

Defining the Risk-Release Relationship of *Daphnia magna*, a Non-native Aquatic Species in the Environment of Duluth-Superior Harbor

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## Abstract

Understanding how inoculation characteristics (propagule pressure) affect establishment outcome of a non-native, invading species in the context of abiotic and biotic factors (ecological resistance) is a recent area of emphasis in ecology given an urgent need to prevent and manage invasive species globally. The overarching objective of this dissertation research is to quantify how a non-native species (*Daphnia magna*) establishes in response to different levels of propagule pressure in the context of a Laurentian Great Lakes port receiving system. Specifically, I described spatial and temporal patterns in the density and diversity of crustacean zooplankton in the context of abiotic factors of the Duluth-Superior Harbor and St. Louis River Estuary (DSHSLRE) and used those measures to develop establishment criteria for the propagule pressure experiments. Next, I evaluated the ability of a surrogate invader, *D. magna* to successfully colonize 200-L mesocosm aquaria by manipulating propagule pressure and several components of ecological resistance. Special attention was paid to the density of 10 individuals  $m^{-3}$  as this is the current standard suggested by the International Maritime Organization for density of permissible non-native species in ballast water discharge from oceangoing vessels. Lastly, I re-analyzed the results from the propagule pressure experiments to evaluate how different establishment criteria, which vary in rigor, influence the perceived outcome. Results demonstrate notable spatial and seasonal patterns in the abiotic conditions and zooplankton diversity and density measured at nine locations sampled biweekly during 2007 and 2008 in the DSHSLRE. Abiotic characteristics of upstream sites were indicative of littoral habitats and changed more dramatically within a year and from year to year. Overall 49 distinct taxa were identified, half at the genus level Copepoda and half Cladocera. Colonization of the mesocosm aquariums by *D. magna* populations ranged from 50-80% in the spring and fall experiments but never achieved success greater than 33% in the summer experiments. Only when the background assemblage of zooplankton was first removed from the tanks in the summer was *D. magna* able to

establish suggesting that ecological resistance related to resource competition was prohibitive. Risk of establishment evaluated by different threshold criteria influenced the perceived outcome of the mesocosm experiments. This study demonstrates the multi-faceted nature of drivers that determine establishment success of a non-native species and the complexities of evaluating standards on permissible biological pollution using an experimental, mesocosm venue.

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## Chapter 1: Introduction

Freshwater ecosystems are highly vulnerable to invasions by non-native species because of their close association with human activity, including use for municipal and industrial water supplies, natural resource development, commercial navigation, and recreation (Ricciardi and MacIsaac 2000; Colautti et al. 2003). Many aquatic non-native species are causing changes to freshwater food web structure and water quality, and are imposing high economic costs in a variety of ways (Colautti et al. 2003; Leung and Mandrak 2007; Bailey et al. 2009). An introduced species that is non-native to a particular ecosystem is termed invasive when it begins to cause environmental or economic harm with respect to that ecosystem.

Dreissenid mussels (zebra mussel: *Dreissena polymorpha*; quagga mussel: *Dreissena bugensis*) are noteworthy examples of invasive non-native aquatic species. Dreissenids have changed the food web structure of the Laurentian Great Lakes and inland water bodies in North America by removing organic material from the water column and enhancing deposition rates of organic matter to the bottom, benefitting the benthic invertebrate community (Hecky et al. 2004; Holeck et al 2007). They have also caused an increase in water clarity due to their filtering activity, leading to increases in light penetration and growth of benthic algae (Hecky et al. 2004; Holeck et al. 2007; Fishman et al. 2009; Kissman et al. 2010). Fish communities of the Great Lakes have also responded to non-native dreissenids. For example, dramatic springtime shifts in the distributions of alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) have occurred in response to changes in water clarity; populations of native lake whitefish (*Coregonus clupeaformis*) have dwindled; and the portioning of energy to fish in the presence of dreissenids has become more complex, especially in near shore habitats (Holeck et al. 2007).

In addition to environmental damage, non-native species can impose significant economic burden. It has been estimated that damages and additional maintenance caused by non-

native species in the U.S. an annual cost of \$120 billion or \$100 per household (Pimentel et al. 2000). Considering zebra mussels alone, the estimated net impacts over 10 years to intake pipes, water filtration equipment and power plants in the Laurentian Great Lakes is at \$3.2 billion (Cataldo 2001).

Ballast water ferried by ships used to correct imbalance in cargo is the leading dispersal agent of coastal aquatic non-native species (including dreissenid mussels) in North America (Ruiz et al. 2000). Foreign ships arriving in U.S. ports discharge in excess of 70 million metric tons of liquid ballast annually (Minton et al. 2005), representing a massive ongoing courier of aquatic non-native species into the country. Since the 1800s, at least 180 non-native species have become established in the Great Lakes proper (Mills et al. 1993; Ricciardi 2001 and 2006) and most are believed to have arrived by ships' ballast water. Many of these species are characteristic of lentic systems, including a broad size, taxonomic, and functional (e.g., benthic and pelagic) spectrum of organisms from phytoplankton to fish. The reported establishment rate of new aquatic non-native species introduced via ballast water was 0.6 species per year between 1959 and 1989, but increased to 1.8 species per year between 1989 and 2001 (Colautti et al. 2003).

In an effort to prevent additional species introductions via ballast water exchange, the U.S. Congress passed and reauthorized legislation in the 1990s that required vessels to manage their ballast water in one of two ways. Ships were required either to carryout Ballast Water Exchange (BWE) by flushing ballast tanks with sea water in the open ocean or to perform Ballast Water Treatment (BWT) by proactive decontamination. Although BWE may be effective at reducing total population densities of non-native freshwater biota (Gray et al. 2007; Briski et al. 2013), the policy suffers from enforcement loopholes (Grigorovich et al. 2003; Duggan et al. 2005) and ignores the possibility that saltwater-tolerant life stages of some species will survive. As a result, researchers are currently developing and testing ballast-water treatment technologies that will kill organisms upon entrance or exit from ballast-holding tanks.

It is widely recognized that no BWT technology can be expected to perform with 100% effectiveness all of the time. Consequently, accepted standards will allow a certain level of biological pollution (viable aquatic organisms) to escape in the post-treated water. The post-treatment standards required of BWT technologies will be guided by standards agreed upon by the International Maritime Organization (IMO). The IMO is the United Nations body that administers the international regulatory regime for shipping. The IMO has currently recognized discharge standards that future technologies will have to meet. One component of these standards states that for organisms greater than 50  $\mu\text{m}$  length in minimum dimension no more than 10 viable individuals may be discharged per one cubic meter of ballast water (Gollasch et al. 2007).

An assumption in the development of a numeric-discharge standard is that there is a direct and quantifiable relationship between the density of individuals released in ballast discharge and the probability of their establishment, also known as the risk-release relationship (Ruiz and Carlton 2003). The original individuals that arrive during an invasion event (comprising the founding population) are often referred to as propagules. A propagule is an individual organism or structure (e.g. egg) that may give rise to an individual organism. Propagule pressure refers to the number of propagules released per event (propagule size) and the number of release events (propagule number) for a species in a location (Lockwood et al. 2005; Colautti et al. 2006; Britton-Simmons and Abbott 2008). Propagule pressure is considered a key component of the risk-release relationship for several reasons. Releases of large numbers of propagules (larger propagule size) in any individual release event should enable the introduced species to endure demographic stochasticity related to random events such as an unexplainable death (Lockwood et al. 2005; Simberloff 2009). Frequent releases of groups of propagules (larger propagule number) should enable the introduced species to endure environmental stochasticity related to variation in the resistance of the recipient habitat (Simberloff, 2009). For example, propagules that are spread across an ecosystem spatially or temporally are less likely to



go extinct because adverse environmental conditions in one location or time may not affect individuals in another location or time (Hanski 1989; Lockwood et al. 2005; Duggan et al. 2005; Bailey et al. 2009). Although propagule pressure may explain why some populations establish and others fail (Elton 1958; Drake et al. 2005; Verling et al. 2005; Colautti et al. 2006; Britton-Simmons and Abbott 2008), the relationship between propagule pressure and establishment success has rarely been tested experimentally across variation in the abiotic and biotic recipient community (Lockwood et al. 2005; Von Holle and Simberloff 2005).

The overarching objective of this dissertation research is to quantify how a surrogate non-native species (*Daphnia magna*) establishes in response to different levels of propagule pressure in the context of different seasonal biotic and abiotic characteristics of the recipient ecosystem where it is released. This research addresses a basic information gap in the discipline of invasion ecology and an applied information gap regarding the development of ballast-water discharge standards. In Chapter 2, I describe the open-water seasonal abiotic and biotic characteristics of the Duluth-Superior Harbor and St. Louis River Estuary from which water and organisms were collected to create the contrived (experimental) recipient ecosystem. These data were used to characterize the dynamics (e.g., seasonality) of the recipient community and to develop establishment criteria for the interpretation of the mesocosm experimental results. In Chapter 3, I determine the shape and magnitude of the relationship between establishment success (risk) and propagule pressure (release) of *D. magna* in an indoor venue with replicate 231-L mesocosm aquaria stocked with water and organisms from the Duluth-Superior Harbor. I used propagule densities that bracketed the current IMO standard for organisms greater than 50  $\mu\text{m}$  length in minimum dimension. In Chapter 4, I evaluate how several different plausible *Daphnia magna* establishment criteria influence the outcome from Chapter 3.

## **Chapter 2: Density, Diversity, and Distribution of Crustacean Zooplankton in the Duluth-Superior Harbor and St. Louis River Estuary of Lake Superior.**

### **Introduction**

The Duluth-Superior Harbor, located on the western end of Lake Superior, is one of the major bulk cargo ports in the Laurentian Great Lakes (hereafter Great Lakes). During 2000-2009, the Harbor handled a yearly average of 1,078 vessel visits and moved a yearly average of 38,723,000 metric tons of cargo making it the busiest commercial shipping port in the Great Lakes (Duluth Seaway Port Authority, 2012). Owing to the significant tonnage of cargo moved, and the export-dominant nature of its market (primarily as iron ore, coal, and grain), the Duluth-Superior Harbor receives a large number of inbound ships partially or fully laden with ballast water, the majority of which is discharged commensurate with cargo loading. During 2005-2007, the fleet of ships that operated exclusively within the Great Lakes and St. Lawrence River west of 63° W (hereafter referred to as Lakers; Rup et al. 2010) were estimated to have delivered a yearly average 19.6 metric tons of ballast water to the Duluth-Superior Harbor making it the top recipient of interregional (between lake) ballast water among Great Lakes ports (Rup et al. 2010). In addition to Lakers, the Duluth-Superior Harbor annually receives a smaller number of commercial vessels from transoceanic locations. During 2005-2007, approximately 1% of bulk carriers and general cargo vessels arriving in the Duluth-Superior Harbor originated from overseas ports (National Ballast Information Clearinghouse 2009).

The overall magnitude of shipping and deballasting activity in the Duluth-Superior Harbor makes the location a likely hub for the arrival (invasion) and export (donation) of aquatic biota within the Great Lakes, including non-native taxa. The Duluth-Superior Harbor and associated St. Louis River Estuary (*see methods*) were the locations of the first North American sightings of Eurasian ruffe (*Gymnocephalus cernuus*) and the first Lake Superior sightings of zebra mussel

(*Dreissena polymorpha*), quagga mussel (*Dreissena bugensis*), *Daphnia lumholtzi* (Crustacea: Cladocera), round goby (*Neogobius melanostomus*), white perch (*Morone americana*) and many other non-native species (Simon and Vondruska 1991; Bronte et al. 2003; Grigorovich et al. 2008; Trebitz et al. 2010). Transport by ship ballast water was likely responsible for the arrival of many of these invaders (Mills et al. 1993; Ricciardi 2006). The close proximity of the Duluth-Superior Harbor and St. Louis River Estuary to thousands of inland water bodies in Wisconsin and Minnesota, coupled with its heavy use by recreational boaters and waterfowl, make it a strong potential donor site for overland secondary spread of non-native biota.

Recent species inventories of benthic and pelagic invertebrates in Lake Superior and the Duluth-Superior Harbor and St. Louis River Estuary were done by Grigorovich et al. (2003) and Trebitz et al. (2010). However, estimates of crustacean zooplankton density and diversity in the Duluth-Superior Harbor and St. Louis River Estuary are needed. The last quantitative open-water surveys of crustacean zooplankton were conducted by Balcer (1978) and Hargis (1984). A current record of crustacean zooplankton is useful for several reasons. It can provide reference points for the determination of invasion timelines for non-native species, benchmarks for comparisons of density and diversity with historical data, and an inventory list to identify taxa that could potentially be exported by ballast water and other vectors. The survey samples were also needed in order to develop density and biomass criteria that could be used to assess the establishment status of the surrogate invader in the propagule-pressure experiments (Chapter 3).

To this end, my objectives were to 1) describe spatial and temporal patterns in the density and diversity of crustacean zooplankton in the Duluth-Superior Harbor and St. Louis River Estuary; 2) analyze the zooplankton population patterns in the context of spatial and temporal patterns in temperature, chlorophyll and other environmental factors; 3) identify probable windows of high and low establishment opportunity for putative zooplankton invaders based on local community measures of zooplankton density and diversity, physiochemical conditions, and

arrival times of propagules in ballast water; and 4) collect samples that could be used to develop establishment criteria for the propagule pressure experiments (Chapter 3).

## **Methods**

### *Site description*

The Duluth-Superior Harbor is a complex hydrologic system (Fig. 1). The main inlet to the harbor is the St. Louis River that drains approximately 5,800 km<sup>2</sup> (Breneman et al. 2000) of northeastern Minnesota and northwestern Wisconsin. Before reaching the Harbor, the St. Louis River widens and becomes the St. Louis River Estuary which comprises approximately 4,900 ha (Breneman et al. 2000). Upstream portions of the estuary retain relatively intact wetland and forested riparian habitats whereas downstream portions of the estuary, inclusive of the Duluth-Superior Harbor, have been modified substantially through dredging and shoreline development (e.g., armored break walls, piers, and impervious surfaces) to accommodate shipping traffic and land-based commerce (Trebitz et al. 2010). The St. Louis River and estuary reach the Harbor on its western end. On its eastern end, the Duluth-Superior Harbor receives water from a secondary inlet, the Nemadji River. On both its eastern and western ends the Harbor also periodically receives water from Lake Superior (Jordan et al. 1981; Hoffman et al. 2010) through channels that serve as Minnesota (city of Duluth) and Wisconsin (city of Superior) ship entrances. Environmental conditions in the harbor and estuary range widely and support a variety of benthic, pelagic, and littoral habitats.

### *Field sampling*

I operationally divided the Duluth-Superior Harbor and St. Louis River Estuary (hereafter DSHSLRE) into three regions defined by shipping activity (Fig. 1). I coded them as upstream (region 1), inner harbor (region 2), and outer harbor (region 3). Cargo ships are active in regions

2 and 3 but not in region 1. Trebitz et al. (2010) partitioned the DSHSLRE in a similar fashion for the assessment of benthic invertebrates. Trebitz et al. (2010) used a point-based, random probability design to identify sampling locations within each region (Stevens and Olsen 2004). For this study, I selected a set of three of their sampling locations within each region (J. Kelly, personal communication, 2007). Each location was sampled biweekly from 20 April to 20 October, 2007, and from 25 April to 18 October, 2008. Typically, all nine sites were sampled on a single day. Using electronic meters, measurements were made at 1-m increments from the surface to 1 m off the bottom for water temperature and dissolved oxygen concentration (YSI 85; YSI Inc.); and for pH, specific conductivity, turbidity, and total *in vivo* chlorophyll concentration as fluorescence (YSI 6600 V2; YSI Inc.). The chlorophyll sensor emitted radiation at 470 nm and measured fluorescent radiation in the region of the spectrum above 630 nm. Three Secchi disk (all white, 13-cm diameter) measurements were collected per site and averaged.

Crustacean zooplankton were sampled by vertical hauls using a standard zooplankton net (0.5-m diameter mouth, 1.5-m length, 100- $\mu$ m mesh). The net also collected planktonic larvae of dreissenids. Even though the dreissenid larvae were not part of the original project objectives, they were analyzed here because they are known to compete with phytoplanktivorous crustacean zooplankton (Thorp and Casper 2002; Kissman et al. 2010). The mesh size would have permitted escape of the smallest dreissenid size classes (Ackerman et al. 1994; Wright et al. 1996), thus results on their densities are conservative. Because one of my objectives was to assess species richness of the crustacean assemblage spatially and temporally, I was sensitive to the fact that census effort (in this case volume searched) can strongly affect richness (Arnott et al. 1998; Pastor et al. 1996). Therefore, at each site, sampling effort was standardized by adjusting the number of vertical tows so that approximately two cubic meters of water was filtered through the plankton net. This sampling effort depended on water depth and necessitated combining between two to six tows per site. All samples were immediately concentrated and preserved in 70%

ethanol final volume. A flow meter (General Oceanics, Inc.) was affixed to the central portion of the net opening and used to estimate net efficiency and adjust absolute collection volumes on all tows. The flow meter was calibrated by towing the net's steel bridle ring, absent of the net mesh, a known distance for 10 replicates.

### *Sample processing*

For this study, 174 zooplankton samples were examined. Zooplankton were counted in a Ward's counting chamber under a Nikon SMZ800 dissecting microscope at 20-40 $\times$  and identified according to several sources (Balcer et al. 1984; Brooks 1959; Evans 1985; Hudson et al. 1998). In cases where higher magnification was required to examine particular characteristics of a single zooplankton, a Nikon Eclipse E200 compound microscope was used. Mature Copepoda and all Cladocera were identified to species level. Because one of my objectives was to assess species richness, I was also sensitive to the influence of subsample size on density precision and taxa richness. Chimney and Bowers (2006) found that taxa richness and the Jaccard similarity coefficient for freshwater zooplankton stabilize at counts of 100-150 individuals per sample. Thus, each zooplankton sample was subsampled using a wide bore pipette and counted until approximately 200 individuals were identified.

The National Ballast Information Clearinghouse (NBIC) database was queried to obtain a proxy measure of ballast water discharged in the Duluth-Superior Harbor. The NBIC is a joint program of the Smithsonian Environmental Research Center and the United States Coast Guard that collects, analyzes, and interprets data on the ballast water management practices of commercial ships that operate in the waters of the United States. The NBIC has been receiving ballast water reporting forms from ships that arrive to United States ports from overseas since 1 July 1999. For the purposes of this study, data were sorted by ship's arrivals to the ports of Duluth, Minnesota, and Superior, Wisconsin, for the years 2007 and 2008. Data from the

categories of volume discharged by source and volume discharged by management method were summed over a two-week period preceding a sampling event.

### *Analyses*

An estimate of water column stability (resistance to mixing) during the stratified period was calculated as the absolute difference between temperature-based water density at the surface and the deepest depth sampled during June and July dates. The availability of dissolved oxygen near the bottom was examined by considering the dissolved oxygen concentration at the deepest depth measured during June and July dates. Measurements of zooplankton density, chlorophyll concentration, and the chemical and physical factors (aside from Secchi depth) were first averaged over the entire water column by site, and thereafter averaged by date and by region. Taxonomic richness by region was computed as the number of discernable unique taxa per region regardless of density or number of sites of detection within a region. Sampling dates were converted to Julian days. Multiple linear regression (critical level of  $P = 0.05$ ) was used to examine the relationships between zooplankton density and physiochemical parameters including temperature, chlorophyll, turbidity, specific conductivity, and pH during 2007 and 2008. Because temperature and dissolved oxygen concentration were auto-correlated, dissolved oxygen concentration was not included in the analysis.

Simple linear regression (critical level of  $P = 0.05$ ) was used to further explore the relationship between zooplankton density and the product of temperature and time (Watson and Wilson 1978) as:

$$\text{Log}_e \text{ zooplankton density} = m (\text{temperature} * \text{days}) + b \quad [1]$$

where day is expressed as Julian day (1 April = day 0) and temperature is the mean water column temperature at a site on that day. This model was used by Watson and Wilson (1978) to describe seasonal variation in crustacean zooplankton density in Lake Superior.

Simple linear regression (critical level of  $P = 0.05$ ) was used to evaluate the effect of search effort on taxonomic richness. All data were analyzed in SYSTAT 13.0.

## **Results**

### *Physical and Chemical*

Physical and chemical conditions in the DSHSLRE varied widely among regions and seasonally from April to October, reflecting strong spatial and temporal variation in the harbor and estuary environments (Table 1). Temperature was generally warmest at region 1 and coldest at region 3, but the distinction was less evident during the earliest and latest dates (Fig. 2). There was a unimodal cycle in temperature with peak values occurring between 29 June (day 90) and 8 August (day 135). Water column stability was consistently lowest at region 1 and highest at region 3 (Table 2). Chlorophyll concentration expressed a bimodal cycle in 2007, peaking in the spring and fall (Fig. 3). In 2008, a similar pattern is suggested by the data but I was unable to measure the full seasonal range that year due to instrument malfunction. In both years, chlorophyll concentration was consistently higher in regions 1 and 2 than in region 3. Secchi transparency was consistently largest at region 3 and inversely correlated with chlorophyll concentration (Table 1, Fig. 3). Turbidity was consistently highest at region 2 and lowest at region 3 that is the region closest to Lake Superior (Table 1, Fig. 1). Dissolved oxygen concentration in the water column (Table 1) and near the bottom (Table 2) was consistently highest at region 3. Within a year, specific conductivity and pH varied much more temporally (see range in values in Table 1) than spatially. Both were higher on average in 2007 than in 2008 (Table 1).

### *Zooplankton Distribution*



Among the 49 distinct taxa that were identified, approximately half at the genus level were Copepoda (Calanoida and Cyclopoida) and half were Cladocera (Table 3). Regional trends were evident in the Copepoda (Table 3). For example, *Eurytemora affinis*, *Leptodiptomus siciloides*, and *Skistodiptomus oregonensis* were present at all sites while *Leptodiptomus ashlandi* and *Leptodiptomus minutus* were present only at sites H and G, respectively (Table 3). *Acanthocyclops vernalis*, *Diacyclops thomasi*, *Mesocyclops edax*, *Microcyclops rubellus*, and *Tropocyclops prasinus mexicanus* were present at all sites, while species of the genera *Eucyclops* and *Macrocylops* were never detected in region 3 and species of the genus *Paracyclops* were detected only in region 1 (Table 3).

Although about half of all Cladocera taxa were present in all three regions, several species were region specific. For example, *Daphnia mendotae* never appeared in region 1, and *Pleuroxus*, *Sida crystallina*, and *Simocephalus* never appeared in region 3 (Table 3).

#### *Zooplankton Density and Seasonality*

The average density of zooplankton ranged widely on a seasonal basis from < 500 to > 100,000 individuals m<sup>-3</sup> (Figs. 4-6). Densities of most groups expressed unimodal cycles of growth and collapse and typically peaked between 29 June (day 90) and 27 September (day 180). Among the major groups, Bosminidae typically dominated the assemblage (Figs. 4-6). Bosminidae were consistently more numerous at regions 2 and 3 than region 1 (Fig. 4). For Copepoda, the densities of all naupliar stages were similar to densities of all copepodid stages suggesting an even age distribution (Fig. 5). There were no major differences in densities among regions within any of the three copepod taxonomic groups (Fig. 5). For Daphnidae, there were contrasting spatial trends between years (Fig. 6). In 2007, densities were highest at regions 1 and 2 and lowest at region 3, while in 2008, densities were lowest at region 1 and highest at regions 2 and 3 (Fig. 6). The group 'other cladocerans,' which included all Cladocera other than

Bosminidae or Daphnidae, was more evenly distributed among regions and between years.

Phenology of the dreissenid veliger larvae generally paralleled the group 'other cladocerans.'

#### *Relationships between Zooplankton Density and Abiotic Factors*

Analysis by multiple linear regression (Tables 4-5) showed that zooplankton density was significantly ( $P < 0.01$ ) predicted by physical and chemical factors in 2007 as:

$$\text{Log}_e \text{ zooplankton density} = 0.108(\text{temperature}) + 1.353(\text{pH}) - 0.174(\text{chlorophyll}) - 1.989; R^2 = 0.69 \quad [2]$$

and in 2008 as:

$$\text{Log}_e \text{ zooplankton density} = 0.164 (\text{temperature}) - 0.412(\text{chlorophyll}) + 11.651; R^2 = 0.46 \quad [3]$$

Neither turbidity nor specific conductivity was a significant predictor ( $P > 0.05$ ) of zooplankton density (Tables 4-5).

To describe the relationship between zooplankton abundance, temperature and time, the natural log of zooplankton abundance and the product of temperature and time was plotted (Fig. 7). Further analysis by simple linear regression showed that zooplankton density as a function of the product of water temperature and Julian day was significant ( $P = 0.01$ ) in 2007:

$$\text{Log}_e \text{ zooplankton density} = 0.001 (\text{temperature} * \text{Julian day}) + 8.24; R^2 = 0.54 \quad [4]$$

and 2008:

$$\text{Log}_e \text{ zooplankton density} = 0.002 (\text{temperature} * \text{Julian day}) + 6.51; R^2 = 0.86 \quad [5]$$

#### *Zooplankton Richness*

Because the taxonomic richness of a species assemblage can be sensitive to search effort, an analysis of the effect of volume of harbor water searched on zooplankton richness was explored (Fig. 8). Results of a best-fit linear regression model indicate that for every 1000 L of

water that was searched, approximately seven new species were found (Fig. 8). The mean volume of harbor water searched was 101 L per sample (n = 176 samples), while the minimum and maximum volumes of harbor water searched were 0.5 and 1079 L, respectively. The samples with volumes of harbor water searched that fell outside the 95% confidence interval of the arithmetic mean (97.5-123.4 L) did not associate with particular sites or dates. Hence, despite the recognized bias associated with these samples, they were left in the analysis of richness. In 2007, average taxon richness ranged from 5-14 taxa per region (Fig. 9), while in 2008, average taxon richness ranged from 8-14 taxa per region. Each year there was a trend toward increasing richness with time in each region (Fig. 9). However, in neither 2007 nor 2008, was there evidence for a strong disparity in richness by region (Fig. 9).

#### *Zooplankton Community Similarity*

Similarity was generally lower between regions 1 and 3 than between either of the other two regional comparisons for both years. The mean similarity between regions was calculated using data from both years and found to be 34% between regions 1 and 3, 39% between regions 1 and 2, and 44% between regions 2 and 3 (Fig. 10). For both years, a temporal trend was observed. Similarity values peaked between 1 August (day 128) and 17 September (day 175) (Fig. 10).

#### *Ballast Water Discharge*

In 2007, estimates of ballast water discharged into the Duluth-Superior Harbor were relatively steady at 1 metric ton every 2 weeks (Fig. 11). By contrast, although in 2008 most estimates of biweekly ballast discharge were about 1 metric ton, these were punctuated by 2-week episodes of higher discharge about every 6 weeks (Fig. 11). Values ranged from a minimum biweekly discharge of 201,760 to a maximum of 1,409,210 metric tons in 2007 and from a minimum of 989,424 to a maximum of 2,451,978 metric tons in 2008 (Fig. 11).

## Discussion

The primary objective of this study was to describe the spatial and temporal patterns of diversity and density of crustacean zooplankton in the DSHSLRE and analyze those patterns in the context of physiochemical factors. The secondary objective of this study was to describe windows of invasion opportunity, if they exist, in the context of the spatial and temporal patterns of both the abiotic and crustacean zooplankton community of the DSHSLRE. Both objectives are discussed below.

