Great Lakes Maritime Research Institute

A University of Wisconsin - Superior and University of Minnesota Duluth Consortium

Developing a Risk Assessment Tool to Predict the Risk of Accelerated Corrosion to Port Infrastructure

Final Report

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Executive Summary

Corrosion of steel infrastructure in the Duluth-Superior Harbor (DSH) and other ports in the Laurentian Great Lakes is a major economic concern. It is estimated that corrosion in the United States costs $275.5 billion per year or 3.14% of our GDP, and in the DSH alone $200-250 million may be needed to replace 20 km of steel structures already impacted by corrosion. The ultimate goal of this study was to develop a risk assessment tool that can be used by businesses and governments to predict whether other ports may experience corrosion similar to that seen in the DSH. Multiple water chemistry parameters were analyzed in 2010 and 2011, used to calculate the Larson-Skold Index of water corrosivity, and their relationship to long-term corrosion rates was evaluated. The lowest long-term corrosion rates of steel structures were observed at the head and mouth of the DSH, while higher corrosion rates were observed in the outer and inner harbor areas. Long-term estimates of steel corrosion were inversely related to dissolved oxygen concentration, positively related to water chloride, alkalinity and conductivity, but not the sulfate concentration or the Larson-Skold Index. The Larson-Skold Index decreased at two of three sites in the DSH from 1972 to 1997 and this index predicted a low risk of corrosion for ten sites visited during 2010 and 2011, indicating that water quality alone may not explain the severe corrosion seen in this harbor.

The abundances of iron-oxidizing bacteria (*Gallionella sp*p.) and sulfate reducing bacteria (SRB) on steel surfaces were estimated at ten sites in the DSH and three other harbors in the western arm of Lake Superior by quantitatively amplifying the 16S rRNA and *dsr*A genes, respectively. Corrosion tubercles in the DSH were enriched with *Gallionella* ssp. compared to biofilm on adjacent steel surfaces and the surrounding water. The abundance of *Gallionella* ssp. on corroded steel surfaces ranged from 10^8 to 10^{10} 16S rDNA gene copies/dry gram of corrosion tubercle. SRB were at least 2 orders of magnitude less abundant within corrosion tubercles than *Gallionella* spp. with abundances ranging from 10^5 to 10^8 *dsr*A gene copies/dry gram of tubercle. In 2010, *Gallionella* spp. abundance was positively related to long-term corrosion rates but not in 2011. SRB abundance was not related to corrosion rates.

While a logistic regression model was not useful for predicting corrosion risk, a multiple linear regression model did predict long-term corrosion rates from alkalinity and SRB abundance. When this model was used to predict corrosion rates at two harbors on the north shore of Lake Superior, it slightly underestimated the measured long-term corrosion rate. Overall, it appears that water chemistry alone is not likely the cause of accelerated corrosion in the DSH, but rather a combination of microbiological and chemical factors appear to influence the corrosion rate of sheet steel structures in this harbor and possibly other areas in the western arm of Lake Superior.

Introduction

Maritime transportation has long been an important historic and economic component of the Laurentian Great Lakes states. Economically, the shipment of products such as iron ore, taconite, coal, grain, and stone aggregates provided 227,000 jobs both directly and indirectly to the United States and total business revenue was $33.6 billion (Martin Associates 2011). Thus, any disruption to the transport of these materials will negatively impact the economy of the Great Lakes states. Accelerated corrosion in the Duluth-Superior Harbor (DSH) has become a major concern and the steel infrastructure in this port is corroding faster than expected (Ray *et al.* 2009). A report by Bushman and Associates in 2006 suggested that water quality and potentially microbiologically influenced corrosion (MIC) may be causes for the corrosive loss of this steel, which they estimated to be 2 to 12 times greater than normal in freshwater.

Water quality is an important factor to consider when examining the corrosion of steel infrastructure in both marine and freshwater environments. Ions such as chloride, sulfate, and oxygen have been implicated to have an aggressive corrosive action on steel surfaces (Huang and Zhang 2005, Montemor *et al.*2003, Simard *et al.* 2001, Larson and Skold 1958). Chloride has been shown to lead to voids in corrosion films and scales, which initially provide some protection against generalized corrosion (Sarin *et al.* 2004), and can lead to pitting of the steel (Montemor *et al* 2003, Simard *et al.* 2001). Sulfate can reduce the effectiveness of corrosion scales made of iron oxy-hydroxides that are known to limit the corrosion reaction by replacing this film with one that is less protective (Al-Tayyib *et al.* 1988). We used the Larson-Skold Index of corrosivity (Larson and Skold 1958) to predict how the corrosive effects of chloride (Cl^-) and sulfate (SO_4^{2-}), and the protective influence of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) may influence corrosion of steel at sites in the DSH.

Corroded steel structures in the DSH have a rusty appearance and corrosion tubercles characteristic of MIC. The raised, hard tubercles are mostly ferric hydroxide but also contain a variety of organic and inorganic compounds that are characteristic of the presence of iron-oxidizing bacteria (Hamilton 1985). The tubercles are often covered in carbohydrate slimes or biofilms produced by iron-oxidizing bacteria (FeOB), sulfate-reducing bacteria (SRB), and a consortia of other microorganisms (Hamilton 1985). Iron-oxidizing species in the genera *Gallionella* and *Leptothrix* are common bacteria that are responsible for the deposition of such ferric oxides (Emerson and Moyer 2002) and can grow in neutral pH and oxic conditions (Emerson *et al.* 2010) typical of the DSH. DNA sequences from bacteria most closely related to *Gallionella* species and *Sideroxydans lithotrophicus* were cloned and identified at sites such as Hallett Dock 5 in the DSH where steel corrosion seems the most severe (Hicks 2009). Biofilms create microenvironments by creating an oxygen gradient and therefore play a critical role in determining the types of microbes present (Hamilton 1985; Lee and Characklis 1993). While oxygen gradients can be established within the microenvironment, local concentrations of chemicals can also change from the outside environment (Lee and Newman 2003).

Not only does sulfate affect the scales produced by the general corrosion reaction, it also supports the metabolism of sulfate-reducing bacteria (Liu *et al.* 2009, Gibson 1990, Hamilton 1985). In the anaerobic zone under corrosion tubercles, sulfate-reducing bacteria (SRB) often produce black precipitates of iron-sulfide (FeS) (Hamilton 1985). The metabolic activities of SRB and other anaerobic bacteria can led to the development of a cathodic region under biofilms

that help establish an electrochemical cell that ultimately leads to pitting of the steel surface (Potekhina *et al.* 1999). Microbial metabolic processes are known to influence corrosion by changing the chemistry around the metal surface, and thus the activities of microorganisms like bacteria may be just as influential as water quality on accelerating metal corrosion. In this study, we estimated the abundances of both *Gallionella* spp. and SRB within corrosion tubercles and adjacent biofilms using quantitative PCR (qPCR).

This research project had several objectives and goals. The first objective was to examine historical water quality data and use it to evaluate the corrosive nature of water in the DSH. Second, we made new measurements of ions in water and estimated the abundances of *Gallionella* spp. and SRB on steel surfaces and water in the DSH and three harbors in the western arm of Lake Superior. These water quality and microbiological measurements were then compared to long-term corrosion rates calculated for this sites by measuring corrosion pit depths and knowing the age the steel structures. The ultimate goal of this project is to develop a risk assessment tool that can be used to predict the risk of corrosion similar to that observed in the DSH in other harbors within Lake Superior. The measurements of water quality parameters, bacterial abundances, and corrosion pit depths were used as the basis to evaluate the influence of water quality on this corrosion process but also construct a risk assessment tool.

