Annapolis River 2009 Annual Water Quality Monitoring Report

including results from the

Annapolis River Guardians Volunteer Water Quality Monitoring Program

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This report is available electronically at *www.annapolisriver.ca*

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Acknowledgements

The Annapolis River Guardians is a volunteer-based program. Without the dedication of the volunteers, the program would not be the success that it is. We would therefore like to extend our thanks to the volunteers who have contributed their time and energy during the 2009 season. The Annapolis River Guardian volunteers include:

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Environment Canada – Atlantic Coastal Action Program

Nova Scotia Environment

The Acadia Centre for Estuarine Research, Acadia University

Human Resources and Skills Development Canada

Executive Summary

In 2009, the Annapolis River Guardians completed their 18th year of continuous water quality monitoring on the Annapolis River. Ten volunteers monitored eight sites over the course of the season, which ran from April to November. Total suspended solids and turbidity were added to the suite of parameters monitored in 2008 and this monitoring was continued in 2009. The other parameters monitored included dissolved oxygen, *E. coli* bacteria, air and water temperature, pH and conductivity, as well as local weather conditions.

E. coli bacteria levels along the Annapolis River during 2009 were slightly higher than those observed in 2008. The 2009 *E. coli* data exhibited higher medians for many locations, most likely due to the wet weather of 2009. Sampling events from May through September often coincided with significant rainfall events, causing the overall bacteria counts to be elevated. Again during 2009, *E. coli* counts increased markedly between the sampling stations at Aylesford Road and Victoria Road, indicating the introduction of fecal material between these two locations. Some additional sampling was performed on the Annapolis River and its tributaries between these two stations in an effort to identify the source of this bacteria contamination. The results were inconclusive due to the variability of the testing method.

Over 18 years of monitoring, mean dissolved oxygen saturation (DOSAT) levels have remained in the range of 80-94%. In 2009, the mean DOSAT level was 85%.

The mean summer water temperature for the Annapolis River during 2009 was 17.8°C, 2.2°C cooler than for the same period in 2008. This value represents the lowest average summer temperature for the Annapolis River since 1997 and the first time the annual reading has been below the 18-year average since 2003. As in previous years, water temperatures during 2009 continued to reach levels stressful to aquatic life regularly during the summer months ($>$20°C).

The pH levels at each of the River Guardians sites were consistently within the recommended range for the protection of aquatic life (6.5-9.0). Mean pH values for the eight monitoring locations along the Annapolis River ranged between 6.8 and 7.4.

Nitrogen and phosphorus levels were measured at two locations along the river in the 2006 to 2008 period, and monitoring was continued at the Wilmot location in 2009. While elevated total nitrogen results were observed, phosphorus remains a significant concern. During the 2006 to 2009 period, 62% of total phosphorus results exceeded the suggested guideline level of 0.030 mg/L. These elevated phosphorus concentrations are believed to have a role in excessive periphyton growth along the main stem of the river and depression of dissolved oxygen levels in the tidal portion of the river.

Working in conjunction with Environment Canada, turbidity and total suspended solids (TSS) samples were collected in 2008 as part of the regular bi-weekly sample collection as well as during high flow precipitation events. This sampling was continued in 2009, as these activities were part of a two-year effort to establish a baseline for turbidity and TSS in the Annapolis watershed and develop a numerical relationship between these parameters.

CARP has collected benthic invertebrate samples in the Annapolis watershed since 2002, using the protocol developed through the Canadian Aquatic Biomonitoring Network (CABIN). There has been no significant change in the Family Biotic Index at the Paradise location over the period of 2005 to 2008. For the Wilmot location, the Family Biotic Index

improved slightly over the period of 2006 to 2008. The results for 2009 CABIN monitoring had not been processed at the time of writing and were not included in this report.

As part of CARP's Quality Assurance Project Plan, regular quality control samples were collected. The accuracy of River Guardian dissolved oxygen readings were estimated at +/- 0.38 mg/L, compared with 0.094 mg/L recorded in 2008. Travel blank samples, collected to check for cross contamination, consistently had *E. coli* counts of 0 cfu/100ml. *E. coli* split samples had a Relative Percent Difference of 29%.

Introduction

History

The Annapolis River Guardians volunteer monitoring program began collecting water quality data in the Annapolis River watershed in 1992. The Clean Annapolis River Project (CARP) initiated the program as a public awareness project, and has had numerous volunteer sample collectors over the years. It is one of the longest running and most extensive volunteer based water quality programs in Eastern Canada. It is also CARP's longest running and only ongoing project. At least 90 volunteers from the Annapolis Valley community have participated in the program over the years, with over 3500 water samples being collected and analyzed.

The program was initiated in the early 1990's by Dr. Graham Daborn and Dr. Mike Brylinsky of the Acadia Centre for Estuarine Research (ACER). Many groups were involved in the planning process for the program, including staff with the Nova Scotia Department of Health, the Nova Scotia Department of Environment, Nova Scotia Community College, and CARP. Although the program has undergone slight changes over the last eighteen years, its core has remained the same.

The initial program design called for 11 sites to be monitored by 17 volunteers. The initial response from the community was excellent and the project was significantly expanded between 1992 and 1994. In 1994, 38 sites were monitored by 43 River Guardians from 36 households (Pittman *et al*/2001). This intensity of monitoring placed considerable strain on the capacity of CARP. While some of the initial enthusiasm surrounding the program has diminished, a core group of 8 to 15 dedicated volunteers has been maintained over the past number of years.

Program Objectives

The Annapolis River Guardians program has four objectives:

- To establish and support a regular observation system that provides an early warning of environmental problems.
- To provide a long-term record of the river's health.
- To develop interest in the Annapolis River and community stewardship to ensure a viable resource for future generations.
- To provide a knowledgeable group of local individuals who can promote the preservation, rehabilitation, and use of these aquatic resources in the future.

Overview of 2009 Monitoring Season

The first sample collection for 2009 occurred on May 3rd and samples were collected on a biweekly basis until November 1st. The parameters that were monitored were *E. coli* bacteria, dissolved oxygen content, water temperature, air temperature, pH, conductivity, total suspended solids (TSS) and turbidity. The last two parameters, TSS and turbidity, were introduced in 2008 and continued in 2009. They were added as part of a joint project between CARP and Environment Canada to determine baseline levels in the Annapolis River and to establish a mathematical relationship between the two variables. The purpose of this was to allow for the calculation of the water quality index (WQI) for the River, which would be useful in the annual reporting of the data.

Eight stations were sampled along the Annapolis River. Further information on these sampling locations is contained in Appendix B. The monitoring sites for 2009 were all within the freshwater portion of the Annapolis River (Figure 1). The data collected by the volunteers is stored in an in-house Microsoft Access database at the CARP office.

