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## *Executive Summary*

This multi-year project addresses the issue of ballast water treatment by examining the efficacy of the standards that will be applied concerning permissible levels of biological pollution. The over-arching objective of the project is to quantify the relationships between propagule pressure and the colonization success of zooplankton in the Duluth-Superior Harbor and St. Louis Estuary through dose-gradient experiments that bracket International Maritime Organization standards.

The main objective of the first and second years of work is to characterize the density and diversity of crustacean zooplankton in the Duluth-Superior Harbor and St. Louis Estuary, a necessary first step in developing the experimental context. Twelve locations were selected for sampling based on a simple random design but constrained in such a way to ensure wide geographic coverage spanning from the Oliver Bridge to the Duluth Harbor entry. On each of 10 May-October dates in 2007 and 10 May-October dates in 2008, 12 locations were sampled during the day for crustacean zooplankton and a suite of physio-chemical variables.

Our preliminary analysis of the 2007 data indicates that patterns of zooplankton density at the study sites peak in midsummer, coincident with peak summer water temperatures, reduced discharge volumes on the St. Louis River, and peaks in ship ballast water discharge volumes in the harbor. The likelihood of cause-effect relationships between these factors requires further analysis, which will include the incorporation of 2008 data, and a detailed analysis of the species diversity of crustacean zooplankton among study sites and between years. The results are providing valuable information for the development of experiments in the coming year that will evaluate the relationships between propagule pressure and colonization success by invasive species.

## ***Introduction***

Burgeoning human transportation and trade networks are disrupting the natural range boundaries of flora and fauna on a global scale. Ballast water ferried by ships and used to correct imbalance in cargo is believed to be the leading dispersal agent of coastal non-native aquatic species in North America (Ruiz et al. 2000). Foreign ships arriving in U.S. ports alone discharge in excess of 70 million metric tons of liquid ballast annually (Minton et al. 2005), representing a massive potential ongoing courier of non-native aquatic species into the country.

In an effort to prevent additional species introductions via this vector, the U.S. Congress passed and reauthorized legislation in the 1990s that requires vessels to manage their ballast water in one of two ways. Ships are required either to carryout Ballast Water Exchange (BWE) by flushing ballast tanks in the open ocean or to perform Ballast Water Treatment (BWT) by proactive decontamination. BWE 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. Researchers are currently developing and testing ballast water treatment technologies that will kill organisms upon entrance or exit from ballast holding tanks. Ballast water treatment promises effective interception of dispersing non-native organisms but demands thorough evaluation of the physiochemical thresholds of a wide diversity of organisms before it can be enacted.

It is widely recognized that no BWT technology can be expected to perform with 100% effectiveness all of the time. Hence, accepted standards will still allow a certain level of biological pollution (viable non-native 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 IMO.

Both theoretical and conceptual models predict that higher numbers of viable organisms in post-treatment discharge (propagule pressure) increase the likelihood of colonization success (MacIsaac et al 2002, Minton et al. 2005, Colautti and MacIssac 2004, Colautti et al. 2006). Nonetheless, few experimental data are available from which to quantify levels of invasion risk associated with specific levels of propagule pressure (MacIsaac et al 2002). This presents a serious challenge in identifying target permissible pollution thresholds for ballast water treatment technologies that will prove to be environmentally protective (Minton et al. 2005).

The Great Ships Initiative recently secured funding to establish a Ballast Water Testing Facility in the Duluth-Superior Harbor. Scientists at the facility will carryout scientific testing of BWT technologies at the bench, pilot and shipboard scales. It is anticipated that results will expedite identification of BWT technologies that meet IMO standards of biological pollution.

In an effort to enhance the work of the GSI, and more broadly inform ballast water treatment issues on a global scale, we need to address a gap in our basic scientific understanding which regards the functional relationship between numbers of viable organisms that escape destruction (propagule pressure) and their eventual colonization success (Colautti et al. 2006) in recipient ecosystems. Our project begins to fill this gap and should provide valuable experimental-based information that can guide the IMO regarding post-treatment standards for BWT technologies.

