud
Achillea millefolium L.	2008	In bud
Achillea millefolium L.	2008	In bud
Achillea millefolium L.	2009	-
Achillea millefolium L.	2011	Flowering
Achillea millefolium L.	2011	In bud
Achillea millefolium L.	2013	Vegetative
Achillea ptarmica L.	1939	Vegetative
Agrostemma githago L.	1897	In bud
Agrostis capillaris L.	1983	Vegetative
Alchemilla subcrenata Buser	1988	Vegetative
Alchemilla subcrenata Buser	1993	Vegetative
Alchemilla subcrenata Buser	2007	Flowering
Alchemilla subcrenata Buser	2011	Flowering
Alchemilla subcrenata Buser (*A. filicaulis*)	1957	Flowering
Alchemilla wichurae (Buser) Stefánsson	1939	Vegetative
Alchemilla sp.	1996	Vegetative
Allium cepa L.	2008	Vegetative
Alopecurus geniculatus L.	2013	Flowering
Alopecurus myosuroides Huds.	1939	Flowering
Alopecurus pratensis L.	1939	Flowering
Alopecurus pratensis L.	1988	Flowering
Alopecurus pratensis L.	2011	In bud
Anthriscus sylvestris (L.) Hoffm.	2007	Flowering
Anthriscus sylvestris (L.) Hoffm.	2007	Flowering
Anthriscus sylvestris (L.) Hoffm.	2008	Flowering
Anthriscus sylvestris (L.) Hoffm.	2011	Fruiting
Artemisia absinthium L.	1988	Vegetative
Atriplex hortensis L.	1992	Vegetative
Atriplex sagittata Borkh.	1988	Vegetative
Avena sativa L.	1928	In bud
Avena sativa L.	1928	Vegetative
Avena sativa L.	1939	-
Barbarea stricta Andrz.	2011	Vegetative
Barbarea vulgaris W.T.Aiton	1935	Flowering
Barbarea vulgaris W.T.Aiton	1936	Flowering
Barbarea vulgaris W.T.Aiton	1960	Vegetative
Barbarea vulgaris W.T.Aiton	1961	Fruiting
Barbarea vulgaris W.T.Aiton	1988	Flowering
Barbarea vulgaris W.T.Aiton	1988	Fruiting
Barbarea vulgaris W.T.Aiton	1990	Flowering

Barbarea vulgaris W.T.Aiton	1991	Flowering
Barbarea vulgaris W.T.Aiton	1993	Fruiting
Barbarea vulgaris W.T.Aiton	1996	Fruiting
Barbarea vulgaris W.T.Aiton	1998	Flowering
Barbarea vulgaris W.T.Aiton	2007	Flowering
Barbarea vulgaris W.T.Aiton	2008	Fruiting
Barbarea vulgaris W.T.Aiton	2008	Fruiting
Barbarea vulgaris W.T.Aiton	2011	Flowering
Brassica sp.	1988	In bud
Buglossoides arvensis (L.) I.M.Johnst. (*Lithospermum arvense* L.)	1897	Flowering
Capsella bursa-pastoris (L.) Medik.	1920	Vegetative
Capsella bursa-pastoris (L.) Medik.	1921	Flowering
Capsella bursa-pastoris (L.) Medik.	1958	Fruiting
Capsella bursa-pastoris (L.) Medik.	1965	Fruiting
Capsella bursa-pastoris (L.) Medik.	1988	-
Capsella bursa-pastoris (L.) Medik.	1993	Fruiting
Carum carvi L.	1939	Vegetative
Cerastium fontanum subsp. *vulgare* (Hartm.) Greuter & Burdet	2011	-
Cerastium fontanum subsp. *vulgare* (Hartm.) Greuter & Burdet (*Cerastium vulgatum* L.)	1897	Vegetative
Cerastium fontanum subsp. *vulgare* (Hartm.) Greuter & Burdet (*Cerastium vulgatum* L.)	1939	Flowering
Chenopodium album L. s. str.	1928	Vegetative
Chenopodium album L. s. str.	1928	Vegetative
Chenopodium album L. s. str.	1928	Vegetative
Chenopodium album L. s. str.	1928	Vegetative
Chenopodium album L. s. lat.	1897	Vegetative
Chenopodium album L. s. lat.	1939	-
Chenopodium album L. s. lat.	1960	Vegetative
Chenopodium album L. s. lat.	1988	Vegetative
Chenopodium album L. s. lat.	1993	Vegetative
Chenopodium album L. s. lat.	1998	Vegetative
Chenopodium album L. s. lat.	2000	Vegetative
Chenopodium album L. s. lat.	2011	Vegetative
Chenopodium album L. s. lat. (*Chenopodium sp.*)	1898	Vegetative
Conringia orientalis (L.) Dumort.	1928	In flower
Dactylis glomerata L.	2000	Vegetative
Deschampsia cespitosa (L.) Beauv.	1923	Flowering
Deschampsia cespitosa (L.) Beauv.	1928	Flowering
Deschampsia cespitosa (L.) Beauv.	1939	Flowering
Deschampsia cespitosa (L.) Beauv.	1939	Flowering
Deschampsia cespitosa (L.) Beauv.	1939	-
Deschampsia cespitosa (L.) Beauv.	1939	Flowering
Deschampsia cespitosa (L.) Beauv.	1939	Flowering
Deschampsia cespitosa (L.) Beauv.	1957	Flowering
Deschampsia cespitosa (L.) Beauv.	1957	Flowering
Deschampsia cespitosa (L.) Beauv.	1957	Flowering
Deschampsia cespitosa (L.) Beauv.	1958	Flowering
Deschampsia cespitosa (L.) Beauv.	1964	-
Deschampsia cespitosa (L.) Beauv.	1967	Vegetative
Deschampsia cespitosa (L.) Beauv.	1983	In bud
Deschampsia cespitosa (L.) Beauv.	1984	-
Deschampsia cespitosa (L.) Beauv.	1988	Flowering
Deschampsia cespitosa (L.) Beauv.	1993	Flowering
Deschampsia cespitosa (L.) Beauv.	2006	-
Deschampsia cespitosa (L.) Beauv.	2007	In bud
Deschampsia cespitosa (L.) Beauv.	2008	In bud
Deschampsia cespitosa (L.) Beauv.	2011	Flowering
Deschampsia cespitosa (L.) Beauv.	2011	Flowering
Descurainia sophia (L.) Webb	1988	In bud
Descurainia sophia (L.) Webb	1992	In bud
Descurainia sophia (L.) Webb	1992	Vegetative
Elytrigia repens (L.) Gould	2008	In bud
Elytrigia repens (L.) Gould	2011	In bud
Erodium cicutarium (L.) L'Hér. ex. Aiton	1988	Vegetative