Methods

Site Descriptions –
Ten sites in the Duluth-Superior Harbor and Lake Superior (Duluth, MN and Superior, WI) ranging from low to severely corroded were selected for this study in 2010 and 2011 (Figs. 1 and 2). The Superior Entry located at N 46^{o} 71.014' W 092^{o} 00.661', Duluth Entry located at N 46^{o} 77.987' W 092^{o} 08.829', and Oliver Bridge located at N 46^{o} 65.673' W 092^{o} 20.225' represented sites known to have less severe corrosion. Moderately corroded sites included Cargill (now known as White Box Dock) at N 46^{o} 76.948' W 092^{o} 10.529', the Army Corps of Engineers dock at N 46^{o} 77.519' W 092^{o} 09.234', the Duluth Seaway Port Authority Berth 4 at N 46^{o} 75.724' W 092^{o} 09.571', Graymont (formerly known as Cutler Magner) located at N 46^{o} 73.332' W 092^{o} 07.468', and Cenex Harvest States dock at N 46^{o} 74.288' W 092^{o} 10.116'. Severe corrosion has been observed at two sites that were sampled; Midwest Energy Resources Company dock at N 46^{o} 74.232' W 092^{o} 11.546' and Hallett Dock 5 located at N 46^{o} 74.563' W 092^{o} 13.223'. Docks and other structures at all of these sites are constructed of the same type of A328 sheet steel (Chad Scott, pers. comm.).

In 2011, six of the ten original sample sites from 2010 were resampled in the Duluth-Superior Harbor (Fig. 1). A new area of the Duluth Entry on the harbor side of the entry, which is moderately corroded, was also sampled in 2011 to compare with samples taken from the Lake Superior side of this entry in 2010. Additional sites in four harbors along the north shore of Lake Superior were also sampled in 2011 to gain a better understanding of the geographic extent of corrosion of steel infrastructure in this lake (Fig 2). The four sample locations included the Knife River Marina (N 46^{o} 56.773' W 091^{o} 46.923'), Two Harbors (N 47^{o} 01.032' W 091^{o} 40.391'), Silver Bay (N 47^{o} 17.030' W 091^{o} 15.668'), and Taconite Harbor (47^{o} 52.24′ N, 90^{o} 92.88′ W).

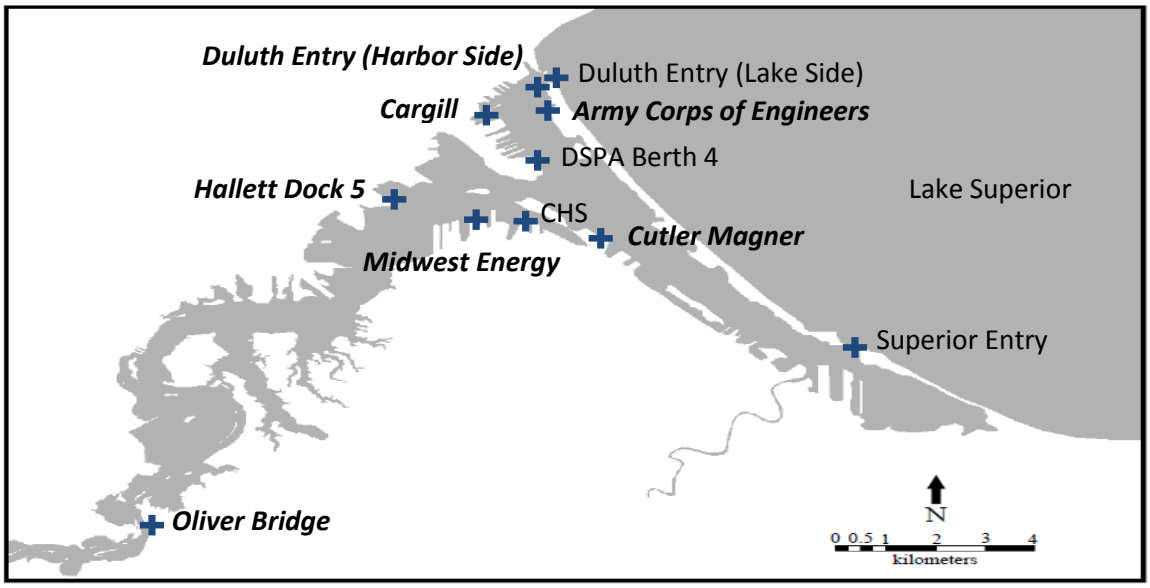

Fig. 1. Map of locations that were sampled in the Duluth-Superior Harbor during August 2010 and July 2011. Sites shown in bold italicized text were sampled both years, while other sites were sampled in either 2010 or 2011.

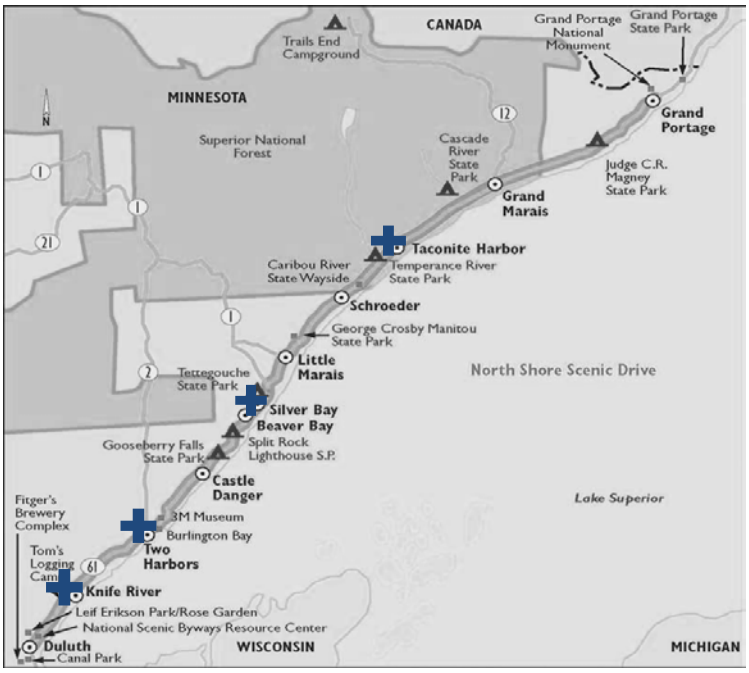

Fig. 2. Locations at harbors sampled along the north shore of Lake Superior in July 2011. Map courtesy of Discovery Communications, 2011.

Sampling –

Bulk water, corrosion tubercles, and biofilm adjacent to tubercles were collected at the sample sites in the DSH and Lake Superior on August 9-10, 2010 and July 26-27, 2011. All samples

were collected by hardhat divers from AMI Consulting Engineers, P.A. (Superior, WI) from sheet steel and pilings at these sites. Bulk water at each site was collected in triplicate using 1 L Nalgene bottles that had been prewashed, rinsed with 10% HCl, and rinsed with sterile Milli-Q water. Water was collected approximately 1 m from the steel surfaces, 1 to 2 m below the water line, and prior to tubercle and biofilm collection at all sites. The collected water was stored in a cooler on ice until held at 4^{o}C in the laboratory before being sent to Trace Analytical Laboratories Inc. for water quality analyses. In addition to the 1 L bottles, 5-gallon carboys of water were collected in 2011 from each site. These water samples were filtered through Duropore membrane filters (142 mm diameter, 0.22 µm pores size) to collect microbial cells for subsequent extraction of bacterial DNA for qPCR analyses.

