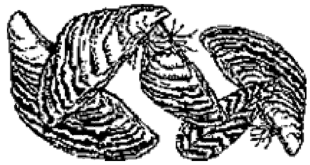

# Zebra Mussels, Shipwrecks
## and the
# Environment

Final Report
May 2001


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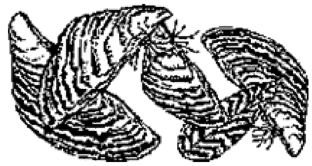

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# EXECUTIVE SUMMARY

Since 1993, zebra mussels (*Dreissena polymorpha*) have rapidly spread throughout Lake Champlain. We believe that this invasion may have the most profound effects on Lake Champlain since the arrival of human beings. From both an ecological and a human and cultural heritage perspective, the lake will simply never be the same. In order to explore these changes, we initiated an integrated research program in 1999. Over the long term, we hope to develop a comprehensive data set that can be used for two major purposes: (1) to predict the implications of the zebra mussel infestation for wooden shipwrecks in various underwater conditions, and (2) to explore natural and other control mechanisms that might be associated with the environmental characteristics of this lake and others.

Our experimental design for the 2000 field season included five major elements: (1) corrosion studies on six shipwreck sites located throughout Lake Champlain, (2) laboratory experiments testing the growth and survival of zebra mussels in water with various concentrations of calcium, (3) settlement experiments designed to measure the physical and chemical effects of zebra mussel colonization on eight different substrate types, (4) recovery, analysis and conservation of an ornate iron grate from the Pot Ash canal boat, and (5) a pilot study of fish predation on one specific wreck in Bulwagga Bay.

Our results clearly show that the zebra mussel population has continued to expand across the bottom of Lake Champlain. All of our sites have mature, well-developed colonies on most available surfaces. Like last year, more zebra mussels were found on vertical surfaces than on horizontal surfaces. All surfaces are also accumulating a layer of organic and inorganic particles that are allowing bacteria to flourish and are attracting a variety of small invertebrates.

The data set that we have collected over the last two field seasons strongly suggests that zebra mussels are accelerating the corrosion of the iron fasteners and fittings on the submerged shipwrecks in Lake Champlain. As zebra mussel colonies have grown and a thick layer of organic matter accumulates under the mat, a complex community of bacteria has become established. These microorganisms are likely facilitating the corrosion process.

Our water quality data definitively document the loss of iron into the water column above the surface of zebra mussel colonies on the wrecks. We always find more iron above mussel colonies than away from colonies on the wrecks. The pattern of iron in the overlying water suggests that loss rates are substantial. Our microbial assays showed a well-developed community of microorganisms under the zebra mussel mat. This community includes several bacterial groups that are known to enhance corrosion.

Zebra mussels settled on all of the substrates we provided in the settlement experiments although they clearly preferred some to others. The wrought iron and steel rods seemed to be the most attractive materials. Physical damage to the wood pieces was minimal compared to the metal. All of the iron pieces subjected to detailed analysis showed some degree of corrosion. For most of the samples, the corrosion began as pitting corrosion on the outer surface of the sample. Deep pits were apparent in the cross sections of some samples. After just one year, we cannot project

an annual corrosion rate in Lake Champlain, however, we did measure a thick scale layer in many areas and pits of greater than 1 mm.

When we recovered the historic cast iron grate from the Pot Ash canal boat, it was completely covered with zebra mussels, sometimes in mats that were more than two inches thick. The scale on the grate was also thick and easily flaked off the surface of the metal. Chemical tests on the iron underneath the zebra mussels documented the presence of reduced sulfur compounds.

The implications of these findings for the structural integrity of the wrecks are still not clear. Because of the patchiness of the chemical environment and the large array of biological processes and chemical reactions at work, additional research is necessary to project an iron loss rate. However, the condition of the cast iron material comprising the Pot Ash Canal Boat window grate may partially forecast the fate of all exposed iron on the wrecks. The interplay between wood and iron will always make projections about overall wreck survival difficult.

The railroad drawboat in Bulwagga Bay near Port Henry, NY was the focus of the fish predation pilot studies following the observation of a large quantity of crushed and broken zebra mussel shells on the deck of the boat. Using a Benthos Minirover remotely operated vehicle we observed and documented fish behavior on this site.

With this technology, we witnessed predation by freshwater drum (*Aplodinotus grunniens*), yellow perch (*Perca flavescens*), and pumpkinseed (*Lepomis gibbosus*) on zebra mussels on the wreck. To our knowledge, this is the only site where direct field documentation of substantial zebra mussel predation has occurred. There are no other published (or unpublished to our knowledge) reports of large collections of crushed mussel shells in the natural environment.

Freshwater drum may be responsible for the crushed shells documented on the railroad drawboat. If drum are eating zebra mussels throughout the lake, and zebra mussels are an increasingly important part of their diet, then this predation may begin to have a measurable effect on the overall abundance of zebra mussels in the lake. The implications of this consumption for the drum are unclear; some research has suggested that individuals that rely on zebra mussels for a significant portion of their diet show declining size at age.

No previous work has suggested that yellow perch might be routinely feeding on significant numbers of zebra mussels. Because yellow perch lack the jaw structure necessary to crush zebra mussels, they must swallow them whole. It is unlikely, therefore, that yellow perch are contributing to the masses of crushed shells observed on the drawboat. Furthermore, no other investigators have found significant consumption of zebra mussels by yellow perch because ingestion is limited to small individuals by the size of their throat. In fact, previous research has suggested the yellow perch feed predominantly on the benthic invertebrates associated with zebra mussel colonies, and not the mussels themselves.

The documentation of significant fish predation on the railroad drawboat suggests a very real evolution in the feeding relationships in Bulwagga Bay. If these changed behaviors are becoming prevalent throughout Lake Champlain, the food web and biodiversity of the Lake will be significantly different in the years to come.

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Mr. Frederick Doane and Paradigm Analytical Laboratories, Wilmington, NC, analyzed all the water samples for iron and calcium at no cost to the project and also provided the remotely operated vehicle for the fish predation observations. This support greatly enhanced the scope of our work.

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# INTRODUCTION

In 1993, zebra mussels (*Dreissena polymorpha*) were discovered in Lake Champlain. Since then, they have rapidly spread and today are found in all sections of the lake. We believe that zebra mussels may have the most profound impact on Lake Champlain since the arrival of human beings. Because zebra mussels will attach to any hard surface, they have already required costly retrofitting of water intake systems. Swimmers and waders can no longer enter the water without shoes to protect their feet from the accumulating shells. Even more significantly, the ecological processes of the lake have begun to change in profound ways. Without exaggeration, the lake's trophic structure and complex of organisms will never be the same. Finally, zebra mussels have begun to colonize most of Lake Champlain's underwater shipwrecks, arguably the best preserved collection of historical wooden ships in North America. There is no greater research challenge than to understand how these organisms will alter the ecological and cultural environment of the lake.

This report builds on the work documented in our previous final report, which summarized the 1999 field season (Watzin et al. 2000). Broadly stated, our research goal is to quantify the effects of zebra mussels on the environment of Lake Champlain. Over the long term, we hope to develop a comprehensive data set that can be used for two purposes: (1) to predict the implications of the zebra mussel infestation for wooden shipwrecks in various underwater conditions, and (2) to explore natural and other control mechanisms that might be associated with the environmental characteristics of this lake and others.

Lake Champlain's underwater heritage spans ten thousand years of human history in the Champlain Valley. It includes canoes used by Native Americans, gunboats and other naval craft used by the French, British and American navies through three military eras, and steam boats, sail boats, canal boats, and tug boats of all description used in commerce and transport in the late 18th, 19th and early 20th century. Study of this legacy of wooden ships began more than two decades ago and to date has produced more information about nautical archaeology, historic ship design, and the history of technology and commerce, than any other region in America.

When zebra mussels colonize these historic shipwrecks, they quickly obliterate the construction details and structure of the wreck site. In reviewing the zebra mussel literature, we noticed that as mussels colonize steel retaining walls and water intake pipes, the steel degrades at a significantly more accelerated rate than when zebra mussels are not present. On the shipwrecks of Lake Champlain, we have also noticed that zebra mussels are particularly attracted to the iron fasteners and other metal structures on the wrecks. The wooden ships on the bottom of Lake Champlain are primarily iron fastened, and the fastenings contribute to the vessel's structural integrity, therefore, anything which affects that integrity could significantly affect the projected life-span of these underwater treasures. In designing this study we focused on gathering data that could be used directly by managers to help make decisions about the future of these priceless cultural artifacts. Our objective was to gain enough information to quantify the effect of zebra mussel colonization on the expected life span of submerged cultural sites composed of wood and fastened with iron.

Using the shipwrecks as locations to study the ecology of the lake as well, our second objective is to understand and project the effects of the zebra mussels on the Lake Champlain ecosystem. In doing so, we are especially interested in changing trophic dynamics and the potential for predators like fish to reduce the overall abundance of the mussels in some sections of Lake Champlain.

## STUDY SITES

Seven shipwrecks scattered throughout Lake Champlain (Figure 1) comprise our study sites. All are in shallow water (less than 10 m) where zebra mussel densities are greatest and SCUBA divers have unlimited bottom time to implement the rather complex underwater sampling protocol.

We chose four primary sites, two in the vicinity of Burlington, VT (North Beach Wreck, *General Butler*), and two in the vicinity of Basin Harbor, VT (Diamond Island Stone Boat, *Champlain II*). These two lake regions differ in average calcium concentration, which may influence mussel growth rates and densities. Because zebra mussels invaded Lake Champlain from the south, the Basin Harbor sites also have a history of infestation at least one year longer than the two northern sites. Two additional sites were selected at points south and north of the primary sites. These were the Pot Ash Canal Boat, near Panton, VT, and the railroad barge at Rouse Point, NY. Details about each of these six sites appear in last year's report (Watzin et al. 2000).

In 1999, as part of its survey to locate and document historic shipwrecks in the lake, the Lake Champlain Maritime Museum discovered a "railroad drawboat" lying intact in about 25 feet of water in Bulwagga Bay, near Port Henry, New York. This unnamed vessel is 250-feet long, 34-ft feet wide and is in "like new" condition, apparently abandoned after only one season of use. It is believed to have been built in the early 1870s for a railroad trestle crossing from Crown Point to Port Henry. Railroad drawboats, or floating drawbridges, were designed to provide rail line connections while leaving a channel open for water traffic. This rail line was probably built to carry iron ore from the Port Henry mines to the furnaces at Crown Point.


*Figure 1. Map of Lake Champlain showing shipwreck study sites.*

When divers first examined the shipwreck, a significant number of crushed zebra mussel shells were noticed on its deck. Therefore, in 2000, we added the drawboat as a seventh research site to follow up on this observation and to determine if fish could now be eating significant numbers of zebra mussels in this part of the lake. This is the only site that we know where substantial zebra mussel predation may be occurring. If the fishes feeding on the railroad drawboat are common and abundant, there may be evidence for the evolution of some level of biological control of zebra mussel densities in Lake Champlain.

## STUDY DESIGN

Our experimental design for the year 2000 included five major elements: (1) corrosion studies on the six primary shipwreck sites described above, (2) laboratory experiments testing the growth and survival of zebra mussels in various concentrations of calcium in lake water, (3) settlement experiments designed to measure the physical and chemical effects of zebra mussel colonization on eight different substrate types, (4) recovery, analysis and conservation of an ornate iron grate from the Pot Ash canal boat, and (5) a pilot study of fish predation on the Bulwagga Bay railroad drawboat.

The corrosion studies built on the water quality sampling we began last year. We sampled the water above the zebra mussel colonies to look for the presence of dissolved iron, which would indicate the loss of wrought iron from the wrecks. We also measured the amount of organic matter that zebra mussels accumulate around themselves on the wreck and collected mussels to determine the density and age distribution of the zebra mussel population. This year, we also added a series of microbial assays. We used these assays to screen for the presence of those bacterial groups known to enhance the iron corrosion process. These include aerobic bacteria, acid-producing species, iron-depositing species, sulfate-reducing species, and anaerobic bacteria. The latter two are only found in situations where oxygen is absent and where the iron is actively moving into the dissolved state.

The laboratory experiments looked at zebra mussel shell growth and survival in water with a variety of concentrations of calcium. Calcium concentrations in Lake Champlain are low and may contribute to thin shells, and eventually, poorer survival of mussels in some locations.

The settlement experiments used the materials we placed into the field in 1999. One experimental table was recovered and documented from each of our six sites in order to document zebra mussel recruitment and the physical effects of zebra mussel colonization on the various substrate materials. Iron substrates from a subset of these sites were also subjected to detailed corrosion analysis.

An ornate iron window grating, 18 inches by 36 inches, on the Pot Ash Point Canal Boat was also recovered during the field season in a restoration effort by the LCMM. The grating was completely covered with zebra mussels and totally unrecognizable. Our goal was to examine an actual 19$^{th}$ century artifact to see if we could detect a corrosion process directly linked to zebra mussel encrustation. Water and zebra mussel samples were taken in a process similar to the main sampling procedure. A bacterial analysis was also performed.

The fish predation pilot study included behavioral observations using a remotely operated vehicle with a high-resolution underwater camera, and collection of a small sample of fish for examination of the gut contents.

# SPECIFIC METHODS

## CORROSION STUDIES

The corrosion studies continued on the same six shipwreck sites we used last year. Initial samples were collected between May 30 and June 8, 2000. Samples were collected again at the four primary research sites from July 27-28, 2000. Finally, samples were collected at all six sites between September 7 and 29, 2000. At each site, we first photodocumented zebra mussel colonization on the surface of the wrecks. We then collected water samples to measure basic water quality characteristics and iron and calcium concentration in a vertical gradient above the zebra mussel colonies. At the same locations where the water samples were taken, we also collected zebra mussel feces and pseudofeces and the zebra mussels themselves from the surface of the wrecks.

**Photodocumentation**. The first component of our experimental design involved the video documentation of current zebra mussel colonization on the six shipwreck sites using a Sony Hi8 TR101 video camera. Video images of eight 15 cm by 15 cm marked quadrats (Figure 2) were taken before and after zebra mussels were removed from the quadrats. Eight new quadrats were established during each visit, four with a horizontal orientation and four with a vertical orientation.

*Figure 2. Vertical sampling frame on the Rouse's Point shipwreck filled with zebra mussels.*

Video images were converted to digital format and National Institutes of Health Scion Image software was used to analyze the colonization of each sample frame. After calibrating the size of the image to the known size of the sample frame, the area of the frame covered by zebra mussels was measured. From these area measurements, the percent of each sample frame covered by zebra mussels was calculated. The computer images were also helpful in noting characteristics of the substrate of each sample frame.

**Water Sampling.** A hand-held, plastic, bow-like sampling device was designed and used to simultaneously collect 5 water samples at 0, 2, 4, 10 and 20 cm above the zebra mussel colonies on the wreck surface. Each sample was collected in a 30 ml syringe. On each sampling date and at each site, 6 such sets of samples were collected above horizontal wreck surfaces covered with zebra mussel colonies, and 6 such sets were collected above horizontal surfaces not colonized by zebra mussels. These stations away from zebra mussel colonies served as reference stations. All water samples were brought to the surface where they were transferred to 30 ml sample bottles. The samples were then preserved with 1 ml concentrated nitric acid and refrigerated for later analysis of iron and calcium concentration by Paradigm Analytical Laboratories (Wilmington, NC) using Inductively Coupled Plasma Atomic Emission Spectrometry (EPA Method 6010B).

An additional water sample was collected in a 60 ml syringe at each of the six locations above mussel colonies to determine pH and alkalinity. Once on the surface, the pH of each sample was measured using a Zellweger WP4007 field meter and datalogger. Samples were then returned to the lab, where they were analyzed for alkalinity by titrating 0.02N hydrochloric acid (HCl) into a solution of sample water and two drops of bromcresol green indicator (APHA 1989).

A total of 960 water samples, 96 alkalinity samples and 120 zebra mussel and feces/pseudofeces samples were collected over the entire study period.

**Zebra mussel feces and pseudofeces.** Zebra mussel feces and pseudofeces were collected using 60ml luer-lock syringes with an attached 3cm of tubing to elongate the syringe tip. Eight samples of feces/pseudofeces were taken, four from a horizontal surface and four from a vertical surface. The four horizontal samples were taken from four of the six water sampling locations above mussel colonies described above. The four vertical samples were taken from nearby vertical surfaces. To collect the feces/pseudofeces, the divers slowly pulled back the plunger of the syringe while moving the syringe across the mussels within the sample frame to collect all visible particulate material within the interstices of the zebra mussel colony. These samples were brought to the surface and filtered through pre-rinsed, pre-weighed Whatman 1.5 µm glass microfiber filters. The filtered water was sent to Paradigm Analytical Laboratories and analyzed for iron and calcium concentrations as described above. The filters were returned to the lab, oven dried at 105ºC for 1 hour and weighed to the nearest 0.1 mg using a Sartorius 1773MP8 analytical balance. To determine the organic matter content of the samples, the filters were then ignited at 550ºC for 20 minutes in a muffle furnace and re-weighed.

**Zebra mussel recruitment.** Zebra mussel samples were collected on the same sampling dates as the water and feces/pseudofeces samples and from the same eight horizontal and vertical surfaces described above. The mussels were collected from the sample frames where the feces/pseudofeces samples were taken in order to investigate correlations between zebra mussel colony density and age structure and iron loss.

All of the mussels inside the 15 cm by 15 cm sample frame were scraped from the wreck surface with a putty knife and collected in Ziploc bags. These samples were brought to the surface and frozen for later analysis. Zebra mussels were counted and maximum shell length was measured along the anterior-posterior axis (at the umbo) to the nearest 0.1 mm with a caliper. These measurements were used to graph the age structure and calculate the median shell length of each zebra mussel colony.

