Non-indigenous species in Northern Europe and the Great Lakes-St. Lawrence River: the importance of geographic origin

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Summary

Ecosystems all over the world are continuously invaded by new species, which become non-indigenous species (NIS) in the new location. Increasing ship traffic raises the chances for relocations of aquatic species to new regions since shipping is identified as the major transport vector. External factors such as climate change, globalization, pollution and other anthropogenic influences favor the invasion of aquatic ecosystems. For the establishment success of a species a high propagule pressure (i.e. number of introduced individuals) in conjunction with beneficial life history traits is crucial. Many species fail to establish a viable population, but some of those that succeed might cause severe harm to native communities or ecosystem functioning. Hence it is of particular interest to protect ecosystems from this threat. In my thesis I focused on NIS from the North and Baltic Seas characterized by a mostly saline ecosystem with several large brackish to freshwater estuaries, and the Great Lakes-St. Lawrence River region, a predominantly freshwater environment. Both regions are connected via frequent transatlantic ship traffic and highly invaded by well documented NIS.

In the first chapter I explored taxonomic composition and origins of NIS from both regions, and compared observed to expected numbers of NIS from major donor regions. My results confirm that the Ponto-Caspian region (i.e. Black, Azov and Caspian Seas) is a major donor of NIS in both regions, however with much higher species numbers than expected by using a model calculation based on the available species pool from donor regions, frequency of shipping transit, and an environmental match between donor and recipient regions. This poses the question what makes species, especially Ponto-Caspian species, so successful invaders.

In the second chapter I followed up on the lists of NIS and investigated seven life history traits of NIS in order to find common traits beneficial for invasion success. Successful invaders have to pass through the following stages: transport, introduction, establishment and spread. For the establishment and spread stages r-selected strategies, dormancy, and the ability to regenerate were important traits. However, the benefit of traits might depend on several factors such as the stage of the invasion process and biotic and abiotic environmental conditions.

In the third chapter I investigated temperature tolerance of three amphipod species. Global warming as part of a predicted climate change will most likely also hit the Baltic Sea and might become a hazard for the survival of native species, especially in coastal zones which is the habitat of amphipods. I compared mortality rates of the native species *Gammarus oceanicus* with the non-indigenous species *Gammarus tigrinus* and the

Ponto-Caspian species *Pontogammarus maeoticus*, which has an invasion history in freshwater areas of Turkey, but is absent in the Baltic Sea. The results reveal that *G. oceanicus*, which evolved under lower temperatures of the Baltic Sea, performed well in the control (16 °C) and decreased temperature (6 °C) treatments, but did not survive the increased temperature (26 °C) for more than five days. The Ponto-Caspian species *P. maeoticus* on the other hand was the most tolerant species at all tested temperatures. This indicates that the Ponto-Caspian species might have an advantage over native species and the potential to colonize the Baltic Sea in the future.

In conclusion, species originating from the Ponto-Caspian region invaded both regions, the North and Baltic Seas and the Great Lakes-St. Lawrence River region, in higher numbers than expected most likely due to beneficial life history traits and a wide environmental tolerance. Hence, with predicted global warming Ponto-Caspian species might be able to compete against native species in the Baltic Sea.

Zusammenfassung

Ökosysteme auf der ganzen Welt werden ständig von neuen Arten besiedelt, die am neuen Standort zu nicht heimischen Arten (NIS: non-indigenous species) werden. Der zunehmende Schiffsverkehr erhöht die Umsiedlungschancen aquatischer Arten in neue Regionen, da die Schifffahrt der wichtigste Transportvektor ist. Externe Faktoren wie Klimawandel, Globalisierung, Umweltverschmutzung und andere anthropogene Einflüsse begünstigen das Eindringen fremder Arten in aquatische Ökosysteme. Für den Etablierungserfolg einer Art ist eine hohe Einschleppungs-Intensität (d. h. die Anzahl eingeführter Individuen) in Verbindung mit vorteilhaften Eigenschaften entscheidend. Viele Arten schaffen es nicht, eine lebensfähige Population zu etablieren, aber einige erfolgreiche Arten könnten den heimischen Gemeinschaften oder dem Ökosystem schweren Schaden zufügen. Daher ist es von besonderem Interesse, Ökosysteme vor dieser Bedrohung zu schützen. Der Fokus meiner Dissertation liegt auf NIS aus der Nord- und Ostsee, charakterisiert durch ein überwiegend salines Ökosystem mit mehreren großen Brackwasser- bis Süßwassermündungen und aus der Great Lakes-St. Lawrence River Region, überwiegend eine Süßwasserregion. Beide Regionen sind durch häufigen transatlantischen Schiffsverkehr miteinander verbunden und wie zahlreiche Studien belegen stark mit NIS besiedelt.

Im ersten Kapitel habe ich die taxonomische Zusammensetzung und die Herkunft der NIS aus beiden Regionen untersucht und die ermittelte mit der erwarteten Anzahl von NIS aus wichtigen Ursprungsregionen verglichen. Meine Ergebnisse bestätigen, dass die Ponto-Kaspische Region (Schwarzes, Asowsches und Kaspisches Meer) für NIS in beiden Regionen eine wichtige Ursprungsregion ist, jedoch mit wesentlich höheren Artenzahlen als erwartet, basierend auf verfügbaren Artenpools aus Ursprungsregionen, der Häufigkeit des Schiffsverkehrs und der Ähnlichkeit der Umweltbedingungen zwischen Ursprungs- und Einwanderungsregionen. Das wirft die Frage auf, wodurch vor allem Ponto-Kaspische Arten zu so erfolgreichen Eindringlingen werden.

Im zweiten Kapitel habe ich sieben biologische Merkmale von den bereits ermittelten NIS aus beiden Regionen untersucht, um Gemeinsamkeiten zu finden, die bei einem Invasionserfolg von Vorteil sind. Erfolgreiche Eindringlinge müssen folgende Phasen durchlaufen: Transport, Einführung, Etablierung und Verbreitung. Für die Etablierung und Verbreitung waren die r-Strategie, Überdauerungsstadien und die Regenerationsfähigkeit wichtige Merkmale. Der Vorteil von Merkmalen könnte jedoch von mehreren Faktoren abhängen, wie z. B. dem Stadium des Invasionsprozesses oder biotischen und abiotischen Umweltbedingungen.

Im dritten Kapitel habe ich die Temperaturtoleranz von drei Amphipodenarten untersucht, die zu den Krebstieren gehören und in vielen Küstenregionen vorkommen. Die globale Erwärmung als Teil des prognostizierten Klimawandels wird sehr wahrscheinlich auch die Ostsee betreffen und könnte eine Gefahr für das Überleben heimischer Arten, insbesondere in Küstengebieten, darstellen. Ich habe die Mortalitätsraten der heimischen Art *Gammarus oceanicus*, der nicht heimischen Art *Gammarus tigrinus* und der Ponto-Kaspischen Art *Pontogammarus maeoticus* miteinander verglichen. Letztere hat einen Invasionshintergrund in Süßwassergebieten der Türkei, kommt in der Ostsee aber nicht vor. Die Ergebnisse zeigen, dass die Art *G. oceanicus*, die sich bei relativ niedrigen Temperaturen in der Ostsee entwickelt, mit den Kontroll- und kalten Temperaturbedingungen (16 bzw. 6 °C) gut zurechtkommt, aber die warme Temperatur (26 °C) auf Dauer nicht überlebt. Die Ponto-Kaspische Art *P. maeoticus* dagegen war bei allen getesteten Temperaturen die toleranteste Art. Das deutet darauf hin, dass die Ponto-Kaspische Art einen Vorteil gegenüber heimischen Arten und das Potenzial zur zukünftigen Besiedlung der Ostsee haben könnte.

Zusammenfassend ist festzuhalten, dass Arten aus der Ponto-Kaspischen Region beide Regionen, die Nord- und Ostsee und die Great Lakes-St. Lawrence River Region, in einer höheren Anzahl besiedeln als erwartet, wahrscheinlich aufgrund von vorteilhaften biologischen Merkmalen und einer hohen Toleranz gegenüber Umweltbedingungen. Daher könnten Ponto-Kaspische Arten durch die vorhergesagte Erderwärmung gegen heimische Arten in der Ostsee konkurrieren.

Introduction

Invasion ecology

Climate change, globalization, pollution, habitat destruction and other anthropogenic influences facilitate introductions of aquatic species to new environments, accounting for great changes of ecosystems (Olden et al. 2004; Hellmann et al. 2008; Hulme 2009; Capinha et al. 2015). This might lead to a homogenization of biodiversity worldwide with an increase of biodiversity in some regions, but a loss of biodiversity in other regions where the native biota become replaced by non-indigenous species (NIS) through e.g. competition, predation, grazing or parasitism (Mack et al. 2000; Olden et al. 2004). Besides ecological impacts, some NIS may have negative economic impacts, e.g. by overgrowing surfaces such as hulls in enormous abundance or by having direct or indirect impacts on commercial fisheries (Ruiz et al. 1997).

More than 90 % of global trade occurs via shipping, which is the leading mechanism for the spread of aquatic NIS worldwide (Ricciardi 2006; Molnar et al. 2008). Besides the unintentional transport of species in ballast water or attached to the ship hull, invasion can also occur via intentional introduction, e.g. for aquaculture or biocontrol (Lockwood et al. 2007; Hulme et al. 2008). Once a species is entrained into one of these transport vectors it first of all has to overcome the mostly adverse environmental conditions during transport while passing through the invasion process.

The invasion process can be categorized into multiple stages which are the basis for the stage-based invasion model (Williamson and Fitter 1996; Kolar and Lodge 2001; Colautti and MacIsaac 2004; Blackburn et al. 2011). The basic model is one of the most accepted concepts in invasion ecology and consists of four stages comprising transport, introduction, establishment and spread (Fig. 1; Kolar and Lodge 2001; Colautti and MacIsaac 2004; Lockwood et al. 2007; Blackburn et al. 2011). According to this model, a NIS becomes entrained into a transport vector, survives transport conditions, is introduced to a new environment, and tolerates the conditions of the new environment including ecological interactions in the new community such as competition and predation (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Blackburn et al. 2011).

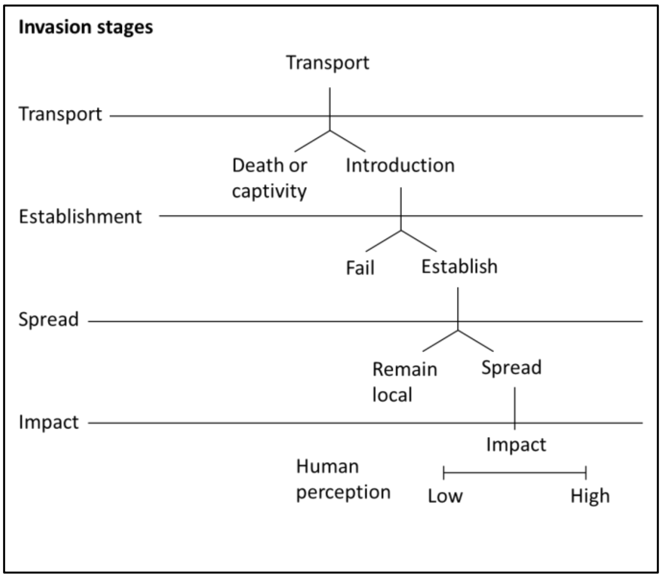

Figure 1. Invasion process model modified from Lockwood et al. (2007).

Many species fail at one of the stages and will therefore not be able to establish a viable population in the new environment. Successful individuals may spread to surrounding areas and may impact local species communities, ecosystem functioning, and/or services to human society (Carlton and Geller 1993; Chapin et al. 2000; Olden et al. 2004; Simberloff et al. 2013). Population characteristics such as phenotypic plasticity, a high genetic diversity and beneficial life history traits as well as a high propagule pressure might promote a successful transition of species through the different stages of the invasion process (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Lockwood et al. 2005; Dlugosch and Parker 2008; Hayes and Barry 2008; Simberloff 2009; Blackburn et al. 2011; Lande 2015).

Propagule pressure and life history traits

Propagule pressure is identified as an important factor for the establishment success of NIS and is defined as the number of introduced individuals in a release event (propagule size) linked with the number of release events (propagule number; Lockwood et al. 2005; Colautti et al. 2006; Jeschke and Strayer 2006; Hayes and Barry 2008; Simberloff 2009: Blackburn et al. 2015). With more individuals that are introduced and more release events, the probability of a successful invasion increases. However, numerous other parameters such as the duration of transport or an environmental match between the donor and recipient regions influence invasion probabilities (Williamson 2006). Population characteristics such as phenotypic plasticity, pre-adaptation to fluctuating environmental conditions and certain life history traits may further maintain a high propagule pressure (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Blackburn et al. 2011; Lande 2015). A strong interaction between propagule pressure and life history traits is distinctive for many successful invaders (Colautti et al. 2006). A good example is the zebra mussel *Dreissena polymorpha* that produces a high number of planktonic larvae that can be easily transported in ballast tanks of ships (Briski et al. 2014).

For different stages of the invasion process different life history traits may be beneficial. To overcome adverse environmental conditions during transport or in new regions, the ability to produce dormant stages (i.e. temporarily inactive but viable) may be of great advantage (Briski et al. 2011). Furthermore, asexual reproduction, elevated fecundity or a wide trophic range may increase the probability of a successful establishment (Kolar and Lodge 2001; Bøhn et al. 2004; Ribeiro et al. 2008). However, it may be difficult to generalize beneficial life history traits due to environmental variations among regions or phenotypic plasticity of NIS (Rosecchi et al. 2001; Sakai et al. 2001; Ribeiro et al. 2008).

The studied regions

The most frequently used shipping routes are located in the Northern Pacific and Northern Atlantic (Fig. 2). Due to a rapidly growing economy, global ship traffic will continue to increase in the future and this will most likely lead to a rise in invasion rates (Leppäkoski and Olenin 2000). The transatlantic cargo ship traffic between the North and Baltic Seas and the Great Lakes-St. Lawrence River is relatively high and of similar intensity in both directions (Fig. 2; Kaluza et al. 2010). Both regions are highly invaded and their NIS are well documented (AquaNIS; GLANSIS).

The North and Baltic Seas, and the Great Lakes-St. Lawrence River regions are geologically young water bodies formed thousands of years ago by glaciations (Leppäkoski et al. 2002; Reid and Orlova 2002). The North and Baltic Seas are characterized by a mostly saline ecosystem with several large brackish to freshwater estuaries, while the Great Lakes-St. Lawrence River region is predominantly a freshwater environment until the river broadens into a huge brackish to saline St. Lawrence River estuary and Gulf of St. Lawrence (Fig. 3; Pocklington 1986; Reid and Orlova 2002; Antonov et al. 2006; Environment Canada 2013). The systems are both marginal water bodies of the North Atlantic Ocean (Pocklington 1986). The salinity conditions in the Baltic Sea with a decreasing salinity gradient from about 24 psu in the

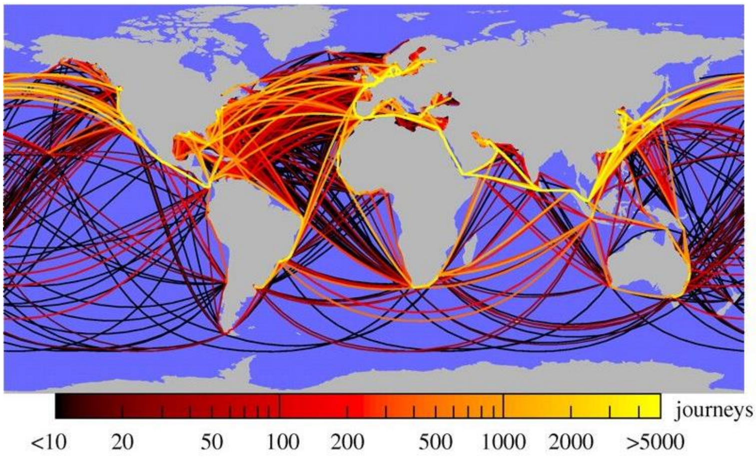

Figure 2. The trajectories of all cargo ships bigger than 10 000 gross tonnage during 2007. The colour scale indicates the number of journeys along each route. Ships are assumed to travel along the shortest paths on water (modified from Kaluza et al. 2010).

western Kattegat to about 2 psu in the eastern and northern basins represent a challenging environment for many species. They often reach the border of their general distribution there (Arndt 1989; Leppäkoski et al. 2002; Gräwe et al. 2013). The salinity of the St. Lawrence River, which consists of freshwater in large parts of the river stretch, starts to increase from Quebec City (5 psu) reaching 24 to 32 psu in the Gulf of St. Lawrence (Pocklington 1986; Environment Canada 2013). When comparing salinity patterns of the studied systems, the salinities of the Baltic Sea and the Lower St. Lawrence River are relatively similar.

Both, the saline as well as the freshwater environment, are highly invaded by NIS and several studies have stated that the Ponto-Caspian region which consists of the Black, Azov and Caspian Seas is one major donor region of these species (Ricciardi and MacIsaac 2000; Leppäkoski et al. 2002). Ponto-Caspian species spread and became abundant in freshwater and estuarine ports of Northern Europe after the openings of the Rhine-Main-Danube, Volga-Don and Volga-Baltic Canals in the 19th and 20th centuries that link the North and Baltic Seas with the Black and Caspian Seas (Ricciardi and MacIsaac 2000; Leppäkoski et al. 2002). Previous studies suggest that the establishment of many Ponto-Caspian species in the Laurentian Great Lakes is a result of secondary introductions from Northern Europe, i.e. the stepping stone hypothesis (Ricciardi and MacIsaac 2000; Leppäkoski et al. 2002). Some of the Ponto-Caspian invaders can have high impact on local communities and ecosystem functioning such as the zebra mussel, *Dreissena polymorpha,* which has become a severe biofouling pest in North America (Ricciardi et al. 1998).

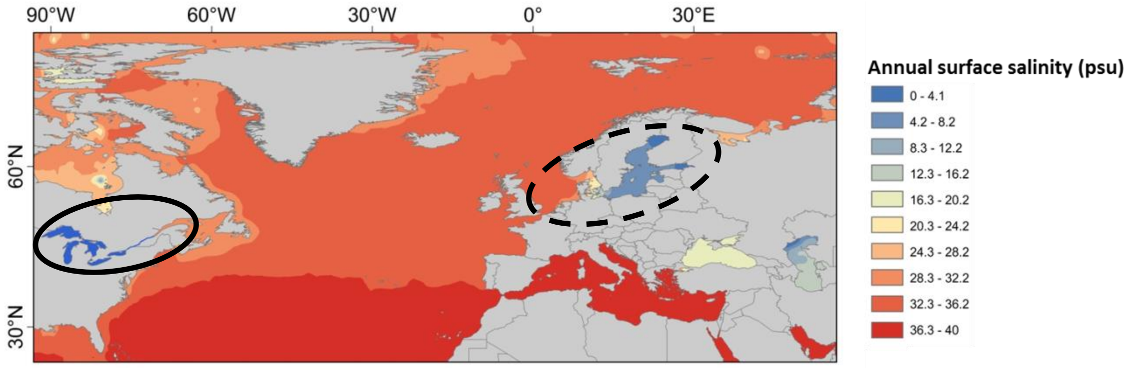

Figure 3. Salinity of the Great Lakes-St. Lawrence River (black ellipse), North Atlantic Ocean, North and Baltic Seas (dashed ellipse), Mediterranean, Black, Azov, and Caspian Seas, constructed using average annual salinity data with a 1° x 1° spatial resolution from the World Ocean Atlas database (Antonov et al. 2006). The salinity of the Great Lakes is below 0.5 psu (i.e. fresh water).

Due to increasing extremely high anthropogenic emissions of greenhouse gases the global climate is changing drastically (IPCC 2014). One major concern is the increase of sea surface temperature that potentially impairs growth, reproduction, and survival of some organisms and, consequently, may lead to migrations to new and more favourable environments (Bulnheim 1979; Sainte-Marie 1991; Neuparth et al. 2002). Changes in species communities and environments as a result of predicted climate change may therefore open new niche opportunities for continuously arriving species (Holopainen et al. 2016). The Baltic Sea is a highly variable semi-enclosed brackish water body and will most likely be subject to future climate change. Reductions in average salinity by about 2 psu and increases of average water surface temperatures by about 2 to 4 °C are predicted by the end of the twenty-first century (IPCC 2007; Gräwe et al. 2013; Holopainen et al. 2016). Previous studies have shown that temperature and salinity are key environmental factors that affect the physiology, growth, reproduction, and survival of organisms (Sutcliffe 1968; Einarson 1993; Neuparth et al. 2002; Bedulina et al. 2010; Delgado et al. 2011). Physiological tolerance is an important trait in determining invasion success and it is therefore of importance to investigate species' tolerance limits to potentially predict and prevent new introductions.

The studied organisms

Gammarids consist of freshwater, brackish and marine species and belong to the order Amphipoda and subphylum Crustacea. They are important keystone species in aquatic ecosystems where they often appear in high abundance in rivers and shallow coastal zones (Gerhardt et al. 2011). *Gammarus* spp. are of high ecological relevance due to amongst others a wide trophic range, high reproductive capacity and migration ability, which allows them to easily colonize new ecosystems (Gerhardt et al. 2011). Furthermore, they play an important role in the detritus cycle and microbial loop and constitute a significant diet source for many fish and water birds (Giller and Bij de Vaate 2000; Gerhardt et al. 2011). To be protected from predators gammarids tend to hide between algae, mussel beds and stone fields (Kolding and Fenchel 1979). In the Baltic Sea species have a high adaptation capacity to cope with the fluctuating salinities. Gammarids are osmoregulators and hence able to maintain intracellular ion and osmolyte homeostasis at varying external water conditions, however it is an energy demanding process (Werntz 1963).

Crustaceans represent a major group of NIS in aquatic systems and many amphipod species have spread to new environments and become successful NIS (Hänfling et al. 2011). Some of them have negative impacts on local communities and ecosystem functioning such as the Ponto-Caspian species *Echinogammarus ischnus* which replaced native amphipod species in North America and Western Europe (Dermott et al. 1998; Leppäkoski et al. 2002; Cristescu et al. 2004). *Pontogammarus robustoides* has spread in the Baltic Sea and has negative impacts on native species e.g. by competitive exclusion (Arbaciauskas and Gumuliauskaite 2007). *Gammarus tigrinus* can outcompete native amphipods in some regions and have predatory impacts (Pinkster 1975).

Figure 4. The three studied species: *Gammarus oceanicus* (a), *Gammarus tigrinus* (b) and *Pontogammarus maeoticus* (c). Each scale bar equals 1 mm.

The focus of this study was set on three gammarid species from different origins.

Gammarus oceanicus is native to the Baltic Sea where it is distributed in the entire area (Fig. 4a; Segerstråle 1947). It is also widespread along the North Atlantic coast up to subarctic regions and thrives at salinities of approximately 2 to 30 psu (Segerstråle 1947; Bulnheim 1979).

Gammarus tigrinus is non-indigenous in Northern Europe including the Baltic Sea and native to the Northwest Atlantic (Fig. 4b; Daunys and Zettler 2006). It occurs at salinities of < 0.5 to 30 psu (Paiva et al. 2018). Studies of *G. tigrinus* revealed that it has a relatively wide range of salinity and temperature tolerance and a greater reproductive capacity compared to native gammarid species in the Baltic Sea (Pinkster 1975; Wijnhoven et al. 2003).

Pontogammarus maeoticus is native to the Caspian Sea and distributed in the Azov and Black Seas where it occurs at a salinity range of 0 to 18 psu (Fig. 4c; Soldatova 1986). It is not present in the Baltic Sea, but it has been chosen due to its invasion history in freshwater areas of Turkey (Ozbek 2011).

Thesis outline

This thesis is divided into three chapters addressing characteristics of NIS from the North and Baltic Seas and the Great Lakes-St. Lawrence River region such as geographic origin, life history traits and environmental tolerance.

In the first chapter I explored taxonomic compositions and geographic origins of observed NIS from the North and Baltic Seas and the Great Lakes-St. Lawrence River region, and compared observed to expected numbers of NIS from major donor regions. The expected numbers were calculated by using a model based on the available species pool from donor regions, frequency of shipping transit, and an environmental match between donor and recipient regions.