Corrosion tubercles and biofilms adjacent to the tubercles were collected by a professional diver on steel surfaces 1 to 2 m below the water line where corrosion was observed to be the most severe. Sterile 60-ml syringes modified by attaching brushes to the syringe plungers were used to scrape off tubercles or biofilm adjacent to corrosion tubercles (Ksoll *et al.* 2007). Sample material was then drawn into the body of the syringe, capped with a silicone stopper and brought to the surface, where the samples were then transferred to Whirlpak® bags, sealed, and placed on ice. All samples were frozen (-20^{o}C) at the laboratory prior to extracting DNA. Corrosion pit depths and diameters were measured at each site by the diver using a manual pit depth gauge. Thirty total pit depths and diameters were taken at each site at the water line (n=10), three feet below the water line (n=10), and at six feet below the water line (n=10). Pit diameters were measured on the same corrosion pits that pit depth was measured using a ruler on the manual pit depth gauge. The long-term corrosion rate of the steel structure sampled at these sites was estimated by dividing the average corrosion pit depth by the in-service age of the steel structure.

Water Quality Measurements –
Water chemistry measurements were performed by Trace Analytical Laboratories, Inc. (Muskegon, MI). Alkalinity was measured using titration method SM 2320 and chloride using ion chromatography (EPA 300.0; Pfaff 1993). Sulfate and dissolved copper measurements were made using ion chromatography (EPA 300.0; Pfaff 1993) and inductively coupled plasma-atomic emission spectrophotometry (EPA 200.7; Martin et al. 1994), respectively. Dissolved oxygen, conductivity, and temperature measurements were made using a YSI 85 probe at all sample sites. A YSI 63 probe was used to make pH measurements and verify conductivity and temperature measurements. Dissolved organic carbon (DOC) was analyzed using the procedure of Burdige and Homstead (1994) using a Shimadzu TOC-Vcsh/TN Analyzer. Water samples (20 ml) were acidified to pH 2 with 6M HCL in glass vials that were pre-combusted to remove inorganic carbon.

Prokaryotic Cell Counts –
Total prokaryotic cell abundance was counted using a DAPI staining method developed by Porter and Feig (1980). Tubercle and biofilm samples (0.025 to 0.035 g each) were preserved in 1.9 ml of nuclease free water and 100 µl 37% formaldehyde (2% formaldehyde final concentration) and then stored at 4^{o}C for less than two weeks. Subsamples were stained with DAPI (10 µM final conc.) and prokaryotic cells were counted using a Nikon Eclipse 80i epifluorescence microscope. Three aliquots of the preserved samples were stained for 5 minutes and filtered onto black polycarbonate filters (Poretics, 25 mm dia., 0.22 µm pore). Using UV illumination, fluorescent prokaryotic cells were counted under a total magnification of 1,000x.

Ten fields of view were then counted for each of the three filters per site and were converted to prokaryotic cells per dry gram.

DNA Extraction –

DNA from tubercles, biofilm, and filtered water was extracted using the PowerSoil® DNA isolation kit from MoBio Laboratories, Inc (Carlsbad, CA). Prior to the corrosion tubercle and adjacent biofilm samples being frozen, 0.25 to 0.35 g of material were utilized for each total DNA extraction. Two extractions of each sample were performed and the two extracts were concentrated through one silica spin filter. Afterwards, the sample was then eluted in 50 µl of TE buffer to obtain a final DNA concentration greater than 2 ng/µl that could be used for quantitative PCR. One-eighth of the Duropore (142 mm dia.; 0.2 µm pore) membrane filter for each water sample were also extracted using the same PowerSoil® DNA isolation kit. Total DNA was quantified (ng/µl) using a Thermo Scientific (Wilmington, DE) NanoDrop 3300 full spectrum fluorospectrometer. DNA samples were fluorescently stained using Quant-iT™ PicoGreen® dsDNA reagent from Invitrogen™ Molecular Probes, Inc (Eugene, OR). Extraction efficiencies were also performed in triplicate using autoclaved corrosion tubercle material amended with DNA from *E. coli* cells. Approximately 500 ng of *E. coli* DNA was extracted through the PowerSoil® DNA isolation kit and re-measured on the NanoDrop fluorospectrometer.

Quantitative PCR –
Two quantitative PCR assays were used to estimate the abundance of *Gallionella* spp. and sulfate-reducing bacteria. All PCR primers and probes for these assays (Table 1) were synthesized by Integrated DNA Technologies (Coralville, IA).

Table 1. PCR primers and probes used for qPCR analyses of FeOB and SRB abundances.

Primer/probe	Sequence (5'→ 3')	Specificity	[Final Primer]	Reference
Gal1f	CGA AAG TTA CGC TAA TAC CGC ATA	*Gallionella* spp.	200 nM	Li *et al.*, 2010
Gal1r	CTC AGA CCA GCT ACG GAT CGT	*Gallionella* spp.	200 nM	Li *et al.*, 2010
Gal1p	CCT CTC GCT TTC GGA GTG GCC G	*Gallionella* spp.	120 nM	Li *et al.*, 2010
DSR1f+	ACS CAC TGG AAG CAC GGC GG	SRB	400 nM	Kondo *et al.*, 2004
DSR-R	GTG GMR CCG TGC AKR TTG G	SRB	400 nM	Kondo *et al.*, 2004

Iron-oxidizing bacteria (*Gallionella* spp.) qPCR. The primers, probe, and qPCR method developed by Li et al. (2010) were used to estimate the abundance of *Gallionella* spp. in DNA from tubercles, biofilms, and water samples. QPCR was performed in a reaction volume of 25 µl consisting of 12.5 µl of Brilliant II Master Mix (Agilent Technologies), 0.5 µl of 10 µM solutions of the appropriate forward and reverse primers (Table 1), 1.0 µl of a 3 µM solution of the Gal1p probe, 2.0 µl of 10 mg/ml bovine serum albumin, 0.5 µl of ROX reference dye, 3 µl of nuclease-free water and 5 µl of DNA template (10 ng total). The 5' end of the Gal1p probe was fluoresecently labeled 6-carboxyfluorescein (FAM) and the 3' end with the quencher 6-carboxytetramethylrhodamine (TAMARA). Amplification was performed on a Rotor-Gene 3000 (Corbett Life Sciences) qPCR thermal cycler following the protocol: 10 min at 95^{o}C followed by 40 cycles of 95^{o}C for 30 sec and 58^{o}C for 40 sec. A 16S RNA gene clone isolated from the Duluth-Superior Harbor (HD5-Clone 113) containing PCR fragment with a maximum sequence

identity of 97% to a *Gallionella* species was cultured and the 16S rRNA gene fragment was reamplified as an authentic standard to construct standard curves. A standard curve ranging from 200 pg to 2 ag of HD5-Clone113 DNA that was amplified with the Gal1F/Gal1R primer set. All qPCR was performed in triplicate for each sample.