### *Spatial patterns*

Spatially, the results indicate that the DSHSLRE has both lentic and lotic habitats with diverse physiochemical conditions. The upper reaches of the study area (region 1) retain relatively unmodified coastal and submerged habitat that includes intact wetlands and forested riparian zones. By contrast, the lower reaches (regions 2 and 3) consist of large portions of dredged river bottom and a substantial amount of residential and commercial shoreline development. Region 1 stations were the shallowest, warmest, most productive (Chl concentration), and least stable (most lotic-like) while region 3 stations were the coolest, least productive, least turbid (lowest turbidity, highest Secchi transparency), and most stable (most lentic-like) of the three regions (Tables 1 and 2). Variables such as pH and specific conductivity varied minimally across space while other variables such as dissolved oxygen and turbidity displayed interesting spatial patterns.

Dissolved oxygen concentrations were generally uniform from surface to sediment with little indication of gradients in concentration. At no location or time did I detect anoxic or even hypoxic ( $< 2 \text{ mg L}^{-1}$  dissolved oxygen) conditions at the sediment surface. These data indicate that benthic species and the benthic life stages of species will find sufficient oxygen conditions in the DSHSLRE at all depths to complete their life cycles.

Although turbidity and transparency varied spatially, turbidity was generally greater upstream (region 1) than downstream (region 3), whereas water transparency (Secchi depth) increased from region 1 to region 3. Despite this general trend, there was tremendous variation in turbidity in region 1 and 2 owing to the fact that parts of these regions are quite shallow and strongly influenced by riverine inputs, whereas region 3 sites are deeper, less influenced by wind and wave action, and flushed more continuously with Lake Superior (e.g., Duluth, MN and Superior, WI entries).

The effects of turbidity (clay and silt) on community structure of zooplankton have been studied in both the laboratory and field settings (McCabe and O'Brien 1983; Kirk and Gilbert 1990; Kirk 1991). Results from laboratory studies showed that high concentrations (50 – 100 mg/L) of coarse clay (Kirk and Gilbert 1990) and turbidity levels between 10-30 NTUs (McCabe and O'Brien 1983) caused reductions in growth and feeding rates of cladocerans. Field observations found that the average body length of zooplankton in Olathe Lake, Kansas, a moderately turbid (5-15 NTUs) reservoir was twice as long compared to zooplankton in Perry State Lake, Kansas, a consistently high turbid (20-50 NTUs) reservoir. Also, two large body daphnids (*Daphnia pulex* and *D. galeata mendotae*) were only found in Perry State Lake (McCabe and O'Brien 1983). Taken together, the above findings suggests turbidity may play a role in the structure of daphnid communities. The turbidity levels described in the DSHSLRE (Table 1) are similar to those described above and may also play a role in the observed patterns of distribution of several species of daphnids (Table 3).

There was a signal in the data that suggests species richness is greater in region 1, which is upstream of Spirit Lake (sites A-C) than region 3 (contiguous with Lake Superior, sites G-I). Species richness in region 2 was typically higher than in region 3; in only two cases did region 2 richness fall below region 3. This pattern in species richness may reflect exchange with Lake Superior's assemblage of zooplankton through the Duluth and Superior entries. Consistent with these factors, the region 1 assemblage of crustacean zooplankton was dominated by littoral genera

including *Camptocercus*, *Macrothrix*, *Monospilus*, *Pleuroxus*, *Sida*, and *Simocephalus*. While the study sites for this region were located in the pelagic-like main river channel with water depths from 4-6 m, large and relatively shallow embayments (e.g., Pokegama Bay) that contained both submerged and emergent vegetation may have provided source habitat for some taxa in this littoral-rich assemblage. Two genera of Cyclopoida, *Eucyclops* and *Macrocylops*, that were found in regions 1 and 2 but not region 3, prefer shallow littoral habitats (Balcer 1978) consistent with the littoral nature of region 1. Research by Walseng et al. (2006) suggested species richness is typically higher in littoral regions than pelagic regions due to greater habitat heterogeneity, which potentially creates higher niche opportunities. This may also be the case in DSHSLRE as suggested by the higher richness in region 1.

In contrast to region 1, the crustacean zooplankton in regions 2 and 3 more strongly reflect the species composition in Lake Superior (Balcer et al. 1984, Brown and Branstrator 2004). Lake Superior is overwhelmingly dominated by calanoid copepods, particularly species of the genus *Leptodiatomus* (Watson and Wilson 1978; Megard 1997; Zhou et al. 2001; Brown and Branstrator 2004). In Lake Superior, cladoceran zooplankton (e.g., *Bosmina*, *Daphnia*) are also common but occur predominantly seasonally and in relation to warmer water temperature (Watson and Wilson 1978). It is possible the occurrence of Lake Superior zooplankton found in regions 2 and 3 resulted from a mass of Lake Superior water mixing with DSHSLRE in regions 2 and 3 (Hoffman et al. 2010) thus creating a zooplankton community that resembles both areas (Balcer 1978).

#### *Temporal patterns*

Temperature varies dramatically both spatially and temporally in the DSHSLRE. It appears to be an excellent predictor of zooplankton abundance (Fig. 7), probably because zooplankton are ectothermic and their growth rate is determined to a large extent by enzyme activity (Vijverberg 1980; Shuter and Ing 1997). Previously, water temperature has been demonstrated to explain 69% (Watson and Wilson 1978) and 54% (Zhou et al. 2001) of the

variation in zooplankton density in Lake Superior. A correlation of 92% between water temperature and zooplankton density has been reported for several North American large lakes (Patalas 1990). The timing of buildup in zooplankton densities in this study closely reflected increasing water temperatures, and often lagged by about one month. These patterns are broadly consistent with the hypothesis that water temperature is a major environmental factor controlling growth potential and overall phenology in density of crustacean zooplankton in the DSHSLRE (Watson and Wilson 1978).

*In vivo* chlorophyll concentration is an indicator of phytoplankton standing crop. The seasonal patterns of chlorophyll concentration in the DSHSLRE are broadly consistent with a general pattern that has been described for mesotrophic or eutrophic lakes in the northern hemisphere (Sommer et al. 1986). In general, phytoplankton in moderately to highly productive lakes express a spring peak followed by a fall peak (Sommer et al. 1986). During 2007 and 2008, this pattern was observed in all three regions of the DSHSLRE (Fig. 3). The late spring, early summer low is usually described as a result of loss to zooplankton grazing, sinking owing to a stabilized water column, and sometimes nutrient limitation (particularly silica) that reduces the abundance of diatoms (Sommer et al. 1986). The timing of the build up of crustacean zooplankton in this study is consistent, in a correlative sense, to grazer losses.

Indeed, a strong-negative relationship (Tables 4-5) between zooplankton abundance and chlorophyll concentration was observed at the DSHSLRE study sites suggesting that herbivorous crustacean zooplankton is exerting grazing control on phytoplankton. Fahnenstiel et al. (1998) also observed a negative relationship between zooplankton abundance and phytoplankton abundance in Lake Michigan. Fahnenstiel et al. (1998) suggested a critical link for controlling phytoplankton abundance was the presence of an abundant large body cladoceran, *Daphnia pulicaria*. Perhaps, in the DSHSLRE the cladoceran responsible for the majority of grazing pressure on phytoplankton is *Bosmina longirostris* (Fig. 4). The density of *Bosmina longirostris* in the DSHSLRE exceeds those of other taxa identified by 2-5 times. *Bosmina longirostris*'s

peak density often followed the chlorophyll maximum and overlapped with the midsummer chlorophyll minimum (Figs. 3-4).

#### *Windows of Invasion Opportunity*

Conceptually, once the propagules (defined here as a structure that gives rise to a new organism) of an invading organism arrive to a new location, what constitutes a window of invasion opportunity (Shea and Chesson 2002) is determined by the invading organism's tolerance of the physiochemical environment (Moyle and Light 1996; Von Holle and Simberloff 2005) and interactions with native species (DeRivera et al 2005; Davis et al. 2000). The interactions with native species includes the biotic resistance hypothesis (Elton 1958), which states that an invader is hindered by biological interactions instead of the physiochemical conditions. It is unclear if the physiochemical properties, biotic interactions, or some combination of these determines the establishment success of an invader. The balance between the roles of the physiochemical environment and biotic interactions influencing the results of invader establishment outcome has been of interest to many ecologists. This balance is of a particular interest when the invading habitat varies both spatially and temporally, such as the DSHSLRE. The spatial and temporal heterogeneity of both the physiochemical and biotic, specifically crustacean zooplankton, components of the DSHSLRE may create windows of invasion opportunity for putative zooplankton invaders.

Several studies have indicated that if the physiochemical conditions are suitable for an invader it is likely the invader will establish regardless of the biota present (Moyle and Light, 1996; Ricciardi 2001; Holway et al. 2002; Von Holle and Simberloff 2005; DeRivera et al. 2005). In the case of my research, if physiochemical conditions matter in the outcome of the invader's success both the spatial and temporal heterogeneity and gradient of habitats in the DSHSLRE will provide multiple sites for colonization thus creating many windows of opportunity for invasion when solely considering physiochemical conditions. Both spatially and temporally the DSHSLRE has a gradient of habitats ranging from warm, productive, littoral habitats (region 1) to cooler,



less productive, pelagic like habitats downstream (regions 2 and 3) thus creating numerous suitable habitats for an arriving invader.

The biotic resistance hypothesis suggests that species-rich communities are more resistant to invasion than species-poor communities (Elton 1958). This hypothesis simply states that communities with rich diversity of native species can provide resistance to invasion through strong biological interactions such as abundant native species, strong native competitors, and efficient native predators (Elton 1958; Ricciardi 2001; DeRivera et al. 2005). Spatial and temporal patterns in density and richness of the crustacean zooplankton were observed in the DSHSLRE. Crustacean zooplankton richness was greatest in region 1 and consisted mainly of littoral taxa while region 3 typically had the lowest richness with an assemblage that reflected taxa from Lake Superior. Densities of crustacean zooplankton varied with the highest densities occurring at regions 1 and 2 and lowest at region 3 in 2007, while the opposite occurred in 2008. Notable spatial patterns occurred, such as Bosminidae were consistently more numerous at regions 2 and 3 than region 1 and no major differences in copepod taxonomic group densities among regions were detected. Though notable patterns of richness and density were observed in the DSHSLRE it is difficult to define windows of invasion opportunity based solely on spatial patterns of richness and density for the following reasons. First, many factors such as nutrient availability, climate, and disturbance events covary and influence changes in richness and density of the native community as well as the invader's establishment success (Von Holle and Simberloff 2005). Second, little evidence exists that addresses the influence of predators and competitors within the native community on establishment outcomes (Baltz and Moyle 1993; Miller et al. 2002). Last, it has been noted in several studies that biotic resistance plays more of a role in physiochemical conditions that are benign and less of a role under stressful physiochemical conditions (Ricciardi 2001; Miller et al. 2002; Dethier and Hacker 2005; DeRivera et al. 2005). Contrary to the biotic resistance hypothesis, the rate of invasion is increasing in the Great Lakes including the DSHSLRE (Ricciardi 2001, Grigorovich et al. 2003).

### Arrival of Propagules

Lastly, the overall magnitude of shipping and deballasting activity in the DSHSLRE, hence the arrival and addition of aquatic non-native species makes this location a likely hub for invasions to occur. The onset of ballast discharge in the DSHSLRE typically occurs towards the end of April and ends late fall. The discharge of ballast water into the DSHSLRE is relatively constant over the shipping season; however, periodic pulses of ballast water were observed in 2008 (Fig. 10). The continual supply of non-native aquatic taxa within the discharged ballast water throughout the shipping season does overlap with windows of invasion opportunity, such as diverse lentic and lotic habitats of the DSHSLRE, periods of high availability of food resources, and periods of low biotic resistance. Occurrence of new, non-native aquatic species in the DSHSLRE is consistent with patterns of ballast water discharge from ships (Grigorovich et al. 2003).

### **Conclusion**

In conclusion, there were notable spatial and temporal patterns in the physical and chemical conditions and the density and diversity of crustacean zooplankton in the DSHSLRE. Patterns of crustacean-zooplankton growth closely reflected water temperatures and were likely responsible for the negative relationship observed with the chlorophyll concentrations. Yearly and regional variations showed that the DSHSLRE is not a static environment; physiochemical and biological conditions within region 1 changed more dramatically than regions 2 and 3 from year to year and within a given year. While each of the individual and biological processes described above played a role in identifying probable windows of high and low establishment opportunity for zooplankton invaders it is ultimately the interaction of these events that will likely lead to high establishment opportunities (Davis et al. 2005).

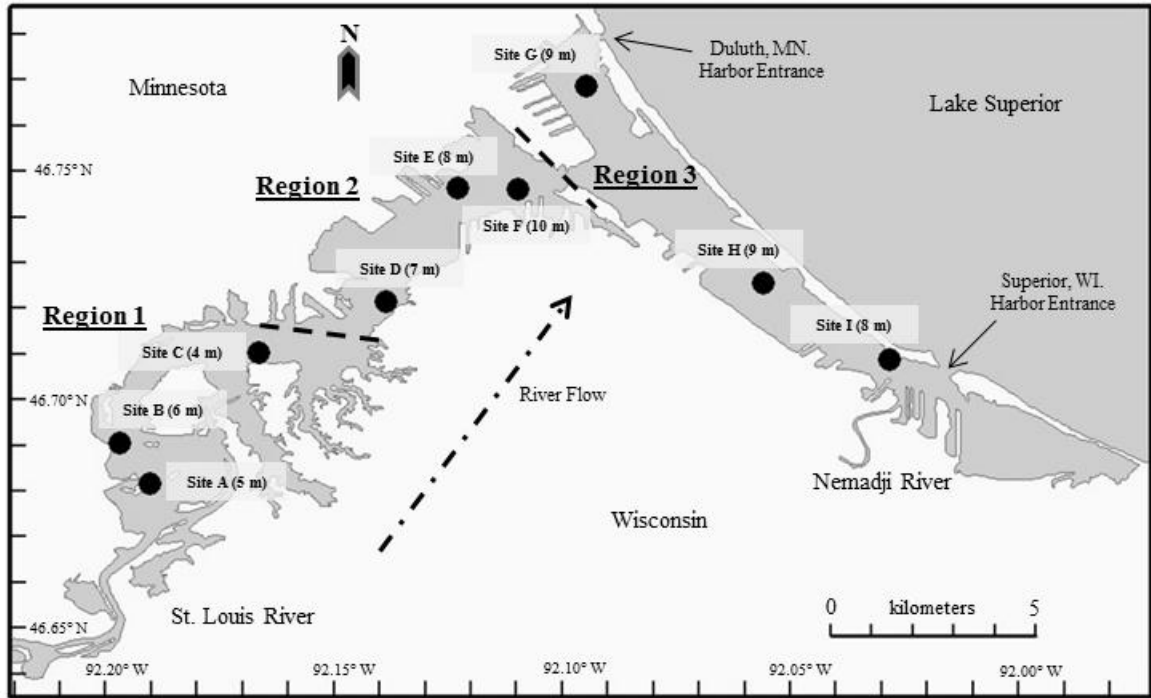


Figure 1. Schematic of the 9 sampling locations (sites A-I) in the Duluth-Superior Harbor and St. Louis River Estuary. Site depth is given in parentheses after the site code. Dashed lines depict regional boundaries (regions 1-3) used in the analysis. The large dashed arrow indicates the predominant direction of water flow.

Table 1. Average (min-max) water column temperature, chlorophyll concentration, Secchi depth, turbidity, dissolved oxygen concentration, specific conductivity, and pH by year and region (see Fig. 1) in the DSHSLE for the period April 20 to Oct 20, 2007 and April 25 to Oct 18, 2008.

Year	Region	Temperature (°C)	Chlorophyll (µg/L)	Secchi Depth (m)	Turbidity (NTU)	Dissolved Oxygen (mg/L)	Specific Conductivity (µS/cm)	pH
<b>2007</b>	<b>1</b>	18.4 (8.0-26.1)	11.8 (6.7-21.8)	1.1 (0.5-1.9)	8.3 (3.1-23.6)	8.8 (6.7-12.4)	190 (126-257)	7.85 (7.58-8.40)
	<b>2</b>	16.9 (8.6-22.2)	11.3 (5.2-19.5)	0.9 (0.4-1.5)	11.2 (4.2-27.0)	8.1 (7.4-11.6)	205 (138-265)	7.84 (7.49-8.26)
	<b>3</b>	16.3 (10.0-19.9)	8.2 (3.4-20.0)	1.4 (0.5-2.4)	5.2 (1.8-11.6)	9.3 (8.4-11.8)	191 (155-266)	7.82 (7.64-8.16)
<b>2008</b>	<b>1</b>	16.3 (7.7-24.3)	8.5 (5.3-11.0)	0.8 (0.4-1.4)	6.1 (4.6-7.2)	8.2 (5.2-12.0)	172 (98-253)	7.72 (7.41-8.16)
	<b>2</b>	16.0 (7.7-22.3)	9.1 (5.5-10.5)	0.8 (0.5-1.0)	9.1 (6.5-11.3)	8.3 (4.8-11.7)	171 (100-257)	7.71 (7.50-8.11)
	<b>3</b>	14.8 (7.2-20.6)	5.0 (3.8-7.4)	1.1 (0.6-1.6)	4.4 (2.6-6.5)	8.9 (4.8-11.7)	164 (116-289)	7.75 (7.49-7.98)

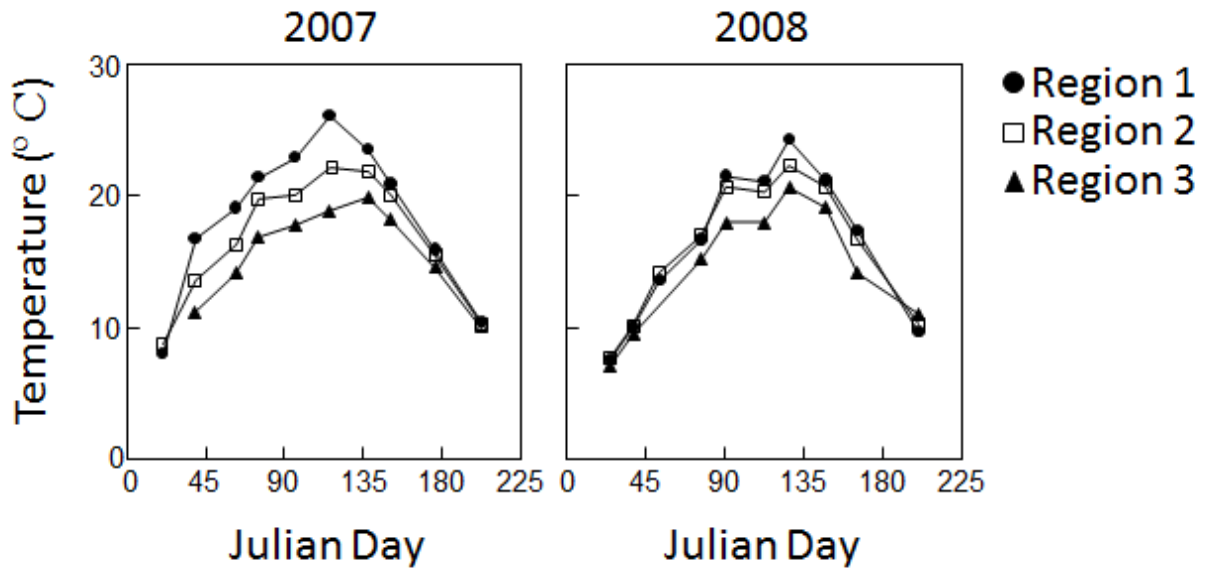


Figure 2. Integrated water column temperature as a function of date (Julian Day, where day 0 = April 1st and day 203 = October 20) shown by region.

Table 2. Average site depth, average  $\pm$  SD density difference between the deepest measurement and the surface, and average  $\pm$  SD dissolved oxygen concentration for the deepest measurements during June and July by year and region.

<b>Year</b>	<b>Region</b>	<b>Average Site Depth (m)</b>	<b>Average <math>\pm</math> SD Density Difference (kg/m<sup>3</sup>)</b>	<b>Average <math>\pm</math> SD Dissolved Oxygen Concentration (mg/L)</b>
<b>2007</b>	<b>1</b>	5	0.310 $\pm$ 0.02	7.42 $\pm$ 0.08
	<b>2</b>	8	0.516 $\pm$ 0.08	7.80 $\pm$ 0.39
	<b>3</b>	9	0.765 $\pm$ 0.13	8.87 $\pm$ 0.54
<b>2008</b>	<b>1</b>	5	0.052 $\pm$ 0.03	6.74 $\pm$ 0.28
	<b>2</b>	8	0.362 $\pm$ 0.23	6.88 $\pm$ 0.20
	<b>3</b>	9	0.622 $\pm$ 0.17	7.65 $\pm$ 0.39

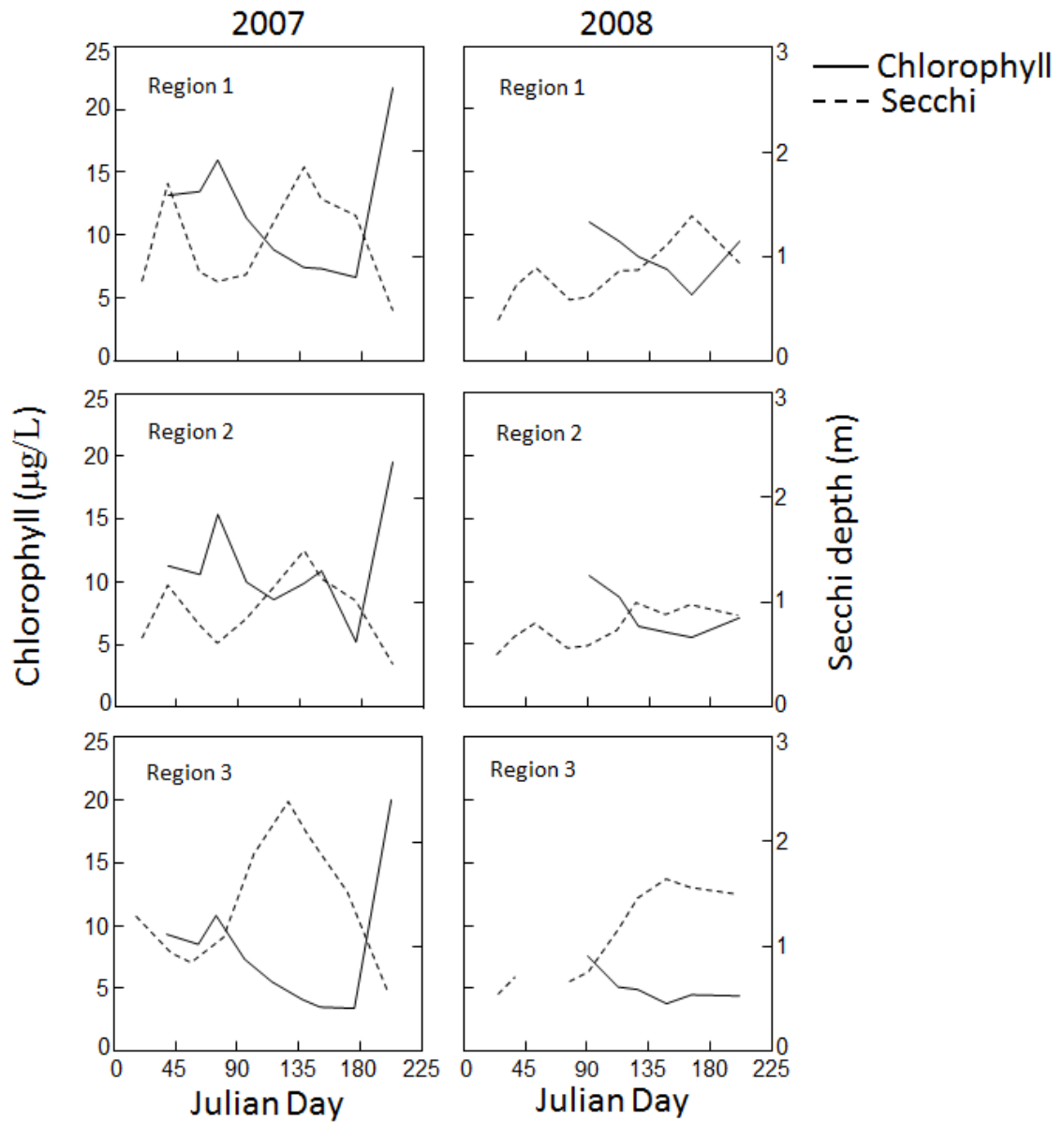


Figure 3. Integrated water column chlorophyll ( $\mu\text{g/L}$ ) and average Secchi depth (m) as a function of date (Julian Day, where day 0 = April 1 and day 203 = October 20) shown by region.

Table 3. The presence (x) of crustacean zooplankton taxa by region and site for 2007 and 2008 combined.