**Statistical Analyses**. Statistical analyses were used to explore differences and patterns in the data. All variables except percent cover were log transformed to stabilize variances and approximate a normal distribution. Percent cover was stabilized with an arcsine square root transformation. Simple analysis of variance was used to compare differences in iron concentrations between sample locations over zebra mussel colonies and those away from colonies, and to look for differences in colony abundances between sites. Two- and three-way analyses of variance were used to compare differences in mean iron concentrations over time, across sites, and between orientations (vertical vs. horizontal surface) on the wreck. Pearson's product moment correlations were used to evaluate relationships between variables. All statistical analyses were run on SAS software (Statistical Analysis Systems Institute, 1992) using an experiment-wide error rate of 0.05.

## CALCIUM TOLERANCE EXPERIMENTS

Experiments were set up in the lab to measure the calcium tolerances of zebra mussels. We established a series of concentrations in reconstituted water by adding specific amounts of calcium carbonate, magnesium sulfate, sodium bicarbonate and potassium chloride to Type I purified water. In order to keep the pH, alkalinity, and hardness approximately equal in all treatments, we changed the amount of magnesium sulfate added in an inverse proportion to the calcium carbonate added. The treatments of reconstituted water were divided into four 1000 ml beakers per concentration. In our first experiment, we established treatments with calcium concentrations of 10 mg/L, 20 mg/L and 60 mg/L. In our second experiment, we established four treatments: 10 mg/L, 15 mg/L, 20 mg/L and 60 mg/L.

Zebra mussels were harvested from Shelburne Bay in Shelburne, VT and brought to the lab. Beakers were set up with flat rocks as a settling substrate and equipped with an airline and air stone. The beakers were covered to reduce evaporation. Forty mussels were placed in each beaker. We fed the mussels every day with a solution of green algae (dried *Chlorella*) and water. The water in the beakers was changed every other day to insure that calcium concentrations remained constant. Dead mussels were removed as they were noticed.

After the experiment was completed, the mussels were collected and frozen. We dissected the mussels to determine the percent of the total body mass that was shell. Mussel tissue was separated from the shell and both were dried and weighed. These weights were then compared to get a shell percentage.

## SETTLEMENT EXPERIMENTS

The intent of these experiments was to examine zebra mussel preferences for different substrate types and the effects of zebra mussel colonization on the substrates over time. Substrates were selected to mimic those found on the underwater wrecks in the lake.

All materials were exposed in the lake on a specially constructed table (Figure 3). Each 4-foot by 6-foot table was built with a rebar frame and legs and a table surface of 1-inch diameter mesh plastic VEXAR sheeting. Three replicates of each of eight materials were attached to the table surface in a random order with plastic cable ties. A horizontal array of the substrate types was chosen because that is the primary surface available on most wrecks, and that is the location where the accumulation of fecal and pseudofecal material was assumed to be greatest. Wood and iron materials were assembled in combinations likely to be found on wrecks. Steel plates and rods were included to test for the effect of substrate size and shape.

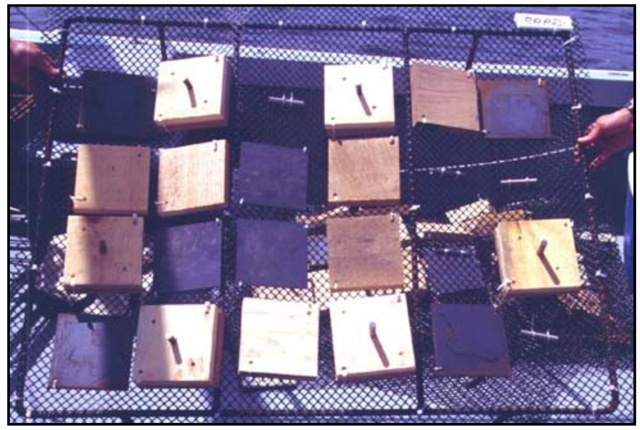

*Figure 3. Settlement table with substrates before deployment.*

The materials used as settling substrates in the experiment included:

1. 15 cm x 15 cm x 2.5 cm blocks of white oak (hardwood).
2. 15 cm x 15 cm x 2.5 cm blocks of white pine (softwood).
3. 15 cm x 15 cm x 5 cm "sandwiched" blocks of hard and soft wood, secured with a wrought iron rod extending about 3 cm above the surface of the soft wood top block.
4. 15 cm x 15 cm x 5 cm "sandwiched" blocks of hard and soft wood, secured with a steel rod extending about 3 cm above the surface of the soft wood top block.
5. 15 cm x 15 cm x 0.64 cm blocks of gray polyvinyl chloride (PVC), a standard monitoring material.
6. 15 cm x 15 cm x 0.5 cm plates of hot-rolled steel.
7. 3-inch rods of wrought iron recovered from Lake Champlain.
8. 3-inch rods of hot rolled steel.

Additional information about these materials appears in last year's report (Watzin et al. 2000). Six tables were deployed at each of the six wreck sites at the beginning of the summer of 1999. Each table was placed so that the horizontal surface of the table was parallel to and about 30 cm off the bottom. The intent was to recover one table at the end of the summer over each of the next 6 years.

This year, one table was recovered at each of the six sites on the final sampling visit in September. Each table was brought aboard the research vessel and photographed completely. The top and bottom of the table and each individual substrate were photographed. Extra photographs were taken of interesting pairings or stark differences. The VEXAR sheeting was then cut around the perimeter of each substrate, removing it from the table. The substrate and VEXAR were put in the Ziploc bag and frozen for further analysis.

In the lab, the remaining substrates were thawed for analysis of settling preference and effects of colonization. The mussels were removed and counted. As they were being removed, the appropriate surface to which they were attached was noted (top of substrate, bottom, sides, post, hard wood vs. soft wood where applicable). This was done in an attempt to determine not only preference of substrate for settling, but also orientation on that substrate. The totals of each surface were then combined for a grand total of each substrate.

**Detailed Analysis of Corrosion on Selected Iron Pieces.** Iron corrosion analysis was performed on three substrates from each of the four main sampling sites (*General Butler*, North Beach, *Champlain II*, and Diamond Island). Two iron rods and one soft wood/hard wood "sandwich" with an iron post were separated from the settlement substrates and submerged in lake water in Tupperware containers. These substrates were then taken to Massachusetts Materials Research in Worcester, MA for analysis of the corrosion.

The iron rod and post samples were analyzed through a series of examinations: a visual examination on a macro scale, a binocular examination under a microscope at a magnification of up to 50x, a metallurgical evaluation under an optical microscope, and an x-ray diffraction analysis (XRD). For the visual and binocular examinations, all samples were analyzed. The metallurgical evaluation was only performed on two samples from each location. The XRD was also performed on two samples. For the metallurgical evaluation, the samples were cross-sectioned in the transverse direction, taking care not to disrupt the scale on the surface of the iron. The cross sections were then encased in plastic, ground and polished to facilitate the optical microscope examination. Deposits were removed from each of these samples for analysis by energy dispersive x-ray spectroscopy (EDS). EDS is a semi-quantitative micro-chemical analysis technique for determining elemental composition. EDS uses equipment attached to a scanning electron microscope (SEM). The analyses were performed in both the standard and "light element" (LE) modes. The LE mode is more sensitive to elements with lower atomic weights. Two layers of scale were taken from each sample for the EDS, the outermost layer and the layer closest to the base metal (MMR 2001).

**Bacterial Analysis.** Water samples for bacterial analysis were also taken at the *General Butler* and the *Champlain II* during the settlement table recovery. *General Butler* and *Champlain II* were chosen because divers believed they could more easily take samples from the zebra mussel mat covering pieces of iron on these sites as opposed to the North Beach and Diamond Island sites. Two bacterial samples were taken at each site by the process described in the feces/pseudofeces section above. On the *General Butler*, one sample was taken from the stern steering gear and the other from the bow windless. On the *Champlain II*, one was taken from the sternpost and the other from the X-frame for the engine mount. These samples were taken as close to the surface of the iron as possible in the interstices of the zebra mussel colony. One sample was also taken at each site approximately 50 centimeters above the mussel colony for a water column control.

Once on the surface, bacterial culture media provided by CC Technologies (Dublin, OH) was inoculated with the water collected from the mussel colonies in a series of dilutions. Media included standard preparations for each of the following five bacteria types: aerobic, acid-producing, anaerobic, iron-depositing and sulfate-reducing. The five bottles for each bacteria type contained cultures specific to that type of bacteria.

Using a sterile syringe, 1 ml was drawn from the water samples. This 1 ml subsample was injected into the first bottle in an inverted position. After mixing by repeated movement of the syringe plunger, 1 ml of the media was removed from the first bottle and injected into the second bottle. This process was continued sequentially to inoculate the three remaining bottles. A new sterile syringe was used for each set of bacteria culture bottles (CC Technologies 2000).

The bottles were then packaged and shipped to CC Technologies where they were incubated. All bottles were incubated at room temperature for a minimum of 28 days to allow cultures to develop. CC Technologies recorded the bacterial count and the elapsed days for a positive response observation for each bottle.

**Statistical Analyses.** Statistical analyses were used to explore differences in recruitment patterns between substrate types. In order to make direct comparisons between substrates types of different sizes, counts were converted to densities. Densities were log transformed to stabilize variances and approximate a normal distribution. Sites were initially combined in a two-way analysis of variance using site and substrate as the main effects. Because of some significant interactions, sites were also analyzed separately using one-way analysis of variance followed by SNK to separate the substrate types. Again, all analyses were run on SAS software (Statistical Analysis Systems Institute, 1992) using an experiment-wide error rate of 0.05.

## POT ASH GRATE RECOVERY

Before the grate was disturbed, water samples and feces/pseudofeces samples were collected through the processes described in the sections above. Additional interstitial water samples were collected to culture bacteria as described above. A fragment of scale, collected from the grate once it was on the surface, was also used to establish bacterial cultures.

**Recovery.** To remove the grate from the wreck, a plastic tray was carefully slipped under the grate (Figure 4). The tray and grate were then lifted by ropes attached at each corner of the lifting tray. Additional care was taken in lifting the grate from the water onto the deck of the research vessel *Melosira*. The transition from water to air is often the most dangerous time in the recovery of a waterlogged artifact.

*Figure 4. Divers prepare to recover the grate from the Pot Ash wreck.*

Once on the deck of the *Melosira*, the grate was submerged in lake water and thoroughly photographed. It was completely covered with mussels (Figure 5). To test for the presence of iron sulfide corrosion products, and thus microbially mediated anaerobic corrosion, a few drops of 12 M hydrochloric acid (HCl) was dripped onto the grate after a patch of zebra mussels was removed. An odor of hydrogen sulfide indicates the presence of the sulfate.

Once the grate was returned to the LCMM, all of the zebra mussels were removed from the grate. All of the mussels were saved and weighed to estimate the total amount of biomass on the grate. Both wet weight and dry weight were determined. A sample of the mussels was separated to determine mussel colony density and age structure. Once the mussels were removed, the thickness and extent of the scale was estimated.

*Figure 5. Pot Ash iron grate after removal from wreck. Note complete coverage of the surface by zebra mussels.*

**Restoration.** Staff at the LCMM performed the restoration of the grate itself. The long-term preservation of historic material recovered from submerged sites is a vital component of the archaeological process. Objects that have been underwater for a long period of time undergo dramatic changes when they are once again exposed to an air environment. Unless they are properly treated after their recovery, important information can be lost. In the case of the Pot Ash Point grate, two materials, the wooden frame and the cast iron grate, required two separate conservation treatments to preserve the artifact.

**Wooden Frame Conservation.** The conservation of waterlogged wood is a particularly delicate matter. Dramatic changes occur in the cellular structure of wood that is submerged for long periods of time. Bacteria and water action break down and carry away the interior structures of wood cells leaving only the lignin framework of the wood behind. This wood can still retain substantial strength as long as it is kept wet. However, if allowed to dry, the lignin structure often collapses causing dramatic shrinkage and warping of the artifact.

In order to counter this effect it is necessary for conservators to introduce a substance into the cellular structure of the wood that will support it when the water is removed. Preservation of the wood from the Pot Ash Point grate involved bulking the wood with pine rosin dissolved into isopropyl alcohol. This treatment required that the artifact first be completely dehydrated by placing it into several baths of alcohol. When it was determined that all water in the wood cells was replaced with alcohol, chunks of pine rosin were dissolved into the alcohol until a solution of 67% rosin (by weight) was reached. After soaking in this solution for a number of weeks, the wood was removed and the excess rosin solution was wiped from the surface with clean rags. As the remaining alcohol in the wood evaporated, the rosin hardened and supported the structure of the wood. This process typically produces an artifact that is very strong and stable.

**Cast Iron Grate Conservation.** On examination of the grate aboard the research vessel, the extreme fragility of the artifact became apparent. In attempting to turn the grate over, the iron portion separated from the wooden frame. Unsupported by its original structure, the artifact began to come apart, and was soon in several pieces, a problem that would surface repeatedly throughout the grate's conservation.

The cast iron portion of the artifact underwent an entirely different set of treatments. It was at this stage that the poor quality and condition of the cast iron became most apparent. Cast iron from the mid-nineteenth century was of notoriously poor quality, and its preservation on submerged sites is rarely as good as wrought iron of the same period. Over time cast iron slowly breaks down and turns into very brittle graphite. Within the grate itself areas were found that retained substantial strength and metal content while others had converted almost entirely to graphite. Even when being most careful and patient it was not unusual for a section of the iron to fracture.

The long term preservation of iron focuses on removing any corrosion present and treating the metal to prevent the formation of rust in the future. Preliminary conservation involved the careful removal of the thick layer of corrosion present on all metal surfaces of the grate. This was very delicate work involving the use of dental picks and soft bristle brushes.

Following the removal of the corrosion layer from the surface of the metal it was treated with tannic acid. Tannic acid reacts with iron forming a thin black layer on the surface of the metal. This coating (ferric tannate) acts as a barrier, isolating the remaining metal from the oxygen and moisture that could cause future corrosion outbreaks. An additional protective coating was added to the iron portions of the grate by submerging them into molten wax that had been heated to 300 degrees Fahrenheit. This high temperature rapidly evaporated any moisture remaining in the artifact, and when allowed to cool the wax formed an additional moisture/oxygen barrier. If stored in a climate controlled facility and carefully handled, an artifact treated in this manner will remain stable for many years.

## FISH PREDATION PILOT STUDY

Two trips were made to the railroad drawboat site in Bulwagga Bay in August 2000. A Benthos Minirover remotely operated vehicle (ROV) provided by Mr. Fred Doane and Paradigm Analytical Laboratories was used to observe fish on the site (Figure 6). For a three-day period, the ROV was deployed at dawn and dusk, common feeding times for most fish species. The ROV is equipped with a closed-circuit, high resolution, low light video camera that allowed us not only to see what the ROV saw, but also to record it. The ROV was positioned on the deck of the draw barge near a clump of zebra mussels. We then completely shut down the thrusters, and turned off the navigation lights. We then simply recorded what the camera saw in an attempt to document fish behavior and record which fish might be eating the zebra mussels.

*Figure 6. Benthos Minirover remotely operated vehicle (ROV).*

Using hook and line, we also caught fish on site and checked the gut contents. We did this to try to find evidence of zebra mussel feeding in the guts of fish in the area. A gill net was also deployed later in the season to target fish not collected by hook and line.

# RESULTS

## CORROSION STUDIES

**Photodocumentation.** As we reported last year, zebra mussels substantially covered the shipwrecks at all six sampling sites. Although the distribution of mussels on the wrecks was patchy, it also showed several patterns (see sections below), including concentrations of mussels on and around metal fittings and fastenings, and generally greater cover on vertical surfaces than on horizontal surfaces.

**Water Sampling.** No significant differences were found in pH or alkalinity among the six sampling sites. The pH ranged from 7.06 to 8.28 with no clear pattern in relation to sampling date or site. Alkalinity ranged from 40 to 72 mg $CaCO_3$/L, with the higher values more commonly found in the southern sections of the lake.

Calcium concentrations were generally higher at the southern sampling sites as compared to those in the north. Concentrations ranged from 11.3 to 21.0 mg/L. There was no pattern across the sampling dates or based on position in the water column.

Iron concentrations varied greatly between locations above zebra mussel colonies and those away from colonies and with position in the water column. Iron concentrations were always higher above zebra mussel colonies than above surfaces not colonized by mussels. Concentrations at both locations were almost always highest in the lowest water sample, collected immediately above the wreck surface. Water samples collected at 2, 4, 10, and 20 cm above the wreck generally had progressively lower concentrations than those measured at 0 cm.

A three-way ANOVA relating iron concentration to sampling date, site (wreck location) and location on the wreck (above zebra mussels vs. away from zebra mussels) was significant for all three main effects (Table 1). Location relative to zebra mussels was highly significant.

| Source | DF | F Value | Pr > F |
|---|---|---|---|
| Model | 31 | 3.29 | <.0001 |
| Date | 2 | 6.17 | 0.0027 |
| Site | 5 | 2.46 | 0.0360 |
| Type | 1 | 30.36 | <.0001 |
| Date*Site | 8 | 2.68 | 0.0090 |
| Site*Type | 5 | 1.21 | 0.3052 |
| Date*Site*Type | 10 | 1.55 | 0.1266 |

*Table 1. Results of overall analysis of variance relating iron concentration to date, site (wreck location) and type (above colony or away).*

Like last year, the concentrations of iron above the wreck surfaces colonized by zebra mussels was significantly greater than that found above surfaces not colonized by mussels (Figure 7). Across all sites, iron concentrations above mussel colonies averaged 1.85 mg/L, while iron concentrations above sites away from zebra mussels averaged 0.54 mg/L. All four of our main wreck sites showed this pattern (Figure 8), and the differences were statistically significant at three of the four sites (*General Butler* p=0.0037, *Champlain II* p=0.0370, and Diamond Island p=0.0004).