Sulfate-reducing bacteria qPCR. A modified procedure from Schippers and Neretin (2006) was used to estimate the abundance of sulfate-reducing bacteria by quantifying copies of the *dsr*A gene. Quantitative PCR was performed in a 25 µl reaction volume consisting of 12.5 µl Brilliant II SYBR Green Master Mix (Agilent Technologies), 1.0 µl of 10 µM forward and reverse primers (Table 1), 2.0 µl of 10 mg/ml bovine serum albumin, 0.5 µl ROX reference dye, and 3.0 µl nuclease-free water and 5 µl of DNA template (10 ng total) on a Rotor-Gene 3000 (Corbett Life Science) qPCR thermal cycler. A standard curve was developed using *Desulfovibrio vulgaris* subsp. *vulgaris* genomic DNA (ATCC 29579D-5) amplified with the DSR1f+ and DSR-R primer set. The standard curve ranged from 2 pg to 2 ag (8 to 8 x 10^9 copies of the *dsr*A gene) of this genomic DNA. The cycling conditions used in this SYBR green qPCR method were: 10 min at 95^{o}C initial denaturation and enzyme activation, 40 cycles of 15 sec at 95^{o}C, 60 sec and an additional data acquisition step of 15 sec at 85^{o}C. Finally, a melt-curve analysis was performed with a melt ramp from 72-95^{o}C to detect primer dimers and other non-specific binding. The additional step at 85^{o}C reduced baseline noise in the melt curve analysis and allowed for more accurate data acquisition around our expected product size of 221 bp. qPCR analyses were performed in triplicate on each sample.

Statistical Analyses –

Linear regression was used to evaluate relationships between water quality parameters, bacterial abundances and long-term corrosion rates estimated from corrosion pit depths. A multiple linear regression model was constructed to predict corrosion rates and used water chemistry and bacterial abundance measurements from the DSH to estimate regression parameters. A forward selection was applied to each parameter, where only parameters that were significant at a $p<0.05$ level were selected to remain in the model. Forward selection enters the variable that is most correlated with corrosion rate first, then continues this processes with the other variables. This selection processes recalculates the significance of each variable based on the partial F-statistic, thus building the model based on the variables that have already been entered into the model. A final model with statistical significance ($p<0.05$) using alkalinity and total SRB measurements was used to predict corrosion rates. This model was parameterized with data collected from the DSH in 2010 and 2011 and then applied to data collected during 2011 at three harbors on the north shore of Lake Superior. All simple linear and multiple linear regressions were made using the JMP™ version 9.0 statistical software package from SAS Institute, Inc (Cary, NC).

Results and Discussion

Historical Changes in Water Quality –

Historical changes in water chemistry were evaluated at three sites in the DSH; the Oliver Bridge (OB), Bascule Bridge Fishing Pier (BB), and Burlington Northern Rail Road Bridge (BNRR) (Fig. 1). Dissolved oxygen concentrations (DO) remained relatively stable at the Bascule Bridge Fishing Pier (BB) from 1987 to 1996 averaging 10.9 mg/L, whereas significant increases in dissolved oxygen were observed at the BNRR and OB sites. At the BNRR, dissolved oxygen increased significantly ($p<0.05$) between 1973 and 1987 from around 5.0 mg/L to 11.0 mg/L. At

the OB site, DO steadily increased from approximately 5 mg/L in 1972 to 10 to 12 mg/L in 1996. DO was lower during the summer months and greater during the winter, reflecting the dependence of DO concentration on water temperatures. A seasonal analysis indicated DO increased ($p<0.05$) during all seasons over this period.

Dissolved oxygen may play a diminishing role in the corrosion process after the initial formation of corrosion products such as lepidicrocite. After the development of corrosion scale and as DO decreases, the black precipitate magnetite becomes the dominant corrosion product (Huang and Zhang 2005) under the corrosion scale. This is relevant because the tubercle anatomy seen in the DSH typically contains a brownish-orange outer shell made of goethite and lepidocrocite and a hard shell containing magnetite under this surface layer (Ray *et al.* 2010). This change indicates that as the inner part of the tubercle becomes anoxic, dissolved oxygen may not be directly involved in the pitting that occurs under the tubercle.

Three dissolved ions found in water, chloride, sulfate, and carbonate (measured as alkalinity), are of particular interest because chloride and sulfate are somewhat corrosion to steel while carbonate provides some protection from corrosion. At the OB, chloride concentrations were higher in the 1970's but remained relatively constant since the early 1980's with an average of 8.3 mg/L. More recently, chloride concentrations at this site were similar to those in the 1980's, ranging from 9.4 to 7.5 mg/L in 2010 and 2011, respectively. Much like chloride, dissolved sulfate at the OB peaked in the mid-1970's, ranging from 15.0 mg/L to 29.0 mg/L. Significant declines have occurred since then to 10.0 mg/L in 1996. In September 2006, sulfate was 14.0 mg/L at the OB. However, concentrations of 49.0 and 10 mg/L were measured in 2010 and 2011, indicating the variability of sulfate concentration that is possible at this site in the most upper part of the DSH. Alkalinity, measured as calcium carbonate ($CaCO_3^{2-}$) remained constant since the early 1970's at the OB with no significant change from about 55 mg/L.

Historical data for the Bascule Bridge Fishing Pier (BB) spanned 10 years from 1987 to1996. There was no significant change in any of the water quality parameters analyzed during this period. Chloride at BB showed no significant change over time. Sulfate remained constant averaging 12.8 mg/L and the average alkalinity was 58.8 mg/L. Similarly, there was no change ($p's>0.05$) in chloride, sulfate, and alkalinity concentrations from 1973 to 1987 at the Burlington Northern Rail Road bridge (BNRR), which is 0.3 miles further upstream from the BB and closer to WLSSD. Alkalinity as $CaCO_3$ averaged 54.4 mg/L, average chloride was 12.2 mg/L, and the average sulfate concentration was 13.8 mg/L.

The Larson-Skold Index of water corrosivity, which relies on chloride, sulfate, and alkalinity concentrations, was calculated for each of these three sites to evaluate the effect of water chemistry on steel corrosion. From 1972 to 1997, the Larson-Skold Index decreased ($p < 0.05$) at the OB (Fig. 2, panel C), whereas this index remained unchanged at the BB and BNRR sites (Fig. 2, panels A and B). It is important to note that among these three sites and of the 478 Larson-Skold calculations, there were only 37 instances (7.7% of the time) when the Larson-Skold Index was above the 0.8 threshold considered an intermediate risk for corrosion during this 25 year period. The Larson-Skold Index was above the 1.2 threshold only three times and only at the OB from 1972 to 1997, which indicates a potentially high risk for corrosion.

In summary, the analysis of water quality from the early 1970's to the late 1990's in the DSH indicated dissolved oxygen increased during this period at two sites, but there were few major

changes in water quality parameters associated with corrosion. In fact, the corrosivity of the water, as indicated by the Larson-Skold Index, remained unchanged at one site (BNRR) and appeared to decrease from the 1970's to the 1990's at the upper most part and lower most part of the DSH (OB and BB). We interpret this to mean that changes in water quality alone may not be responsible for the appearance of severe and possibly accelerated corrosion of steel structures that was recently discovered in this harbor. Bostrom (2010) reached a similar conclusion but suggested that water chemistry may influence the composition of bacterial communities found on these structures.