Taxon	Sampling Region and Site								
	Region 1			Region 2			Region 3		
	A	B	C	D	E	F	G	H	I
<b>Calanoid Copepods</b>									
<i>Epischura lacustris</i>	X			X	X	X	X	X	X
<i>Eurytemora affinis</i>	X	X	X	X	X	X	X	X	X
<i>Leptodiaptomus ashlandi</i>								X	
<i>Leptodiaptomus minutus</i>							X		
<i>Leptodiaptomus sicilis</i>		X	X			X	X		X
<i>Leptodiaptomus siciloides</i>	X	X	X	X	X	X	X	X	X
<i>Skistodiaptomus oregonensis</i>	X	X	X	X	X	X	X	X	X
<b>Cyclopoid Copepods</b>									
<i>Acanthocyclops vernalis</i>	X	X	X	X	X	X	X	X	X
<i>Diacyclops thomasi</i>	X	X	X	X	X	X	X	X	X
<i>Diacyclops sp.</i>		X	X	X					
<i>Eucyclops agilis</i>	X	X	X	X	X	X			
<i>Eucyclops elegans</i>			X	X	X				
<i>Eucyclops prionophorus</i>	X	X	X	X		X			
<i>Eucyclops sp.</i>	X	X	X	X	X	X			
<i>Macrocyclus albidus</i>	X			X					
<i>Macrocyclus sp.</i>				X					
<i>Mesocyclops americanus</i>	X	X				X			
<i>Mesocyclops edax</i>	X	X	X	X	X	X	X	X	X
<i>Microcyclops rubellus</i>	X	X	X	X	X	X	X	X	X
<i>Paracyclops chittoni</i>	X		X						
<i>Paracyclops poppei</i>		X							
<i>Tropocyclops prasinus mexicanus</i>	X	X	X	X	X	X	X	X	X
<b>Cladocera</b>									
<i>Alona sp.</i>	X	X	X	X	X	X	X	X	X
<i>Alonella sp.</i>						X			
<i>Bythotrephes longimanus</i>							X		
<i>Bosmina longirostris</i>	X	X	X	X	X	X	X	X	X
<i>Eubosmina coregoni</i>		X			X	X	X		
<i>Camptocercus rectirostris</i>	X						X		
<i>Camptocercus sp.</i>	X	X	X	X	X			X	
<i>Ceriodaphnia sp.</i>	X	X	X	X	X	X	X	X	X
<i>Chydorus sp.</i>	X	X	X	X	X	X	X	X	X
<i>Daphnia ambigua</i>					X				
<i>Daphnia galeata mendotae</i>				X	X	X	X	X	X
<i>Daphnia parvula</i>			X		X				
<i>Daphnia pulex</i>									X
<i>Daphnia retrocurva</i>	X	X	X	X	X	X	X	X	X
<i>Daphnia sp.</i>	X	X	X		X	X		X	
<i>Diaphanosoma sp.</i>	X	X	X	X	X	X	X	X	X
<i>Eurycercus sp.</i>	X		X	X					
<i>Graptoleberis sp.</i>		X							
<i>Holopedium gibberum</i>	X					X	X	X	X
<i>Ilyocryptus sp.</i>		X	X						
<i>Leptodora kindtii</i>	X	X	X	X	X		X	X	X
<i>Leydigia sp.</i>	X	X	X	X	X	X		X	
<i>Macrothrix sp.</i>	X	X	X			X			
<i>Monospilus sp.</i>	X	X	X		X			X	
<i>Pleuroxus sp.</i>	X								
<i>Sida crystallina</i>	X	X	X	X	X				
<i>Simocephalus sp.</i>	X		X	X	X				



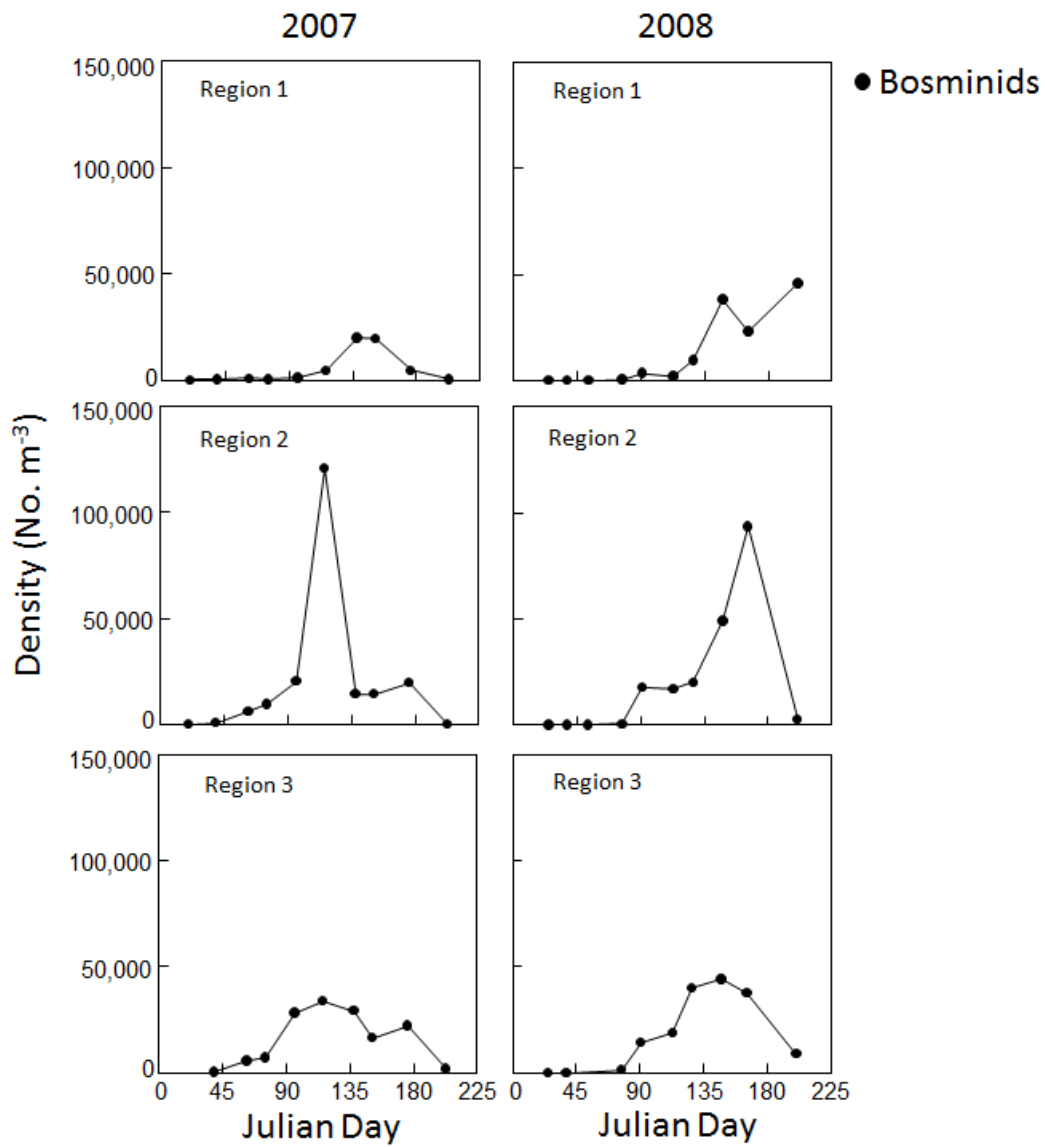


Figure 4. Average water column densities (No. m<sup>-3</sup>) of bosminid cladocerans (*Eubosmina coregoni* and *Bosmina* sp.) as a function of date (Julian Day, where day 0 = April 1 and day 203 = October 20) shown by region.

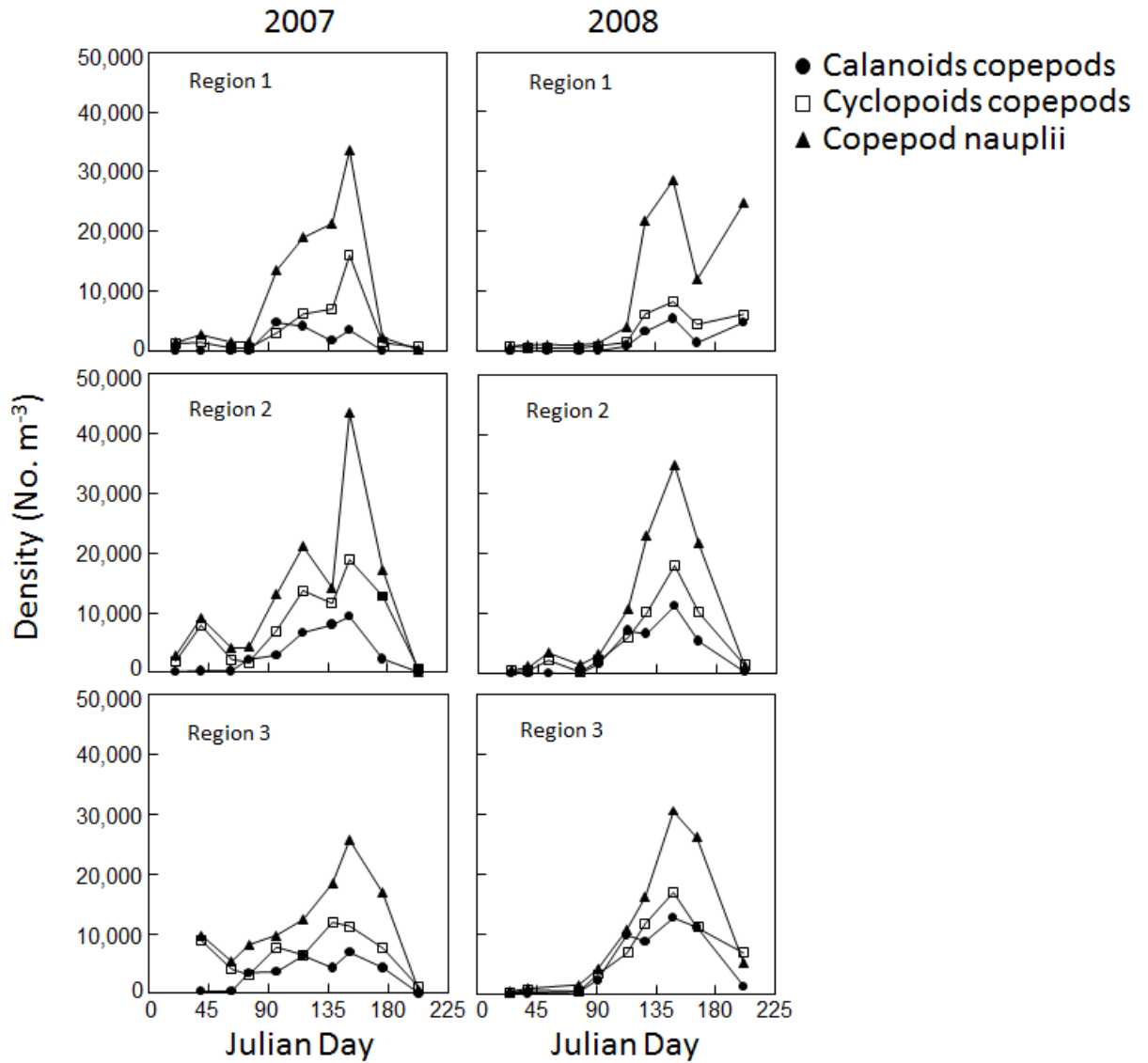


Figure 5. Average water column densities (No. m<sup>-3</sup>) of calanoids (immature and adult stages), cyclopoids (immature and adult stages) and copepod nauplii as a function of date (Julian Day, where day 0 = April 1 and day 203 = October 20) shown by region.

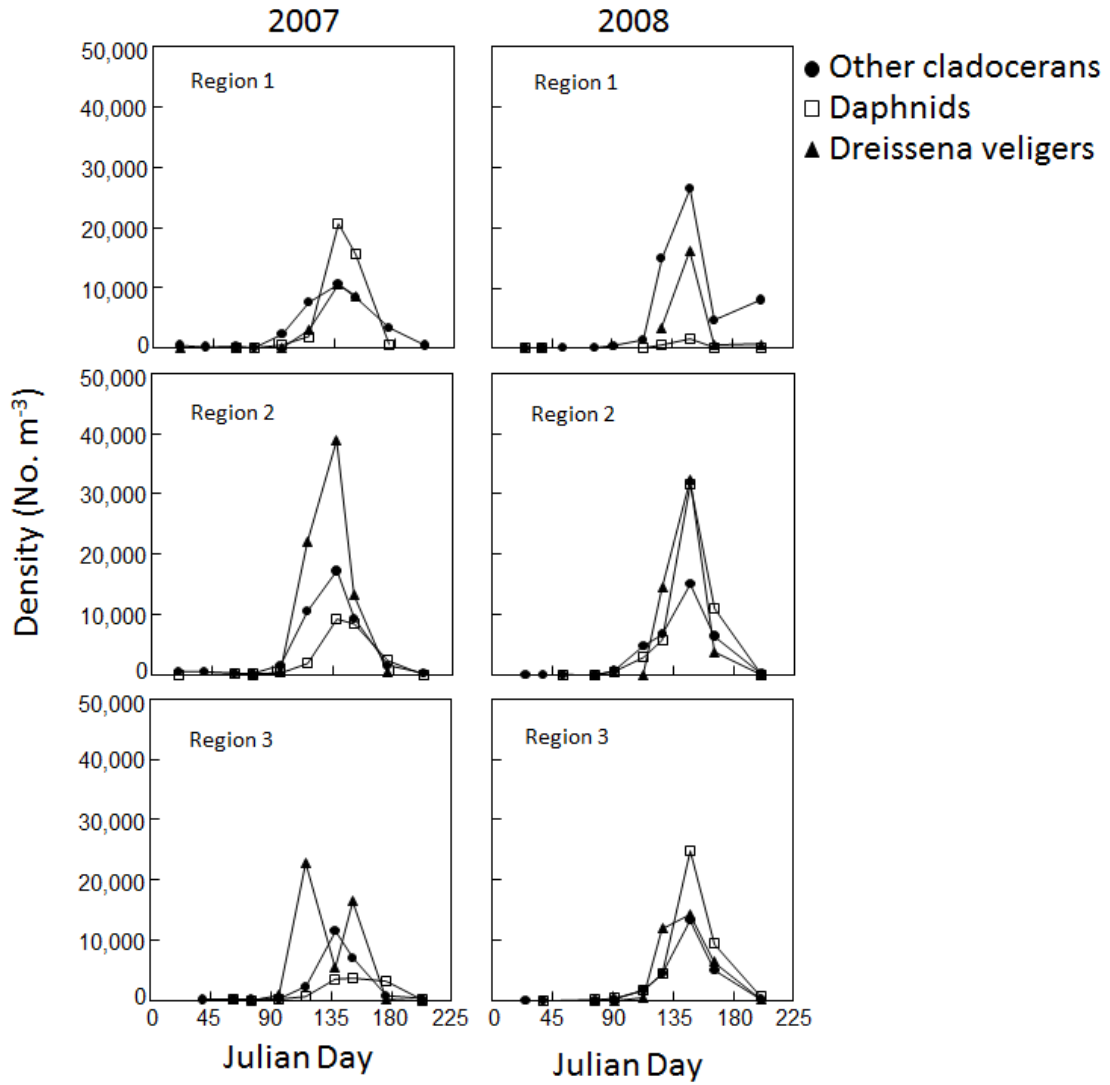


Figure 6. Average water column densities (No. m<sup>-3</sup>) of non-bosminid cladocerans, dreissena veligers and daphnia as a function of date (Julian Day, where day 0 = April 1 and day 203 = October 20) shown by region.

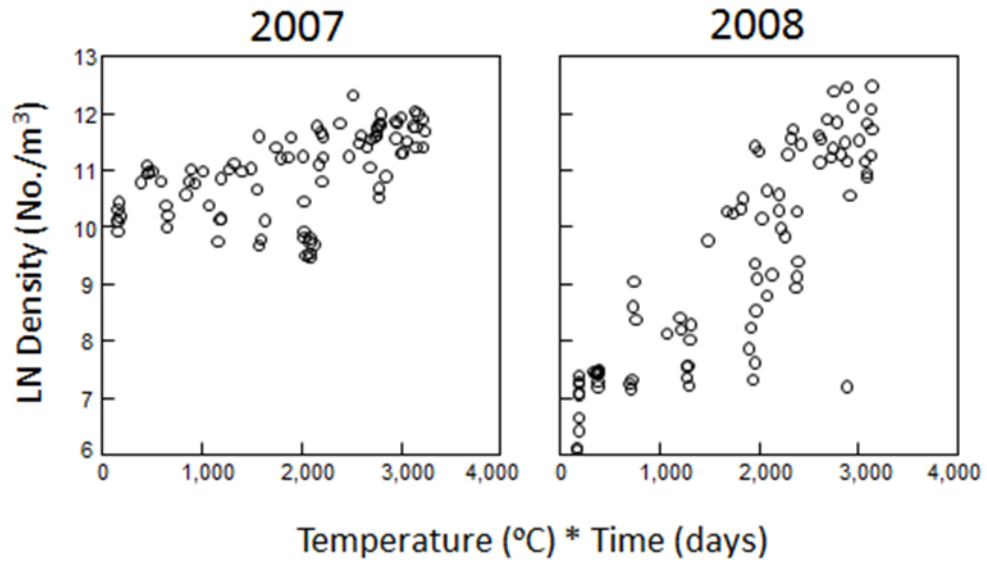


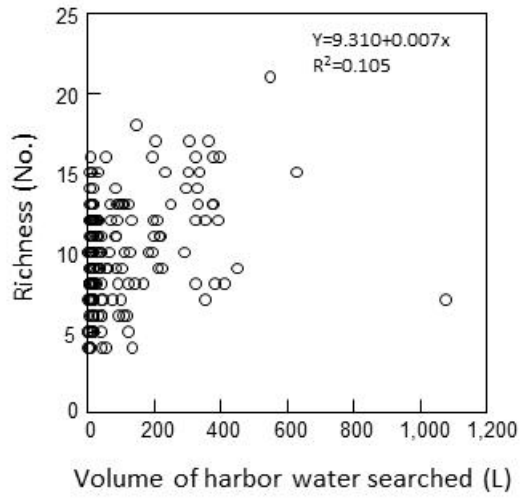
Figure 7. The growth relationship between temperature, time, and zooplankton abundance. Data points represent individual site density values per sampling event for the years of 2007 and 2008.

Table 4. Multiple linear regression coefficients and analysis of variance describing the relationship between mean water column parameters and zooplankton abundance and strength of model for 2007. Data points represent site values.

<b>Regression Coefficients <math>B=(X'X)^{-1}X'Y</math></b>					
<b>Effect</b>	<b>Coefficient</b>	<b>Standard Error</b>	<b>P-value</b>		
Constant	-1.989	3.975	0.618		
Temperature	0.108	0.028	0.000		
Turbidity	0.032	0.025	0.194		
Chlorophyll	-0.174	0.029	0.000		
Specific Conductivity	0.005	0.003	0.056		
pH	1.353	0.484	0.007		
R-squared = 0.69; N=79					
<b>Analysis of Variance</b>					
<b>Source</b>	<b>SS</b>	<b>df</b>	<b>Mean Squares</b>	<b>F-ratio</b>	<b>P-value</b>
Regression	112.6	5	22.5	32.1	0.000
Residual	51.2	73	0.7		

Table 5. Multiple linear regression coefficients and analysis of variance describing the relationship between mean water column parameters and zooplankton abundance and strength of model for 2008. Data points represent site values.

<b>Regression Coefficients <math>B=(X'X)^{-1}X'Y</math></b>					
<b>Effect</b>	<b>Coefficient</b>	<b>Standard Error</b>	<b>P-value</b>		
Constant	11.651	10.533	0.274		
Temperature	0.164	0.049	0.002		
Turbidity	0.060	0.078	0.445		
Chlorophyll	-0.412	0.097	0.000		
Specific Conductivity	0.000	0.004	0.933		
pH	-0.205	1.283	0.874		
R-squared = 0.46; N=52					
<b>Analysis of Variance</b>					
<b>Source</b>	<b>SS</b>	<b>df</b>	<b>Mean Squares</b>	<b>F-ratio</b>	<b>P-value</b>
Regression	44.7	5	8.9	7.9	0.000
Residual	51.8	46	1.1		



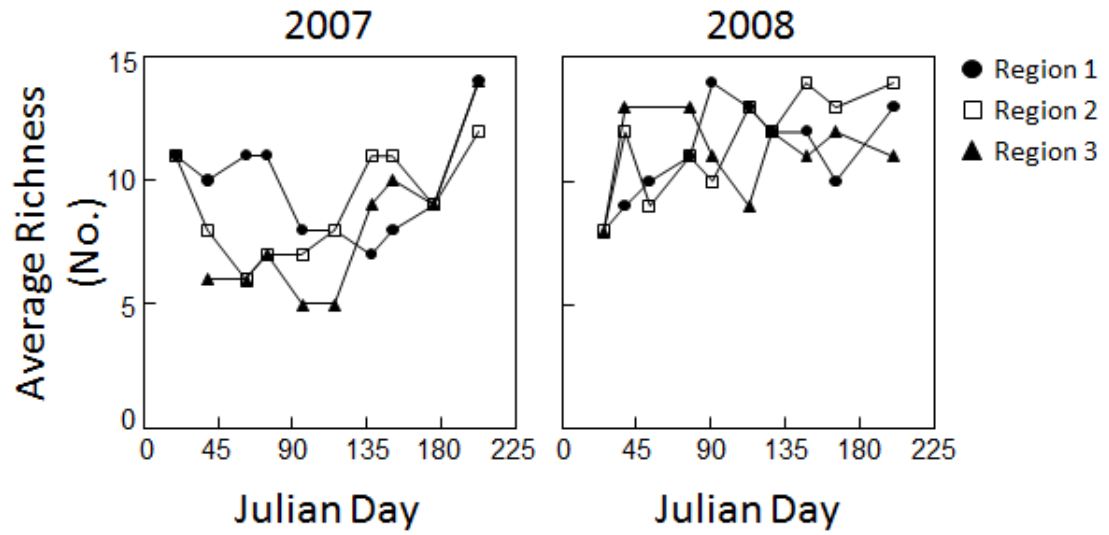


Figure 9. Taxon richness (number of unique taxa present) of crustacean zooplankton as a function of date (Julian Day, where day 0 = April 1 and day 203 = October 20) shown by region.



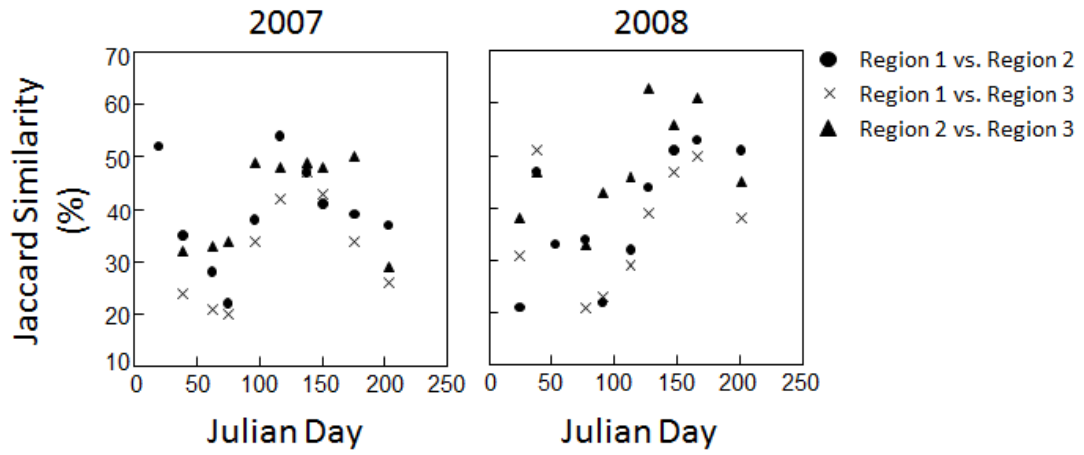


Figure 10. Jaccard similarity (%) of crustacean zooplankton communities between regions as a function of date (Julian Day, where day 0 = April 1 and day 203 = October 20) shown by region.

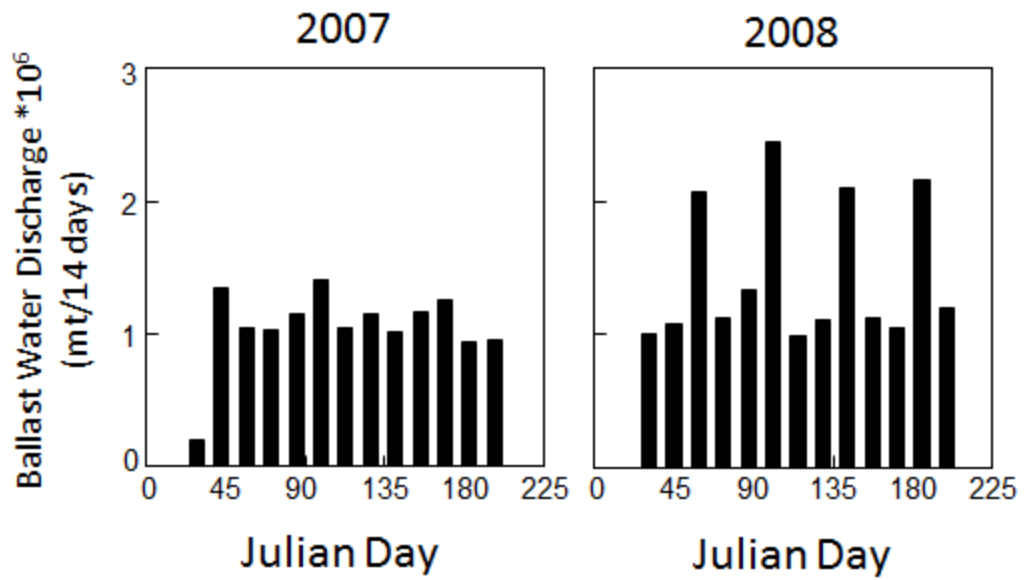


Figure 11. Ballast water discharge (metric ton / 14 days) of ships entering the Duluth-Superior Harbor and St. Louis River Estuary as a function of date (Julian Day, where day 0 = April 1 and day 203 = October 20).

# **Chapter 3: Assessing the Relationship between Propagule Pressure and Colonization Success of an Invasive Species, *Daphnia magna*, in the Environment of Duluth-Superior Harbor Water.**

## **Introduction**

The human-mediated spread of non-native organisms to regions of the world they could never reach by natural means has had great impacts on the environment and the economy (Holeck et al. 2007; Pimentel et al. 2000). In both freshwater and marine coastal regions, these invasions have been mediated commonly by the uptake and subsequent release of ballast water in ships (Ricciardi and MacIsaac 2000; Ricciardi 2006). On average, a typical ballasted ocean going ship entering the Laurentian Great Lakes (hereafter Great Lakes) carries about three million liters of water, which is either partially or fully discharged during subsequent cargo operations (Ricciardi and MacIsaac 2000). Surveys of organisms in ballast water and associated sediments reveal a variety of live planktonic and benthic species (Duggan et al. 2005; Bailey et al. 2005) which span orders of magnitude in size and concentration (Mills et al. 1993; Holeck et al. 2004; Bailey et al. 2011). While more than 180 non-native species may be present in the Great Lakes (Ricciardi 2006), that estimate is likely overinflated (Reid and Hudson 2008; Bailey et al. 2011). Nonetheless, more non-native species are anticipated to enter the Great Lakes in the coming years because of increasing world trade activity, a growing shipping fleet, and larger and faster vessels. This has prompted national and international interest in ballast water management.

Over the past ten years, ballast water exchange has been used by ships entering the Great Lakes to reduce densities of organisms possibly imported from other regions of the world in ballast water (Bailey et al. 2005; Briski et al. 2013). Although when done correctly, thorough ballast water exchange can serve to reduce the concentrations of live organisms in imported ballast water, and reduce the viability of surviving organisms through the delivery of osmotic

shock; however, the method has limitations. For example, it has been reported that mid-ocean exchange efficiencies for reducing live organisms range from 48% to 99% (Dickman and Zhang 1999; Wonham et al. 2001; McCollin et al. 2007; Taylor et al. 2007). Factors such as depth of water, season, method of exchange, and age of ship can influence exchange efficiencies (McCollin et al. 2007). In an effort to go beyond the protectiveness afforded by the practice of ballast water exchange, the U.S. Environmental Protection Agency (EPA), the U.S. Coast Guard (USCG) and the International Maritime Organization (IMO) are developing numeric standards that limit the density of organisms in ballast water discharged to U.S. waters (Albert et al. 2013)

An assumption in the development of a numeric standard for live organisms allowed per unit volume of ballast water discharged is that a direct and quantifiable relationship exists between the densities of individuals released in ballast water (release) and the probability of their eventual establishment (risk) (Ruiz and Carlton 2003). While a relationship between inoculum density and establishment probability may exist, many other factors also affect establishment success in aquatic systems (Wonham et al. 2013).

In general, each arriving propagule meets what Elton (1958) termed “ecological resistance” to its establishment, which is considered to consist of abiotic resistance, biotic resistance, and demographic stochasticity. Multiple studies (Baltz and Moyle 1993; Holway et al. 2002; Miller et al. 2002; DeRivera et al. 2005; Von Holle and Simberloff 2005; Dethier and Hacker 2005; Von Holle, 2005), consisting of a wide range of species have begun to explore “ecological resistance” by examining the effects of abiotic factors (e.g. temperature, water flow, chemistry), biotic factors (e.g. prey availability, competition, predation) and demographic stochasticity (e.g. propagule pressure and allele effects) on invasion outcome. It is abundantly clear that significantly reducing propagule pressure will reduce the probability of successful invasion, or overcoming ecological resistance. There is both strong theoretical and empirical support for this, across a diverse range of habitats, geographic regions and types of organisms (Davis and Pelsor 2001; Lockwood et al. 2005; Von Holle and Simberloff 2005; Colautti et al.

2006; Leung and Mandrak 2007; Bailey et al. 2009). However, the precise nature of the risk-release relationship can vary enormously over species, time, pathways and environments. There has been no effort to collect and integrate invasion data to provide a robust analysis of the risk-release relationship associated with ballast-water discharge, and specifically not in association with a particular standard. Moreover, existing experimental and field data that could be analyzed are limited in scope and not relevant to the Great Lakes.