In the second chapter I followed up on the lists of NIS from the North and Baltic Seas and the Great Lakes-St. Lawrence River region and investigated seven life history traits of NIS in order to find common traits beneficial for invasion success. The main focus of the traits was on reproduction type, dormancy, regeneration and feeding type.

In the third chapter I investigated temperature tolerance of three amphipod species. I compared mortality rates of the species *Gammarus oceanicus*, native to the Baltic Sea, with the non-indigenous species *Gammarus tigrinus* and the Ponto-Caspian species *Pontogammarus maeoticus*, which has an invasion history in freshwater areas of Turkey, but is absent in the Baltic Sea. I performed the tests at two different salinities, 10 and 16 psu, and three different temperature treatments including one at a constant temperature of 16 °C, one gradually increasing from 16 to 26 °C, and one gradually decreasing from 16 to 6 °C.

Chapter I

Importance of geographic origin for invasion success: a case study of the North and Baltic Seas *versus* the Great Lakes-St. Lawrence River region

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Abstract

Recently, several studies indicated that species from the Ponto-Caspian region may be evolutionary predisposed to become non-indigenous species (NIS), however origin of NIS established in different regions have rarely been compared to confirm these statements. More importantly, if species from certain area/s are proven to be better colonizers, management strategies to control transport vectors coming from those areas must be more stringent, as prevention of new introductions is a cheaper and more effective strategy than eradication or control of established NIS populations. To determine if species evolved in certain areas have inherent advantages over other species in colonizing new habitats, we explored NIS established in the North and Baltic Seas and Great Lakes-St. Lawrence River regions – two areas intensively studied in concern to NIS, highly invaded by Ponto-Caspian species and with different salinity patterns (marine vs. freshwater). We compared observed number of NIS in these two regions to expected numbers of NIS from major donor regions. The expected numbers were calculated based on the available species pool from donor regions, frequency of shipping transit and an environmental match between donor and recipient regions. A total of 281 NIS established in the North and Baltic Seas and 188 in the Great Lakes-St. Lawrence River. Ponto-Caspian taxa colonized both types of habitats, saltwater areas of the North and Baltic Seas and freshwater of the Great Lakes-St. Lawrence River, in much higher numbers than expected. Propagule pressure (i.e. number of introduced individuals or introduction effort) is of great importance for establishment success of NIS; however in our study either shipping vector or environmental match between regions did not clarify the high numbers of Ponto-Caspian taxa in our study areas. Although we cannot exclude the influence of other transport vectors, our findings suggest that the origin of the species plays an important role for the predisposition of successful invaders.

Introduction

Anthropogenic introductions of species to new areas increase due to globalization and climate change, leading to homogenization of biodiversity worldwide (Olden *et al*., 2004; Hellmann *et al*., 2008; Hulme, 2009; Capinha *et al*., 2015). Species are incidentally transported with commercial travel and trade, such as in ships' ballast water, wood packing materials, and horticultural soils, or are intentionally introduced like for games or biocontrol (Lockwood *et al*., 2007; Hulme *et al.*, 2008; Briski *et al*., 2013). Many species fail to establish a viable population after arriving to a new environment, but those that succeed may have significant consequences for local communities, ecosystem functioning and/or services to human society (Carlton & Geller, 1993; Chapin *et al.,* 2000; Olden *et al*., 2004; Simberloff *et al*., 2013). Though empirical and statistical evidence suggests that propagule pressure (i.e. number of introduced individuals) is of crucial importance for establishment success (Hayes & Barry, 2008; Simberloff, 2009), population characteristics such as phenotypic plasticity and pre-adaptation to cope with changeable environmental conditions, may keep a high propagule pressure of species while passing through the stages of the invasion process (i.e. transport, introduction, establishment, and spread; Colautti & MacIsaac, 2004; Lande, 2015). Moreover, species evolved in regions known as more geologically and environmentally disturbed and challenged, may possess life history traits, higher phenotypic plasticity or adaptational and evolutionary capacity which would enable them to be more successful invaders (Reid & Orlova, 2002). If species from certain area/s are proven to be better colonizers, management strategies to control transport vectors coming from those areas must be more stringent, as prevention of new species introductions is a cheaper and more effective strategy than eradication or control of established NIS populations (Lodge *et al.* 2006; Lockwood *et al*. 2007; Hulme *et al*. 2008).

After the opening of canals that link the North and Baltic Seas with the Black and Caspian Seas (the Rhine-Main-Danube, Volga-Don and Volga-Baltic Canals), species from the Ponto-Caspian region (i.e. Black, Azov and Caspian Seas; Fig. 1) spread and became abundant in freshwater and estuarine ports of Northern Europe (Ricciardi & MacIsaac, 2000; Leppäkoski *et al.,* 2002). The invasion history of the Laurentian Great Lakes tells a more intriguing story, with many Ponto-Caspian species establishing in the region after invasion of Europe (Ricciardi & MacIsaac, 2000; Leppäkoski *et al.,* 2002). Shipping is a leading mechanism for the spread of aquatic non-indigenous species (NIS) globally (Ricciardi, 2006; Molnar *et al.*, 2008), and as ship transit between the North and Baltic Seas and the Great Lakes-St. Lawrence River is relatively high and of similar intensity in both directions (Kaluza *et al*., 2010), one would expect a similar ratio of NIS from the Great Lakes-St. Lawrence River in the North and Baltic Seas, and *vice versa*. However, recent studies stated that the transfer of species has been asymmetrical, with only a small number of species from the Great Lakes having invaded Northern European waters (Reid & Orlova, 2002; Leppäkoski *et al.,* 2010).

The North and Baltic Seas, and the Great Lakes-St. Lawrence River regions are intensively explored systems, and probably the most studied areas with regards to aquatic NIS globally (Reise *et al*., 1999; Ricciardi, 2006; Pyŝek *et al*., 2008; Gollasch *et al.*, 2009; AquaNIS, 2015; DAISIE, 2015; GLANSIS, 2015). Both regions are geologically young water bodies formed by glaciations (Leppäkoski *et al.,* 2002; Reid & Orlova, 2002). Their habitat types represent an interesting inverse mirror image with the North and Baltic Seas being mostly marine ecosystem with several large brackish to freshwater estuaries, while the Great Lakes-St. Lawrence River region is predominantly a freshwater environment with a huge brackish to saline St. Lawrence River estuary and Gulf of St. Lawrence (Fig. 1; Pocklington, 1986; Reid & Orlova, 2002; Antonov *et al*., 2006; Environment Canada, 2013). Both systems are marginal water bodies of the North Atlantic Ocean (Pocklington, 1986). Despite their opposing salinity patterns, some parts of the systems are rather alike: in particular the Baltic Sea and the Lower St. Lawrence River. The Baltic Sea is a large semi-enclosed brackish water area characterized by a strong salinity gradient ranging between 2 and 24 ppt (Leppäkoski *et al*., 2002), while the salinity of the St. Lawrence River, though freshwater in large part of the river stretch, starts to increase from Quebec City (5 ppt) reaching 24 to 32 ppt in the Gulf of St. Lawrence (Pocklington, 1986; Environment Canada, 2013). The climate in the Baltic Sea and St. Lawrence River is also similar, ranging from maritime temperate to continental sub-arctic climate (Pocklington, 1986).

Both, the North and Baltic Seas, and the Great Lakes-St. Lawrence River regions are heavily invaded by Ponto-Caspian taxa, while at the same time the systems are different in concern to salinity (marine vs. freshwater), in this study we explored origin and taxonomic composition of NIS in these two regions to determine if Ponto-Caspian taxa have inherent advantages over other species in colonizing new areas. We compared observed numbers of NIS in these two regions (i.e. established) to expected numbers of NIS from major donor regions. The expected numbers of NIS were estimated based on the available species pool from donor regions, frequency of shipping transit and an environmental match between donor and recipient regions. We tested the hypothesis that there is no difference between expected and observed numbers of NIS in the two regions. We also tested the hypotheses that there is no difference in i) number of established NIS; ii) geographic origin of NIS; and iii) taxonomic composition of NIS between the two regions.

Material and Methods

Observed numbers of NIS, their origin and taxonomic composition

Lists of aquatic NIS were compiled for the North and Baltic Seas, and the Great Lakes-St. Lawrence River region, respectively. The North and Baltic Seas NIS list (Appendix S1) was assembled using data from AquaNIS - the information system on aquatic non-indigenous and cryptogenic species (AquaNIS, 2015), Reise *et al.* (1999), Bij de Vaate *et al.* (2002) and Gollasch *et al*. (2009). The region was defined as the area affiliated to the Baltic Sea and North Sea, and was confined by a line between Dover and the Belgian border and a line between the Shetland Islands to Norway (AquaNIS, 2015). The Great Lakes-St. Lawrence River's NIS alien species list (Appendix S2) was assembled from de Lafontaine and Costan (2002), Ricciardi (2006), and the Great Lakes Aquatic Nonindigenous Information System (GLANSIS) database (GLANSIS, 2015). The region was defined as the area of the Great Lakes basin and its ordinarily attached channels, wetlands and waters, and the St. Lawrence River until the Gulf of St. Lawrence River (Ricciardi, 2006; GLANSIS, 2015). The Gulf of St. Lawrence River was not included.

To examine if species from the Ponto-Caspian region are more common NIS and whether an opposing salinity pattern of the two systems has an effect on established taxa, geographic origin of NIS and their taxonomic composition were determined. Geographic origin of species was assigned based on AquaNIS (2015) for the North and Baltic Seas NIS, while that of the Great Lakes-St. Lawrence River NIS was based on GLANSIS (2015). If information was not available on these two websites, a general internet search engine was conducted. Geographic origin was assigned to one or more groups: north-east Atlantic, north-west Atlantic, south-east Atlantic, south-west Atlantic, north-east Pacific, north-west Pacific, south-east Pacific, south-west Pacific, North Sea, Baltic Sea, the Great Lakes-St. Lawrence River region, Mediterranean Sea, Eurasia (inland freshwaters except Yangtze River), Mississippi River, Yangtze River, Arctic, Australia (inland freshwaters), New Zealand (inland freshwaters), Indo-Pacific (Indian Ocean and the archipelago of Indonesia, Malaysia, and Pilipinas), Africa (inland freshwaters), North America (inland freshwaters except the Laurentian Great Lakes, St. Lawrence and Mississippi Rivers), South America (inland freshwaters), Ponto-Caspian region and unknown region. If a species was native to two or more regions, its contribution was counted as a ratio of “one” over the number of regions that the species was native to. For example, if a species was native to two regions, the value of 0.5 has been assigned to each region. However in figure 2, if a species was native to two or more regions it was shown as two or more flows in the plot. Taxonomic assignments were based on several websites (e.g. Barcode of Life Database (BOLD), European Nature Information System (EUNIS), World Register of Marine Species (WORMS) and ZipcodeZoo). Species were assigned to kingdom, phylum, and class. Due to a high number of Tracheophyta species (vascular plants) established in the Great Lakes-St. Lawrence River region, and the fact that Tracheophyta is a mostly terrestrial and freshwater phylum with rare representatives in marine habitats (Les & Cleland, 1997; Bell & Hemsley, 2000), the results of our study are shown with and without this phylum.

Expected numbers of NIS

To calculate expected numbers of NIS from major donor regions for the North and Baltic Seas and Great Lakes-St. Lawrence River region, we first estimated average species richness for major donor regions using derived global species richness data from Tittensor *et al*., (2010). We calculated average species richness for a particular donor region by adding derived species richness of all coastal grids (880-km resolution equal-area grid) of that region, and then divided this total derived species richness with the number of coastal grids in that region. The average species richness of coastal grids was used to avoid overestimation of species richness due to a potential overlap of the same species from neighbouring grids. Tittensor *et al*. (2010) data did not provide species richness for the Black and Caspian Seas nor for the Great Lakes-St. Lawrence River region, therefore we calculated an average species richness for these regions using total species richness data from the European Environment Agency (EEA, 2016), and National Oceanic and Atmospheric Administration (NOAA, 2016). As the north-west Atlantic data was available in both, in Tittensor *et al*. (2010) as per 880-km resolution equal-area grids and in Marine Species Registers for the Northwest North Atlantic Ocean (MSRNNAO, 2016) as total species richness, we used these data to derive the correction factor. The correction factor was then applied to the total species richness data from the European Environment Agency (EEA, 2016) and National Oceanic and Atmospheric Administration (NOAA, 2016) to calculate average species richness for the Black and Caspian Seas and the Great Lakes-St. Lawrence River region compatible to the rest of our data (i.e. per 880-km resolution equal-area grids). The correction was necessary as total species richness per regions were approximately ten times higher than species richness per 880-km resolution equal-area grids.

In the second step, the obtained estimated average species richness was multiplied by the probability of invasion between regions to get the expected numbers of NIS transported from a donor to a recipient region. The expected number of NIS was used as a null model of NIS exchanges irrespective of species' traits, which can be compared with the observed NIS exchanges. The invasion probabilities were calculated using the statistical model of Seebens *et al.* (2013). The model integrates global ship movement data, biogeographical similarity and environmental conditions of ports worldwide to obtain the likelihood that a NIS is transported in ballast water from a donor port, released in a recipient port and able to establish a new population there.

According to Seebens *et al.* (2013), the model consists of three independent probabilities each denoting an important step of the invasion process: first, the ballast water released at site *j* may contain species from all regions previously entered by the ship including NIS but also species, which are native to the recipient site *j*. This is accounted for by the probability to be non-indigenous

$$P_{ij}\left(Non\text{-}indigenous\right) = (1 + \frac{\gamma}{d_{ij}})^{-\beta} \ ,$$

describing the probability that a species native at donor port *i* is non-indigenous in recipient port *j*. P_{ij}(Non-indigenous) is a sigmoidal function of geographic distance d_{ij} between the ports, with β and γ being constants, and can be interpreted as the proportion of NIS inoculated in the ballast water of a ship and transported over a certain distance.

Second, the probability of introduction describes the likelihood that a species is introduced from port *i* to port *j* on ship route *r*:

$$\mathrm{P}_r(Intro) = \left(1 - e^{-\lambda B_r}\right) e^{-\mu \, \Delta \, t_r}.$$

It increases with the amount of released ballast water B_r that originates from port *i* on ship route *r* and decreases with mortality rate μ and travel time Δt_r between *i* and *j*. Ship routes were established from nearly 3 Mio. port calls (arrival and departure dates at ports) of 32,511 ships during 2007-2008. The arrival and departure dates as well as ship specific information were reported by the Automatic Identification System (AIS) and provided by Lloyd's Register Fairplay (www.ihs.com). For each port call of a ship, B_r was calculated depending on the ship type, ship size, the mean ballast water tank volume and the past route of the ship: For a certain ship type and ship size a mean volume of discharged ballast water was calculated from 717,250 ballast water release protocols provided by the National Ballast Information Clearinghouse for the USA (NBIC 2012). Although these data are restricted to the USA, they represent by far the most comprehensive collection of ballast water release protocols currently available. To estimate the amount of discharged ballast water originating from port *i*, we require the mean ballast water tank volumes for different size classes and ship types, which were obtained from the American Bureau of Shipping (ABS 2011). While assuming a constant release of ballast water at each port of call, we were then able to calculate for each port call of a ship the mean ballast water volume B_r originating from port *i* and discharged at port *j*. Travel times Δt_r were extracted from these ship routes.

Third, the probability of establishment describes the likelihood that a species native at port *i* is able to establish a population in the recipient port *j*:

$$P_{ij}(Estab) = \alpha e^{-\frac{1}{2}\left[\left(\frac{\Delta T_{ij}}{\sigma_T}\right)^2 + \left(\frac{\Delta S_{ij}}{\sigma_S}\right)^2\right]}$$

P_{ij}(Estab) is a Gaussian function of differences in water temperatures T and salinities S normalized by standard deviations σ_T and σ_S, and α being a constant.

The product of the three probabilities gives the probability of invasion P_{ij}(Inv). To obtain invasion probabilities between regions, the invasion probabilities from all ports *a* in the donor region *A* to all ports *b* in the recipient region *B* were aggregated according to $P_{A,B}(\mathrm{Inv})=1-\Pi_{a,b}(1-P_{a,b}(\mathrm{Inv}))$. The parameter setting was adopted from Seebens *et al.* (2013), where more details of the underlying data, the model itself, model validation and a sensitivity analysis can be found. The calculated invasion probabilities were multiplied with the estimated average species richness of a donor region to get the expected number of NIS from a particular donor region to a particular recipient region. Eurasia (inland freshwaters except Yangtze River) and North America (inland freshwaters except the Laurentian Great Lakes, St. Lawrence and Mississippi Rivers), two major donor regions for the Great Lakes-St. Lawrence River region, were excluded from this analysis due to a lack of shipping data. We emphasize here that our expected numbers of NIS from major donor regions to recipient regions could not be taken as absolute numbers of NIS in the recipient regions, but only as rough estimates due to the possibility that other vectors than shipping may operate between the regions and due to temporal changes in vector strength (e.g. number of arriving ships per time). A necessary time for species to have a chance to be transported and established in the recipient regions was also not taken into account in our calculations. Therefore, by considering only one transport vector, we underestimated expected numbers of NIS, but at the same time by not including time necessary for species to be transported and established we overestimated those numbers. Finally, observed and expected numbers of NIS from each major donor region to each recipient region were statistically compared using Chi-square tests (Performed in R version 3). Additional Chi-Square tests were performed for each of the ten pairs of donor and recipient region separately.

Additionally, to better illustrate salinity patterns of the two systems and their connections to the Ponto-Caspian region, we constructed a salinity map showing the Great Lakes-St. Lawrence River, North Atlantic Ocean, North, Baltic, Mediterranean, Black, Azov, and Caspian Seas (Fig. 1). The map was constructed using average annual salinity data with a 1° x 1° spatial resolution from the World Ocean Atlas database of the *National Oceanic and Atmospheric Administration* (NOAA)'s National Oceanographic Data Centre (NODC) United States Department of Commerce (Antonov *et al*., 2006) by ArcGIS, ESRI Inc.

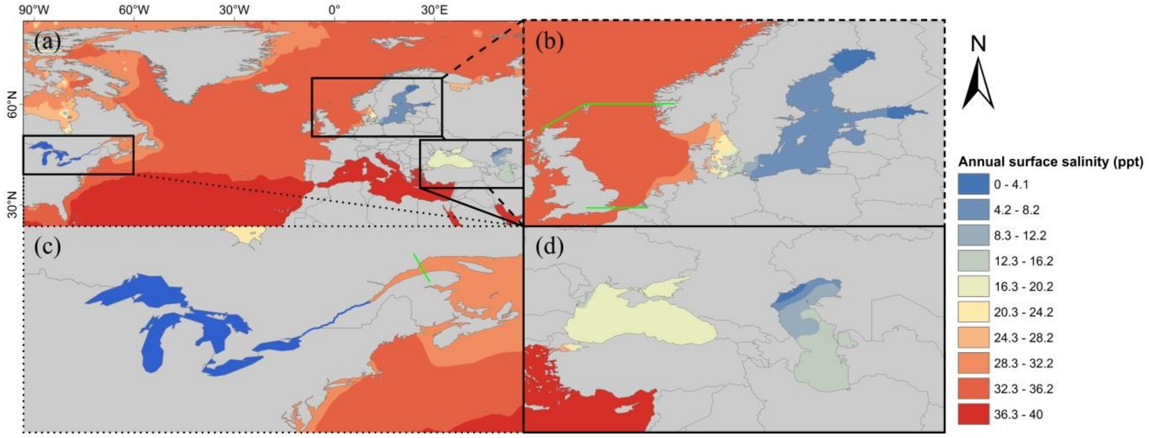

Fig 1. Salinity of the Great Lakes-St. Lawrence River, North Atlantic Ocean, North, Baltic, Mediterranean, Black, Azov, and Caspian Seas, constructed using average annual salinity data with a 1° x 1° spatial resolution from the World Ocean Atlas database (Antonov *et al*., 2006)(a). Close-up maps of the North and Baltic Seas (b), the Great Lakes-St. Lawrence River (c), and the Black, Azov and Caspian Seas (d) are shown, as well. The green lines mark the boundaries of the studied areas. Though, the salinity of the Great Lakes is shown in the range from 0.0 to 4.1 (i.e. dark blue), the salinity of the Great Lakes is under 0.5 ppt (i.e. freshwater).

Results

Observed numbers of NIS, their origin and taxonomic composition

A total of 281 NIS established in the North and Baltic Seas region, of which 156 established only in the North Sea and 53 only in the Baltic Sea; the establishment of 72 species overlap in the two water bodies (Table S1). In the Great Lakes-St. Lawrence

River region, 188 NIS established with104 only in the Great Lakes, three only in the St. Lawrence River, and 81 in both areas (Table S2). NIS occurring only in the St. Lawrence River were two fishes, *Oncorhynchus clarkii* and *Tinca tinca*, and one crustacean *Oronectes limosus* (Table S2).

Geographic origins of NIS differed between the two regions (Fig. 2). While for 19% of NIS in the North and Baltic Seas region donor areas were unknown (54 species), the next dominant donors were the north-west Pacific (17%, 49 species), north-west Atlantic (16%, 44 species), and Ponto-Caspian region (15%, 42 species; Fig. 2, Tables 1 and S1). Contrary to the North and Baltic Seas, the most dominant donors for the Great Lakes-St. Lawrence River region were Eurasia (47%, 88 species) followed by 17% of species from unknown areas (31 species), 12% from the Ponto-Caspian region (23 species), and nine percent from North America (17 species; Fig.2, Tables 1 and S2). After excluding Tracheophyta from the datasets, the most dominant donor regions did not change in either of the two regions (Fig. 2). Yet, percentages of donors for the Great Lakes-St. Lawrence River region changed due to a high number of Tracheophyta NIS in that region (56 species; Table S2), which mostly originated from one region (86% from Eurasia); the new donor region percentages were 30, 23, 17, and 12% for Eurasia, unknown region, Ponto-Caspian region, and North America, respectively (Fig. 2). Twenty-five species were recorded in both the North and Baltic Seas region and the Great Lakes-St. Lawrence River region (Tables S1 and S2). Eleven of these were from the Ponto-Caspian region, four from both the north-east and north-west Pacific, while the rest originated from Eurasia, north-west Atlantic, North America, north-east Pacific, Yangtze River, New Zealand, or unknown region (Tables 1, S1 and S2).

Even though only 25 species established in both the North and Baltic Seas and Great Lakes-St. Lawrence River regions, the taxonomic composition of NIS was similar between the regions (Tables S1 and S2). The largest distinction was the phylum Tracheophyta; this phylum represented only 2% of species in the North and Baltic Seas region, but 30% in the Great Lakes-St. Lawrence River region. In the North and Baltic Seas region the most abundant phyla were Arthropoda (25%, 69 species), Chordata (17%, 48 species), Mollusca (11%, 30 species), Annelida (10%, 27 species) and Ochrophyta (9%, 25 species), while those in the Great Lakes-St. Lawrence River region were Tracheophyta (30%, 56 species), Chordata (17%, 31 species), Arthropoda (13%, 24 species), Ochrophyta (10%, 19 species) and Mollusca (10%, 18 species; Fig. 3). After excluding Tracheophyta from the datasets, the percentages of the most dominant phyla in the Great Lakes-St. Lawrence River region were 24, 18, 14 and 14% for Chordata, Arthropoda, Ochrophyta and Mollusca, respectively (Fig. 3).

Twenty-four species that established in both the North and Baltic Seas region and the Great Lakes-St. Lawrence River region were Animalia, dominated by Actinopterygii (fishes); beside Animalia, there was one bacterium (*Aeromonas salmonicida*). Forty-four percent of those species were from the Ponto-Caspian region (eleven species; Tables 1, S1 and S2). More than two dozen of Arthropoda, Chordata, Cnidaria and Mollusca that originated from the Ponto-Caspian region and established in the North and Baltic Seas were not recorded in the Great Lakes-St. Lawrence River region. In contrast, Ponto-Caspian Chromista established only in the Great Lakes-St. Lawrence River region (Tables 1, S1 and S2). Furthermore, taxonomic composition of NIS from Eurasia (after excluding Tracheophyta) was similar in the two regions, though only two species established in both regions (Tables S1 and S2).