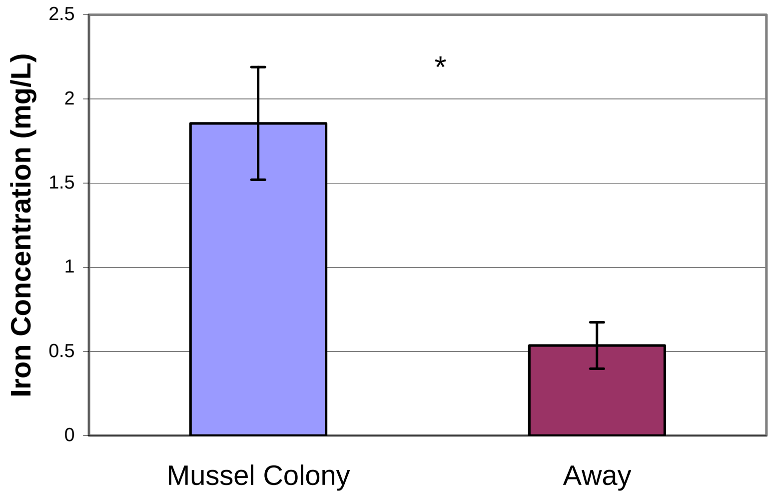


*Figure 7. Comparison of the mean concentrations of iron above wreck surfaces colonized by mussels ("mussel colony") and surfaces not colonized by mussels ("away"). Error bars represent one standard error above and below the mean. Means were significantly different at p=0.0001.*

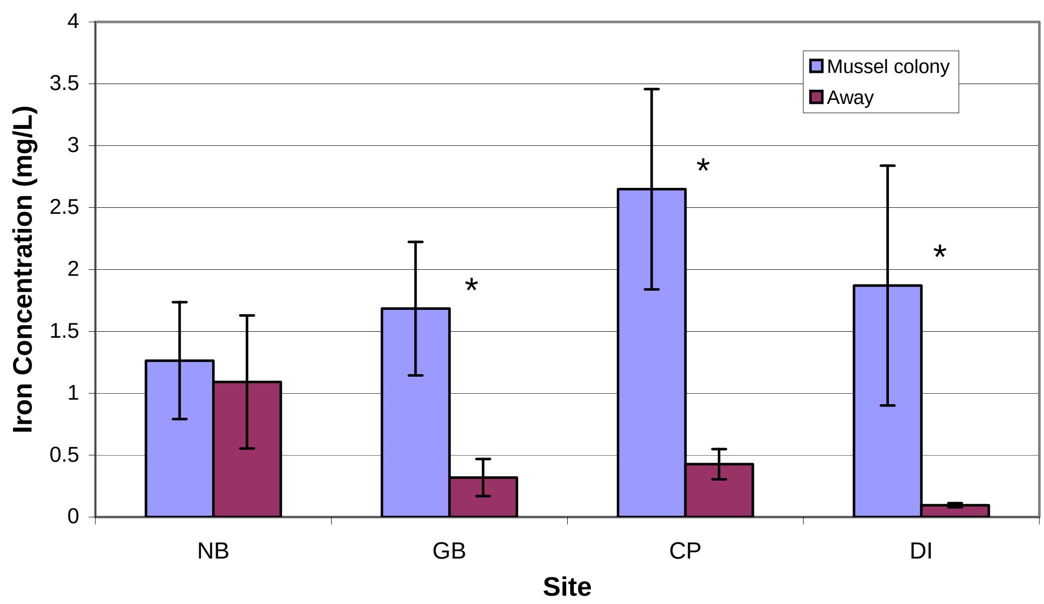


*Figure 8. Comparison of the mean iron concentrations above wreck surfaces colonized by zebra mussels ("mussel colony") and surfaces not colonized by mussels ("away") for each of the four main sampling sites. Error bars represent one standard error above and below the mean. Means were significantly different at the General Butler (p=0.0037), the Champlain II (p=0.0370) and Diamond Island (p=0.0004). NB = North Beach, GB = General Butler, CP = Champlain II, and DI = Diamond Island.*

A comparison of the iron concentrations found in the 0 cm water samples collected in 1999 to those collected in 2000 is presented in Figure 9. While both years showed significant differences between locations above zebra mussel colonies and those away from zebra mussel colonies (p=0.0001 for both), the iron concentrations measured in 2000 were much higher for both locations.

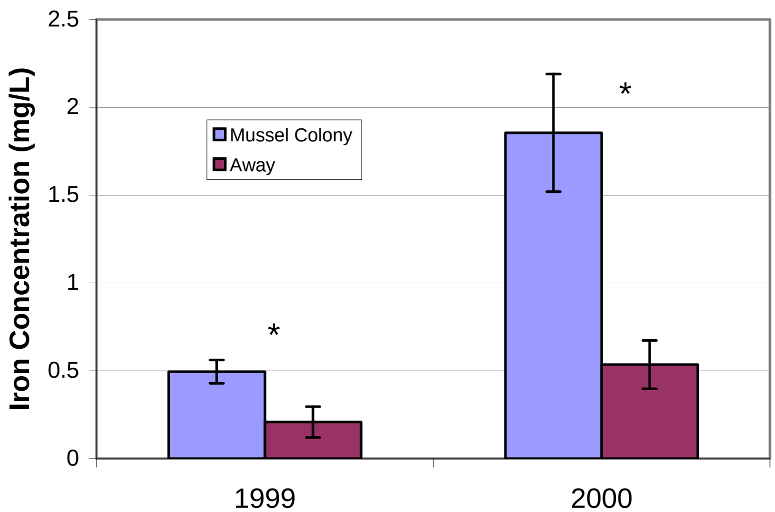


*Figure 9. Comparison of mean iron concentrations above wreck surfaces colonized by mussels ("mussel colony") and surfaces not colonized by mussels ("away") from 1999 to 2000. Error bars represent one standard error above and below the mean. Means were significantly different at p=0.0001 for 1999 and 2000.*

The differences in iron concentrations measured at 0, 2, 4, 10, and 20 cm above the surface of the wreck are still being analyzed. A vertical gradient of iron concentrations was observed for both sampling types (Figure 10). Gradients were clearer and steeper for samples collected above zebra mussel colonies. Most of the concentration gradients declined to background levels that were near the detection limit for iron (0.03 mg/L) by or before 10 cm above the wreck surface.

The purpose of collecting these samples was to estimate an iron loss rate. Such estimation requires a large number of assumptions about the behavior of iron under the mussel mat and the conditions in the water above the mussels. To be most accurate, it also requires three or more measurements above background in each profile. In many cases, we did not meet this requirement. We are currently working with an environmental chemist to develop an iron behavior model for each site and apply it to the data as appropriate.

**Zebra Mussel Feces and Pseudofeces.** Analysis of total suspended solids (TSS) and organic matter loss-on-ignition (LOI) revealed higher concentrations of suspended sediments and organic matter on horizontal surfaces than on vertical surfaces. However, orientation (horizontal versus vertical) was only significant for TSS (p=0.0125). Orientation was not significant for LOI.

In a Pearson's Correlation analysis (Table 2), TSS and LOI were significantly correlated (p=0.0001) on both horizontal and vertical surfaces. TSS and LOI were also correlated with the iron concentrations measured in the water collected with the feces/pseudofeces samples. However, neither TSS nor LOI were significantly correlated with the number of zebra mussels collected within each frame, mean major axis length of zebra mussels in the frame, or percent cover of each frame by zebra mussels.

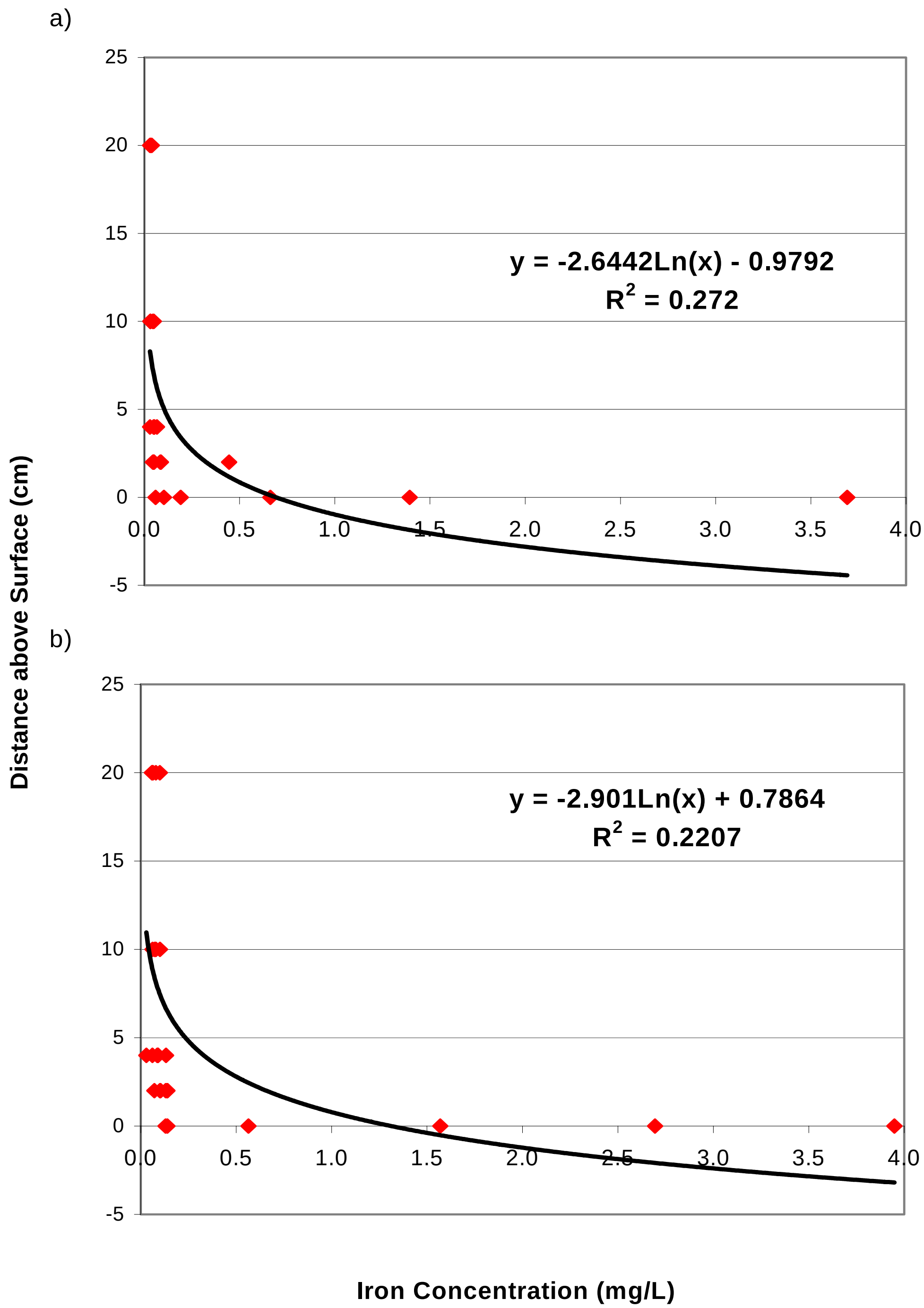


*Figure 10. Example concentration gradients for iron measured in water samples collected above surfaces of wrecks colonized by mussels. Each point represents a single concentration at a given point above the surface. Trendlines were fit to the individual points as a whole and are not based on linked gradients for individual sets of samples. a) North Beach, July 2000, b) Diamond Island, July 2000.*

The correlation analysis was also performed with the iron concentrations from the 0 cm water samples, rather than the iron concentrations measured from the feces/pseudofeces samples. These samples were not subjected to any filtering and were, therefore, a more accurate measurement of iron concentrations in the water. Iron concentration was again significantly correlated with TSS and LOI at p=0.0001, but was not correlated with total number of mussels, mean length or percent cover.

*Table 2. r-values, significance levels and sample sizes associated with Pearson's correlations between iron concentration, total suspended solids (TSS), organic matter loss-on-ignition (LOI), number of mussels per frame (number), mean length of mussels per frame (length), and the percent of the frame covered by zebra mussels (% cover) for horizontal and vertical frames. Significant correlations at the p=0.0500 level are shown in bold text.*

| | Horizontal | | | | | | Vertical | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Iron** | **TSS** | **LOI** | **Number** | **Length** | **% Cover** | **Iron** | **TSS** | **LOI** | **Number** | **Length** | **% Cover** |
| **Iron** | 1.00000 | 0.67011 | 0.61674 | -0.00621 | -0.10056 | -0.12780 | 1.00000 | 0.65860 | 0.38034 | 0.12085 | 0.15317 | 0.08987 |
| | 0.0000 | 0.0001 | 0.0001 | 0.9681 | 0.5160 | 0.4320 | 0.0000 | 0.0001 | 0.0070 | 0.4082 | 0.2934 | 0.5572 |
| | 44 | 44 | 44 | 44 | 44 | 40 | 49 | 49 | 49 | 49 | 49 | 45 |
| **TSS** | **0.67011** | 1.00000 | 0.95611 | -0.15787 | 0.15593 | -0.29801 | **0.65860** | 1.00000 | 0.77156 | -0.07162 | 0.03290 | 0.00231 |
| | **0.0001** | 0.0000 | 0.0001 | 0.2735 | 0.2796 | 0.0443 | **0.0001** | 0.0000 | 0.0001 | 0.6175 | 0.8187 | 0.9877 |
| | **44** | 50 | 50 | 50 | 50 | 46 | **49** | 51 | 51 | 51 | 51 | 49 |
| **LOI** | **0.61674** | **0.95611** | 1.00000 | -0.10413 | 0.04832 | -0.27236 | **0.38034** | **0.77156** | 1.00000 | -0.11724 | -0.04851 | 0.03942 |
| | **0.0001** | **0.0001** | 0.0000 | 0.4717 | 0.7389 | 0.0671 | **0.0070** | **0.0001** | 0.0000 | 0.4126 | 0.7353 | 0.7925 |
| | **44** | **50** | 50 | 50 | 50 | 46 | **49** | **51** | 51 | 51 | 51 | 47 |
| **Number** | -0.00621 | -0.15787 | -0.10413 | 1.00000 | -0.38799 | 0.75415 | 0.12085 | -0.07162 | -0.11724 | 1.00000 | 0.18638 | 0.32885 |
| | 0.9681 | 0.2735 | 0.4717 | 0.0000 | 0.0045 | 0.0001 | 0.4082 | 0.6175 | 0.4126 | 0.0000 | 0.1859 | 0.0225 |
| | 44 | 50 | 50 | 52 | 52 | 48 | 49 | 51 | 51 | 52 | 52 | 48 |
| **Length** | -0.10056 | 0.15593 | 0.04832 | **-0.38799** | 1.00000 | 0.13943 | 0.15317 | 0.03290 | -0.04851 | 0.18638 | 1.00000 | 0.34386 |
| | 0.5160 | 0.2796 | 0.7389 | **0.0045** | 0.0000 | 0.3446 | 0.2934 | 0.8187 | 0.7353 | 0.1859 | 0.0000 | 0.0167 |
| | 44 | 50 | 50 | **52** | 52 | 48 | 49 | 51 | 51 | 52 | 52 | 48 |
| **% Cover** | -0.12780 | **-0.29801** | -0.27236 | **0.75415** | 0.13943 | 1.00000 | 0.08987 | 0.00231 | 0.03942 | **0.32885** | **0.34386** | 1.00000 |
| | 0.4320 | **0.0443** | 0.0671 | **0.0001** | 0.3446 | 0.0000 | 0.5572 | 0.9877 | 0.7925 | **0.0225** | **0.0167** | 0.0000 |
| | 40 | **46** | 46 | **48** | 48 | 48 | 45 | 49 | 47 | **48** | **48** | 48 |

**Zebra mussel recruitment.** Zebra mussel recruitment varied substantially across sites and with orientation. The maximum number of mussels found in one frame was 809, while some frames contained as few as 14 mussels. In a three-way ANOVA (Table 3), both site and orientation were highly significant (p=0.0006 and p=0.0001, respectively) while date was not significant in explaining differences in the number of mussels per frame. In general, the southern wreck sites had more mussels than the more northerly sites.

When each site was analyzed individually, mean number of mussels per frame was higher for the vertical orientation for every site except *Champlain II*. Orientation was highly significant for both North Beach and *General Butler* (p=0.0001 and p=0.0004, respectively). Diamond Island, Pot Ash, and Rouse's Point all had higher means on the vertical orientation, but the differences were not significant (Figure 11).

| Source | DF | F Value | Pr > F |
|---|---|---|---|
| Model | 31 | 3.04 | <.0001 |
| Date | 2 | 0.09 | 0.9181 |
| Site | 5 | 4.78 | 0.0006 |
| Orientation | 1 | 18.35 | <.0001 |
| Date*Site | 8 | 0.60 | 0.7750 |
| Date*Orientation | 2 | 0.45 | 0.6376 |
| Site*Orientation | 5 | 7.12 | <.0001 |
| Date*Site*Orientation | 8 | 0.95 | 0.4784 |

*Table 3. Results of three-way analysis of variance relating number of individuals per 15 x 15 cm frame to date, site and orientation (horizontal or vertical).*

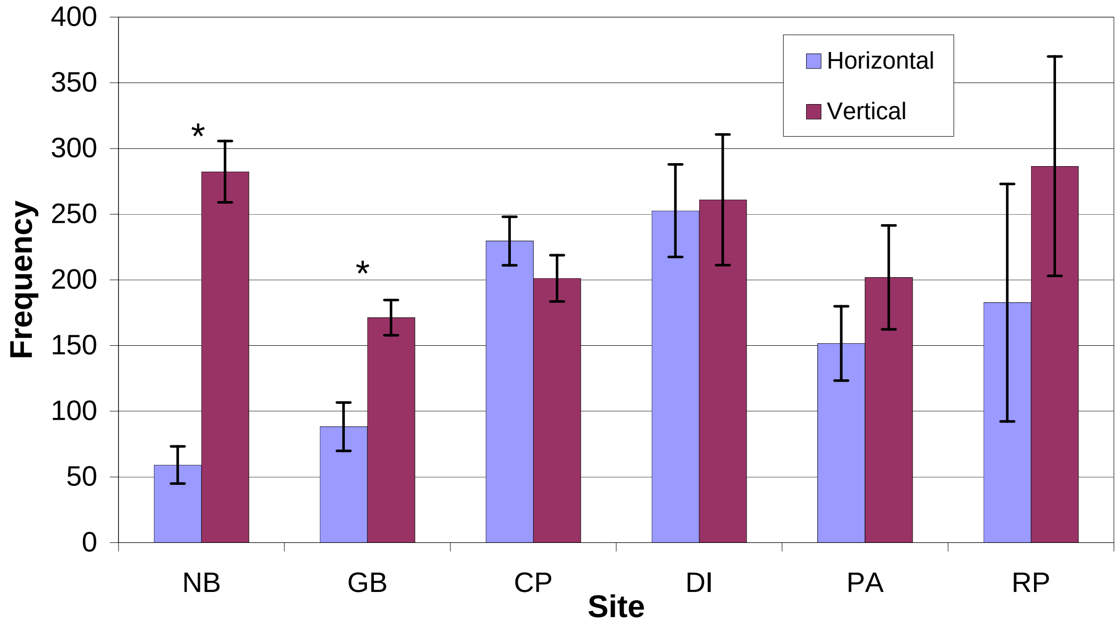


*Figure 11. Comparison of mean number of mussels per frame on horizontal and vertical surfaces of wrecks for all sites. Error bars represent one standard error above and below the mean. Means were significantly different at North Beach (p=0.0001) and General Butler (p=0.0004). Note higher numbers of mussels on vertical surfaces at all sites except Champlain II. NB = North Beach, GB = General Butler, CP = Champlain II, DI = Diamond Island, PA = Pot Ash Point, and RP = Rouse's Point.*

The same patterns are apparent when analyzing the percent of the 15 cm x 15 cm frames covered by zebra mussels. In a one-way analysis of variance (ANOVA) using data from all six sampling sites, percent cover on vertical surfaces was significantly greater than percent cover on horizontal surfaces (p=0.0001, Figure 12). Orientation was also significant for each of the six sampling sites when tested individually.