In this chapter, I evaluate the ability of a surrogate invader, *Daphnia magna*, to successfully colonize mesocosm aquariums by manipulating the propagule pressure and several components of “ecological resistance.” Prior research (Chapter 2) showed that the natural concentrations and species compositions of crustacean zooplankton, as well as water quality parameters, change seasonally in the Duluth-Superior Harbor and St. Louis River Estuary. This phenology in community and habitat was used to create a range of “ecological resistance” treatments against which to test the experimental invader’s colonization success.

One important aspect of the study was to quantify the relationship between propagule pressure and risk of colonization as it relates to the efficacy of permissible post-treatment concentration limits suggested by the International Maritime Organization (IMO). The IMO is the United Nations body that administers the international regulatory regime for shipping. The IMO has currently recognized discharge standards that future technologies will have to meet. The particular component of these standards, as it relates to this study, states that no more than ten viable organisms, each greater than 50  $\mu\text{m}$  length in minimum dimension, may be discharged per one cubic meter (1000 L) of ballast water (Gollasch et al. 2007). Thus, this research is both basic and applied (i.e. ballast water introductions) invasion ecology.

## **Methods**

Experiments were conducted at the University of Wisconsin-Superior (UWS) during 2010 and 2011 in a room with plumbing constructed specifically for work on aquatic, non-native species. All effluent water leaving the room through floor drains and sinks is pretreated by chlorination prior to being discharged to the municipal sewer.

Mesocosm aquaria consisted of 230-L square polyethylene containers (61 cm L x 46 cm W x 91 cm H) equipped with air stones to promote gentle mixing and gas exchange with the atmosphere. The tanks were administered a 16:8 hour light:dark cycle by overhead fluorescent lights. The average illuminance, measured with a Fisher Scientific Traceable Light Meter (Model: 06-662-64), above the water surface was 980 lux (average of 6 trials over 2 years) and held constant over all trials. The testing room air temperature for a particular eight-week exposure period was set to match the ambient temperature of the harbor water used to fill the tanks. The goal was to maintain this initial ambient temperature over the 8-week trial.

*Daphnia magna* (Crustacea: Cladocera) served as the surrogate invader. *D. magna* is found worldwide in the Northern Hemisphere in freshwater habitats, brackish waters of lakes and ponds, ditches, and small eutrophic reservoirs (Hanski and Ranta 1983) but it is not native to northern Wisconsin, Minnesota, or Lake Superior and it is not currently found there. *D. magna* range in total length from about 0.5 mm as neonates to 3 mm as adults. As adults, they are among the largest extant species of daphnids known. *D. magna* occupy a key position in aquatic communities, as herbivores that eat algae and bacteria, and as a major prey item of fish (Dodson and Frey 2001). *D. magna* serves as a “worse-case” scenario experimental invader in aquatic ecosystems because it is capable of rapid population growth following the introduction of a single individual. Rapid population growth is accomplished through cyclical parthenogenesis, which couples periods of clonal, asexual reproduction (spring and summer) with sexual reproduction (fall) when diapausing eggs are produced. The average life span for *D. magna* is 40 days at 25 °C and 56 days at 20 °C (Eaton et al. 2005). Animals used for stocking mesocosms were selected

from the UWS in-house cultures, which have been maintained since 1995 and are routinely used for environmental toxicity testing. All *D. magna* used for experiments were standardized for age (0-24 hr) across treatments.

To begin an experiment, 18 tanks (230 L aquaria) were filled with raw water collected and transferred from the Duluth-Superior Harbor in plastic carboys (20-L capacity). Water was collected by pumps (ABS, model ROBUSTA 100-TS, 0.25 hp) from Montreal Pier located on the Wisconsin shoreline of the Duluth-Superior Harbor at the site of the Ballast Water Testing Facility (22 19<sup>th</sup> Ave East, Superior, WI). Carboys were continuously randomly assigned to the 18 tanks to help homogenize any possible patchiness in water quality or ambient organism density or species composition. Tanks were filled to 200 L to allow enough head space for periodic physiochemical measurements and zooplankton collection. Starting with the July 27, 2010 trial, three of the 18 tanks were randomly selected to receive filtered harbor water (FWC). Prior to filling the tanks, FWC was created at the moment of filling by pouring carboy water through a 50-micron mesh filter, which removed the majority of crustacean and rotifer zooplankton but not protists and bacteria.

Tanks were subsequently stocked with *D. magna* on the day after filling. Tanks were randomly assigned and stocked with either zero, one, two, three, or four individuals per tank (200 L). This equates to zero, five, 10, 15, and 20 individuals per cubic meter (1000 L) of water. The FWC water treatments were stocked with the maximum inoculum of four individuals.

Each experiment ran for eight weeks during which physiochemical conditions of the tanks as well as densities of *D. magna* and other zooplankton were measured. Physiochemical conditions were measured weekly at mid-depth in each tank and included water temperature, dissolved oxygen concentration, pH, specific conductivity, turbidity, and total *in vivo* chlorophyll concentration as fluorescence (YSI 6600 V2; YSI Inc.). The chlorophyll sensor emits radiation at

470 nm and measures fluorescent radiation in the region of the spectrum above 630 nm. Chlorophyll was always measured immediately prior to the lights turning on, hence the chlorophyll was dark-adapted (Maxwell and Johnson 2000).

Weekly estimates of *D. magna* density in each tank were made by gently stirring each tank, subsampling 1.0 L of water using a clear plastic tube at mid-depth, and scanning the subsample visually for *D. magna*. Samples were scanned on a back-lit table against which *D. magna* could be quickly identified and counted. All *D. magna* found were returned to their respective tank, but the other zooplankton in the subsample were concentrated and preserved in 70% ethanol final volume for later analysis. On the last day of each trial the entire contents of the tank were passed through a 20-micron mesh filter, concentrated, and preserved with 70% ethanol final volume.

In 2011, whole water samples from each experimental tank were collected weekly throughout the experiment to determine the carbon:nitrogen ratio of particles less than 50 microns in size. This was done as a means to estimate the food quality for *D. magna* and other herbivorous crustaceans and rotifers in the tank (Hecky et al. 1993). Whole water samples were collected from gently-mixed tanks, inspected for *D. magna*, prefiltered to remove particles greater than 50 microns in size, and a known volume was filtered through a preconditioned 934-AH glass microfiber filter (Whatman). The filters were packaged in tin capsules and analyzed for total carbon and total nitrogen at the University of California-Davis Stable Isotope Facility using a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Germany).

#### *Zooplankton sample processing*

The entire, preserved sample collected from each mesocosm at the end of a trial was inspected. Crustacean zooplankton were counted under a Nikon SMZ800 dissecting microscope at 40× and identified according to several sources (Balcer et al. 1984; Brooks 1959; Evans 1985;



Hudson et al. 1998). All *D. magna* were enumerated. Other taxa were counted until approximately 200 crustacean zooplankton and 200 rotifer zooplankton were identified. In cases where higher magnification was required to examine particular characteristics of a single zooplankton, a Nikon Eclipse E200 compound microscope was used. Mature Copepoda and all Cladocera were identified to species level. Rotifer zooplankton were counted under a Nikon Eclipse CI compound microscope at 40x-200x and identified according to several sources (Edmondson 1959; Stemberger 1979; Haney 2013).

### *Data Analyses*

The determination of establishment success by a non-native species is usually linked to evidence of long-term population presence that is self-sustaining, that is without ongoing inoculation from external propagule sources. In my short-term (8-week) experimental trials, evidence for this was unattainable and therefore an alternative measure of establishment success had to be developed. As a proxy for establishment success, I compared the biomass of *D. magna* achieved in a mesocosm tank against the mean total biomass of five native daphnid species (*D. ambigua*, *D. parvula*, *D. pulex*, *D. mendotae*, and *D. retrocurva*) present in the Duluth Superior Harbor and St. Louis River Estuary during 2007 and 2008 (Chapter 2).

The proxy threshold for establishment success was developed as follows. Crustacean zooplankton samples were collected biweekly from nine locations throughout the Duluth Superior Harbor and St. Louis River Estuary to obtain estimates of mean densities of the five native daphnids (Chapter 2). To reflect the relative presence of organisms as biomass, the mean densities were also converted to biomass (as dry-weight) using formulas relating body length to body weight (EPA 2003). Each of the biweekly mean density and biomass values from the nine sites was assigned one of three groups that corresponded to the season (spring, summer, or fall) that each experiment was initiated. Each seasonal grouping was then averaged to calculate a

single-seasonal establishment threshold (density or biomass). The average of week's 6-8 *D. magna* density and biomass in the experimental trials were compared against the establishment thresholds for a corresponding season.

Results were scored for each tank by assigning a value of one when the average of week's 6-8 *D. magna* density and/or biomass exceeded the establishment criteria or zero when the average of week's 6-8 *D. magna* density and/or biomass did not exceed the establishment criteria. This binary outcome permitted analysis of the results by logistic regression (see below).

Physical and chemical characteristics are reported as averages of replicate tanks for each inoculum treatment. Densities of crustacean and rotifer taxa in the recipient communities at the beginning and end of each experiment were converted to biomass. All biomass values for both the *D. magna* and the recipient communities reported are expressed as dry-weight, which was calculated from formulas relating linear measurement, usually body length to body weight. Formulas are of the general form:

$$\ln w = \ln a + b \ln L \quad [1]$$

where  $\ln a$  and  $b$  are species specific constants, and  $\ln L$  is the geometric mean length of measured individuals. Formulas, mean lengths, species-specific constants and conversions from wet-weight to dry-weight were derived from the Sampling and Analytical Procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes (EPA 2003). Zooplankton densities and biomass values were averaged among triplicates within an inoculum treatment. Differences between mean starting dry-weights among trials within a year were further explored by ANOVA (critical level of  $P = 0.05$ ). Post hoc analysis on mean starting densities among trials within a year was conducted given any statistical significance, specifically Games-Howell Test (critical level of  $P = 0.05$ ) was used given the data was heteroscedastic and non-normal. The differences between starting dry-weights among inoculum treatments within trial was explored

using Kruskal-Wallis ANOVA Test (critical level of  $P = 0.05$ ). Finally, differences between the initial and final dry-weights within a trial were explored using a paired t-test (critical level of  $P = 0.05$ ).

Binary logistic regression is a statistical tool used to describe the relationship between a categorical response variable and a set of predictor variables. The categorical outcome is binary (e.g., successful or unsuccessful colonization) while the predictor variables may be continuous or categorical. Binary logistic regression analysis was used to model those parameters (temperature, chlorophyll, dissolved oxygen, specific conductivity, turbidity, pH, initial rotifer and crustacean biomass, and *D. magna* stocking density) thought to be significant predictors of *D. magna* colonization success. It was also of interest to explore further the parameters found to be significant predictors from the full data set. For these variables, further exploration was done using subsets of data sorted by year and season of trial initiation. Nagelkerke  $R^2$  is a measure of deviance explained by the model, analogous to variance explained in linear regression models. It describes the strength of the relationship between dependent and independent variables (Nagelkerke, 1991) and is reported for each binary regression analysis. All data analyses were conducted using SYSTAT 13.

## **Results**

### *Physical and Chemical*

The physiochemical conditions of the mesocosm tanks varied between trials within a year and between years (Figs. 1-2). Initial temperatures over both years ranged from approximately 12 to 23 °C (Figs. 1-2) and closely reflected seasonal trends in the Duluth Superior Harbor St. Louis River Estuary (Chapter 2). Generally, over the course of eight weeks, temperatures were warmest in the July trials (Figs. 1-2). Temperatures were quite variable at times in 2011; for example, the May trial temperature was the lowest (12 °C) at the start of the trial, climbed above the October

trial for several weeks and then finished coolest of the three trials (Figs. 2). Some of the variation in average temperatures observed can be attributed to brief periods of malfunction of the climate control unit in the lab space, but were never outside suitable ranges (15-30° C, Goss and Bunting 1983) for *D. magna*.

In general, dissolved oxygen concentrations correlated inversely with temperature values and were more variable in 2010 (Figs. 1-2). Dissolved oxygen concentrations never dropped below 8.0 mg/L in any of the trials (Figs. 1-2).

Notable patterns of initial total chlorophyll concentrations were detected that closely reflected seasonal and interannual variation of the Duluth Superior Harbor St. Louis River Estuary (Figs. 1-2). In May 2010, initial chlorophyll concentration was approximately 1.0 µg/L, which was ten-fold less than October 2010 (Fig. 1). Chlorophyll concentrations in May and October 2010 were constant during the eight weeks with the exception of an increase during the last week in October (Fig. 1). During July 2010, there was a within-trial drop from approximately 30 µg/L to less than 5 µg/L over three weeks (Fig. 1). May and July 2011 trials had chlorophyll concentrations of approximately 11 µg/L, which remained relatively constant (Fig. 2). Unlike October 2010, the average total chlorophyll concentration was 2 µg/L during the October 2011 trial (Fig. 2).

In 2010 and 2011 the initial turbidity levels (ranged from <1-4.5 NTU) varied but typically declined to less than 1 NTU within the first week (Figs. 1-2). The notable exception occurred during the May 2010 trial, which had a similar initial pattern to the other trials but then increased to approximately three NTUs and remained constant for the next five weeks (Fig. 1).

The average specific conductivity values were consistently highest in the October and lowest in the July 2010 trials (Fig. 1) while the opposite was true in the 2011 (Fig. 2). In both

2010 and 2011 the average specific conductivity increased overtime and was likely caused from replenishing the tanks with reserved harbor water as evaporation occurred (Figs. 1-2).

No apparent temporal patterns within a particular trial or seasonal patterns between trials of average pH values were detected in both years (Figs. 1-2). The minimum (7.03) and maximum (9.17) pH values were recorded in the October 31, 2011 trial (Fig. 2). Average pH values per trial as a function of time for 2010 and 2011 ranged from 7.90 to 8.16 (Fig. 1-2).

Carbon to nitrogen ratios of particles less than 50 microns were measured in 2011. In general, C:N ratios were less than 8.3 with the exception of the last two data points in May trials (Fig 3). These data suggests there is not a nitrogen deficiency of the particles measured (Fig. 3).

#### *Biomass and richness of the recipient zooplankton community*

There were strong seasonal and yearly differences in biomass and richness of the background zooplankton assemblage (Tables 1-2). Differences between mean starting biomass (dry-weights) among trials within a year were observed. The initial rotifer dry-weight was highest in the Oct. 2010 trial, while the crustacean dry-weight was highest in the July 2010 trial (Table 1). However, in 2011 both the initial rotifer and crustacean dry-weights were the highest in the Oct. 2011 trial (Table 2). All statistical pairwise comparisons of initial dry-weights among trials within years 2010 and 2011 indicated significant ( $P < 0.05$ ) differences thus suggesting that none of the trials within a year started with the same biomass (Tables 1-2). Also, no statistically significant ( $P > 0.05$ ) differences between starting rotifer or crustacean dry-weights among inoculum treatments with a trial were detected suggesting the randomization of filling the mesocosm tanks lead to homogenous recipient zooplankton communities at the start of each trial (Tables 1-2). In general, the final dry-weights for both the rotifer and crustacean was significantly ( $P < 0.05$ ) greater than the initial dry-weights in Oct. 2010 trial and all 2011 trials (Tables 1-2). Only the final rotifer dry-weight was significantly ( $P < 0.05$ ) greater than the initial

dry-weight and no difference in crustacean dry-weights was detected for trials July 2010 and Oct. 2011 (Tables 1-2).

Tables 3 and 4 document presence of rotifer (Table 3) and crustacean (Table 4) zooplankton taxa by trial and year. Richness (number of species) ranged from 7-13 for rotifers (Table 3) and 4-21 for crustaceans (Table 4). No strong patterns of increase or decrease were noted between initial and final richness values for either group (Tables 3-4). However, members of *Polyarthra* were not present by the end of 8 weeks in October 2010 or 2011 (Table 3). In five of six trials, *Synchaeta sp.* was present at the start but never the end (Table 3). Cyclopoid copepods were more diverse than calanoid copepods both years, likely reflecting the littoral nature of the ambient water collection site (Table 4).

#### *Establishment of Experimental Invader, Daphnia magna*

In the spring and fall experiments, *D. magna* populations, described as either density or biomass, demonstrated establishment success during the 8-week period in many of the trials (Figs. 4-5). By contrast, in the summer experiments *D. magna* rarely achieved levels that were high enough to be considered established by either density or biomass criteria (Figs. 4-5). Only when the background assemblage of zooplankton was first removed (FWC) from the tanks in the summer was *D. magna* able to achieve densities above the threshold (Figs. 4-5). The FWC results suggest that the establishment barrier in the summer trials was probably biotic and not abiotic (Figs. 4-5).

Establishment success was analyzed in the context of permissible post-treatment concentration limits currently stipulated by the IMO (10 individuals per 1000L) by combining trials for both years and examining the average risk of establishment by inoculation density and season. Results indicate that establishment was greatest in spring and fall trials and lowest in summer trials (Fig. 6). There was measurable risk of establishment at a stocking density of even

one individual per 200 L for spring and fall trials but not for summer trials (Fig. 6). One individual per 200 L represents a density well below the acceptable discharge standard (10 individuals per 1000 L) currently upheld by the IMO as a target standard for environmental safety that can reduce the chance of establishment success.

#### *Relationships between D. magna Colonization Success and Ecological Resistance Factors*

The results from the binary logistic regression analysis of the complete data set of *D. magna* colonization success and several ecological resistance factors indicate a significant (P=0.007) negative relationship between establishment success and the initial rotifer biomass and a significant (P < 0.05) positive relationship between establishment success and *D. magna* inoculum density (Fig. 7 and Table 5). The estimated model is obtained from the following equation:

$$\ln\left(\frac{p}{1-p}\right) = 8.776 - 0.117 * \text{initial rotifer biomass} + 0.887 * D. magna \text{ inoculum} \quad [2]$$

where  $p$  is the estimated probability of an outcome. Crustacean biomass, temperature, specific conductivity, pH, dissolved oxygen, total chlorophyll, and turbidity were not significant predictors of *D. magna* colonization success.

*Daphnia magna* colonization success varied seasonally (Fig 7); therefore, binary logistic regression analysis was conducted on a subset of predictors (*D. magna* inoculum density and initial rotifer biomass) grouped by season (spring, summer, and fall, Table 6). The spring and fall trials indicated a significant (P = 0.008 and P = 0.010, respectively) positive relationship between *D. magna* colonization success and *D. magna* inoculum while the summer trials indicated a significant (P = 0.010) negative relationship between *D. magna* colonization success and initial rotifer biomass (Table 6) and the spring trials indicated a marginally significant (P = 0.059) trend in this direction.

## Discussion

To uncover the potential mechanism(s) governing the relationship between the introduction of a non-native freshwater zooplankton invader and colonization of an environment I tested the following components of ecological resistance: propagule pressure, abiotic resistance and biotic resistance. Multiple studies consisting of a wide range of species have begun to explore ecological resistance by examining the effects of abiotic resistance (e.g. temperature, water flow, and water chemistry), biotic resistance (e.g. prey availability, competition, and predation) and components of demographic stochasticity (e.g. propagule pressure and allele effects) on invasion outcome (Baltz and Moyle 1993; Holway et al. 2002; Miller et al. 2002; DeRivera et al. 2005; Von Holle and Simberloff 2005; Dethier and Hacker 2005; Von Holle, 2005). However, the precise nature of the response can vary enormously over species, time, and environments. The approach I used to explore these ideas was novel in the sense that ecological resistance, both the abiotic and biotic components, were manipulated by exposing the surrogate invader to natural Duluth-Superior Harbor and St. Louis River Estuary water that varied on a seasonal basis over two years. That is, different inoculum concentrations of *D. magna* were exposed to different “real-world” and Great Lakes relevant environmental conditions. The results of this study demonstrate that colonization success of *D. magna* can be strongly influenced by both the number of arriving individuals (propagule pressure) and the timing of arrival.

### *Propagule pressure – one component of demographic stochasticity*

The combination of total number of arriving individuals as well as the number of arrival events, often referred to as propagule pressure, has been receiving considerable attention in biological invasions and is likely to be a key determinant of establishment success of non-native species (Lockwood et al. 2005). Widespread and growing experimental and observational evidence across a broad section of plant and animal species supports this idea. In general, experimental studies indicate the higher the propagule pressure, the greater the probability of



successful colonization or establishment by a non-native invader (Ahloth et al. 2003; Von Holle and Simberloff 2005; Colautti et al. 2006; Simberloff 2009; Zenni and Simberloff 2013). For example, studies in which insects were released to control invasive weeds showed that the probability of establishment was positively correlated with release size (Grevstad 1999; Memmott et al. 2005). Likewise, in a plant study by Von Holle and Simberloff (2005) in which they manipulated three determinants of invasion outcome (resident diversity, abiotic conditions, and propagule pressure) the strongest determinant of establishment success was number of propagules that arrived at an invasion site. The abiotic conditions and resident diversity in the Von Holle and Simberloff (2005) study had negligible impact on invasion outcome. Results from my study also indicate that increasing concentrations of *D. magna* inoculum during the spring and fall trials increased the probability of *D. magna* to successfully colonize the mesocosms. However, inoculum concentration was not the strongest determinant of colonization outcome during the summer trials.

### *Ecological resistance*

While it is clear that a relationship between propagule pressure and colonization success exists, many other local factors and processes (i.e., non-native invader excluded from site via abiotic or biotic conditions) also affect colonization success in aquatic systems (Elton 1958; Ruiz and Carlton 2003; Simberloff 2009; Wonham et al. 2013). Colonization success is strongly dependent upon the quality of the environment (environmental resistance) and in many ways can be described as a first order filter for preventing colonization (Keddy 1992; Ricciardi 2001). In studies by Baltz and Moyle (1993) and Moyle and Light (1996), invading fishes in California streams appear to colonize a new reach of stream if abiotic conditions are suitable for the invader, regardless of other biota present. Likewise, in a study by Ahloth et al. (2003) the quality of environment (i.e. stream depth) determined the colonization success of waterstriders. That is, as stream depth increased, hatching of waterstrider eggs increased ensuring a better chance of

colonizing a stream. My study was designed to test *D. magna*'s colonization ability against a broad range of abiotic conditions that reflected the natural conditions of the Duluth- Superior Harbor and St. Louis River Estuary. This was achieved by capturing the abiotic seasonal variation of the Duluth-Superior Harbor and St. Louis River Estuary during the different water collection periods. In my study, the abiotic conditions varied widely but were not significant determinants of *D. magna*'s colonization success.

Although resource availability (food quantity and quality) is not an abiotic parameter its role in influencing invasion outcomes is generally discussed along with environmental resistance. The effects of resource levels on invasion have been studied. In a plant community study, Burke and Grime (1996) found as fertilizer application increased so did the levels of invasive species biomass. Likewise, invasion in the pitchers of the pitcher plant, *Sarracenia purpurea*, depended upon resource availability and presence of predators (Miller et al. 2002). Frequency of invasion by a protozoa and a chrysomonad was increased by the addition of dead prey into the pitchers (Miller et al. 2002). In my study, food quality did not appear to be limiting during the summer trials when *D. magna* failed to establish. This was supported by the low C:N ratio of particles less than 50  $\mu\text{m}$  in size (Hecky et al. 1993). However, P was not measured in any of the major components (e.g. water, food items, *D. magna*) of these trials. In general, freshwater phytoplankton and bacteria are able to adjust their P concentrations relative to their surrounding concentrations but zooplankton cannot and therefore are not able to store excess nutrients (Hessen 1990; Sterner 1990; Sterner et al. 1992). If P was limiting during my summer trials it could be possible that *D. magna* experienced reduced reproduction (Sterner 1990). The growth of *D. magna* in the treatments in which the recipient background community was removed (FWC treatments) does not support that P, other nutrients or food quantity were limiting during the summer trials as *D. magna* exceeded the colonization threshold in this treatment.

A possibility exists such that when environmental conditions are suitable for a new invader, biotic resistance may serve as a second filter in the prevention of a new species from colonizing. Biotic resistance can arise from native community diversity (Elton 1958; Stachowicz et al. 1999), native predators (DeRivera et al. 2005), or native competitor interactions (Tilman 1997). In a study of invasibility of oak savanna plants, Tilman (1997) determined that the proportion of added plant species in a onetime addition was negatively correlated with initial species richness of the test plots. This suggests that species-rich plots were more resistant to invasion. However, when plants were sorted into major functional groups (e.g. perennial grasses, legumes) it was found that their ability to invade was different, suggesting that local biotic interactions were also at play (Tilman 1997). Limits of the southern distribution of the invasive European green crab *Carcinus maenas* along the eastern United States appear to be affected by predation from the native blue crab *Callinectes sapidus* (DeRivera et al. 2005). DeRivera et al. also speculated that other factors may also operate in conjunction with predation to set the southern range limit. Dzialowski et al. (2007) experimentally manipulated native zooplankton community structure, the presence or absence of an invertebrate predator, and nutrient supply in a series of mesocosm experiments to explore how different community attributes influence *Daphnia lumholtzi*'s establishment success. Dzialowski et al. (2007) results indicated that only when biomass and diversity of native zooplankton were significantly reduced was *D. lumholtzi* able to invade the mesocosms (Dzialowski et al. 2007). The presence of the invertebrate predator and additional nutrients modified the native zooplankton community but had no bearing on establishment of *D. lumholtzi*.

The results from my study are consistent with the biotic resistance hypothesis, that is in the spring and fall experiments, *D. magna* populations demonstrated clear episodes of colonization success. By contrast, in the summer experiments the population densities of *D. magna* rarely achieved levels that were high enough to be considered successful colonization.

Only when the background assemblage of zooplankton was first removed from the tanks in the summer was *D. magna* able to achieve densities above colonization criteria. This suggests that the establishment barrier in the summer trials is probably biotic and not abiotic. More specifically, the initial rotifer biomass of the recipient community appears to be the major driving component in determining the outcome of *D. magna*'s ability to colonize the mesocosms under these test conditions.

Considerable research including both observational (Neill 1984; Vanni 1986; Fussman 1996) and experimental (Gilbert 1985; Gilbert and Stemberger 1985; Burns and Gilbert 1986; MacIsaac and Gilbert 1989) studies have revealed a dynamic relationship between the abundances of co-inhabiting cladocerans and rotifers, with cladocerans negatively impacting rotifer populations. One axis of this relationship involves exploitative competition because rotifers and cladocerans share overlapping preferences for algal particles. In general, cladocerans are considered more efficient grazers (Brooks and Dodson 1965; Hall et al. 1976) and prefer food particles in size from 1-50  $\mu\text{m}$ , which overlaps the food particle size preference of 1-20  $\mu\text{m}$  for rotifers (Allan 1976; Gilbert 1985). Gilbert (1985) showed that two species of rotifer, *Brachionus calyciflorus* and *Keratella cochlearis*, could be excluded by *Daphnia pulex* after 2-3 weeks, after starting with initial densities of 100-2500 rotifers and only 2-3 *Daphnia*. *D. pulex* had a greater impact on available food in competition experiments and grown alone after day 10 then compared to rotifers grown alone suggesting the decline of both types of rotifers was largely by exploitative competition (Gilbert 1985).

Negative interaction between rotifers and *Daphnia* may also extend to interference competition. Rotifers can be swept into the branchial chamber of large bodied cladocerans and can result in damage or death to the rotifer (Gilbert 1985; Gilbert and Stemberger 1985). Investigators have shown that cladocerans, similar to *D. magna*, are able to increase mortality on many rotifer species through interference competition (Gilbert and Stemberger 1985; Burns and

Gilbert 1986). Specifically, Gilbert and Stemberger (1985) demonstrated that rotifer mortality can occur through interference competition with *Daphnia* over a wide range of *Keratella* densities (220-1400 L<sup>-1</sup>) and *Daphnia* densities (22-75 L<sup>-1</sup>) under both food-limited and food-unlimited conditions. Burns and Gilbert (1986) examined the effects of rotifer (*Keratella*) density on interference competition with *Daphnia pulex* and *Daphnia galeata mendotae*. Interference competition exerted by both daphnid species at densities of 15-100 L<sup>-1</sup> was density-independent over the range of rotifer densities (125-1000 individuals L<sup>-1</sup>) tested (Burns and Gilbert 1986).

