Project Objective:

The objective of our project is to answer the following questions:

1) Can scallop shells be used to improve the condition of water obtained from various sources including the McIntosh Runs (a local river)?

2) Can we identify the organism(s) and materials responsible for this effect?

3) What type of contaminants will this system (scallop shell and organism) reduce or eliminate, and under what conditions?

Introduction:

Urban rivers are important socially, culturally, and historically. Until the expansion of Halifax into the Super City, the McIntosh Runs was its only river. The McIntosh Runs spreads itself through the city from lake to sea. As a result, many urban toxins and pollutants have seeped into the water. Some examples are: road salt, acid rain, car wash fluid, sewage, as well as commercial and industrial wastes and by-products.

Throughout history, mankind's primary need has been for food and clean water. This project focuses on an alternative method to improve water conditions using biological-based technologies. Thus far, any clean-up attempts for the McIntosh Runs have simply consisted of the removal of rubber tires, shopping carts, and other eyesores. This does improve the look of the river, but does nothing towards improving the water quality itself. In order to significantly improve water quality, dissolved cations from metals, dissolved particles, and other contaminants must be removed or reduced.

There is a need for an inexpensive, user-friendly, and non-intensive labour methodology that actually works and uses locally available materials. Such an answer was found in bivalve shells, specifically scallop shells. This idea may have been used by Aboriginals. Over the past few decades archaeologists have discovered several shell middens in places such as Wales, Mexico and coastal British Columbia. "Midden" means rubbish dump and the shell midden is where shell-fish debris has accumulated. Until recently, the shell middens were believed to be where aboriginals dumped their unwanted shells. These shell middens are usually found near river banks, lakes, or swamps. Distinctive depressions were found along side of these middens which led archaeologists to believe that huts were built on them. However, this may be unlikely because the middens would attract large predators and neighbouring tribes. We speculate that these depressions were not actually where huts were built, but were used as a place to store water. Water from a nearby river or lake was channelled through the shell middens and left to sit in the storage area.

A similar method was used in this century by a pig farmer in Japan. Serendipitously, he discovered that oyster shells could be used to sanitize a pond that had been repeatedly soiled by his pigs. In an attempt to get rid of oyster shells accumulated from his personal consumption, he

deposited them into his pond. After passing by the pond a few days later, he noticed that the water had visibly cleared.

We expanded on this discovery using *in vitro* and *in vivo* experiments. Examination of this biological system at selected sites on the McIntosh Runs confirmed the empirical findings of the farmer. Controlled experiments with the shell system and water from the McIntosh Runs within the laboratory enabled us to examine the effect of this shell system on selected water quality parameters.

In addition, we used innovative technologies to transmit data from one site to another via wireless technology. This has educational benefits in enhancing and expanding the use of Pasco probes which are already used in many schools. In the future, this technology could be used for similar projects or to enhance scientific educational and community related enquiries.

Materials:

Macintosh ® base station modem and Airport card
Pasco ® Probes – pH, temperature, light and conductivity

Pasco ® Explorer	Scallop Shells
Graphical Analysis	Data Studio
Chicken wire	Metal stakes and broom handles
6 x 500 ml beakers	9 x 4 litre buckets
Digital camera	Macintosh ® G4 Titanium PowerBook
Wireless antenna	Wave guide
Homemade fountain	Sewer water
Commercial Bleach	Used household dishwater
River water	

Methods:

The combination of *in vitro* and *in vivo* testing helped to maximize the projects testing area and results. For each experiment, we measured light, conductivity, pH, and temperature using the Pasco Probes. Data were then recorded using the PowerBook and stored for later analysis.

Lab Experiments (*in vitro*)

Experiment #1- dish water

This initial laboratory experiment was conducted in the J. L. Ilsley chemical laboratory on February 28. 350 ml of murky, brown dishwater was placed in four 600ml clear, glass beakers. Two were with scallop shells and the remaining two were controls (just dish water). Original readings were taken from each of the beakers before the shells were added. Shells were then added and data from the probes was collected.

The same method was then repeated but tap water was used instead of the dish water.

Experiment #2
This experiment entailed the plating of the organism(s) on/in the scallop shells at the National Research Council's Institute for Marine Biosciences (NRC/IMB) on two different media: blood agar and tryptic soy agar with added NaCl. A Gram stain was also done on water and swabs from the shells.

Experiment #3-1
This experiment involved using water from the McIntosh Runs. For this experiment, nine containers of equal size, volume, and density were filled with 3 litres of river water each. These nine containers were divided into three groups of 3: 3 containers as controls, 3 containers with sterilized shells (shells that have been cooked to eliminate all organisms that might have been causing the results in the previous experiments), and 3 containers with the system (shells that have not been altered, through cooking or other methods - they contained the organism(s)). The containers with sterilized shells and the system had 4 shells per container. The river water was left to sit in the containers for a 24 hour period before added the shells. Readings were taken every day using the Pasco probes.

Experiment # 3-2
The next experiment utilized the same method as experiment 3-1 except bleach was added to the river water after 24 hours. Bleach was used because it is a contaminate found in many rivers. Then the shells were added to this solution 24 hours after the bleach was added. Readings were then taken every day using the Pasco probes.