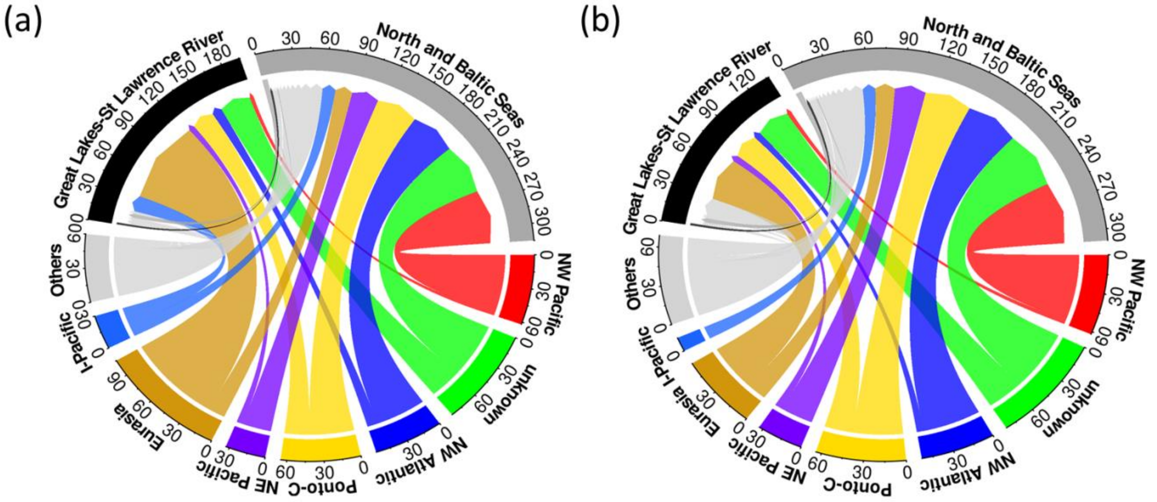

Fig 2. Flows of aquatic non-indigenous species from different regions to the North and Baltic Seas and the Great Lakes-St. Lawrence River region including Tracheophyta (a) and excluding Tracheophyta (b). The arrows at the end of the flows show towards the recipient region. If a species is native to two regions it is shown as two flows in the plot. Each region is a colour assigned and represented by a circle segment: north-west (NW) Pacific, unknown region, north-west (NW) Atlantic, Ponto-Caspian region (Ponto-C.), north-east (NE) Pacific, Eurasia (inland freshwaters except Yangtze River), North (N) America (inland freshwaters except the Laurentian Great Lakes, St. Lawrence and Mississippi Rivers), Indo-Pacific (I-Pacific; Indian Ocean and the archipelago of Indonesia, Malaysia, and Pilipinas). Others include: north-east Atlantic, Mediterranean Sea, south-east Atlantic, south-west Pacific, Arctic, south-west Atlantic, Africa (inland freshwaters), Baltic Sea, New Zealand (inland freshwaters), North Sea, south-east Pacific, Yangtze River, Mississippi River, Australia (inland freshwaters), South America (inland freshwaters).

Table 1. List of Ponto-Caspian species established in the North and Baltic Seas, and the Great Lakes and the St. Lawrence River regions, and their taxonomic assignment.

Taxon	Species	North Sea	Baltic Sea	Great Lakes	St. Lawrence River
Animalia					
Annelida					
Clitellata	*Paranais frici*		X		
	Potamothrix bedoti		X	x	
	Potamothrix heuscheri		X		
	Potamothrix moldaviensis			x	
	Potamothrix vejdovskyi		X	x	
Polychaeta	*Hypania invalida*	x	X		
Arthropoda					
Branchiopoda	*Cercopagis pengoi*		X	x	
	Cornigerius maeoticus		X		
	Evadne anonyx		X		
Malacostraca	*Chelicorophium (=Corophium) curvispinum*	x	X		
	Chelicorophium robustum	x			
	Dikerogammarus haemobaphes	x	X		
	Dikerogammarus villosus	x	X		
	Echinogammarus ischnus		X	x	x
	Echinogammarus warpachowskyi		X		
	Hemimysis anomala	x	X	x	
	Jaera istri	x			
	Limnomysis benedeni		X		
	Obesogammarus crassus	x	X		
	Paramysis (=Mesomysis) intermedia		X		
	Paramysis (=Serrapalpisis) lacustris		X		
	Pontogammarus robustoides		X		

		Pseudocuma (=Stenocuma) graciloides		X		
	Maxillopoda	*Eurytemora affinis*			x	x
		Nitocra hibernica			x	
		Nitocra incerta			x	
		Schizopera borutzkyi			x	
Bryozoa						
	Gymnolaemata	*Victorella pavida*	x	X		
Chordata						
	Actinopterygii	*Acipenser gueldenstaedtii*	x	X		
		Acipenser oxyrinchus		X		
		Acipenser ruthenus	x	X		
		Acipenser stellatus		X		
		Cyprinus carpio		X	x	x
		Huso huso		X		
		Neogobius fluviatilis	x	X		
		Neogobius kessleri	x			
		Neogobius melanostomus	x	X	x	x
		Proterorhinus marmoratus		X	x	
Cnidaria						
	Hydrozoa	*Cordylophora caspia*	x	X	x	
		Maeotias marginata		X		
		Moerisia (=Ostroumovia) inkermanica	x			
		Pachycordyle navis	x	X		
Mollusca						
	Bivalvia	*Dreissena rostriformis bugensis*	x	X	x	x
		Dreissena polymorpha	x	X	x	x
	Gastropoda	*Lithoglyphus naticoides*		X		
		Theodoxus pallasi		X		
		Viviparus acerosus	x			

	Myxozoa			
		Myxosporea	*Sphaeromyxa sevastopoli*	x
	Platyhelminthes			
		Trematoda	*Ichthyocotylurus pileatus*	x
			Neascus brevicaudatus	x
Chromista				
	Cercozoa		*Psammonobiotus communis*	x
		Gromiidea	*Psammonobiotus dziwnowi*	x
			Psammonobiotus linearis	x
	Ciliophora			
		Phyllopharyngea	*Acineta nitocrae*	x

Table 2. Estimated average species richness for major donor regions (per 880-km resolution equal-area grids), probabilities of invasion [P(Inv)] for species likely to be transported by ballast water from these regions and established in the North and Baltic Seas or the Great Lakes-St. Lawrence River region, estimated expected number of non-indigenous species (NIS) from major donor regions in the recipient regions, observed number of NIS from major donor regions in the recipient regions, and statistical comparisons of expected and observed numbers of NIS (i.e. Chi-square and p-values) are shown. Significant p-values are presented in bold.

Donor region	Recipient region	Estimated average species richness	Invasion risk [P(Inv)]	Expected number of NIS	Observed number of NIS	Statistical comparison	
						Chi- square	p-value
North-west Atlantic	North and Baltic Seas	~570	0.16172	92	44	25.04	< **0.001**
Great Lakes-St. Lawrence River	North and Baltic Seas	~320	0.04665	15	2	11.27	< **0.001**
North-east Pacific	North and Baltic Seas	~450	0.03669	17	17	0	1
North-west Pacific	North and Baltic Seas	~1 200	0.03509	42	49	1.17	0.279
Ponto-Caspian region	North and Baltic Seas	~500	0.00514	3	42	507	< **0.001**
North and Baltic Seas	Great Lakes-St. Lawrence River	~250	0.04849	12	4	5.33	**0.021**
Ponto-Caspian region	Great Lakes-St. Lawrence River	~500	0.00014	0.07	23	7511	< **0.001**
North-west Atlantic	Great Lakes-St. Lawrence River	~570	0.00006	0.03	10	3313	< **0.001**
North-west Pacific	Great Lakes-St. Lawrence River	~1 200	0.00003	0.04	2	96.04	< **0.001**
North-east Pacific	Great Lakes-St. Lawrence River	~450	0.00001	0.005	4	3192	< **0.001**

Expected numbers of NIS and their comparison to observed numbers of NIS

Estimated average native species richness for major donor regions ranged from 250 to 1200 species (Table 2). Invasion risks between major donor regions and recipient regions varied greatly, with at least one or two orders of magnitude higher risks from donor regions to the North and Baltic Seas than to the Great Lakes-St. Lawrence River region. Expected numbers for both regions, the North and Baltic Seas and the Great Lakes-St. Lawrence River, were significantly different from observed numbers (Chi-square tests, $p < 0.05$). Estimated expected numbers of NIS from major donor regions were up to four orders of magnitude higher for the North and Baltic Seas than for the Great Lakes-St. Lawrence River region (Table 2). Observed numbers of NIS in the North and Baltic Seas from the north-east Pacific and north-west Pacific were similar to expected numbers from these regions ($p > 0.5$; Fig. 4, Table 2). However, expected numbers of NIS from the north-west Atlantic and Great Lakes-St. Lawrence River region to the North and Baltic Seas were two and seven times higher, respectively, than observed numbers from these regions ($p < 0.05$; Fig. 4, Table 2). The observed number of NIS from the Ponto-Caspian region in the North and Baltic Seas was 14 times higher than expected ($p < 0.05$; Fig. 4, Table 2). In the case of the Great Lakes-St Lawrence River, observed numbers of NIS from the North and Baltic Seas were three times lower than expected numbers ($p < 0.05$; Fig. 4, Table 2). The numbers of observed NIS in the Great Lakes-St Lawrence River from all other donor regions were higher than expected ones, with Ponto-Caspian species being more than 300 times higher ($p < 0.05$; Fig. 4, Table 2).

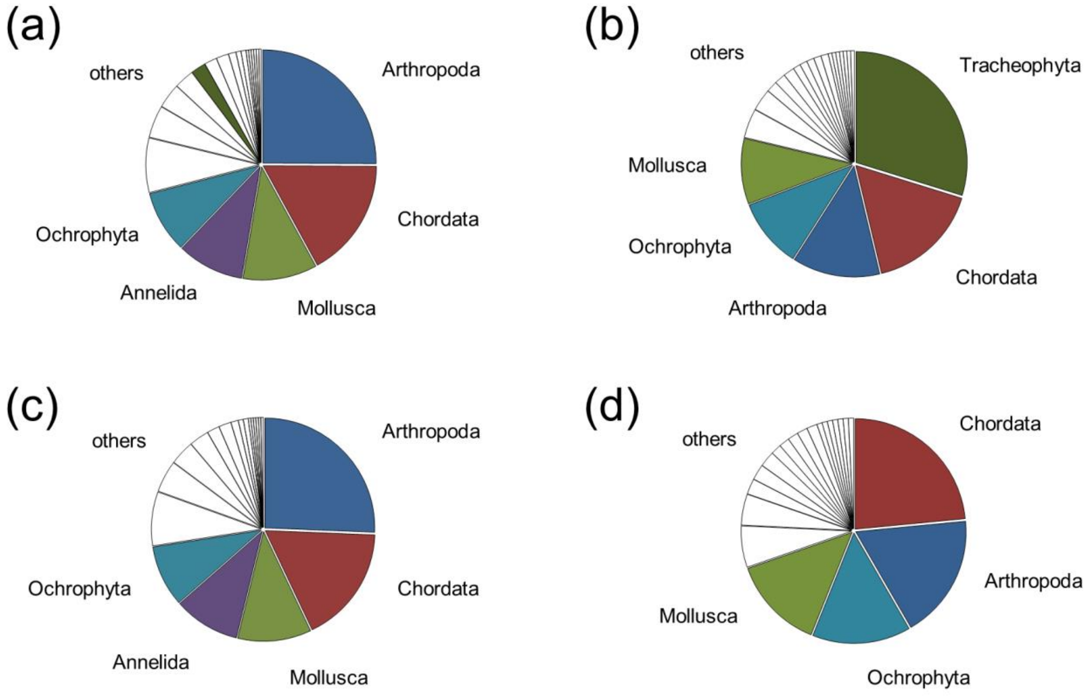

Fig 3. Phyla of aquatic non-indigenous species in the Baltic and North Sea including Tracheophyta (a) and excluding Tracheophyta (c) and in the Great Lakes-St. Lawrence River region including Tracheophyta (b) and excluding Tracheophyta (d). White fields in descending order show: Rhodophyta, Cnidaria, Myzozoa, Platyhelminthes, Bryozoa, Chlorophyta, Cercozoa, Ctenophora, Porifera, Acanthocephala, Ascomycota, Charophyta, Heterokontophyta, Nematoda, Proteobacteria (a and c) and Platyhelminthes, Annelida, Cercozoa, Chlorophyta, Virus, Cnidaria, Microsporidia, Myxozoa, Proteobacteria, Rhodophyta, Actinobacteria, Bryozoa, Charophyta, Ciliophora, Cyanobacteria, Euglenida, Haptophyta (b and d).

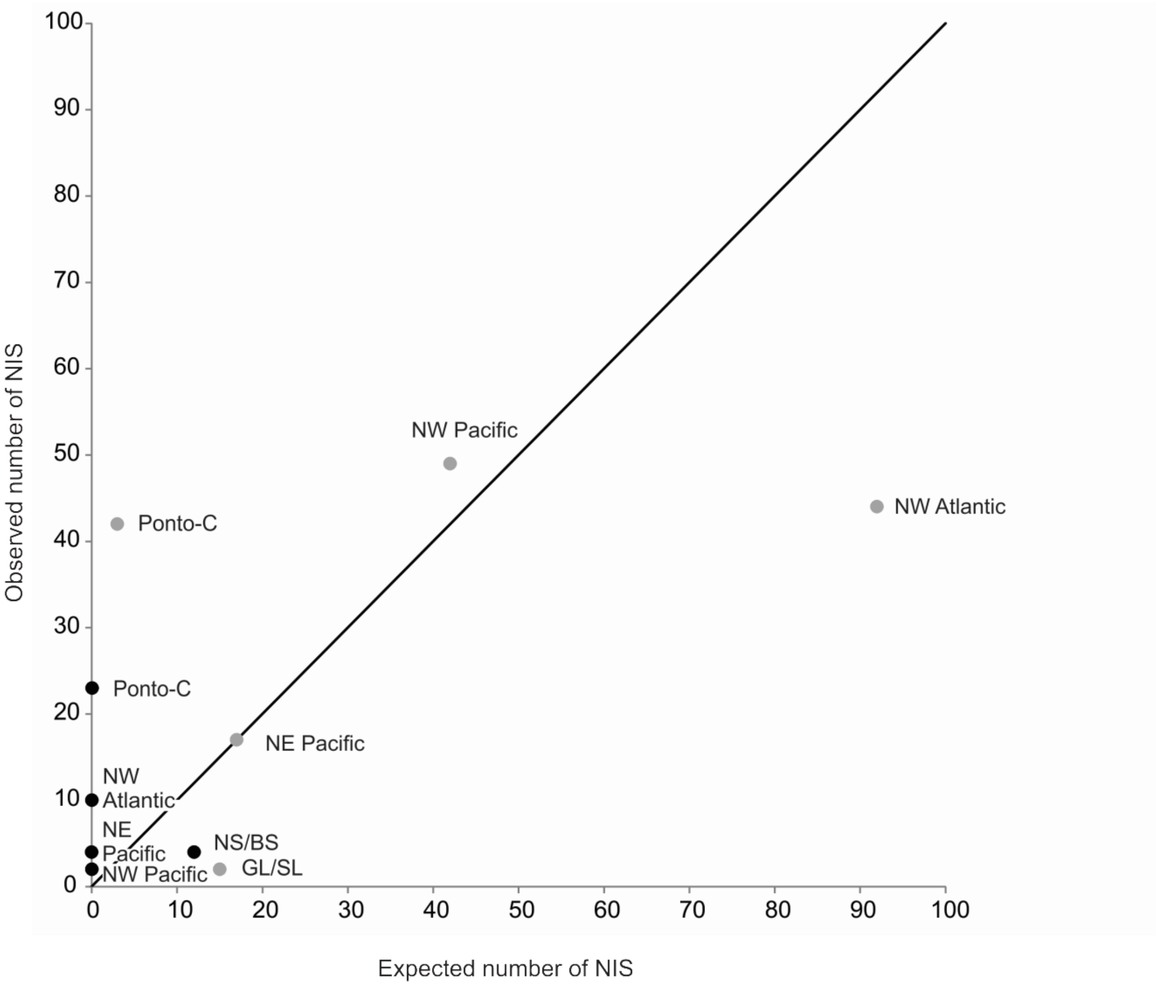

Fig 4. Scatter plot with number of expected non-indigenous species (NIS) in the North and Baltic Seas and the Great Lakes-St. Lawrence River region on x-axis and number of observed NIS in these regions on y-axis. Gray dots show the North and Baltic Seas region as the recipient region, while black dots show the Great Lakes-St. Lawrence River region as the recipient region. NW Pacific, NE Pacific, NW Atlantic, Ponto-C, NS/BS, and GL/SL denote north-west Pacific, north-east Pacific, north-west Atlantic, Ponto-Caspian region, North and Baltic Seas, and Great Lakes-St. Lawrence River region as donor regions of NIS, respectively. The line of unity is included.

Discussion

Several studies pointed out that species evolved in the Ponto-Caspian region may be evolutionary predisposed to become NIS (Ricciardi & MacIsaac, 2000; Reid & Orlova, 2002; Bij de Vaate *et al*., 2002; Leppäkoski *et al*., 2002, 2010), however the origin of NIS established in different regions have rarely been compared to confirm these statements. In this study, we explored origin of NIS established in the North and Baltic Seas and Great Lakes-St. Lawrence River regions – two areas intensively studied in concern to NIS, highly invaded by Ponto-Caspian species and with different salinity patterns (marine vs. freshwater). We compared established (observed) to expected numbers of NIS from donor regions and confirmed that there are many more Ponto-Caspian species in both regions than expected based on the available pool of species from the Ponto-Caspian region, frequency of shipping transits and an environmental match between regions. Additional vectors, in particular the canals that connect the North and Baltic Seas with the Black and Caspian Seas, might explain a higher number of Ponto-Caspian taxa in Northern Europe (this study; Bij de Vaate *et al*., 2002). Though, interestingly, the North and Baltic Seas are not dominant donor areas of NIS in the Ponto-Caspian region (Shiganova, 2011). Contrary, there is no such vector that would explain two orders of magnitude higher observed than expected numbers of Ponto-Caspian taxa in the Great Lakes-St. Lawrence River. Previous studies suggested that the most probable pathway for Ponto-Caspian species to the Great Lakes is a secondary introduction from Northern Europe (Ricciardi & MacIsaac, 2000, Leppäkoski *et al*., 2002), though some species such as the quagga mussel *Dreissena rostriformis bugensis* likely came to the Great Lakes directly from the Black, Azov or Caspian Sea (Spidle *et al*., 1994). As half of the Ponto-Caspian species in the Great Lakes are not established in the North and Baltic Seas, the potential stepping stone dynamics via this region do not provide a parsimonious explanation. Northern European rivers however were not included in our study and therefore we cannot confidently disregard the stepping stone hypothesis. Species characteristics and their environmental tolerance, such as the production of dormant stages, r-reproductive strategy (Briski *et al*., 2011, 2012) or high phenotypic plasticity, may also explain colonization success of Ponto-Caspian taxa under a lower propagule pressure scenario (i.e. low introduction effort).

Nevertheless, we show here that the Ponto-Caspian region was one of the major donors for both the North and Baltic Seas and the Great Lakes-St. Lawrence River regions, and the main donor of NIS in both regions, only eleven out of 56 species from that area established in both regions (i.e. 44 established in the North and Baltic Seas and 23 in the Great Lakes-St. Lawrence River). Bij de Vaate *et al*. (2002) described chronological spread for several Ponto-Caspian species following the Danube and/or Dniepr rivers and migrating to the Rhine, Ems, Weser, Elbe, Oder, Vistula and Neman rivers, with some of those species establishing in these rivers and their river mouths, but not spreading further to the North or Baltic Sea. In addition, the majority of Ponto-Caspian NIS are established in the lower salinity areas of the Baltic, but not in the North Sea's higher salinity habitats (this study; Paavola *et al.,* 2005). All the above mentioned may point to several directions: i) even though the Ponto-Caspian region is a donor region for both freshwater and marine habitats, all Ponto-Caspian species are not able to equally thrive in both types of habitat; ii) Ponto-Caspian species established in European rivers that are not in the North or Baltic Sea might spread to those areas in the future; iii) species established in Northern Europe, but not in the Great Lakes-St. Lawrence River region, might also reach the region in the future; iv) species established in the Great Lakes-St. Lawrence River but not in Northern Europe, arrived to North America directly from the Ponto-Caspian region, not as secondary introduction from Northern Europe; or v) a combination of those above. To determine which of the above is correct or more significant, further studies on Ponto-Caspian species, transport vectors, pathways and propagule pressure regarding the Ponto-Caspian area are needed.

Beside Ponto-Caspian taxa, species from the north-west Atlantic, north-west and north-east Pacific were also established at higher numbers than expected in the Great Lakes-St. Lawrence River region. However, almost all of these species were intentionally introduced fishes (Ricciardi, 2006). In contrast, the number of established north-west Atlantic taxa in the North and Baltic Seas was lower than predicted. The number of the Great Lakes-St. Lawrence River taxa in the North and Baltic Seas, and vice versa, was also lower. The lower number of the North and Baltic Sea's taxa in the Great Lakes-St. Lawrence River region is quite intriguing as the shipping vector until the early 1980s was stronger than during 2007-2008 which were used for our probability estimations, and mainly unidirectional delivering the Baltic water to the Great Lakes while transporting wheat to the USSR (Kelly *et al*., 2009). Underestimating the number of NIS might be a more common error due to unknown transport vectors that might operate between two regions, while overestimating is harder to explain because of a high propagule pressure between regions. An overestimated number of species from a certain region indicates that taxa from that region might be less suitable for colonization of new habitats. Therefore, taxa from the Great Lakes-St. Lawrence River, North and Baltic Seas, and north-west Atlantic seemed to be evolutionary less predisposed to be colonizers than Ponto-Caspian taxa.

Previous studies stated that the transfer of species between different salinity habitats is asymmetrical, with a colonization of freshwater habitats by marine and brackish species becoming increasingly common in recent years, but not *vice versa* (Grigorovich *et al*., 1998; Lee & Bell, 1999; Sylvester *et al*., 2013). Ponto-Caspian species originating from brackish areas, with a salinity gradient from freshwater in the east to more saline in the west, accompanied by strong salinity fluctuations (Reid & Orlova, 2002) colonized both freshwater habitats of the Great Lakes and European major rivers, and brackish habitats of the St. Lawrence River and the North and Baltic Seas. However, the Ponto-Caspian area is not an important donor of NIS to the Mediterranean Sea (CIESM, 2015). The Ponto-Caspian region is geologically old and has several times undergone large-scale environmental changes from fully marine environments, while it was a part of the Tethys Sea, to almost freshwater habitats as Sarmatian Sea (Zenkevitch, 1963; Reid & Orlova, 2002). Further, during the Pleistocene Epoch and the Ice Age, the majority of the Ponto-Caspian area dried out, followed by freshwater flooding after ice melting at the end of the Ice Age. Later, a few more geological connections and disconnections of the whole or parts of the region with the Mediterranean Sea caused several additional changes in salinity, with Ponto-Caspian low-saline taxa surviving increases in salinity in surrounding rivers and spreading out to the basins once again when salinity dropped (Zenkevitch, 1963; Reid & Orlova, 2002). Hence, species evolved in this region may be freshwater taxa adapted to low saline habitats, accompanied later by Mediterranean taxa adapted to brackish environments. Therefore, colonization of the Great Lakes and European rivers by Ponto-Caspian species should not be surprising. If endemic Ponto-Caspian species are evolutionarily freshwater taxa, which are today highly euryhaline, then the statements about recent numerous colonization of freshwater habitats by marine and brackish species are not surprising. However, further experimental and evolutionary studies are required to confirm this hypothesis.