This project has three interrelated objectives:

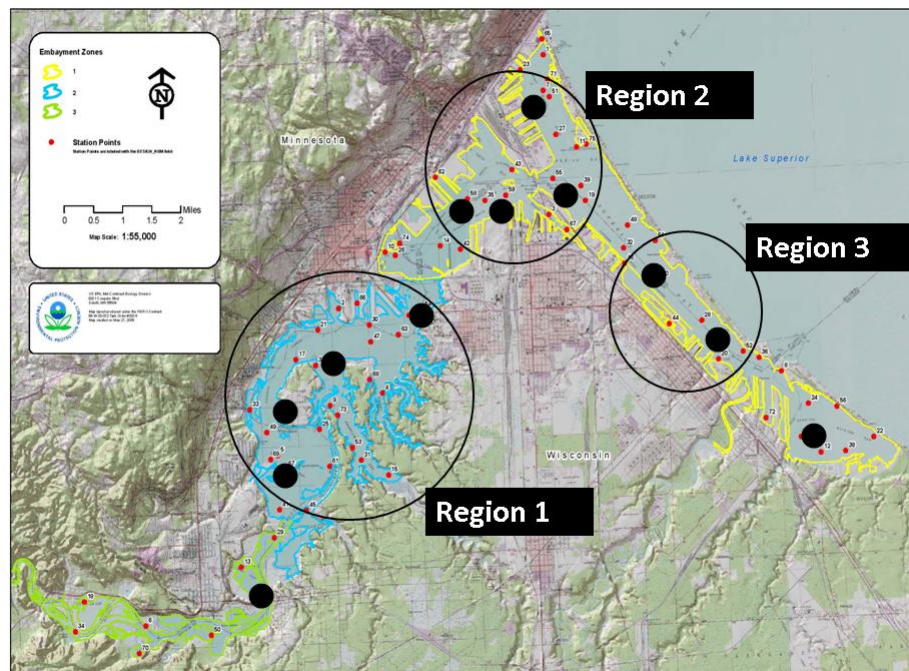
1. Assess the seasonal density and diversity of zooplankton at the species level in the Duluth-Superior Harbor and its connected waters including the St. Louis Estuary and Lake Superior adjacent to the Duluth-Superior Harbor.

2. Test the hypothesis that seasonal density and diversity of zooplankton in the Duluth-Superior Harbor (a measure of colonization success) as determined under Objective 1, is temporally and spatially correlated with seasonal shipping traffic and ballast discharge (volume, port of origin) statistics (a measure of propagule pressure).
3. Quantify relationships between propagule pressure and colonization success of zooplankton in the Duluth-Superior Harbor through dose-gradient experiments that bracket International Maritime Organization (IMO) standards.

This interim report addresses data collected under Objectives 1 and 2 which were the focus of the 2008 work plan.

### ***Report Body***

Objective 1 required the selection and establishment of sampling sites in the Duluth-Superior Harbor and St. Louis Estuary for periodic monitoring. Site selection was addressed in the 2007 interim report and resulted in the sampling grid given in Figure 1.