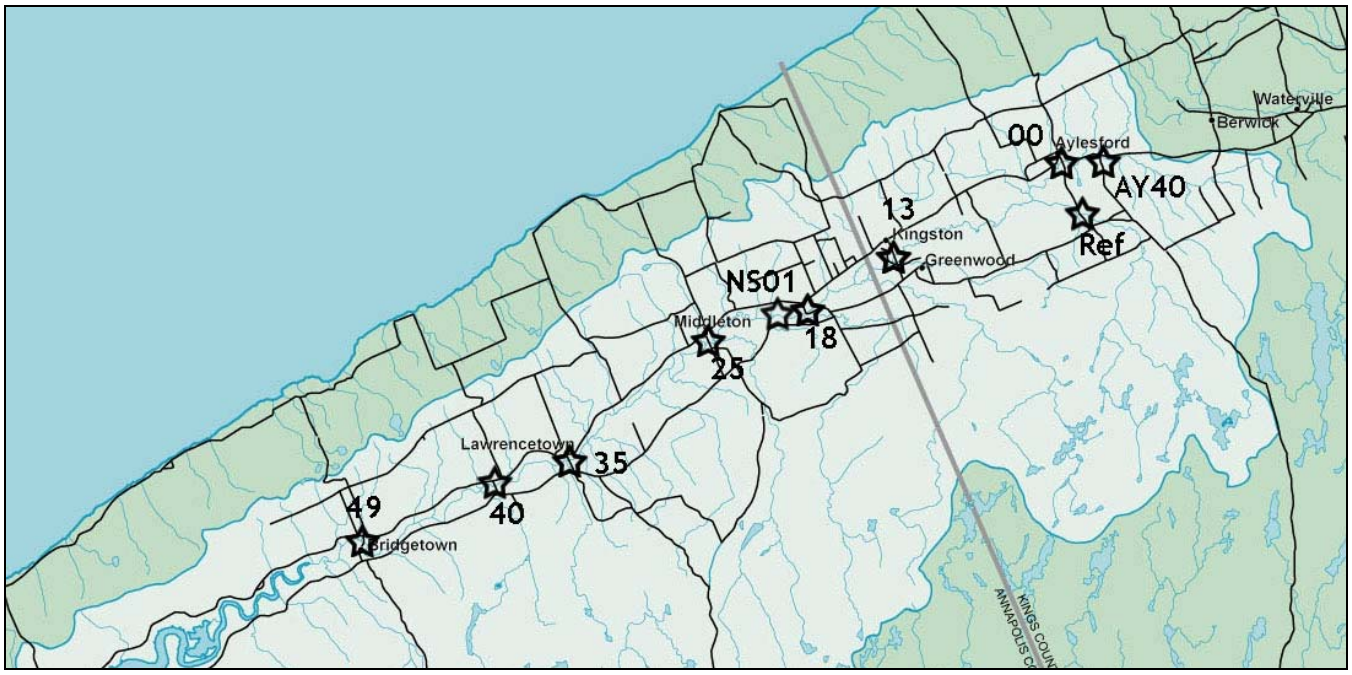

Figure 1. Annapolis watershed with 2009 River Guardian monitoring sites identified by stars. Sites NS01 and Ref, which were used for nutrient and turbidity/TSS monitoring are also shown on this map.

The 2009 River Guardian sampling locations (with their identification numbers) were:

49 – Bridgetown	40 – Paradise	35 – Lawrencetown	25 – Middleton
18 – Wilmot	13 – Kingston	00 – Victoria Road, Aylesford	AY40 – Aylesford Road, Aylesford

All sample sites were located on the main stem of the Annapolis River. With the exception of Aylesford Road (Site AY40), each location has a large River Guardians sign (Figure 2) that indicates *E. coli* contamination and overall water quality trends for that location. The signs are updated by the volunteers every two weeks and are on display from May through to November.

In addition to the regular River Guardians sites, site NS01 (Bayard Road in Wilmot) and Ref (South Annapolis River at Millville) are shown in Figure 1. These sites were not monitored by the River Guardians, but they were used for the monitoring of nutrients by Environment Canada and for turbidity/total suspended solids sampling.

As part of CARP's Quality Assurance/Quality Control (QA/QC) plan (Sharpe and Sullivan, 2006), additional samples were taken to ensure good data quality. The QA/QC measures taken are detailed in appendix C. At the time of writing, CARP's QA/QC plan is in draft form.

Figure 2. River Guardians sign displaying the date, latest bacteria count and overall water quality trend.

In the autumn of 2005, CARP was alerted by a member of the community of foul odours in the vicinity of Middleton's Riverside Park. Subsequent investigation and collection of water samples from Lily Lake Brook, a tributary of the Annapolis River, indicated very elevated *E. coli* levels ($>$20,000 cfu/100 ml). The problem was traced back to limitations in the Town's sewage infrastructure. When heavy rains occurred, the water was collected through the combined sewer system and exceeded the capacity of the sewage treatment plant (STP). This resulted in the discharge of untreated waste to the Lily Lake Brook and the Annapolis River.

In the autumn of 2007, a temporary repair to the STP was made to ensure that untreated waste would not be released into the Lily Lake Brook during peak flow events. During 2009, CARP continued to work with the Town of Middleton to address issues concerning the sewage treatment plant. The Town has received funding from federal and provincial governments for the construction of a new four-cell lagoon sewage treatment plant. Construction at the site commended in 2009, with completion of the project expected late in 2010.

CARP would like to acknowledge and congratulate the Town of Middleton for its persistence in pursuing a solution to its sanitary waste challenges. This case serves as an example of how community water quality monitoring programs, such as River Guardians, can help to identify water quality issues, motivate regulators and polluters to address the problem and work with all parties to ensure the long-term health of a watershed.

<u>Recommendations for CARP:</u>

- Complete the Quality Assurance Project Plan for all of CARP's Water Quality monitoring programs.

2009 Monitoring Results

E. coli Bacteria

Introduction

Escherichia coli (*E. coli*) are rod-shaped, aerobic, lactose fermenting bacteria. They are Gram-stain negative, thermotolerant and appear as dark blue colonies when cultured in the laboratory. The predominant sources of *E. Coli* bacteria in the watershed include poorly maintained on-site septic systems, malfunctioning central sewage treatment plants, aquatic wildlife, domestic animals, and livestock. Because they occupy the same ecological niche as many human pathogens, such as *Cryptosporidium*, *E. coli* are used as indicators for the possible presence of other potentially dangerous pathogens. *E. coli* levels have been identified in the past as a major cause of concern in the Annapolis River watershed (Pittman *et al*/2001).

Many factors in a particular ecosystem affect the abundance of *E. coli* in rivers. These include the type of source, the transport mechanism with which the *E. coli* is deposited, and precipitation. The result is that *E. coli* densities in surface waters can be highly variable. Their survival in surface waters is not well understood, and is dependent on many factors. These include predation by other organisms, amount of sunlight, salinity of the water, temperature, as well as composition and abundance of sediment (Davies *et al*/1995). There is a range of estimates for the survival times of the commonly monitored *E. coli* in various media:

Cow pats: 49 days at 37°C, 70 days at 5°C (also dependent on moisture content) (Chalmers *et al*/2000)
Drinking water: Between 28 and 84 days (Edberg *et al*/2000)
Soil cores with grass roots: 130 days (Chalmers *et al*/2000)
Freshwater sediment: 57 days (Davies et al 1995)

Over the period of 1992 to 2009, numerous initiatives have been undertaken which have contributed to the improvement of water quality in the Annapolis River. For example, in the winter of 1994, 14 Wing Greenwood discontinued the discharge of untreated aircraft wash-water into a tributary of the Annapolis River. In August 1998, the base discontinued the operation of its own sewage treatment plant, redirecting its waste to the Greenwood municipal facility. As was mentioned above, in 2009 the Town of Middleton commenced construction of a new sewage treatment plant.

While the core River Guardian monitoring program has been maintained over the period of 1992 to 2009, a number of modifications have been made. For example, in 1996, the collection of *E. coli* samples was standardized to a fortnightly basis. During the period of 1997 to 2002, fecal coliform numbers were determined using the IDEXX Colilert procedure, which specifically identifies *E. coli*. With the change to a new laboratory, the 2003 and 2004 samples were analyzed using the Membrane Filtration procedure, which enumerates fecal coliforms (see Appendix A). In 2005, the Science Advisory Committee for the Annapolis River Guardians advised that bacteria monitoring be switched from fecal coliforms to *E. coli*, to bring the program more in line with current guidance at a national level. To ensure the continuity of the historic dataset, it was decided to collect split samples for the first two months of the season, to allow parallel testing for fecal coliform and *E. coli*. This process confirmed that the two methods do not give statistically different results. Further information on the parallel testing and statistical analysis can be found in the 2005 Annual Report for the Annapolis River Guardians (Beveridge *et al*/2006).

Canadian Water Quality Guidelines

Various government agencies have developed water quality guidelines to protect the safety of the general public. Health Canada is responsible for the guidelines for drinking and recreational waters. The Canadian Council of Ministers of the Environment (CCME) has incorporated these guidelines in the comprehensive Canadian Water Quality Guidelines (CCME 2002). There have been several different guidelines developed for different possible water uses, such as protection of aquatic life, agricultural uses, drinking or recreation. CARP has summarized some of these guidelines for fecal bacteria contamination into a single table for public awareness purposes (Table 1).