Experiment #4
For this experiment we took one of our scallop shells to Dalhousie University and analysed the interior surface using an atomic force microscope. This experiment was done to determine if the structure of the shell itself was responsible for the trapping of sediment that could be housing the organism.

<u>Flowing Water Experiments</u>
Experiment #5 (sewage)
This experiment was done with the use of a small home-made water fountain that was used to circulate sewage water. This was done to mimic the conditions flowing water from an urban area in a lab situation. For the experiment 500 ml of sewage water was circulated for 24 hours before adding the shells (one whole shell was used). Readings were taken during this 24 hour period and then again 24 hours after the water had circulated through the shell. Both samples (control and shell-system) were taken to the Water Bacteriology Department of the QEII Health Sciences Centre for total and fecal coliform counts.

<u>Field Experiment</u> (*in vivo*)
Experiment #6
For the next part of our project, experiments were conducted in the field: the McIntosh Runs. Cages made of chicken wire were filled with 150 shells each. Four cages were made and set into the river. The river was divided into 3 different sections: two sections with cages and one upstream from the other two as the control. Each of the two sections in which the cages were

placed had 2 cages. The cages were both set on the same side of the river and they were approximately 6 feet apart. A length of approximately 2 river widths separated the sections. Readings were taken every day at the same time of day, for 1 week following the day that the cages were put in. An original profile was taken of the river before the cages were added using the same Pasco probes.

Use of Wireless Technology

This experiment included the use of new, break-through technology in order to complete experiments 1 – 6. The new Pasco Passport probes were used to gather our data. We also used them in new ways. For example, the light probe was used to measure the clarity of the water. Also, wireless data transmission was used in order to send our data from the river to the school. This was made possible by the use of a Macintosh laptop, airport card, base station, and the Pasco probes. We were able to send the readings we obtained from the river and live video to a computer in our school instantaneously and without any wires. This process is still being tested and we were the first to use it in such a way.

Results:

For each experiment four graphs were plotted: temperature, pH, light, and conductivity.

These graphs as well as the data tables can be seen on our display board.

In experiments 1, 3, 5, and 6 many changes occurred. In all of the experiments the pH level increased or decreased to approach 7.0 after addition of the shells.
In addition, there was a decrease in conductivity in experiments 1, 3-2, 5, and 6. The light readings in experiments 1, 3, 5, and 6 decreased when the shells were added. The temperature stayed constant between the controls and the shells. In all of the lab experiments there was a visible clearing in the solutions with the shells. All of these changes occurred over a 24 hour period.

For experiment 2 several different types of bacteria grew on each medium. The majority of these organisms found from the Gram stain were Gram positive diplo bacilli.

For experiments 3-1 and 3-2 the same results were seen for the cooked shells as well as the system. The only difference observed was the containers with the cooked shells had a lower conductivity than that of the system. This is because the system contains more organic matter. The results from experiment 4 follow up to this. The shell, after being examined under an atomic force microscope, appeared to have micro-channels on the surface of the shell. The expert who had taken the photographs speculated these micro-channels were an advantage to the filtering process.

In experiment 5, the results were very similar to that of the other experiments, except that the results occurred more rapidly. Instead of taking 24 hours, results were seen within one hour. This was because the fountain circulated the water continuously and there is more force on the shell itself. The force that is applied on the shell causes the filtering process to occur quicker. Sewer water taken before and after the shell was analysed for total and fecal coliforms at the QEII

Health Sciences Centre. The results from the count before the shell was added was "too numerous to count". The technical specialist explained: "Too numerous to count means that there are too many bacteria to count at any dilution unless the sample was, say, to be diluted a million times!" Furthermore, using his professional judgement he remarked the least number of fecal coliform bacteria present was 5000 cfu (colony-forming units)/100ml. After the sewer water had been exposed to the shell system for 24 hours, it was again taken to the QEII Health Sciences Centre. The fecal coliforms drastically deceased to 130 cfu/100ml of the sewer water. The acceptable limit for recreational water is 200 cfu/100ml. The total coliform count decreased to 5500 cfu/100ml.

In experiment 6, the results obtained were very similar yet more subtle than those conducted in the laboratory. After one day the pH of the water that passed through the cages in the still section was 5.8, compared to the mere 5.0 of the control. The flowing section with the shell cages changed to 5.3, which was still significant. Throughout the week, the still section remained at approximately 5.9, while the flowing stayed at approximately 5.7. The control's pH changed as the temperature did, because temperature affects pH. The light readings in the still and flowing shell cage sections decreased dramatically from approximately 3.40 lux to as low as 1.45 lux, averaging about 2.00 lux. Conductivity within the still and flowing sections dropped from approximately 2000 µS/cm to an average of 1850 µS/cm. The control section of the river maintained a conductivity measure of approximately 2100 µS/cm, which is clearly higher than the water with the shell cages.