While it is clear that *Daphnia* can impose mortality on rotifers through either exploitative or interference competition, it is not clear if *Daphnia* benefits from these interactions. Thus far, only one study reported in the literature adequately explored the effects of a rotifer (*Keratella cochlearis*) on the growth and survival of *Daphnia pulex* (Wickham et al. 1993). Experimental results from Wickham et al. (1993) indicated that large body (mean length 2.17 mm) *D. pulex* growth increased by approximately 11% when rotifers were added. However, a decrease in survivorship of small body (mean length 1.79 mm) *D. pulex* was observed when 300 *Keratella* per liter were present, compared to when *Keratella* were absent. *D. magna* stocked in my mesocosm tanks were neonates 0-24 hrs in age and were likely to be less than 1.0 mm length. Also, average (n=15) rotifer densities (not reported here) by trial, excluding FWC treatments, for 2010 and 2011 were 1119 and 2058 L<sup>-1</sup>, respectively. The combination of a single inoculum event that ranged between 1-4 individuals, the initial small body size of *D. magna*, and the large numbers of competitors likely created a biological filter that *D. magna* could not overcome.

#### *Risk of establishment in relationship to ballast water discharge standards*

These results suggest that the IMO standards, as currently defined, may be sufficiently restrictive under some, but not all, natural circumstances to prevent colonization of non-native zooplankton such as *D. magna* in the Duluth-Superior Harbor. Analyses of these results suggest

that the risk of establishment of *D. magna* in the Duluth-Superior Harbor is higher in spring and fall than it is during summer months because of lower densities and fewer zooplankton competitors in the spring and fall periods.

## **Conclusion**

The principal limitation with an experimental approach is the potential inability to capture all of the relevant variables, spatial scales, and taxonomic groups that is necessary to obtain a picture of the risk-release relationship. Mesocosms are artificial settings that cannot precisely mimic real-world conditions, despite every effort to do so. However, with mesocosm experiments, it is possible to know and control the propagule pressure of an invader, control the many abiotic and biotic conditions used to challenge the surrogate invader, and measure the colonization outcome. Unlike observation studies, experiments can be replicated across a range of taxonomic groups and environmental conditions and provide robust data in a short time horizon relative to observational data. Therefore, these experiments are a good first approach to understanding the risk-release relationship.

Further analysis and refinement of the risk-release relationship for non-native species in ballast water discharge is critical. Standards that are too lenient may pose serious, long-term threats to the environment and economy. Standards that are too strict may require unnecessary investments in time, labor, and materials necessary to meet the standards. Empirical justification of accepted standards should strengthen support among stakeholders and encourage timely and sustained compliance.

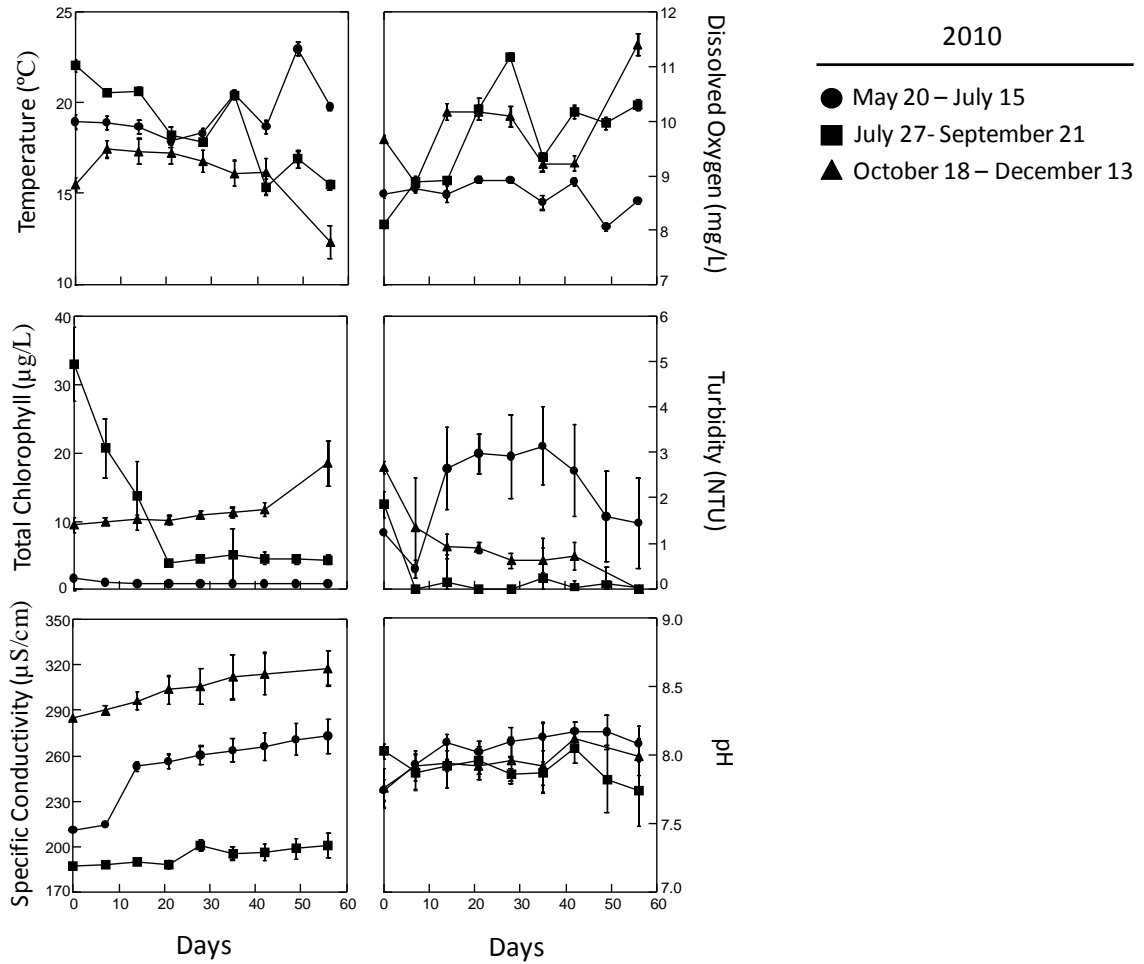


Figure 1. Average temperature, dissolved oxygen, total chlorophyll, turbidity, specific conductivity, and pH as a function of days shown by trial for 2010. Error bars represent standard error.

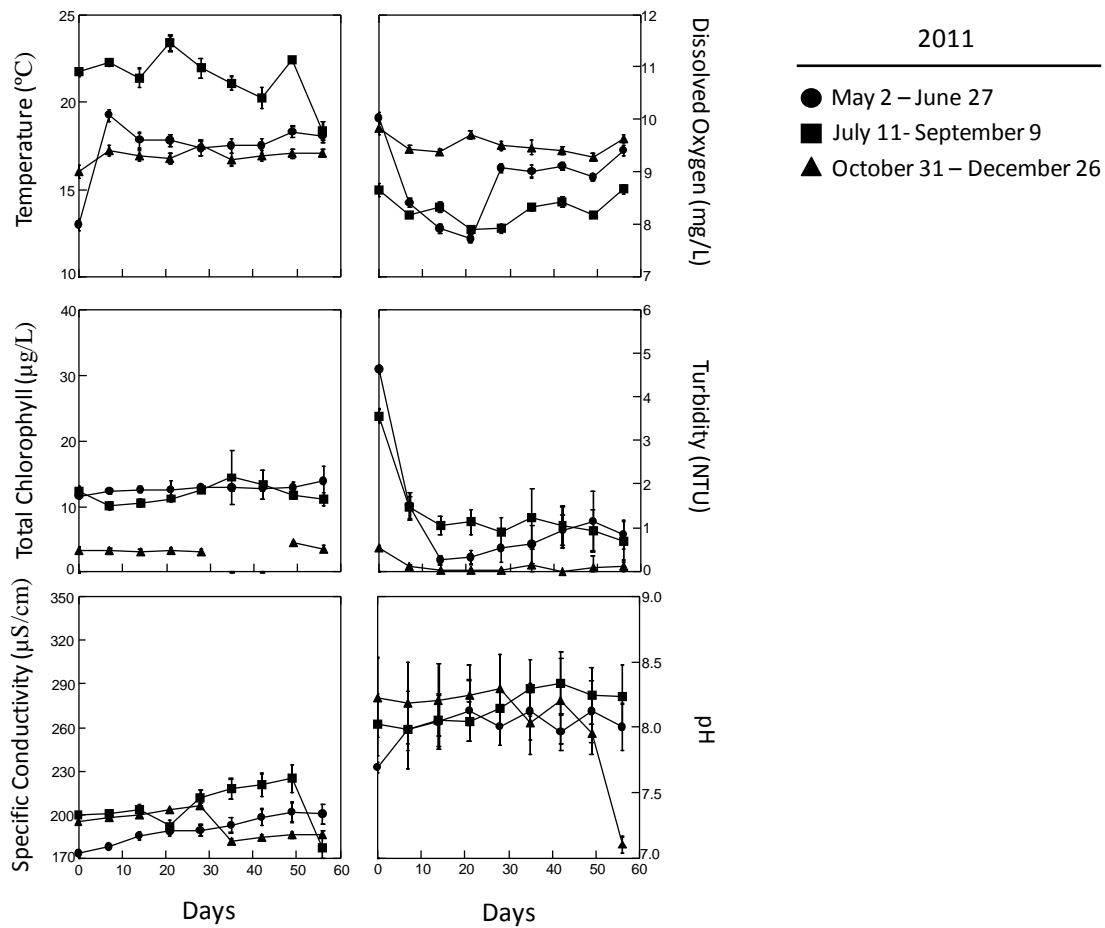


Figure 2. Average temperature, dissolved oxygen, total chlorophyll, turbidity, specific conductivity, and pH as a function of day shown by trial for 2011. Error bars represent standard error.



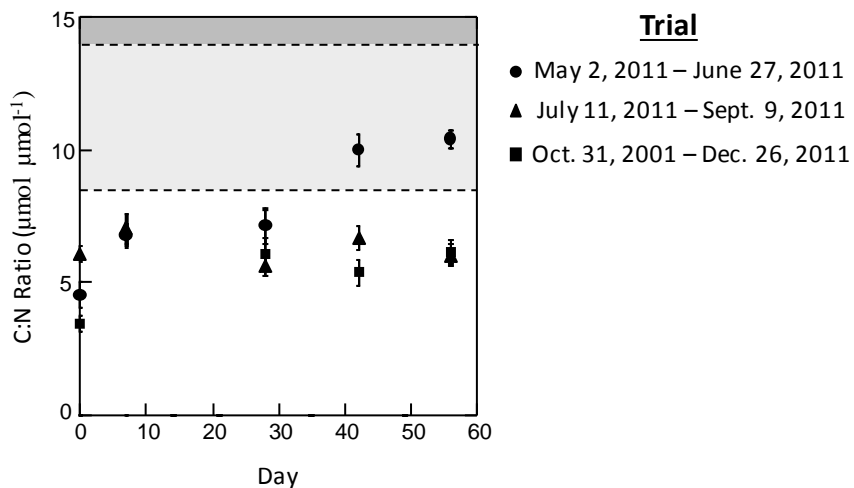


Figure 3. Average carbon to nitrogen ratio of particles less than 50 microns in size as a function of day shown by trial for 2012. Error bars represent standard error. Shaded areas represent zones of nitrogen deficiency with dark grey area representing severe, light grey area representing moderate deficiency and no shading representing no deficiency.

Table 1. Mean  $\pm$  standard deviation dry weight estimate of crustacean biomass ( $\mu\text{g L}^{-1}$ ), excluding *D. magna*, and rotifer biomass ( $\mu\text{g L}^{-1}$ ) in mesocosms ( $n = 3$  mesocosm per treatment) at the start (initial) and termination (final) of experiments sorted by *D. magna* inoculum density within experimental trial for 2010. Italicized values are mean trial dry-weight estimates  $\pm$  standard deviations.

Experimental Trial	<i>D. magna</i> inoculum (No. 200 L <sup>-1</sup> )	Equivalent No. of <i>D. magna</i> (m <sup>-3</sup> )	Crustacean		Rotifer	
			Initial	Final	Initial	Final
May 20-July 15	0	0	44 $\pm$ 3	97 $\pm$ 72	15 $\pm$ 2	27 $\pm$ 18
	1	5	62 $\pm$ 17	119 $\pm$ 110	12 $\pm$ 4	52 $\pm$ 17
	2	10	30 $\pm$ 7	61 $\pm$ 47	12 $\pm$ 6	30 $\pm$ 6
	3	15	31 $\pm$ 7	33 $\pm$ 18	13 $\pm$ 2	33 $\pm$ 6
	4	20	54 $\pm$ 140	56 $\pm$ 19	16 $\pm$ 3	24 $\pm$ 18
				<i>44 <math>\pm</math> 16</i>	<i>73 <math>\pm</math> 63</i>	<i>14 <math>\pm</math> 4</i>
July 17-Sept. 21	0	0	21 $\pm$ 12	136 $\pm$ 163	36 $\pm$ 13	18 $\pm$ 6
	1	5	21 $\pm$ 9	93 $\pm$ 86	54 $\pm$ 21	24 $\pm$ 14
	2	10	23 $\pm$ 2	91 $\pm$ 67	42 $\pm$ 6	34 $\pm$ 13
	3	15	26 $\pm$ 10	120 $\pm$ 80	33 $\pm$ 24	19 $\pm$ 12
	4	20	24 $\pm$ 1	24 $\pm$ 23	48 $\pm$ 16	24 $\pm$ 5
	4*	20	0	21 $\pm$ 18	3 $\pm$ 2	5 $\pm$ 5
			<i>19 <math>\pm</math> 11</i>	<i>98 <math>\pm</math> 104</i>	<i>36 <math>\pm</math> 21</i>	<i>21 <math>\pm</math> 12</i>
Oct. 18-Dec. 13	0	0	11 $\pm$ 3	254 $\pm$ 325	4 $\pm$ 1	19 $\pm$ 4
	1	5	12 $\pm$ 8	264 $\pm$ 107	4 $\pm$ 2	12 $\pm$ 3
	2	10	10 $\pm$ 3	157 $\pm$ 155	3 $\pm$ 2	8 $\pm$ 6
	3	15	5 $\pm$ 6	256 $\pm$ 64	4 $\pm$ 1	13 $\pm$ 5
	4	20	10 $\pm$ 6	279 $\pm$ 280	5 $\pm$ 1	11 $\pm$ 5
	4*	20	0	57 $\pm$ 92	0	3 $\pm$ 3
			<i>8 <math>\pm</math> 6</i>	<i>228 <math>\pm</math> 183</i>	<i>3 <math>\pm</math> 2</i>	<i>11 <math>\pm</math> 6</i>

\* *D. magna* were added to Duluth-Superior Harbor water that was pretreated by passing through a 20 micron mesh net prior to filling tank.

Table 2. Mean  $\pm$  standard deviation dry weight estimate of crustacean, excluding *D. magna*, and rotifer biomass ( $\mu\text{g L}^{-1}$ ) in mesocosms ( $n = 3$  mesocosm per treatment) at the start (initial) and termination (final) of experiments sorted by *D. magna* inoculum density within experimental trial for 2011. Italicized values are mean trial dry-weight estimates  $\pm$  standard deviations.

Experimental Trial	<i>D. magna</i> inoculum (No. 200 L <sup>-1</sup> )	Equivalent No. of <i>D. magna</i> (m <sup>-3</sup> )	Crustacean		Rotifer	
			Initial	Final	Initial	Final
May 2 –June 27	0	0	1 $\pm$ 2	12 $\pm$ 5	2 $\pm$ 1	14 $\pm$ 9
	1	5	0	16 $\pm$ 10	2 $\pm$ 2	18 $\pm$ 9
	2	10	0	9 $\pm$ 7	2 $\pm$ 1	15 $\pm$ 12
	3	15	0	83 $\pm$ 69	1 $\pm$ 1	25 $\pm$ 19
	4	20	0	59 $\pm$ 32	1 $\pm$ 2	21 $\pm$ 7
	4*	20	3 $\pm$ 6	195 $\pm$ 143	3 $\pm$ 1	18 $\pm$ 5
			<i>1 <math>\pm</math> 2</i>	<i>62 <math>\pm</math> 87</i>	<i>2 <math>\pm</math> 1</i>	<i>19 <math>\pm</math> 10</i>
July 11 –Sept. 9	0	0	156 $\pm$ 14	62 $\pm$ 38	56 $\pm$ 22	27 $\pm$ 11
	1	5	100 $\pm$ 66	123 $\pm$ 155	39 $\pm$ 14	17 $\pm$ 9
	2	10	187 $\pm$ 15	47 $\pm$ 13	56 $\pm$ 10	22 $\pm$ 14
	3	15	137 $\pm$ 36	237 $\pm$ 226	61 $\pm$ 11	16 $\pm$ 6
	4	20	88 $\pm$ 71	97 $\pm$ 29	70 $\pm$ 20	27 $\pm$ 22
	4*	20	3 $\pm$ 3	34 $\pm$ 25	7 $\pm$ 5	15 $\pm$ 7
			<i>112 <math>\pm</math> 70</i>	<i>100 <math>\pm</math> 119</i>	<i>48 <math>\pm</math> 25</i>	<i>21 <math>\pm</math> 12</i>
Oct. 31 –Dec. 26	0	0	52 $\pm$ 33	73 $\pm$ 87	4 $\pm$ 1	5 $\pm$ 4
	1	5	67 $\pm$ 17	47 $\pm$ 72	4 $\pm$ 3	4 $\pm$ 3
	2	10	56 $\pm$ 22	91 $\pm$ 91	6 $\pm$ 2	3 $\pm$ 3
	3	15	60 $\pm$ 7	40 $\pm$ 34	5 $\pm$ 2	8 $\pm$ 4
	4	20	53 $\pm$ 15	118 $\pm$ 27	6 $\pm$ 1	11 $\pm$ 9
	4*	20	5 $\pm$ 9	117 $\pm$ 99	0	6 $\pm$ 3
			<i>49 <math>\pm</math> 26</i>	<i>81 <math>\pm</math> 69</i>	<i>4 <math>\pm</math> 3</i>	<i>6 <math>\pm</math> 5</i>

\* *D. magna* were added to Duluth-Superior Harbor water that was pretreated by passing through a 20 micron mesh net prior to filling tank.

Table 3. The presence (x) or absence and taxon richness (number of unique species present) of individual rotifer zooplankton species at the beginning and finish of each trial for both years of 2010 and 2011.

Taxon	2010						2011					
	May 20 – July 15		July 27 – Sept. 21		Oct. 18 – Dec. 13		May 2 – June 27		July 11 – Sept. 9		Oct. 31 – Dec. 26	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
<b>Rotifera</b>												
<i>Brachionus sp.</i>	X											
<i>Cephalodella sp.</i>	X	X	X	X		X		X		X		X
<i>Collotheca sp.</i>										X	X	X
<i>Conochilus unicornis</i>			X		X		X	X		X	X	X
<i>Euchlanis sp.</i>	X	X		X	X	X		X	X	X		
<i>Filinia sp.</i>	X			X								
<i>Lepadella sp.</i>	X	X	X	X	X	X		X		X		X
<i>Kellicottia longispina</i>			X	X	X		X			X	X	X
<i>Keratella crassa</i>		X	X						X	X		
<i>Keratella cochlearis</i>	X		X	X	X	X	X	X	X	X	X	X
<i>Keratella earlinae</i>									X		X	X
<i>Keratella hiemalis</i>							X					
<i>Lecane sp.</i>	X	X	X	X		X	X	X		X		X
<i>Monostyla sp.</i>	X	X	X	X	X	X	X	X	X	X	X	X
<i>Notholca sp.</i>	X			X	X	X	X					
<i>Notommata sp.</i>		X		X	X		X	X	X			
<i>Ploesoma hudsoni</i>	X		X									
<i>Ploesoma truncatum</i>			X				X		X			
<i>Polyarthra vulgaris</i>	X	X		X	X			X	X		X	
<i>Polyarthra major</i>					X						X	
<i>Polyarthra remata</i>			X		X				X		X	
<i>Synchaeta sp.</i>	X		X		X		X		X		X	
<i>Testudinella sp.</i>		X		X						X		
<i>Trichocera similis</i>		X	X	X	X				X			
<i>Trichotria sp.</i>	X											
<b>RICHNESS</b>	13	11	13	13	12	7	8	9	12	10	10	8

Table 4. The presence (x) or absence and taxon richness (number of unique species present) of individual crustacean (not including *D. magna*) zooplankton species at the beginning and finish of each trial for both years of 2010 and 2011.

Taxon	2010						2011					
	May 20 – July 15		July 27 – Sept. 21		Oct. 18 – Dec. 13		May 2 – June 27		July 11 – Sept. 9		Oct. 31 – Dec. 26	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
<b>Copepod nauplii</b>	X	X	X	X	X	X	X	X	X	X	X	X
<b>Copepodites</b>												
<i>Cyclops</i>	X	X	X	X	X	X	X	X	X	X	X	X
<i>Mesocyclops</i>	X			X				X				X
<i>Tropocyclops</i>					X	X						X
<i>Diaptomus</i>		X	X	X	X	X		X	X	X	X	X
<i>Epischura</i>			X	X	X	X		X	X	X	X	
<i>Eurytemora</i>	X		X	X	X				X	X	X	
<b>Calanoid Copepods</b>												
<i>Epischura lacustris</i>						X						
<i>Eurytemora affinis</i>			X			X						
<i>Leptodiaptomus minutus</i>				X								
<i>Leptodiaptomus sicilis</i>				X							X	X
<i>Leptodiaptomus siciloides</i>							X					
<i>Limnocalanus macrurus</i>				X								
<i>Skistodiaptomus oregonensis</i>									X	X	X	
<b>Cyclopoid Copepods</b>												
<i>Acanthocyclops brevispinosus</i>		X	X	X		X		X	X	X	X	X
<i>Acanthocyclops robustus</i>								X	X	X	X	X
<i>Diacyclops thomasi</i>			X					X	X	X	X	X
<i>Eucyclops agilis</i>		X		X	X	X		X	X	X	X	X
<i>Eucyclops sp.</i>	X											X
<i>Macrocyclus albidus</i>		X						X	X			
<i>Mesocyclops edax</i>		X				X			X			
<i>Microcyclus rubellus</i>	X					X	X					
<i>Tropocyclops prasinus mexicanus</i>				X	X	X		X				X
<b>Cladocera</b>												
<i>Alona sp.</i>	X	X		X				X	X	X		
<i>Bosmina sp.</i>	X	X	X	X	X	X		X	X	X	X	X
<i>Camptocercus sp.</i>		X		X								
<i>Ceriodaphnia sp.</i>	X	X	X	X				X	X			
<i>Chydorus sp.</i>	X	X	X	X	X	X		X	X	X		X
<i>Daphnia galeata mendotae</i>						X					X	X
<i>Daphnia retrocurva</i>			X					X				
<i>Diaphanosoma birgei</i>		X	X	X				X	X			X
<i>Eurycercus sp.</i>	X		X						X			
<i>Holopedium gibberum</i>											X	
<i>Ostracod sp.</i>		X	X	X	X	X			X			
<i>Pleuroxus sp.</i>		X		X					X			
<i>Sida crystalline</i>		X		X				X	X			
<i>Scapholebreis sp.</i>									X			
<i>Dreissena sp. veligers</i>	X	X	X			X	X					
<b>RICHNESS</b>	12	17	16	20	10	17	4	14	14	21	12	17

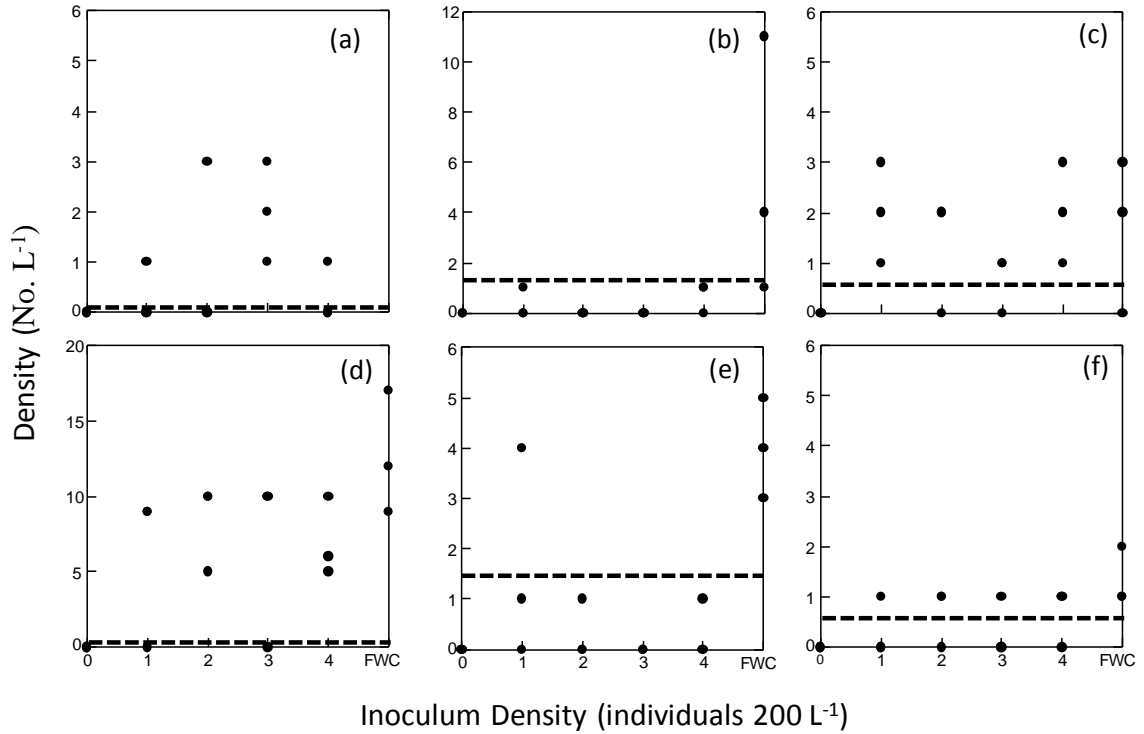


Figure 4. Densities (No. L<sup>-1</sup>) of *Daphnia magna* per tank integrated over weeks 6-8 sorted by trial: (a) May 20 – July 15, 2010, (b) July 27-September 21, 2010 (c) October 18-December 13, 2010, (d) May 2-June 27, 2011, (e) July 11-September 9, 2011, and (f) October 31-December 26, 2011. Filtered harbor water control (FWC) is a treatment in which *Daphnia magna* were added at an initial density of four per 200 L to Duluth-Superior Harbor water that was pretreated by passing through a 20 micron mesh net prior to filling mesocosm. Dashed line represents establishment criteria threshold.