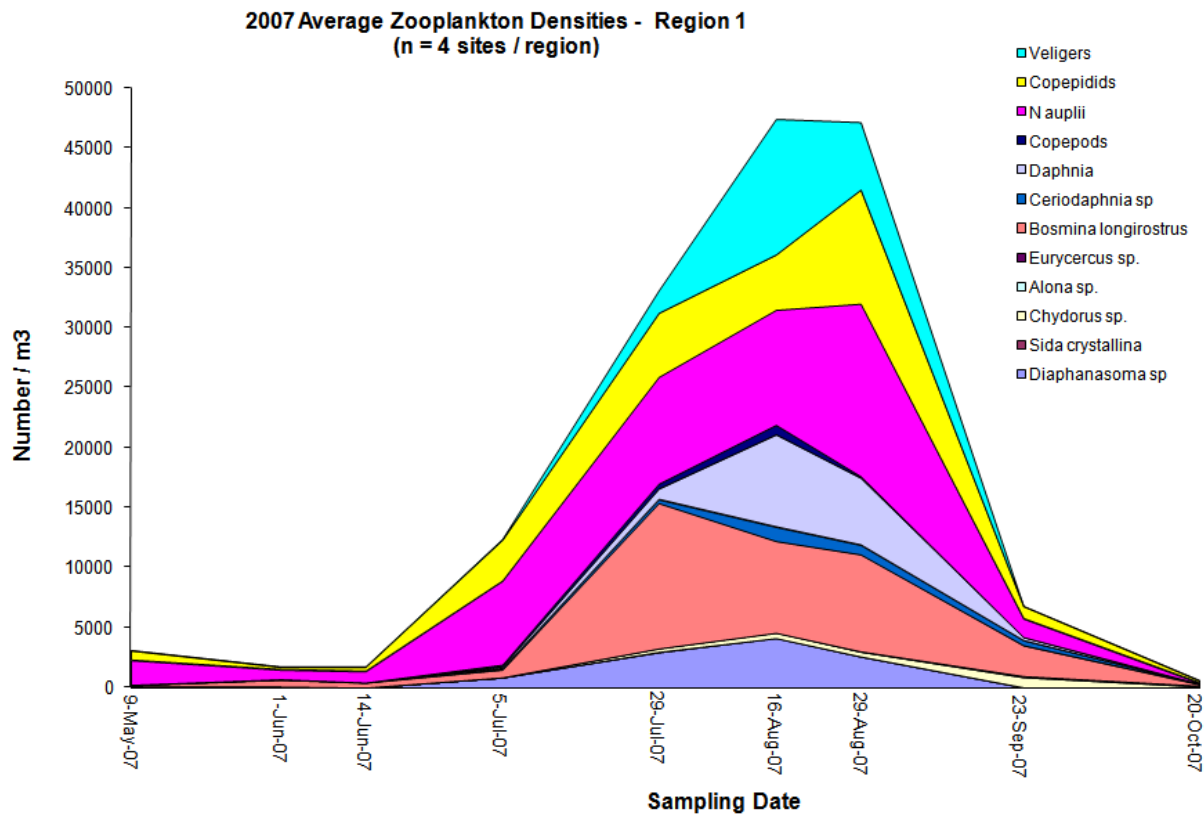


**Figure 1. Schematic of the 12 sampling sites (black circles) in the Duluth-Superior Harbor and St. Louis Estuary. Circles define sets of sites (regions) used in the analysis (see text).**

Sampling was successfully completed on 10 dates spanning May-October in each of 2007 and 2008. General sampling methodologies for 2007 were documented in the 2007 interim report and did not change during the 2008 field season. In the 2007 interim report we described the general composition of the zooplankton assemblage in the Duluth-Superior Harbor and St. Louis Estuary and noted some spatial and temporal trends in the zooplankton composition, zooplankton density, and physio-chemical values among sampling sites. In this 2008 interim report we further develop our analysis of the 2007 data. The results presented herein were summarized in an oral presentation at the Minnesota Invasive Species Conference (Duluth, MN) on October 26-29, 2008.