Discussion /Conclusion:

Throughout this project we have learned that biological-based technologies (scallop shells) can safely be used to purify water as opposed to a more expensive chemical process. It is much more environmentally friendly and uses materials which are in excess and normally discarded as waste. It is proven to be a quick, user-friendly process. Only 24 hours were required at the maximum to have its full effect. Furthermore, it has proven not only to raise the pH of acids but to also lower the pH of substances which are too basic. But it does not only affect the pH - it also removes metal cat-ions from liquid substances. This can be seen by the decrease in conductivity. Also, the shells have proven to decrease the amount of dissolved solids in liquids. This can be deduced from the decrease in the light readings, showing that there is far less sediment present. Furthermore, the sterilized shells maintained the same effects. Thus, the shell itself was responsible for the changes. Moreover, using the temperature readings obtained, it can be concluded that the shells do not have any effect on the temperature.

The shells have not only improved the abiotic factors in the liquids - they have remarkably lowered the presence of harmful biotic coliforms as well. The shells decrease the number of fecal (harmful bacteria) as well as total coliforms in a sewage sample from too numerous to count (over 5000 cfu/100ml of fecal coliform) to 130 cfu per 100ml and 5500 total cfu of sewer water, respectively. This falls within the acceptable limit (200 cfu/100ml) for recreational water (swimming, shower, etc.). The most probable reason for this is the micro-filter present on the surface of the shell and the chemical make up of the shell itself. There are many holes the size of bacteria within the filter and that is why this is speculated. In essence, one shell can transform half a litre of sewage water into legal recreational swimming water in only one day.

The decrease in conductivity shows that the amount of metal cat-ions in the water also decreased after the shells were added). The reason why the conductivity in experiment 3-1 increased was because of the calcium carbonate in the shells.

The decrease in light readings when the shells were added means that less light was reflected off of sediments in the water. Thus, it is proven quantitatively as well as qualitatively that there was a drastic decrease in sediment in the containers with shells as well as in the sections of the river with the shells. The most probable cause for this is that the shell's structure traps sediment in its micro-filter.

Furthermore, the results from the experiment conducted on the McIntosh Runs show that the shells are effective in the field as well as in the lab. The shells which were placed in the stiller section had superior results than the more rapid section. This is because the water was able to sit in the shells for a longer period of time, leaving the calcium carbonate in the shells with more time to disperse into the water. It also gave more time for the filtration process to occur. The results from this experiment were not as dramatic as the ones in the lab because there is a lower shell to water ratio. However, it is still a phenomenal achievement to remediate water in such a dynamic situation.

Applications of this project can be used to better biotechnological means. This is a safe alternative to chemicals that are frequently being used. The Halifax harbour is a prime example of a situation where a biological, environment-friendly process (the shells) could be used as opposed to a multi-million dollar, harmful chemical procedure. This innovative solution could save the city of Halifax millions of dollars and could improve the water conditions of the harbour naturally instead of possibly harming it more chemically. The river that this project has been applied to could also use this environmentally safe process. Every year the McIntosh Run has annual clean up to remove components from the river - unfortunately solely the eyesores. Shopping carts, garbage bags, even spare tires end up in the river. Yet the community does not do anything about the chemical imbalance of the substances in the water. This shell procedure could improve the condition of the river water's internal components and produce a safe and healthy habitat for new organisms to develop, such as fish. From a local river that needs cleaning, or to a third world country that has polluted water, this safe, inexpensive, environmentally friendly procedure of using shells to purify water could benefit plants, animals, and human beings for many years to come.

Item 10.3.1

From: Dr. Michael Simonich and Dr. Katharine Field
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Oregon State University
Corvallis, OR 97331
Ph: 541-737-2572

To: Shalom Mandaville
[redacted]

Date: January 31, 2003

Subject: Report of results for human and dog fecal markers in lake samples.

Summary:

Twenty-one samples (18 filters and 3 blanks) were extracted for total DNA content and analyzed in duplicate by PCR with 2 human and 1 dog host-specific fecal markers for presence or absence of the marker. The samples were also analyzed by PCR with a non-specific marker of fecal contamination. A measurable quantity of DNA was recovered from 17 of the 18 filters. The filter from which no DNA was recovered (269081) was also negative in all PCR analyses. Of the 17 filters, all were positive for the non-specific fecal marker. Nine of the 17 filters were positive for one or both human markers. None of the filters was positive for the dog marker. A geographical limitation of the markers, or, fecal pollution from sources other than human and dog are suggested.

Method of analysis:

DNA extraction

The 18 filters were received in guanidine isothiocyanate (GITC) buffer, a nucleic acid preservative and cell lysing agent. The 3 blanks were GITC without a filter. Each filter held the filtrate from 100 mls of lake water sample. The sample tubes were vortexed for several minutes upon arrival to ensure maximum removal of filter-adhered cellular debris. A 0.2 ml aliquot of each of the 21 lysates was extracted using a Fast DNA kit (Q Biogene, Carlsbad, CA) according to the manufacturer's protocol. The DNA of each extract was eluted at 60°C for 5 min. in 0.1 ml 10 mM Tris-HCl, pH 8.0 and stored in siliconized microcentrifuge tubes at −20°C. The DNA concentration in each eluate was determined flourometrically with the double strand DNA binding dye Pico Green (Molecular Probes, Eugene, OR) according to the manufacturer's protocol.