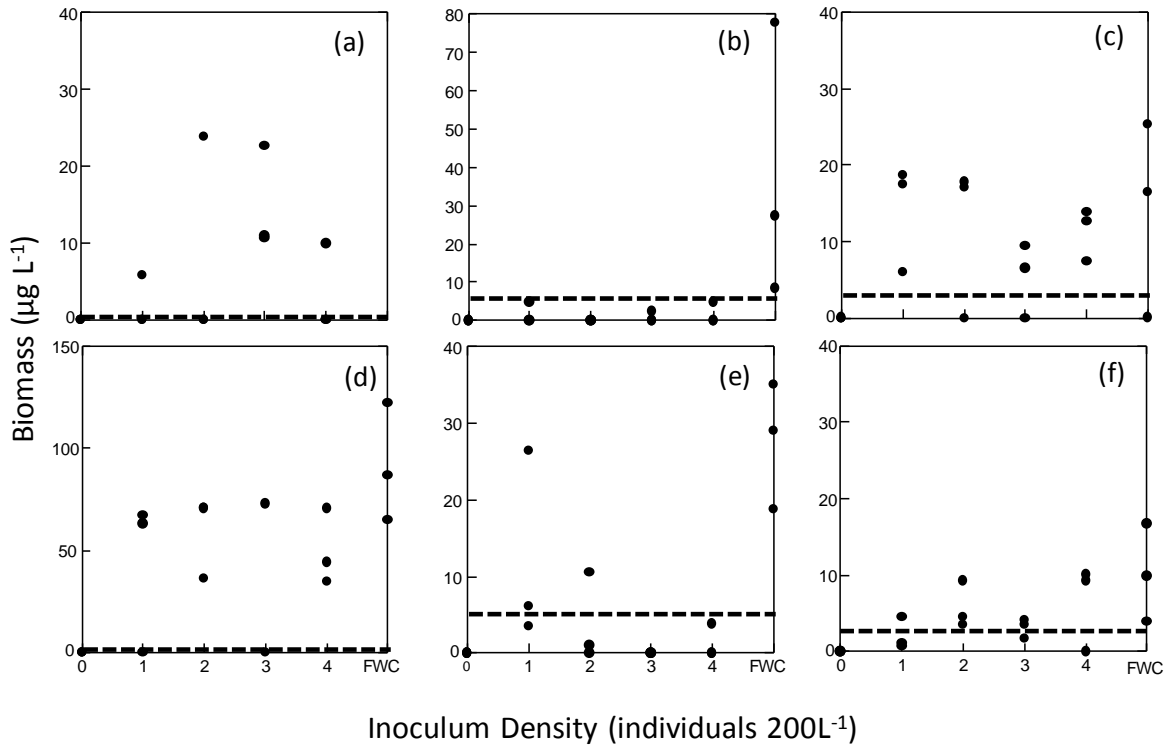


Figure 5. Biomass as dry weight ( $\mu\text{g L}^{-1}$ ) estimates of *Daphnia magna* per tank by stocking density integrated over weeks 6-8 sorted by trial: (a) May 20 – July 15, 2010, (b) July 27-September 21, 2010 (c) October 18-December 13, 2010, (d) May 2-June 27, 2011, (e) July 11-September 9, 2011, and (f) October 31-December 26, 2011. Filtered harbor water control (FWC) is a treatment in which *Daphnia magna* were added at an initial density of four per 200 L to Duluth-Superior Harbor water that was pretreated by passing through a 20 micron mesh net prior to filling mesocosm. Dashed line represents establishment criteria threshold.

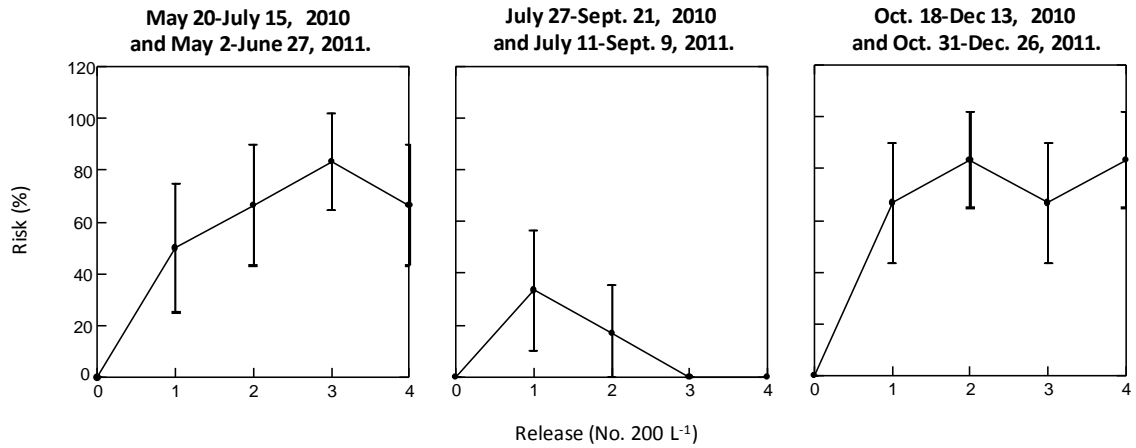


Figure 6. Relationship between risk of establishment (% of mesocosm tanks with established *D. magna* populations) as a function of inoculum density (individuals 200 L<sup>-1</sup>) of the species into the mesocosm tanks. Each data point represents the combined result of six experimental tanks (three in 2010 and three in 2011). Error bars represent standard error.



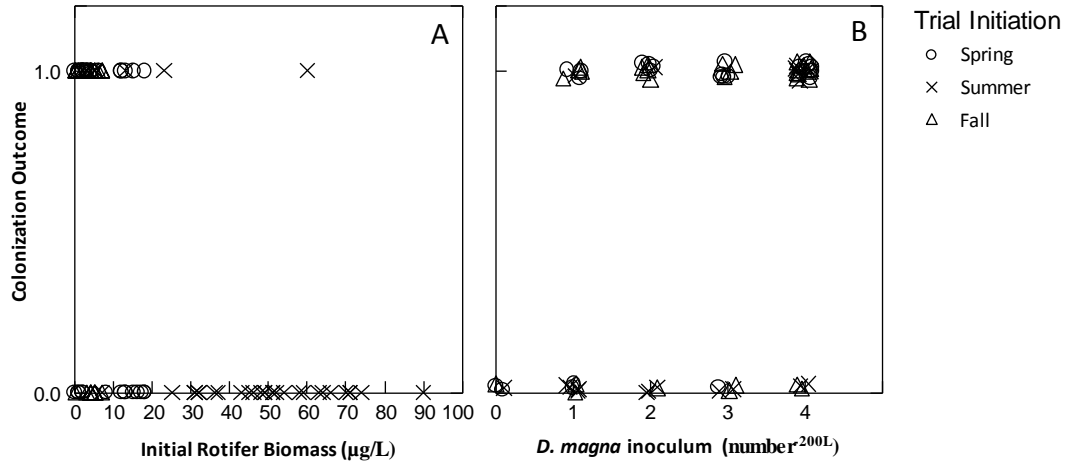


Figure 7. Scatter plots of variables found to be statistically significant ( $P < 0.05$ ) predictors of *D. magna* colonization outcomes per tank sorted by trial initiation of spring, summer and fall. Panel A represents a plot of the initial rotifer biomass expressed as dry-weight ( $\mu\text{g/L}$ ) and panel B represents the *D. magna* inoculum (number<sup>-200L</sup>) versus the colonization outcomes.

Table 5. Final model summary from binary logistic regression analysis including the parameter, estimate, standard error, P-value and Naglekerke's R-Square. Data points represent values for all trials from both years.

<b>Logistic equation = <math>\ln\left(\frac{p}{1+p}\right) = \beta_0 + \beta_1 X + \beta_2 X</math></b>			
<b>Parameter</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>P-value</b>
Constant	8.776	24.638	0.787
Initial rotifer biomass	-0.117	0.044	0.007
<i>D. magna</i> inoculum	0.877	0.223	0.000
Naglekerke's R-Squared = 0.58			

Table 6. Model summaries from binary-logistic regression analysis including the parameter, estimate, standard error, P-value and Naglekerke's R-Square. Data points represent values for trials grouped by season.

<b>Logistic equation = <math>\ln\left(\frac{p}{1+p}\right) = \beta_0 + \beta_1 X + \beta_2 X</math></b>			
<b>Spring (May 2010 and 2011)</b>			
<b>Parameter</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>P-value</b>
Constant	-0.646	0.855	0.450
Initial rotifer biomass	-0.149	0.079	0.059
<i>D. magna</i> inoculum	1.008	0.377	0.008
Naglekerke's R-Squared = 0.47			
<b>Summer (July 2010 and 2011)</b>			
<b>Parameter</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>P-value</b>
Constant	0.580	1.763	0.742
Initial rotifer biomass	-0.087	0.034	0.010
<i>D. magna</i> inoculum	0.387	0.425	0.362
Naglekerke's R-Squared = 0.61			
<b>Fall (October 2010 and 2011)</b>			
<b>Parameter</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>P-value</b>
Constant	-0.597	1.075	0.579
Initial rotifer biomass	-0.104	0.196	0.597
<i>D. magna</i> inoculum	0.741	0.289	0.010
Naglekerke's R-Squared = 0.30			

## **Chapter 4: An Evaluation of How Establishment Criteria Influence the Perceived Outcome of Invasion Experiments.**

### **Introduction**

Understanding the process of biological invasions by non-native species are the focus of ever-increasing attention by ecologists (Sakai et al. 2001). Some characteristics of species that make them effective invaders are high fecundity, habitat generality, and high propagule mobility (Rejmanek and Richardson 1996, Williamson and Fitter 1996). Propagule pressure may also be an important component of biological invasions (Chapter 3). Studies on invasive plants consistently support dual roles for propagule pressure and ecological resistance (competition, herbivory, disturbance), and highlight their interactions as barriers to establishment (Von Holle and Simberloff 2005, Lambrinos 2006, Thomsen et al. 2006, Sanders et al. 2007, Britton-Simmons and Abbott 2008, Houseman et al. 2014). Studies on invasive animals demonstrate that propagule pressure can be very important (Grevstad 1999, Ahlroth et al. 2003, Memmott et al. 2005, Bailey et al. 2009, Hedge et al. 2012). However, in many cases it is not clear what factors facilitate or prevent invasions which leads to the unpredictable nature of this process. Most of our current knowledge of the invasion process comes from either observational or theoretical studies. Despite its prevalence, the invasion process, and particularly the establishment stage, is difficult to study in nature because an invasion is often well underway before it becomes evident (Ricciardi 2001, Lee and Chown 2009) making experimental studies on the topic essential.

In the invasion literature, ‘establishment success’ is typically discussed but usually poorly defined. Ecologists often use the term ‘establishment’ to describe one of the stages of the invasion process (Carlton 1985, Williamson and Fitter 1996, Richardson et al. 2000, Kolar and Lodge 2001), yet this term carries no uniform meaning in describing the stage. For example, Williamson and Fitter (1996) define establishment as a “self-sustaining population, naturalized.” Richardson et al. (2000), in the context of describing the invasion of plants, defined establishment

as “survival and not reproduction.” Kolar and Lodge (2001) build upon Richardson’s definition and explicitly define establishment as “a species with a self-sustaining population outside of its native range.” Finally, Davis (2009) devotes one entire chapter in his book *Invasion Biology* to exploring establishment and defines the term in the very first sentence as “persisting long enough in the new environment to reproduce.” This lack of uniformity and clear description of the term ‘establishment’ complicates the interpretation and understanding of invasion results.

A strategic approach that may provide more clarity to the issue is to define the term establishment in the context of its use. This may include clearly describing 1) the objective(s) of an experiment, 2) the stage or stages of the invasion process, or 3) the interpretation of experimental or observational results. Establishment may mean different things depending on the life history of the species at hand and may mean different things to scientists versus people working on public policy (e.g. ballast water discharge standards).

To begin to unravel the general use of the term establishment and the influence of establishment criteria on the perceived outcomes of invasion experiments I conducted a literature survey of invasion-ecology experiments. To provide a tangible example of how establishment criteria influence the perceived outcomes of invasion experiments; I re-analyzed my results from Chapter 3 in light of increasingly more stringent establishment criteria to determine the influence establishment criteria may have on the outcome (interpretation) of invasion experiments. Specifically, I tested the hypothesis that no difference in establishment outcome (risk) exists as the establishment criteria become more rigorous.

## **Methods**

### *Survey of Literature*

Using literature values, data were compiled on inoculum taxa, inoculum age, and establishment criteria from both plant and animal invasion-ecology experiments. Observational

and theoretical studies were not included in this survey. When possible, I included the experimental organism's common and Latin name, inoculum age as described by major age-class (e.g. seedling, larvae, juvenile, adult, mated females), and establishment criteria. Papers selected were explicit in describing their establishment criteria.

### *Density and Growth Rate of D. magna (Chapter 3)*

The experimental results of Chapter 3 were re-analyzed. In that study, six experiments were conducted over the course of three seasons (spring, summer, fall) and two years (2010-2011). The *D. magna* density per tank was calculated weekly. The inoculum treatments (either 0, 1, 2, 3, or 4) were the number of *D. magna* added per tank (200 L) at the start of the experiment. A filtered water control (FWC) was utilized in which the maximum inoculum was added. All density results were described as number per liter. For detailed experimental methods, see methods section of Chapter 3.

From the weekly density data, I calculated weekly growth rates of *D. magna* populations in each tank using a fundamental population growth parameter, lambda ( $\lambda$ ). Lambda is equal to the finite rate of increase of the population in one time step and is given by the equation:

$$\lambda = \frac{N_t}{N_{t-1}} \quad [1]$$

where  $N$  equals the number of individuals at time step  $t$  and  $t$  equals one week. The finite rate of growth of *D. magna* per week per individual tank was calculated.

### *Selection and Development of Experimental Establishment Criteria*

To estimate establishment probabilities of *D. magna* in Chapter 3, I used the probability of passing a threshold criteria based on density and biomass of native daphnids found in the ambient test water. That is, I compared the density and biomass of *D. magna* achieved in a mesocosm tank against the mean total density and biomass of five native daphnid species (*D.*

*ambigua*, *D. parvula*, *D. pulex*, *D. mendotae*, and *D. retrocurva*) present in the Duluth-Superior Harbor and St. Louis River Estuary during 2007 and 2008 (Chapter 2). These criteria are included as part of the evaluation.

The following establishment threshold criteria were used to score the establishment outcomes of *D. magna* (Chapter 3) and are novel in the sense that they were not used to evaluate the results in Chapter 3. The criteria were broken into two major measures, density and growth as described by lambda. The threshold criteria used were:

- a density of *D. magna* greater than or equal to 10 times the inoculum at any time point during the experiment (density  $\geq 10x$  inoculum),
- a density of *D. magna* greater than or equal to 10 times the inoculum for any two consecutive weeks (density  $\geq 10x$  inoculum for 2 cons. weeks),
- a density of *D. magna* greater than or equal to 10 times the inoculum for any five consecutive weeks (density  $\geq 10x$  inoculum for 5 cons. weeks),
- any lambda value that showed growth during any time point (any  $\lambda \geq 1$ ),
- any lambda value that showed growth for two consecutive weeks (any  $\lambda \geq 1$  for 2 cons. weeks),
- any lambda value that showed growth for any three weeks (any  $\lambda \geq 1$  for any 3 weeks).

The establishment threshold criteria were used to evaluate the outcome of individual tanks using a binary approach. That is, those populations of *D. magna* that exceeded the threshold criteria were assigned a value of 1 and those populations of *D. magna* that did not exceed the threshold criteria were assigned a value of 0. This binary approach led to the calculation of risk of *D. magna* establishment described as percentage of all tanks that surpassed the criteria. The average (n=6) and standard error of establishment by experimental trial was calculated and plotted by the different criteria. Statistical differences in establishment probability among criteria were determined by conducting a Kruskal-Wallis One Way Analysis of Variance

on Ranks with a significance level of  $P < 0.05$ . To isolate a group or groups that differ from the others, a Student-Newman-Keuls nonparametric method of all pairwise comparisons at a significance level of  $P < 0.05$  was used. Lastly, the influence of trial on the overall risk of establishment was explored by plotting the risk of establishment by the different criteria for each trial.

## **Results**

### *Survey of Literature*

Establishment criteria were not well defined in many of the experiments reviewed and thus not included in Table 1. In general, establishment criteria ranged from simple metrics such as monitoring for presence/absence and density-based measures (e.g., final density greater than inoculum) to more complicated measures such as the probability of reaching a species-specific critical reproduction threshold density (Table 1). Plant-invasion experiments generally compared either biomass or density of the test species to those of the native community as a measure of establishment (Table 1). By contrast, animal-invasion experiments generally used measures of reproduction or presence of offspring at some time post-inoculation (Table 1).

### *Density and Growth Rate of *Daphnia magna**

Strong seasonal and yearly differences in *D. magna* density were observed (Fig. 1). Growth started earlier in the summer trials than in either spring or fall trials for both years; however, overall density was higher in the spring trials (Fig. 1). The FWC treatment (removal of zooplankton competitors) reached greater densities in both summer trials compared to other inoculum treatments within those trials (Fig. 1). However, during the spring 2011 and both the fall 2010 and 2011 trials, generally no differences between the FWC and other inoculum treatments were observed (Fig. 1).



Lambda values between zero and one in early time steps for each trial indicate a decrease in growth (Fig. 2); however, it is likely the 1-L subsample was not adequate to sample the sparse populations early on. The onset of positive growth ( $\lambda > 1$ ) was detected at week 3 in both the 2010 and 2011 summer trials; this was one week earlier than found for both the spring and fall trials (Fig. 2). More tanks had positive-growth rates in the spring trials compared to those in the summer and fall trials (Fig. 2). Patterns of higher growth rate were associated with *D. magna* inoculum of 3, 4, and FWC in all trials (Fig. 2).

#### *Risk of establishment evaluated by different threshold criteria*

Eight different criteria were used to evaluate the risk of establishment of *D. magna* in the mesocosm experiments from Chapter 3. Results indicate the risk of establishment described by the average established by experiment ranged from 18% to 65% (Fig. 3). The greatest risk of establishment in all trials occurred with the least stringent criterion of *D. magna* density  $\geq 10x$  the inoculum concentration while the smallest risk of establishment occurred with the most rigorous criterion of any positive-growth for any 3 weeks (Fig. 3). Statistical results indicate four major groups of criteria, that is establishment criteria with like letters are not significantly ( $P > 0.05$ ) different from each other (Fig. 3).

Differences in risk of establishment described as the percentage of tanks established evaluated by the different threshold criteria by each trial were explored (Fig. 4). The risk of establishment for the spring 2011 trial was very similar across the different criteria and ranged from 67-78% (Fig. 4). By contrast, the risk of establishment for the fall 2011 trial varied greatly (0%-78%) across the different criteria (Fig. 4).

## **Discussion**

To begin to unravel the influence of establishment criteria on the perceived outcomes of invasion experiments I utilized a two-pronged approach: 1) an assessment of establishment

criteria used in invasion ecology experiments and 2) re-analysis of my experimental data from Chapter 3 using establishment criteria that range in rigor.

### *Survey of Literature*

The expanding field of invasion ecology has seen growth and with it an increasing use of various terms and definitions to describe similar ideas and concepts (Richardson et al 2000, Colautti and Richardson 2009, Blackburn et al 2011). Sparked by the idea of attempting to understand and define ‘establishment’ in my experiments (Chapter 3) I focused my attention on this term and its associated concepts. Several authors (Carlton 1985, Williamson and Fitter 1996, Richardson et al. 2000, Kolar and Lodge 2001) have used the term ‘establishment’ in proposing the intermediate stage that links ‘introduction’ to ‘invasion’ but often do not provide enough clarity regarding the definition and use of the term. Likewise, many theoretical, observational, and experimental studies use and discuss the term ‘establishment’, yet again with no clarity on establishment criteria. While the debate (Richardson et al. 2000, Colautti and MacIsaac 2004, Colautti and Richardson 2009, Blackburn et al. 2011) on terminology may be semantic, it is inefficient and can greatly complicate our ability to cross-compare experimental results and make predictions that connect the relevancy of experimental results to natural settings.

As suggested by Colautti and Richardson (2009), it may not always be appropriate to be strictly impartial in defining and using invasion ecology terms and concepts in experiments because different ecologists studying different species in different systems will need to use different criteria in determining establishment outcomes. By contrast, Blackburn et. al (2011) propose a unified framework that assimilates key concepts and phases of invasion ecology which can be useful to all invasions, regardless of species, systems, or establishment criteria, to minimize the confusing range of terms, concepts, and definitions.

### *Density and Growth Rate of *Daphnia magna**

In all experiments, some tanks maintained growing populations at the end of eight weeks. This demonstrates that the mesocosms were not always prohibitive to “long-term” occupancy by the invader. In other words, these trials demonstrate that *D. magna*, if the environment were right, could grow and persist for at least the duration of the trial in the mesocosm tanks, which underscores the suitability of the tank environment for this type of study. At the same time, it tends to suggest that many of the factors were not good enough for long term growth and long term persistence in the tanks and that the reason was likely biological or chemical or both and not something physically prohibitive about the tanks themselves as physical conditions (e.g., light, temperature,) were largely controlled among tanks. Having high growth for long periods is one form of positive control.

Temperature and resource dependent characteristics are key biological factors that help to determine invasion success of a particular species either in the natural environment or during invasion ecology experiments. In general, zooplankton respond (i.e. increase in density and growth rate) to temperature according to species’ tolerance and optima (Watson and Wilson 1978, Sommer et al. 1986, Lennon et al. 2001). Recall from the mesocosm experiments that generally temperature was the warmest in the summer trials followed by the spring trials and then the fall trials (Chp. 3, Figs. 1-2). Likewise, total chl concentrations followed a similar pattern (Chp. 3, Figs. 1-2). *D. magna* population densities and growth rates were measurable one week earlier in both summer trials likely due to the warmer temperatures and higher initial chl concentrations.

Other studies have shown that temperature and food concentration can have direct effects on the rate of growth of *Daphnia*. Foran (1986) found that both temperature and food concentration had significant effects on the intrinsic rate of population growth ( $r$ ) of both *D. magna* and *D. laevis*, a temperate and subtropical species, respectively. Specifically, rate of growth increased as temperature rose from 15 to 27°C at high food concentrations (*C. reinhardi* at  $5 \times 10^5$  cells mL<sup>-1</sup>). However at lower food concentrations (*C. reinhardi* at  $1 \times 10^4$  cells mL<sup>-1</sup>) it

was not until 21°C that an increase in growth rate was observed (Foran 1986). These trends and patterns are consistent for *D. parvula* (Orcutt and Porter 1983). Lennon et al. (2001) showed that *D. lumholtzi* had a net positive growth rate between 11 and 38°C with optimal performance at 24°C. Also, *D. lumholtzi* reproductive rates also occurred between 15 and 30 °C with the highest offspring output at 20°C (Lennon et al. 2001). Similar to results from others, *D. magna* populations achieved some net growth ( $\lambda > 1$ ) during the experiments even if it failed to establish.

#### *Risk of establishment evaluated by different threshold criteria*

It is clear that the risk of establishment evaluated by different threshold criteria influenced the perceived outcome. Despite low growth rates in the fall compared to spring and even summer, the criteria used to conclude establishment were different enough to have a different conclusion about risk. The original criteria, Chp. 3 density and Chp. 3 biomass, were part of a set of criteria that were not statistically different from each other and were moderately liberal criteria, relative to others. Only one other criterion, any density  $\geq 10x$  inoculum, was more liberal while all others were more conservative. Interestingly, the original criteria (i.e. Chp. 3 biomass and Chp. 3 density) developed in Chapter 3 were comprehensive, meaning they included both spatial and temporal growth patterns of similar daphnia found in the ambient experimental test water and reflect “real-world” conditions; plus they require a minimum of one to two years of sampling and taxonomic effort. By contrast, the other establishment criteria in the same group used to re-analyze the results of Chapter 3 were much less intensive to develop, as they only required creative thought and minor post-hoc data manipulation and analysis.

The establishment criteria used to evaluate the outcome of my experimental work was based on life history characteristics (e.g. parthenogenic reproduction, life span of approximately 40 days) of *D. magna*, water quality parameters (e.g. temperature, chlorophyll, zooplankton

competitors) of the Duluth-Superior Harbor and St. Louis River Estuary are specific to the objectives of my research (i.e. evaluation of propagule pressure). In comparison, others who have used experiments and similar daphnid species to study invasion –ecology concepts have used a variety of establishment criteria. For example, while trying to parse out the types of ecological process and community attributes that contribute to biotic resistance Dzialowski et al. (2007) based establishment of *D. lumholtzi* on a final density greater than the inoculum density in their mesocosm experiments. Drake et al. (2005) used a simple presence/absence at the end of a bench-top experiment that used populations of parthenogenetic *D. magna* to determine how inoculum size and inoculum number influence population persistence. More sophisticated modeling approaches such as using a modified diffusion approximation to calculate establishment probabilities as the probability of reaching a species-specific reproduction threshold density necessary to initiate sexual reproduction have also been utilized (Bailey et al. 2009).

Currently, confusion around the term “establishment” complicates the interpretation and understanding of invasion results. Short-term establishment success in experiments does not necessarily translate into long-term persistence but may serve as the best available approach for predicting long-term persistence. Similar to my experiments, the establishment criteria used in animal invasion-ecology experiments only evaluate the initial establishment of the founding propagules, their subsequent survival through a juvenile period to some reproductive status and then maybe production of one to several generations of offspring. In essence, these experiments only explore the founding propagules initial establishment after drop-off and short-term persistence. By contrast, many of the plant invasion-ecology experiments evaluate the initial establishment of seeds or seedlings, some species-specific period of growth and survival, some period of short-term (1-2 year) reproduction, and periods of longer (4-10 years) survival, growth, reproduction and species change.

## **Conclusion**

The realized invasion risk clearly depends upon the life history of the species, their survival as it relates to both the abiotic and biotic environments, propagule pressure (Elton 1958, Lockwood et al 2005, Blackburn et al 2011) and the use of establishment criteria in both observational and experimental studies. The risk of establishment and its clear understanding applies broadly to basic (e.g. understanding of invasion-ecology concepts) and applied (e.g. numeric-ballast-water discharge standards) questions of population establishment. Challenges remain in converting mesocosm results into management thresholds (e.g. early response programs and ballast water discharge standards). Because this research was set up as a scientific experiment designed to test specific hypotheses, it has provided considerable information about the introduction of a particular species and associated establishment events. It has also shed light on areas that are in need of further research such as the criteria used to determine a successful outcome.

Table 1. Results of literature review on experimental studies of establishment listed as inoculum taxa, inoculum age, establishment criteria, and reference number.

Inoculum Taxon (species)	Inoculum Age	Establishment criteria	Reference
<i>Plant</i>			
aquatic ( <i>H. verticillata</i> )	seedling	greater biomass (dry-weight) than dominant species	10
native and nonnative	seedling; adult	number that survive to end of experiment	6
legume, daisy ( <i>D. canadense</i> , <i>D. purpurea</i> , <i>R. hirta</i> )	seed; seedling	percentage cover of plant by seed introduction; number of survivors for seedlings	7
18 pine species	seedlings	survive not reproducing; survive producing offspring; survive produce offspring 100 m from parent	15
legume ( <i>L. cuneate</i> )	seed	stem density	14
54 species	seed	change of species present over 4 years	16
<i>Animal</i>			
oyster ( <i>C. gigas</i> )	larvae	observation of one individual at first census	8
orange tunicate ( <i>B. diegensis</i> )	juvenile	inoculum disappeared or reproduction	1
whitefly ( <i>B. tabaci</i> )	adult	production of offspring by inoculum	9
cladocerans and rotifers	mixed age	present in inoculum and appeared in treatments throughout experiment	2
waterstrider ( <i>A. najas</i> )	mated females	mature offspring present at inoculum site	11
waterflea ( <i>D. magna</i> )	unknown age	population extant at end of experiment	12
waterflea ( <i>D. lumholtzi</i> )	adult	final density greater than stocking density	3
broom psyllid ( <i>A. Spartiophila</i> )	adult	any colony that survives past the first year	4
beetle ( <i>G. pusilla</i> , <i>G. californiensis</i> )	adult	if at least one adult beetle was found during fourth summer	5
six species of cladocerans	mixed-age	probability of reaching species-specific critical reproduction threshold density	13

<sup>1</sup>Stachowicz et al. 1999; <sup>2</sup>Shurin 2000; <sup>3</sup>Dzialowski et al. 2007; <sup>4</sup>Memmott et al. 2005; <sup>5</sup>Grevstad 1999; <sup>6</sup>Von Holle and Simberloff 2005; <sup>7</sup>Davis and Pelsor 2001; <sup>8</sup>Hedge et al. 2012; <sup>9</sup>Hufbauer et al. 2013; <sup>10</sup>Chadwell and Engelhardt 2008; <sup>11</sup>Ahloth et al. 2003; <sup>12</sup>Drake et al. 2005; <sup>13</sup>Bailey et al. 2009; <sup>14</sup>Houseman et al 2014; <sup>15</sup>Zenni and Simberloff 2013; <sup>16</sup>Tilman 1997.