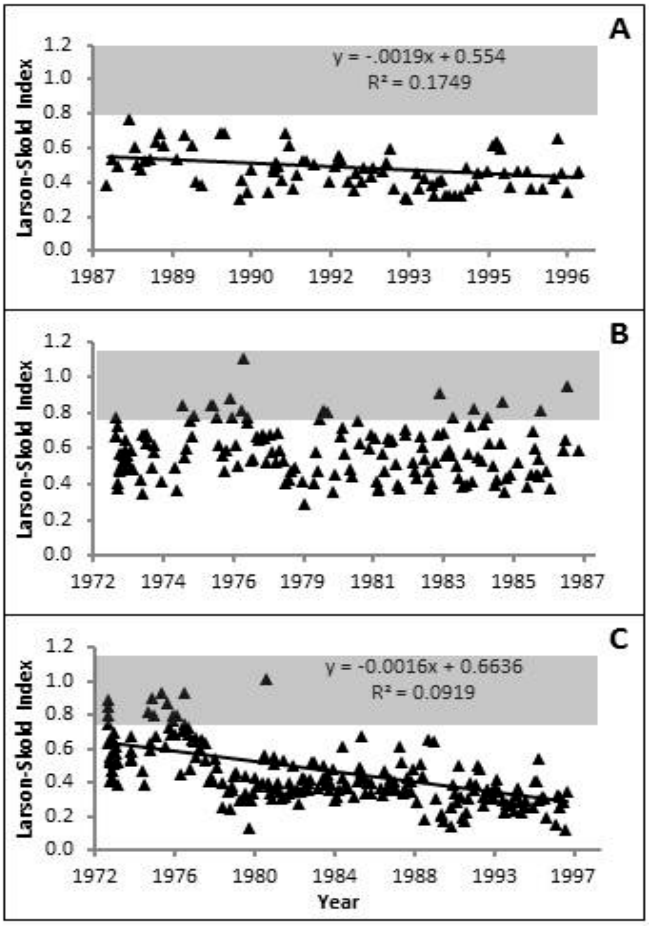

Fig. 3. Long term change in the Larson-Skold Index of water corrosivity for the (A) Bascule Bridge, (B) Burlington Northern Railroad Bridge, and (C) Oliver Bridge in the Duluth-Superior Harbor from 1972 to 1997.

Estimates of Long-Term Steel Corrosion Rates in the DSH –

The steel corrosion rates estimated in 2010 and 2011 represent long-term corrosion occurring over 30 to 100 years. These corrosion rates varied among sites in the Duluth-Superior Harbor and the north shore of Lake Superior (Fig. 4) but were similar between years at sites where measurements were made in both 2010 and 2011. The lowest corrosion rates were observed at the head (OB site) and mouth of the harbor (Duluth and Superior entries), while higher rates were observed in the outer and inner harbor areas where corrosion appears more severe. Wiener

and Salas (2005) determined that average corrosion rate in marine systems varies between 0.1 to 0.3 mm/year. At several sites in the outer (Cargill, DSPA Berth 4) and inner (Hallett Dock 5, Midwest Energy Resources) harbor, the long-term steel corrosion rates were within this range (Fig. 4). A typical rate of corrosion for freshwater ecosystems may be an order of magnitude lower than in seawater, between 0.01 to 0.04 mm/year (Bushman and Associates 2006). The long-term corrosion rates at all outer and inner harbor sites in 2010 and 2011 were above this typical rate of corrosion in freshwater.

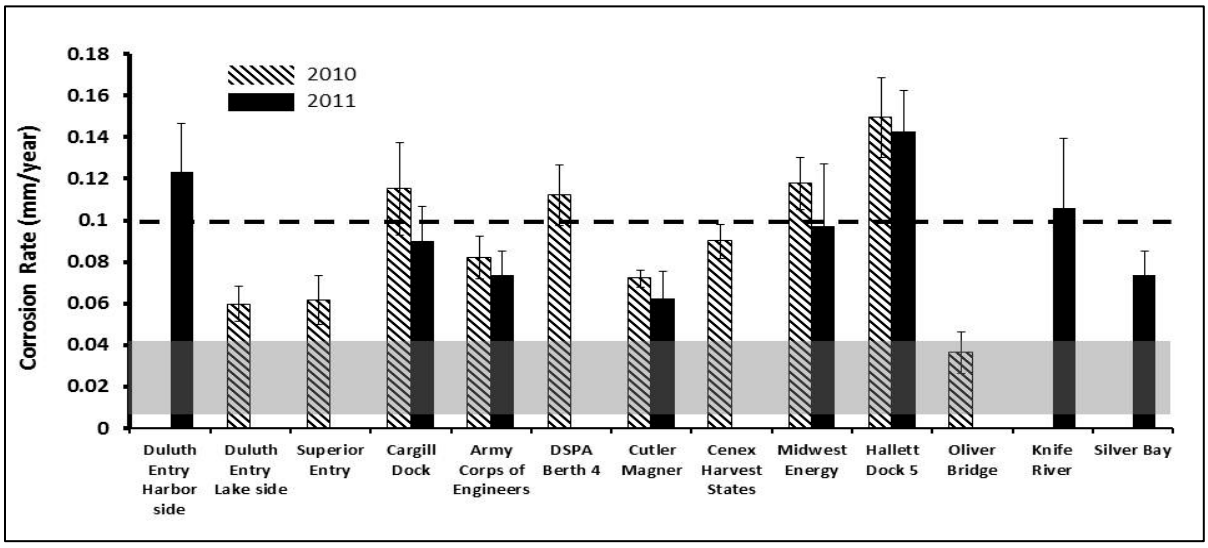

Fig. 4. Long-term steel corrosion rates in the Duluth-Superior Harbor and along the north shore of Lake Superior estimated in 2010 and 2011. Gray box indicates corrosion rates estimated for freshwater environments (0.01-0.04 mm/year) and the dashed horizontal line indicates a corrosion rate that is considered normal in marine ecosystems (0.1-0.3 mm/year).

Longitudinal Changes in Water Quality in the DSH during 2010 and 2011 –
Several water quality parameters were measured (see Appendix) at sites throughout the DSH (Fig. 1) during August 2010 and July 2011 to evaluate gradients and relate these measurements to long-term rates of corrosion. Dissolved oxygen increased from the head of the harbor (8.8 to 10.4 mg/L) to the Duluth and Superior entries to the DSH, where DO concentrations were 11.2 and 11.3 mg/L, respectively. DO was lowest at Hallet Dock 5 (8.43 mg/L) in 2010. Interestingly, the long-term estimates of steel corrosion were inversely related to the dissolved oxygen concentration – corrosion rates were typically higher at sites with lower DO concentrations (Table 2).

Chloride was relatively constant throughout the harbor with a median of 12.0 mg/L and average of 10.2 mg/L, but was positively related ($p<0.05$) to the long-term rate of corrosion at these sites (Table 2) - as chloride increased the rate of corrosion also increased. Sulfate concentration was more variable among sites and there was no significant relationship between sulfate and the long-term steel corrosion rate (Table 2). The highest sulfate concentration was found at the Oliver Bridge (49.0 mg/L) in August 2010 and the lowest at the Duluth (3.6 mg/L) and Superior (7.4 mg/L) entries to the DSH. The higher concentration at the OB might have been due to a rain

event the night prior to sampling. The sulfate concentration at other sites ranged from 14.0 to 35.0 mg/L in 2010 and 2011.

There was an indication of a slight alkalinity gradient in the harbor in 2010 and 2011, with higher alkalinity in the inner harbor and lower concentrations near the harbor entrances. The highest alkalinity measurements were typically found at sites noted to be the most severely corroded such as Midwest Energy Resources Company (73 to 91 mg/L) and Hallett Dock 5 (70 to 95 mg/L). In 2010, alkalinity was positively related to the long-term corrosion rate at these sites in the DSH (Table 2).