PCR analysis

The markers are PCR primers and are also referred to here as such. The non-specific fecal markers Bac32F/Bac708R comprise a forward and reverse priming pair. The 2 human source specific fecal markers HF134F and HF183F and the dog marker are routinely paired with Bac708R as only one member of the pair need be host-specific. Bernhard and Field, 2000, previously described the non-specific and 2 human markers (Appl. Environ. Microbiol. 66: 1587-1594) and (Appl. Environ. Microbiol. 66: 4571-4574). The dog specific fecal marker was recently developed in our laboratory and is not yet published. One microliter of the eluted DNA was used in replicated PCR reactions for each marker. PCR reaction conditions were 1X TaKaRa ExTaq PCR buffer (Panvera, Madison, WI), 200 uM each dNTP, 0.2 uM forward primer and 0.2 uM Bac708R, 0.06% BSA, 0.63 U TaKaRa ExTaq and 1 ul sample DNA eluate. The total reaction volume was 25 ul. All samples were also diluted 10 fold in pure water and run in duplicate PCR assays to check for inhibition of PCR. A positive control consisting of human or dog fecal DNA was included with the appropriate marker PCR set as verification of the PCR performance. Thermocycler conditions were 94°C denature, 3 min, 1 cycle; 94°C denature, 1 min, anneal 1 min, 72°C extend, 1.5 min, 40 cycles; 72°C, 7 min final extend, 1 cycle; 4°C hold. The annealing temperature was 53°C for the non-specific marker, 63°C for both human specific markers, and 64°C for the dog marker. Completed PCR reactions were scored for presence/absence of the correct size product for each marker on 2.2% agarose-TAE gels stained with ethidium bromide.

Results and conclusions:

PCR scores are tabulated below.

1 = positive for marker; 0 = negative for marker

Sample ID	Bac32	HF134	HF183	Dog
264776	1	0	0	0
264777	1	0	0	0
265544	1	0	0	0
265545	1	1	0	0
265802	1	0	0	0
265803	1	0	0	0
266028	1	0	0	0
266029	1	1	1	0
266746	1	1	1	0
266747	1	0	0	0
267600	1	1	1	0
269081*	0	0	0	0
269803	1	1	0	0
270311	1	1	1	0
270680	1	1	1	0
106	1	1	0	0
282	1	0	0	0
504	1	1	1	0
Blank 1	0	0	0	0
Blank 2	0	0	0	0
Blank 3	0	0	0	0

* No detectable DNA in extract

In all instances the positive PCR control amplified well indicating no underlying problems with the PCR reaction components. Moreover, detection of the non-specific fecal marker in all samples for which DNA was recovered in the extraction step suggests that integrity of the samples for PCR amplification was not compromised. PCR analysis with each sample diluted 10 fold yielded identical results, indicating that significant inhibition was not present in the 1X eluates.

The ready detection of non-specific fecal contamination by PCR is in agreement with significant fecal coliform counts (per 100 ml) reported for all 18 samples by James Halliday. One possibility for finding human marker in only half the samples and dog in none when these two sources were communicated to be the most likely source of contamination is a geographical limitation of the markers. Fecal DNA sequences that the host-specific markers detect may be more variant over North America than our sequence database reflects. Thus, the markers may not always detect human and dog fecal contamination even though its presence is suggested by prior knowledge of likely sources and the non-specific marker data. While certainly possible for the dog marker, which has been less extensively tested, this explanation is unlikely for the 2 human markers. Both human markers have been tested over much of North America with no indication that both markers ever missed human fecal pollution above the PCR detection limit of the non-specific marker.

Another possibility is that a different source of fecal pollution is impacting the lake. In the obvious absence of farm animal impact, the most likely source is waterfowl. The non-specific fecal marker readily detects waterfowl fecal contamination. We are currently developing gull and goose/duck specific markers and hope to employ them in 2003.

Sincerely,

[redacted]

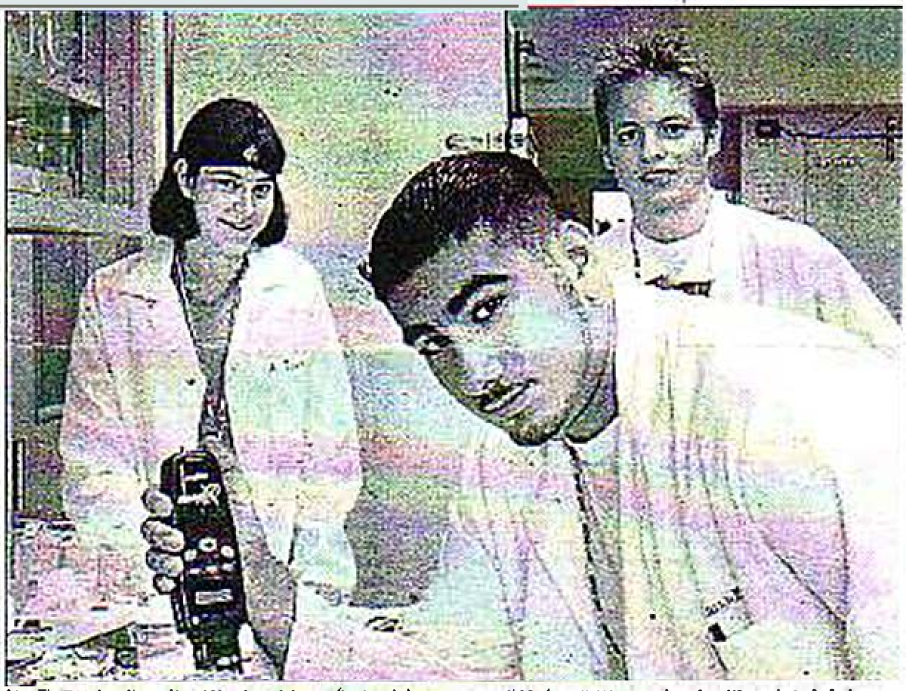
Michael Simonich, Ph.D. Katharine Field, Ph.D., Associate Professor