The main difference in taxonomic composition of established NIS in the two regions was in the number of established plants. Tracheophyta was the most represented phylum of NIS established in the Great Lakes-St. Lawrence River (i.e. 30%, 56 species), most likely transported as seeds with solid ballast that was used prior to ballast water (e.g. sand, rocks and mud; Mills *et al*., 1993; Ricciardi, 2006). However, the phylum was negligible in the North and Baltic Sea region. Lambdon *et al*. (2008), taking into account the entire area of Europe, also stated that marine habitats are much less invaded by plants than inland waters. Beside the fact that the biodiversity of marine plants is much lower than that of freshwater plants (Les & Cleland, 1997), seeds of the latter are also highly resistant to harsh environments and drying conditions compared to those of the former (Cook *et al*., 1974; Leck, 1989; Orth *et al*., 2000; Larkum *et al*., 2007). Environmental tolerance, often dormancy of freshwater seeds, and dates of species discoveries support further the assumptions of solid ballast being the main vector for introduction of these taxa to aquatic habitats (Ricciardi, 2006). After replacement of solid ballast with ballast water at the beginning of the 20th Century, fewer introductions of plants were recorded in the Great Lakes (Ricciardi, 2006).

Taking into account numerous Ponto-Caspian species established in the Great Lakes and Northern Europe, the areas which are greatly connected by shipping to Eastern Asia and coastal North America (Kaluza *et al.* 2010; Seebens *et al*., 2013), one would expect Ponto-Caspian species spreading practically all around the world. However, as the

Ponto-Caspian region was not the main donor for the Mediterranean Sea (CIESM, 2015), nor did Ponto-Caspian species establish in high salinities of the North Sea (this study; Paavola *et al.,* 2005), we doubt that Ponto-Caspian taxa may colonize highly saline marine habitats. We suspect that Ponto-Caspian species would colonize big river mouths and estuaries, such as Chesapeake Bay, San Francisco Bay, Yangtze River, and Rio de la Plata. In addition to a salinity match among those regions and the Ponto-Caspian region, very large shipping ports are also located in those areas. Comparative assessment of NIS in multiple regions around the world, including freshwater, brackish and marine habitats, in connection to transport vectors (i.e. as a proxy for propagule pressure, or introduction effort) and species characteristics would elucidate further if Ponto-Caspian species are better colonizers than species evolved in other regions. However, this comparison would require a huge amount of work and sampling effort to establish reliable and complete lists of NIS, which are lacking for many areas around the world.

Authors' contributions

E.B. designed the study, I.C. collected the data, I.C. and E.B. drafted the manuscript, and H.S. conducted the model calculations. All authors revised the manuscript and gave final approval for publication.

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Chapter II

Beneficial life history traits for invasion success revisited

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Submitted to *Aquatic Invasions*

Abstract

One of the most dominant concepts in invasion ecology is the stage-based invasion model, consisting of transport, introduction, establishment and spread. Many species fail at one of the stages, with propagule pressure (i.e. number of introduced individuals) identified as a principal factor affecting establishment success. Population characteristics such as phenotypic plasticity and beneficial life history traits may facilitate successful transition of species through different stages of the process; however, studies on the latter are not so common. Based on the stage-based invasion model, we hypothesized different life history traits that may be beneficial for different stages of invasion. To test our model, we determined life history traits for established aquatic non-indigenous species (NIS) in the North and Baltic Seas (i.e. saline environment) and Great Lakes-St. Lawrence River regions (i.e. freshwater environment). The main focus of our model testing was on reproduction type, dormancy, regeneration and feeding type. An r-strategy, dormancy, and the ability to regenerate were determined as important traits for establishment and spread stages. Approximately, 60% of NIS established in these regions have also spread within the regions. Specific life history traits that resulted as the most beneficial, when combined together, reduce demographic and environmental stochasticity during the invasion process. This finding accords with previous studies that emphasize the interaction between propagule pressure and life history traits.

Introduction

Anthropogenic introductions of species to new areas are facilitated by globalization and climate change (Lockwood et al. 2007; Hellmann et al. 2008; Hulme et al. 2008; Hulme 2009; Capinha et al. 2015). One of the most dominant concepts in invasion ecology is the stage based invasion model (Williamson and Fitter 1996; Kolar and Lodge 2001; Colautti and MacIsaac 2004; Blackburn et al. 2011). The basic model consists of stages including transport, introduction, establishment and spread (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Blackburn et al. 2011). Accordingly, a non-indigenous species (NIS) is first entrained into a transport vector, should survive the transport conditions, being released into a new region, and tolerates the environmental conditions of the new region (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Blackburn et al. 2011). If the NIS establishes a population, it may then spread to surrounding areas and impact the new community (Carlton and Geller 1993; Chapin et al. 2000; Olden et al. 2004; Simberloff et al. 2013). However, many species fail at one of the stages, with low propagule pressure (i.e. number of introduced individuals) identified as a key limiting factor (Lockwood et al. 2005; Colautti et al. 2006; Jeschke and Strayer 2006; Blackburn et al. 2015). Phenotypic plasticity and certain life history traits may facilitate maintenance of high propagule pressure, thereby increasing the probability of successful invasion (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Blackburn et al. 2011; Lande 2015).

Several studies have tried to determine which life history traits would favour species invasiveness (reviewed in Colautti et al. 2006). To do so, they mostly compared characteristics of NIS with those of species native to the invaded regions, with species from the same source regions as the NIS, with their global congeners, or with other NIS in the invaded areas (Colautti et al. 2006). However, many of the studies based their conclusions on small number of species or groups of species, or they used data of intentionally introduced species, which might be biased towards healthier, bigger or more adapted individuals (see Colautti et al. 2006). Furthermore, it may be difficult to generalise beneficial life history traits owing to variation among regions or if NIS exhibit phenotypic plasticity (Rosecchi et al. 2001; Sakai et al. 2001; Ribeiro et al. 2008). Many of these studies failed to account for propagule pressure (see Colautti et al. 2006). Consequently, clear studies highlighting the role of life history traits to invasion success are rare.

Dormancy - the ability to remain in a temporarily inactive but viable stage (Cáceres 1997; Gyllström and Hansson 2004) - may be of great advantage to overcome adverse environmental conditions during transport or in new regions (Briski et al. 2011). Furthermore, asexual reproduction, elevated fecundity or dietary generalism may increase the probability of successful reproduction by decreasing demographic stochasticity and increasing the chances of finding available resources during the establishment stage (Kolar and Lodge 2001; Bøhn et al. 2004; Ribeiro et al. 2008). As pointed above there is also a strong interaction between propagule pressure and some life history traits and this is distinctive for many successful invaders (Colautti et al. 2006). A good example is the zebra mussel *Dreissena polymorpha* (Pallas, 1771), an r-strategist, that produces a high number of planktonic veliger larvae that can be easily transported, such as in ships' ballast tanks (Briski et al. 2012, 2014a).

In this study, based on the stage-based invasion model (Colautti and MacIsaac 2004; Colautti et al. 2006; Blackburn et al. 2011), we hypothesized life history traits that may increase the probability of successful transition between stages while considering different invasion scenarios (i.e. environmental conditions of transport vectors and in new habitats). To support our prior hypotheses – conceptual model, we used lists of aquatic NIS established in the North and Baltic Seas (i.e. saline environment) and the Great Lakes-St. Lawrence River regions (i.e. freshwater environment; Casties et al. 2016) and determined life history traits of NIS in these two regions. These regions were chosen as they are intensively explored systems and probably the most studied areas with regards to aquatic NIS globally (Reise et al. 1999; Ricciardi 2006; Gollasch et al. 2009). We acknowledge that our study would be stronger if we would compare the proportion of each trait present in the invaded region to the proportion of that trait in the native region of NIS, or to the proportion of that trait represented by total biodiversity in the invaded region. But as this kind of study is highly laborious, and with all our effort was not accomplishable due to unavailable data for species biodiversity for many regions. We acknowledge also that physiological tolerance is an important trait in determining invasion success and is, therefore, included in our model. However, due to unavailable data on physiological tolerance for many species, the main focus of our model testing lay on reproduction type, dormancy, regeneration and feeding type.

Material and Methods

Development of the conceptual model

Based on diversified invasion scenarios (i.e. environmental conditions of transport vectors and new habitats), as well as numerous taxonomic groups reported as aquatic NIS globally (e.g. Casties et al. 2016; Galil et al. 2016; Daisie (2017; http://www.europe-aliens.org/); GISD (2017; http://www.iucngisd.org/gisd/); NEMESIS (2017; https://invasions.si.edu/nemesis/)), we extended the stage-based invasion model (Colautti and MacIsaac 2004; Colautti et al. 2006; Blackburn et al. 2011) by hypothesizing life history traits that may increase the probability of species transiting between the stages. Beneficial life history traits were hypothesised based on Colautti et al.'s (2006) literature review of generalized invasiveness traits, and augmented by consulting Ruppert et al. (2004) for invertebrates, Lee (2008) and Kim (2011) for algae, Moyle and Cech (2003) for fish, Nabors (2003) for plants, and Tortora et al. (2012) for microorganisms. We acknowledge that the life history traits hypothesized in our conceptual model are not exhaustive. As all listed traits are not beneficial for all transport vectors, potential vectors were suggested for each trait of the transport stage (stage I); four transport vectors were suggested based on their importance for species introductions to saline (Molnar et al. 2008) and freshwater habitats (Ricciardi 2006).

Supporting the conceptual model by life history traits of established and spread NIS

To support our conceptual model, we used two lists of aquatic NIS assembled by Casties et al. (2016): i) 281 NIS in the North and Baltic Seas (i.e. saline environment); and ii) 188 NIS in the Great Lakes-St. Lawrence River regions (i.e. freshwater environment), and determined life history traits of those species. Seven life history traits were determined for each species: reproduction mode, number of broods per year, r- or K-strategy, ability for dormancy and/or regeneration, feeding type, and feeding mode. Reproduction mode was determined as asexual, sexual or both; number of broods as less than one brood per year, one brood, or more than one brood per year; dormancy as yes or no; regeneration as yes or no; and feeding mode as carnivore, herbivore, omnivore, filter feeder, suspension feeder, or parasite. When information for specific life history traits could not be determined, the trait was assigned as unknown. Though our conceptual model hypothesized beneficial life history traits for each stage of the invasion process, model validation was based only on the establishment and spread stages owing to unavailable or incomplete data for taxa that passed the transport and introduction stages but did not reach the establishment stage. Validation of the establishment stage was based on life history traits of all NIS established in the two regions, while that of the spread stage used only traits of NIS that have been reported in at least three locations in one of the regions. In the case of spread, we assumed that establishment at three or more locations in a system was secondary from original point of establishment. However, we acknowledge that multiple introductions from different regions are also possible.

To determine life history traits for each species, Clarivate Analytics' Web of Science and Google Scholar were searched by using combinations of keywords including species name and a certain life history trait, for example "*Gammarus tigrinus*" and "reproduction". Additional information was obtained from the online databases FishBase (2016; www.fishbase.org), the Great Lakes Aquatic Nonindigenous Information System (GLANSIS, 2014; https://nas.er.usgs.gov/queries/default.aspx) and Aquatic Nonindigenous and Cryptogenic Species (AquaNIS, 2015; http://www.corpi.ku.lt/databases/index.php/aquanis/), as well as from Ruppert et al. (2004) for invertebrates, Lee (2008) and Kim (2011) for algae, Nabors (2003) for plants, Tortora et al. (2012) for microorganisms, or from general internet search engines. Life history traits were summarized for all taxa per studied region (i.e. saline and freshwater habitat), as well as for phylum; in the latter case, only phyla with three or more species in both regions were summarized.

We consulted a variety of data sources to determine the status of NIS spread in the North and Baltic Seas (e.g. Gollasch et al. 2009, HELCOM 2012, AquaNIS - the information system on aquatic non-indigenous and cryptogenic species (http://www.corpi.ku.lt/databases/index.php/aquanis/, 2017), DAISIE - Delivering Alien Invasive Species Inventories for Europe (http://www.europe-aliens.org/, 2017), NOBANIS - European Network on Invasive Alien Species (https://www.nobanis.org/, 2017) and OBIS Ocean Biogeographic Information System (http://www.iobis.org/, 2017)). In the case of the Great Lakes-St. Lawrence River region, data were collected from GLANSIS - Great Lakes Aquatic Nonindigenous Information System (http://nas.er.usgs.gov/, 2017). The primary dataset including non-indigenous species and their life history traits is available at https://doi.org/10.1594/PANGAEA.887396.

Results

Life history traits in the conceptual model

In our conceptual model, r-reproduction and several broods per year were hypothesized as associated with NIS entrainment in a transport vector in their native region (i.e. transition from stage 0 to stage I; Fig.1). To survive transport and be released in a new habitat (i.e. transition from stage I to stage II), asexual and r-reproduction, dormancy, and regeneration were hypothesized as beneficial life history traits for all four transport vectors assessed in the model (i.e. aquaculture, ballast tanks, hull fouling and canal constructions), omnivorous feeding and broad physiological tolerance were beneficial for ballast tanks, hull fouling and canal constructions, and autotrophic feeding for hull fouling and canal constructions (Fig. 1). The transition from stage II to stage III (i.e. NIS establishing self-sustaining populations in a new habitat), asexual and r-reproduction, regeneration, dormancy, omnivorous, autotrophic and predatory feeding, and a broad physiological tolerance have been hypothesized as being beneficial. Finally, life history traits enhancing the spread of species in a new habitat (i.e. transition from stage III to stage IV) resulted as asexual and r-reproduction, more than one brood per year, dormancy, omnivorous, autotrophic and predatory feeding were hypothesized (Fig. 1).

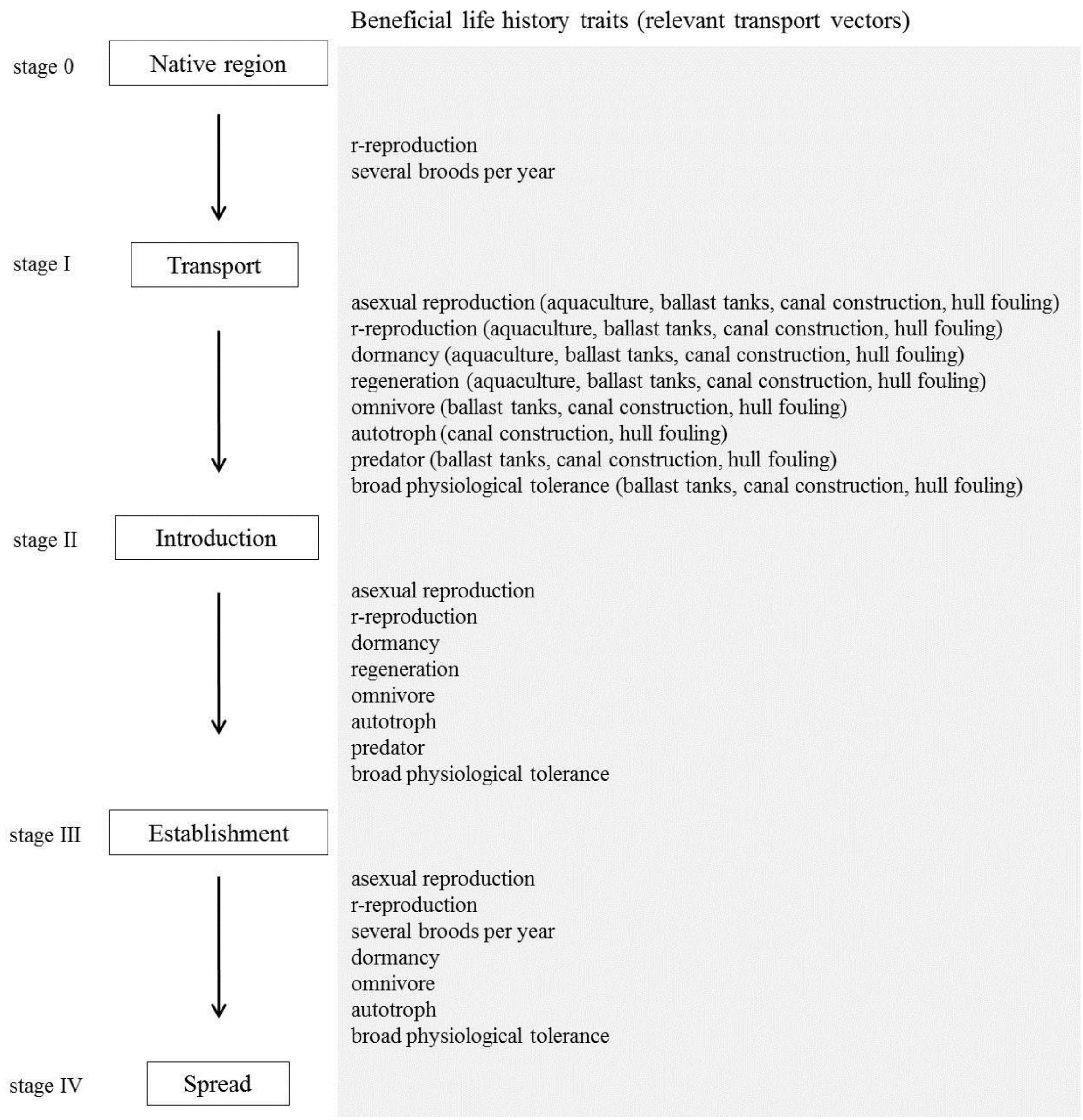

Fig 1. Conceptual model of life history traits beneficial for transition between stages of the invasion process. Potential transport vectors are assigned to each trait of the transport stage (stage I). Modified after Colautti and MacIsaac (2004).

Life history traits of established and spreading NIS

Seven life history traits were analyzed for 281 NIS established in saline (i.e. the North and Baltic Seas) and 188 in freshwater (i.e. the Great Lakes-St. Lawrence River) habitats. Reproduction mode was similar in both regions with almost equal percentages of sexual and asexual/sexual species (i.e. 57.7 and 35.9% of sexual and asexual/sexual species in the saline, and 40.4 and 49.5% in the freshwater habitat, respectively; Fig. 2). While the number of broods per year was unknown for the majority of species in both regions (i.e. 80.8 and 79.3% for the saline and freshwater habitat, respectively), 12.5 and

16.5% of species had more than one brood per year, respectively (Fig. 2). r-strategy was the predominant type of reproduction in both regions with 94.4 and 98.4% of species, respectively. The ability to produce dormant stages was unknown for many species (i.e. 89.7 and 48.4% for the saline and freshwater habitat, respectively); however, those for which this trait was determined, dormancy was far less important in saline than freshwater habitats (i.e. 9.6 and 51.6%, respectively; Fig. 2). Regeneration capability was determined for 35.2 and 43.6% of NIS in the saline and freshwater habitats, respectively; for the remaining species, capability of regeneration was not specified (Fig. 2). The three biggest groups of feeding types were autotrophs (i.e. 21.4 and 43.6% for the saline and freshwater habitat, respectively), omnivores (37 and 16%, respectively) and carnivores (21.1 and 21.2%, respectively; Fig. 2). The majority of species in both regions were non-predaceous (i.e. 80.4 and 83.5% for the saline and freshwater habitat, respectively), with fishes representing the majority of predators in both regions (Fig. 2). In summary, life history traits were associated with an r-strategy and regeneration capability in both types of habitats at the establishment stage, while dormancy was important only in freshwater habitats (Fig. 2). Asexual and asexual/sexual reproduction, number of broods per year, and different feeding types were equally important at the establishment stage in both habitats, while predation was not in any habitat (Fig. 2).

When life history traits were assessed for major phyla at the establishment stage, sexual reproduction was dominant for Arthropoda, Mollusca and Chordata in saline and freshwater habitats (i.e. 91.3 and 75%, 93.3 and 94.4%, 97.9 and 100%, respectively), and for Platyhelminthes in the freshwater habitat (100%; Fig. 2). Both sexual and asexual/sexual reproduction were similarly represented for Annelida in both habitats (i.e. 59.3 and 40.7%, 33.3 and 16.7%, respectively) and for Platyhelminthes in the saline habitat (62.5 and 37.5%), while asexual/sexual reproduction was dominant for Ochrophyta and Tracheophyta in both habitats (i.e. 100% for both in both habitats; Fig. 2). The number of broods was unknown for the majority of phyla, but more than one brood was reported for Ochrophyta in both habitats (80.8 and 100%, respectively; Fig. 2). r-strategy was characteristic (i.e. 100%) for all phyla except Chordata in the saline environment and Platyhelminthes in the freshwater one (97.9 and 75%, respectively; Fig. 2). While dormancy was unknown for the majority of phyla, it represented 100% of species in the Tracheophyta phylum in both habitats, 50 and 89.5% of Ochrophyta in the saline and freshwater habitat, respectively, and 70.8% of Arthropoda in the freshwater habitat (Fig. 2). Regeneration was unknown for the majority of species in Arthropoda, Mollusca and Chordata. However, it represented 37.5 and 37.5% of species of Platyhelminthes, and 50 and 10.5% of Ochrophyta in the saline and freshwater habitat, respectively, and 100% of species in Annelida and Tracheophyta in both habitats (Fig. 2). Feeding type was diverse among phyla, with Ochrophyta and Tracheophyta being 100% autotrophs in both habitats. The majority of predators were Chordata (64.6 and 71% in the saline and freshwater habitat, respectively), with less representatives in Arthropoda (5.8 and 16.7% in the saline and freshwater habitat, respectively), Mollusca (16.7% in the saline habitat) and Platyhelminthes (12.5% in the freshwater habitat; Fig. 2).

Of the 281 and 188 NIS established in the North and Baltic Sea region and in the Great Lakes-St. Lawrence River region, 168 and 122 have subsequently spread to three or more new locations. Phyla with the highest percentage of spread were Tracheophyta (83.3 and 82.1% in the saline and freshwater habitats, respectively), Chordata (70.8% in the saline habitat), Annelida (59.3 and 83.3% in the saline and freshwater habitats, respectively) and Mollusca (63.3 and 72.2% in the saline and freshwater habitat, respectively). Life history traits of NIS that have spread were similar to those of NIS that established in the two regions (Figs. 2 and 3).

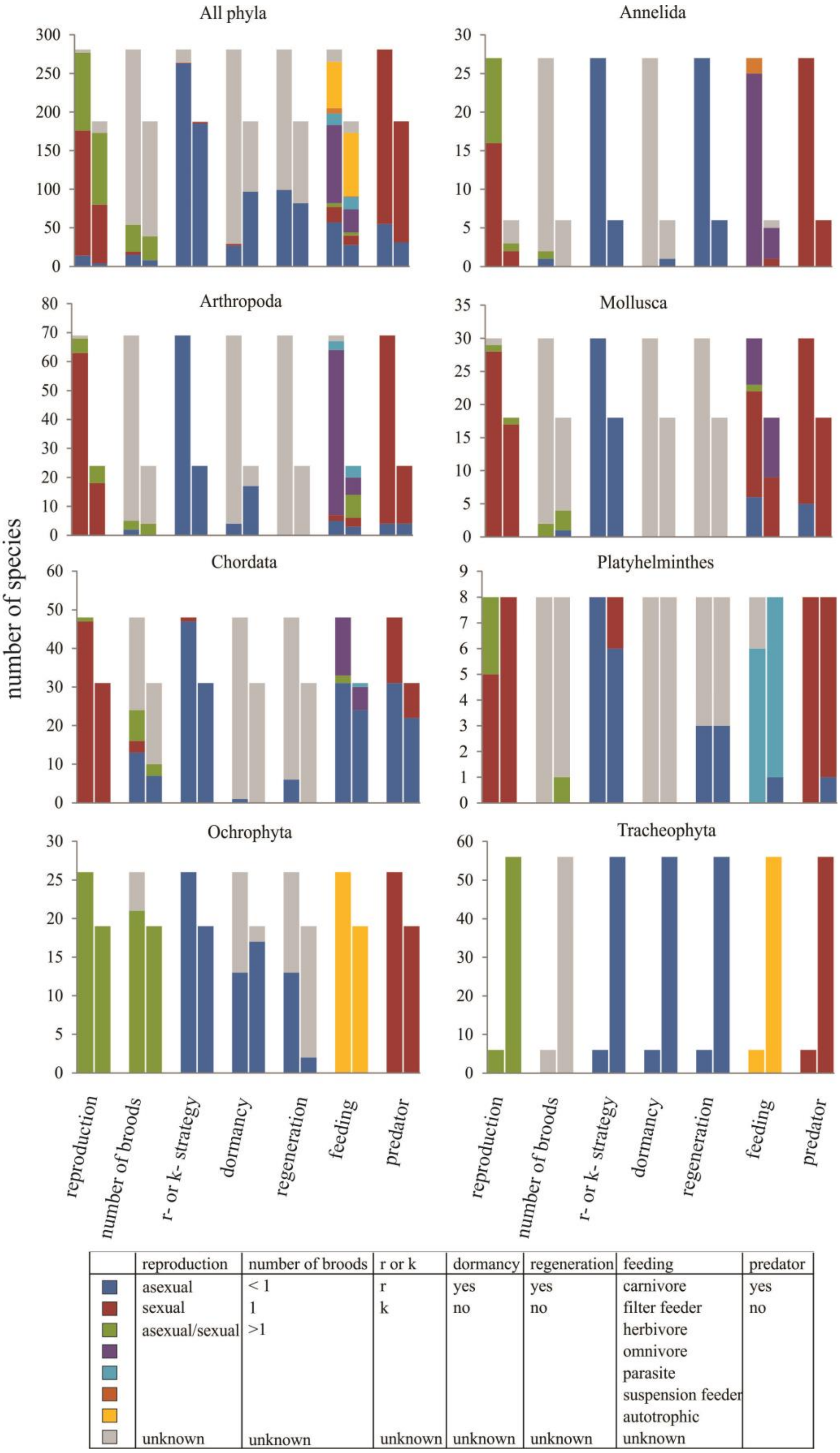

Fig 2. Life history traits of all non-indigenous species established in the North and Baltic Seas (left bar for each life history trait) and Great Lakes-St. Lawrence River region (right bar for each life history trait), and of each phylum separately (only phyla with at least three species in each region were shown).