Table 1. Summary of water quality guidelines for fecal coliforms.

cfu*/100ml	Water Use	Explanation/Source
0	Acceptable for drinking	CCME/Health Canada, fecal coliforms/100ml.
1-50	Acceptable for livestock watering	Interpretation of CCME narrative "high-quality water given to livestock" (total coliforms).
50-100	Acceptable for food crop irrigation	Tentative Maximum Concentration. CCME Guidelines (fecal coliform bacteria/100ml).
100-200	Acceptable for recreational use	CCME/Health Canada, Geometric Mean of 5 samples taken during a period not to exceed 30 days, should not exceed 200 cfu/100 ml.
$>$200	Unacceptable for human contact	
cfu = colony forming units		

Monitoring Results

The high variability of fecal bacteria measurements presents a number of challenges with respect to data analysis. Samples collected from a single site, on separate occasions, can vary by two and sometimes three orders of magnitude (e.g. 3 cfu/100 ml to 3000 cfu/100 ml). The use of standard data analysis methods, such as calculating and comparing mean values, inadequately describes the distribution of fecal bacteria results. The following analysis is therefore based on the proportion of samples analysed that exceed particular water quality thresholds. This approach was chosen as it best presents to decision-makers and resource managers whether the water at a site is unsuitable for particular uses.

While this approach eliminates the bias of calculating means with highly variable data, it presents another type of bias. If the majority of samples one year fall slightly below a guideline threshold (e.g. 200 cfu/100 ml), a small increase in fecal coliform concentration the next year may cause the proportion of samples above 200 cfu/100 ml to increase significantly. This would give the appearance that the water quality had worsened considerably, when in fact the mean coliform concentration may have only increased slightly. In order to ensure the differences observed in the following analysis are real, a box-whisker plot was prepared to compare the distribution of the 2008 and 2009 *E. coli* results (Figure 3). The box plot shows the 25th and 75th percentiles as well as the median for each site. The minimum and maximum results are also shown. Note that the y-axis of the graph is plotted using a logarithmic scale (Log *E. coli*) and that the data is artificially capped at 2420 cfu/100mL, as this is the maximum possible value with the IDEXX Colilert testing system. From 1992 to 2009, approximately 3% of the data have exceeded this cap value.

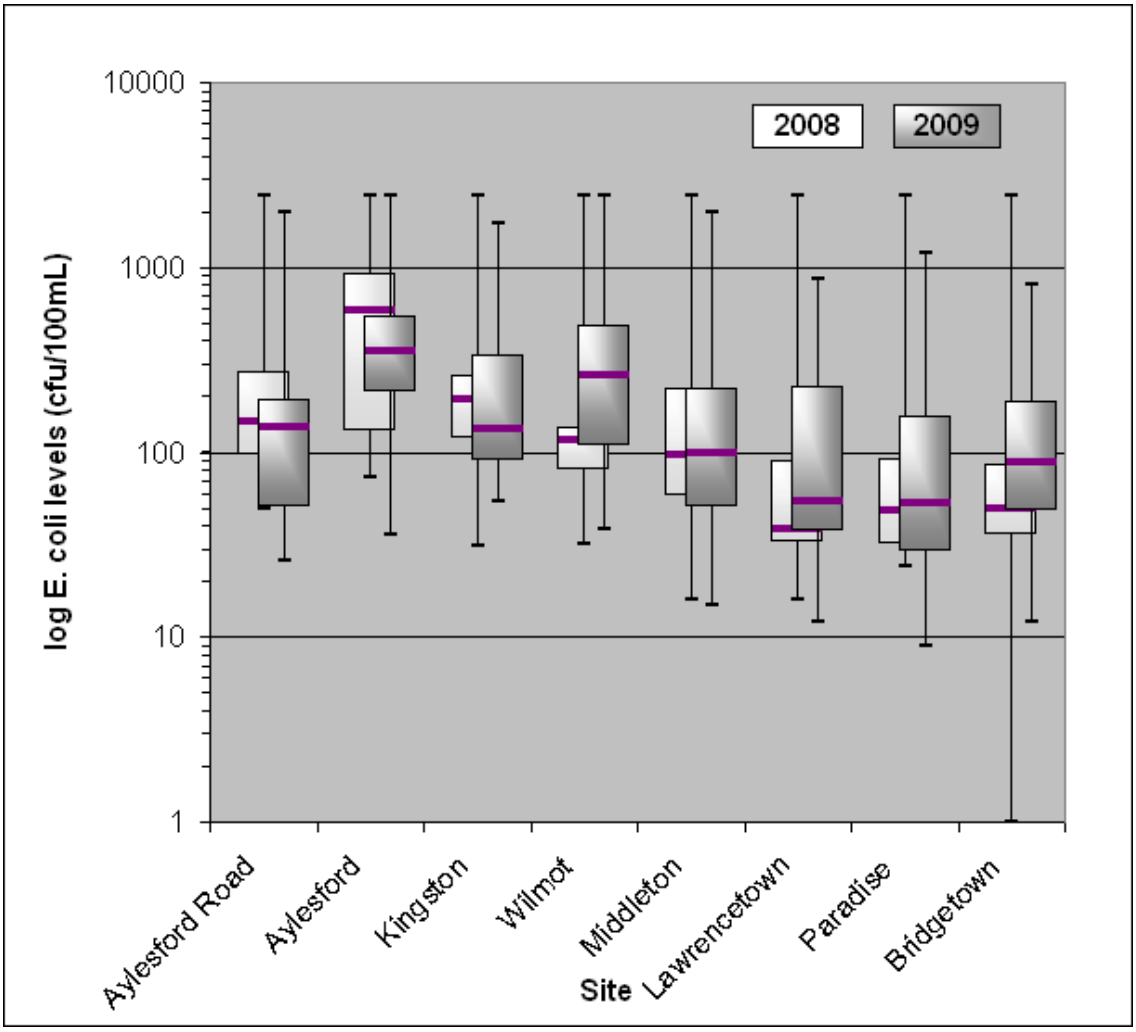

Figure 3. Box and whisker plot of Annapolis River Guardian *E. coli* bacteria results for 2008 and 2009.

In 2009, the median *E. coli* values for Aylesford Road, Aylesford and Kingston were lower than the 2008 respective medians while Wilmot, Lawrencetown, Paradise and Bridgetown all showed higher median values in 2009. The Middleton median remains unchanged from the previous year. Also in 2009, every site except Aylesford showed greater variability when compared to 2008. This may be due to the fact that there was more frequent precipitation in 2009 than in 2008, causing more extreme values to appear.

The *E. coli* data for each River Guardians location was calculated as the percentage of samples that fall within each of the ranges specified in table 1 (Tables 2 through 9). This allows one to easily see how the *E. coli* readings have fluctuated and changed for each station since CARP began monitoring the Annapolis River. All of the *E. coli* ranges are in units of cfu/100mL.

Table 2. *E. coli* percentages for Aylesford Road samples.

Year	% 0 - 50	% 51 - 100	% 101 - 200	% >200
1992				
1993				
1994				
1995				
1996				
1997				
1998				
1999				
2000				
2001				
2002				
2003	20	40	20	20
2004				
2005	33	13	27	27
2006	29	6	6	59
2007	20	20	33	27
2008	8	23	38	31
2009	29	14	36	21

Table 3. *E. coli* Percentages for Aylesford, Victoria Road.

Year	% in 0 - 50	% 51 - 100	% 101 - 200	% >200
1992	0	0	50	50
1993	9	9	27	55
1994	17	17	17	49
1995	66	0	17	17
1996	62	0	0	38
1997	14	14	29	43
1998	15	8	23	54
1999	9	18	27	46
2000	40	0	20	40
2001	25	19	31	25
2002	6	11	33	50
2003	16	16	57	11
2004	6	0	23	71
2005	29	7	7	57
2006	8	23	8	61
2007	6	6	12	76
2008	0	23	8	69
2009	7	14	0	79

Table 4. *E. coli* percentages for Kingston.

Year	% in 0 - 50	% 51 - 100	% 101 - 200	% >200
1992	67	33	0	0
1993	21	21	35	21
1994	33	17	0	50
1995	86	0	0	14
1996	50	19	6	25
1997	19	38	31	12
1998	27	27	27	17
1999	35	18	18	29
2000	40	20	33	7
2001	24	29	18	29
2002	38	28	17	17
2003	13	13	41	33
2004	7	14	43	36
2005	33	7	33	27
2006	7	29	14	50
2007	14	29	14	43
2008	16	0	46	38
2009	0	29	42	29

Table 5. *E. coli* percentages for Wilmot.