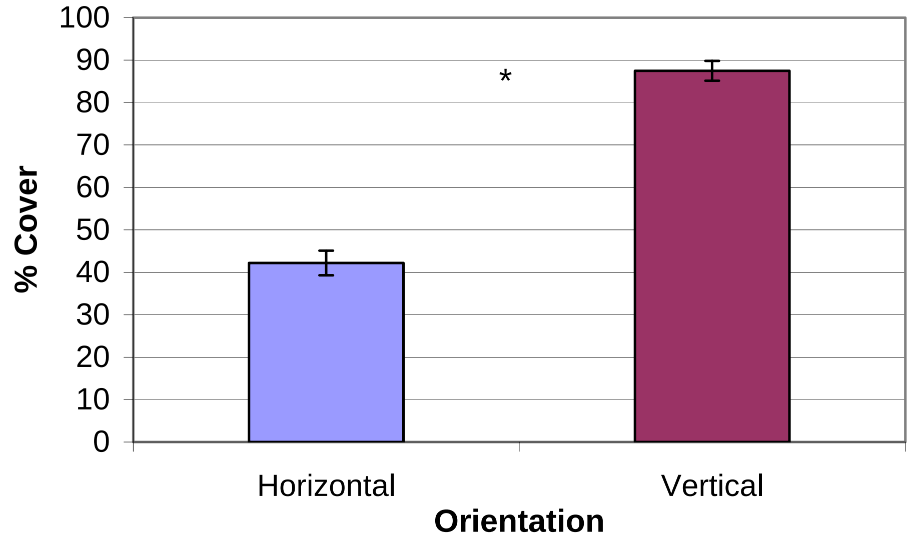


*Figure 12. Comparison of mean percent cover on horizontal and vertical surfaces using data from all six shipwreck sites. Error bars represent one standard error above and below the mean. Means were significantly different at p=0.0001.*

The Pearson's Correlation analysis described above revealed a significant correlation between the number of individuals and percent cover at both orientations. In horizontal samples, total number of mussels per frame was highly correlated with percent cover at p=0.0001. In the vertical orientation, mussels per frame and percent cover were significantly correlated at p=0.0225.

The size distribution of mussels was found to vary across date, site and orientation. The length of mussels ranged from 1.2 mm to 45.2 mm. Mussels under 10 mm in size represent mussels settled from larvae in the summer of 2000. Mussels larger than 38-40 mm are probably at least four years old. In Lake Champlain, zebra mussels seem to live 4-5 years, although mortality is significant in every year class.

In a three-way ANOVA examining mean lengths per frame, date, site and orientation were all highly significant (p=0.0001, p=0.0001, and p=0.0010, respectively) in explaining differences in mean length (Table 4). Mean lengths per frame were calculated through the measurement of all mussels collected in each frame. The smallest mussels were generally seen at Rouse's Point, the northernmost site.

Generally, larger mussels were present in vertical frames than horizontal frames. A comparison of mean shell length per horizontal frame versus mean shell length per vertical frame using data pooled across dates and sites was marginally significant at p=0.0604.

| Source | DF | F Value | Pr > F |
|---|---|---|---|
| Model | 31 | 11.37 | <.0001 |
| Date | 2 | 31.04 | <.0001 |
| Site | 5 | 38.84 | <.0001 |
| Orientation | 1 | 11.48 | 0.0010 |
| Date*Site | 8 | 2.67 | 0.0107 |
| Date*Orientation | 2 | 1.50 | 0.2289 |
| Site*Orientation | 5 | 6.56 | <.0001 |
| Date*Site*Orientation | 8 | 1.13 | 0.3533 |

*Table 4. Results of three-way analysis of variance relating mean length of zebra mussels per 15 x 15 cm frame to date, site and orientation.*

Mussel samples collected showed an obvious difference in size distribution across the three sampling dates. While the mean lengths were not necessarily different for each date, the distribution of those lengths resulted in clear patterns. Mussel lengths generally showed two peaks in size distributions (Figure 13), one of mussels between 5 and 15 mm and one between 20 and 30 mm. For each site and date, all samples were pooled to give a representative view of the size distribution at that site and date. In Figure 13, an increase in numbers at larger sizes is noticeable as the date progresses towards the end of the summer. In the graph for the final sampling date, a spike at the 0-4 mm length is clear, signaling the presence of the newest year class of zebra mussels.

## CALCIUM TOLERANCE EXPERIMENTS

The calcium tolerance experiments are on-going and no results or interpretation have been generated as of this time. Shell and tissue weights and analyses will continue into the summer. When this work is completed, we will start statistical analysis and interpretation. Based on the interpretation of results at that time, we will decide what additional follow-up is warranted.

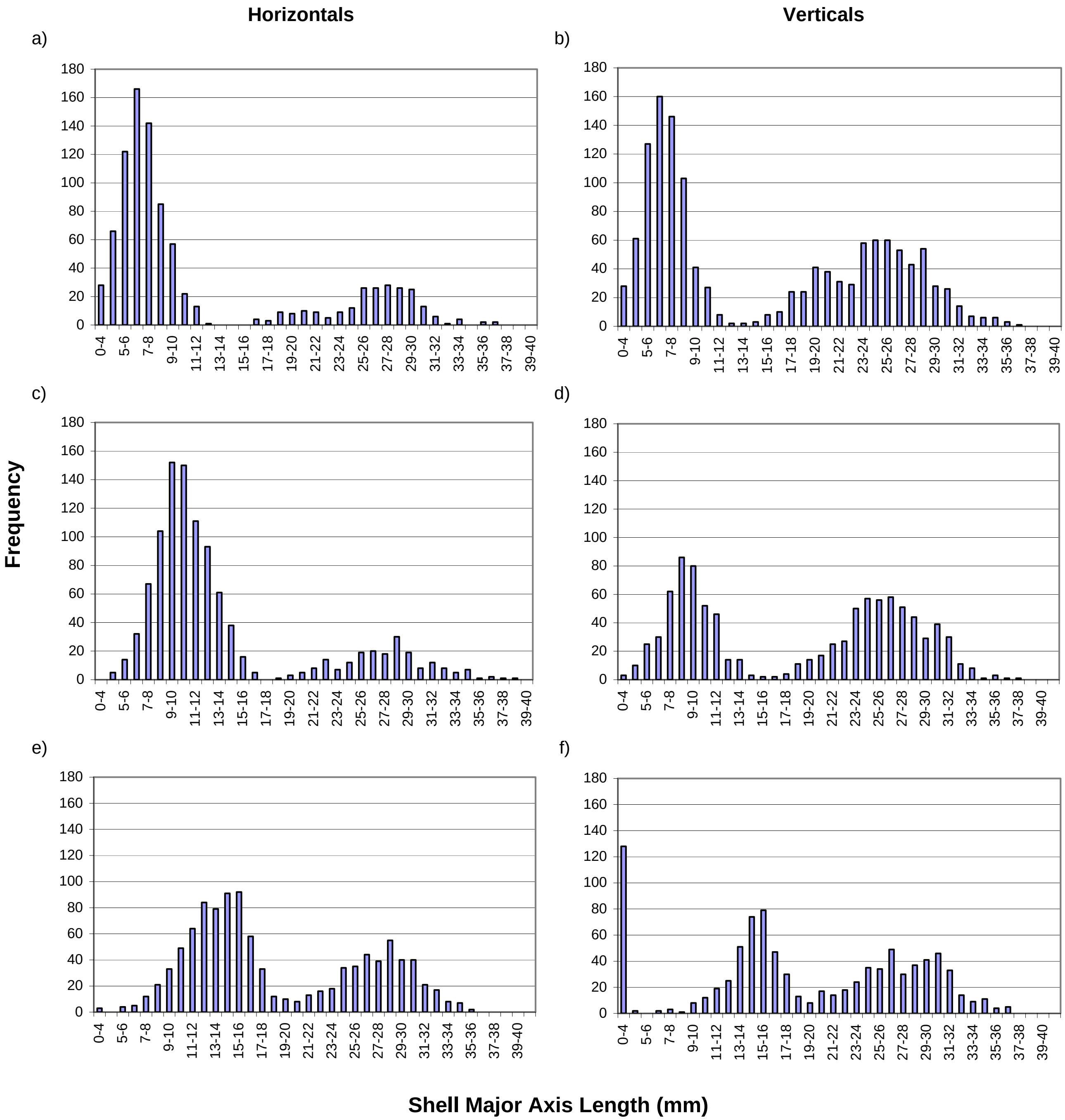


*Figure 13. Size distribution over time of zebra mussels from Diamond Island stone boat. Graphs on the left (a, c, e) represent the sum of the horizontal 15 x 15 cm frames collected at Diamond Island for each sampling date. Graphs on the right (b, d, f) represent the sum of the vertical frames collected at Diamond Island for each date. a) horizontal frames from June 2000, b) vertical frames from June 2000, c) horizontal frames from July 2000, d) vertical frames from July 2000, e) horizontal frames from September 2000, and f) vertical frames from September 2000. Note general increase in numbers of larger sizes over the course of the summer. Vertical frames generally had more mussels of larger size. Also note the large number of mussels in the 0-4 mm length range in f.*

## SETTLEMENT EXPERIMENTS

**Table Recovery and Substrate Differences.** Four of the sites, North Beach, *General Butler*, Diamond Island, and Rouse's Point, showed substantial zebra mussel settlement on the experimental tables. The tables at the Pot Ash and *Champlain II* sites were not well settled. The table at the Pot Ash wreck site had sunk into the mud, which obviously interfered with recruitment. The table at the *Champlain II* site, although not sunk into the substrate, had also become substantially covered with mud. The total number of mussels settled on all substrates was over 12,000 for North Beach, *General Butler*, and Diamond Island (Figure 14) and close to 10,000 for Rouse's Point. The total number of mussels was only 350 at Pot Ash and most of the substrates had no mussels on them at all. This was inconsistent with the data collected on the wreck itself, therefore, we removed the settlement data from Pot Ash from most of the statistical analyses. Mussels on the *Champlain II* table numbered close to 4,000 so it was included in many analyses.

*Figure 14. Diamond Island settlement table.*

Settlement densities were calculated for each site and substrate (for examples, see Figure 15). A two-way ANOVA relating settlement densities to site and substrate was highly significant (Table 5a). Because the interaction between site and substrate was highly significant, an ANOVA was run on each site individually. In these analyses, substrate was highly significant in explaining settlement differences at all sites except *Champlain II*. Substrate was only marginally significant at *Champlain II* (Table 5b).

To determine differences and similarities between settlement densities, the wood sandwiches were separated from the six other substrates. This was done because the sandwiches are composites of three different substrate materials, hard wood, soft wood and either steel or iron rods. Therefore, these materials were compared separately while the six remaining uniform substrates, steel rod, iron rod, hard wood, soft wood, plastic (PVC) plate and steel plate, were analyzed together.

In general, steel and iron rods showed higher densities than the other substrates. Hard wood, soft wood, and PVC all had densities of approximately 1 mussel per square centimeter. Density on the steel plate was significantly lower than all of the other substrates.

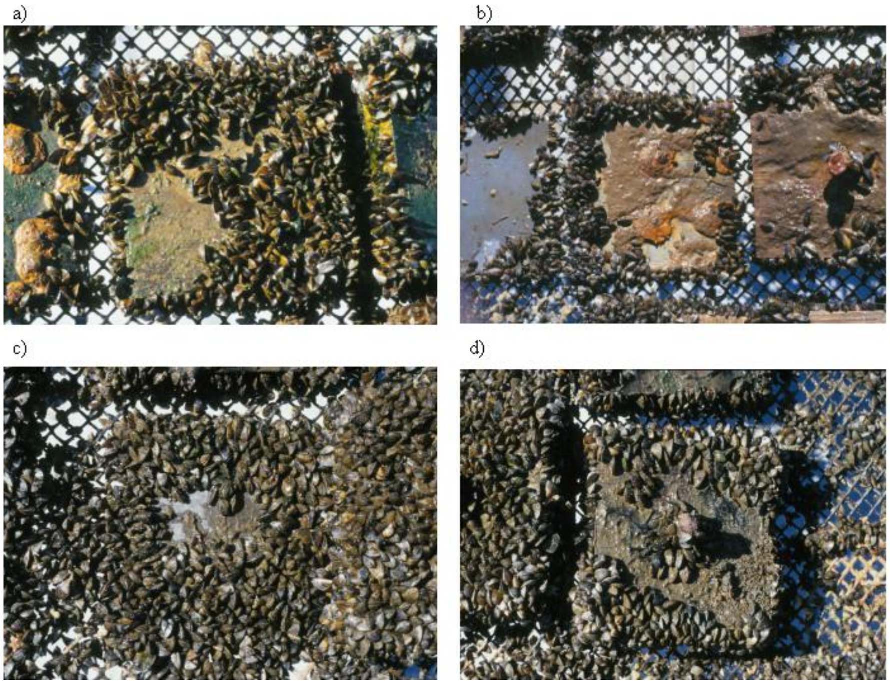


*Figure 15. Examples of settlement patterns on a) soft wood from North Beach, b) steel plate from General Butler, c) PVC plate from Diamond Island, d) wood sandwich with steel post from Diamond Island.*

**a) Sites Pooled**

| Source | DF | F Value | Pr > F |
|---|---|---|---|
| Model | 47 | 36.77 | <.0001 |
| Site | 5 | 284.43 | <.0001 |
| Substrate | 7 | 21.65 | <.0001 |
| Site*Substrate | 35 | 4.42 | <.0001 |

**b) Sites Individually**

| Source | DF | F Value | Pr > F |
|---|---|---|---|
| Substrate | | | |
| a) North Beach | 7 | 5.81 | 0.0017 |
| b) *General Butler* | 7 | 23.22 | <.0001 |
| c) *Champlain II* | 7 | 2.44 | 0.0665 |
| d) Diamond Island | 7 | 10.60 | <.0001 |
| e) Pot Ash Point | 7 | 16.65 | <.0001 |
| f) Rouse's Point | 7 | 3.82 | 0.0126 |

*Table 5. a) Results of analysis of variance relating settlement density to site and substrate. b) Results of analysis of variance relating settlement density to substrate for each site.*

At North Beach and the *General Butler*, steel rods had higher densities than iron rods (Figure 16). At Diamond Island, Rouses Point, and *Champlain II* (not graphed because densities were very low), iron rods had the highest densities (Figure 17). At all sites, the steel plates had the lowest settlement density, while densities on hard wood, soft wood and PVC were very similar.