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Erysimum cheiranthoides L.	1928	In bud
Erysimum cheiranthoides L.	1988	Vegetative
Erysimum strictum P. Gaertn., B.Mey. & Scherb. (*Erysimum hieraciifolium* L.)	1897	Flowering
Fagopyrum esculentum Moench (*Polygonum fagopyrum* Moench)	1897	Vegetative
Fallopia convolvulus (L.) Á.Löve (*Polygonum convolvulus* L.)	1928	Vegetative
Fallopia convolvulus L.	1988	Vegetative
Fallopia convolvulus L.	1993	Vegetative
Festuca rubra L. cf. ssp. *megastachys* Gaudin	2008	In bud
Festuca rubra L. cf. ssp. *rubra*	1965	Flowering
Festuca rubra L. ssp. *rubra*	1992	Flowering
Festuca rubra L. ssp. *rubra*	1993	Flowering
Festuca rubra L. ssp. *rubra*	2006	-
Festuca rubra L. ssp. *rubra*	2007	Flowering
Festuca rubra L. ssp. *rubra*	2008	Flowering
Festuca rubra L. ssp. *rubra*	2009	Flowering
Festuca rubra L. ssp. *rubra*	2010	Flowering
Festuca rubra L. ssp. *rubra*	2011	Flowering
Festuca rubra L. ssp. *rubra*	2011	Flowering
Festuca rubra L. ssp. *rubra*	2011	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1928	Fruiting
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1928	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1936	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1937	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1939	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1939	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1939	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1939	Fruiting
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1939	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1949	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1957	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1960	Flowering
Festuca rubra L. ssp. *rubra* (*Festuca rubra*)	1960	Flowering
Galeopsis tetrahit L.	1897	Vegetative
Galeopsis tetrahit L.	1920	Vegetative
Galeopsis tetrahit L.	1939	-
Galeopsis tetrahit L.	1958	Vegetative
Galeopsis tetrahit L.	1960	Vegetative
Galeopsis tetrahit L.	1988	Vegetative
Galeopsis tetrahit L.	1993	Vegetative
Galium aparine L.	1897	Flowering
Galium aparine L.	1988	Vegetative
Galium mollugo L. ssp. *erectum* Syme	2008	Vegetative
Galium mollugo L. ssp. *erectum* Syme	2011	In bud
Helianthus annuus L.	1988	Vegetative
Hieracium vulgatum agg.	1939	-
Hordeum vulgare L.	1897	Vegetative
Hordeum vulgare L.	1988	Vegetative
Lappula squarrosa (Retz.) Dumort.	1928	In bud
Lappula squarrosa (Retz.) Dumort.	1988	Flowering
Lappula squarrosa (Retz.) Dumort.	1993	Flowering
Lapsana communis L.	1939	-
Lathyrus pratensis L.	2011	Flowering
Lepidium densiflorum Schrad.	1988	Vegetative
Lepidotheca suaveolens (Pursh) Nutt.	1965	In bud
Lepidotheca suaveolens (Pursh) Nutt. (*Matricaria discoidea* DC.)	1988	Flowering
Malus × domestica Mill.	1988	Vegetative
Malus × domestica Mill.	1993	Vegetative
Medicago lupulina L.	1988	Vegetative
Medicago polymorpha L. (*Medicago hispida* Gaertn.)	1928	Vegetative
Melilotus officinalis (L.) Lam.	1928	Vegetative
Myosotis arvensis (L.) Hill	1883	Vegetative
Myosotis arvensis (L.) Hill	1939	-
Myosotis arvensis (L.) Hill	1939	-