Year	% in 0 - 50	% 51 - 100	% 101 - 200	% >200
1992	0	33	0	67
1993	19	12	19	50
1994	13	0	31	56
1995				
1996				
1997	28	11	44	17
1998	60	30	10	0
1999	31	25	19	25
2000	49	17	17	17
2001	25	31	25	19
2002	29	35	12	24
2003	20	47	13	20
2004	0	21	58	21
2005	27	7	59	7
2006	21	36	14	29
2007	27	27	27	19
2008	23	8	54	15
2009	15	8	23	54

Table 6. *E. coli* percentages for Middleton.

Year	% in 0 - 50	% 51 - 100	% 101 - 200	% >200
1992	0	33	0	67
1993	14	14	43	29
1994	9	9	27	55
1995				
1996	40	10	20	30
1997	13	24	50	13
1998	50	0	25	25
1999	50	8	25	17
2000	60	20	7	13
2001	40	18	24	18
2002	65	29	6	0
2003	36	29	14	21
2004	15	23	39	23
2005	54	20	13	13
2006	43	21	7	29
2007	19	27	27	27
2008	14	36	21	29
2009	29	21	21	29

Table 7. *E. coli* percentages for Lawrencetown.

Year	% in 0 - 50	% 51 - 100	% 101 - 200	% >200
1992	0	34	33	33
1993	7	14	21	58
1994	24	6	41	29
1995	42	0	29	29
1996	13	13	33	41
1997	29	35	29	7
1998	42	25	25	8
1999	40	30	30	0
2000	53	20	7	20
2001	56	25	13	6
2002	50	11	17	22
2003	53	20	7	20
2004	21	29	21	29
2005	47	33	20	0
2006	40	7	13	40
2007	58	14	7	21
2008	54	23	8	15
2009	50	14	7	29

Table 8. *E. coli* percentages for Paradise.

Year	% in 0 - 50	% 51 - 100	% 101 - 200	% >200
1992	0	0	67	33
1993	14	14	36	36
1994	14	29	0	57
1995	62	0	13	25
1996	29	18	12	41
1997	50	36	7	7
1998	22	45	22	11
1999	42	25	25	8
2000	33	17	8	42
2001	35	18	29	18
2002	58	6	18	18
2003	40	20	27	13
2004	14	21	21	44
2005	36	36	21	7
2006	33	7	13	47
2007	53	27	7	13
2008	54	23	15	8
2009	44	21	14	21

Table 9. *E. coli* percentages for Bridgetown.

Year	% in 0 - 50	% 51 - 100	% 101 - 200	% >200
1992				
1993				
1994	14	21	21	44
1995	45	11	22	22
1996	29	18	18	35
1997	35	12	47	6
1998	43	38	13	6
1999	33	22	28	17
2000	60	27	13	0
2001	70	18	0	12
2002	41	35	12	12
2003	33	27	13	27
2004	14	7	50	29
2005	39	47	7	7
2006	27	20	20	33
2007	54	13	0	33
2008	50	29	7	14
2009	29	29	13	29

In 2009, only Lawrencetown showed similarity between the percentage of samples (a difference of less than five percentage points) within the 0 – 50 cfu/100mL range compared to 2008. Bridgetown, Paradise, Wilmot and Kingston all decreased in the number of samples within this range while Middleton, Aylesford and Aylesford road all showed an increase in the percentage. In the 51 – 100 cfu/100mL range, sample percentages increased for Kingston, decreased for Lawrencetown, Middleton, Aylesford and Aylesford Road and did not change significantly for Bridgetown, Paradise and Wilmot. For the 101 – 200 cfu/100mL range, sample percentages for Aylesford and Wilmot decreased, increased for Bridgetown while the percentage of samples for Aylesford Road, Kingston, Middleton, Lawrencetown and Paradise did not change significantly. The percentages of samples falling into the >200 cfu/100mL range increased for Aylesford, Wilmot, Lawrencetown, Paradise and Bridgetown; the percentage dropped for Aylesford Road and Kingston, and remained unchanged for Middleton. The increase in the percentage of samples falling into this range was especially pronounced for Wilmot, with an increase of 39%.

The percentage of samples falling into the >200 cfu/100mL category increased in 2009 when compared to 2008, while all of the other categories showed fewer results. This is not entirely unexpected, as the summer of 2009 was significantly wetter than that of 2008, causing *E. coli* levels to spike frequently. The percentage of data falling into each of these categories for all locations was compiled (Figure 4) as well as the number of samples taken in each year (Table 10).

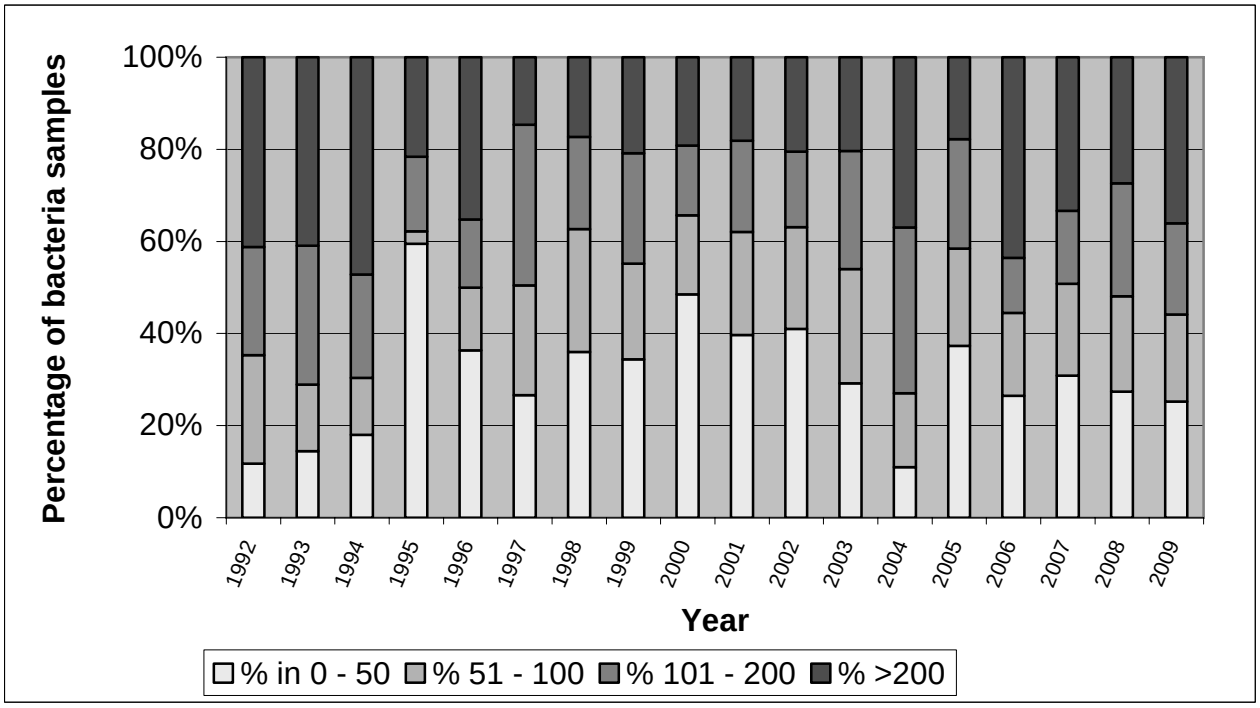

Figure 4. The percentages of fecal bacteria samples that fall in each water quality category by year.

Table 10. The number of *E. coli* or fecal coliform samples taken each year.

Year	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Sample Count	17	83	89	37	88	109	75	96	99	116	122	113	100	118	117	120	106	111

It is important to note that in 1992 and 1995, a relatively small number of samples were collected, meaning results for these years may not be as representative as for other years. 1992 showed an extreme low for sample proportion falling into the 0 – 50 cfu/100mL range, while 1995 showed an extreme high. However, due to the fact that there were so few samples taken in those years, the results may not reflect actual water quality for those years.