Table 2. Statistical relationship between the long-term rate of steel corrosion (mm/yr) and various water quality parameters measured during 2010 at ten sites in the Duluth-Superior Harbor.

Regression Parameter (x)	**Regression Equation**	**P-value**	**Significance**
Alkalinity	y = 0.0014x – 0.0103	0.005	Significant
Chloride	y = 0.0055x + 0.0382	0.02	Significant
Sulfate	y = 0.0008x + 0.0735	0.23	Not Significant
Larson-Skold Index	y = 0.0372x + 0.0751	0.40	Not Significant
Conductivity	y = 0.0004x + 0.0052	0.04	Significant
pH	y = -0.0155x + 0.2113	0.29	Not Significant
DOC	y = 0.0016x + 0.0683	0.08	Not Significant
Dissolved Copper	y = 3.785x + 0.0803	0.43	Not Significant
Dissolved Oxygen	y = -0.0151x + 0.2306	0.04	Significant Negative

The Larson-Skold Index (Fig. 5) indicated low water corrosivity at all sites in the DSH in 2010 and 2011, except at the OB in August 2010. There was no relationship ($p>0.05$) between the Larson-Skold Index calculated at sites in 2010 and the estimated long-term steel corrosion rate (Table 2). These data support the conclusion reached earlier by calculating the Larson-Skold Index using long-term historical water quality measurements - water chemistry alone may not be directly responsible for the accelerated corrosion observed in the DSH. Rather, water chemistry may influence the types of communities of bacteria present on these steel structures (Bostrom 2010) and the activities of these microbial consortia may increase the corrosion rates.

The water pH was in the neutral (7.71) to slightly alkaline (8.17) range at all DSH sites in August 2010 and it was not related to the long-term steel corrosion rate (Table 2). In both 2010 and 2011, water conductivity was typically higher at sites such as Midwest Energy Resources (215-239 µS/cm), Hallett Dock 5 (191-240 µS/cm), and Cenex Harvest States (248 µS/cm) where extensive corrosion has been observed than at sites like the Duluth Entry (101 µS/cm) where less corrosion has been observed. In 2010, water conductivity was positively related to the long-term steel corrosion rate (Table 2).

Dissolved copper in water ranged from 0.8 to 8.3 µg/L considering 2010 and 2011, which overlaps of the range of dissolved copper (0.4-1.6 µg/L) reported in unpolluted freshwater ecosystems in Europe (Roussel *et al.* 2007). Dissolved copper was not related to estimates of the long-term corrosion rate (Table 2). Copper has been shown to precipitate in the pits of corroding steel in the DSH, which may ultimately lead to galvanic coupling and more aggressive corrosion (Ray *et al.* 2009).

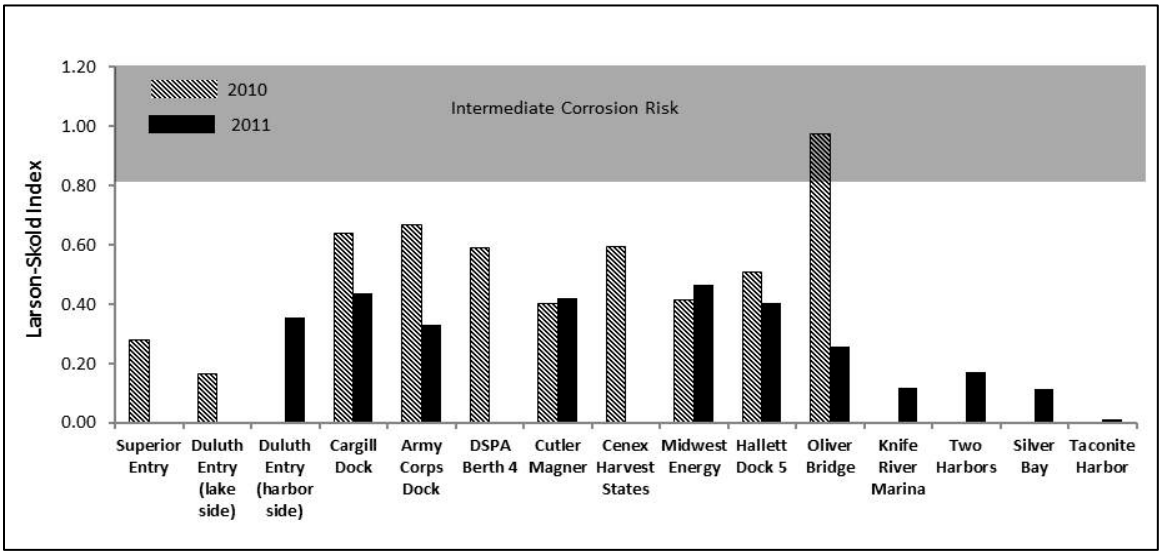

Fig. 5. Larson-Skold Index of water corrosivity for sites sampled during 2010 and 2011 in the Duluth-Superior Harbor and three harbors on the north shore of Lake Superior. Larson-Skold value 0.8-1.2 indicates an intermediate corrosion risk, > 1.2 indicates a high corrosion risk.

Dissolved organic carbon (DOC) exhibited a strongly decreasing gradient from head of the harbor at OB (~19 mg/L) to the entries of the DSH (2 to 6 mg/L; see Appendix). This DOC gradient is typical of riverine systems that dump into large receiving waters. These organic inputs to the St. Louis River and DSH are most likely due to surrounding wetlands and terrestrial runoff (Stephens and Minor 2010).

When 2010 and 2011 water quality data were considered together, there was no significant relationship between any water chemistry parameter and the estimated long-term corrosion rate. These corrosion rates represent long-term corrosion occurring over 30 to 100 years. Considering that the concentration of chemical ions can vary from day-to-day, individual measurements on a particular day may not accurately represent their effect on steel corrosion over several years. So, it may be more informative to look for relationships between long-term corrosion rates and the average or maximum concentration of chemicals in long-term data sets.

Estimates of Iron-Oxidizing and Sulfate-Reducing Bacterial Abundances within Corrosion Tubercles –

Iron-oxidizing bacteria (FeOB) have been implicated in the corrosion of steel surfaces (Emerson 2010, Emerson and Moyer 2002, Lee and Characklis 1993, Hamilton 1985). Previous studies have found bacterial cells similar in morphology to *Gallionella* species within tubercles (Ray *et al.* 2010, Ray *et al.* 2009). Bacterial DNA most similar to iron-oxidizing bacteria like *Gallionella spp.* and *Sideroxydans lithotrophicus* have been found in 16S rRNA gene clone libraries constructed from microbial DNA extracted from corrosion tubercles in the DSH (Hicks 2009, Hick *et al*., unpublished data). And, a bacterium tentatively identified as *S. lithotrophicus* has even been isolated from corrosion tubercles in the DSH and cultivated (Hicks 2009).

Although the abundance of *Gallionella* spp. within corrosion tubercles varied between sites in the DSH and from year to year at sites where these estimates were made in August 2010 and July 2011 (Table 3), the highest abundances each year (122 to 324 x10^7 16S rRNA gene copies/dry g) were found at sites like Hallett Dock 5, and Midwest Energy Resources where steel corrosion appears more severe. DNA from *Gallionella* spp. was not detected on the steel surface at the Lake Superior side of the Duluth Entry in August 2010 where no corrosion tubercles were observed, and very low *Gallionella* abundances (2 to 3 x10^7 16S rRNA gene copies/dry g) were measured on steel surfaces in other harbors along the north shore of Lake Superior in July 2011. Overall, *Gallionella* spp. abundance represented 2% to 34% of the total prokaryotic cells counted in tubercle samples at most sites (Table 3). DNA from *Gallionella* ssp. was not present in the adjacent water surrounding corroded steel surfaces in the DSH, indicating that corroded steel surfaces are enriched with *Gallionella.* The abundance of *Gallionella* spp. within corrosion tubercles was not related to the long-term corrosion rates at sites in the DSH (Table 4).