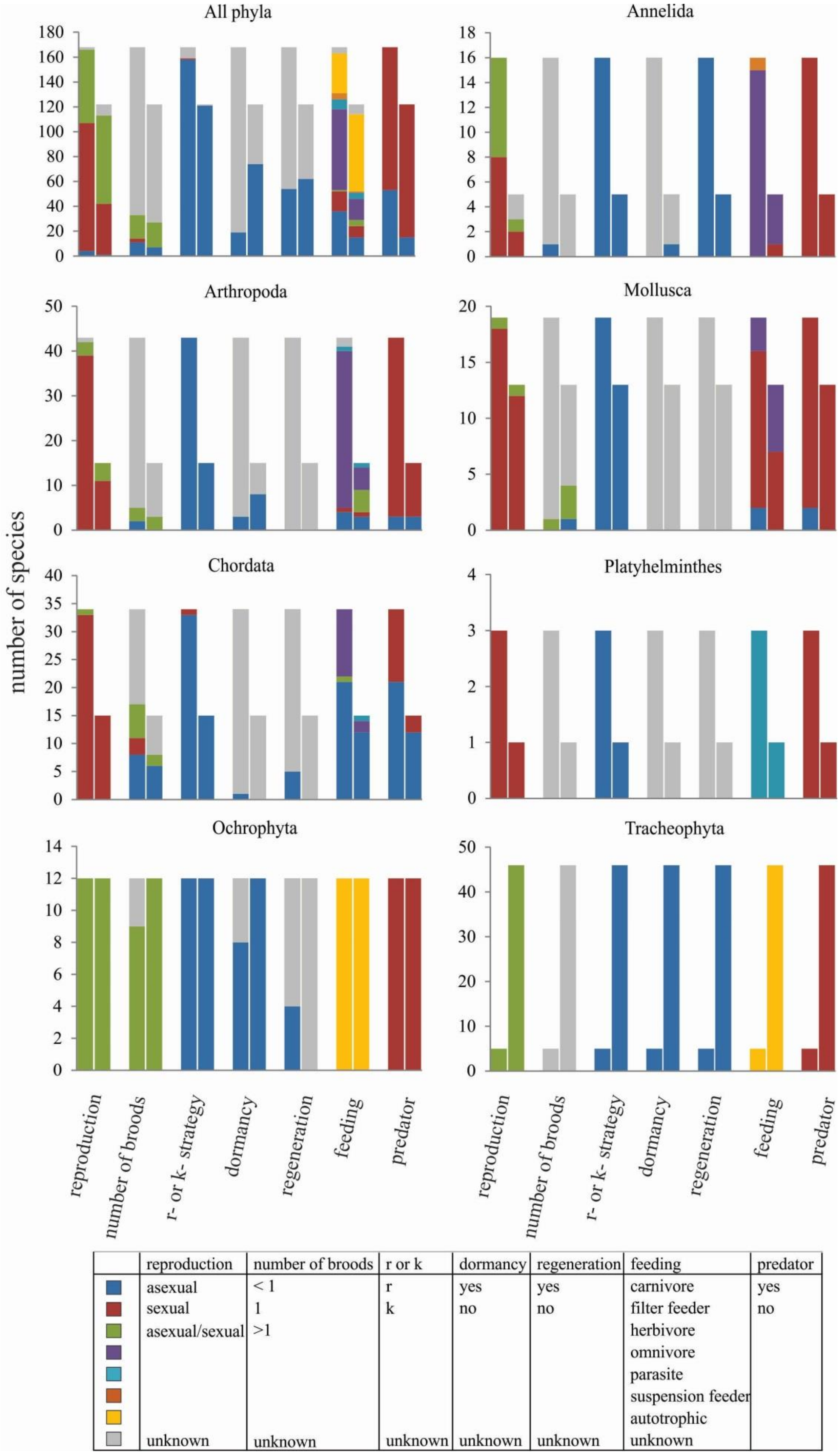

Fig 3. Life history traits of all non-indigenous species that have spread to three or more locations in the North and Baltic Seas (left bar for each life history trait) and Great Lakes-St. Lawrence River region (right bar for each life history trait), and of each phylum separately (only phyla with at least three species in each region were shown).

Discussion

Life history traits may play an important role in invasion success (Sol et al. 2012). In this work, we extended on previous studies conducted on life history traits and investigated which life history traits would be favorable during the transition through the various invasion stages in two different aquatic regions - the North and Baltic Seas, mostly saline ecosystems with several large brackish to freshwater estuaries, and the Great Lakes-St. Lawrence River region, which is predominantly a freshwater system. Approximately 60% of NIS established in these regions have also spread to three or more subsequent locations in the same regions. Our model evaluation confirmed that r-strategy, dormancy and the ability to regenerate are important traits at the establishment stage, while an r-strategy and dormancy are important to the spread stage. Feeding type was very diverse among species, though autotrophs and omnivores were dominant, which was consistent with our conceptual model. Specific life history traits that resulted as the most beneficial, when combined together, reduce demographic and environmental stochasticity during the invasion process. This finding accords with previous studies that emphasize the interaction between propagule pressure and life history traits.

Propagule pressure (Lockwood et al. 2005; Hayes and Barry 2008; Simberloff 2009) and high genetic diversity (Dlugosch and Parker 2008) have previously been identified as critical to establishment success, which is in concordance with our model and the empirical confirmation of the model as our study determined r-strategy as the most important trait for both the established and spread NIS in the systems. A number of recent studies investigated which component of propagule pressure – high propagule size (i.e. number of introduced individuals per event) or frequent propagule number (i.e. number of introduction events) – was more important to establishment success (Wittmann et al. 2014; Sinclair and Arnott 2016; Drolet and Locke 2016). It has been assumed that high propagule size would increase the probability of establishment by diminishing demographic stochasticity and Allee effects, while increased propagule number would diminish environmental stochasticity. The above mentioned studies concluded that a single introduction event with high propagule size posed a greater invasion risk than multiple introductions with lower propagule size. As the majority of species in our study were r-strategists, which are characterized by a large number of offspring, high growth rate and low parental investment in offspring, our findings are consistent with the view that a single, high-propagule size introduction event would pose a greater invasion risk. Dlugosch and Parker (2008) also identified single introductions with high genetic diversity as riskier than multiple introductions with low genetic diversity.

Finally, shipping is a major vector for species introductions to aquatic habitats (Molnar et al. 2008; Hulme 2009); ship-based introductions typically involve transport of whole communities and high abundances of species, many of which are r-strategists (Kaluza et al. 2010; Briski et al. 2013, 2014b).

Introduction events of low propagule size might also result in establishment success (Memmott et al. 2005), particularly if species can reproduce asexually. Since on average half of the species considered in our study were able to reproduce both sexually and asexually, we emphasize the importance of this trait for reducing Allee effect during early phases of establishment. We believe that those events are rare as many other components of Allee effect would still be present, such as density dependence and inbreeding depression (Courchamp et al. 2008). Species that reproduce asexually avoid inbreeding depression by different physiological processes versus their sexual counterparts (Bengtsson 2003). However, as many species in our study use both reproduction modes (i.e. asexual and sexual), multiple introduction events (i.e. high propagule number) may bring fresh genetic material to prevent inbreeding and rescue small unstable populations when each introduction event is characterized by low propagule size (Simberloff 2009). The studies of Wittmann et al. (2014) and Drolet and Locke (2016) did not consider asexual reproduction in their models, nor did Sinclair and Arnott (2016) study asexually or asexually/sexually reproducing species. Inclusion of reproductive modes in future modeling and experimental studies appears particularly important considering the high number of NIS that are capable of it.

While demographic stochasticity and Allee effect are diminished by r-strategy and asexual reproduction, environmental stochasticity can be reduced by dormancy, another important trait identified by our model. Environmental conditions during the transport stage, as well as in the new environment, may be harsh or temporally unfavourable for survival (Klein et al. 2010; Seiden et al. 2011; Simard et al. 2011). To avoid unfavourable conditions, the production of dormant, diapausing, or resting eggs, cysts or statoblasts would be advantageous as most of these stages are resistant to harsh environments (Cáceres 1997; Schröder 2005; Briski et al. 2011). Though we were not able to determine presence or absence of dormancy for the majority of marine species (i.e. established in the North and Baltic Seas), at least half of NIS in the freshwater system (i.e. the Great Lakes-St. Lawrence River) were characterized by dormant stages. In general, freshwater habitats are more prone to strong environmental fluctuations, such as complete freezing or drying out compared to marine environments, leading to evolution of dormancy in more freshwater than marine taxa (Cáceres 1997; De Stasio

2007). As dormancy is the ability to stay in a temporarily inactive highly resistant stage until environmental conditions improve (Cáceres 1997; Gyllström and Hansson 2004; Briski et al. 2011), our findings suggest that freshwater and estuarine habitats might be at higher invasion risk than marine ones. This risk may be exacerbated when one considers that the majority of shipping ports worldwide are located in rivers and river mouths (Kaluza et al. 2010).

The transport stage may be very harsh (Klein et al. 2010; Seiden et al. 2011; Simard et al. 2011), and transported individuals are often physically injured before they are introduced to a new habitat (Briski et al. 2011, 2014a). Therefore, regeneration - the ability to renew lost appendages and/or body parts - might be of great importance. More than one third of species in both regions are capable of regeneration. However, we were not able to determine from available data if many species possess this trait. Furthermore, regeneration is a very energy-demanding process (Fielman et al. 1991), and might be difficult during periods of food limitation, such as during transport. It is possible that regeneration may not only save the injured individual but also increase the number of introduced individuals (i.e. propagule size). One example is the ctenophore *Mnemiopsis leidyii* (Agassiz, 1865), which has the capacity to regenerate to such extent that when the body is cut in half both damaged parts can become whole functioning individuals again (Henry and Martindale 2000). Unfortunately, there is a lack of knowledge about regeneration capability for many species, and further studies are needed to improve the understanding of that trait in the invasion process.

Our study determined that three life history traits - r-strategy, dormancy and the ability of regeneration - could reduce demographic and environmental stochasticity during the invasion process. This finding accords with previous work emphasizing the interaction between propagule pressure and life history traits (Colautti et al. 2006; Jeschke and Strayer 2006; Blackburn et al. 2015). We were not able to evaluate beneficial traits for the transport and introduction stages, and emphasize that some of the traits that proved beneficial at the establishment and spread stages may also be necessary for the first two stages. Furthermore, the prediction of potential NIS might be complicated if rapid evolution of favoured life history traits occurs in NIS populations (Bøhn et al 2004; Tayeh et al. 2015), where properties of the environment, community and species are important keys in a successful transition across invasion stages (Hayes and Barry 2008; Rosecchi et al. 2001). In addition, environmental tolerance of species is of great importance; however it is not explored in our study. For example the notorious invaders the freshwater mussels *Dreissena* spp. and *Limnoperna fortune* (Dunker, 1857), have similar life history traits, but established in different geographic regions in spite of high shipping frequency among the regions (i.e., northern and southern hemisphere, respectively; Karatayev et al. 2007; Kaluza et al. 2010), which might be due to differences in their mineral requirements. Early invasion stages are important for NIS management (Kolar and Lodge 2001), and even though we cannot state with confidence that our identified most beneficial traits for late stages of invasion are also important earlier on, they ought to be considered in future management efforts. Finally, while our study covered only aquatic environments, we believe that the finding can be extrapolated to terrestrial habitats as the majority of NIS in latter habitats are plants and insects (DAISIE 2017), which are characterized by the same three beneficial life history traits identified here (Nabors 2003; Ruppert et al. 2004).

Authors' contributions

I.C. and E.B. designed the study, I.C. collected the data, I.C. and E.B. drafted the manuscript. Both authors revised the manuscript and gave final approval for publication.

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Chapter III

Will non-indigenous species win under global warming scenario?

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Abstract

Numerous regions worldwide are highly impacted by anthropogenic activities and globalization, with climate change and species introductions being among the greatest stressors to biodiversity and ecosystems. A main donor region of non-indigenous species (NIS) for numerous European water bodies, as well as in the North American Great Lakes is the Ponto-Caspian region (i.e., Black, Azov and Caspian Seas), with some of those species having significant impact on local communities and ecosystem functioning. To determine environmental tolerance and probability of replacement of native species by related NIS under current and future global warming scenario of the Baltic Sea, we conducted common garden experiments to test temperature tolerance of three euryhaline gammarid species: one Baltic Sea (*Gammarus oceanicus*), one Ponto-Caspian (*Pontogammarus maeoticus*) and one North American species (*Gammarus tigrinus*) in two different salinities. Our results determined that mortality of *P. maeoticus* in all temperature treatments (i.e. increased, control, and decreased) at the end of both experiments (i.e. conducted in salinities of 10 and 16 g/kg) was lower when compared to mortality of *G. oceanicus* and *G. tigrinus*. The highest mortality was observed for *G. oceanicus*, reaching 100% in both experiments in the increased temperature treatment. Due to the high environmental tolerance of the Ponto-Caspian species tested in this study, in addition to the history of invasions and ecological impact of Ponto-Caspian taxa in the Baltic Sea, as well as geological history of the Ponto-Caspian region, we suggest that Ponto-Caspian taxa might have inherent advantages over native species and species from other regions in the Baltic Sea. Consequently, this may lead to new invasions from the Ponto-Caspian region, which in the best case scenario may increase biodiversity of the Baltic Sea. However, if notorious invaders arrive, they may have significant impact on local communities and ecosystem functioning.

Introduction

Ecosystems all over the world are progressively invaded by non-indigenous species (NIS) due to globalization and climate change (Lockwood *et al*., 2007; Hellmann *et al*., 2008; Hulme *et al*., 2008; Hulme, 2009; Capinha *et al*., 2015). A main donor region of NIS for numerous European water bodies as well as in the North American Great Lakes is the Ponto-Caspian region (i.e., Black, Azov and Caspian Seas; Ricciardi & MacIsaac, 2000; Leppäkoski *et al*., 2002; Casties *et al*., 2016). As Ponto-Caspian species established in the North and Baltic Sea and Great Lakes-St. Lawrence River region in much higher numbers than expected based on introduction effort (i.e. shipping frequency) and environmental conditions between these regions, it has been suggested that Ponto-Caspian taxa are inherently better colonizers than species from other regions (Ricciardi & MacIsaac, 2000; Leppäkoski *et al*., 2002; Casties *et al*., 2016). Some of those Ponto-Caspian species, such as the zebra mussel *Dreissena polymorpha*, the amphipod *Echinogammarus ischnus* and the round goby *Neogobius melanostomus*, can have high impact on local communities and ecosystem functioning (Dermott *et al.*, 1998; Ricciardi *et al*., 1998; Charlebois *et al*., 2001; Ojaveer *et al*., 2015). Changes in species communities and environments may open new niche opportunities for continuously introduced species and might be enhanced by predicted future climate change (IPCC, 2014; Holopainen *et al*., 2016).

Beside introduction of NIS, other anthropogenic stressors have serious impact on biodiversity, ecosystems and environment, such as eutrophication and loss of habitats, as well as high emissions of greenhouse gases that change the global climate drastically (Sala *et al*., 2000; IPCC, 2014). Several previous studies determined temperature and salinity having strong influence on the distribution of organisms in aquatic environments (Sutcliffe, 1968; Neuparth *et al*., 2002; Einarson, 1993; Delgado *et al*., 2011).Therefore, one of the major concerns is the increase in sea surface temperature that potentially impairs growth, reproduction and survival of organisms and might lead to migrations of taxa to new and more favorable habitats (Bulnheim, 1979; Sainte-Marie, 1991; Neuparth *et al*., 2002). As global climate models predict warming and acidification of the oceans worldwide (IPCC, 2014) while species’ resistance to multiple stressors facilitates establishment of NIS in new habitats, it is of great importance to investigate species’ environmental tolerance limits, and in particular in regard to introduction of NIS.

The Baltic Sea is a highly variable brackish water body which has already been invaded by 125 NIS (Casties *et al*., 2016), and will most likely be subject to future climate change. An increase in average surface water temperature by approximately 2 - 3 °C and reduction in average salinity by approximately 2 g/kg have been predicted by the end of the twenty-first century (IPCC, 2007, 2014; Gräwe *et al.*, 2013; Holopainen *et al*., 2016). Native species in the Baltic Sea have a high phenotypic plasticity and adaptation capacity, however, some of their populations often inhabit areas characterized by environmental conditions at the limit of their environmental tolerance (Arndt, 1989; Gräwe *et al*., 2013). Amphipods are important keystone species in the Baltic Sea and other aquatic ecosystems where they often appear in high abundance in shallow coastal zones (Gerhardt *et al*., 2011). They constitute a significant diet source for many zoobenthivorous fish and waterbirds. Furthermore, amphipods are successful invaders and known NIS in many regions worldwide due to their wide trophic range, high reproductive capacity and migration ability (Gerhardt *et al*., 2011). The replacement of native amphipods by a related NIS has been well documented in Europe and the Great Lakes (Pinkster *et al*., 1977, 1992; Witt *et al*., 1997; Dermott *et al*., 1998; Kotta *et al*., 2011, 2013; Jänes *et al*., 2015). They are osmoregulaters able to maintain intracellular ion and osmolyte homeostasis at varying external water conditions, yet restricted by their individual salinity limits (Werntz, 1963).

To determine environmental tolerance and probability of replacement of native species by related NIS under current and future global warming scenario of the Baltic Sea, in this study we conducted common garden experiments to test stress tolerance of three euryhaline gammarid species. We tested temperature tolerance of one Baltic Sea (*Gammarus oceanicus*), one Ponto-Caspian (*Pontogammarus maeoticus*) and one North American species (*G. tigrinus*) in two different salinities (i.e. 16 g/kg - ambient salinity of *G. oceanicus*, and 10 g/kg - ambient salinity of *P. maeoticus* and *G. tigrinus*). To determine performance of these species during the experiments, we followed mortality and conducted RNA/DNA ratio measurements on tested individuals. We tested the hypotheses that there is no difference in temperature tolerance: i) among different species in salinity of 10 g/kg (i.e. ambient salinity of NIS); ii) among different species in salinity of 16 g/kg (i.e. ambient salinity of native species); and iii) among different treatments within each species (i.e. increased, control, and decreased temperature treatments).

Material and Methods

Specimen collection

Three species were collected, transported in their ambient water to GEOMAR in Kiel, Germany, and acclimatized before the experiments were conducted. Two species were collected in their native range (*G. oceanicus* - Kiel Fjord, Western Baltic Sea, Germany and *P. maeoticus* - the South Caspian Sea, Iran), and one in its non-indigenous range (*G. tigrinus* - Dassow Bay, Germany). Specimens of *P. maeoticus* were collected in October 2014, while those of *G. oceanicus* and *G. tigrinus* in May 2015 and May 2016, respectively. The choice of the species was based on their native and non-indigenous status: i) *G. oceanicus* is native to the Baltic Sea, where it occurs in its entire area in salinities from 2 to 30 g/kg. It is also widespread along the coasts of the North Atlantic up to subarctic regions (Segerstråle, 1947; Bulnheim, 1979). It was not reported outside its native range (Paiva *et al*., 2018); ii) *P. maeoticus* is native to the Ponto-Caspian region in salinities from 0.5 to 30 g/kg (Paiva *et al*., 2018). It is NIS in freshwater areas of Turkey, but not in the Baltic Sea (Ozbek, 2011; Casties *et al*., 2016); and *G. tigrinus* is native to the Atlantic coast of North America in salinities from < 0.5 to 30 g/kg (Paiva *et al*., 2018), while it is NIS in Northern Europe including the Baltic Sea (Daunys & Zettler, 2006). During the period until the experiments commenced, all three species were kept at the temperature and salinity of their respective collection sites (*i.e*., 16 °C, 16 g/kg for *G. oceanicus*, 18 °C, 10 g/kg for *P. maeoticus*, and 16 °C, 10 g/kg for *G. tigrinus*). The water used was filtered water from the surrounding area of the institute (i.e. Kiel Fjord; filtered through 5 µm filter); salinity was adjusted by adding artificial seawater (Instant Ocean®) or potable tap water.

Laboratory experiments

Two common garden experiments were performed in the GEOMAR facilities in June 2016 and September 2016. The first experiment was conducted in a salinity of 10 g/kg giving an advantage to *P. maeoticus* and *G. tigrinus*, while the second one was conducted in 16 g/kg giving an advantage to *G. oceanicus*. The experimental design of both experiments consisted of three temperature treatments: i) control; ii) increased; and iii) decreased. Each treatment in each experiment was tested in four replicates, containing 14 adult individuals of each species per replicate in the first experiment and 13 individuals in the second experiment. Three 1-litre beakers having mesh on two vertical sides to allow water exchange, each containing one species, were submerged in a common 8-litre tank (i.e. one replicate). Species were separated in 1-litre beakers to prevent cannibalism among different species. Both experiments started from 16 °C. During the experiments, the temperature was increased/ decreased by 2 °C every three days until reaching 26 and 6 °C respectively. Mortality was checked daily throughout the experiments. When the values of 26 and 6 °C were reached, mortality was followed for two more weeks in these temperature conditions. Water in each 1-litre beaker and a common 8-litre tank was exchanged every three days for the first two weeks using temperature pre-equilibrated water. Afterwards the water was exchanged once per week until the end of the experiments. During the experiments, each 1-litre beaker was continuously aerated, animals were fed ad libitum with *Fucus vesiculosus*, *Ulva lactuca* and a mixture of algae flakes, while the light/dark cycle was 12:12 hours. Temperature and salinity were measured daily using a WTW Cond 3110 salinometer and a Tetracon 325 probe (Xylem Analytics Germany Sales GmbH & Co. KG, WTW, Germany). Primary dataset containing experimental results is available at: XXX.