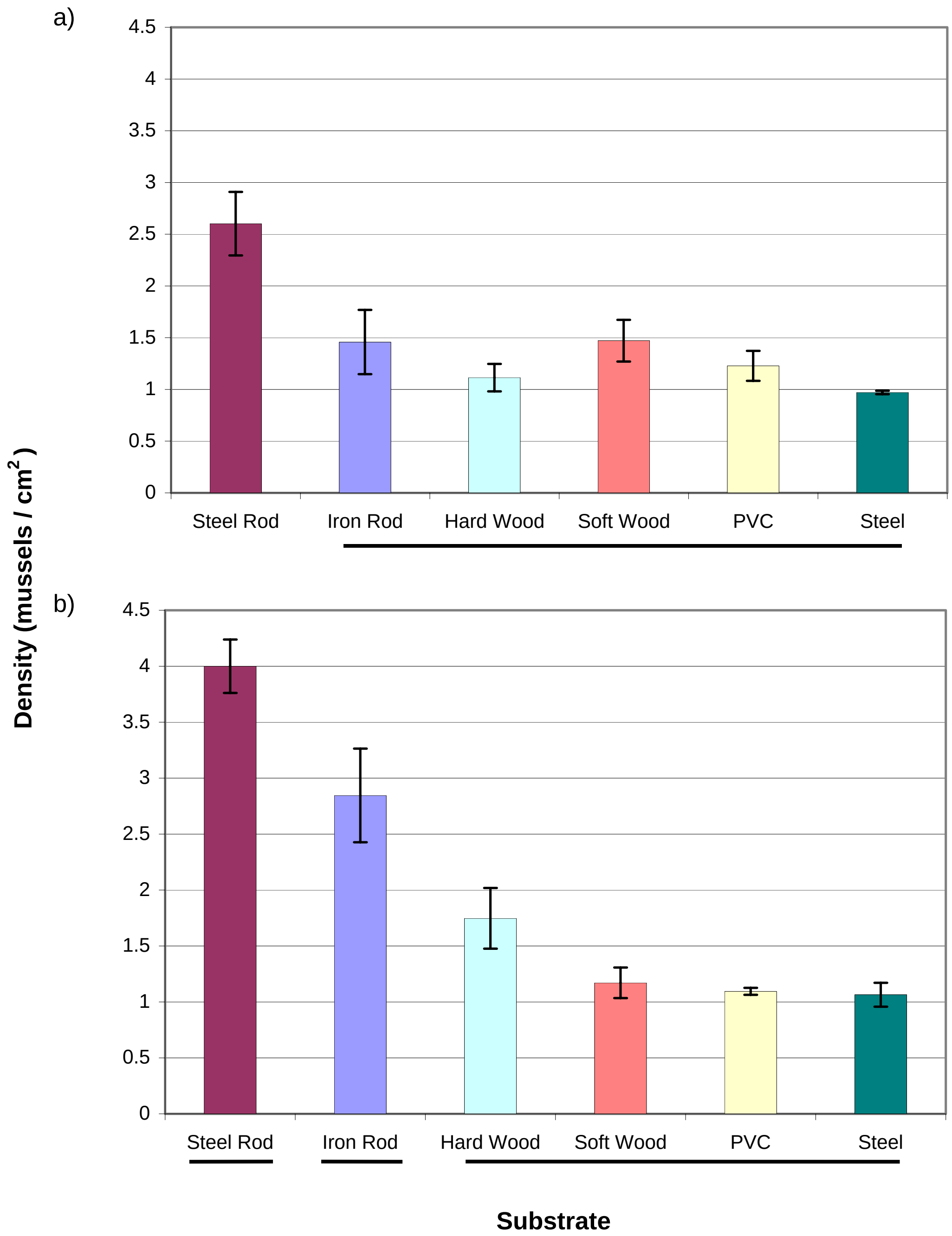


*Figure 16. Comparison of settlement density on the six uniform substrates at a) North Beach and b) General Butler. Error bars represent one standard error above and below the mean. Bars below the x-axis connect substrates that were not significantly different in SNK. Note highest densities on steel and iron rods.*

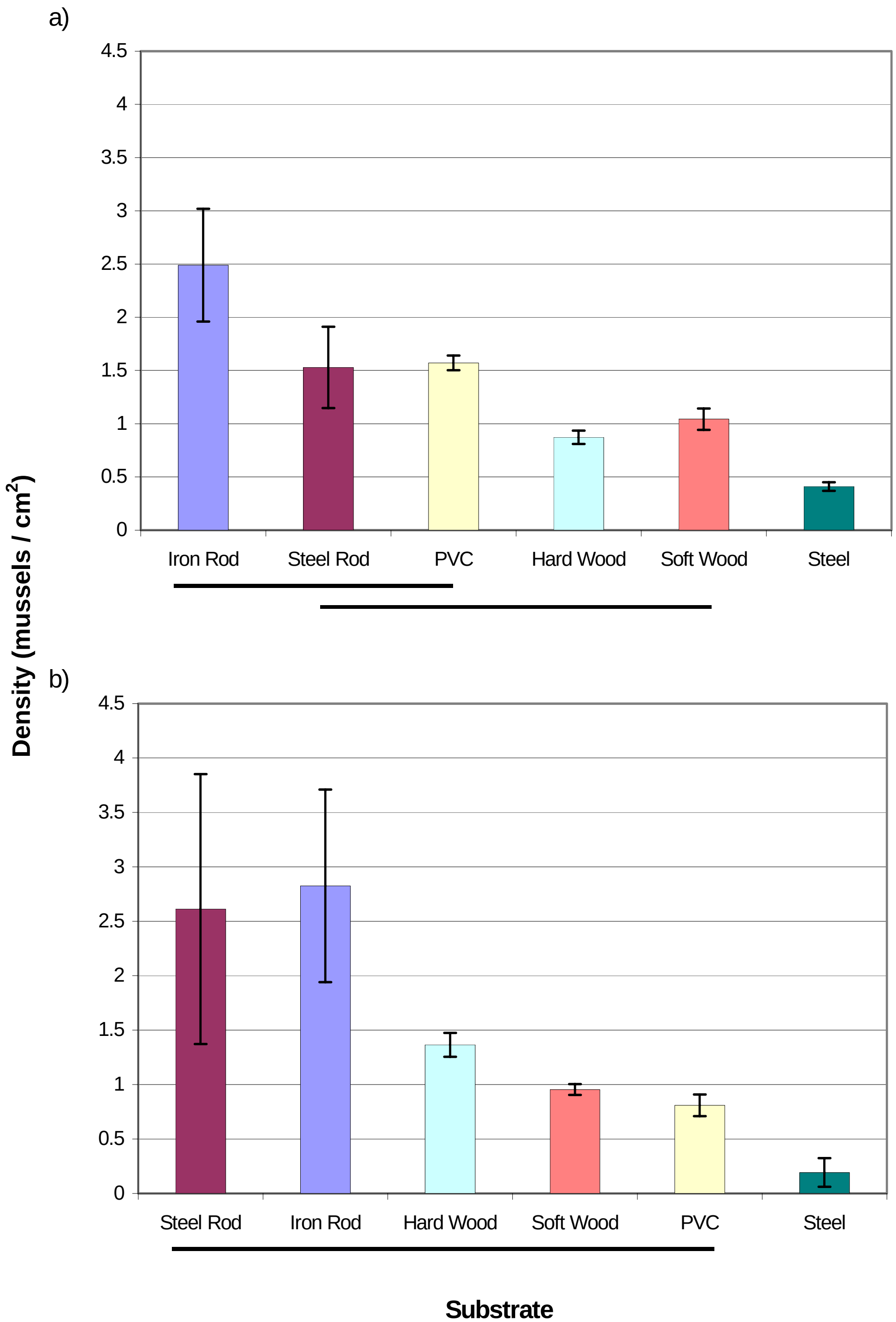


*Figure 17. Comparison of settlement density on the six uniform substrates at a) Diamond Island and b) Rouse's Point. Error bars represent one standard error above and below the mean. Bars below the x-axis connect substrates that were not significantly different in SNK. Note highest densities on steel and iron rods.*

Settlement densities were also compared on the tops and bottoms of substrates to determine if zebra mussels settled more densely on one or the other. Hard wood, soft wood, PVC and steel plates were used for these analyses because all four had equal surface areas available on the top and the bottom. In a three-way ANOVA (Table 6), site (excluding Pot Ash), substrate type and surface (top or bottom) were all highly significant in explaining settlement differences.

When we compared top and bottom densities on a site-by-site basis, we found that bottom density was almost always higher than top density, especially at the shallower sites (Figure 18). Rouse's Point was the exception to this pattern; here the top density was higher for every substrate, with significant differences on hard wood and soft wood.

| Source | DF | F Value | Pr > F |
|---|---|---|---|
| Model | 27 | 7.36 | <.0001 |
| Site | 4 | 20.34 | <.0001 |
| Substrate | 3 | 10.62 | <.0001 |
| Surface | 1 | 19.43 | <.0001 |
| Site*Substrate | 13 | 3.00 | 0.0022 |
| Substrate*Surface | 3 | 0.22 | 0.6641 |

*Table 6. Results of analysis of variance relating settlement density to site, substrate, and surface (top or bottom).*

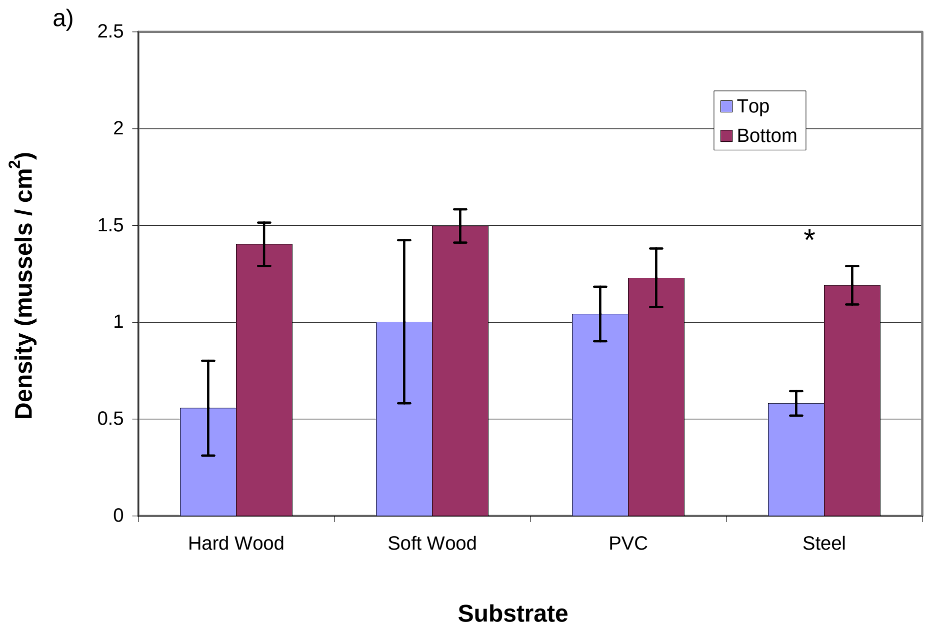


*Figure 18a. Comparison of settlement density on tops and bottoms of the four substrates with equal top and bottom surface area at North Beach. Note that bottom density is generally higher on all substrates. Error bars represent one standard error above and below the mean.*
** indicates a significant difference between top and bottom density for that substrate.*

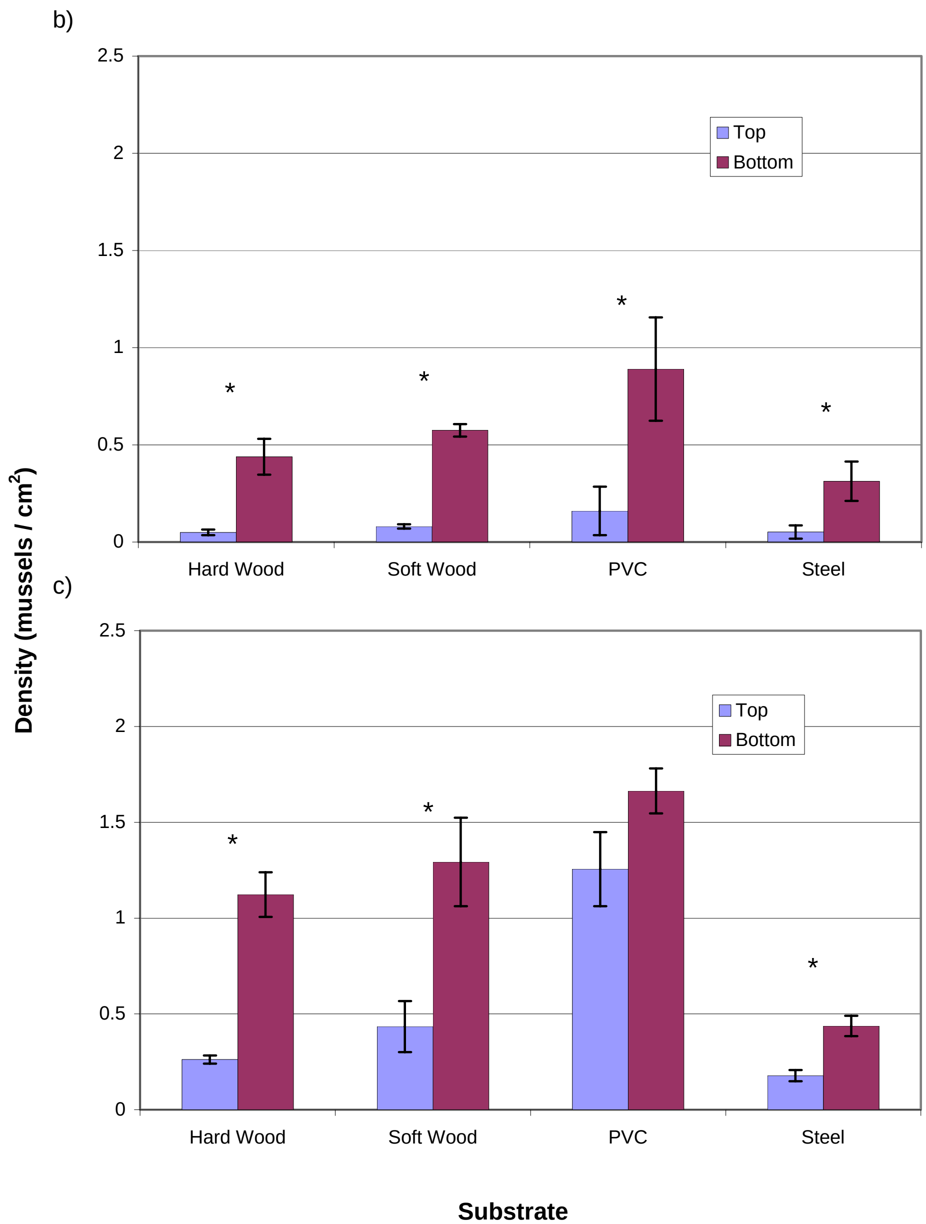


*Figure 18b, 18c. Comparison of settlement density on tops and bottoms of the four substrates with equal top and bottom surface area at b) Champlain II and c) Diamond Island. Note that bottom density is generally higher for all and substrates. Error bars represent one standard error above and below the mean. * indicates a significant difference between top and bottom density for that substrate.*

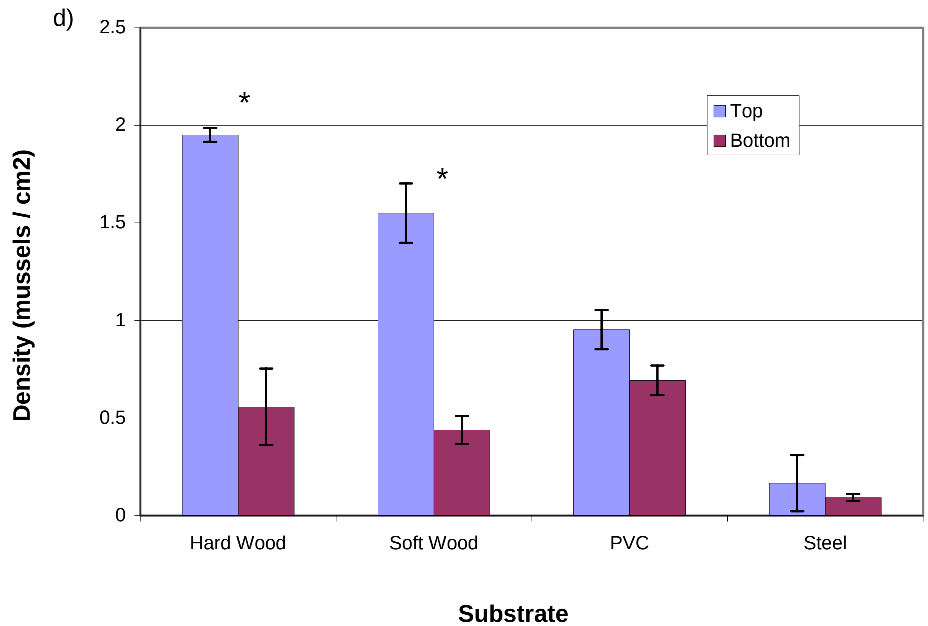


*Figure 18d. Comparison of settlement density on tops and bottoms of the four substrates with equal top and bottom surface area at Rouse's Point. Note that top density is higher on all substrates. Error bars represent one standard error above and below the mean. * indicates a significant difference between top and bottom density for that substrate.*

The wood sandwiches presented an opportunity to compare settlement on the horizontally oriented iron and steel rods to the vertically oriented iron and steel posts. Densities were generally higher on the rods than the posts. A two-way ANOVA comparing orientation (post versus rod) and material (steel versus iron) was run on each site individually. Orientation was significant in explaining differences in settlement densities at *General Butler* ($p=0.0023$), with more mussels settling on the rods than the posts. Material was marginally significant at *Champlain II* ($p=0.0705$), with more mussels on iron posts than steel posts and more mussels on steel rods than iron rods.

Because patterns with material appeared to be opposite on posts and rods, separate one-way ANOVAs were performed by site on the posts and rods to measure the significance of material. *Champlain II* was the only site where material was significant in explaining differences for the posts. Material was marginally significant in explaining settlement differences on the rods at North Beach and *General Butler*. No other site showed significant differences in either orientation or material, but in general, densities were higher on iron posts and than steel posts (Figure 19a) and higher on steel rods than iron rods (Figure 19b).