Item 10.3.1

School science fair winners pioneers in water clean-up research

By Catherine Roberts
MPWWR

[illegible]



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Project Objective:

The objectives of the continuation to our project are to answer the following questions:

1) What is the saturation point of shell and mass of shell to volume of water ratio.

2) Can other types of shells produce effects similar to those from our previous experiments?

3) If the shell is crushed into a fine powder, will the effects differ, and how?

4) Can the shells produce the same results on a large scale basis? (i.e. the Springfield Lake Sewage Treatment Plant)

5) To analyze any changes within the shell after the reaction occurs. Possibly with an atomic force microscope to verify that the structure of the shell is causing this process. Also, to mass the shell before and after to confirm that the shell is absorbing sediment and heavy metals.

Introduction:

Previously to last year, the notion of scallop shells being utilized to purify water was unknown and absurd. Normally discarded, the research conducted discovered that this "sea waste product" was capable of significantly reducing amounts of bacteria, dissolved cations from metals, dissolved particles – even odour – in various kinds of water. It was discovered that it was not only the chemical make-up of the shell but the surface of the shell itself that was contributing to this effect. Furthermore, it could counteract the effects of pH imbalance, resulting from chemical spills or acid rain, by altering the pH in a trend towards seven. Perhaps the most astonishing of the results obtained was that this shell was capable of producing these results within a mere 24 hours (and in our experiment using the McIntosh Run, less than a week). Having concluded this project, it became evident that there was an immense need for a methodology such as this throughout the world.

In fact in Japan, scallop shells are being used in other innovative ways. Utilizing a fine scallop shell ceramics powder, it was discovered that it had the ability to decompose chemical materials, plastics, reduce chemicals, and even repel insects. (http://www.pow.hi-tech.ac.jp/kenkyu/koyama/english/scallop_e.html).

Another type of shell that is abundant in this region is clam shells. Clam shells have been used and are still being used for purposes such as road construction and to enrich soil in gardens; however, they are normally just thrown away not unlike what is done with scallop shells. Both scallop shells and clam shells are inexpensive and locally available materials. Even though clam shells have some common uses, they have never been tested for any water purification properties prior to this.

While practical usage can be located throughout the world, it can also be found as close to home as the Halifax harbour. Sewage plants require tremendous funding to be built, to maintain, and often the water emitted is still harmful. It was clear, scallops shells could be of great aid in the cleaning of sewage water. It was found that single scallop shells could clean large amounts of water in a very short period of time, but the exact amount of water that could be cleaned was still unknown. Moreover, there were many other questions left unanswered.

To our phenomenal luck, others in the community became aware of this research and offered their gracious help – Mayor Peter Kelly offered us summer jobs to continue, and Dr. George Iwama awarded us each "Youth Innovation Fellowships" entitling us to lab space and library access at the NRC. We whole-heartedly took this opportunity to expand our knowledge and, as a result, have taken another step forward to fully understanding the extraordinary properties that shells contain in the purifying of water.

Methods:

The combination of *in vitro* and *in vivo* testing helped to maximize the projects testing area and results. For each experiment, we measured light, conductivity, pH, and temperature using the Pasco Probes.

Lab Experiments (*in vitro*)
In all of our laboratory experiments readings were taken of the following: temperature, light, conductivity, pH, mass, and fecal coliform count.

Experiment #1 More Detailed Sterilized Shell Experiment

Objective: To determine if the sterilized shell produces the same effect as the system pertaining to fecal coliform decrease and odour removal.

Materials: 4 x Scallop Shells
3600ml Sewage Water (from Springfield Sewage Plant)
Funnel, Filter Paper
6 x 1500ml Beakers
Oven
Pasco Probes
Balance Scale

Method: This laboratory experiment was conducted at the National Research Council, using the designated space. To begin, the four scallop shells were cleaned (with tap water) and massed to ensure they are of equal mass. Two were then baked in an oven set at 350°C for 30 minutes, eliminating any organisms present on the shell.

The sewage water was filtered, by means of the funnel and filter paper. Next, 600 ml of this solution was poured into each of the 1500ml beakers. Readings were taken for temperature, light, conductivity, and pH.

Following this, the baked shells were added to two of the six beakers, while the non-sterilized shells were added to two others.

After a time period of 12 to 24 hours, readings of temperature, light, conductivity, and pH were taken. This process was continued for a period of 15 days. However readings were not able to be taken on weekends or holidays. After this period had ended 100ml of the sewage water from control 1, baked 1, and system 1 were poured into sample bottles, labelled and immediately taken for fecal coliform testing at the Water Bacteriology department of the Queen Elizabeth II Health Sciences Centre.

The shells were then removed concluding this experiment.