Table 3. Prokaryotic cell, *Gallionella* spp. (FeOB), and sulfate-reducing bacterial (*dsr*A) abundances within corrosion tubercles on corroding steel structures at various sites in the Duluth-Superior Harbor and along the north shore of Lake Superior during August 2010 and July 2011. *Gallionella* and sulfate-reducing bacterial abundances were estimated by quantitative PCR measurements of *Gallionella*-specific 16S rRNA and *dsr*A gene copies, respectively, per dry gram of tubercle material. nd = not determined.

	2010			2011		
Location	Prokaryotes (10^7 cells/g)	FeOB (10^7 copies/g)	SRB	Prokaryotes (10^7 cells/g)	FeOB (10^7 copies/ g)	SRB
Oliver Bridge	636	43	0.5	782	58	3.1
Hallet Dock 5	700	122	2.2	1,010	99	1.9
Midwest Energy Resources	448	44	0.02	1,080	324	0.8
Cenex Harvest States	495	169	0.8	nd	nd	nd
Cutler Magner	318	82	0.02	616	43	0.2
DSPA Berth 4	432	16	0.7	nd	nd	nd
Army Corps Dock	724	206	1.1	822	143	25
Cargill Dock	378	127	0.6	578	22	3.8
Duluth Entry (outer harbor)	nd	nd	nd	628	32	57
Duluth Entry (lake exposed)	nd	0	0	nd	nd	nd
Superior Entry	313	193	0.02	nd	nd	nd
Knife River Marina	nd	nd	nd	148	2.4	2.1
Two Harbors	nd	nd	nd	201	3.1	3.7
Silver Bay	nd	nd	nd	130	2.6	2.3

Table 4. Relationship between long-term corrosion rate (mm/year) and bacterial abundances measured on steel surfaces at ten sites in the Duluth-Superior Harbor during 2010.

Regression Parameter (x)	Regression Equation	P-value	Significance
SRB Abundance	y = 0.2784x + 0.0801	0.37	Not Significant
Gallionella ssp. Abundance	y = 2E-11x + 0.0773	0.12	Not Significant

Sulfate-reducing bacteria were at least 2 orders of magnitude less abundant within corrosion tubercles than *Gallionella* spp. (Tables 3) and varied from site to site and between 2010 and 2011 at sites where measurements were made each year. The greatest SRB abundances each year were often found at sites in the DSH with more severe corrosion such as Hallett Dock 5 (1.9 to 2.2 x 10^7 dsrA gene copies/dry g). The harbor side of the Duluth Entry, which was moderately corroded in 2011, had the highest SRB abundance (57 x 10^7 dsrA gene copies/dry g). The abundance of SRBs on steel surfaces was lowest on the Lake Superior side of the Duluth and Superior entries to the DSH (0 to 0.02 x 10^7 dsrA gene copies/dry g) where corrosion is less severe than in the DSH (Table 3). It is important to note that SRB require anoxic conditions to grow. If there are only small anoxic areas within the porous corrosion tubercles found in the DSH, then SRB abundances might be expected to be lower compared to the abundance of aerobic iron-oxidizing bacteria like *Gallionella* and *Sideroxydans*.

The abundance of SRBs within corrosion tubercles were not related to the long-term corrosion rate of steel estimated at ten sites in the DSH (Table 4). Although, SRB abundance was not related to the long-term corrosion rate and accounted for less than 1% of the total prokaryotic cells within corrosion tubercles at most sites examined (Table 3), their presence within corrosion tubercles in the DSH may still be of concern. SRB are known to cause pitting of steel structures (Hamilton 1985). Their abundance may not be as important as their activity within corrosion tubercles. Thus, it is important to know if they are present within corrosion tubercles in the DSH.

Corrosion Risk Assessment Tool –

While a logistic regression model was not useful for predicting corrosion risk, a multiple linear regression model could predict long-term corrosion rates in the Duluth-Superior Harbor from water chemistry and microbiological parameters together. Multiple linear regression analysis indicated (Fig. 5) that alkalinity, sulfate, and the abundance of Log_{10} SRB taken together, can be used to predict the long-term corrosion rate with some confidence ($p<0.05$) but this model could only explain 61% of the variability seen in the long-term (actual) corrosion rate estimates in the DSH. The model predicted by this multiple regression is:

Predicted Corrosion Rate (mm/year) = (0.0021*Alkalinity (mg/L) + (0.015* Log_{10} SRB (*dsr*A gene copies/dry g) + (-0/0014 * Sulfate (mg/L))) – 0.008413

Although the abundance of *Gallionella* spp. was related to the long-term corrosion rate in 2010, it is interesting that it was not included as a significant parameter in the multiple regression model.

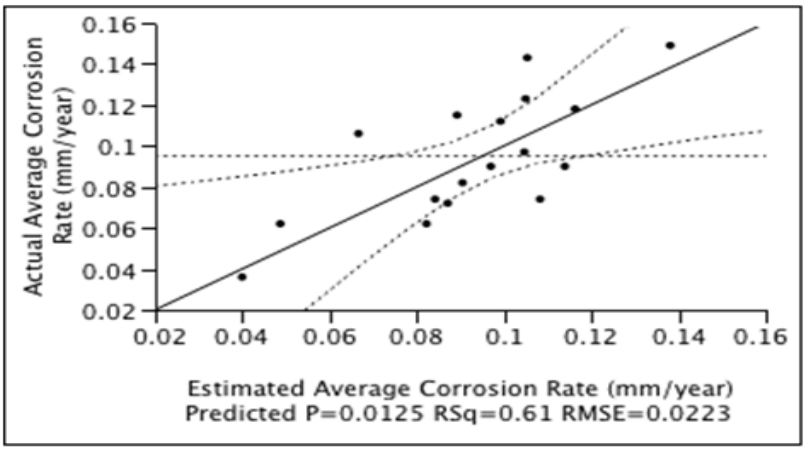

Fig. 6. Multiple regression analysis that uses alkalinity and SRB abundance to predict the long-term corrosion rate (mm/year) of steel structures in the DSH. Plot shows predicted corrosion rates versus actual *in situ* long-term corrosion rates (mm/year).

When this model was used to predict corrosion rates at two harbors on the north shore of Lake Superior, the measured long-term corrosion rate at Knife River Marina (0.106mm/year) was slightly underestimated by the model at 0.067 mm/year (Table 6). At Silver Bay the actual measured long-term corrosion rate was 0.074 mm/year and the estimated corrosion rate by the model was 0.084 ± 0.012 mm/year, which is within the 95% confidence interval. However, it is important to note that while this model may underestimate the actual corrosion rate, severity of corrosion seen in the harbors along the North Shore is different. The distribution of corrosion tubercles is more sparse than in the DSH, where tubercles can sometimes overlap each other, and the pitting under the DSH tubercles seems to be more extensive. Extensive means there were often corrosion pits within pits, and corrosion pits were larger in diameter and depth in the DSH. Unlike the DSH, there was usually a single relatively deep corrosion pit under smaller diameter corrosion tubercles in harbors along the north shore of Lake Superior. Further validation of this model is needed to determine its true applicability in estimating corrosion rates in other harbors, therefore, we conclude that this model should be tested in areas similar to the Duluth Harbor or at sites in the DSH which have not been studied in the past. It is clear, however, that water chemistry and microbial agents together may be more important than either alone in the corrosion of steel infrastructure in the DSH and possibly other areas in the western arm of Lake Superior.