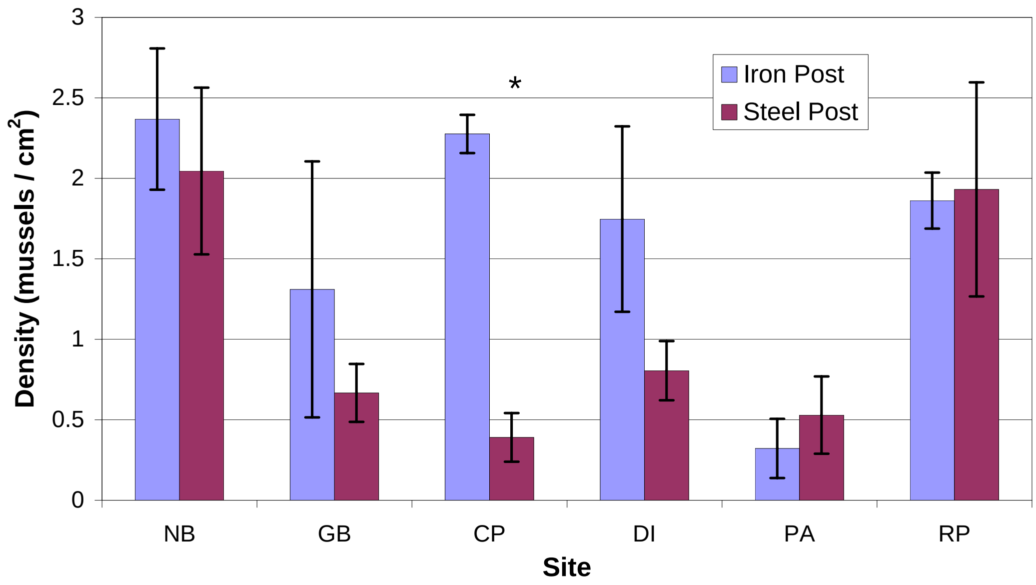


*Figure 19a. Comparison of mean settlement densities on iron posts versus steel posts by site. Note the generally higher concentrations on iron posts. Error bars represent one standard error above and below the mean. Means were significantly different for Champlain II at p=0.0066.*

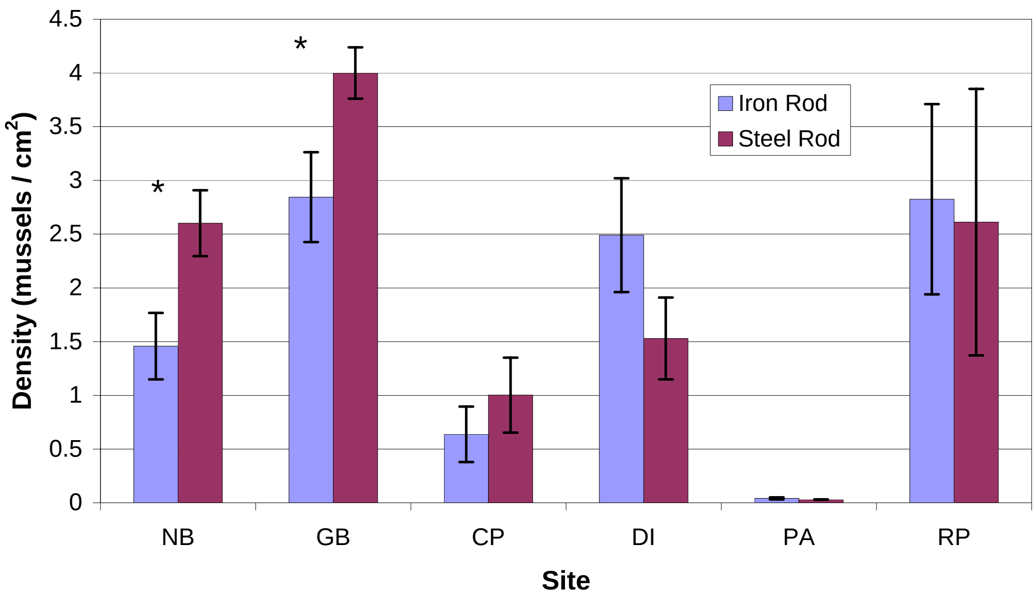


*Figure 19b. Comparison of mean settlement densities on iron rods versus steel rods by site. Note the generally higher concentrations on steel rods. Error bars represent one standard error above and below the mean. Means were marginally significantly different for North Beach at p=0.0674 and General Butler at p=0.0779.*

**Detailed Iron Corrosion Analysis.** Massachusetts Materials Research, Inc. (MMR) of Worcester, MA provided detailed analyses of the corrosion present on selected iron posts and rods. Two iron rods and one sandwich with an iron post from each of the four main sampling sites (*General Butler*, North Beach, *Champlain II*, and Diamond Island) were sent for examination.

A summary of the findings of the visual, binocular microscope, and metallurgical optical microscope examinations for each iron sample follows (MMR 2001).

*General Butler* # 1 – Iron Rod

- Visual Examination – Most of the rod was covered with zebra mussels.
- Binocular Examination – In general, the entire rod was rust colored and covered with thick deposits and films. Some pitting was observed in random locations (Figure 20).
- Metallurgical Examination – The general corrosion was not severe and most likely started as pitting corrosion and then became general attack type corrosion. The deepest depth of corrosion was measured to be 0.010 in. The microstructure of the material consisted of ferrite grains with random inclusions (Figure 21).

*Figure 20. General Butler #1 iron rod with pitting corrosion. Magnification 6.5x.*

*General Butler* # 8 – Iron Rod

- Visual – The rod was almost completely covered with mussels.
- Binocular – Rust colored deposits were present over the surface of the entire rod. Byssal threads and whitish crystal deposits were observed on the surface.
- Metallurgical – No metallurgical analysis was performed on this sample.

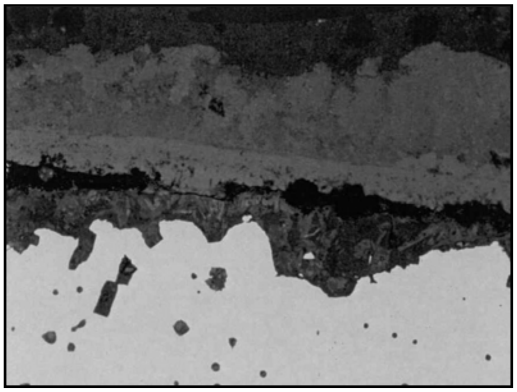

*Figure 21. Cross section of General Butler #1 iron rod. Note the three layers present in the scale. Magnification 500x.*

*General Butler* # 9 – Wood Sandwich with Iron Post

- Visual – The iron post was covered with mussels.
- Binocular – The post was bumpy and covered with thick, rust-colored corrosion deposits.
- Metallurgical – There was general corrosion all around the surface of the post. The corrosion started as pitting and then became general corrosion. The maximum depth of corrosion was 0.010 in. The microstructure mostly consisted of ferrite grains with areas of both ferrite and pearlite structure.

North Beach # 4 – Iron Rod

- Visual – Most of the mussels were attached to the top of the rod.
- Binocular – A significant amount of corrosion was present on the entire surface of the rod. The corrosion deposits varied from rust colored to dark gray color.
- Metallurgical – No metallurgical analysis was performed on this sample.

North Beach # 11 – Wood Sandwich with Iron Post

- Visual – The entire post was covered with mussels.
- Binocular – Significant corrosion was present on the iron post. The color varied from rust to dark gray. Heavy gray deposits were present at the interface between the iron post and the wood. Byssal threads and white crystals were present on the rust colored areas of the post.
- Metallurgical – The post had general type attack corrosion initiated as pitting corrosion. Many inclusions were present at the surface. A thick oxide layer (0.017 in) was present. The general microstructure consisted of ferrite grains with inclusions. Maximum pitting depth was 0.010 in (Figure 22).

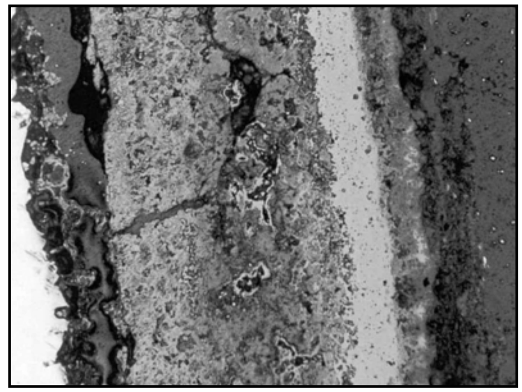

*Figure 22. Cross section of North Beach #11 iron post. Note the layers of scale present. Magnification 200x.*

North Beach # 15 – Iron Rod

- Visual – More mussels were present on the top than the bottom.
- Binocular – Significant amounts of rust-colored deposits were present over the entire surface of the rod. Some light yellow colored deposits were observed on the front of the rod. The overall corrosion appeared to be in the longitudinal direction.
- Metallurgical – There were many deep pits on the rod. The general surface showed less severe corrosion than other samples. The maximum depth of pitting was 0.030 in. The scale was not significantly thick. The microstructure revealed some areas of ferrite grains, some areas of ferrite mixed with pearlite, and some inclusions.

*Figure 23. Champlain II #17 iron rod. Arrow points to corrosion in the longitudinal direction under the scale. Magnification 6.5x.*

*Champlain II* # 17 – Iron Rod

- Visual – Very few mussels attached to the rod.
- Binocular – Rust colored and grayish colored deposits were present. The crusts were significantly thick on the surface. Different layers of deposits were noticeable (Figure 23).

- Metallurgical – There was a significant amount of corrosion and many deep pits. Many of the pits had depths of 0.030 in. Many large inclusions were present all over the sample. The microstructure consisted of ferrite grains with large inclusions/slags (Figure 24).

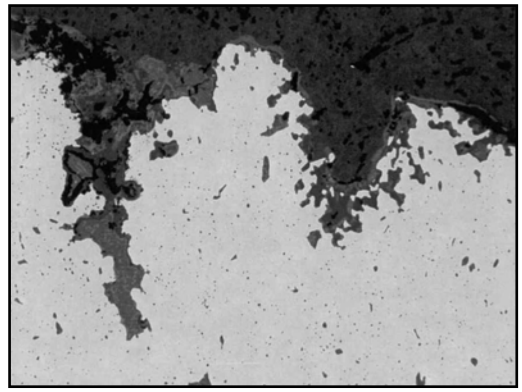

*Figure 24. Cross section of Champlain #17 iron rod showing pitting and general corrosion. Magnification 100x.*

*Champlain II* # 19 – Wood Sandwich with Iron Post

- Visual – The post was almost completely covered with mussels.
- Binocular – A thick crust of rust colored and grayish colored deposits were present all over the post.
- Metallurgical – There was not a significant amount of corrosion at the surface. Many small areas of pitting were observed along with many large inclusions. The microstructure consisted of ferrite grains with large random inclusions. In a few areas, a mixture of ferrite and pearlite grains was observed (Figure 25).

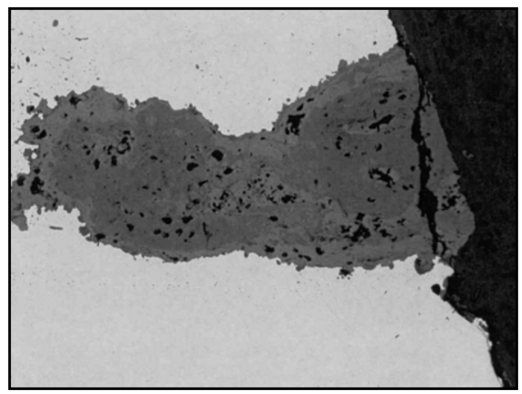

*Figure 25. Deep pitting type corrosion on surface of Champlain #19 iron post. Magnification 100x.*

*Champlain II* # 22 – Iron Rod

- Visual – The top of the rod was covered with mussels while the bottom was not.
- Binocular – Rust colored and grayish colored deposits were present on the rod but the crust was thinner than in most other samples.
- Metallurgical – No metallurgical analysis was performed on this sample.

Diamond Island # 5 – Wood Sandwich with Iron Post

- Visual – The post and wood were completely covered with mussels.
- Binocular – The sample had an overall dark gray appearance. Thick, rust colored deposits were present at the post and wood interface. The top layer of the deposits was rust colored and the deposit on the base metal was darker red. Small white crystals and byssal threads were present all over the post.
- Metallurgical – There was general pitting attack on the surface to depths of 0.030 in.

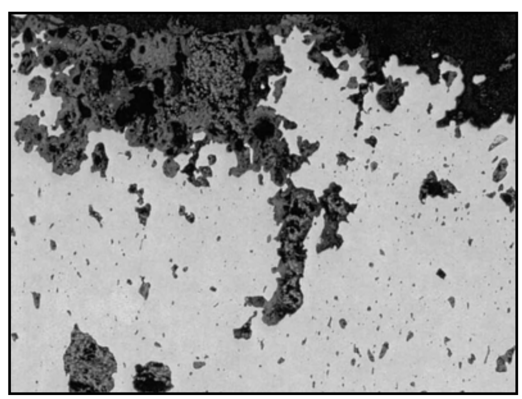

*Figure 26. Cross section of Diamond Island #5 iron post. Note pitting and general attack corrosion. Magnification 100x.*

in many areas. Many small inclusions were present all over the surfaces. There were three layers of thick scale, about 0.05 inches in many areas. The microstructure consisted of ferrite grains with small random inclusions (Figure 26).

Diamond Island # 11 – Iron Rod

- Visual – Most of the mussels were on the sides and bottom of the rod.
- Binocular – The rod had rust colored to light yellow and light gray colored deposits all over. In some areas, dark reddish deposits were observed.
- Metallurgical – The rod had less corrosion than other samples. Large pits were only observed in a few isolated areas but had a maximum depth of 0.050 in. The corrosion appears to have started as a general attack on the entire surface and not as pitting. The microstructure consisted of ferrite grains, some pearlite and some small inclusions.

Diamond Island # 16 – Iron Rod

- Visual – The top of the rod was covered with only a few mussels on the bottom.
- Binocular – Dark, rust colored deposits were present all over the rod. In some areas, the thick crust had broken off.
- Metallurgical – No metallurgical analysis was performed on this sample.

**X-ray Diffraction Analysis.** The two samples analyzed by XRD were Diamond Island #5 iron post and North Beach #11 iron post. The two samples showed similar patterns of scale and gave similar XRD results. Iron oxides and hydroxides were the primary compounds detected in the scale of both samples. The different variations of iron oxides and hydroxides are consistent with iron material exposed to an aqueous environment. Generally two forms of FeO(OH) were observed that differed only in their structure. Similarly, two forms of $Fe_3O_4$ were found with cubic structures but different symmetry patterns (MMR 2001).

Semi-quantitative analyses of the XRD results for the Diamond Island sample are given below. Scale on the North Beach sample revealed similar results.

| | |
|---|---|
| FeO(OH) – Lepidocrocite | 42% +/- 5% weight fractions |
| FeO(OH) – Goethite | 23% +/- 5% weight fractions |
| $Fe(OH)_3$ | 21% +/- 5% weight fractions |
| $Fe_3O_4$ – cubic fcc form | 6% +/- 3% weight fractions |
| $Fe_3O_4$ – diamond form | 5% +/- 3% weight fractions |

Only trace amounts of iron sulfide(s) were found in the corrosion debris analyzed. The EDS elemental analysis confirmed this finding. MMR was unable to make a final conclusion on the thermodynamics of the formation of the different forms of the oxides/hydroxides detected in the scale deposits (MMR 2001).

**Bacterial Analysis.** Results of the bacterial cultures from samples taken over both the *General Butler* and the *Champlain II* are summarized in Tables 7a and 7b. At both sites, aerobic, anaerobic, and acid-producing bacteria were common (greater than 1000 bacteria per milliliter in culture). For all three of these groups, numbers were substantially higher in the mussel mat samples than in the respective control samples ("Water Column"). Only the *General Butler* sample contained small numbers of sulfate-reducing bacteria (between 1 and 10 per milliliter of water). The control samples for each site were similar except that the *General Butler* site had more acid-producing bacteria and less aerobic bacteria than the *Champlain II* samples. Iron-depositing bacteria were absent or rare at both sites and all locations (CC Technologies 2000).

*Table 7a. Summary of results of bacteria culture inoculations from the General Butler following 30 days of incubation. Incubation and bacteria counts performed by CC Technologies (2000).*

| **Type of Bacteria** | **Water Column Bacteria/mL; (days)** | ***General Butler* # 1 Bacteria/mL; (days)** | ***General Butler* # 2 Bacteria/mL; (days)** |
|---|---|---|---|
| Aerobic | 1 - 10 (2)<br>10 - 100 (9) | > 1000 (2) | > 1000 (2) |
| Acid-Producing | 100 - 1000 (2) | > 1000 (2) | > 1000 (2) |
| Anaerobic | 100 - 1000 (2) | > 1000 (2) | > 1000 (2) |
| Iron-Depositing | < 1 (30) | < 1 (30) | < 1 (30) |
| Sulfate-Reducing, (SRB) | < 1 (30) | 1 - 10 (15) | < 1 (30) |

*Table 7b. Summary of results of bacteria culture inoculations from the Champlain II following 30 days of incubation. Incubation and bacteria counts performed by CC Technologies (2000).*

| **Type of Bacteria** | **Water Column Bacteria/mL; (days)** | ***Champlain II* # 1 Bacteria/mL; (days)** | ***Champlain II* # 2 Bacteria/mL; (days)** |
|---|---|---|---|
| Aerobic | 1 - 10 (1)<br>> 1000 (7) | 100 - 1000 (1)<br>> 1000 (7) | 100 - 1000 (1)<br>> 1000 (7) |
| Acid-Producing | 1 - 10 (1)<br>10 - 100 (7) | > 1000 (7) | > 1000 (7) |
| Anaerobic | 1 - 10 (1)<br>10 - 100 (7)<br>100 - 1000 (21) | > 1000 (7) | > 1000 (7) |
| Iron-Depositing | < 1 (30) | < 1 (30) | < 1 (30) |
| Sulfate-Reducing, (SRB) | < 1 (30) | < 1 (30) | < 1 (30) |

## POT ASH IRON GRATE RECOVERY

All of the mussels were removed from the grate and weighed (Figure 27). The wet weight of all the mussels on the grate was 6.534 kg, the dry weight was 3.440 kg. The size distribution of the sample of mussels taken from the grate indicated a well-established colony dominated by older individuals (Figure 28). There was a higher percentage of larger mussels on the grate than in the samples collected from the wooden surfaces of the wreck where the iron appears only as fasteners. In separate t-tests, the mean size of the mussels on the grate was compared to the mean size of the mussels collected from the shipwreck during the initial and final sampling dates. In both cases, the mussels from the grate were significantly larger than the mussels from the shipwreck (p=0.0001 for both).