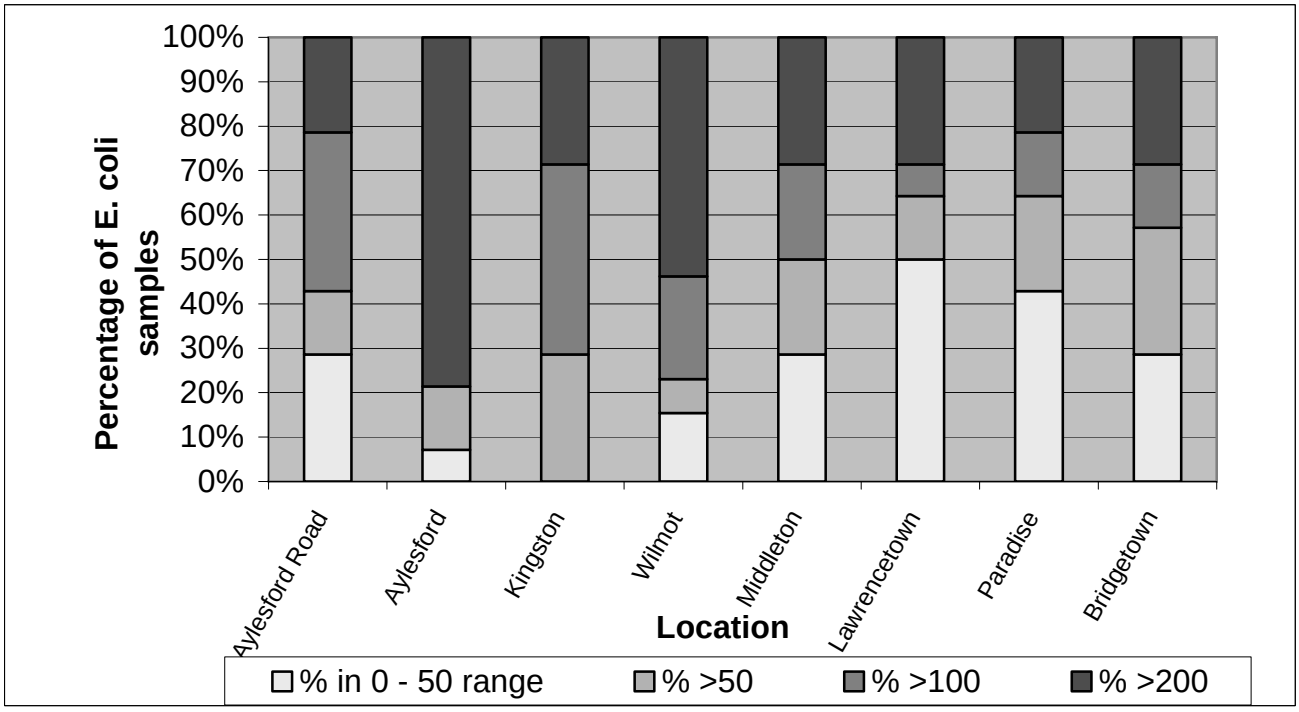

Figure 5. The percentages of 2009 samples falling into the different cfu/100mL ranges, organized by location.

Following a similar pattern as 2008, the highest bacteria counts occurred in Aylesford on Victoria road, while the lowest occurred at the Bridgetown, Paradise and Lawrencetown locations (Figure 5). There seems to be a source of contamination between the Aylesford Road and Aylesford sites, which may be coming in from one of several tributaries that join the main river between these two sites. In 2009, an attempt was made to identify possible sources of this bacterial contamination. These efforts are detailed in the chapter of this report labelled 'Aylesford *E. coli* Investigation.'

Recommendations

- Continue regular River Guardian *E. coli* monitoring at the eight main river sample locations.

Dissolved Oxygen

Introduction

Dissolved oxygen (DO) is a widely used and important general indicator of the health of a river system (Addy *et al* 1997). Aquatic organisms require oxygen in solution for internal respiration. Oxygen in the atmosphere, which is readily available to terrestrial organisms, must be dissolved into the water and is present at much lower concentrations. Wind, wave action, rainfall, and photosynthesis help aerate waterways and increase dissolved oxygen levels. Sewage, lower rates of photosynthesis, eutrophication and limited diffusion from the atmosphere due to ice cover can all lead to decreased oxygen levels.

As the temperature of water decreases, a greater concentration of oxygen is able to dissolve in the water. DO levels are also dependent to a lesser degree on atmospheric pressure and water salinity. The amount of oxygen in water can be reported in two ways, either as a concentration measurement (mg/L) or as percent saturation. Water reaches its saturation point when it can no longer dissolve any additional oxygen for a given temperature. High levels of photosynthesis or turbulent conditions can "supersaturate" the water, resulting in saturation levels greater than 100%. Dissolved oxygen levels below 60% saturation are known to cause stress to aquatic life, particularly cold-water fish species (Mackie 2004).

Monitoring Results

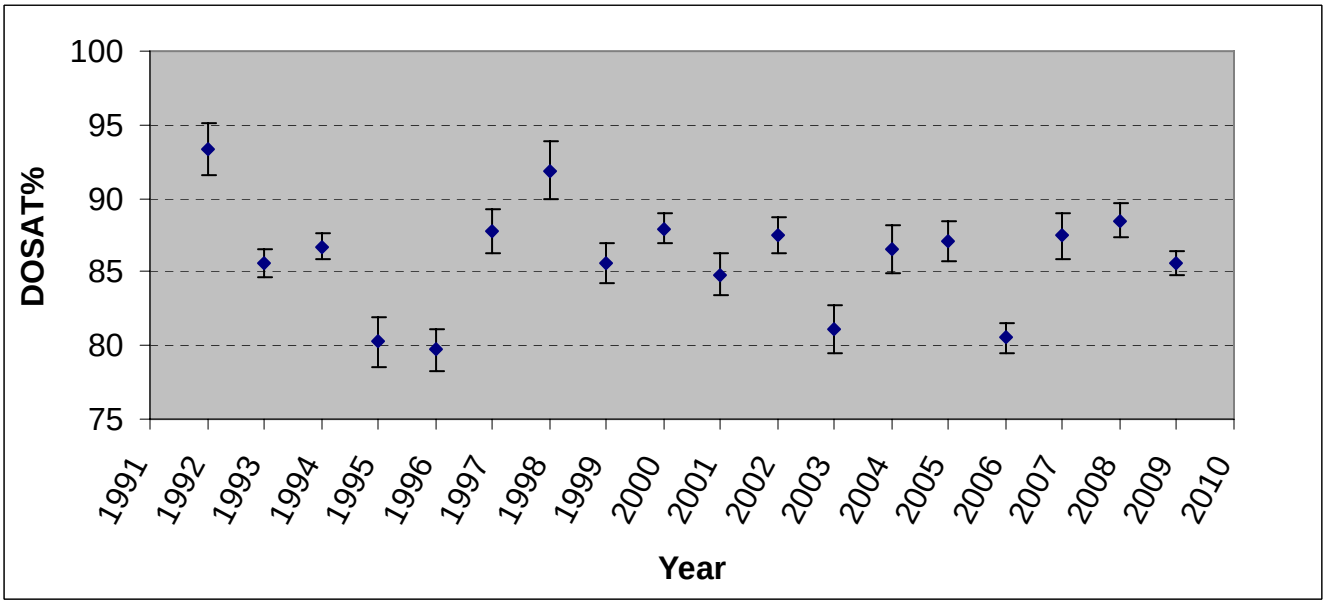

Figure 6. Mean dissolved oxygen saturation (DO SAT) by year, 1992 to 2009 (showing standard error of the mean).

During the period of 1992 to 2009, annual mean dissolved oxygen (percent saturation) levels have varied from a high of 94% in 1992, to a low of 80% in 1996 (Figure 6). For the values recorded during 2009, the mean dissolved oxygen saturation was 85%, compared with 89% in 2008. This value is within the normal range of variability observed for the Annapolis River. The standard error of the mean is shown with error bars and they indicate that there was less variability in the data in 2009.

The 16-year mean dissolved oxygen (percent saturation) values for each of the main river monitoring sites were calculated (Figure 7). The standard error of this mean is shown with error bars. This is overlaid with the mean values for the 2009 monitoring season. With the exception of Lawrencetown, all the results fell within the normal DO range, as shown by the bars indicating standard error of the mean. Note that the average for Aylesford Road is only for 6 years, and that the Middleton and Wilmot averages are missing some data from1995 and 1996.