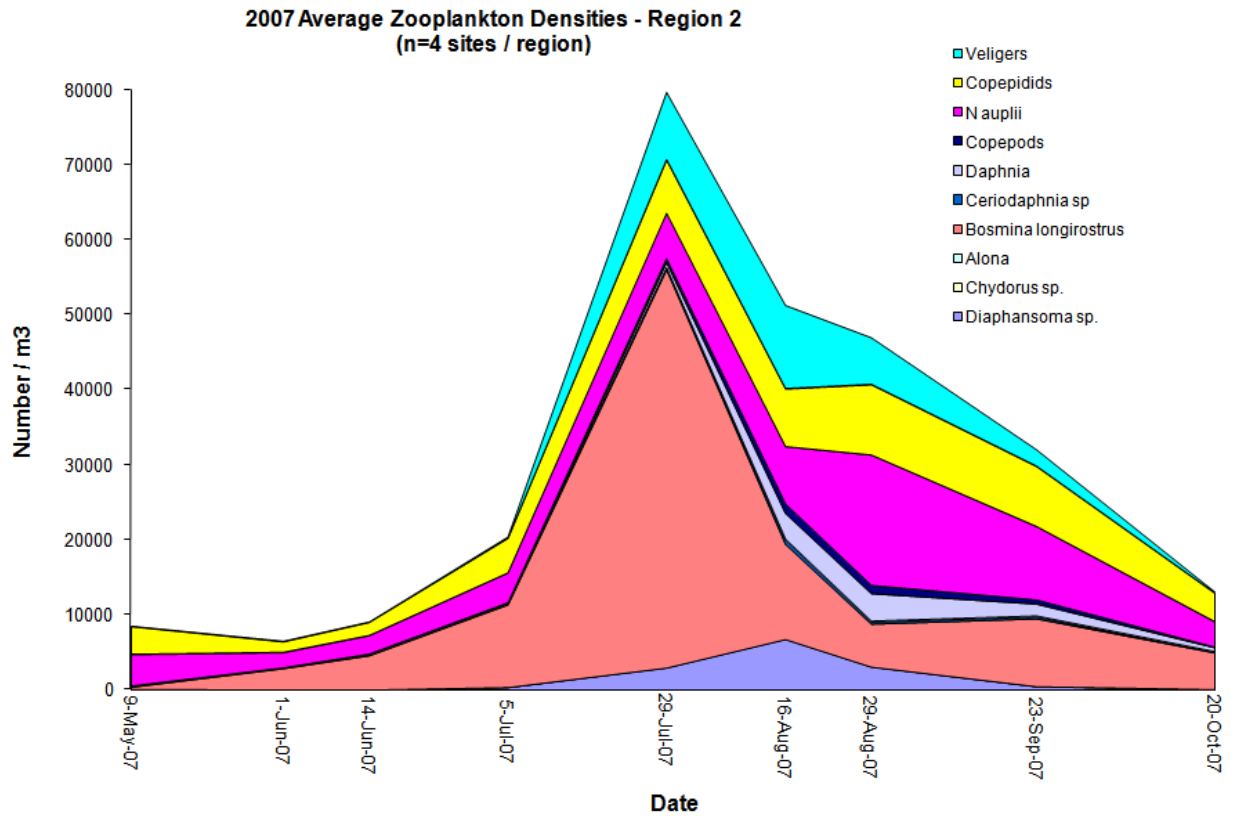
For analysis, the 2007 sampling sites were aggregated into 3 general regions based on their proximity to one another and their general position either upstream or downstream of large ship deballasting activity. (Figure 1). Region 1 sites are upstream of deballasting activity, Region 2 sites are within the heart of deballasting activity, and Region 3 sites are downstream of Region 2 sites. Two sampling sites were excluded from analysis because of their exceptional physio-chemical conditions on particular dates.

Zooplankton total mean densities (number  $m^{-3}$ ) expressed strong variation on a seasonal cycle in each of the three regions. At Region 1, mean densities peaked in August at abundances 10 times greater than those obtained in May (Figure 2). Mean densities collapsed during September, and by October were comparable to May densities.