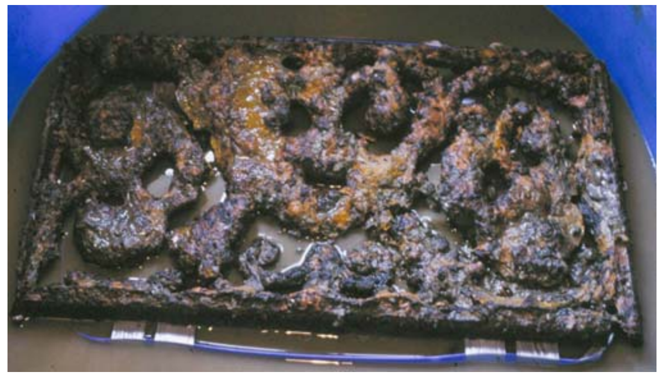

*Figure 27. Pot Ash iron grate after all zebra mussels were removed.*

The iron concentrations in the water above the Pot Ash grate showed a strong gradient with elevation above the material (Figure 29). The highest iron concentrations were seen in the 0 cm samples.

The bacterial assays showed similar abundances and groups of bacteria to those found on the Champlain II and General Butler (Table 8). For most of the bacteria types, the mat and scale samples contained more viable bacteria than the water column sample. The mat sample contained over ten times more aerobic and acid-producing bacteria than the water column sample. It also contained colonies of sulfate-reducing bacteria. The scale sample contained higher concentrations of aerobic and anaerobic bacteria than the control sample. Again, no iron-depositing bacteria were found in any of the samples.

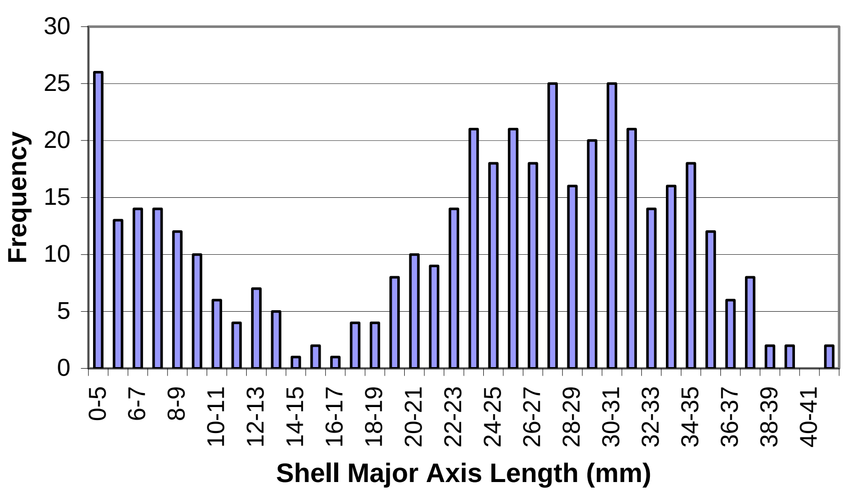


*Figure 28. Size distribution of a sample of zebra mussels collected from the surface of the Pot Ash iron grate.*

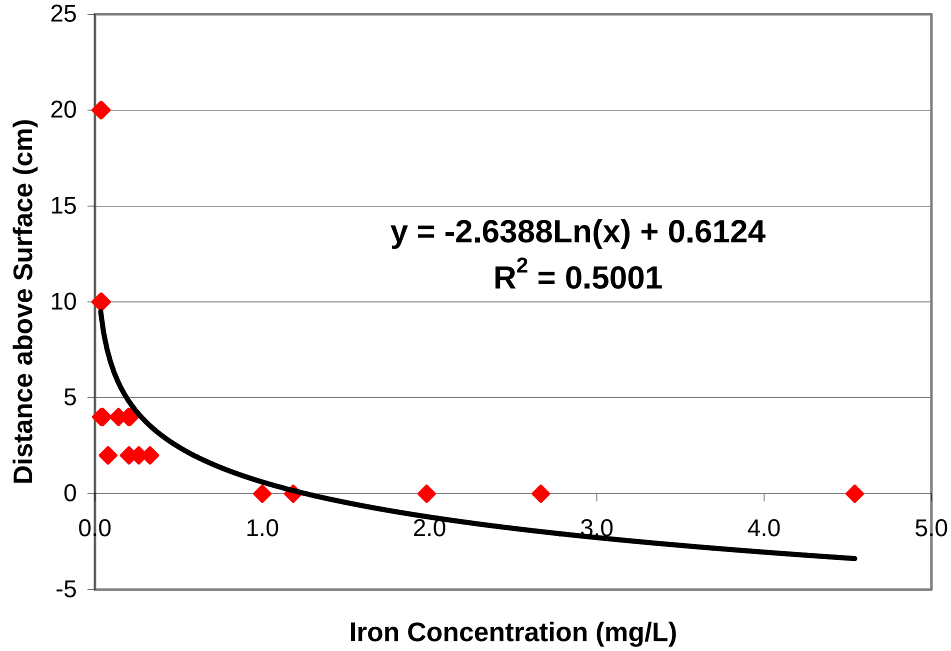


*Figure 29. Concentration gradient for iron measured in water samples collected above the surface of the grate. Each point represents a single concentration at a given point above the surface. The trendline was fit to all of the points as a whole and is not based on linked gradients for individual sets of samples.*

*Table 8. Summary of results of bacteria culture inoculations from the Pot Ash Point iron grate following 36 days of incubation. Incubation and bacteria counts performed by CC Technologies. * Based on 1 gram of wet deposit, assuming extraction in 10 mLs solution.*

| **Type of Bacteria** | **Water Column Bacteria/mL; (days)** | **Mat Bacteria/mL; (days)** | **Scale Bacteria/gram*; (days)** |
|---|---|---|---|
| Aerobic | 10 - 100 (2)<br>100 - 1000 (7) | > 1000 (2) | 1000 - 10,000 (2)<br>> 10,000 (18) |
| Acid-Producing | 100 - 1000 (2) | > 1000 (2) | 1000 - 10,000 (2) |
| Anaerobic | 10 - 100 (2) | > 1000 (2) | 1000 - 10,000 (2) |
| Iron-Depositing | < 1 (36) | < 1 (36) | < 10 (36) |
| Sulfate-Reducing, (SRB) | <1 (36) | 1 - 10 (14) | < 10 (36) |

When a drop of concentrated HCl was placed on the surface of the grate, a strong odor of hydrogen sulfide was produced and lingered for several minutes, indicating an abundance of reduced sulfur corrosion products.

The grate is still undergoing final treatment by LCMM conservationists. Figure 30 shows the center portion of the grate as it appears after final restoration. As is apparent in the picture, the grate was in multiple pieces throughout the restoration process. Upon completion, the grate will be exhibited for the public at the Lake Champlain Maritime Museum.

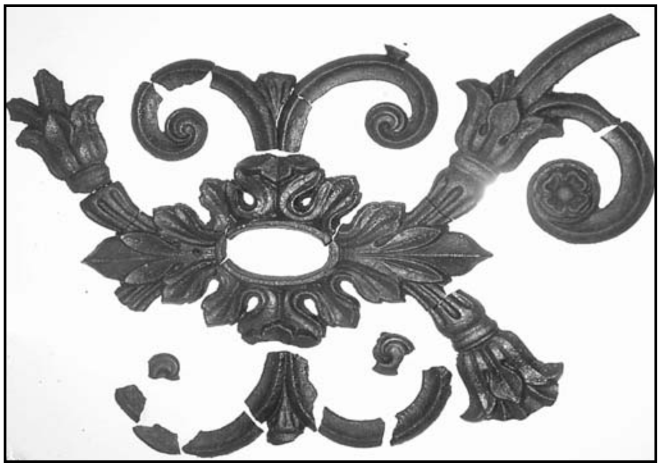

*Figure 30. Reconstruction of the central portion of the iron grate. Note the multiple pieces.*

**FISH PREDATION PILOT STUDIES**

Numerous fish representing a variety of species were observed over the Bulwagga Bay draw boat. We witnessed and recorded several instances of sheepshead (a freshwater drum, *Aplodinotus grunniens*) striking mussel clumps, chewing the mussels, and then spitting out pieces of shell (Figure 31a). Sheepshead was the primary species that was seen feeding on the mussels, although both yellow perch (*Perca flavescens*) and pumpkinseed (*Lepomis gibbosus*) were also observed eating mussels (Figure 31b). Yellow perch took smaller zebra mussels

a) b)

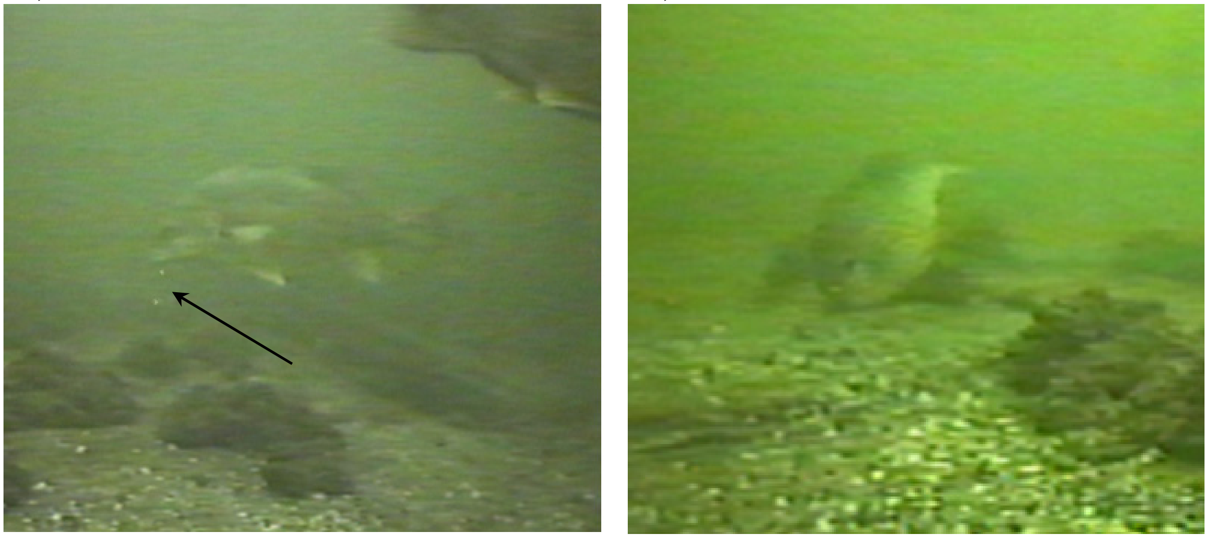

*Figure 31. Deck of the Bulwagga Bay shipwreck. a) Sheepshead above zebra mussel clumps. Arrow points to shell fragments falling from its mouth. b) Pumpkinseed striking the shell debris. Note the broken shells on the deck in each photo.*

because they are not capable of crushing the shells so must swallow the mussels whole. Pumpkinseeds may be able to crush the shells, but we did not witness the shell-spitting behavior with this fish. All strikes and feeding moments witnessed were captured on video. Video footage was converted to digital images by the LCMM.

Twenty-one fish were caught on site (8 yellow perch, 5 pumpkinseed, 7 bluegill, and 1 bass). Gut contents were taken for all fish. All the yellow perch and pumpkinseed had zebra mussel shells and zebra mussel tissue in their stomachs and intestines. The bluegill and bass had no zebra mussel shells or tissue in their guts.

We were unable to catch any fish in the gillnet deployed on the site in late September.

# DISCUSSION

## CORROSION STUDIES

The zebra mussel population has continued to expand across the bottom of Lake Champlain. All of our sites have mature, well-developed colonies on most available surfaces. Like last year, more zebra mussels were found on vertical surfaces than on horizontal surfaces. All surfaces are also accumulating a layer of organic and inorganic particles that are allowing bacteria to flourish and are attracting a variety of small invertebrates.

The accumulation of organic matter around zebra mussels makes their attachment sites very active zones biologically and chemically. As this material decomposes, it uses up oxygen, creating a high biochemical oxygen demand (Call 1996) and changing the redox potential of the environment surrounding the colony. The reducing environment among the byssal threads can support the growth of sulfur-reducing bacteria that also lower the pH on the substrate surface (Claudi and Evans 1993) and can greatly increase rates of corrosion (Little et al. 2000).

The increased iron concentrations measured above the zebra mussel colonies suggest that biochemical processes associated with the mat are facilitating the corrosion of the metal underneath. The fact that we were able to demonstrate a decreasing profile of iron in the water column above the wrecks suggests that iron corrosion and dissolution rates are substantial. The higher concentrations in the water in 2000 compared to 1999 may reflect the fact that the population density of zebra mussels has continued to increase. As zebra mussel colonies have continued to expand, it has become more and more difficult to find patches without zebra mussels for the “away” or reference water samples. The proximity of zebra mussels to the away samples may explain the increase in iron concentrations at both locations in the year 2000.

The positive correlation between iron concentration measured in the water immediately above the zebra mussel colony and TSS and LOI suggests that the fecal and pseudofecal material is contributing to the loss of iron. The positive correlations between iron in the interstitial water and the amount of accumulated fecal and pseudofecal material (TSS and LOI) suggests that biochemical oxygen demand is high, and that anoxic conditions may exist in the microlayer at the surface of the wreck. It seems unlikely that we would have measured such high concentrations of iron in the water column if only oxidative corrosion was occurring.

We did not find a significant correlation between the number of zebra mussels and LOI and TSS in the year 2000, while in 1999, we did (Watzin et al. 2000). We believe this may be the result of a sampling artifact. There is so much accumulated particulate matter under the colonies now that it is difficult for the divers to collect it all in the syringes we used for sampling. In future efforts, a different method of sampling should be devised.

The biofilm generated by microbes in an organically rich wet environment includes a complex mixture of bacteria. One group uses the metabolic byproducts of another. The thickness of the biofilm is never uniform resulting in patches of corrosion of different types and rates. In a well-developed mat, microbially mediated oxidation and reduction reactions can take place in various layers and patches. The deepest layers contain the obligate anaerobic bacteria including the sulfate-reducing bacteria. The sulfate-reducing bacteria use sulfate as an energy source, producing hydrogen sulfide as a byproduct. Hydrogen sulfide both acidifies and accelerates a process known as hydrogen embrittlement, which results in stress crack formation. Iron sulfide minerals also form on the surface of the metal as a byproduct of these reactions (Videla 1996, NACE 1997, Little et al. 2000).

Our bacterial assays showed the presence of aerobic, anaerobic, acid-producing, and occasionally, sulfate-reducing bacteria in the mussel mat at the locations where we measured it. This strongly suggests that microbially influenced corrosion is occurring. The fact that we did not find large numbers of sulfate-reducing bacteria may simply reflect our inability to sample in the extremely thin layer directly on the metal surfaces on the wrecks. This is where those bacteria would be found.

**Zebra mussel colony structure.** We found more larger mussels in 2000 than we did in 1999. The average life span of a mussel is 4-5 years, corresponding to a shell length of > 35 mm. A significant number of mussels greater than that, indeed > 40 mm in size were found this year at our three southern sites. In the coming year, significant mortality of mature individuals should begin to occur.

## CALCIUM TOLERANCE EXPERIMENTS

Calcium concentrations measured at our six primary research sites were similar to those recorded last year. At 11.3 to 21.0 mg/L, we still expect to see calcium limitation emerge, especially at the northern sites, where concentrations are lowest. In general, zebra mussels do not flourish unless the average calcium concentration exceeds 28 mg/L (Ramcharan *et al.* 1992). In fact, as we reported last year, Lake Champlain has the lowest calcium environment in North America known to support significant adult zebra mussel populations (Eliopoulos and Stangel, 1999). Although adult zebra mussels have now been found in Lake George, which has even lower average calcium concentrations than Lake Champlain, the mussels do not appear to be successfully reproducing in that lake (NY DEC, personal communication). Because embryos and veliger larvae need a higher concentration of calcium than the adults need later in life, low calcium concentrations may first appear as the loss of new recruits in a colony. In laboratory experiments, Sprung (1987) found that calcium concentrations lower than about 40 mg/L will increase the number of crippled larvae. In field and laboratory experiments using Great Lakes water, veliger production dropped to zero when calcium concentrations were lower than 20 mg/L (Hincks and Mackie 1992). In these experiments, loss of calcium from the shells of adult mussels was also observed at low calcium concentrations. A model based on data from over 250 lakes in Europe predicts that zebra mussel densities in Lake Champlain should be constrained by its calcium concentration (Ramcharan *et al.* 1992).

Our laboratory tolerance experiments are designed to determine natural tolerances of the Lake Champlain zebra mussel population to various calcium concentrations. It is obvious that the Lake Champlain population has high tolerance to low calcium concentrations but calcium concentration may only begin to serve as a natural control on population growth at higher colony densities. If a localized zone of calcium depletion is created at the shell-water interface, it may disappear as older mussels die. Then the colony may again be able to attract new recruits. Only a longer term data set will be able to determine whether such "boom-bust" population dynamics may be coming in Lake Champlain.

## SETTLEMENT EXPERIMENTS

Zebra mussels settled on all of the substrates we provided although they clearly preferred some to others. The hot-rolled steel plates were the least attractive to zebra mussels perhaps because of the carbon layer on the surface of this material. The wrought iron and steel rods seemed to be the most attractive. In other experiments, zebra mussels have been shown to settle on wood, iron, steel and polypropylene and polyvinylchloride plastics while avoiding glass, teflon, aluminum, copper, zinc and other galvanized metal surfaces (Walz 1973, Kilgour and Mackie 1993, Gu *et al*. 1997).

We found more mussels on the bottom than the tops of our hardwood, softwood, plastic, and steel plates. This difference was particularly pronounced at our shallowest sites, and may have occurred as mussels avoid full sun (Walz 1973, Call 1996). More sediment collected on the tops of the plates, which may also have contributed to the lower settlement densities there. Also, the bottoms of the plates were directly attached to the plastic VEXAR sheeting. This created a rougher surface on the bottom, perhaps creating more attractive sites and allowing for easier settlement. Our plastic plates are the same material used in many monitoring programs. Because mussels like the undersides of these plates, this surface is often used to measure recruitment (Marsden 1992, Eliopoulos and Stangel 1999). Our results suggest that this surface does generally give a higher estimate of abundance.