Myosotis arvensis (L.) Hill		1939	-
Myosotis arvensis (L.) Hill		1958	Vegetative
Myosotis arvensis (L.) Hill		1960	In bud
Phleum pratense L.		1928	Vegetative
Phleum pratense L.		1928	In bud
Phleum pratense L.		1993	In bud
Phleum pratense L.		2008	In bud
Pisum sativum L.		1895	Vegetative
Pisum sativum L.		1928	Vegetative
Pisum sativum L.		1928	Vegetative
Pisum sativum L.		1939	-
Plantago major L.		1928	Vegetative
Plantago major L.		1988	Vegetative
Plantago media L.		1988	Flowering
Plantago media L.		2011	Flowering
Poa alpina L. var. *alpina*		1939	Flowering
Poa alpina L. var. *alpina*		1939	Flowering
Poa alpina L. var. *alpina*		1957	Flowering
Poa alpina L. var. *alpina*		1983	Flowering
Poa annua L.		1958	Flowering
Poa annua L.		1988	Fruiting
Poa annua L.		1993	Flowering
Poa annua L.		2013	Flowering
Poa cf. *annua* L.		2008	Vegetative
Poa palustris L.		1993	Flowering
Poa palustris L.		2008	In bud
Poa pratensis L. ssp. *pratensis*		2008	In bud
Poa pratensis L. ssp. *pratensis*		2008	Flowering
Poa pratensis L. ssp. *pratensis*		2011	Flowering
Poa pratensis L. ssp. *pratensis*		2013	In bud
Poa pratensis L. ssp. *pratensis*		2013	In bud
Poa pratensis L. ssp. *pratensis*		2013	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* "cultura" (Lindm.) Hiitonen)		1915	-
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1928	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1928	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1928	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1928	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1928	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1931	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1932	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1932	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1936	In bud
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1957	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1957	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1958	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1958	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1958	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1967	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1975	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1983	Fruiting
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm)		1984	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	-
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	-
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	-
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	-
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	-
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	-
Poa pratensis L. ssp. *pratensis* (*Poa alpigena* (Fr.) Lindm. ssp. *domestica* (Laest.) Hadač)		1939	-
Poa pratensis L. ssp. *pratensis* (*Poa pratensis* L.)		1965	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa pratensis* L.)		1965	Flowering
Poa pratensis L. ssp. *pratensis* (*Poa pratensis* L.)		1975	Flowering
Poa pratensis L. ssp. *irrigata* (Lindm.) H. Lindb.		2009	Flowering

Poa pratensis L. ssp. *irrigata* (Lindm.) H. Lindb.	2009	Flowering
Poa pratensis L. ssp. *irrigata* (Lindm.) H. Lindb.	2009	Flowering
Poa pratensis L. ssp. *angustifolia* (L.) Gaudin	2011	Flowering
Poa trivialis L.	1939	Flowering
Poa trivialis L.	1988	Fruiting
Poa trivialis L.	1992	In bud
Polygonum aviculare L. ssp. *boreale* (Lange) Karlsson	1928	Vegetative
Polygonum aviculare L. ssp. *boreale* (Lange) Karlsson	1939	Vegetative
Polygonum aviculare L. ssp. *boreale* (Lange) Karlsson	1958	Vegetative
Polygonum aviculare L. ssp. *boreale* (Lange) Karlsson	1960	In bud
Polygonum aviculare L. ssp. *boreale* (Lange) Karlsson	1993	In bud
Polygonum aviculare L. ssp. *boreale* (Lange) Karlsson (*Persicaria sp.*)	2000	Vegetative
Polygonum aviculare L. s. lat.	1897	Vegetative
Polygonum aviculare L. s. lat.	1920	Vegetative
Polygonum aviculare L. s. lat.	1939	-
Polygonum aviculare L. s. lat.	1988	Vegetative
Polygonum aviculare L. s. lat.	2011	-
Prunus domestica L.	2006	Vegetative
Prunus domestica L.	2006	Vegetative
Prunus domestica L.	2009	Vegetative
Ranunculus acris L. ssp. *acris*	1939	Flowering
Ranunculus acris L. ssp. *acris*	2008	Flowering
Ranunculus acris L. ssp. *acris*	2011	Fruiting
Ranunculus acris L. ssp. *friesianus* (Jord.) Syme	1988	Flowering
Ranunculus acris L. s. lat.	1939	-
Ranunculus acris L. s. lat.	1993	Flowering
Ranunculus acris L. s. lat.	1996	Vegetative
Ranunculus acris L. s. lat.	2007	Flowering
Ranunculus acris L. s. lat.	2011	-
Ranunculus auricomus L. coll	1988	-
Ranunculus repens L.	1939	Vegetative
Ranunculus repens L.	1988	Vegetative
Ranunculus repens L.	1993	Vegetative
Ranunculus repens L.	2007	-
Ranunculus repens L.	2008	Flowering
Ranunculus repens L.	2011	In bud
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1928	Vegetative
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1928	Vegetative
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1928	Vegetative
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1936	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1939	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1957	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1957	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1957	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1958	In bud
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1958	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1958	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1960	Flowering
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1964	Vegetative
Ranunculus subborealis Tzvelev ssp. *villosus* (Drabble) Elven	1967	Vegetative
Ranunculus sp.	1898	Vegetative
Raphanus raphanistrum L. ssp. *raphanistrum*	1928	In bud
Raphanus raphanistrum L. ssp. *raphanistrum*	1928	Vegetative
Raphanus raphanistrum L. ssp. *raphanistrum*	1928	In bud
Raphanus raphanistrum L. ssp. *raphanistrum*	1988	In bud
Rorippa palustris (L.) Besser	1988	Vegetative
Rorippa palustris (L.) Besser	1993	Fruiting
Rorippa sylvestris (L.) Besser	1988	Vegetative
Rumex acetosa L. ssp. *acetosa*	1897	Vegetative
Rumex acetosa L. ssp. *acetosa*	1898	Vegetative
Rumex acetosa L. ssp. *acetosa*	1928	Vegetative
Rumex acetosa L. ssp. *acetosa*	1939	-
Rumex acetosa L. ssp. *acetosa*	1939	-