RNA/DNA ratio measurements

As fitness level of animals under challenging environmental conditions can be characterized by the RNA/DNA ratio as a biochemical variable to measure growth in many marine organisms including amphipods (Moss, 1994; Malzahn *et al*., 2003; Chícharo & Chícharo, 2008; Tong *et al*., 2010), beside mortality we conducted RNA/DNA measurements to determine the performance of our species. Therefore, at the end of the experiments, all survived individuals were stored at -20 °C. The specimen were prepared for the measurement by dry freezing for 18 h (Alpha1-4 freeze dryer, Christ GmbH, Germany) and subsequently weighed using a microbalance (SC2, Sartorius AG, Germany). Dried tissue was extracted from the exoskeleton and quantification of RNA and DNA was performed following the method of Malzahn *et al*. (2003). To obtain reference values for the evaluation of the nutritional condition for each species, an additional food experiment was conducted where animals were kept in their natural salinity and either fed with algae ad libitum or kept under food deprivation for 18 days. This reference experiment was conducted in five replicates per species (i.e. one individual per replicate to prevent cannibalism among individuals), the temperature of 16 °C, and ambient salinity of each species. All surviving individuals were used for RNA/DNA measurements.

Statistical Analysis

We tested for differences in the onset and rate of mortality between species in each treatment and between treatments for each species. The increased treatment was compared to the decreased treatment of the same species to determine if the species equally tolerates higher and lower temperature stress. To test for differences between and within species, a mortality curve was constructed for each species for each temperature treatment using data from the four replicates, described by the equation (Briski *et al*., 2008, 2011):

$$y=100/1+e^{-Z(t-Q)} \tag{1}$$

where Z is the slope of the mortality rate, t is temperature and Q is the onset of mortality. The model was then expanded to compare the slope and onset of the mortality rate between two curves using the equation:

$$y=100/1+e^{-(Z_1+Z_2)(t-Q_1-Q_2)} \tag{2}$$

where Z_1 and Z_2 are the slopes of the mortality rates and Q_1 and Q_2 the onsets of mortality, for the first and second curves respectively. All pairs of curves were statistically compared by the Fit Nonlinear Model using Generalized Least Squares. Significance levels for statistical comparisons of estimated parameters Z_1 and Z_2, and Q_1 and Q_2, were adjusted for multiple pairwise comparisons by Bonferroni-type correction to guard against inflating the Type I error rate. The family-wise error rate of 0.05 was used. All tests were performed using S-Plus 6.1 (S-Plus ® 6.1, 2002; Insightful Corp., Seattle, WA, USA).

In addition, to test for differences in mortality among different species on day 17 (in the middle of experiments) and 32 (end of experiments), a one-way analysis of variance (ANOVA) was conducted. Twelve separate tests were conducted, each for one experiment (i.e. conducted in salinities of 10 and 16 g/kg), one temperature treatment (i.e. increased, control, and decreased), and one time point (i.e. after 17 and after 32 days). Additionally, post hoc Bonferroni tests were applied. Finally, to test for differences in the RNA/DNA ratios among temperature treatments (i.e. increased, control and decreased), additional ANOVA was conducted. Four different tests were conducted, each for one experiment (i.e. conducted in salinities of 10 and 16 g/kg) and one species (i.e. *P. maeoticus* and *G. tigrinus*). In the case of *G. oceanicus*, independent *t*-test was applied as all individuals in the increased treatment died until the end of the experiments. Two separate tests were conducted, one for the experiment conducted in a salinity of 10 g/kg and one for the experiment in16 g/kg. The RNA/DNA reference values of each species were tested using again independent *t*-test. Two separate tests were conducted, each for one species (i.e. *G. oceanicus* and *P. maeoticus*). In the case of *G. tigrinus*, independent *t*-test was not applied as only one individual survived the end of the reference experiment in the food deprived treatment. ANOVAs and independent *t*-tests were performed using SYSTAT® version 11 (SYSTAT Software 2004).

Results

Mortality of experimental individuals

In general, average mortality of *P. maeoticus* in all treatments (i.e. increased, control, and decreased) at the end of both experiments (i.e. conducted in salinities of 10 and 16 g/kg) was lower when compared to average mortality of *G. oceanicus* and *G. tigrinus* (Table 1; Fig. 1). The highest mortality was observed for *G. oceanicus*, reaching 100% in both experiments in the increased temperature treatment (Table 1; Fig. 1). In general, mortalities of all three species were highest in the increased than in the control and decreased treatments (Table 1; Fig. 1). In the middle of the experiment conducted in a salinity of 10 g/kg in both the increased and decreased temperature treatments, mortality of *G. tigrinus* was significantly higher than those of *G. oceanicus* and *P. maeoticus* ($p < 0.05$; Table 2; Fig. 1). The onset of mortality of *G. tigrinus* started significantly faster in all three temperature treatments compared to the other two species ($p < 0.05$; Table 3; Fig. 1). In the increased temperature treatment, however faster mortality rate of *G. oceanicus* and *P. maeoticus* than that of *G. tigrinus* ($p < 0.05$; Table 3; Fig. 1), resulted in similar mortality among all three species at the end of experiment ($p > 0.05$; Table 2; Fig. 1). But, due to slower mortality rates of the former two species in the control and decreased temperature treatments (Table 3; Fig. 1) there was significant difference among all three species at the end of the experiment, with *G. tigrinus* having the highest and *P. maeoticus* the lowest mortality ($p < 0.05$; Table 2; Fig. 1). When the increased temperature treatment was compared to the decreased treatment for each species, both the onset of mortality and mortality rates were significantly different for *G. oceanicus* and

P. maeoticus (*p* < 0.05; Table 4; Fig. 1), demonstrating that these two species better tolerate lower than higher temperature stress in 10 g/kg salinity experiment. In the case of *G. tigrinus*, there was difference only in the onset of mortality but not in the rate of mortality, indicating that *G. tigrinus* is equally sensitive to both lower and higher temperature stress (Table 4).

In the middle of the second experiment (i.e. conducted in a salinity of 16 g/kg) in the increased temperature treatment, mortality of *P. maeoticus* was significantly lower than those of *G. oceanicus* and *G. tigrinus* (*p* < 0.05; Table 2; Fig. 1). In the control temperature treatment, mortality of *P. maeoticus* was significantly lower than that of *G. tigrinus*, while in the decreased treatments mortality of *P. maeoticus* was significantly lower than that of *G. oceanicus* (*p* < 0.05; Table 2; Fig. 1). The onset of mortality was significantly different among all three species in all treatments, with that of *P. maeoticus* being the slowest in the increased and decreased treatments, and that of *G. oceanicus* in the control treatment (*p* < 0.05; Table 3; Fig. 1). The mortality rate was also significantly different among all three species in all treatments (*p* < 0.05; Table 3; Fig. 1), resulting in significantly lower mortality of *P. maeoticus* in the increased and decreased treatments at the end of the experiment when compared to *G. oceanicus* and *G. tigrinus* (*p* < 0.05; Table 2; Fig. 1). In the control treatment, mortality of *G. tigrinus* was significantly higher than those of *G. oceanicus* and *P. maeoticus* (*p* < 0.05; Table 2; Fig. 1). When the increased treatment was compared to the decreased treatment for each species, both the onset of mortality and mortality rates were significantly different for *G. oceanicus* and *G. tigrinus* (*p* < 0.05; Table 4; Fig. 1), demonstrating that these two species better tolerate lower than higher temperature stress in 16 g/kg salinity. In the case of *P. maeoticus*, there was difference only in the onset of mortality but not in the rate of mortality, indicating that *P. maeoticus* equally tolerates both lower and higher temperature stress (Table 4).

Table 1. Average percentage mortality of three gammarid species at the end of experiments conducted in 10 and 16 g/kg salinity in the increased, control and decreased temperature treatments.

Species	Salinity [g/kg]	Mortality after 32 days [%]		
		Increased	Control	Decreased
Gammarus oceanicus	10	100	48	38
	16	100	44	40
Pontogammarus maeoticus	10	70	29	34
	16	31	40	23
Gammarus tigrinus	10	75	75	66
	16	85	69	46

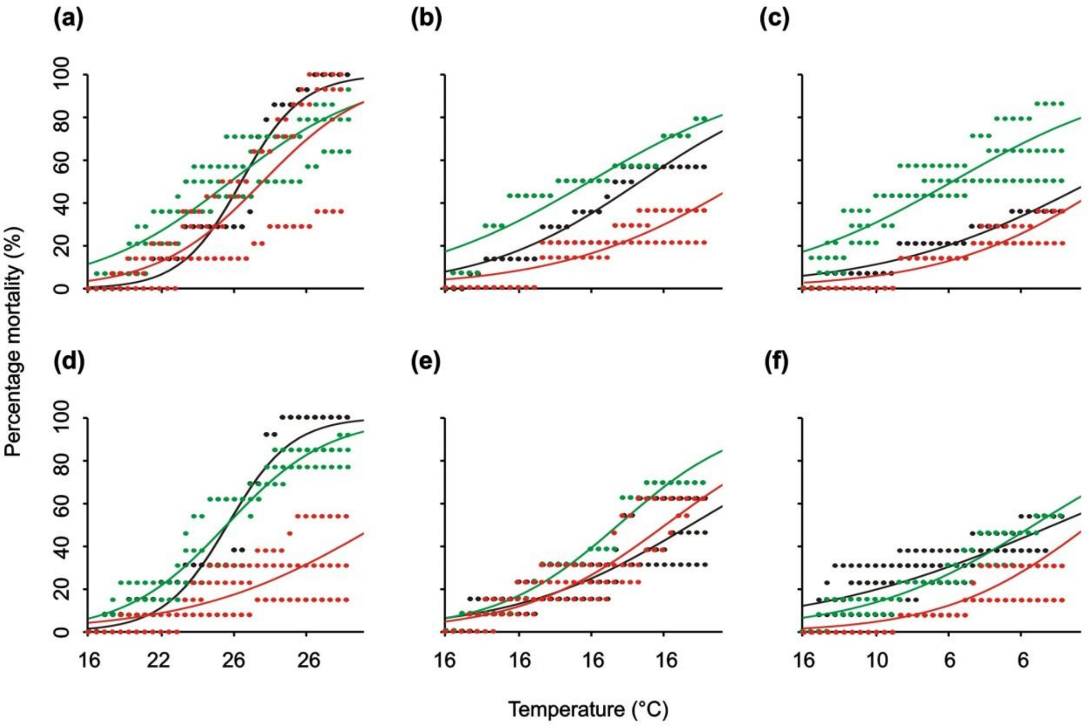

Fig. 1 Mortality rates (%) of three gammarid species: *G. oceanicus* (black), *G. tigrinus* (green) and *P. maeoticus* (red) in experiments conducted in 10 g/kg salinity in increased (a), control (b) and decreased temperature treatment (c), and in experiments conducted in 16 g/kg salinity in increased (d), control (e) and decreased temperature treatment (f). The curves were constructed using pooled data from four replicates.

Table 2. Results of analyses of variance (ANOVAs) addressing differences in mortality among three gammarid species after 17 and 32 days of experiments conducted in 10 and 16 g/kg salinity in increased, control and decreased temperature treatments.

Experiment	Temperature treatment	Day 17			Day 32		
		df	*F*	*p*	Df	*F*	*p*
Salinity of 10 g/kg	Increased	11	8.491	**0.008**	11	2.637	0.125
	Control	11	11.226	**0.004**	11	15.566	**0.001**
	Decreased	11	72.894	**< 0.0001**	11	10.425	**0.005**
Salinity of 16 g/kg	Increased	11	6.996	**0.015**	11	49.573	**< 0.0001**
	Control	11	6.125	**0.021**	11	4.956	**0.035**
	Decreased	11	9.863	**0.005**	11	5.870	**0.023**

Table 3. Statistical comparisons of parameters between pairs of fitted curves for the species in experiments conducted in 10 and 16 g/kg salinity in increased, control and decreased temperature treatments. The *t*-test incorporated in the Fit Nonlinear Model using Generalized Least Squares was used to test for differences between estimated parameters - rate of mortality (Z_1 and Z_2) and onset of mortality (Q_1 and Q_2). Significant *p*-values are presented in bold. Bonferroni-type protection to guard against inflating the Type I error rate and family-wise error rate of 0.05 were used for pairwise statistical comparisons.

Species compared	Salinity [g/kg]	Temperature treatment	*p* -value of mortality onset	*p* -value of mortality rate
Gammarus oceanicus - Pontogammarus maeoticus	10	Increased	**0.0001**	**< 0.0001**
		Decreased	0.4741	0.0602
	16	Increased	**< 0.0001**	**< 0.0001**
		Decreased	**< 0.0001**	**0.0068**
Gammarus oceanicus - Gammarus tigrinus	10	Increased	**< 0.0001**	**< 0.0001**
		Decreased	**< 0.0001**	0.2819
	16	Increased	**< 0.0001**	**< 0.0001**
		Decreased	**0.0239**	**< 0.0001**
Gammarus tigrinus - Pontogammarus maeoticus	10	Increased	**< 0.0001**	**0.002**
		Decreased	**< 0.0001**	0.3091
	16	Increased	**< 0.0001**	**< 0.0001**
		Decreased	**< 0.0001**	**0.0033**

Table 4. Statistical comparison of parameters between pairs of fitted curves for the different temperature treatments for each species in experiments conducted in 10 and 16 g/kg salinity. The increased treatment was compared to the decreased treatment of the same species to determine if the species equally tolerates higher and lower temperature stress. The *t*-test incorporated in the Fit Nonlinear Model using Generalized Least Squares was used to test for differences between estimated parameters - rate of mortality (Z_1 and Z_2) and onset of mortality (Q_1 and Q_2). Significant *p*-values are presented in bold. Bonferroni-type protection to guard against inflating the Type I error rate and family-wise error rate of 0.05 were used for pairwise statistical comparisons.

Species	Salinity [g/kg]	Temperature treatments compared	*p* -value of mortality onset	*p* -value of mortality rate
Gammarus oceanicus	10	Control - Increased	**< 0.0001**	**< 0.0001**
		Control - Decreased	**< 0.0001**	0.3378
		Increased - Decreased	**< 0.0001**	**< 0.0001**
	16	Control - Increased	**< 0.0001**	**< 0.0001**
		Control - Decreased	0.1300	**0.0016**
		Increased - Decreased	**< 0.0001**	**< 0.0001**
Pontogammarus maeoticus	10	Control - Increased	**< 0.0001**	**0.0004**
		Control - Decreased	0.0744	0.1802
		Increased - Decreased	**< 0.0001**	**0.0049**
	16	Control - Increased	0.1300	0.3378
		Control - Decreased	**0.0003**	0.7319
		Increased - Decreased	**0.0316**	0.1815

Gammarus tigrinus	10	Control - Increased	**< 0.0001**	**0.0007**
		Control - Decreased	**< 0.0001**	**0.0011**
		Increased - Decreased	**< 0.0001**	0.2685
	16	Control - Increased	0.1300	**0.0016**
		Control - Decreased	**< 0.0001**	**< 0.0001**
		Increased - Decreased	**< 0.0001**	**< 0.0001**

RNA/DNA ratio measurements

At the end of the common garden experiments, the average RNA/DNA ratios ranged from 2.1 to 3.8, from 1.6 to 3.7, and from 2.4 to 3.9 in the increased, control and decreased treatments, respectively (Fig. 2). There was no significant difference among tested treatments (i.e. increased, control and decreased) for any species in any experiment (i.e. conducted in 10 g/kg and 16 g/kg; $p > 0.05$).

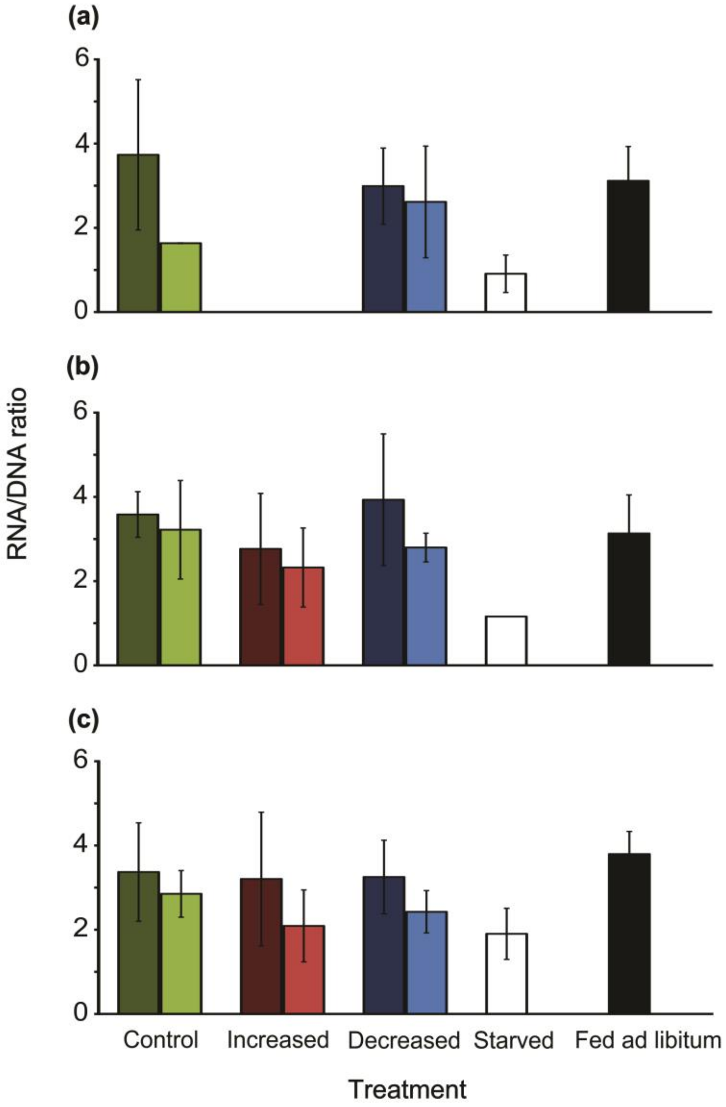

Fig. 2 RNA/DNA ratios of *G. oceanicus* (a), *G. tigrinus* (b) and *P. maeoticus* (c) at the end of experiments conducted in 10 (left bar) and 16 g/kg salinity (right bar) in the control, increased and decreased temperature treatments, as well as in the reference treatments (i.e. starved and fed ad libitum). The error bars show the standard deviation.

During the reference experiment, out of five individuals tested four, five and one individual of *G. oceanicus*, *P. maeoticus* and *G. tigrinus* survived in the food deprived treatment, respectively, and five, four and five in the fed ad libitum treatment. The average RNA/DNA ratio of *G. oceanicus* and *P. maeoticus* in the food deprived treatment were 0.9 and 1.9, respectively, while those in the fed ad libitum treatment were 3.1 and 3.8 (Fig. 2). The RNA/DNA ratio of *G. tigrinus* in the food deprived treatment was 1.1, and the average RNA/DNA ratio in the fed ad libitum treatment was 3.1 (Fig. 2). RNA/DNA ratios of the fed and starved treatments were significantly different ($p < 0.05$) for *G. oceanicus* and *P. maeoticus*. The independent *t*-test was not performed for *G. tigrinus* as only one individual survived the food deprived treatment.

Discussion

Temperature and salinity are two important environmental parameters influencing life of aquatic organisms and limiting their distribution (Sutcliffe, 1968; Neuparth *et al*., 2002; Einarson, 1993; Delgado *et al*., 2011). In this study, we compared temperature tolerance in two different salinities of three gammarid species originating from three different regions - Northern Europe, Ponto-Caspian and Great Lakes-St. Lawrence River regions - to determine if native species would have environmental advantage over NIS under current and future global warming conditions. Our results demonstrated that the native species *G. oceanicus*, which evolved under temperatures of the Baltic Sea, performed well in the control and decreased temperature treatments, but was not able to survive in the increased temperature treatment. On contrary, the Ponto-Caspian species *P. maeoticus*, not only performed well in the ambient salinity and temperature of the Baltic Sea and in the decreased treatment, but also performed slightly better in those conditions than the native species and significantly better in the increased treatment. The second NIS, *G. tigrinus*, did not perform well in any treatment. Due to high environmental tolerance of the Ponto-Caspian species tested in this study, in addition to the history of invasions and ecological impact of Ponto-Caspian species in the Baltic Sea, as well as geological history of the Ponto-Caspian region, we suggest that Ponto-Caspian taxa might have evolutionary advantages over native species and species from other regions in environments of the Baltic Sea, which may lead to new invasions from that region. Those invasions may, in the best case scenario, increase biodiversity of the Baltic Sea. However, if notorious invaders establish, they may have significant impact on local communities and ecosystem functioning (Dermott *et al*., 1998; Ricciardi *et al*., 1998; Charlebois *et al*., 2001).

The high mortality of the native species in the increased temperature treatment indicates that *G. oceanicus* probably would not be able to survive in some locations of the Baltic Sea where water temperature would increase due to future climate change (IPCC, 2014; Holopainen *et al*., 2016). In Kiel Fjord, the sampling location of *G. oceanicus*, water temperature fluctuates during different seasons, with summer temperatures currently reaching values between 18 °C and 21 °C in 1.8 m depth (GEOMAR). In shallow coastal areas, however, where many gammarid species are found temperature is reaching even higher values. Since a sea surface temperature rise of 2 - 4 °C is predicted by the end of the century, the maximum tested temperature of 26 °C is likely to be reached in many shallow locations in the future (Holopainen *et al*., 2016). If such extreme temperatures only occur during heat waves for some days (Hobday *et al*., 2016) but not for an extended period of time, the tested species might be able to survive in those habitats. However, if those increased temperatures would last for a longer period of time, or some NIS arrive, it is questionable if *G. oceanicus* would be able to withstand those stressful environmental conditions and/or compete with newcomers. The replacement of native gammarids by a related NIS has already been documented in the Baltic Sea (Kotta *et al*., 2011, 2013; Jänes *et al*., 2015). Therefore, we advise further comparative studies using different species and taxonomic groups are urgently needed for determining environmental tolerance and competitive ability of the Baltic Sea taxa, as well as of taxa in other regions, to be able to predict diversified anthropogenic impact on the ecosystems, and in general, to preserve biodiversity globally.

The wide temperature and salinity tolerance of the Ponto-Caspian species *P. maeoticus* (results of this study, Fig. 1; Paiva *et al*., 2018), in addition to shipping and canal connectivity between Northern Europe and the Ponto-Caspian region (Bij de Vaate *et al*., 2002; Kaluza *et al*., 2010), imply that there is a potential that this species, as well as some others from the Ponto-Caspian region invade the Baltic Sea under future global warming scenario. Due to wide salinity and temperature tolerance, non- indigenous amphipods are predicted to become more widespread and abundant along the coasts of the northern Baltic Sea (Holopainen *et al*., 2016). Species originating from the Ponto-Caspian region are generally known to be successful invaders in freshwater as well as marine environments (Ricciardi & MacIsaac, 2000; Casties *et al*., 2016). In the Baltic Sea, the Ponto-Caspian region is one of the major donor regions of NIS, and out of seven non-indigenous gammarid species, six of them originate from the Ponto-Caspian area (Casties *et al*., 2016). In general, likely reason for high environmental tolerance and amazing colonizing success of species evolved in the Ponto-Caspian region may be due to the geological history of the region (Zenkevitch, 1963; Dumont, 1998; Reid & Orlova, 2002; Cristescu *et al*., 2003). The region is geologically old and underwent numerous drastic changes in sea-level, salinity and temperature, with periods when it almost completely dried out during the Glacial Maxima (Zenkevitch, 1963; Dumont, 1998; Reid & Orlova, 2002; Cristescu *et al*., 2003). The species that have persisted and thrived despite these complex changes and successions over millions of years are probably highly adaptable to diverse environmental conditions, and in particular to environmentally fluctuating habitats, making them excellent candidates to become NIS.

The Northwest Atlantic species, *G. tigrinus*, did not perform well in our laboratory experiments, having high mortality in the control treatments as well. However, the species is highly successful invader that established in majority of Northern European freshwater and brackish habitats, where in some areas it replaced native species and altered community compositions (Pinkster *et al*., 1992; Kelly *et al*., 2006; Kotta *et al*., 2011, 2013; Jänes *et al*., 2015). In addition, several previous studies reported that *G. tigrinus* can tolerate temperatures up to 30 °C in brackish environments, and it thrives in heavily polluted waters (Ruoff, 1968; Pinkster *et al*., 1977; Savage, 1982; Wijnhoven *et al*., 2003), while Paiva *et al*. (2018) determined different environmental tolerance among populations of that species and five others. In the population used in our experiments dark spots were regularly observed on animals, therefore we suspect that the population was infected by parasite or some disease, reducing the immune system of individuals. Kestrup *et al.* (2011) observed high mortality of amphipods in the St. Lawrence River caused by parasitic oomycete (water mold), while microphallid trematodes and microsporidia were also reported in amphipods (MacNeil *et al*., 2003; Mouritsen *et al*., 2005). Another explanation could be cannibalism among individuals within replicates (Wijnhoven *et al*., 2003). However, the individuals were fed ad libitum to compensate for higher metabolic activity due to increased temperatures (Gillooly *et al*., 2001), and we did not detect any signs of cannibalism during the experiments. Consequently, based on our and previous studies, we are not able to provide clear conclusions considering environmental tolerance of this species.