Table 6. Actual long-term corrosion rates (mm/year) in three harbors along the north shore of Lake Superior compared to corrosion rates predicted by the multiple regression model.

Location	Predicted Corrosion Rate (mm/year)	Actual Corrosion Rate (mm/year)
Knife River Marina, MN	0.067 ± 0.015	0.106 ± 0.058
Two Harbors, MN	0.066 ± 0.012	Not Determined
Silver Bay, MN	0.084 ± 0.012	0.074 ± 0.020

Impacts of this Study

The corrosion of steel infrastructure is a serious economic concern for many sectors of the United States' economy. It has been estimated that corrosion in the United States costs an astounding $275.5 billion per year or approximately 3.14% of our entire GDP (Furbeth and Schutze 2009). It might cost $200-250 million to replace or repair the 20 km of steel currently affected in the Duluth-Superior Harbor alone (Marsh *et al.* 2005, Larsen 2008, Hicks 2009). The Duluth-Superior Harbor and other ports in the Laurentian Great Lakes rely on the transportation of many products including taconite, grain, coal, and stone aggregates for their local economies. These areas have created 227,000 jobs related to the shipping industry (Martin Associates 2011). Thus, any disruption to the shipment of these goods could potentially have negative consequences for the Great Lakes region.

The ultimate goal of this project was to provide businesses and government agencies a tool that could assist them in determining whether their facilities may experience severe corrosion similar to what has been seen in the DSH. Developing a risk assessment tool using logistic regression was not successful, but a multiple linear regression approach provided a better way to predict corrosion rates. Having estimated corrosion rates, even ones that are slightly underestimated, may be helpful to maritime businesses and government agencies for determining the expected longevity of their steel structures. With this knowledge, they can plan for resources needed to replace corroded steel structures that are already impacted or may become corroded. From this study, it appears that water chemistry alone is not likely the cause of accelerated corrosion in the DSH, but rather a combination of microbiological and chemical factors appear to influence the corrosion of steel structures in this harbor.

Dissemination of Results

1. Publications
   none to date

2. Presentations
   Oster, R. J. and R. E. Hicks. Phase II- Developing a Risk Assessment Tool to Predict the Corrosive Loss of Port Infrastructure. GLMRI University Affiliates Meeting, September 23, 2011, Duluth, MN. (oral presentation)

   Oster, R. J. and R. E. Hicks. Using Microbiological and Chemical Factors to Assess the Risk of Corrosion to Port Infrastructure. 2011 IAGLR Conference, May 30-June 3, 2011, Duluth, MN. (oral presentation)

   Hicks, R. E. Rusting Away: Water Quality and Microbial Aspects of Corrosion in the Duluth-Superior Harbor. October 27, 2010. EPA Mid-Continent Ecology Laboratory, Duluth, MN. (oral presentation)

   Hicks, R. E. and R. J. Oster. Developing a Risk Assessment Tool to Predict the Accelerated Corrosive Loss of Port Transportation Infrastructure. GLMRI University Affiliate Meeting, September 25, 2010, Duluth, MN. (oral presentation)

   Oster, R. J. and R. E. Hicks. Harbor and North Shore Corrosion Sampling Summer 2010. US Army Corps of Engineers Technical Advisory Committee, September 15, 2010, Duluth, MN. (oral presentation)

   Randall Hicks, Ryan Oster, and Jonathan Bostrom participated in US Army Corps of Engineers Technical Advisory Committee meeting, May 12, 2010. Randall Hicks provided a review of past projects and an overview of the current project.

   Oster, R. J. and R. E. Hicks. Historic Water Quality Changes in the Duluth-Superior Harbor. US Army Corps of Engineers Technical Advisory Committee, May 12, 2010, Duluth, MN. (oral presentation)

3. Graduate Theses and Dissertations
   Oster, R. J. Developing a Model Using Chemical and Microbiological Factors to Assess the Risk of Accelerated Corrosion of Port Structures. M.S. Thesis, Water Resources Science Graduate Program, University of Minnesota (in progress)

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Appendix

Table 7. Water quality measurements made from 9-10 August 2010 in the Duluth-Superior Harbor.

Sample Site Location	Alkalinity (mg/L)	Chloride (mg/L)	Sulfate (mg/L)	Larson Skold Index	Conductivity (µS/cm)	pH	DO (mg/L)	DOC (mg/L)	Dissolved Copper (mg/L)
Duluth Entry (lake side)	44	2.4	3.6	0.16	101.0	8.17	11.17	1.77	0.00082
Superior Entry	54	5.2	7.4	0.28	123.4	7.93	11.33	5.59	0.0012
Cargill Dock	77	12	31	0.64	219.6	7.80	8.93	12.66	0.0015
Army Corps	77	12	33	0.67	215.4	7.72	9.12	12.95	0.0030
DSPA Berth 4	80	12	29	0.59	195.2	7.86	9.80	12.23	0.0083
Cutler Maner	75	11	14	0.40	220.1	8.04	9.27	13.30	0.0019
Cenex Harvest States	90	12	35	0.59	247.5	7.96	8.78	15.62	0.0018
Midwest Energy	91	14	17	0.41	239.0	7.94	8.75	16.08	0.0016
Hallett Dock 5	95	12	30	0.51	240.1	7.87	8.43	16.18	0.0018
Oliver Bridge	68	9.4	49	0.97	176.3	7.71	8.80	18.99	0.0020

Table 8. Water quality measurements made from 26-27 July 2011 in the Duluth-Superior Harbor and three harbors on the north shore of Lake Superior.

Sample Site Location	Alkalinity (mg/L)	Chloride (mg/L)	Sulfate (mg/L)	Larson Skold Index	Conductivity (µS/cm)	pH	DO (mg/L)	DOC (mg/L)	Dissolved Copper (mg/L)
Duluth Entry (harbor side)	57	7	10	0.36	146.2	6.88	8.7	9.41	0.0013
Cargill Dock	64	9.6	14	0.44	177.1	6.79	7.46	13.01	0.0015
Army Corps	61	7	10	0.33	161.4	6.83	7.64	11.02	0.0023
Cutler Magner	65	9.9	13	0.42	186.7	6.90	8.01	13.37	0.0016
Midwest Energy	73	13	15	0.47	215.3	7.07	7.29	17.66	0.0016
Hallett Dock 5	70	9.7	14	0.40	190.5	7.48	6.78	17.43	0.0017
Oliver Bridge	81	7.5	10	0.26	203.6	6.71	7.01	19.27	0.0013
Knife River Marina	45	1.4	3.3	0.12	83.4	8.00	10.85	1.30	0.0029
Two Harbors	43	2.7	3.5	0.17	84.8	7.63	10.28	1.20	0.0011
Silver Bay	53	1.8	3.5	0.12	83.2	6.83	10.11	1.18	<0.0008
Taconite Harbor	380	1.4	3.4	0.01	48.0	7.05	10.38	1.83	0.0015