Rumex acetosa L. ssp. *acetosa*	1957	Flowering
Rumex acetosa L. ssp. *acetosa*	1957	Flowering
Rumex acetosa L. ssp. *acetosa*	1957	Flowering
Rumex acetosa L. ssp. *acetosa*	1957	Flowering
Rumex acetosa L. ssp. *acetosa*	1958	Vegetative
Rumex acetosa L. ssp. *acetosa*	1958	Vegetative
Rumex acetosa L. ssp. *acetosa*	1964	Flowering
Rumex acetosa L. ssp. *acetosa*	1964	-
Rumex acetosa L. ssp. *acetosa*	1967	Flowering
Rumex acetosa L. ssp. *acetosa*	1983	Vegetative
Rumex acetosa L. ssp. *acetosa*	1984	-
Rumex acetosa L. ssp. *acetosa*	2008	Vegetative
Rumex acetosella L. ssp. *acetosella*	1992	Vegetative
Rumex acetosella L. ssp. *acetosella*	1993	Vegetative
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1883	Vegetative
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1920	Vegetative
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1928	Vegetative
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1928	Vegetative
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1928	Vegetative
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1936	In bud
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1939	In bud
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1939	-
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1960	Vegetative
Rumex acetosella L. ssp. *acetosella* (R. *acetosella* L.)	1988	Vegetative
Rumex cf. *crispus* L.	2011	In bud
Rumex longifolius DC.	1988	Vegetative
Rumex longifolius DC.	1993	Vegetative
Rumex longifolius DC.	2007	Vegetative
Rumex longifolius DC.	2008	Vegetative
Rumex longifolius DC.	2011	-
Rumex longifolius DC.	2011	Vegetative
Rumex longifolius DC. (*Rumex domesticus* Hartm.)	1928	Vegetative
Rumex longifolius DC. (*Rumex domesticus* Hartm.)	1928	Vegetative
Rumex longifolius DC. (*Rumex domesticus* Hartm.)	1928	Vegetative
Rumex longifolius DC. (*Rumex domesticus* Hartm.)	1939	-
Rumex longifolius DC. (*Rumex domesticus* Hartm.)	1939	-
Rumex longifolius DC. (*Rumex domesticus* Hartm.)	1958	Vegetative
Rumex longifolius DC. (*Rumex domesticus* Hartm.)	1960	Vegetative
Saussurea alpina (L.) DC	1954	Vegetative
Saussurea alpina (L.) DC	1960	In bud
Saussurea alpina (L.) DC	1960	In bud
Saussurea alpina (L.) DC	2008	Vegetative
Secale cereale L.	1897	In bud
Senecio vulgaris L.	1898	Vegetative
Silene latifolia Poir. ssp. *alba* (Mill.) Greuter & Burdet	1988	Vegetative
Sinapis arvensis L.	1928	Vegetative
Sinapis arvensis L.	1988	Flowering
Sinapis arvensis L.	1993	Flowering
Sisymbrium altissimum L.	1939	-
Sonchus oleraceus L.	1898	Vegetative
Stellaria graminea L.	1988	Vegetative
Stellaria graminea L.	1988	Flowering
Stellaria graminea L.	1993	Vegetative
Stellaria graminea L.	2008	Vegetative
Stellaria media (L.) Vill.	1898	Vegetative
Stellaria media (L.) Vill.	1921	Vegetative
Stellaria media (L.) Vill.	1928	Fruiting
Stellaria media (L.) Vill.	1928	Fruiting
Stellaria media (L.) Vill.	1928	Flowering
Stellaria media (L.) Vill.	1928	Flowering
Stellaria media (L.) Vill.	1928	Flowering
Stellaria media (L.) Vill.	1939	-
Stellaria media (L.) Vill.	1939	Vegetative