When we removed the zebra mussels from the surface of our wood plates, one of two things happened: either the byssal threads were left behind, or a fragment of wood was removed with the zebra mussels. Fragments of wood were more often removed on the hard wood than the soft wood, however, the significance of these losses after just one year was relatively minor, resulting only in some surface roughness. If the damage to wood as a result of mussel colonization continues to worsen, then the manual removal of zebra mussels from shipwrecks as a preservation method may not be appropriate.

All of the iron pieces subjected to detailed analysis showed some degree of corrosion. For most of the samples, the corrosion began as pitting corrosion on the outer surface of the sample. Deep pits were apparent in the cross sections. After just one year, we cannot project an annual corrosion rate in Lake Champlain, however, we did measure a thick scale layer in many areas and pits of greater than 1 mm after just one year.

In several studies of corrosion on sheet metal (stainless and mild steel) conducted for the U.S. Army Corp of Engineers (1998), general corrosion was found to increase slightly under zebra mussels, but the rate of pitting corrosion increased substantially, up to 10 times the rate normally expected. Pitting corrosion rates of 0.1-0.3 mm per year were observed. In another study comparing corrosion on two adjacent sheet steel pilings, one colonized by zebra mussels and one not, the same pattern was found (Simkins and Jones 1997). The piling covered with zebra mussels showed scale 2-5 mm thick and large corrosion pits over 2 mm deep.

The perforation associated with pitting corrosion reduces the structural integrity of the metal and increases the likelihood of failure. The smaller the metal part, the greater the concern about this type of corrosion (U.S. Army Corp of Engineers 1998, ASTM 1995). If wooden ships on the bottom of Lake Champlain are primarily iron fastened, and the fastenings contribute to the vessels' structural integrity, it follows that anything which might affect that integrity could significantly affect the projected life-span of these cultural resources.

Analysis of the corrosion deposits by EDS and XRD showed predominantly iron oxides and hydroxides. This suggests that in the first year of exposure, mostly oxidation reactions were occurring on the surface of the iron. Many of the deposits showed three distinct layers representing different forms of iron oxides and hydroxides. It is common to find these different products in layers as iron corrodes in water (MMR 2001).

Corrosion mounds or tubercles were also common on some of the samples. Tubercles can be produced by microbially influenced corrosion (MMR 2001); they frequently contain aerobic slime forming bacteria. These aerobic bacteria were very common in our bacterial assays.

There was no real evidence of a reducing environment under the zebra mussels on the iron rods. Very little sulfur was found in the EDS analyses. This is not particularly surprising with just a single year of exposure and mostly small zebra mussels attached to the iron surfaces. All sulfate-reducing bacteria are obligate anaerobes requiring significant organic matter accumulation and isolation from oxygen in a mature biofilm (NACE 1986, Little et al. 2000, MMR 2001). As the zebra mussel mat and biofilm continue to develop, it is likely these groups of bacteria will become more abundant. Because rates of corrosion will increase with sulfate-reducing bacteria, it is important to determine when this will happen.

## POT ASH IRON GRATE RECOVERY

The Pot Ash grate recovery gave us the opportunity to gather information over a site known to be primarily cast iron. Cast iron from the mid-nineteenth century is of notoriously poor quality and over time, slowly breaks down and turns into very brittle graphite. Despite the fact that significant amounts of the cast iron had been converted to graphite, we still measured a strong signature of iron in the overlying water column. The strong odor of hydrogen sulfide with acidification clearly indicates that iron sulfide corrosion products were abundant on the metal implicating the sulfate-reducing bacteria. Sulfate-reducing bacteria were found in the cultures, although abundances were not high. Again, this is probably because we were not able to sample immediately on the surface of the metal, where these bacteria are found.

The larger average size of the mussels removed from the grate reinforces the notion that zebra mussels settle preferentially on metal surfaces. There is nothing about the position of the grate on the wreck that would give a feeding advantage to zebra mussels found there as opposed to other locations on the wreck, so the most logical explanation for the size difference is early settlement there.

### Brief History of Canal Boats on Lake Champlain

The Pot Ash canal boat can be placed within an intriguing era in the history of the Lake Champlain Basin. The opening of the Champlain Canal profoundly affected economic development in the region. Extractive industries, particularly timber cutting, stone quarrying, and iron mining, experienced a surge of activity as entrepreneurs hastened to take advantage of the new unrestricted domestic market for their products. Agricultural surpluses of apples, potatoes, grain, butter, cheese, and other semi-perishables could quickly and inexpensively be shipped to urban centers along the Eastern Seaboard. The Champlain Canal also provided residents of Vermont and northeastern New York with manufactured goods and raw materials that had previously cost a great deal to ship overland or import from Canada. Many lakeside merchants advertised themselves as forwarding agents that could take responsibility for the safe and rapid transportation of freight from one destination to another. As shipping lines developed, the work of forwarding products was simplified since freight could now be under the supervision of one company from its origin to its destination. The year 1823 marked the end of the Champlain Valley's relative isolation from the outside world and its entry into the national economy.


*Figure 32. A group of standard canal boats is towed by a steamship on Lake Champlain .*

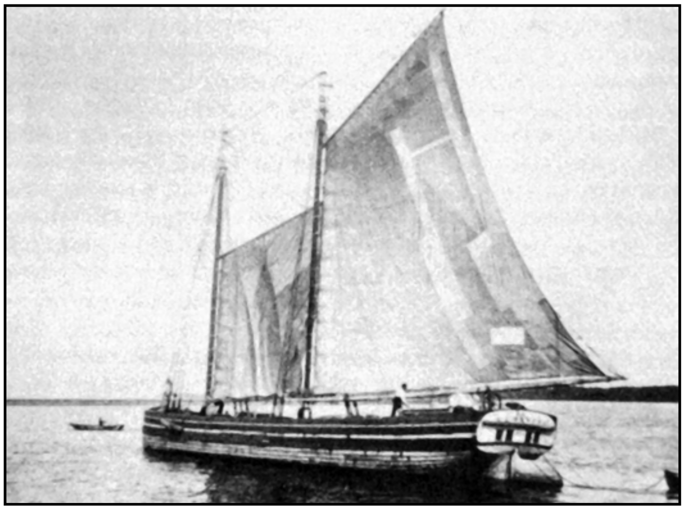

*Figure 33: Sailing Canal Boat on Lake Champlain*

The number and types of vessels that passed over Lake Champlain's waters greatly increased after 1823. The canal's shallow channels, low bridges, and narrow locks were too restrictive for nearly all of the existing lake merchant craft, so large numbers of long, narrow, shallow-draft boats were constructed for canal service. Three types of canal vessels were employed during the early years of the canal: standard canal boats, sailing canal boats, and packets. All of these craft were towed through the canal by teams of mules or horses. By 1833, there were 232 cargo- and passenger-carrying canal boats registered at towns along Lake Champlain and the canal. Shipyards that specialized in the building of standard canal boats and packets appeared in the southern portion of Lake Champlain and at towns along the Champlain Canal. Shipbuilders at the northern end of the lake occasionally constructed sloop- or schooner-rigged canal boats that could sail up to Whitehall, unstep their masts, raise a centerboard or leeboards, and pass through the canal.

The effect of the sailing canal boat on other types of merchant craft was considerable. The construction of sloops and schooners declined very rapidly after 1842, and those that remained in service were relegated to secondary roles such as carrying stone, lumber, and other bulky cargoes between lake ports. In order to compete with the sailing canal boats, owners of standard canal boat lines also dispensed with the unnecessary freight handling by building steam tugboats for canal service and a different style of tugboat for lake service, which towed rafts of standard canal boats. The elimination of trans-shipment at each end of the Champlain Canal lowered freight rates and increased the profitability of bulk cargoes.

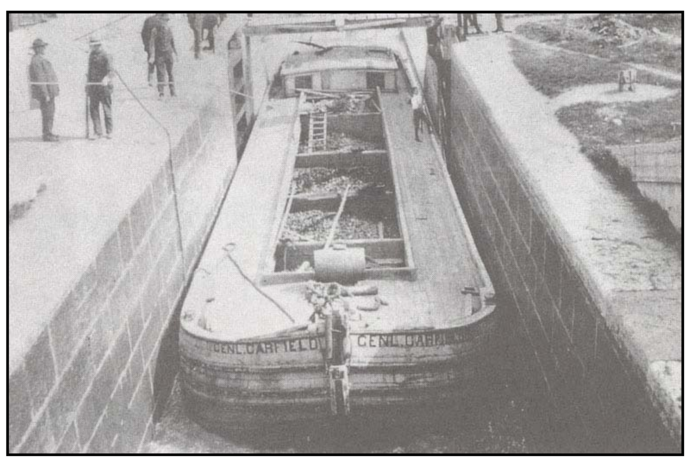

*Figure 34: Canal boats were constructed for maximum carrying capacity allowed by the locks.*

As commerce on the Champlain Canal continued to grow in the decades following its opening the canal itself was expanded on two occasions in the 19th century to accommodate large vessels. The first canal expansion was completed in 1841, and the second in 1862. With each expansion of the lock system new and larger vessels were built to take maximum advantage of carrying capacity. Conveniently for archaeologists, the canal expansion and corresponding growth of the vessels has led to the creation of a rough typology for canal boats, this system allows them to be classified as 1823 class, 1841 class, or 1862 class based on their general dimensions.

The Pot Ash Point Wreck was first located in 1984 by members of the local dive community lying in approximately 30 feet of water. Preliminary measurements of the site suggest that it is a canal boat of the 1841 class, though this has not been confirmed by other dating techniques. The hull of the vessel is partially disarticulated but fairly well preserved. The bow and stern of the canal boat are present and stand proud of the bottom. However, the sides and decking of the central hull have collapsed revealing the canal boats cargo of stone. The iron grate recovered for analysis and conservation was located near the stern cabin where the vessel's crew resided. It is assumed that it was used to protect one of the windows of this cabin. Unfortunately, the identity of the vessel and the manner/cause of its sinking are still unknown. We hope that future archaeological examination and historical research may reveal these facts.

## FISH PREDATION PILOT STUDY

The Bulwagga Bay railroad drawboat is a unique find ecologically as well as archeologically. We documented predation by freshwater drum (*Aplodinotus grunniens*), yellow perch (*Perca flavescens*), and pumpkinseed (*Lepomis gibbosus*) on zebra mussels on the wreck. To our knowledge, this is the only site where direct field documentation of substantial zebra mussel predation has occurred. All previous conclusions about fish predation on zebra mussels is the result of morphological analysis of predators (French and Bur 1993, French 1993), limited gut content analysis of fish captured by trawl or gill net (Morrison et al. 1997, Marsden 1997, Molloy et al. 1997), or feeding trials in enclosures or aquaria (Nagelkerke and Sibbing 1996, Fullerton et al. 1998). The level of predation by any of these predators, including drum, has not been thought to be sufficient to act as a population control (French 1995). There are no other published (or unpublished to our knowledge) reports of large collections of crushed zebra mussel shells in the natural environment.

Freshwater drum are probably responsible for the crushed shells documented on the railroad drawboat. If drum are eating zebra mussels throughout the lake, and zebra mussels are an increasingly important part of their diet, then this predation may begin to have a measurable effect on the overall abundance of zebra mussels in the lake. The implications of this consumption for the drum are unclear; some research has suggested that individuals that rely on zebra mussels for a significant portion of their diet show declining size at age (French and Bur 1996).

No previous work has suggested that yellow perch might be routinely feeding on significant numbers of zebra mussels. Because yellow perch lack the jaw structure necessary to crush zebra mussels, they must swallow them whole. It is unlikely, therefore, that yellow perch are contributing to the masses of crushed shells observed on the drawboat. Morrison et al. (1997) have shown that yellow perch only take small zebra mussels because ingestion is limited by the size of the pharyngeal gape. They supplement their diet with zebra mussels primarily during the early spring, when other soft-bodied prey is scarce. Morrison et al. (1997) did not find substantial consumption of zebra mussels during other times of the year, nor have any other investigators.

In fact, previous research by Watzin and other investigators (Richardson and Bartsch 1997, Thayer et al. 1997, González and Downing 1999, Watzin and Cobb 2000) has suggested the yellow perch feed predominantly on the benthic invertebrates associated with zebra mussel colonies, and not the mussels themselves. Because the abundance of benthic invertebrates increases by as much as several orders of magnitude around zebra mussel colonies in the patches located on natural bottoms (Wisenden and Bailey 1995, Howell et al. 1996, Silver Botts et al. 1996, Stewart et al. 1998, González and Downing 1999), yellow perch might benefit from this increased biomass if they are able to effectively forage in the spatially complex colonies. In laboratory experiments, Watzin and Cobb (2000) showed that yellow perch foraging efficiency decreases among zebra mussel colonies compared to bare substrates, but the overall quantity of prey caught may increase because of the much greater abundance of invertebrates around the mussels.

Yellow perch is the most-caught fish in Lake Champlain; over 800,000 fish are taken from the lake each year (Vermont Fish and Wildlife Department, personal communication). The yellow perch population in Lake Champlain is very large. It also shows a reduced size at age, suggesting that food limitation has led to stunted growth (Cobb and Watzin 1998). If the yellow perch in Lake Champlain are actively foraging in zebra mussel patches, we first predicted that they might be getting more food, and thus grow a little faster because of the increased densities of invertebrates in the colonies (Watzin and Cobb 2000).

Although we only collected a few yellow perch for gut content analyses in Bulwagga Bay in the summer of 2000, the fact that all eight of these fish had predominantly zebra mussels in their gut suggests that a diet switch may be occurring, at least in this location. Because the zebra mussels passed into the gut intact, they are only minimally digested, which begs the question of the nutritional return of a diet comprised primarily of zebra mussels.

The small size at age of yellow perch in Lake Champlain is a recurring and significant concern among the angling community. The State of Vermont convened a special task force to address this question in 1997. Very logical arguments in the scientific literature have suggested both potentially beneficial and detrimental effects of zebra mussels on yellow perch and other bottom-feeding fishes. The nutritional value of zebra mussels themselves for yellow perch has not been evaluated. Management decisions about the yellow perch fishery must be based on an understanding of its entire prey base. The additional work we are undertaking in this area will shed light on these questions.

## FUTURE DIRECTIONS

We are now more firmly convinced than ever that our future work must concentrate on the bacterial layer, or biofilm, underneath the zebra mussel mat. The biogeochemical processes that are facilitating the loss of iron from the wrecks are occurring on a microscale and can only be understood by sampling at this scale. Therefore, to take our work to the next step, we need to develop a sampling protocol for use with microprobes that could be inserted among the zebra mussels, measuring parameters in the biofilm on the surface of the wreck.

In the coming field season, we would like to focus our activities on three objectives:

1. An intense investigation of the extent of fish predation in the lake, its potential to reduce the abundances of zebra mussels in the coming years, and the implications of a diet of zebra mussels to the fish populations themselves.

2. A continuation of our long-term settlement experiments, documenting the changes in wood and metal surfaces as mussels accumulate on these materials. The data we are gathering in these experiments are directly relevant to predicting the management challenge on the historic shipwrecks in the lake (and beyond).

3. New studies of the microbial mat that develops under mature mussel communities and the changes in chemistry that lead to the loss of iron under these communities. These studies would include laboratory experiments under controlled conditions and field measurements in selected locations. These studies are dependent upon the purchase of a microprobe system, and, for use by the divers using the probes for the field studies, an underwater communication system.

The microprobe system is designed to be submerged. A supporting rod and micromanipulator for the probes and an underwater picoammeter that can cycle between the three sensors must be assembled for our specific application. The divers would use the probes to measure pH, oxygen, and hydrogen sulfide in the zebra mussel colonies at two or three of our wreck sites. The microprobes would also be used in a series of controlled laboratory experiments. Laboratory experiments are key to understanding the rates of iron loss. The Rubenstein Ecosystem Science Laboratory has the capability to support controlled laboratory experiments under various experimental treatments. We would establish aquaria with wood, iron, and steel surfaces in them, and cover these materials with zebra mussels. We would also establish reference tanks without zebra mussels.

On the iron and steel, we would use our microprobe system to follow the development of the biofilm and changes in the surface chemistry. After a reasonable period of exposure, we would also recover the materials and measure aspects of their structural integrity. Standard methods, either using dissecting microscopes or scanning electron microscopy are available for measuring pitting density and severity (size, depth and pattern of the pit). Mass loss is generally not a very effective measure of corrosion; however, x-ray diffraction analysis can be used to analyze the forms of iron present after corrosion occurs. Corrosion should lead to an increase in the amount of iron oxide, iron sulfide, and/or other reaction products in the metal sample. If warranted, in future years we could enclose well-developed zebra mussel colonies in environmental chambers where we could construct a mass balance for key chemicals and calculate the rate of iron complexation and/or dissolution under zebra mussel colonies. Such a system would be designed after we have experience with simple tank experiments.

# CONCLUSIONS

The data set that we have collected over the last two summers strongly suggests that zebra mussels are accelerating the corrosion of the iron fasteners and fittings on the submerged shipwrecks in Lake Champlain and probably elsewhere as well. As zebra mussel colonies have grown and a thick layer of organic matter accumulates under the mat, a complex community of bacteria has become established. These microorganisms are likely facilitating the corrosion.

The implications of these findings for the structural integrity of the wrecks are still not clear. Because of the patchiness of the chemical environment and the large array of biological processes and chemical reactions at work, additional research is necessary to project an iron loss rate.

The condition of the cast iron material comprising the Pot Ash Canal Boat window grate may partially forecast the coming fate of all exposed iron on the wrecks. The interplay between wood and iron will always make projections about overall wreck survival difficult.

The documentation of significant fish predation on the railroad drawboat suggests a very real evolution in the feeding relationships in Bulwagga Bay. If these changed behaviors are becoming prevalent throughout Lake Champlain, the food web and biodiversity of the Lake will be significantly different in the years to come.

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