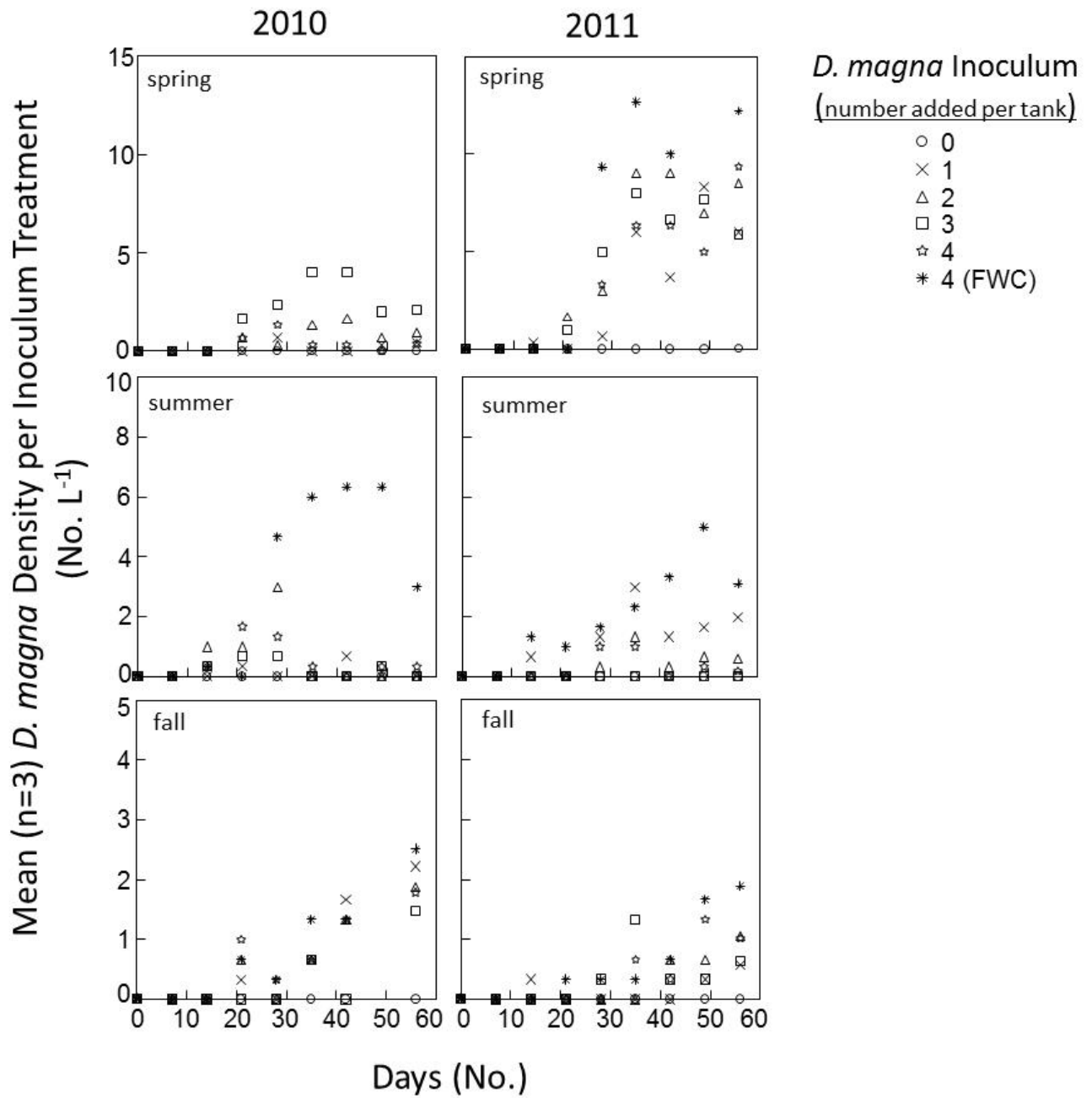


Figure 1. Average *Daphnia magna* density per inoculum concentration over time sorted by trial and year.



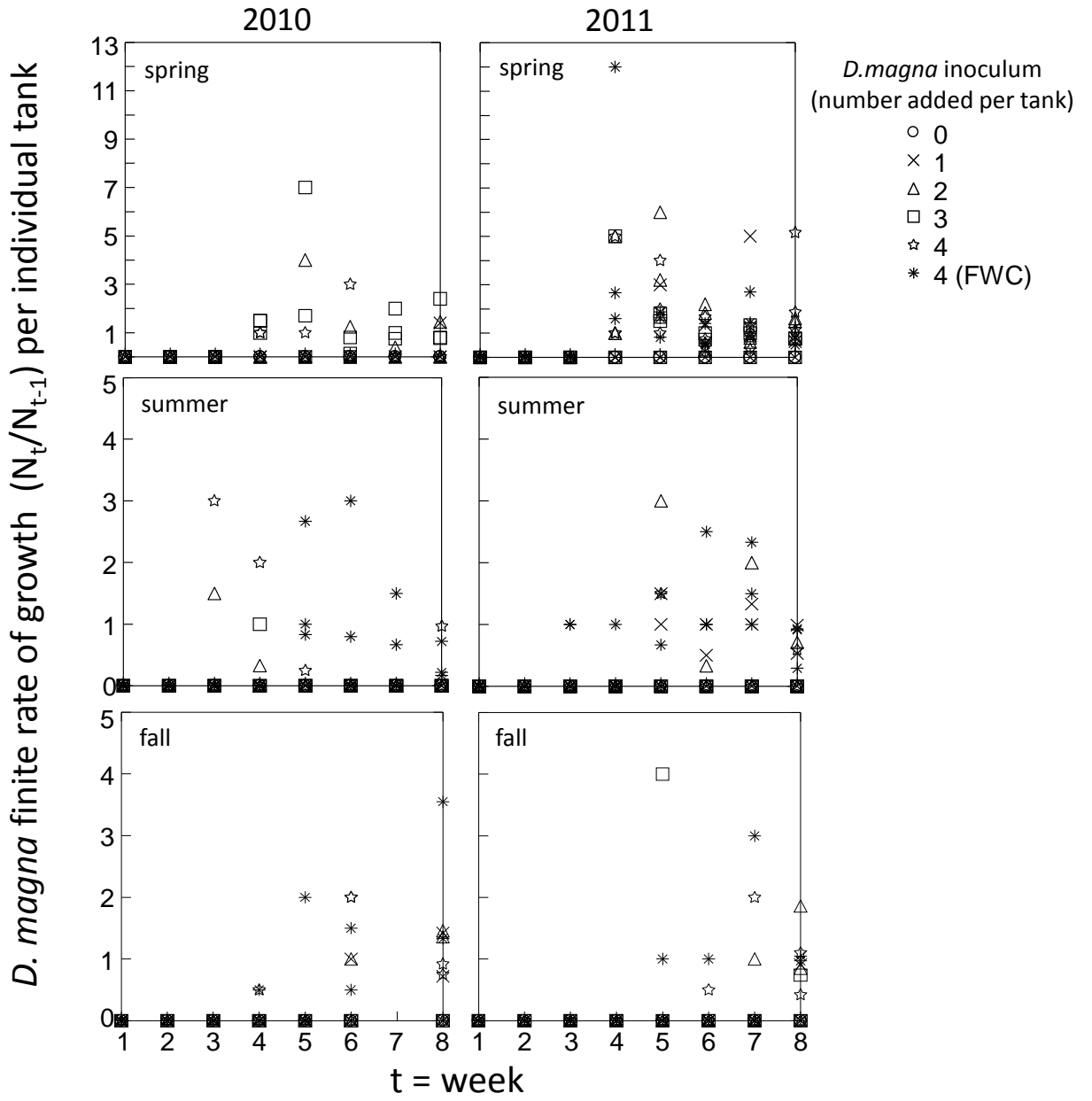


Figure 2. Finite *Daphnia magna* growth rate ( $\lambda$ ) per individual tank sorted by inoculum concentration and trial.

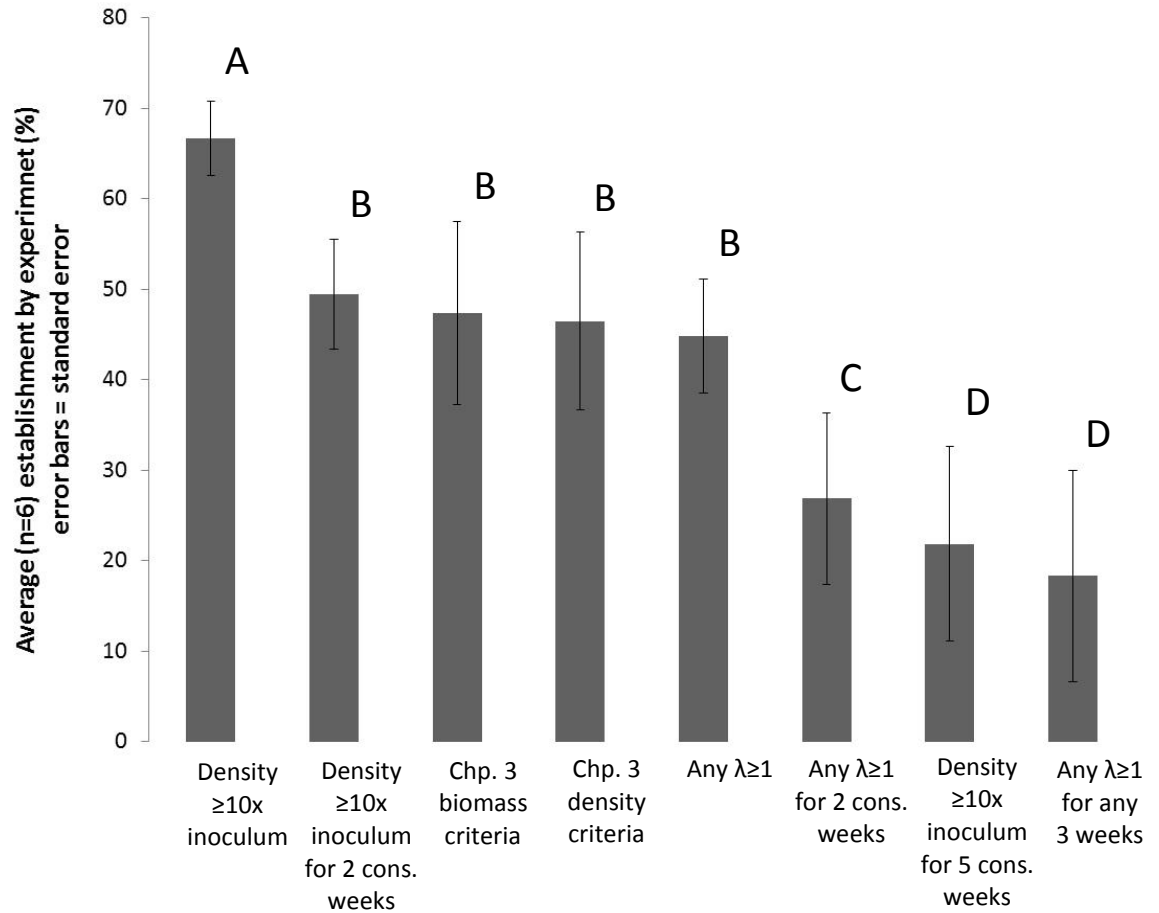


Figure 3. Risk of establishment described as the average of experimental trials sorted by different threshold criteria. Threshold criteria with like letters indicate no statistically significant ( $P < 0.05$ ) difference between criteria.

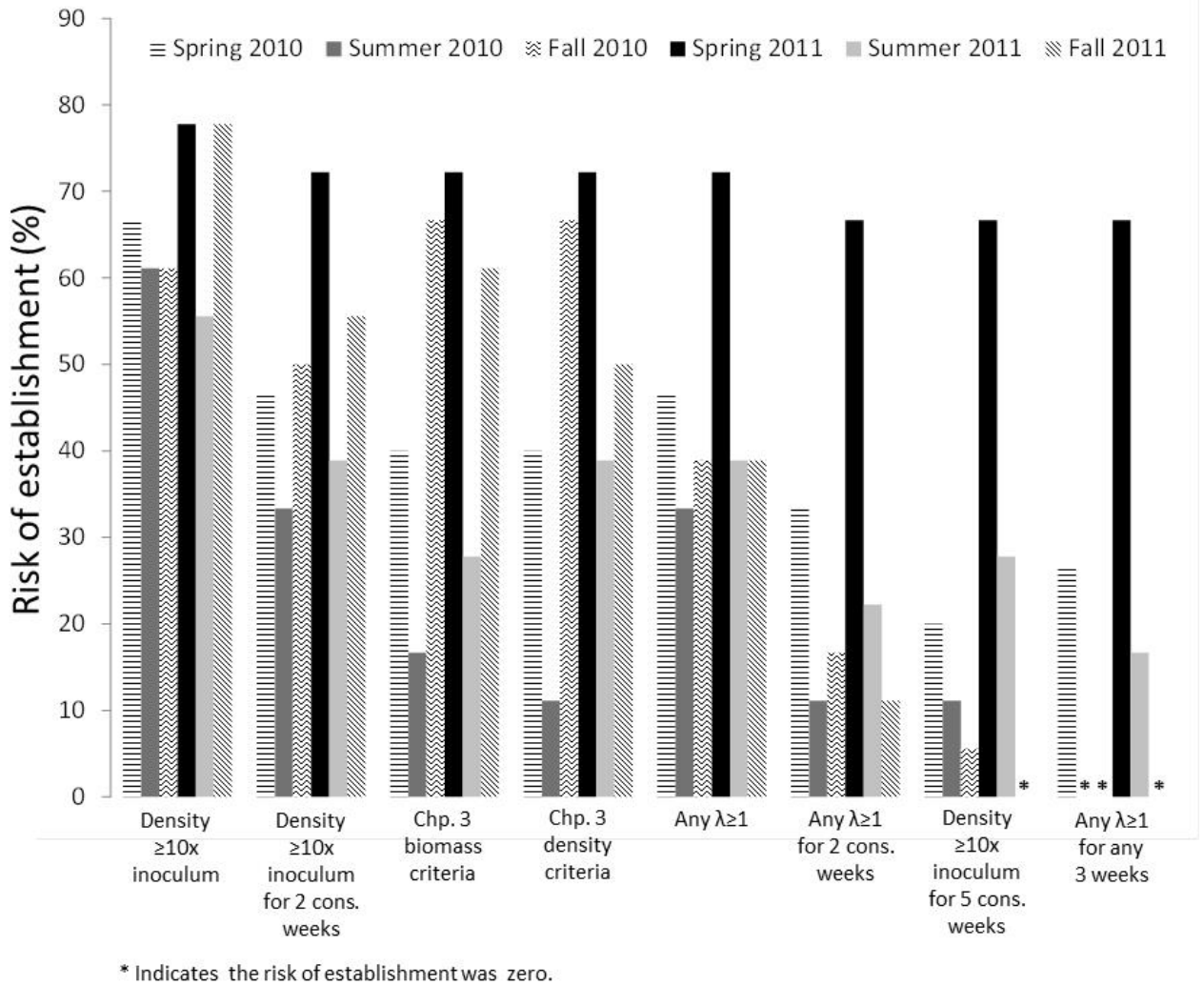


Figure 4. Risk of establishment described as the percentage of tanks established evaluated by different threshold criteria and sorted by individual trials.

## **Chapter 5: Understanding the Factors that Determine Invasion Success of *Daphnia magna*.**

Invasive species have been common targets of recent experimental studies as researchers test various approaches for managing the impacts of these organisms. Conceptual models of the invasion process (Keddy 1992, Moyle and Light 1996, Sakai, et al. 2001) can be useful tools for A) providing a framework to describe the steps during invasion events, and B) cross-comparing data and outcomes from invasion experiments. As a means to summarize my dissertation results, here I propose a conceptual model. First, I introduce the model and then I summarize the major findings from my work as they relate to the model.

My conceptual model of the invasion process (Fig. 1) by non-native organisms depicts a series of three steps -- arrival, establishment, and spread -- that must be completed. It starts with a source pool of non-native organisms, sometimes referred to as donor biota (Fig. 1). For a non-native organism to complete the arrival step it must be transported from the source pool to the new environment.

To complete the establishment step, a non-native organism must overcome what Charles Elton (1958) called ecological resistance. I broke ecological resistance into two major subcomponents called environmental resistance and demographic resistance (Fig. 1). Environmental resistance includes such factors as environmental tolerance to the physical and chemical factors of the recipient environment, vulnerability to predation, and competition with native organisms. Demographic resistance includes such factors as the non-native organism's reproductive strategies, birth and death rates, and genetic variation that may limit the non-native organism from establishing. Generally, propagule pressure, as depicted in my conceptual model, can help overcome both environmental and demographic resistance.

Propagule pressure is considered a key component of the risk-release relationship (Lockwood et al. 2005). Frequent releases of groups of propagules should enable the introduced species to overcome environmental resistance related to variation in the resistance of the recipient habitat (Simberloff, 2009). Releases of large numbers of propagules in a single release event should enable the introduced species to endure demographic resistance related to random events such as an unexplainable death (Lockwood et al. 2005; Simberloff 2009). Propagules spread across an ecosystem spatially or temporally are less likely to go extinct because adverse environmental conditions in one location or time may not affect individuals in another location or time (Hanski 1989; Lockwood et al. 2005; Duggan et al. 2005; Bailey et al. 2009).

To successfully complete the spread step (Fig. 1), an established non-native species must expand its range in the new ecosystem. Subtle changes in the recipient ecosystem may occur to permit or deny this final step. Only a small percentage (<10%) of successful invasive species spread significantly to create major changes (e.g. become pests to humans, cause local extinction of native organisms) in the receiving systems (Williamson and Fitter 1996). Most introduced non-native organisms result in failed invasions (lack of establishment) which makes it extremely difficult to study this last step.

In my dissertation, I focused on the initial establishment step, not to be confused with population persistence. In Chapter 2, I used an aggressive survey approach to characterize the spatial and temporal variation in the physical, chemical, and biological properties of the Duluth Superior Harbor and St. Louis River Estuary environment over a 2-year period. This provided a context for understanding possible seasonal and spatial variation in ecological resistance that could prevent establishment in this ecosystem. In Chapter 3, I experimentally evaluated components of ecological resistance and interactions between ecological resistance and propagule pressure. Ecological resistance and propagule pressure were manipulated by exposing different

densities of a surrogate invader (*Daphnia magna*) to natural Duluth-Superior Harbor and St. Louis River Estuary water in 200-L mesocosms that varied on a seasonal basis over two years .

The results of the field survey demonstrate that there is significant variation in the potential factors that comprise environmental resistance across spatial and seasonal scales in the Duluth-Superior Harbor and St. Louis River Estuary. Hence, one should expect different potential outcomes in the capacity of introduced propagules to overcome establishment related to where and when they are introduced in the ecosystem.

The results of the mesocosm experiments demonstrate that colonization success of *D. magna* can be strongly influenced by both environmental resistance (specifically, biotic factors associated with the timing of arrival of individuals) and demographic resistance (e.g. the number of arriving individuals). In my research, abiotic conditions varied widely but were not significant determinants of *D. magna*'s colonization success. The results from my study are consistent with the biotic resistance hypothesis. During the spring and fall experiments, *D. magna* populations demonstrated clear episodes of colonization success. By contrast, in the summer *D. magna* rarely achieved levels that were high enough to be considered successful colonization. Only when the background assemblage of zooplankton was first removed from the tanks in the summer was *D. magna* able to achieve densities above colonization criteria. Initial rotifer biomass of the recipient community appears to be the major driving component of environmental resistance in determining the outcome of *D. magna*'s ability to colonize the mesocosms under these test conditions.

Results from the mesocosms also indicate that increasing concentrations of *D. magna* inoculum (increasing propagule pressure) during the spring and fall trials increased the probability of *D. magna* to successfully colonize the mesocosms, overwhelming demographic resistance. However, during summer trials, even the highest inoculum concentration was not able

to overcome ecological resistance. Hence, my results revealed strong interaction between propagule pressure and ecological resistance. These results suggest that predicting invasion success depends on characteristics of the invading propagule and the receiving system.

The realized invasion risk clearly depends upon the life history of the species, their survival as it relates to both the abiotic and biotic environments, propagule pressure (Elton 1958, Lockwood et al 2005, Blackburn et al 2011) and the use of establishment criteria in both observational and experimental studies. Also, it is clear that the risk of establishment evaluated by different threshold criteria influenced the perceived outcome (Chp. 4). The original criteria, Chp. 3 density and Chp. 3 biomass, were part of a set of criteria that were not statistically different from each other and were moderately liberal criteria, relative to others (Chp. 4). Only one other criterion, any density  $\geq 10x$  inoculum, was more liberal while all others were more conservative (Chp. 4). Interestingly, the original criteria developed in Chapter 3 included both spatial and temporal growth patterns of similar *Daphnia* found in the ambient experimental test water and reflect “real-world” conditions. By contrast, the other establishment criteria used to re-analyze the results of Chapter 3 were much less intensive to develop, as they only required creative thought and minor post-hoc data manipulation and analysis.

Currently, confusion around the term “establishment” complicates the interpretation and understanding of invasion results. Short-term establishment success in experiments does not necessarily translate into long-term persistence but may serve as the best available approach for predicting long-term persistence. Similar to my experiments, the establishment criteria used in many animal invasion-ecology experiments only evaluate the initial establishment of the founding propagules, their subsequent survival through a juvenile period to some reproductive status and then maybe production of one to several generations of offspring. In essence, these experiments only explore the founding propagules initial establishment after drop-off and short-term persistence.

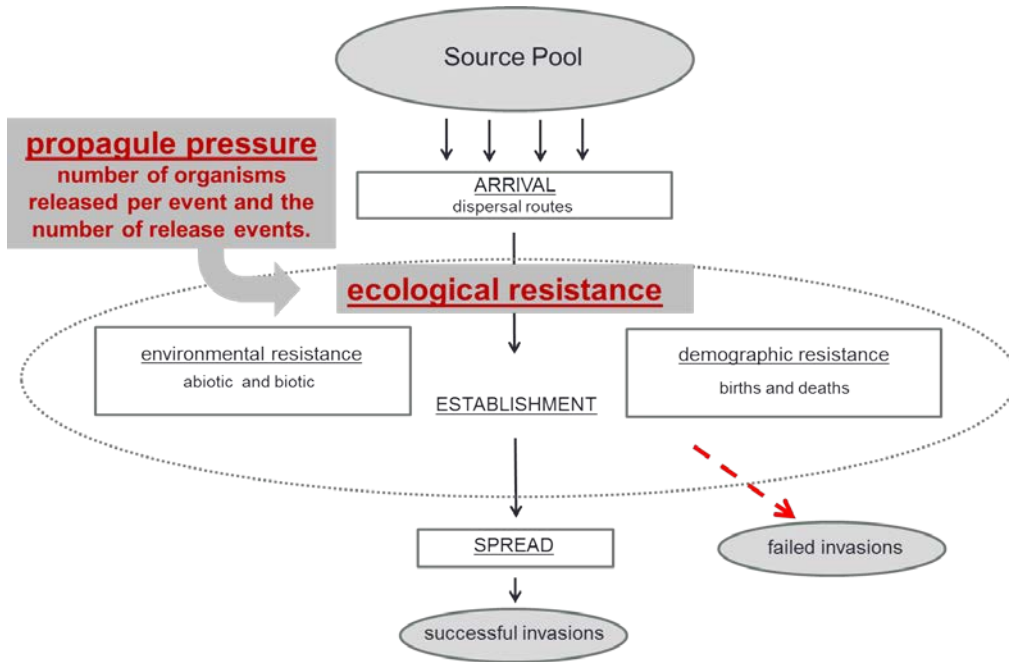


Figure 1. Basic conceptual model describing the general steps of an invasion by a non-native organism.



## Works Cited

- Ackerman, J. D., Sim, B., Nichols, S. J., and Claudi, R. (1994). A review of the early life history of zebra mussels (*Dreissena polymorpha*): comparisons with marine bivalves. *Canadian Journal of Zoology*, 72, 1169-1179.
- Ahlroth, P., Alatalo, R. V., Holopainen, A., Kumpulainen, T., and Suhonen, J. (2003). Founder population size and number of source populations enhance colonization success in waterstriders. *Oecologia*, 442, 617-620.
- Allan, J. D. (1976). Life history patterns in zooplankton. *The American Naturalist*, 110, 165-180.
- Armour, A. F., Tsou, J. L., and Wiancko, P. M. (1993). Zebra Mussels: The Industrial Impact." Toronto: Third International Zebra Mussel Conference.
- Arnott, S. E., Magnuson, J. J., and Yan, N. D. (1998). Crustacean zooplankton species richness: single- and multiple-year estimates. *Canadian Journal of Fisheries and Aquatic Science*, 55, 1573-1582.
- Bailey, S. A., Deneau, M. G., Jean, L., Wiley, C. J., Leung, B., and MacIsaac, H. J. (2011). Evaluating efficacy of an environmental policay to prevent biological invasions. *Environmental Science and Technology*, 45, 2554-2561.
- Bailey, S. A., Duggan, I. C., Jenkins, P. T., and MacIsaac, H. J. (2005). Invertebrate resting stages in residual ballast sediment of transoceanic ships. *Canadian Journal of Fisheries and Aquatic Science*, 62, 1090-1103.
- Bailey, S. A., Velez-Espino, L. A., Johannsson, O. E., Koops, M. A., and Wiley, C. J. (2009). Estimating establishment probabilities of Cladocera introduced at low density: an evaluation of the proposed ballast water discharge standards. *Canadian Journal of Fisheries and Aquatic Sciences*, 66, 261-276.
- Balcer, M. D. (1978). *Crustacean zooplankton of the Duluth-Superior Region of Lake Superior*. University of Wisconsin. Madison: University of Wisconsin.
- Balcer, M. D., Korda, N. L., and Dodson, S. I. (1984). *Zooplankton of the Great Lakes A guide to the Identification and Ecology of the Common Crustacean Species*. Madison: University of Wisconsin Press.
- Baltz, D. M., and Moyle, P. B. (1993). Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications*, 3(2), 246-255.
- Bergstrom, M. A., Evrard, L. M., and Mensinger, A. (2008). Distribution, abundance, adn range of the Round Goby, *Apollina melanostoma*, in the Duluth-Superior Harbor and St. Louis River Estuary, 1998-2004. *Journal of Great Lakes Research*, 34, 535-543.