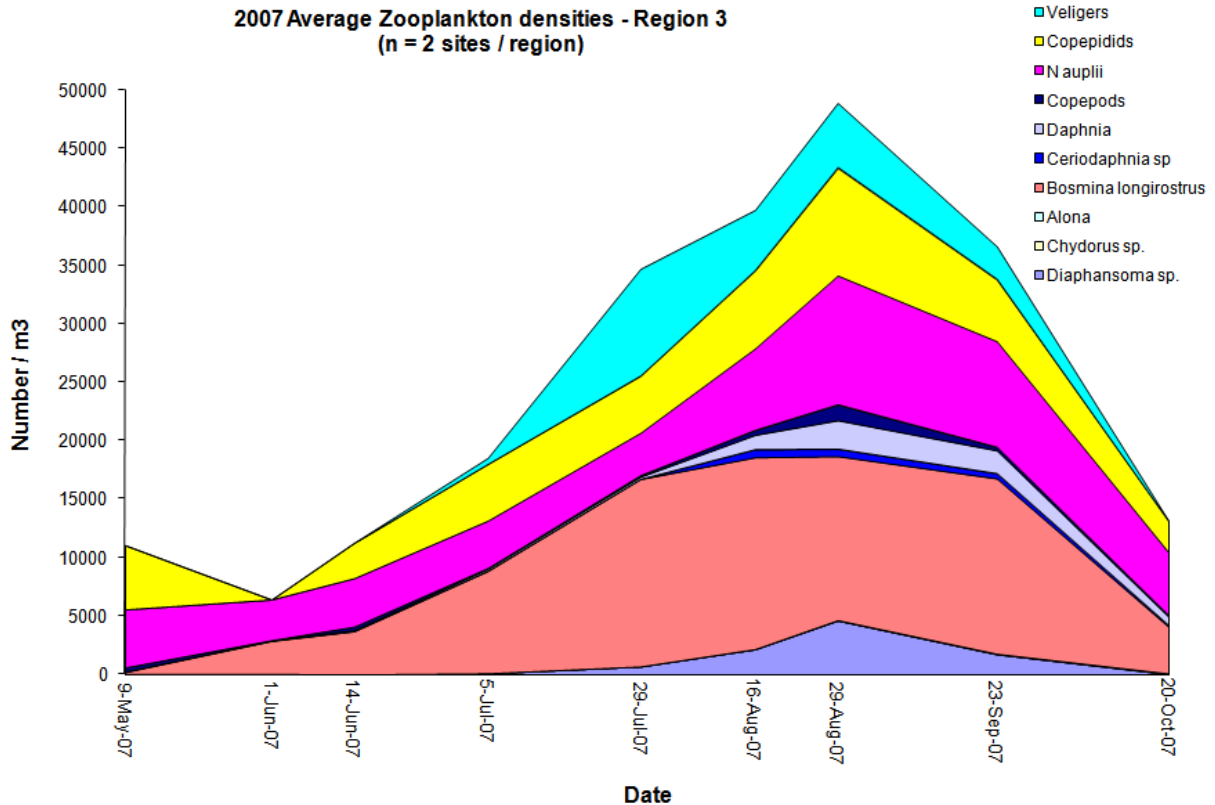
**Figure 2. Seasonal mean densities of the most abundant taxa of crustacean zooplankton occurring at Region 1 during 2007. Note x-axis indicates actual sampling dates.**

At Region 2, mean densities achieved in July were nearly 10 times greater than those obtained in May (Figure 3). Mean densities collapsed during September, and by October were comparable to May densities.



**Figure 3. Seasonal mean densities of the most abundant taxa of crustacean zooplankton occurring at Region 2 during 2007. Note x-axis indicates actual sampling dates.**

At Region 3, mean densities peaked in August at abundances nearly 10 times greater than those obtained in May (Figure 4). Mean densities collapsed during September, and by October were comparable to May densities.