Stellaria media (L.) Vill.	1939	-
Stellaria media (L.) Vill.	1939	-
Stellaria media (L.) Vill.	1939	Flowering
Stellaria media (L.) Vill.	1958	Fruiting
Stellaria media (L.) Vill.	1960	Flowering
Stellaria media (L.) Vill.	1961	Vegetative
Stellaria media (L.) Vill.	1964	-
Stellaria media (L.) Vill.	1965	Fruiting
Stellaria media (L.) Vill.	1972	-
Stellaria media (L.) Vill.	1988	Vegetative
Stellaria media (L.) Vill.	1993	Flowering
Stellaria media (L.) Vill.	1993	Fruiting
Stellaria media (L.) Vill.	2011	Flowering
Tanacetum vulgare L.	2011	In bud
Taraxacum sect. *Ruderalia*	1928	-
Taraxacum sect. *Ruderalia*	1928	Vegetative
Taraxacum sect. *Ruderalia*	1939	-
Taraxacum sect. *Ruderalia*	1960	Vegetative
Taraxacum sect. *Ruderalia*	1988	Vegetative
Taraxacum sect. *Ruderalia*	1996	Flowering
Taraxacum sect. *Ruderalia*	2008	Fruiting
Taraxacum sect. *Ruderalia*	2011	Fruiting
Thlaspi arvense L.	1897	Fruiting
Thlaspi arvense L.	1920	Flowering
Thlaspi arvense L.	1921	Fruiting
Thlaspi arvense L.	1928	Fruiting
Thlaspi arvense L.	1928	Fruiting
Thlaspi arvense L.	1928	Flowering
Thlaspi arvense L.	1928	Flowering
Thlaspi arvense L.	1928	Flowering
Thlaspi arvense L.	1930	Fruiting
Thlaspi arvense L.	1988	Fruiting
Thlaspi arvense L.	1993	Flowering
Trifolium hybridum L.	1928	Vegetative
Trifolium hybridum L.	1928	Vegetative
Trifolium hybridum L.	1939	-
Trifolium pratense L.	1928	Vegetative
Trifolium pratense L.	1928	Vegetative
Trifolium pratense L.	1928	Vegetative
Trifolium pratense L.	1939	-
Trifolium pratense L.	1960	Vegetative
Trifolium pratense L.	1961	Vegetative
Trifolium pratense L.	2011	Flowering
Trifolium repens L.	1988	-
Trifolium repens L.	1993	Vegetative
Trifolium repens L.	1998	Flowering
Trifolium repens L.	2011	Flowering
Trifolium repens L.	2011	Vegetative
Tripleurospermum inodorum (L.) Sch.Bip.	1998	Vegetative
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *phaeocephalum* (Rupr.) Hämet-Ahti	1924	Flowering
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *phaeocephalum* (Rupr.) Hämet-Ahti	1958	Flowering
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *phaeocephalum* (Rupr.) Hämet-Ahti	1965	Flowering
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *subpolare* (Pobed.) Hämet-Ahti	1928	Flowering
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *subpolare* (Pobed.) Hämet-Ahti	1928	Flowering
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *subpolare* (Pobed.) Hämet-Ahti	1936	In bud
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *subpolare* (Pobed.) Hämet-Ahti	1958	Flowering
Tripleurospermum maritimum (L.) W.D.J.Koch ssp. *subpolare* (Pobed.) Hämet-Ahti	1960	Flowering
Tripleurospermum sp.	1993	Vegetative
Tripleurospermum sp.	2000	Vegetative
Tripleurospermum sp.	2011	Vegetative
Tripleurospermum sp. (*Matricaria inodora* L.)	1920	Vegetative
Tripleurospermum sp. (*Matricaria inodora* L.)	1926	Vegetative
Tripleurospermum sp. (*Matricaria inodora* L.)	1928	-

Tripleurospermum sp. (*Matricaria inodora* L.)	1928	Flowering
Tripleurospermum sp. (*Matricaria inodora* L.)	1930	Flowering
Tripleurospermum sp. (*Matricaria inodora* L.)	1939	-
Tripleurospermum sp. (*Matricaria inodora* L.)	1939	-
Tripleurospermum sp. (*Matricaria inodora* L.)	1958	Vegetative
Tripleurospermum sp. (*Matricaria maritima* L.)	1961	Flowering
Tripleurospermum sp. (*Tripleurospermum inodorum* (L.) Sch.Bip.)	1988	Flowering
Tripleurospermum sp. (*Tripleurospermum inodorum* (L.) Sch.Bip.)	1988	Vegetative
Tussilago farfara L.	1988	Vegetative
Urtica dioica L.	1960	Vegetative
Urtica dioica L.	2011	Flowering
Veronica longifolia L.	2008	-
Veronica longifolia L.	2011	In bud
Vicia sativa L. s. lat.	1988	Vegetative
Vicia sativa L. s. lat. (*Vicia angustifolia* L.)	1897	Flowering

Appendix 2

2.1 Environmental data

For future conditions, we consider a single projection performed using the EC-Earth climate model participating in CMIP5. The simulation starts from a spin-up climate quasi equilibrium state followed by historical simulations from 1850 up to 2006. Following this, the model was forced by the RCP8.5 scenario (RCP stands for representative concentration pathway, the number 8.5 represents the net Top Of Atmosphere imbalance at the year 2100 in W/m2, *sensu* Moss et al. 2010) over the period 2006-2100. The version (V2.2) of EC-Earth is a fully coupled Atmosphere Ocean General Circulation Model (AOGCM), with oceanic (Nucleus for European Modelling of the Ocean, NEMO), sea ice (LIM2) and land surface (HTESSEL) components having been coupled to the IFS atmospheric forecast model through the OASIS3 coupler (Sterl et al. 2012).