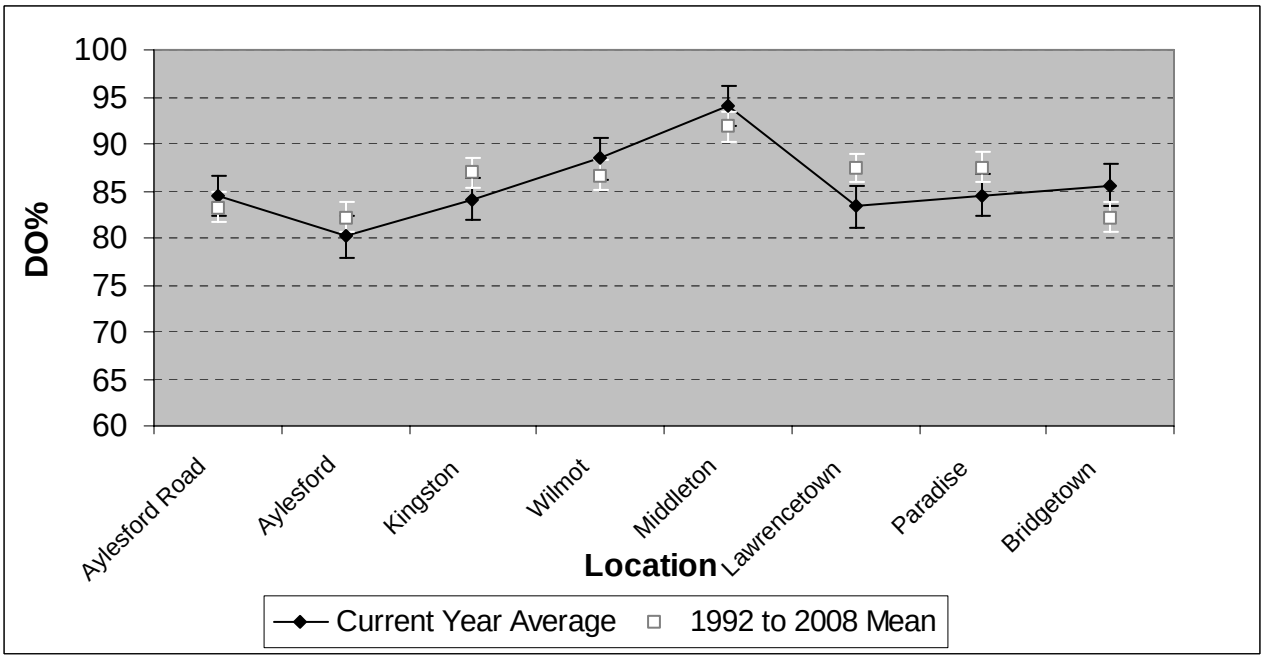

Figure 7. DOSAT results for 2009 as well as mean dissolved oxygen saturation (DO SAT) from 1992 to 2008, organized by sample site. The error bars show standard error of the mean.

The Canadian dissolved oxygen water quality guideline for the protection of freshwater aquatic life is 5.5 mg/L (CCME 2002). Only two of the 109 water samples analyzed by the Annapolis River Guardians in 2008 had dissolved oxygen levels below this guideline level (Aylesford on August 24th and Kingston on September 7th, both with a reading of 5.42 mg/L) (Table 11). The cause of the depressed oxygen at these locations is not known. The high levels of dissolved oxygen observed consistently at Middleton are likely due to input from the Nictaux River tributary, which is fast-flowing and well oxygenated. The Nictaux River joins with the Annapolis River between the Wilmot and Middleton sites.

Table 11. Dissolved oxygen percent saturation (DOSAT) thresholds for Annapolis River.

Site	Samples less than 60%	Samples within 61-74%	Samples greater than 75%	Total Samples 2008
Aylesford Road	0	2	12	14
Aylesford	1	1	11	13
Kingston	1	0	12	13
Wilmot	0	0	13	13
Middleton	0	0	14	14
Lawrencetown	0	0	14	14
Paradise	0	0	14	14
Bridgetown	0	1	13	14
Totals	2	4	103	109

Dissolved Oxygen Monitoring in the Estuary

From 2004 to 2005, low oxygen levels were observed in the Annapolis River estuary, from Bridgetown to Annapolis Royal. This prompted a further investigation in 2007, the details of which were reported by Sharpe and Sullivan (2007). This monitoring was continued in 2008. DO readings were collected at two different depths from the lower river on three separate occasions (Figure 8).

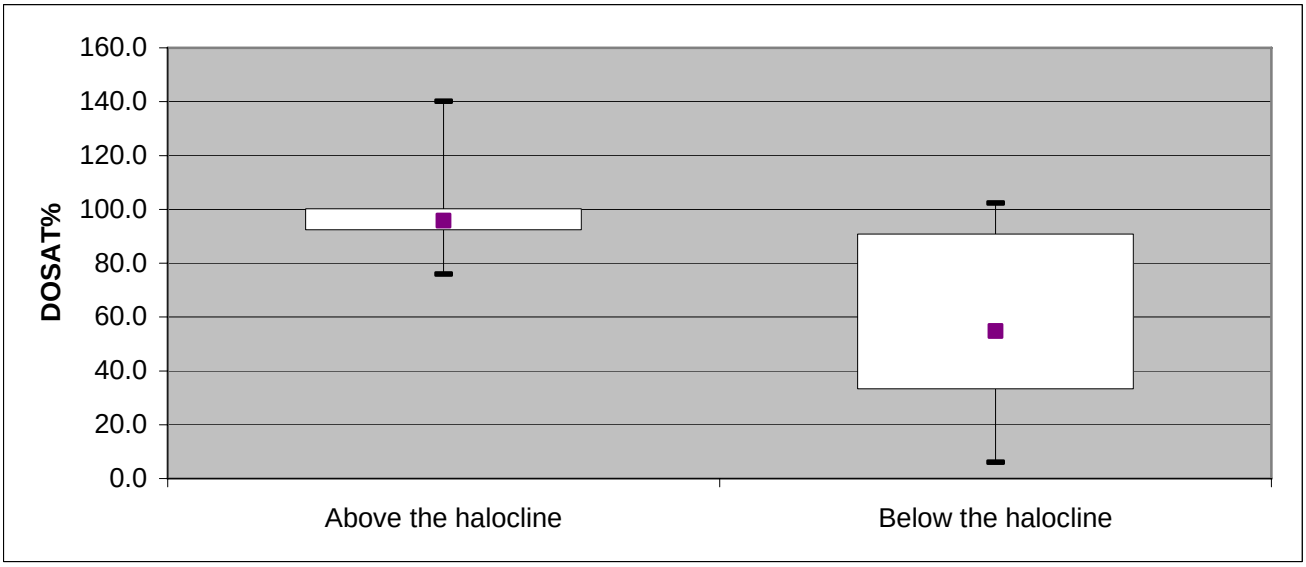

Figure 8. DO levels in the Annapolis River estuary, grouped by depth.

Samples were collected at 8 different locations on August 18th, August 27th, and October 15th, 2008. The lowest DO level observed was 0.50 mg/L, or 6.1%. This value was recorded on August 27th, 2008 at Bridgetown. It occurred at a depth of 3.5 m, below the halocline[1].

The results are similar to those obtained in 2007. DO levels above the halocline are acceptable for supporting aquatic life, while the DO levels below the halocline at times fall to levels that are stressful for aquatic life, and possibly lethal.

Recommendations

- Continue regular River Guardian DO monitoring program at the eight main river sample locations.
- Undertake periodic DO monitoring of the Annapolis River estuary in the late summer and early autumn. These times are most likely to display depressed levels of DO. Depth profiling should be included as part of this monitoring.
- Investigate atmospheric pressure readings to determine whether or not they vary enough to affect dissolved oxygen readings.

[1]Halocline – the layer of water within a river where an abrupt change in salinity occurs.

Temperature

Introduction

Water temperature, like dissolved oxygen, serves as a broad indicator of water quality. The temperature of water has a direct bearing on the aquatic species present and their abundance. For example, trout and salmon species experience stress at water temperatures in excess of 20°C, with lethality occurring after prolonged exposures to temperatures over 24°C (MacMillan *et a*/2005).

Monitoring Results

The mean summer water temperature for the Annapolis River in 2009 was 17.8°C, which is 2.2°C cooler than for the same period in 2008. As in previous years, water temperatures during 2009 continued to reach levels stressful to aquatic life regularly during the summer months, especially near the end of August. For the first time since 2003, however, the average summer temperature was below the total average. In addition, it was the lowest average temperature since 1997 (Figure 9). The mean summer water temperature (July, August, September) values by year for the main eight River Guardian monitoring sites were compared to the 1992 to 2008 mean summer water temperature (18.5 °C).