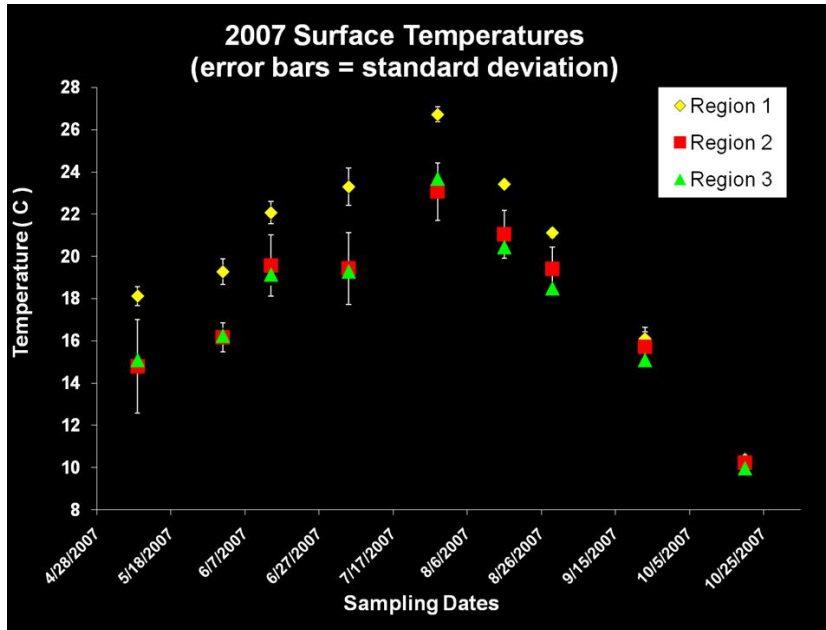


**Figure 4. Seasonal mean densities of the most abundant taxa of crustacean zooplankton occurring at Region 3 during 2007. Note x-axis indicates actual sampling dates.**

Among regions, the data showed strong convergence in the seasonal timing of density peaks and valleys. May, June, and October were characteristically periods when crustacean zooplankton populations were at their lowest seasonal densities, whereas July and August were consistently periods of peak, or near peak, seasonal densities.

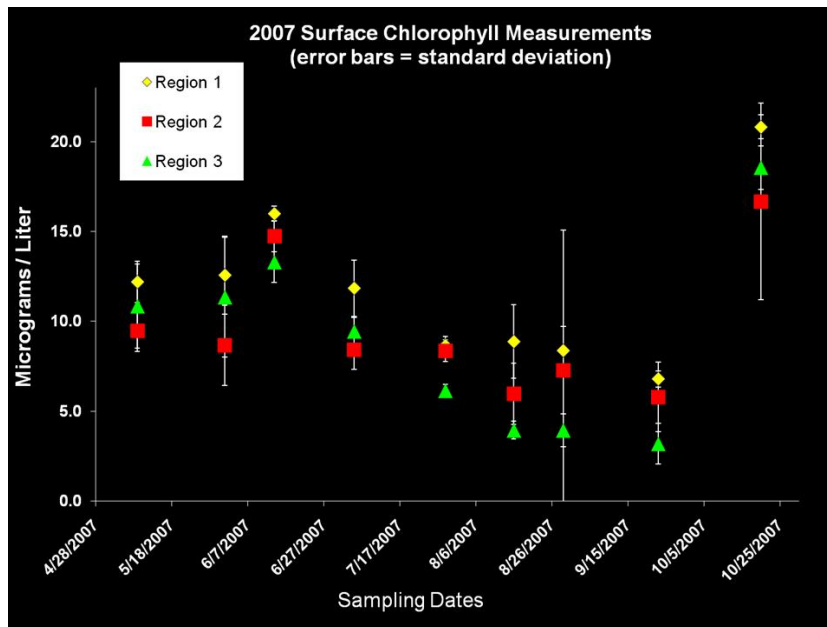
These results offer a strong basis for planning the timing of propagule pressure experiments to be carried out under Objective 3 in the coming year. In those experiments, the background zooplankton communities against which invasive propagules will be challenged will be developed from natural assemblages in the harbor. The information in Figures 2-4 will permit us to plan effectively for the timing of experimental set up to control the densities (low or high) of the background assemblages.

The patterns in zooplankton densities among regions in 2007 (Figures 2-4) coincided positively with water temperatures (Figure 5) which tended to peak in midsummer. This is not surprising as temperature is generally an excellent predictor of zooplankton abundance. Zooplankton are ectothermic, hence their enzyme activity and growth potential is in direct response to surrounding, environmental temperatures.