The RCP 8.5 scenario chosen for this study corresponds to the pathway with the highest greenhouse gas emissions considered in CMIP5 projections. In EC-Earth, the global average two-meter temperature warming exceeds 4°C by the end of the 21st century, while mean and maximum Svalbard SSTs are projected to increase by 1.8°C and by 2.3°C respectively by 2050 (SST = 4.7°C, and 8.9°C) and by 4.2°C and 5.9°C respectively by 2100 (SST = 8.2°C, and 12.5°C). Predicted salinity changes are small (< ±0.2psu), reflecting a largely unaltered system of ocean exchanges between the north Atlantic and Arctic Ocean. This contrasts the gradual decline of the thermohaline overturning in the mid-latitude Atlantic. The RCP 8.5 scenario assumes high population growth, relatively slow income growth, and modest rates of technological change and energy intensity improvements, that lead in the long term to high energy demand and greenhouse gas emissions in the absence of climate change policies (Riahi et al. 2007). The scenario is most closely akin to the IPCC A2 scenario family (Meehl et al. 2007, Riahi et al. 2007) which represent more extreme estimates of future climate suited to the generation of policy protective of the environment.

From this data set we extracted minimum, maximum and mean annual values for the years 2011, 2050, and 2100 for all ports in the study. Data were extracted for all coastal regions and inland waterways for which data were available. For some of the coastal ports that fell just outside the geographic coverage of the model, data were interpolated from the adjacent grid cells (Therkildsen et al. 2013). This was not possible for inland water-way ports; however, as these ports are typically fresh-water ports (see Keller et al. 2011), environmental match to Svalbard would be low.

To remove errors in calculating Euclidean distances associated with correlated variables, we normalised highly correlated variables. This was done for both minimum and maximum SST and SSS (Pearson's correlation coefficient r reduced from 0.9-0.99 to 0.001-0.003; $P < 0.05$), producing a single variable representing a measure of seasonality for each port. These new variables indicate the deviation in minimum SST and SSS from that expected given the maximum (Floerl et al. 2013; see also Leathwick et al. 2008). The four variables were linearly rescaled to the common data range mean 0 and standard deviation 1 to remove any influence of variable measurement scale. Scaling was based on all data available for global ports so that measurements of environmental distance reflected the full range of similar and dissimilar values (Clarke et al. 2003; Chan et al. 2012). Euclidean distance was then calculated between ports based on the variables seasonal SST, mean annual SST, seasonal SSS, and mean annual SSS for all three time periods (2011, 2050, and 2100). We based calculations of future Euclidean distances on the pattern of movements made by the current shipping network.

We acknowledge that ports visited by vessels may change from year to year, and new connections are possible over coming decades. Accordingly, there is a level of uncertainty in our approach. As the majority of ships visiting Svalbard call at the port of Longyearbyen in Svalbard, and conditions between all four Svalbard ports are similar, we calculated environmental distance only to the port of Longyearbyen extrapolating results to all ports.

Sensitivity analysis was performed using linear regression models of environmental distance against each explanatory variable, and evaluating the difference when calculated without individual parameters. To determine the effect on our data set of calculating environmental match under a different emission scenario, we also obtained data for the year 2050 modelled under the IPCC A1B scenario (IPCC, 2007). This emission scenario entails more moderate assumptions about population growth and technological improvements that serve to reduce expected greenhouse gas emissions compared to that of the high emissions RCP 8.5 scenario. We used Aqua Maps data (Kaschner et al. 2008), which included mean annual values for both salinity and temperature. As only mean annual variables were available, we undertook the comparison using only mean annual values from both data sets.

Our analysis identified two ports that would become environmentally matched to Svalbard by the end of the century (Lisbon and Las Palmas). We consider these to be 'false positives' attributable to the way the environmental distance metric was measured. Specifically, measures of temperature seasonality (the deviation of minimum values from that expected given the maximum) can, be more similar between high- and low-latitudes than high- and mid-latitudes (owing to lower summer-winter temperature changes). As the metric calculates environmental distance of one port relative to another, a low-latitude port such as Las Palmas (with a minimum and maximum SST of 20° C and 24° C respectively) can have a lower environmental distance than a mid-latitude port with a broader (but possibly lower) temperature range. This is a limitation of the way the metric is calculated, and suggests that environmental match from lower latitudes may be artificially high, while those from mid-latitudes may be moderately underestimated. Therefore, consideration of ports and their environmental parameters is necessary when evaluating environmental match.