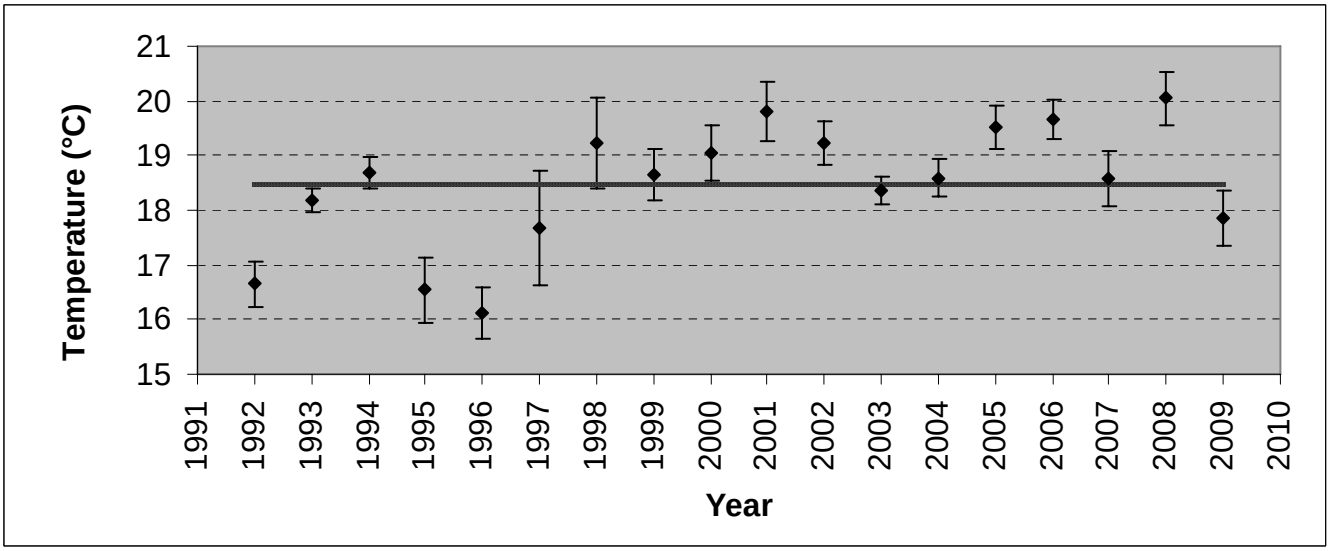

Figure 9. Mean summer water temperature by year (showing standard error of the mean) with 1992-2008 mean shown as a thick line.

The data from previous River Guardians annual reports suggested a gradual increase in temperature in the lower river sites, particularly in the summer data. The mean summer water temperature values along the main Annapolis River in 2009 were compared to the historical averages for those sites (Figure 10). While the temperatures for Kingston were slightly higher than the historical averages, the remainder of the sites were cooler in 2009, some by over 1 degree. With the exception of Aylesford Road, every value falls within the normal historical variability, as indicated by the error bars.

Of the 48 temperature measurements recorded during the months of July, August and September in 2009, 23% exceeded 20°C. The amount that exceeded 20°C in 2008 was 54%. The maximum temperature observed was 24.8°C, recorded at Aylesford on July 27th, 2009.

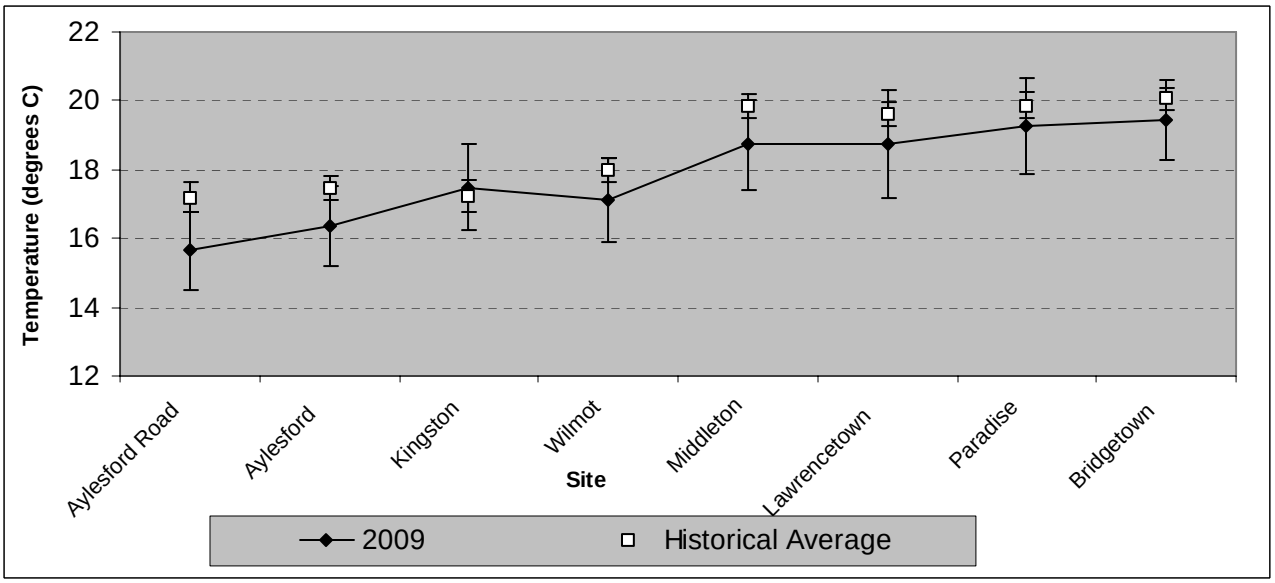

Figure 10. Mean 2009 summer water temperature and historical average temperature (1992 – 2008) by site, with standard error of the mean.

Recommendations

- Continue regular River Guardian temperature monitoring program at the eight main river locations.
- Install temperature loggers in candidate streams to assess for fish habitat improvements.
- Temperature data loggers should be calibrated immediately prior to deployment and at least once *in situ*. These procedures should be added to the QA/QC Project Plan.
- Investigate the temperature increase on the Annapolis River between Aylesford and Lawrencetown. This may include collection of thermal status data on tributaries to the Annapolis River.

pH

Introduction

pH is a measure of the acidic/basic nature of water and is determined by measuring the concentration of the hydrogen ion (H^+). It is expressed on a logarithmic scale from 0 to 14, with zero being the most acidic. As pH is an inverse logarithmic scale, every unit decrease in the pH scale represents a tenfold increase in acidity. To ensure the health of freshwater aquatic life, pH levels should not vary beyond the range of 6.5-9.0 (CCME, 2002). Levels below 5.0 are known to adversely affect many species of fish, including salmon and trout. pH varies naturally depending on the underlying soil type and its buffering capacity; however it can also be influenced by anthropogenic means, such as acid precipitation.

pH values are typically measured on the day following River Guardian collection by CARP staff using the portable HydroLab Quanta water meter (see Appendix A for more details on sampling procedure and meter calibration).

Monitoring Results

pH values all along the Annapolis River are generally good, being only slightly acidic (Figure 11). In total, 103 individual pH measurements were made during 2009 and of these measurements, only 3 fell outside of the 6.5-9.0 healthy range for the protection of aquatic life as indicated by the CCME. A number of the principal tributaries of the Annapolis River pass through the Torbrook geologic formation, which contains limestone that helps buffer rivers and streams in the watershed from acidification. The pH levels seem to be fairly consistent across all the locations on the main stem of the River, with slightly depressed levels at the downstream locations, although the large standard error ranges overlap with many of the other locations' values. The pH drop between Middleton and Lawrencetown may be due to inputs from the Nictaux River, which is highly coloured and has a lower pH.