Experiment #2 Saturation Point & Mass of Shell to Volume of Water Ratio

Objective: To discover the saturation point of the purifying process of a scallop shell and to determine the mass of shell to the volume of water ratio (How much shell is needed in how much water?)

Materials:

- 1 x Scallop Shell
- 4 x 0.4 meters of plastic tubing
- 2 x plastic fish packing containers
- Sink
- Atomic Force Microscope
- 2 x Plastic Plugs
- 16 L of sewage water(Springfield Sewage Plant)
- Pasco Probes
- Filter paper and funnel
- 1 x 1000ml scoop
- 2 x aquarium pumps

Method:

This laboratory experiment was conducted at the National Research Council in the laboratory space provided for us. Initially, the shell was cleaned using tap water to remove any sediment and remnants of the organism. The shell was then massed and recorded. The apparatus for this experiment was set up twice, one as a control and one with the scallop shell as seen in figure 3-1. The Pasco Probes were used to take original readings of the solution.

2 litres of the sewage water were filtered and added to each of the containers. Initial readings were taken and recorded in both the control and the apparatus that would contain the shell. The shell was then added to one of the containers and submerged in the sewage water. The aquarium pumps were then plugged in.

Readings were then taken every 24 hours using the Pasco Probed in order to determine if and when the water had been purified. After this solution has been purified by the shell a 100ml of the water was placed into a sample bottle for immediate fecal coliform testing. The water was then replaced by removing the plug in the corner of the container. This causes the solution to run down the second plastic tube and into the sink. At this time another 2 litres of the solution was added by the same method as above. This process was repeated for the control simultaneously.

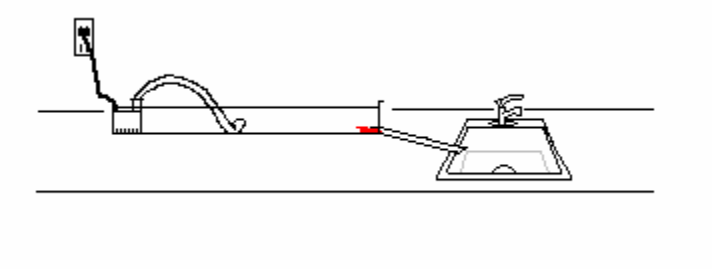
The above methods were repeated until the shell became saturated and no longer purified the solution, at which point no more solution was added. At this time the amount of solution that has been purified will be recorded. The shell will also be massed and recorded. This shell will then be taken to Dalhousie University to have a picture taken of its surface with an atomic force

microscope in an attempt to observe the difference between the surface of a saturated and unsaturated shell. Time will be kept as to determine how long the scallop shell takes to saturate.

The mass of the shell before the experiment began and the amount of solution it purified will be used to calculate the mass of shell to the volume of water ratio. The time it took for the shell to purify the solution and the amount of solution it purified will be used to calculate the saturation point of the shell.

This concludes the experiment.

Figure 3-1



* In this experiment a sample of the water was removed from the beaker in order for the conductivity reading to not be increased by the calcium carbonate in the shell.

Experiment #3-1 Crushed Shell

Objective: To prove if the purifying process occurs if the shells are crushed as opposed to being whole.

Materials:

Scallop Shells 2 Crushed & 2 Whole	3 L of McIntosh Run water
6 x 1000ml Beakers	Pasco Probes
Balance Scale	Hammer
Sifter	

Method: This laboratory experiment will be conducted at the National Research Council in the laboratory space provided for us. 500ml's of contaminated water from the McIntosh Runs was used in each of the six beakers. Two beakers remained as controls in this experiment (just the contaminated water), two beakers contained the contaminated water and the crushed scallop shells, and two beakers contained the contaminated water and the whole scallop shells.

Initially, four scallop shells were cleaned (with tap water) and massed to ensure they are of equal mass. Two of these scallop shells were crushed into a fine power using a hammer and a sifter.

Original readings were taken from each of the beakers before the shells were added. The shells were then added and readings were taken every day for a period of 10 days. After this time period had expired, samples from control 1, crushed 1, and system 1 were taken for fecal

coliform testing at the Water Bacteriology department of the Queen Elizabeth II Health Sciences Centre.

The shells were then removed concluding this experiment.

Experiment # 3-2 Crushed Shell pH Curve

Objective: To determine how quickly the crushed shell can raise pH to a neutral level.

Materials:	1.73g of Crushed Scallop Shell	30ml's of pH Buffer of 2.0
	1 x 60 ml Beaker	Pasco Explorer and pH Probe
	Balance Scale	Hammer
	Sifter	Datastudio

Method: To start, a scallop shell was cleaned using tap water and it was then crushed into a fine powder. The ratio of the amount of crushed shell to volume of water from experiment 3-1 was calculated. Following this same ratio, 1.73g of crushed shell was used for 30ml's of the solution. This mass was found and recorded.

The pH probe was set to take a reading of pH every second after the experiment was started in order to create a pH curve.

The crushed shell was added to the solution and the pH probe began to take readings. The readings continued to be taken until the pH ceased to rise anymore. When this occurred the experiment concluded.

Experiment #4 Clam Shell

Objective: To determine if clam shells possess similar purifying properties to that of scallop shells.