2.2 Characterising propagule pressure

We assume that the probability of entraining an organism (through ballast water uptake or biofouling – $P_{Entrain}$) in a port is perfect by means of ballast water uptake (a value of 1), and between 0.1 and 1 by means of biofouling depending on time spent in a port (< 1 week = 0.1; 1 week – 1 month = 0.5; > 1 month = 1) (Sylvester and MacIsaac, 2010; Sylvester et al. 2011). The rate at which biofouling accumulates varies widely between ports, with the age of vessel antifouling paint, and between seasons. Macrofouling may settle on a ship hull within a day of arrival in port, but generally does not accumulate densely before one week (*sensu* Floerl et al. 2010). As dense biofouling cover can be expected indicate higher diversity and therefore NIS (Floerl et al. 2005), we set a medium probability to an intermediate time frame, beyond which we expect high densities of biofouling. We further assume that season influences the number of captured organisms and apply a scalar to ships calling at ports outside of the period where many organisms undertake recruitment (S_{Season}, June-September = 1; otherwise = 0.5) (Floerl et al. 2010). The probability of survival of biofouling organisms *en route* is assumed to perfect (i.e. 1) whereas the survival of ballast water organisms ($P_{Survival}$) where voyage duration is < 8 days is 0.5 (otherwise = 0.1). This cut-off was selected based on published data suggesting zooplankton abundances and diversity decrease to a minimum after eight days (Gollasch *et al.*,

2000), and conditions may become inhospitable in ballast water tanks owing to oxygen concentration declines thereafter (Klein *et al.*, 2010). We apply a ballast water management scalar of 0.1 (S_{Mngmt}) to vessels that performed BWE to reflect the lowered abundance of NIS (otherwise = 1). BWE has been shown experimentally to remove upwards of 90% of coastal biota between ports (Ruiz and Reid, 2007; Bailey et al. 2011), yet this varies between seasons, ports, and taxa (McCollin et al. 2008; Simard et al, 2011). As such we retain the possibility that ballast water discharge following BWE still result in NIS being introduced. We also apply a probability of organism release constraint ($P_{Release}$) to vessel biofouling (0.1) where vessels call at a Svalbard port for < 12 hours (as is the case for many cruise ships), and a probability of 0.5 for vessels spending 12 hours – 14 days at Svalbard ports (otherwise = 1) (*sensu* Inglis et al. 2012). We note that the likelihood of biofouling NIS spawning under current Svalbard port conditions is expected to be low, yet retain the possibility that introduction occurs via dislodgment, fragmentation, or detachment by mobile taxa. Finally, as propagule pressure is also a function of the number of times species are introduced (Lockwood *et al.*, 2009), we include a parameter ($P_{RepeatVisit}$) that indicates the number of repeat visits a vessel makes to Svalbard from the same port (here assuming that the same species are introduced each visit) (1 visit = 1, 2…$_n$ visits = 2…$_n$). The final model is then:

Propagule pressure = $P_{Entrain} \times S_{Season} \times P_{Survival} \times S_{Mngmt} \times P_{Release} \times P_{RepeatVisit}$

The maximum propagule pressure estimate for either transport mechanism (ballast water or biofouling) is 1 subject to repeat visits, whereas the minimum rating for either mechanism is 0.025. We classified propagule pressure according to outputs of the model accordingly: ≤ 0.05 = low; 0.06 – 0.9 = medium; ≥ 1 = high.

2.3 **NIS known from ecoregions that are environmentally similar (d < 1) to Svalbard presently (*), or will become so following predicted climate change (d < 2.2). Ecoregions are given as in Molnar et al. (2008). '+' indicates whether species are adapted to transport via either ballast water intake or biofouling. Ecoregions are (as per Spalding et al. 2007): 20 - South and West Iceland; 21- Faroe Plateau; 22 - Southern Norway; 23 - Northern Norway and Finnmark; 25 - North Sea; 26 - Celtic Seas; 27 - South European Atlantic Shelf; 29 - Azores Canaries Maderia; 36 - Alboran Sea; 39 - Scotian Shelf.**

Taxa	Species	Ecoregion	Ballast	Biofouling
Algae	*Aglaothamnion halliae*	25	+	+
	Alexandrium minutum	25	+	
	Antithamnionella spirographidis	29	+	+
	Antithamnionella ternifolia	27	+	+
	Asparagopsis armata	27	+	+
	Asparagopsis taxiformis	29	+	+
	Bonnemaisonia hamifera*	21	+	+
	Bryopsis pennata	29	+	+
	Caulerpa racemosa var. cylindracea	29	+	+
	Caulerpa taxifolia	29	+	+
	Chara connivens	25	+	+
	Chattonella aff verruculosa	25	+	+
	Codium fragile ssp tomentosoides*	21		+