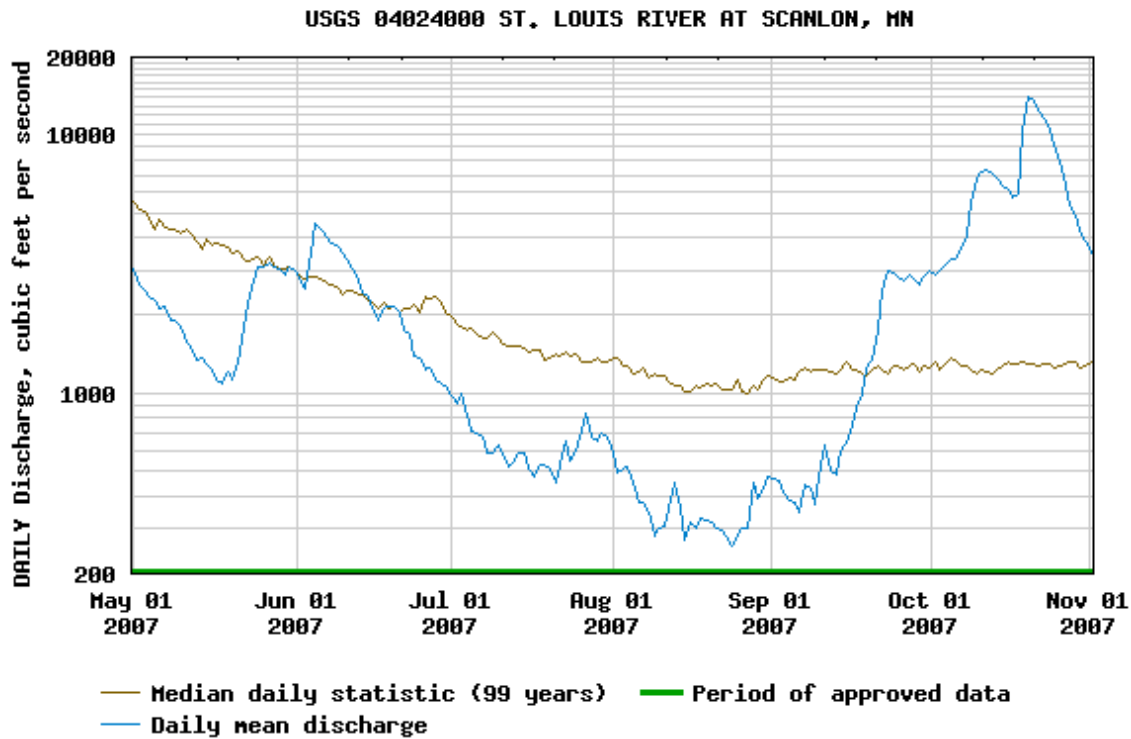
**Figure 5. Seasonal mean surface water temperature (°C) at sampling regions in the Duluth-Superior Harbor and St. Louis Estuary during 2007.**

Zooplankton density patterns also coincided strongly in a negative fashion with phytoplankton density (measured as Chlorophyll *a*), which is the primary food source of the dominant crustacean zooplankton taxa in the ecosystem (Figure 6).