Materials:	6 x 1000ml beakers	9 x Soft Shell Clam Shells
	2 x Scallop Shells	Balance Scale
	3600 ml's litres of Sewage Water	Pasco Probes

Method: This laboratory experiment was conducted at the National Research Council, using the designated laboratory space. 600ml's of sewage water from the Springfield Lake Water Pollution Control Plant was placed into each of the six beakers.
Two scallop shells were cleaned using tap water and massed and recorded to ensure that they had the same mass. Nine clam shells were then cleaned and each one was massed and recorded separately to make sure that there total mass was equal to that of each of the scallop shells.

Original readings were taken of each of the six beakers before the shells were added.

Five clam shells were placed into the beaker labelled clam 1 and 4 clam shells were placed into the beaker clam 2. One scallop shell was placed into each of two beakers and the other two beakers were left as controls.

Readings were then taken every 24 hours for a 7 day period. After this time period had expired samples were taken from control 2, clam 2, and scallop 2 to be tested for fecal coliform.

The shells were then removed and massed again. These masses were then recorded concluding this experiment.

* In this experiment a sample of the water was removed from the beaker in order for the conductivity reading to not be increased by the calcium carbonate in the shell.

Experiment #5 Food Colouring

Objective: To determine if the shell is absorbing coloured solute in a solution.

Materials: 2 x 600 ml Glass Beakers
800 ml of Tap Water (room temperature)
Pasco Explorer using Light Probe *
1 x Scallop Shell.
2x0.5 ml Red Food Dye,
Eyedropper
Camera

Method: Rinsed scallop shell thoroughly with tap water, removing dirt and remnants of the organism, then dried. In setting up the experiment, a sheltered, untouched area without light fluctuations was found. A white towel was put down upon which the beakers and shell were laid.

Filled the beakers with 400 ml of tap water, and took a light reading of each by holding the probe at the rim of the beaker, positioned vertically **. Added food dye and stirred; again light readings were taken. Next, the interior surface of the shell was photographed.

Following this, the shell was added to one beaker, and another light reading was taken. Using the camera, a photograph was taken from the side, and from above. It was left for 24 hours. After this time had elapsed photographs were taken of the two beakers.

The shell was removed from the beaker; any change in colour was looked for and where it was most concentrated. The shell was again photographed. Finally, a light reading was taken of the water, in the beaker that the shell had been removed from. This was the value that was used for comparison.

* Ensured light probe remained at same light intensity level throughout experiment (candle, bulb or sun).
** Note: Light readings were analyzed carefully in this lab to find significance -higher lux readings mean more food dye was present; lower lux readings signify less food dye was present.

Field Experiment (*in vivo*)
Experiment #6 Springfield Lake Water Pollution Control Plant

Objective: To determine how effectively scallop shells can purifying water on a large scale basis.

Materials:

Pasco Probes	Plastic Cable Ties
Utility Net	490 x Scallop Shells
2 x Sterile Sample Bottles	Sample Scoop

Method:

Before initiating the actual experiment, measurements were taken of the trough in the indoor section of the plant. Following this, 7 bags of shells were constructed using the utility nets and plastic cable ties in accordance to the width and depth of the trough; each contained 70 shells.

They were then placed into the trough and spaced 4 feet apart. Using the sample scoop, samples were taken before and after the shell cages, and tested using the Pasco probes. Each consecutive day, accept for the weekends and holidays, samples and readings were taken using the sample bottles and were recorded at the National Research Council. Fecal coliform counts were conducted twice, as budget permitted.

This experiment began on August 1st and concluded on August 26th. At this time, shell cages were removed from the trough.

Results:

For each experiment four graphs were plotted: temperature, pH, light, and conductivity. See attached graphs and tables for quantitative measurements.

In experiments 1, 2, 3, 4, and 6 many changes occurred. In all of the experiments the pH level increased or decreased to approach 7.0 after addition of the shells.
In addition, there was a decrease in conductivity in experiments 2, 4, and 6. There was an increase in conductivity in experiments 1 and 3 because the readings were affected by the calcium carbonate within the shell. In experiments 2, 4, and 6 a water sample was removed from the experiment and readings were taken on this sample. This was done so that readings would not be affected by the Calcium Carbonate in the shell. The light readings in experiments 1, 2, 3, 4, and 6 decreased when the shells were added. The temperature was not affected by the presence of the shells. In all of the lab experiments there was a visible clearing in the solutions with the shells. All of these changes occurred over a 24 hour period.

For experiment 1 a surprising outcome was found. Through the use of fecal counts it was found that a sterilized shell (baked) removed more of the harmful fecal coliform from the water as oppose to a non-sterilized shell (system). The total coliform and E. coli results for the control, system, and sterilized shell can be seen in table 1-5. It was also found that the shells have a

significant affect in a static environment while being used in sewer water. See graphs and tables 1-1 to 1-4. All previous experiments with the use of sewer water were in a fluent environment.

In experiment 2 the goal was to find out what the saturation point and mass of shell to volume of water ratio. It was found that the shell did not saturate in the one and a half month period of time that we worked on this project. Therefore both the saturation point and the mass of shell to volume of water ratio are still unknown. These are promising results because it signifies that the saturation point of scallop shells is high. This experiment will be continued through out the school year and the final data will be collected. The shell continued to clean the water in respects that, the light, conductivity, and fecal coliform readings continued to drop, and the pH rose or dropped to a neutral level.