The amount of RNA is directly proportional to the rate of protein synthesis which is varying with changing environmental conditions, while the amount of DNA in a somatic cell is assumed to be constant (Bulow, 1987). In general, organisms in good conditions have higher RNA/DNA ratios than those in poor conditions (Bulow, 1987; Clemmesen, 1994). In our study, RNA/DNA ratios of all three species were similar among the treatments and experiments, and corresponded to the fed ad libitum values of the reference experiment. One of the reasons that we did not detect any stress using RNA/DNA ratio measurements, might be due to selection that occurred during the experiments leaving alive only the fittest individuals until the end of the experiments. In addition, the surviving individuals were exposed to same environmental conditions in the last two weeks of the experiment (i.e. 6, 16 and 26 °C), and probably acclimatized to the new environment, decreasing a chance of detecting temperature stress. Therefore, we assume that surviving individuals were in relatively good fitness and nutritional condition (Chícharo & Chícharo, 2008).

Numerous regions worldwide are highly impacted by anthropogenic activities and globalization, with climate change and species introductions being among the greatest stressors to biodiversity and ecosystems (Sala *et al*., 2000; IPCC, 2014; Capinha *et al*., 2015). Changes in ecosystems open new opportunities for continually introduced species, which are often more resistant to multiple stressors than many native species in an area, and probably preadapted to anthropogenically impacted habitats (Hufbauer *et al*., 2011; IPCC, 2014; Holopainen *et al.*, 2016). Non-indigenous species often appear to occur with little impact on the recipient community, and may even increase biodiversity in some regions (Zettler, 2008). However, approximately 5 - 20% of established NIS are predicted to have strong effects on recipient environments (Williamson & Fitter, 1996; Ricciardi & Atkinson, 2004). These species may act as predators, parasites, pathogens, or competitors of native species, and their introduction can result in dramatic changes in ecosystems (Simberloff *et al*., 2013). Therefore, with global climate models predicting warming and acidification of the oceans worldwide (IPCC, 2014), it is of great importance to mitigate anthropogenic impacts and try to prevent new introductions of species. Ponto-Caspian species established in the North and Baltic Sea and Great Lakes-St. Lawrence River region in much higher numbers than expected, so it has been proposed that Ponto-Caspian species might be inherently better colonizers than species from other areas (Ricciardi & MacIsaac, 2000; Leppäkoski *et al.*, 2002; Casties *et al.*, 2016). Identifying such donor region, with high rates of potential invaders can be valuable for management strategies to prevent new species introductions.

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Synthesis

Comparing origins of established aquatic non-indigenous species (NIS) in the North and Baltic Seas and the Great Lakes-St. Lawrence River region revealed that both systems are highly invaded by species that originate from the Ponto-Caspian region which consists of the Black, Azov and Caspian Seas (Chapter 1). I further compared observed numbers of established NIS in the two regions to expected numbers of NIS from major donor regions based on the available species pool from donor regions, frequency of shipping transit, and an environmental match between donor and recipient regions. I discovered that Ponto-Caspian taxa colonized both regions in much higher numbers than expected (Chapter 1). Regarding the taxonomic composition of NIS, the taxa distributions of the macrofauna were similar in both regions and dominated by arthropods, chordates and molluscs which reflects the biodiversity data from GBIF (Global Biodiversity Information Facility, www.gbif.org) for freshwater and marine habitats (Chapter 1). A comprehensive study of seven life history traits for each NIS revealed that certain traits such as dormancy, regeneration and r-strategy are potentially beneficial for invasion success (Chapter 2). Environmental tolerance towards changing temperatures, especially rising temperatures, was higher in the Ponto-Caspian gammarid species *Pontogammarus maeoticus* compared to *Gammarus oceanicus*, which is native to the Baltic Sea (Chapter 3). Below, I will emphasize characteristics of the Ponto-Caspian region and the difficulties of making general assumptions about species' traits that could favor invasions.

The Ponto-Caspian region as an important donor region

With progressive globalization and the consequential increase of ship traffic, invasion opportunities for aquatic species are continuously rising (Leppäkoski and Olenin 2000; Lockwood et al. 2007; Hulme 2009; Kaluza et al. 2010; Seebens et al. 2017). The North and Baltic Seas and the Great Lakes-St. Lawrence River regions are invaded to a similarly high extent by Ponto-Caspian species compared to species from other donor regions such as the Northwest Pacific, Northwest Atlantic or Eurasia (Chapter 1). In the North and Baltic Seas 15 % of discovered NIS originate from the Ponto-Caspian region, while in the Great Lakes-St. Lawrence River region it is 12 % (Chapter 1). The numbers of Ponto-Caspian species are much higher than expected considering the available species pool from donor regions, frequency of shipping transit and an environmental match between donor and recipient regions (Chapter 1). This indicates that there are other possible explanations for the invasion success of Ponto-Caspian species. Regarding the connectivity of the Ponto-Caspian region with Northern Europe, canals and rivers between the Baltic Sea and the Black and Caspian Seas can be considered as important transport vectors (Bij de Vaate et al. 2002). However, this transfer of species seems to be unidirectional since the North and Baltic Seas are not dominant donor areas for NIS in the Ponto-Caspian region (Shiganova 2011). Reasons for this are not well examined, but it can be assumed that the transport conditions or the Ponto-Caspian environment are not suitable for European species. Further, propagule pressure, which is of great importance for establishment success of NIS, might be different between the regions. To answer the question of how Ponto-Caspian species reach the Great Lakes-St. Lawrence River region the stepping stone hypothesis could be one explanation indicating a secondary introduction from Northern Europe (Ricciardi and MacIsaac 2000; Leppäkoski et al. 2002). However, neither direct transport from the Ponto-Caspian region, secondary introductions from other regions nor other transport vectors than shipping can be excluded.

In this thesis, I aimed to elucidate why so many species from the Ponto-Caspian region become successful invaders in freshwater as well as saline regions. One assumption is that these species might be evolutionary predisposed to become invaders in various environments due to fluctuating environmental conditions in their geologically old native region. The Ponto-Caspian region was formed millions of years ago and has undergone numerous extensive environmental changes from fully marine to almost completely freshwater environments and vice versa (Zenkevitch 1963; Dumont 1998; Reid and Orlova 2002).

These extensive environmental changes might have led to a high adaptation potential of those species to a wide salinity and temperature range (Bij de Vaate et al. 2002), which would be beneficial since numerous donor ports are characterized by varying salinities due to their location in transition areas from freshwater to marine regions (Sylvester et al. 2013). In laboratory studies, it was found that Ponto-Caspian species have a broad salinity and temperature tolerance, but regarding salinity their tolerance covers rather freshwater and low saline than fully marine waters (Paiva et al. 2018). The assumption that euryhaline species from the Ponto-Caspian region might be evolutionary freshwater taxa (Paiva et al. 2018) would explain why only a few of them have colonized highly saline waters such as the Mediterranean Sea (Shiganova 2010). Thus, the origin of a species plays an important role for the predisposition of successful invaders and in order to find out how this is expressed, I investigated life history traits and environmental tolerance of species from different regions.

Life history traits

The stage-based invasion model is one of the most accepted concepts in invasion ecology and consists of the four stages transport, introduction, establishment and spread (Kolar and Lodge 2001; Colautti and MacIsaac 2004; Lockwood et al. 2007; Blackburn et al. 2011). Life history traits may play an important role for species during the transition through the various invasion stages (Sol et al. 2012; Sakai et al. 2001). In this thesis, the identification of certain life history traits of established and spread NIS in the North and Baltic Seas and the Great Lakes-St. Lawrence River region revealed that r-strategy, dormancy and the ability to regenerate (i.e. the ability to renew lost appendages and/or body parts) were important traits (Chapter 2). During transport when environmental conditions might be unfavourable and species often get physically injured dormant stages or the ability to regenerate might be of advantage for survival (Cáceres 1997; Briski et al. 2011). A complete evaluation of beneficial traits for the transport and introduction stages was not possible, because of the difficulty to gather data of all species in a transport vector or at the introduction events. This would also include species that failed at one of these stages, though especially the numerous failures stay unnoticed in most cases. On the contrary, data of established and spread species are more accessible. However, one should take into account that only an excerpt of a large set of life history traits was investigated and information was lacking about traits of some NIS. This might impede general assumptions about common traits of all NIS.

Previous studies propose that NIS might not necessarily have specific beneficial life history traits in common (Kolar and Lodge 2001; Cardeccia et al. 2016). Instead, traits may be highly specific to taxonomic groups and to different habitats (Sakai et al. 2001). However, some traits are distinct for several NIS such as an earlier maturation compared to native populations e.g. for the jellyfish *Mnemiopsis leidyi* (Jaspers et al. 2017) or compared to native species e.g. for the amphipod *Pontogammarus robustoides* (Grabowski et al. 2007). For a successful transition across invasion stages several factors such as properties of the environment, community and species play important roles which might complicate the prediction of potential NIS (Bøhn et al. 2004; Tayeh et al. 2015; Hayes and Barry 2008; Rosecchi et al. 2001). Populations that are pre-adapted to the new environmental requirements most likely have an advantage (Bij de Vaate et al. 2002). Even though an environmental match is assumed to be beneficial for successful invasions, there are cases where species established in climatic environments that do not match their native region (Broennimann et al. 2007; Capinha et al. 2013). One example is the spotted knapweed *Centaurea maculosa* which tolerates climatic niche shifts (Broennimann et al. 2007). Variations in life history traits within one species and a rapid evolution of these traits are proposed as beneficial for a successful transition across invasion stages (Tayeh et al. 2015; Jaspers et al. 2017).

The reasons for invasion success are not fully understood and ongoing research interests are exploring why some species become non-indigenous, while others with similar properties do not. One obstacle is that NIS can behave differently in the non-native environment than in the native one, for example, when they have the ability to shift between traits such as r- and K-selected strategies. This makes it difficult to predict impacts of species before they establish a viable population that can have minor to major effects on the ecosystem (Gallardo et al. 2015). In the case of a major impact an early detection of new arriving species would be important and could for example be implemented by regular monitoring.

Environmental tolerance

With future global climate change, many aquatic ecosystems are facing changes in abiotic and biotic factors (IPCC 2007; Hellmann et al. 2008). The Baltic Sea is one of these systems with a predicted sea surface warming and desalination (IPCC 2007, 2014; Gräwe et al. 2013; Holopainen et al. 2016). As explored in several previous studies, temperature and salinity strongly influence the development and distribution of organisms in aquatic environments with increasing temperatures leading to an increase in metabolic activities and with this an increase in energy demand (Sutcliffe 1968; Einarson 1993; Neuparth et al. 2002; Paavola et al. 2005; Delgado et al. 2011). To withstand variations of those factors a broad environmental tolerance is a benefit. For some native species with narrow tolerance windows the environment might become unsuitable which provides niches for new invaders resulting in a change of biodiversity (Olden et al. 2004).

The Baltic Sea is considered as an invasion hotspot (Leppäkoski and Olenin 2000; Leppäkoski et al. 2002) with 125 discovered aquatic NIS, of which about 30 % originate from the Ponto-Caspian region including many crustaceans (Chapter 1). The amphipod, *Pontogammarus robustoides* for example has spread in the Baltic Sea after an intentional introduction and became an aggressive invader which has negative impacts on native species e.g. by competitive exclusion (Arbaciauskas and Gumuliauskaite 2007). For the temperature tolerance experiment (Chapter 3) a Ponto-Caspian species from the same genus, *Pontogammarus maeoticus* without an invasion record in the Baltic Sea but a known invader in Turkish waters was examined (Ozbek 2011). It could tolerate water temperatures from as low as 6 °C up to 26 °C. On the other hand, the native species *Gammarus oceanicus* which thrives in the entire Baltic Sea could not tolerate the highest tested temperature of 26 °C for more than five days (Chapter 3). This is in accordance with a study by Bedulina et al. (2010) where 50 % mortality was observed at 26 °C after 48 hours in *G. oceanicus*. With the predicted warming of the seawater, this could imply that the more tolerant Ponto-Caspian species has the potential to invade the Baltic Sea. The non-indigenous, gammarid *Gammarus tigrinus* from the North West Atlantic has established populations along the coast of the Baltic Sea and adjacent water bodies (Kotta et al. 2013). There, it is considered as one major invasive species which outcompetes native gammarid communities in some regions (Kotta et al. 2011, 2013; Jänes et al. 2015). The invasion success of this species is attributed to its wide environmental tolerance e.g. regarding temperature and salinity which has been revealed in several studies (Ruoff 1968; Wijnhoven et al. 2003; Sareyka et al. 2011). However, a wide temperature tolerance could not be observed in the present study where *G. tigrinus* had relatively high mortalities in all treatments which could have been due to laboratory conditions. Hence, even tolerant species might behave differently in the laboratory influenced by non-controlled parameters such as a different ion composition or concentration compared to the natural habitat (Dobrzycka-Krahel and Graca 2018). It has been shown that thermal tolerance ranges can be shifted due to an interaction with other abiotic factors such as salinity changes and ocean acidification (Parker et al. 2017). Furthermore, different populations of one species might be locally adapted (Kuo and Sanford 2009).

Conclusion

Invasive species can cause ecological and economic harm and may lead to a homogenization of biodiversity worldwide. Species which evolved in regions that are more geologically and environmentally challenging might become more successful invaders, because of beneficial life history traits, higher phenotypic plasticity or a higher adaptation capacity (Reid and Orlova 2002). Identifying donor areas such as the Ponto-Caspian region, with high rates of potential invaders, can be a useful tool for future management strategies on selected transport vectors. Transport vectors coming from those areas could be more strictly controlled, since the prevention of new introductions of species is a cheaper and more effective strategy than eradication or control of established NIS populations (Lodge et al. 2006; Lockwood et al. 2007; Hulme et al. 2008).

One important life history trait for invasion success is the ability of species to produce dormant stages that are resistant to harsh environmental conditions for example during transport or in the new environment (Cáceres 1997; Briski et al. 2011). Many aquatic invertebrates have this ability and future studies could set a focus on these species to prevent new introductions. However, the invasion process is enormously complex and population-specific differences make general assumptions difficult about characteristics of one species, its closely allied species or the whole group of NIS.

In the Baltic Sea global warming might lead to unfavourable environments for some native species and to the introduction of new invaders which can tolerate higher temperatures such as Ponto-Caspian species. Both factors, climate change and new species introductions, might result in a displacement of some native species. Comprehensive information about well-established NIS regarding life history traits and environmental tolerance is fundamental to help estimate the invasion potential of new species. Future studies should incorporate NIS from locations worldwide, also including poorly studied but highly invaded regions for example in Asia. However, since a high number of newly discovered NIS does not have a known invasion history elsewhere (Seebens et al. 2018), information not only about NIS but all species are important. Therefore, future studies that gather comprehensive data about origin, life history traits and environmental tolerance of species will lead to a better understanding of the invasion process.

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Appendix

Supporting information for the article in chapter I entitled

“Importance of geographic origin for invasion success: a case study of the North and Baltic Seas *versus* the Great Lakes-St. Lawrence River region”

Isabel Casties, Hanno Seebens and Elizabeta Briski

Supplementary table S1 List of NIS established in the North and Baltic Seas, their geographic origin, and taxonomic assignment. Asterisks mark the NIS that occur in both the North and Baltic Seas and the Great Lakes-St. Lawrence River regions. NS, BS, NW, NE, SW and SE denote the North Sea, Baltic Sea, north-west, north-east, south-west, and south-east, respectively. Eurasia represents inland freshwaters except Yangtze River, Indo-Pacific represents Indian Ocean and the archipelago of Indonesia, Malaysia, and Pilipinas, North America (N America) represents inland freshwaters except the Laurentian Great Lakes, St. Lawrence and Mississippi Rivers, while Australia, New Zealand, Africa and South America (S America) cover all inland freshwaters in these areas.

Taxon	Species	Invaded areas	Origin
Animalia			
Acanthocephala			
Eoacanthocephala	*Paratenuisentis ambiguus*	BS	NE Pacific, NW Atlantic
Annelida			
Clitellata	*Branchiura sowerbyi**	NS, BS	Eurasia
	Limnodrilus cervix	BS	N America
	Paranais botniensis	NS	unknown
	Paranais frici	BS	Ponto-Caspian
	*Potamothrix bedoti**	BS	Ponto-Caspian
	Potamothrix heuscheri	BS	Ponto-Caspian
	*Potamothrix vejdovskyi**	BS	Ponto-Caspian
	Tubificoides pseudogaster	BS	North Sea

	Polychaeta	*Alitta succinea*	BS	NE Atlantic
		Boccardia proboscidea	NS	NE Pacific
		Boccardiella ligerica	NS, BS	unknown
		Clymenella torquata	NS	NW Atlantic
		Ficopomatus enigmaticus	NS, BS	Indo-Pacific
		Hydroides dianthus	NS	NW Atlantic
		Hydroides elegans	NS	unknown
		Hydroides ezoensis	NS	NW Pacific
		Hypania invalida	NS, BS	Ponto-Caspian
		Laonome calida	NS	unknown
		Marenzelleria arctia	BS	Arctic
		Marenzelleria neglecta	NS, BS	NW Atlantic
		Marenzelleria viridis	NS, BS	NW Atlantic
		Marenzelleria wireni	NS	NW Atlantic
		Marphysa sanguinea	NS	unknown
		Neodexiospira (=Janua) brasiliensis	NS	SW Atlantic
		Pileolaria berkeleyana	NS	NW Pacific
		Scolelepis bonnieri	NS	unknown
		Tharyx killariensis	NS	unknown
Arthropoda				
	Branchiopoda	*Cercopagis pengoi**	BS	Ponto-Caspian
		Cornigerius maeoticus	BS	Ponto-Caspian
		Evadne anonyx	BS	Ponto-Caspian
		Penilia avirostris	NS, BS	unknown
	Insecta	*Telmatogeton japonicus*	NS, BS	NW Pacific
	Malacostraca	*Atyaephyra desmarestii*	NS	Eurasia
		Brachynotus sexdentatus	NS	NE Atlantic, SE Atlantic
		Callinectes sapidus	NS, BS	NW Atlantic
		Caprella mutica	NS	NW Pacific
		Chelicorophium (=Corophium) curvispinum	NS, BS	Ponto-Caspian
		Chelicorophium robustum	NS	Ponto-Caspian

Dikerogammarus haemobaphes	NS, BS	Ponto-Caspian
Dikerogammarus villosus	NS, BS	Ponto-Caspian
*Echinogammarus ischnus**	BS	Ponto-Caspian
Echinogammarus warpachowskyi	BS	Ponto-Caspian
Eriocheir sinensis	NS, BS	NW Pacific
*Gammarus tigrinus**	NS, BS	NW Atlantic
Gmelinoides fasciatus	BS	Eurasia
Goneplax rhomboides	NS	NE Atlantic
Hemigrapsus penicillatus	NS	NW Pacific
Hemigrapsus sanguineus	NS	NW Pacific
Hemigrapsus takanoi	NS	NW Pacific
*Hemimysis anomala**	NS, BS	Ponto-Caspian
Homarus americanus	NS, BS	NW Atlantic
Ianiropsis tridens	NS	unknown
Incisocalliope aestuarius	NS	NW Atlantic
Jaera istri	NS	Ponto-Caspian
Jassa marmorata	NS	NW Atlantic, Mediterranean
Limnomysis benedeni	BS	Ponto-Caspian
Melita nitida	NS, BS	NW Atlantic
Monocorophium sextonae	NS	unknown
Monocorophium uenoi	NS	unknown
Obesogammarus crassus	NS, BS	Ponto-Caspian
Orchestia cavimana	BS	unknown
*Orconectes limosus**	BS	N America
Orconectes virilis	NS	N America
Palaemon elegans	BS	NW Atlantic
Palaemon macrodactylus	NS	NW Pacific
Paramysis (=Mesomysis) intermedia	BS	Ponto-Caspian
Paramysis (=Serrapalpisis) lacustris	BS	Ponto-Caspian
Platorchestia platensis	NS, BS	SW Atlantic
Pontogammarus robustoides	BS	Ponto-Caspian

	Proasellus coxalis	NS	Mediterranean
	Procambarus fallax f. *virginalis*	NS	unknown
	Pseudocuma (=Stenocuma) graciloides	BS	Ponto-Caspian
	Rhithropanopeus harrisii	NS, BS	NW Atlantic
	Sinelobus stanfordi	NS	unknown
	Synidotea laticauda	NS	NE Pacific
Maxillopoda	*Acartia tonsa*	NS, BS	NW Atlantic, Indo-Pacific
	Ameira divagans divagans	NS, BS	NW Atlantic
	Amphibalanus (=Balanus) improvisus	NS, BS	NW Atlantic
	Amphibalanus eburneus	NS	NW Atlantic
	Amphibalanus reticulatus	NS	Indo-Pacific
	Amphibalanus variegatus	NS	Indo-Pacific
	Austrominius modestus	NS	SW Pacific
	Balanus amphitrite	NS	Indo-Pacific
	Balanus trigonus	NS	unknown
	Conchoderma auritum	NS	unknown
	Eurytemora americana	NS	NW Atlantic
	Megabalanus coccopoma	NS	SE Pacific
	Megabalanus tintinnabulum	NS	unknown
	Myicola ostreae	NS	NW Pacific
	Mytilicola intestinalis	NS	Mediterranean
	Mytilicola orientalis (=ostreae)	NS	NW Pacific
	*Skistodiaptomus pallidus**	NS	N America
	Solidobalanus fallax	BS	SE Atlantic
Merostomata	*Limulus polyphemus*	NS, BS	NW Atlantic
Ostracoda	*Eusarsiella zostericola*	NS	NW Atlantic
Pycnogonida	*Ammothea hilgendorfi*	NS	NW Pacific
Bryozoa			
Gymnolaemata	*Bugula neritina*	NS	NW Atlantic
	Bugula stolonifera	NS	NW Atlantic
	Smittoidea prolifica	NS	NE Pacific, NW Pacific

	Tricellaria inopinata	NS	unknown
	Victorella pavida	NS, BS	Ponto- Caspian
Chordata			
Actinopterygii	*Acipenser baerii*	NS, BS	Eurasia
	Acipenser gueldenstaedtii	NS, BS	Ponto-Caspian
	Acipenser oxyrinchus	BS	Ponto-Caspian
	Acipenser ruthenus	NS, BS	Ponto-Caspian
	Acipenser stellatus	BS	Ponto-Caspian
	Acipenser transmontanus	NS	NE Pacific
	Carassius gibelio	BS	unknown
	Catostomus catostomus	BS	N America
	Coregonus autumnalis	BS	Arctic
	Coregonus muksun	BS	Eurasia
	Coregonus nasus	BS	Eurasia
	Coregonus peled	BS	Eurasia
	Ctenopharyngodon idella	BS	Eurasia
	*Cyprinus carpio**	BS	Ponto-Caspian
	Huso huso	BS	Ponto-Caspian
	Hypophthalmichthys molitrix	NS, BS	Eurasia
	Hypophthalmichthys nobilis	BS	Eurasia
	Lepomis gibbosus	NS, BS	N America
	Micropogonias undulatus	NS	NW Atlantic
	Micropterus dolomieu	NS, BS	Great Lakes
	Micropterus salmoides	BS	Great Lakes
	Morone saxatilis	NS	N America
	Neogobius fluviatilis	NS, BS	Ponto-Caspian
	Neogobius kessleri	NS	Ponto-Caspian
	*Neogobius melanostomus**	NS, BS	Ponto-Caspian
	*Oncorhynchus clarkii**	NS	NE Pacific
	*Oncorhynchus gorbuscha**	BS	NE Pacific, NW Pacific
	Oncorhynchus keta	BS	NE Pacific, NW Pacific