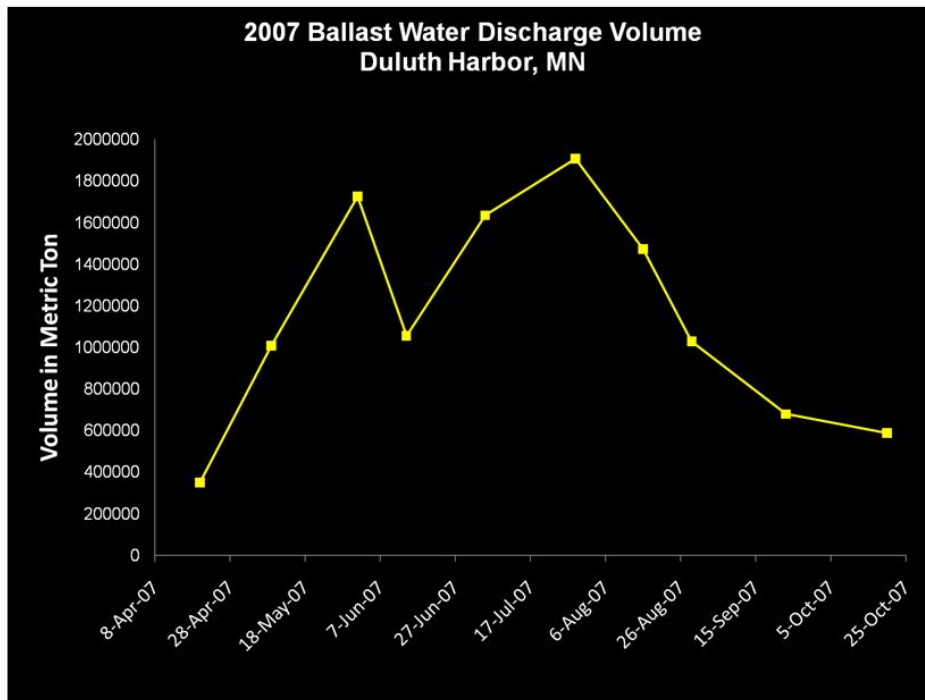
**Figure 6. Seasonal mean Chlorophyll *a* concentration (µg L<sup>-1</sup>) at sampling regions in the Duluth-Superior Harbor and St. Louis Estuary during 2007.**

We assessed whether patterns in zooplankton peak densities in 2007 coincided with patterns in St. Louis River discharge. As an estimate of river discharge we examined the phenology of flow rates measured in Scanlon, Minnesota, by the United States Geological Survey ([www. USGS.gov](http://www.USGS.gov)). Data for 2007 (Figure 7) revealed an inverse correlation between flow rate and zooplankton density (Figures 2-4). One mechanism supporting such a relationship posits that lower discharge during midsummer increases the residence time of zooplankton in the system and enhances population buildup by reducing washout into Lake Superior.



**Figure 7. Daily discharge on the St. Louis River for May-November, 2007, measured by the United States Geological Survey at Scanlon, Minnesota.**

We assessed whether patterns in zooplankton peak densities in 2007 coincided with ballast water discharge volumes (Figure 8). Patterns were somewhat correlated in that one period of peak ballast water discharge occurs midsummer (July and August), contemporaneous with periods of peak crustacean zooplankton densities in the harbor.



**Figure 8. Estimated ballast water discharge volumes in the Duluth-Superior Harbor, summed bi-weekly, from 8 April to 25 October, 2007. Values based on information available through the National Ballast Information Clearinghouse Online Database at <http://invasions.si.edu/nbic/search.html>).**

In the coming year, data from 2008 on the seasonal densities of crustacean zooplankton and physiochemical variables, will be worked up. The combined data set (2007 and 2008) will be analyzed for patterns in zooplankton density, diversity by region, and similarity between regions to test Objective 2.

### **References**

- Colautti, R. I. and H. MacIsaac. 2004. A neutral terminology to define 'invasive' species. *Diversity and Distributions* 10:135-141.
- Colautti, R. I., I. A. Grigorovich and H. J. MacIsaac. 2006. Propagule pressure: a null model for biological invasions. *Biological Invasions* 8: 1023-1037.
- Duggan, I. C., and others. 2005. Invertebrates associated with residual ballast water and sediments of cargo-carrying ships entering the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2463-2474.

- Grigorovich, I. A. and others. 2003. Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences* 6: 740-756.
- MacIsaac, H. J., T. C. Robbins, and M. A. Lewis. 2002. Modeling ships' ballast water as invasion threats to the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1245-1256.
- Minton, M. S., E. Verling, A. W. Miller and G. M. Ruiz. 2005. Reducing propagule supply and coastal invasions via ships: effects of emerging strategies. *Frontiers in Ecology and the Environment* 3: 304-308.
- Ruiz, G. M. and others. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics* 31: 481-531.