- Birch, L. C. (1948). The intrinsic rate of natural increase of an insect population. *Journal of Animal Ecology*, 17(1), 15-26.
- Blackburn, T. M., Pysek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarosik, V., Wilson, J. R. U., and Richardson, D. M. (2011). A proposed unified framework for biological invasions. *Trends in Ecology and Evolution*, 26(7), 333-339.
- Bohonak, A. J., and Jenkins, D. G. (2003). Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters*, 6, 783-796.
- Breneman, D., Richards, C., and Lozano, S. (2000). Environmental influences on benthic community structure in a Great Lake embayment. *Journal of Great Lakes Research*, 26, 287-304.
- Briski, E., Allinger, L., Balcer, M., Cangelosi, A., Fanberg, L., Markee, T., Mays, N., Reid, D., Saillard, H., Schwerdt, T., Schaefer, H., TenEyck, M., Wiley, C., and Bailey, S. (2013). A multidimensional approach to invasive species prevention. *Environmental Science and Technology*, 47, 1216-1221.
- Britton-Simmons, K. H., and Abbott, K. C. (2008). Short- and long-term effects of disturbance and propagule pressure on a biological invasion. *Journal of Ecology*, 96, 68-77.
- Bronte, C. R., Ebener, M. P., Schreiner, D. R., DeVault, D. S., Petzold, M. M., Jenson, D. A., Richards, C., and Lozano, S. J. (2003). Fish community change in Lake Superior, 1970-2000. *Canadian Journal of Fisheries and Aquatic Science*, 60, 1552-1574.
- Brooks, J. L. (1959). Cladocera. In W. T. Edmondson (Ed.), *Freshwater Biology* (2nd ed., pp. 587-656). New York, New York: John Wiley and Sons Inc.
- Brooks, J. L., and Dodson, S. I. (1965). Predation, Body Size, and Composition of Plankton. *Science*, 150, 28-35.
- Brown, J. H., and Kodric-Brown, A. (1977). Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology*, 58, 445-449.
- Brown, M. E., and Branstrator, D. K. (2004). A 2001 survey of crustacean zooplankton in the western arm of Lake Superior. *Journal of Great Lakes Research*, 30, 1-8.
- Burke, J. W., and Grime, J. P. (1996). An experimental study of plant community invasibility. *Ecology*, 77, 776-790.
- Burns, C. W., and Gilbert, J. J. (1986). Effects of daphnid size and density on interference between *Daphnia* and *Keratella cochlearis*. *Limnology and Oceanography*, 31(4), 848-858.

- Capinha, C., Brotons, L., and Anastacio, P. (2013). Geographical variability in propagule pressure and climatic suitability explain the European distribution of two highly invasive crayfish. *Journal of Biogeography*, *40*, 548-558.
- Carlton, J. T. (1985). Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanography and Marine Biology Annual Review*, *23*, 313-374.
- Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Cochran, P. A., Elser, J. J., Elser, M. M., Lodge, D. M., Kretcher, D., He, X., and von Ende, C. N. (1987). Regulation of lake primary productivity by food web structure. *Ecology*, *68*(6), 1863-1876.
- Cataldo, R. (2001). Musseling in on the ninth district economy. *Fedgazette*, *13*(1), 15-17.
- Chadwell, T. B., and Engelhardt, K. A. (2008). Effects of pre-existing submersed vegetation and propagule pressure on the invasion success of *Hydrilla verticillata*. *Journal of Applied Ecology*, *45*, 515-523.
- Chimney, M. J., and Bowers, J. A. (2006). The influence of subsample size in a study of freshwater zooplankton. *Journal of Freshwater Ecology*, *21*, 349-351.
- Colautti, R. I., and MacIsaac, H. J. (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions*, *10*, 135-141.
- Colautti, R. I., and Richardson, D. M. (2009). Subjectivity and flexibility in invasion terminology: too much of a good thing? *Biological Invasions*, *11*, 1225-1229.
- Colautti, R. I., Grigorovich, I. A., and MacIsaac, H. J. (2006). Propagule pressure: a null model for biological invasions. *Biological Invasions*, *8*, 1023-1037.
- Colautti, R. I., Niimi, A. J., van Overdijk, C. D., Mills, E. L., Holeck, K., and MacIsaac, H. J. (2003). Spatial and temporal analysis of transoceanic shipping vectors to the Great Lakes. In G. M. Ruiz, and J. T. Carlton (Eds.), *Invasive Species Vectors and Management Strategies* (pp. 227-246). Washington D.C.: Island Press.
- Davis, M. A. (2009). *Invasion Biology* (1 ed.). New York: Oxford University Press.
- Davis, M. A., and Pelsor, M. (2001). Experimental support for a resource-based mechanistic model of invasibility. *Ecology Letters*, *4*, 421-428.
- Davis, M. A., Thompson, K., and Grime, J. P. (2005). Invasibility: the local mechanism driving community assembly and species diversity. *Ecography*, *28*(5), 696-704.
- Davis, M. D., Grime, J. P., and Thompson, K. (2000). Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, *88*, 528-534.

- DeRivera, C. E., Ruiz, G. M., Hines, A. H., and Jivoff, P. (2005). Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. *Ecology*, *86*(12), 3364-3376.
- Dethier, M. N., and Hacker, S. D. (2005). Physical factors vs. biotic resistance in controlling the invasion of an estuarine marsh grass. *Ecological Applications*, *15*(4), 1273-1283.
- Dickman, M., and Zhang, F. (1999). Mid-ocean exchange of container vessel ballast water 2: Effects of vessel type in the transport of diatoms and dinoflagellates from Manzanillo, Mexico, to Hong Kong, China. *Marine Ecology Progress Series*, *176*, 253-262.
- Drake, J. M., Baggenstos, P., and Lodge, D. M. (2005). Propagule pressure and persistence in experimental populations. *Biology Letters*, *1*, 480-483.
- Duggan, I. C., van Overdijk, C. D., Bailey, S. A., Jenkins, P. T., Limen, H., and MacIsaac, H. J. (2005). Invertebrates associated with residual ballast water and sediments of cargo-carrying ships entering the Great Lakes. *Canadian Journal of Fisheries and Aquatic Science*, *62*, 2463-2474.
- Duluth Seaway Port Authority. (2012). *The largest, farthest-inland freshwater port*. Retrieved March 2012, from <http://www.duluthport.com/port.php>
- Dzialowski, A. R., Lennon, J. T., and Smith, V. H. (2007). Food web structure provides biotic resistance against plankton invasion attempts. *Biological Invasions*, *9*, 257-267.
- Edmondson, W. T. (1959). *Ward and Whipple's Freshwater Biology* (2nd ed.). New York: John Wiley.
- Elton, C. S. (1958). *The Ecology of Invasions by Animals and Plants*. Chicago: University of Chicago Press.
- EPA, U. S. (2003). Standard Operating Procedure for Zooplankton Analysis-LG403. Chicago: US Environmental Protection Agency.
- Evans, M. (1985). The morphology of *Daphnia pulicaria*, a species newly dominating the offshore southeastern Lake Michigan summer *Daphnia* community. *Transactions of the American Microscopical Society*, *104*, 223-231.
- Fahnenstiel, G. L., Krause, A. E., McCormick, M. J., Carrick, H. J., and Schelske, C. L. (1998). The structure of the planktonic food-web in the St. Lawrence Great Lakes. *Journal of Great Lakes Research*, *24*(3), 531-554.
- Fishman, D. B., Adlerstein, S. A., Vanderploeg, H. A., Fahnenstiel, G. L., and Scavia, D. (2009). Causes of phytoplankton changes in Saginaw Bay, Lake Huron, during the zebra mussel invasion. *Journal of Great Lakes Research*, *35*, 482-495.

- Foran, J. A. (1986). A comparison of the life history features of a temperate and subtropical *Daphnia* species. *Oikos*, 46, 185-193.
- Fussman, G. (1996). The importance of crustacean zooplankton in structuring rotifer and phytoplankton communities: an enclosure study. *Journal of Plankton Research*, 18(10), 1897-1915.
- Gilbert, J. J. (1985). Competition between rotifers and *Daphnia*. *Ecology*, 66(6), 1943-1050.
- Gilbert, J. J. (1988). Susceptibilities of ten rotifer species to interference from *Daphnia pulex*. *Ecology*, 69(6), 1826-1838.
- Gilbert, J. J. (1989). The effect of *Daphnia* interference on a natural rotifer and ciliate community: Short-term bottle experiments. *Limnology and Oceanography*, 34(3), 606-617.
- Gilbert, J. J., and Stemberger, R. S. (1985). Control of *Keratella* populations by interference competition from *Daphnia*. *Limnology and Oceanography*, 30(1), 180-188.
- Gollasch, S., David, M., Voigt, M., Dragsund, E., Hewitt, C., and Fukuyo, Y. (2007). Critical review of the IMO international convention on the management of ships' ballast water and sediments. *Harmful Algae*, 6, 585-600.
- Goss, B. L., and Bunting, D. L. (1983). *Daphnia* development and reproduction: responses to temperature. *Journal of Thermal Biology*, 8(4), 375-380.
- Graves, G. A. (2004). A Monte Carlo simulation of biotic metrics as a function of subsample size. *Journal of Freshwater Ecology*, 19, 195-201.
- Gray, D. K., Johengen, T. H., Reid, D. F., and MacIsaac, H. J. (2007). Efficacy of open-ocean ballast water exchange as a means of preventing invertebrate invasions between freshwater ports. *Limnology and Oceanography*, 52, 2386-2397.
- Grevstad, F. S. (1999). Experimental invasions using biological control introductions: the influence of release size on the chance of population establishment. *Biological Invasions*, 1, 313-323.
- Grigorovich, I. A., Colautti, R. I., Mills, E. L., Holeck, K., Ballert, A. G., and MacIsaac, H. J. (2003). Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences*, 6, 740-756.
- Grigorovich, I. A., Kelly, J. R., Darling, J. A., and West, C. W. (2008). The quagga mussel invades the Lake Superior Basin. *Journal of Great Lakes Research*, 34, 342-350.
- Grigorovich, I. A., Korniushev, A. V., Gray, D. K., Duggan, I. C., Colautti, R. I., and MacIsaac, H. J. (2003). Lake Superior: an invasion coldspot? *Hydrobiologia*, 499, 191-210.

- Hall, D. J., Threlkeld, S. T., Burns, C. W., and Crowley, P. H. (1976). The size-efficiency hypothesis and the structure of zooplankton communities. *Annual Review of Ecology and Systematics*, 7, 177-208.
- Haney, J. F. (2013). *An-imaged based key to zooplankton of North America version 5.0*. Retrieved October 3, 2013, from cfb.unh.edu
- Hanski, I. (1989). Matapopulation dynamics: does it help to have more of the same? *Trends in Ecology and Evolution*, 4(4), 113-114.
- Hargis, J. R. (1984). Seasonal primary production and plankton dynamics in the St. Louis River and Harbor. In A. R. Carlson, and N. A. Thomas (Eds.), *Chemical and biological studies related to the water quality of St. Louis Bay of Lake Superior*. (pp. 73-92). Duluth, Minnesota: U.S. EPA.
- Havel, J. E., and Hebert, P. D. (1993). *Daphnia lumholtzi* in North America: another exotic zooplankton. *Limnology and Oceanography*, 38, 1823-1827.
- Havel, J. E., Shurin, J. B., and Jones, J. R. (2002). Estimating dispersal from patterns of spread: spatial and local control of lake invasions. *Ecology*, 83(12), 3306-3318.
- Hecky, R. E., Campbell, P., and Hendzel, L. L. (1993). The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of oceans and lakes. *Limnology and Oceanography*, 38(4), 709-724.
- Hedge, L. H., O'Connor, W. A., and Johnston, E. L. (2012). Manipulating the intrinsic parameters of propagule pressure: implications for bio-invasion. *Ecosphere*, 3(6), 1-13.
- Hessen, D. O. (1990). Carbon, nitrogen and phosphorus status in *Daphnia* at varying food conditions. *Journal of Plankton Research*, 12, 1239-1249.
- Hessen, D. O. (1992). Nutrient element limitation of zooplankton production. *The American Naturalist*, 140(5), 799-814.
- Hoffman, J. C., Peterson, G. S., Cotter, A. M., and Kelly, J. R. (2010). Using stable isotope mixing in a Great Lakes coastal tributary to determine food web linkages in young fishes. *Estuaries and Coasts*, 33, 1391-1405.
- Holeck, K. T., Mills, E. L., and MacIsaac, H. J. (2007). Globalization, biological invasions, and ecosystem changes in North America's Great Lakes. In W. W. Taylor, M. G. Schechter, and L. G. Wolfson (Eds.), *Globalization: Effects on Fisheries Resources* (pp. 156-181). Cambridge, United Kingdom: Cambridge University Press.
- Holeck, K. T., Mills, E. L., MacIsaac, H. J., Dochoda, M. R., Colautti, R. I., and Ricciardi, A. (2004). Bridging troubled waters: biological invasions, transoceanic shipping, and the Laurentian Great Lakes. *BioScience*, 54, 919-929.

- Holway, D. A., Suarez, A. V., and Case, T. J. (2002). Role of abiotic factors governing susceptibility to invasion: a test with Argentine ants. *Ecology*, 83(6), 1610-1619.
- Houseman, G. R., Foster, B. L., and Brassil, C. E. (2014). Propagule pressure invasibility relationships: testing the influence of soil fertility and disturbance with *Lespedeza cuneata*. *Oecologia*, 174, 511-520.
- Hudson, P. L., Reid, J. W., Lesko, L. T., and Selgeby, J. H. (1998). Cyclopoid and harpacticoid copepods of the Laurentian Great Lakes. *Ohio Biological Survey Bulletin (New Ser.)*, 12(2), 1-50.
- Hufbauer, R. A., Rutschmann, A., Serrate, B., Vermeil de conchard, H., and Facon, B. (2013). Role of propagule pressure in colonization success: disentangling the relative importance of demographic, genetic and habitat effects. *Journal of Evolutionary Biology*, 26, 1691-1699.
- Jordan, T. F., Stortz, K. R., and Sydor, M. (1981). Resonant oscillation in Duluth-Superior Harbor. *Limnology and Oceanography*, 26(1), 186-190.
- Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, 3, 157-164.
- Kirk, K. L. (1991). Inorganic particles alter competition in grazing plankton: the role of selective feeding. *Ecology*, 72(3), 915-923.
- Kirk, K. L., and Gilbert, J. G. (1990). Suspended clay and the populations dynamics of planktonic rotifers and cladocerans. *Ecology*, 71(5), 1741-1755.
- Kolar, C. S., and Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution*, 16(4), 199-204.
- Lambrinos, J. G. (2006). Spatially variable propagule pressure and herbivory influence invasion of chaparral shrubland by an exotic grass. *Oecologia*, 147, 327-334.
- Lee, J. E., and Chown, S. L. (2009). Breaching the dispersal barrier to invasion: quantification and management. *Ecological Applications*, 19(7), 1944-1959.
- Lennon, J. T., Smith, V. H., and Dzialowski, A. R. (2003). Invasibility of plankton food webs along a trophic state gradient. *OIKOS*, 103, 191-203.
- Lennon, J. T., Smith, V. H., and Williams, K. (2001). Influence of temperature on exotic *Daphnia lumholzi* and implications for invasion success. *Journal of Plankton Research*, 23(4), 425-434.
- Leung, B., and Mandrak, N. E. (2007). The risk of establishment of aquatic invasive species: joining invasibility and propagule pressure. *Proceedings Of The Royal Society B*, 2603-2609.

- Lockwood, J. L., Cassey, P., and Blackburn, T. (2005). The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution*, 20(5), 223-228.
- Lockwood, J. L., Hoopes, M. F., and Marchetti, M. P. (2007). *Invasion Ecology*. Oxford: Blackwell Publishing.
- Lodge, D. M. (1993). Biological invasions: lessons for ecology. *Trends in Ecology and Evolution*, 8, 133-141.
- Lodge, D. M., Williams, S., MacIsaac, H. J., Hayes, K. R., Leung, B., Reichard, S., Mack, R. N., Moyle, P. B., Smith, M., Andow, D. A., Carlton, J. T., and McMichael, A. (2006). biological invasions: recommendations for U.S. policay and management. *Ecological Applications*, 16(6), 2035-2054.
- Lonsdale, W. M. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology*, 80, 1522-1536.
- MacIsaac, H. J., and Gilbert, J. J. (1989). Competition between rotifers and cladocerans of different body sizes. *Oecologia*, 81, 295-301.
- MacIsaac, H. J., Robbins, T. C., and Lewis, M. A. (2002). Modeling ships' ballast water as invasion threats to the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, 1245-1256.
- Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., and Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and conrol. *Ecological Applications*, 10(3), 689-710.
- Martin Associates. (2001). *Economic Impact Study of the Great Lakes St. Lawrence Seaway System*. Washington D.C.: U.S. Saint Lawrence Seaway Development Corporation.
- Maxwell, K., and Johnson, G. N. (2000). Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany*, 51(345), 659-668.
- McCabe, G. D., and O'Brien, W. J. (1983). The effects of suspended silt on feeding and reproduction of *Daphnia pulex*. *The American Midland Naturalist*, 110(2), 324-337.
- McCollin, T., Shanks, A. M., and Dunn, J. (2007). The efficiency of regional ballast water exchange: Changes in phytoplankton abundance and diversity. *Harmful Algae*, 6, 531-546.
- Megard, R. O. (1997). Spatial distributions of zooplankton during coastal upwelling in Western Lake Superior. *Limnology and Oceanography*, 42, 827-840.
- Memmott, J., Craze, P. G., Harman, H. M., Syrett, P., and Fowler, S. V. (2005). The effect of propagule size on the invasion of an alien insect. *Journal of Animal Ecology*, 74, 50-62.



- Miller, T. E., Kneitel, J. M., and Burns, J. H. (2002). Effect of community structure on invasion success and rate. *Ecology*, 83(4), 898-905.
- Mills, E. L., Leach, J. H., Carlton, J. T., and Secor, C. L. (1993). Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. *Journal of Great Lakes Research*, 19(1), 1-54.
- Minton, M. S., Verling, E., Miller, W. A., and Ruiz, G. M. (2005). Reducing propagule supply and coastal invasions via ships: effects of emerging strategies. *Frontiers in Ecology and the Environment*, 3(6), 304-308.
- Moss, B., McGowan, S., and Carvalho, L. (1994). Determination of phytoplankton crop by top-down and bottom-up mechanisms in a group of English lakes, the West Midland meres. *Limnology and Oceanography*, 39(5), 1020-1029.
- Moyle, P. B., and Light, T. (1996). Fish invasions in California: do abiotic factors determine success? *Ecology*, 77(6), 1666-1670.
- Moyle, P. B., and Light, T. (1996). Fish invasions in California: do abiotic factors determine success? *Ecology*, 77(6), 1666-1670.
- Nagelkerke, N. J. (1991). A note on a general definition of the coefficient of determination. *Biometrika*, 78(3), 691-692.
- National Ballast Information Clearinghouse. (2009). *NBIC Online Database*. (Smithsonian Environmental Research Center and United States Coast Guard) Retrieved 2007 and 2008, from <http://invasions.si.edu/nbic/search.html>
- Neill, W. E. (1984). Regulation of rotifer densities by crustacean zooplankton in an oligotrophic montane lake in British Columbia. *Oecologia*, 61, 175-181.
- O'Neill, C. R., and Dextrase, A. (1994). The introduction and spread of zebra mussel in North America. In *Proceedings of the Fourth International Zebra Mussel Conference* (pp. 433-446). Madison.
- Orcutt, J. D., and Porter, K. G. (1983). Diel vertical migration by zooplankton: Constant and fluctuating temperature effects on life history patterns of *Daphnia*. *Limnology and Oceanography*, 28(4), 720-730.
- Pastor, J., Downing, A., and Erickson, H. E. (1996). Species-area curves and diversity-productivity relationships in beaver meadows of Voyageurs National Park, Minnesota, USA. *OIKOS*, 77, 399-406.
- Patalas, K. (1990). Diversity of the zooplankton communities in Canadian lakes as a function of climate. *Verhandlungen des Internationalen Verein Limnologie*, 24, 360-368.

- Pimentel, D., Lach, L., Zuniga, R., and Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience*, 50, 53-65.
- Reid, J. W., and Hudson, P. L. (2008). Comment on "Rate of species introductions in the Great Lakes via ships ballast water and sediments.". *Canadian Journal of Fisheries and Aquatic Sciences*, 65, 549-553.
- Rejmanek, M., and Richardson, D. M. (1996). What attributes make some plant species more invasive? *Ecology*, 77, 1655-1661.
- Ricciardi, A. (2001). Facilitative interactions among aquatic invaders: is an invasional meltdown occurring in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Science*, 58, 2513-2525.
- Ricciardi, A. (2006). Patterns of invasions in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions*, 12, 425-433.
- Ricciardi, A., and MacIsaac, H. J. (2000). Recent mass invasion of North American Great Lakes by Ponto-Caspian species. *Trends in Ecology and Evolution*, 15, 62-65.
- Richardson, D. M., Pyšek, P., Rejmanek, M., Barbour, M. G., Panetta, F. D., and West, C. J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*, 6, 93-107.
- Ruiz, G. M., and Carlton, J. T. (2003). Invasion vectors: a conceptual framework for management. In G. M. Ruiz, and J. T. Carlton (Eds.), *Invasive Species Vectors and Management Strategies* (pp. 459-504). Washington D.C.: Island Press.
- Ruiz, G. M., Fofonoff, P. W., Carlton, J. T., Wonham, M. J., and Hines, A. H. (2000). Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Reviews of Ecology and Systematics*, 31, 481-531.
- Rup, M. P., Bailey, S. A., Wiley, C. J., Minton, M. S., Miller, A. W., Ruiz, G. M., and MacIsaac, H. J. (2010). Domestic ballast operations on the Great Lakes: potential importance of Lakers as a vector for introduction and spread of nonindigenous species. *Canadian Journal of Fisheries and Aquatic Science*, 67, 256-268.
- Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neill, P., Parker, I. M., Thompson, J. N., and Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics*, 32, 305-332.
- Sanders, N. J., Weltzin, J. F., Crutsinger, G. M., Fritzpatrick, M. C., Nunez, M. A., Oswalt, C. M., and Lane, K. E. (2007). Insects mediate the effects of propagule supply and resource availability on a plant invasion. *Ecology*, 88(9), 2383-2391.

- Shea, K., and Chesson, P. (2002). Community ecology theory as a framework for biological invasions. *TRENDS in Ecology and Evolution*, 17(4), 170-176.
- Shurin, J. B. (2000). Dispersal limitation, invasion resistance, and the structure of pond zooplankton communities. *Ecology*, 81(11), 3074-3086.
- Shuter, B. J., and Ing, K. K. (1997). Factors affecting the production of zooplankton in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, 359-377.
- Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, 40, 81-102.
- Simon, T. P., and Vondruska, J. T. (1991). Larval identification of the ruffe, *Gymnocephalus cernus* (Linnaeus)(Percidae:Percini), in the St. Louis River Estuary, Lake Superior drainage basin, Minnesota. *Canadian Journal of Zoology*, 69, 436-442.
- Sommer, U., Gliwicz, M. Z., Lampert, W., and Duncan, A. (1986). The PEG-model of seasonal succession of planktonic events in fresh waters. *Archives of Hydrobiologie*, 106, 433-471.
- Stachowicz, J. J., Whitlatch, R. B., and Osman, R. W. (1999). Species diversity and invasion resistance in a marine ecosystem. *Science*, 286, 1577-1579.
- Stemberger, R. S. (1979). *A guide to rotifers of the Laurentian Great Lakes*. US EPA, EPA-600/4-79-021.
- Sterner, R. W. (1990). The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. *American Naturalist*, 136, 209-229.
- Sterner, R. W., Elser, J. J., and Hessen, D. O. (1992). Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. *Biogeochemistry*, 17, 49-67.
- Taylor, M. D., MacKenzie, L. M., Dodgshun, T. J., Hopkins, G. A., de Zwart, E. J., and Hunt, C. D. (2007). Trans-Pacific shipboard trials on planktonic communities as indicators of open ocean ballast water exchange. *Marine Ecology Progress Series*, 350, 41-54.
- Thomsen, M. A., D'Antonio, C. M., Suttle, K. B., and Sousa, W. P. (2006). Ecological resistance, seed density and their interactions determine patterns of invasion in a California coastal grassland. *Ecology Letters*, 9, 160-170.
- Tilman, D. (1997). Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology*, 78(1), 81-92.
- Trebitz, A. S., West, C. W., Hoffman, J. C., Kelly, J. R., Peterson, G. S., and Grigorovich, I. A. (2010). Status of non-indigenous benthic invertebrates in the Duluth-Superior Harbor

- and the role of sampling methods in their direction. *Journal of Great Lakes Research*, 36, 747-756.
- Vanni, M. J. (1986). Competition in zooplankton communities: Suppression of small species by *Daphnia pulex*. *Limnology and Oceanography*, 31(5), 1039-1056.
- Verling, E., Ruiz, G. M., Smith, D. L., Galil, B., Miller, W. A., and Murphy, K. R. (2005). Supply-side invasion ecology: characterizing propagule pressure in coastal ecosystems. *Proceedings Of The Royal Society B*, 272, 1249-1257.
- Vijverberg, J. (1980). Effect of temperature in laboratory studies on development and growth of Cladocera and Copepoda from Tjeukemeer, Netherlands. *Freshwater Biology*, 36, 747-756.
- Von Holle, B. (2005). biotic resistance to invader establishment of a southern Appalachian plant community is determined by environmental conditions. *Journal of Ecology*, 93, 16-26.
- Von Holle, B., and Simberloff, D. (2005). Ecological resistance to biological invasions overwhelmed by propagule pressure. *Ecology*, 86(12), 3212-3218.
- Walseng, B., Hessen, D. O., Halvorsen, G., and Schartau, A. K. (2006). Major contributions from littoral crustaceans to zooplankton species richness in lakes. *Limnology and Oceanography*, 51(6), 2600-2606.
- Watson, N. H., and Wilson, B. J. (1978). Crustacean zooplankton of Lake Superior. *Journal of Great Lakes Research*, 4, 481-496.
- Wickham, S. A., Gilbert, J. J., and Berninger, U. G. (1993). Effects of rotifers and ciliates on the growth and survival of *Daphnia*. *Journal of Plankton Research*, 15(3), 317-334.
- Williamson, M., and Fitter, A. (1996). The varying success on invaders. *Ecology*, 77(6), 1661-1666.
- Wonham, M. J., Carlton, J. T., Ruiz, G. M., and Smith, L. D. (2000). Fish and ships: relating dispersal frequency to success in biological invasions. *Marine Biology*, 136, 1111-1121.
- Wonham, M. J., Walton, W. C., Ruiz, G. M., Frese, A. M., and Galil, B. S. (2001). Going to the source: role of the invasion pathway in determining potential invaders. *Marine Ecology Press Series*, 215, 1-12.
- Wonham, M., Byers, J., Grosholz, E., and Leung, B. (2013). Modeling the relationship between propagule pressure and invasion risk to inform policy and management. *Ecological Applications*, 13, 2477-2497.
- Wright, D. A., Setzler-Hamilton, E. M., Magee, J. A., Kennedy, V. S., and McIninch, S. P. (1996). Effect of salinity and temperature on survival and development of young zebra

(*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels. *Estuaries*, 19(3), 619-626.

Zenni, R. D., and Simberloff, D. (2013). Number of source populations as a potential driver of pine invasions in Brazil. *Biological Invasions*, 15(7), 1623-1639.

Zhou, M., Zhu, Y., Putnam, S., and Peterson, J. (2001). Mesoscale variability of physical and biological fields in southeastern Lake Superior. *Limnology and Oceanography*, 46, 679-688.