	Codium webbiana	29	+	+
	Colpomenia peregrine	27	+	+
	Coscinodiscus wailesii*	22	+	
	Dasya baillouviana	25	+	+
	Fucus evanescens	25	+	+
	Grateloupia filicina var. luxurians	27	+	+
	Grateloupia turuturu	27	+	+
	Gymnodinium catenatum	27	+	
	Heterosiphonia japonica*	27	+	+
	Hypnea musciformis	27	+	+
	Odontella sinensis	25	+	
	Pikea californica	26		+
	Pleurosigma simonsenii	25	+	
	Polysiphonia harveyi	27	+	+
	Prorocentrum minimum	25	+	
	Sargassum muticum	27	+	+
	Solieria chordalis	26	+	+
	Stypopodium schimperi	29	+	+
	Womersleyella setacea	29	+	+
	Undaria pinnatifida*	21	+	+
Annelida	*Boccardia proboscidea*	39	+	+
	Clymenella torquate	25		+
	Ficopomatus enigmaticus	27	+	+
	Goniadella gracilis	26	+	+
	Hydroides dianthus	27	+	+
	Hydroides elegans	29	+	+
	Hydroides ezoensis	25	+	+
	Hypania invalida	25	+	+
	Janua brasiliensis	25		+
	Marenzelleria neglecta	25	+	+
	Marenzelleria viridis	25	+	+
	Mytilicola orientalis	26		+
	Pileolaria berkeleyana	25		+
	Polydora ciliate	39	+	+
	Pseudobacciger harengulae	25	+	
	Pseudopolydora paucibranchiata	27	+	+
	Salmacina dysteri*	21		+
	Spirorbis marioni	29	+	+
Bacteria	*Aeromonas salmonicida*	25	+	
Bryozoa	*Amathia distans*	27	-	+
	Schizoporella unicornis	26	+	+
	Tricellaria inopinata	25		+
	Victorella pavida	25		+

Chelicerata	*Ammothea hilgendorfi*	25		+
Cnidaria	*Clavopsella navis*	29	+	
	Cordylophora caspia*	21	+	
	Diadumene lineata	25		+
	Ectopleura crocea	29		+
	Gonionemus vertens	27	+	
	Haliplanella lineata*	21		+
	Maeotias inexspectata	27	+	
	Maeotias marginate	27	+	
Crustacea	*Balanus eburneus*	25	+	+
	Callinectes sapidus	27	+	+
	Caprella mutica*	22	+	+
	Cercopagis pengoi	25	+	
	Elasmopus pectenicrus	27	+	
	Eriocheir sinensis	27	+	+
	Eusarsiella zostericola	25	+	+
	Gammarus tigrinus	25	+	
	Hemigrapsus penicillatus	27	+	+
	Hemigrapsus sanguineus	25	+	+
	Hemigrapsus takanoi	27	+	+
	Monocorophium sextonae	25	+	+
	Orchestia cavimana	27	+	
	Paralithodes camtschaticus*	23	+	+
	Percnon gibbesi	29	+	+
	Rhithropanopeus harrisii	25	+	+
Mollusca	*Acar plicata*	25	+	+
	Balanus Amphitrite	36	+	+
	Ceratostoma inornatum	27	+	+
	Corbula gibba	27	+	+
	Crassostrea gigas*	22	+	+
	Crepidula fornicata*	22	+	+
	Cyclope neritea	27	+	+
	Elminius modestus	27	+	+
	Ensis americanus	25	+	+
	Mercenaria mercenaria	25	+	+
	Mya arenaria*	20	+	+
	Myosotella myosotis	39	+	+
	Ocinebrellus inornatus	27	+	+
	Petricola pholadiformis*	22	+	+
	Rapana venosa	26	+	+
	Ruditapes philippinarum	27	+	+
	Teredo navalis*	20		+
	Urosalpinx cinerea	25		+

	Xenostrobus secures	27	+	+
Tunicata	*Ascidiella aspersa*	36		+
	Botryllus violaceus	25	+	+
	Ciona intestinalis*	21	+	+
	Clavelina oblonga	27	+	+
	Distaplia corolla	29		+
	Styela clava*	21	+	+

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Appendix 3

Table 1 Critical minimum reproductive thresholds, and the number of days required at thresholds, used to estimate habitat suitability for eight non-indigenous species found in the ballast water tanks of ships arriving at Svalbard ports.

Species	Thermal minimum	Salinity minimum	Days required	Stage	Reference
Amphibalanus improvisus	10	30	21	Naupliar, cypris	de Rivera et al. 2011; Nasrolahi et al. 2012
Austrominius modestus	6	30	66	Naupliar, cypris	Harms 1986
Acartia tonsa	10	Marine	41	Hatching – copepodite VI	Leandro et al. 2006
Carcinus maenas	10	Marine	70	Hatching - instar	de Rivera et al. 2007
Crangon crangon	12	Marine	79	Egg and larval development	Temming and Damm 2002
Eurytemora affinis	10	30	24	Naupliar, copepodite V	Devreker et al. 2004; 2007
Hemigrapsus penicillatus	12	Marine	86	Embryonic stages	van den Brink et al. 2012
Podon leuckartii	5	Marine	77	Egg development	Gieskes 1971a; 1971b; Onbé 1990;

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