For experiments 3-1 and 3-2 the purpose was to investigate the purifying properties of a crushed scallop shell. It was found that the shell had very similar effects to that of the whole scallop shell. On average the conductivity readings for the crushed shell were slightly higher than those of the whole scallop shell. The temperature, light, and pH readings were very similar for both the crushed shell and the whole shell. The conductivity readings for the control were lower than both the crushed and whole shell due to the reasons previously stated. The light readings for the crushed and whole shells both dropped from that of the controls. The pH readings for both of these also rose to approach a neutral value while the control remained below the neutral level. See graphs and tables 3-1 to 3-4 for quantitative results. The total coliform and E. coli levels dropped in both the crushed and whole shell. See table 3-5. Experiment 3-2 was designed as a follow-up to experiment 3-1. A pH curve was constructed for the crushed scallop shell and the resulting curve can be seen in graph 3-5.

In experiment 4 it was determined that not only scallop shells, but clam shells as well, have significant purifying capabilities. The clam shell has the ability to drop pH from the basic level, lower the light readings (the amount of sediment), and lower the conductivity level of sewage water. It did not have an effect on the temperature of the solution. The scallop shell also caused the conductivity, light, and pH readings to drop. On average the scallop shell had a greater effect on decreasing the conductivity and light readings. See results in graph 4-1 to graph 4-4 and table 4-1 to table 4-4. The total coliform and E. coli levels dropped in both the clam and scallop shell. See table 4-5.

In experiment 5, it was found that the initial light reading of beaker #1(water plus food colouring) and beaker #2(water, food colouring, and scallop shell) were both 5.45lux. After a period of 27.68 hrs the light reading of beaker #1 was 9.80lux and beaker #2 was 8.75lux. There was a slight drop in the amount of food dye present in the beaker that contained the scallop shell. It was observed that there was no visible colour change in the water. There was a very small amount of red pigment on one side of the shell which was not apparent on the shell prior to the experiment. This pigment was unable to be removed by way of a cloth or rubbing.

In experiment 6, our methods were tested out on a sewage treatment facility at Springfield Lake. It was seen, that even with a high volume of water to mass of shell ratio, that the shells were still having positive effects on the water. The reading taken from the water that had followed through the shells showed a decrease in the amount light and conductivity. The pH had dropped to a

more neutral level than that of the water which had not yet come in contact with the shells. See graphs and tables 6-1 to 6-4. The levels of total coliform and E. coli dropped when the water passed through the shells as seen in table 6-5.

Discussion /Conclusion:

Throughout this project we have learned that biological-based technologies (scallop shells) can safely be used to purify water. It is an environmentally friendly process and uses materials which are normally considered and treated as waste. It is proven to be a swift and comprehensible procedure. After a period of only 24 hours the effects of the shells were already having both visible and quantitative results. Moreover, it has proven to not only raise the pH of acids but to also lower the pH of substances which are too basic such as the sewage water which was primarily used throughout this segment of our research. The effects of the scallop shells are not limited only to pH; they have also proven to reduce the amount of metal cat-ions, sediment, and total coliforms as well as E. coli. Furthermore, similar results were found when parallel testing was conducted using clam shells. In general the clam shells produced the same results as the scallop shells, but to a lesser extent. It raised the pH to approach a neutral level and decreased levels of sediment, conductivity, and E. coli.

Through further testing it was found that the shells lower the presence of harmful biotic coliforms present in various water sources. The shells decrease the number of total coliforms as well as E. coli in sewage and McIntosh Run water samples. It was also remarkably found that sterilized shells reduce more total coliform and E. coli than un-sterilized shells. Through additional testing it was found that clam shells and crushed scallop shells also have the ability to eliminate these harmful bacteria. For quantitative results see tables 1-5, 3-5, 4-5, and 5-5.

The results from the experiment conducted on the sewage treatment plant in Springfield Lake show that the shells are effective in aiding the process of purifying sewage water even in an industrial environment. The shells proved to withstand a constant flow of sewage water for approximately twenty days without showing any sign of a drop in the shells efficiency.

The applications of this project can be used in several areas to improve water quality. In the Halifax area alone, a proposal for secondary treatment plants that use scallop shells to purify water may be formed. Or for the sewer outfalls, it may be proposed that cages of scallop shells be placed at the mouth of the outfall to reduce fecal coliform and sediment that may flow into the harbour. This will eliminate the smell of the water near the outfalls as well. This shell procedure could also be used during the summer swimming season to quickly and efficiently stabilize water that has become elevated, above accepted levels, with fecal coliforms. This process would be less of a hassle then conventional means, as lakes and beaches would not have to be closed down for long periods of time. Finally this method could have far reaching applications. Scallop shells could also be used in third world countries that have unsanitary water. It would be an inexpensive, environmentally friendly, and life saving procedure. So whether it be from the city of Halifax that needs to clean their harbour, or to a third world country that has polluted water, this safe, inexpensive, environmentally friendly procedure of using shells to purify water could benefit plants, animals, and human beings for many years to come.