		*Oncorhynchus kisutch**	NS, BS	NE Pacific, NW Pacific
		*Oncorhynchus mykiss**	NS, BS	NE Pacific
		*Oncorhynchus nerka**	BS	NE Pacific, NW Pacific
		*Oncorhynchus tshawytscha**	BS	NE Pacific, NW Pacific
		Oreochromis niloticus niloticus	NS	Africa
		Perccottus glenii	BS	NW Pacific
		Poecilia (=Lebistes) reticulata	NS	NW Atlantic
		*Proterorhinus marmoratus**	BS	Ponto-Caspian
		Salvelinus fontinalis	NS, BS	N America
		Salvelinus namaycush	NS, BS	N America
		Sander lucioperca	NS	Eurasia
		Sebastes schlegelii	NS	NW Pacific, SW Pacific
		Tetraodon fluviatilis	NS	Eurasia
		Trinectes maculatus	NS	NW Atlantic
	Ascidiacea	*Aplidium glabrum*	NS	unknown
		Botrylloides violaceus	NS	NW Pacific
		Didemnum vexillum	NS	NW Pacific
		Perophora japonica	NS	unknown
		Styela clava	NS, BS	NW Pacific
	Aves	*Branta canadensis*	BS	N America
Cnidaria				
	Anthozoa	*Diadumene cincta*	NS	NE Pacific, NW Pacific
		Diadumene lineata	NS	NW Pacific
		Nematostella vectensis	NS	NW Atlantic
	Hydrozoa	*Bougainvillia macloviana*	NS	unknown
		Bougainvillia rugosa	BS	NE Pacific, NW Atlantic
		*Cordylophora caspia**	NS, BS	Ponto-Caspian
		*Craspedacusta sowerbii**	NS	Yangtze river
		Garveia franciscana	NS	NW Atlantic
		Gonionemus vertens	NS, BS	NW Pacific
		Maeotias marginata	BS	Ponto-Caspian

		Moerisia (=Ostroumovia) inkermanica	NS	Ponto-Caspian
		Nemopsis bachei	NS	NW Atlantic
		Pachycordyle navis	NS, BS	Ponto-Caspian
Ctenophora				
	Nuda	*Beroe ovata*	BS	NW Atlantic
	Tentaculata	*Mnemiopsis leidyi*	NS, BS	NW Atlantic, SW Atlantic
Mollusca				
	Bivalvia	*Corbicula fluminea**	NS	Eurasia
		Crassostrea gigas	NS, BS	NW Pacific
		Crassostrea virginica	NS, BS	NW Atlantic
		*Dreissena rostriformis bugensis**	NS, BS	Ponto-Caspian
		*Dreissena polymorpha**	NS, BS	Ponto-Caspian
		Ensis directus (=americanus)	NS, BS	NW Atlantic
		Mercenaria mercenaria	NS	NW Atlantic
		Mya arenaria	NS, BS	NW Atlantic
		Mytilopsis leucophaeata	NS, BS	NW Atlantic
		Petricolaria pholadiformis	NS, BS	NW Atlantic
		Psiloteredo megotara	NS	unknown
		Rangia cuneata	NS, BS	NW Atlantic
		Ruditapes philippinarum	NS	NW Pacific
		Spisula solidissima	NS	NW Atlantic
		Teredo navalis	NS, BS	Indo-Pacific
	Gastropoda	*Bellamya chinensis*	NS	Eurasia
		Corambe obscura (=batava)	NS	NW Atlantic
		Crepidula fornicata	NS, BS	NW Atlantic
		Ferrissia wautieri	NS	unknown
		Hexaplex (Trunculariopsis) trunculus	NS	unknown
		Lithoglyphus naticoides	BS	Ponto-Caspian
		Ocenebra erinaceus	NS	unknown
		Ocenebra inornata	NS	unknown
		Physella acuta	NS	Eurasia

			*Potamopyrgus antipodarum**	NS, BS	New Zealand
			Rapana venosa	NS	NW Pacific
			Theodoxus pallasi	BS	Ponto-Caspian
			Urosalpinx cinerea	NS	NW Atlantic
			Viviparus acerosus	NS	Ponto-Caspian
			Viviparus viviparus	NS	unknown
	Nematoda				
		Chromadorea	*Anguillicola crassus*	NS, BS	Eurasia
	Platyhelminthes				
		Cestoda	*Dugesia tigrina*	NS	N America
		Monogenea	*Gyrodactylus salaris*	NS	Baltic Sea
			Onchocleidus dispar	NS	NW Atlantic
			Pseudodactylogyrus anguillae	NS, BS	NW Pacific
			Pseudodactylogyrus bini	NS, BS	NW Pacific
		Trematoda	*Pseudobacciger harengulae*	NS	NW Pacific
		Turbellaria	*Euplana gracilis*	NS	unknown
			Imogine necopinata	NS	unknown
	Porifera				
		Demospongiae	*Celtodoryx ciocalyptoides*	NS	unknown
			Chalinula loosanoffi	NS	NE Atlantic
Bacteria					
	Proteobacteria		*Aeromonas salmonicida**	NS	unknown
Chromista					
	Cercozoa				
		Ascetosporea	*Bonamia ostreae*	NS	unknown
			Haplosporidium armoricanum	NS	NE Atlantic, SE Atlantic
			Marteilia refringens	NS	NE Atlantic, SE Atlantic
	Myzozoa				
		Dinophyceae	*Alexandrium leeii*	NS	NE Pacific, NW Pacific
			Dicroerisma psilonereiella	NS	unknown
			Gymnodinium aureolum	NS	NE Pacific, NW Pacific

	Gymnodinium catenatum	NS	unknown
	Karenia mikimotoi	NS, BS	NW Pacific
	Karlodinium veneficum	NS	unknown
	Oxytoxum criophilum	NS	unknown
	Pfiesteria piscicida	NS	NE Pacific, NW Atlantic
	Pfiesteria shumwayae	NS	unknown
	Thecadinium yashimaense	NS	NE Pacific, NW Pacific
Ochrophyta			
Bacillariophyceae	*Coscinodiscus wailesii*	NS, BS	Indo-Pacific
	Lennoxia faveolata	BS	unknown
	Odontella sinensis	NS, BS	Indo-Pacific
	Pleurosigma simonsenii	NS	Indian Ocean
	Stephanopyxis palmeriana	NS	unknown
	Thalassiosira hendeyi	NS	SE Atlantic, SW Atlantic
	Thalassiosira punctigera	NS, BS	NE Pacific, NW Pacific
	Thalassiosira tealata	NS	NE Pacific, NW Pacific
Phaeophyceae	*Asperococcus scaber*	NS	unknown
	Botrytella sp.	NS	unknown
	Colpomenia peregrina	NS, BS	NE Pacific
	Corynophlaea umbellata	NS	NE Pacific, NW Pacific
	Corynophlaea verruculiformis	NS	NW Pacific
	Elachista sp.	NS	unknown
	Fucus evanescens	NS, BS	Arctic
	Leathesia verruculiformis	NS	unknown
	Myriactula sp.	NS	unknown
	Saccharina japonica	NS	unknown
	Sargassum muticum	NS, BS	NW Pacific
	Scytosiphon dotyi	NS	unknown
	Undaria pinnatifida	NS	NW Pacific
Raphidophyceae	*Chattonella marina*	NS	unknown
	Chattonella marina var. *antiqua*	NS	NE Pacific, NW Pacific

	Chattonella verruculosa	NS	NW Pacific
	Fibrocapsa japonica	NS	NE Pacific, NW Pacific
	Heterosigma akashiwo (carterae)	NS	NW Pacific
Fungi			
Ascomycota			
Sordariomycetes	*Claviceps purpurea*	NS	unknown
Plantae			
Charophyta			
Charophyceae	*Chara connivens*	BS	Eurasia
Chlorophyta			
Bryopsidophyceae	*Codium fragile atlanticum*	NS	Indo Pacific
	Codium fragile fragile (tomentosoides)	NS, BS	NW Pacific
	Codium fragile ssp. scandinavicum	NS	Indo-Pacific
Ulvophyceae	*Protomonostroma undulatum*	BS	Indo-Pacific
	Ulva pertusa	NS	NW Pacific
Rhodophyta			
Florideophyceae	*Acrochaetium catenulatum*	NS	NE Pacific, NW Pacific
	Acrochaetium savianum	NS	NW Atlantic
	Agardhiella subulata	NS	unknown
	Aglaothamnion halliae	NS	NW Atlantic
	Anotrichium furcellatum	NS	Mediterranean
	Antithamnion nipponicum	NS	NW Pacific
	Antithamnionella spirographidis	NS	NW Pacific
	Antithamnionella ternifolia	NS	SW Pacific
	Asparagopsis armata	NS	SW Pacific
	Bonnemaisonia hamifera	NS, BS	NW Pacific
	Ceramium cimbricum	NS	unknown
	Colaconema dasyae	NS	unknown
	Dasya baillouviana	NS, BS	NE Atlantic, Mediterranean
	Dasysiphonia sp.	NS	NW Pacific
	Devaleraea ramentacea	NS	unknown

		Gracilaria vermiculophylla	NS, BS	NW Pacific
		Grateloupia doryphora	NS	NE Pacific
		Grateloupia luxurians	NS	Indo-Pacific
		Heterosiphonia japonica	NS, BS	NW Pacific
		Lomentaria hakodatensis	NS	NE Pacific, NW Pacific
		Neosiphonia harveyi	NS, BS	NW Pacific
		Polysiphonia senticulosa	NS	NW Pacific
Tracheophyta				
	Liliopsida	*Elodea canadensis*	BS	N America
		Elodea nuttallii	NS	N America
		Spartina anglica	NS, BS	NE Atlantic
		Spartina townsendii	NS, BS	NE Atlantic
	Magnoliopsida	*Cotula coronopifolia*	NS	SE Atlantic
		Ludwigia grandiflora (Michx.)	NS	unknown

Supplementary table S2 List of NIS established in the Great Lakes-St. Lawrence River region, their geographic origin, and taxonomic assignment. Asterisks mark the NIS that occur in both the North and Baltic Seas and the Great Lakes-St. Lawrence River regions. GL, SL, NW, NE, SW and SE denote the Great Lakes, St. Lawrence River, north-west, north-east, south-west, and south-east, respectively. Eurasia represents inland freshwaters except Yangtze River, Indo-Pacific represents Indian Ocean and the archipelago of Indonesia, Malaysia, and Pilipinas, North America (N America) represents inland freshwaters except the Laurentian Great Lakes, St. Lawrence and Mississippi Rivers, while Australia, New Zealand, Africa and South America (S America) cover all inland freshwaters in these areas.

Taxon	Species	Invaded areas	Origin
Animalia			
Annelida			
Clitellata	*Branchiura sowerbyi**	GL	Eurasia
	Gianius aquaedulcis	GL	Eurasia
	*Potamothrix bedoti**	GL	Ponto-Caspian
	Potamothrix moldaviensis	GL	Ponto-Caspian
	*Potamothrix vejdovskyi**	GL	Ponto-Caspian
	Ripistes parasita	GL, SL	Eurasia
Arthropoda			
Branchiopoda	*Bosmina maritima*	GL	Baltic Sea
	Bythotrephes longimanus	GL	Eurasia
	*Cercopagis pengoi**	GL	Ponto-Caspian
	Daphnia galeata galeata	GL	Arctic
	Daphnia lumholtzi	GL	Australia
	Eubosmina coregoni	GL, SL	Eurasia
Insecta	*Acentropus niveus*	GL	Eurasia
	Tanysphyrus lemnae	GL	Eurasia
Malacostraca	*Echinogammarus ischnus**	GL, SL	Ponto-Caspian
	*Gammarus tigrinus**	GL	NW Atlantic
	*Hemimysis anomala**	GL	Ponto-Caspian
	*Orconectes limosus**	SL	N America
	Orconectes rusticus	GL	N America
Maxillipoda	*Argulus japonicus*	GL	Eurasia

		Cyclops strenuus	GL	unknown
		Eurytemora affinis	GL, SL	Ponto-Caspian
		Heteropsyllus nr. nunni	GL	unknown
		Megacyclops viridis	GL	Eurasia
		Neoergasilus japonicus	GL	Eurasia
		Nitocra hibernica	GL	Ponto-Caspian
		Nitocra incerta	GL	Ponto-Caspian
		Salmincola lotae	GL	Eurasia
		Schizopera borutzkyi	GL	Ponto-Caspian
		*Skistodiaptomus pallidus**	GL	N America
Bryozoa				
	Phylactolaemata	*Lophopodella carteri*	GL, SL	Eurasia, Africa
Chordata				
	Actinopterygii	*Alosa aestivalis*	GL	NW Atlantic
		Alosa pseudoharengus	GL	NW Atlantic
		Apeltes quadracus	GL	NE Atlantic, NW Atlantic
		Carassius auratus	GL, SL	Eurasia
		*Cyprinus carpio**	GL, SL	Ponto-Caspian
		Enneacanthus gloriosus	GL	N America
		Esox niger	GL	NW Atlantic
		Gambusia affinis	GL	N America
		Gymnocephalus cernuus	GL	Eurasia
		Ictiobus cyprinellus	GL	N America
		Lepisosteus platostomus	GL	Mississippi River
		Lepomis humilis	GL	N America
		Lepomis microlophus	GL	N America
		Misgurnus anguillicaudatus	GL	Eurasia
		Morone americana	GL	N America
		*Neogobius melanostomus**	GL, SL	Ponto-Caspian

	Notropis buchanani	GL	Mississippi River
	Noturus insignis	GL, SL	N America
	*Oncorhynchus clarkii**	SL	NE Pacific
	*Oncorhynchus gorbuscha**	GL	NE Pacific, NW Pacific
	*Oncorhynchus kisutch**	GL, SL	NE Pacific, NW Pacific
	*Oncorhynchus mykiss**	GL, SL	NE Pacific
	*Oncorhynchus nerka**	GL	NE Pacific, NW Pacific
	*Oncorhynchus tshawytscha**	GL, SL	NE Pacific, NW Pacific
	Osmerus mordax	GL	N America
	Phenacobius mirabilis	GL	Mississippi River
	*Proterorhinus marmoratus**	GL	Ponto-Caspian
	Salmo trutta	GL, SL	Eurasia
	Scardinius erythrophthalmus	GL, SL	Eurasia
	Tinca tinca	SL	Eurasia
Petromyzonti	*Petromyzon marinus*	GL	N America
Cnidaria			
Hydrozoa	*Cordylophora caspia**	GL	Ponto-Caspian
	*Craspedacusta sowerbii**	GL	Yangtze River
Mollusca			
Bivalvia	*Corbicula fluminea**	GL	Eurasia
	*Dreissena rostriformis bugensis**	GL, SL	Ponto-Caspian
	*Dreissena polymorpha**	GL, SL	Ponto-Caspian
	Lasmigona subviridis	GL	N America
	Pisidium amnicum	GL, SL	Eurasia
	Pisidium henslowanum	GL, SL	Eurasia
	Pisidium moitessierianum	GL	Eurasia
	Pisidium supinum	GL	Eurasia
	Sphaerium corneum	GL, SL	Eurasia
Gastropoda	*Bithynia tentaculata*	GL, SL	Eurasia

			Cipangopaludina japonica	GL	Eurasia
			Cipangopaludina malleata	GL, SL	Eurasia
			Elimia virginica	GL	N America
			Gillia altilis	GL	N America
			*Potamopyrgus antipodarum**	GL	New Zealand
			Radix auricularia	GL, SL	Eurasia
			Valvata piscinalis	GL, SL	Eurasia
			Viviparus georgianus	GL, SL	N America
	Myxozoa				
		Myxosporea	*Myxobolus cerebralis*	GL	unknown
			Sphaeromyxa sevastopoli	GL	Ponto-Caspian
	Platyhelminthes				
		Cestoda	*Bothriocephalus acheilognathi*	GL	Eurasia
			Dugesia polychroa	GL, SL	Eurasia
			Scolex pleuronectis	GL	unknown
		Monogenea	*Dactylogyrus amphibothrium*	GL	Eurasia
			Dactylogyrus hemiamphibothrium	GL	Eurasia
		Trematoda	*Ichthyocotylurus pileatus*	GL	Ponto-Caspian
			Neascus brevicaudatus	GL	Ponto-Caspian
			Timoniella sp.	GL	unknown
Bacteria					
	Actinobacteria		*Renibacterium salmoninarum*	GL	unknown
	Cyanobacteria				
		Cyanophyceae	*Cylindrospermopsis raciborskii*	GL	unknown
	Proteobacteria				
		Gammaproteobacteria	*Aeromonas salmonicida**	GL, SL	unknown
			Piscirickettsia cf. *salmonis*	GL	unknown
Chromista					
	Cercozoa				

		Gromiidea	*Psammonobiotus communis*	GL	Ponto-Caspian
			Psammonobiotus dziwnowi	GL	Ponto-Caspian
			Psammonobiotus linearis	GL	Ponto-Caspian
	Ciliophora				
		Phyllopharyngea	*Acineta nitocrae*	GL	Ponto-Caspian
	Haptophyta				
		Prymnesiophyceae	*Hymenomonas roseola*	GL	Eurasia
	Ochrophyta				
		Bacillariophyceae	*Actinocyclus normanii* fo. *subsalsa*	GL	Baltic Sea
			Biddulphia laevis	GL	unknown
			Chaetoceros hohnii	GL	unknown
			Cyclotella atomus	GL	unknown
			Cyclotella cryptica	GL	unknown
			Cyclotella pseudostelligera	GL, SL	unknown
			Cyclotella woltereki	GL	unknown
			Diatoma ehrenbergii	GL, SL	unknown
			Skeletonema potamos	GL, SL	unknown
			Skeletonema subsalsum	GL, SL	Eurasia
			Stephanodiscus binderanus	GL, SL	Baltic Sea
			Stephanodiscus subtilis	GL	Eurasia
			Thalassiosira baltica	GL	unknown
			Thalassiosira guillardii	GL, SL	unknown
			Thalassiosira lacustris	GL	unknown
			Thalassiosira pseudonana	GL, SL	unknown
			Thalassiosira weissflogii	GL, SL	unknown
		Phaeophyceae	*Sphacelaria fluviatilis*	GL	Eurasia
			Sphacelaria lacustris	GL	unknown
Fungi					
	Microsporidia				

		Microsporea	*Glugea hertwigi*	GL, SL	Baltic Sea
			Heterosporis sp.	GL	unknown
Plantae					
	Charophyta				
		Charophyceae	*Nitellopsis obtusa*	GL, SL	Eurasia
	Chlorophyta				
		Ulvophyceae	*Enteromorpha flexuosa*	GL	unknown
			Enteromorpha intestinalis	GL, SL	NW Atlantic
			Enteromorpha prolifera	GL, SL	unknown
	Rhodophyta				
		Bangiophyceae	*Bangia atropurpurea*	GL	unknown
		Rhodellophyceae	*Chroodactylon ramosum*	GL, SL	unknown
	Tracheophyta				
		Liliopsida	*Agrostis gigantea*	GL, SL	Eurasia
			Alopecurus geniculatus	GL, SL	Eurasia
			Butomus umbellatus	GL, SL	Eurasia
			Carex acutiformis	GL	Eurasia
			Carex disticha	GL, SL	Eurasia
			Echinochloa crusgalli	GL, SL	Eurasia
			Glyceria maxima	GL	Eurasia
			Hydrocharis morsus-ranae	GL, SL	Eurasia
			Iris pseudacorus	GL, SL	Eurasia
			Juncus compressus	GL, SL	Eurasia
			Juncus gerardii	GL	NW Atlantic
			Juncus inflexus	GL	Eurasia
			Najas marina	GL, SL	Eurasia
			Najas minor	GL	Eurasia
			Pistia stratiotes	GL	unknown
			Poa trivalis	GL, SL	Eurasia

	Potamogeton crispus	GL, SL	Eurasia
	Puccinellia distans	GL, SL	Eurasia
	Sparganium glomeratum	GL, SL	Eurasia
	Typha angustifolia	GL, SL	Eurasia
Magnoliopsida	*Alnus glutinosa*	GL	Eurasia
	Cabomba caroliniana	GL	S America
	Chenopodium glaucum	GL, SL	Eurasia
	Cirsium palustre	GL, SL	Eurasia
	Conium maculatum	GL, SL	Eurasia
	Epilobium hirsutum	GL, SL	Eurasia
	Impatiens glandulifera	GL, SL	Eurasia
	Lupinus polyphyllus	GL	N America
	Lycopus asper	GL, SL	Mississippi River
	Lycopus europaeus	GL, SL	Eurasia
	Lysimachia nummularia	GL, SL	Eurasia
	Lysimachia vulgaris	GL	Eurasia
	Lythrum salicaria	GL, SL	Eurasia
	Mentha gentilis	GL, SL	Eurasia
	Mentha piperita	GL, SL	Eurasia
	Mentha spicata	GL, SL	Eurasia
	Myosotis scorpioides	GL, SL	Eurasia
	Myriophyllum spicatum	GL, SL	Eurasia
	Nymphoides peltata	GL, SL	Eurasia
	Polygonum persicaria	GL, SL	Eurasia
	Rhamnus frangula	GL, SL	Eurasia
	Rorippa nasturtium aquaticum	GL, SL	Eurasia
	Rorippa sylvestris	GL, SL	Eurasia
	Rumex longifolius	GL, SL	Eurasia
	Rumex obtusifolius	GL, SL	Eurasia

			Salix alba	GL, SL	Eurasia
			Salix fragilis	GL, SL	Eurasia
			Salix purpurea	GL, SL	Eurasia
			Solanum dulcamara	GL, SL	Eurasia
			Solidago sempervirens	GL	NW Atlantic
			Stellaria aquatica	GL, SL	Eurasia
			Trapa natans	GL, SL	Eurasia
			Veronica beccabunga	GL, SL	Eurasia
		Spermatopsida	*Pluchea odorata* var. *purpurescens*	GL	NW Atlantic
			Pluchea odorata var. *succulenta*	GL	NW Atlantic
		Polypodiopsida	*Marsilea quadrifolia*	GL	Eurasia
Protozoa					
	Euglenida				
		Kinetoplastea	*Trypanosoma acerinae*	GL	Eurasia
Virus			Rhabdovirus carpio	GL	Eurasia
			Novirhabdovirus sp. genotype IV sublineage b	GL	unknown
			Largemouth Bass Virus	GL	unknown

Contribution of authors

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Manuscript 1:

“Importance of geographic origin for invasion success: a case study of the North and Baltic Seas versus the Great Lakes-St. Lawrence River region”

Authors: Isabel Casties (IC), Hanno Seebens (HS) and Elizabeta Briski (EB)

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Contributions: EB designed the study, IC collected the data, HS conducted the model calculations, EB and IC drafted the manuscript, and all authors revised the manuscript.

Manuscript 2:

“Beneficial life history traits for invasion success revisited”

Authors: Isabel Casties (IC) and Elizabeta Briski (EB)

Submitted to *Aquatic Invasions*

Contributions: EB designed the study, IC collected and processed the data, EB and IC drafted the manuscript.

Manuscript 3:

“Will non-indigenous species win under global warming scenario?”

Authors: Isabel Casties (IC), Catriona Clemmesen (CC) and Elizabeta Briski (EB)

Submitted to *Diversity and Distributions*

EB and IC designed the study, IC conducted the experiments and data analyses, CC and IC conducted and analyzed molecular data, EB and IC drafted the manuscript, and all authors approved the last version of the manuscript.

Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation eigenständig, mit der Beratung meiner Betreuerin, angefertigt habe. Die Dissertation ist in Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel und Quellen verwendet. Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden. Diese Dissertation wurde an keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt und ist mein erstes und einziges Promotionsverfahren. Da es sich bei der vorliegenden Arbeit um eine kumulative Dissertation handelt, sind Teile dieser Arbeit in wissenschaftlichen Fachzeitschriften veröffentlicht oder zur Veröffentlichung vorgesehen.

Kiel, den 16.04.2018

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Isabel Casties