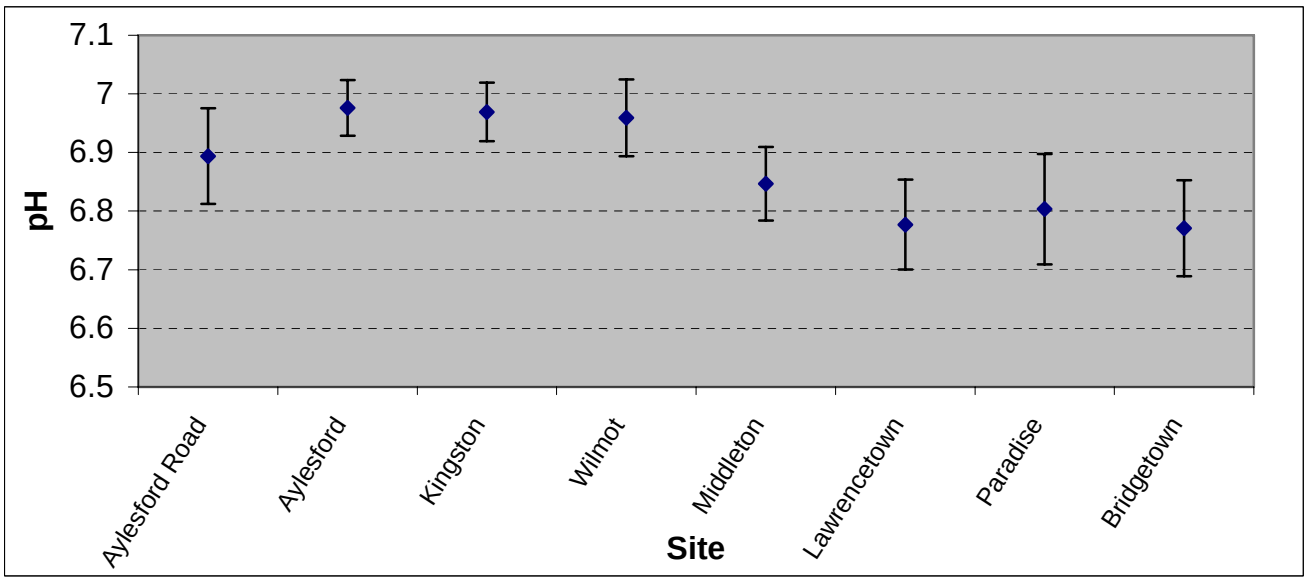

Figure 11. pH 2009 averages for the sampling locations along the Annapolis River, with standard error of the mean.

pH data has been collected from eight main river sites for 2003 to 2009, using the Quanta Hydrolab meter (Figure 12). During the early years of the Annapolis River Guardians program, pH was regularly measured at many of the main river

sample locations. During this period, the mean pH was 6.9, based on 634 individual measurements. This historic pH is similar to that observed during the 2003 to 2009 period.

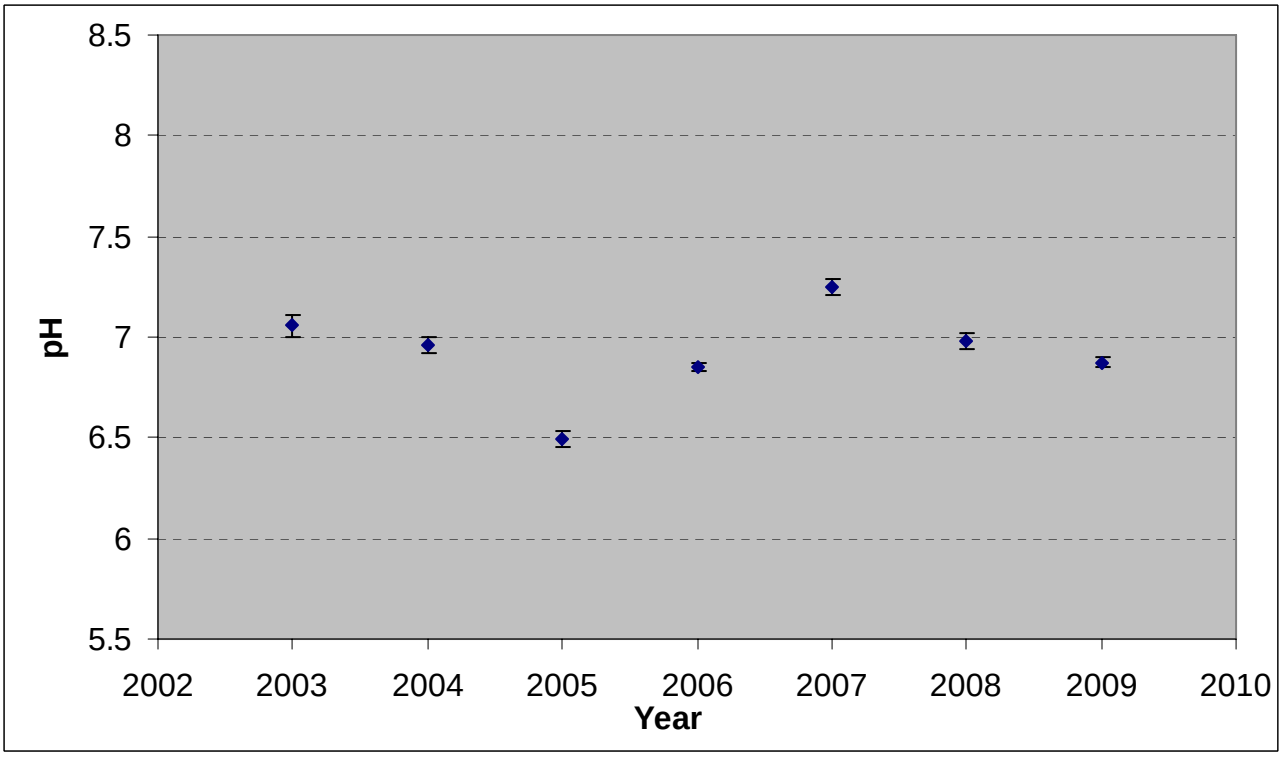

Figure 12. Average pH measurements along the Annapolis River, 2003 to 2008, with standard error of mean.

Recommendations

- Regular pH monitoring should be continued at the eight Annapolis River Guardian monitoring locations.

Nutrients: Nitrogen and Phosphorus

Introduction

Nutrients are naturally occurring substances that are essential for the growth of both plant and animal life. From 2006 to 2007, Environment Canada monitored two locations along the Annapolis River for a large range of water quality parameters, including nitrogen and phosphorus. In 2008, a reference site on the South Annapolis River in Millville was added and in 2009, the Lawrencetown sample site was dropped, and monitoring was carried out in Wilmot and Millville. Although nitrogen and phosphorus are naturally occurring, there are many anthropogenic sources. Any kind of wastewater discharges (domestic, municipal, industrial), agricultural chemicals such as fertilizers and atmospheric deposition can all contribute to elevated nutrient levels in a river system.

Nitrogen and phosphorus occur naturally in very small amounts and thus are often the limiting factor for plant growth. When nitrogen levels rise, they can cause excessive periphyton and macrophyton growth in freshwater systems. Excess phosphorus levels can lead to large algal blooms that, upon dying and decomposing, deplete oxygen to levels that can threaten aquatic life.

There is much disparity between literature sources identifying unacceptable levels of these two nutrients. Dodds and Welch (2000) compiled many different criteria from literature sources for unacceptable levels of both nitrogen and phosphorus. For total nitrogen, depending on the water quality target, the upper limit ranged from 0.25 mg/L to 3.0 mg/L. For dissolved nitrate, the limits are defined to be anywhere from 0.02 mg/L to 1.0 mg/L. The CCME has established a guideline for nitrates at 2.9 mg/L NO_3 as N for the protection of aquatic life.

There appears to be greater consensus for guidelines for phosphorus. The Ontario Ministry of Environment and Energy (OMEE) set a guideline of 0.030 mg/L total P, above which excessive plant growth occurs. Mackie (2004) suggested that total phosphorus levels in excess of 0.030 mg/L indicate that the surface waters are eutrophic. Dodds and Welch (2000) list upper limits ranging from 0.02 mg/L to 0.07 mg/L.

Monitoring results

The nutrient results shown in this section were collected and analyzed by Environment Canada. Environment Canada collects regular water quality samples at one location on the Annapolis River and one location on the South Annapolis River. Grab sampling is performed in Wilmot, near the bridge and gauging station on Bayard Road and in Millville, near the bridge and gauging station on Victoria Road. In the past, Environment Canada has monitored nutrients at a station near the River Guardians site #35 in Lawrencetown. This location was not monitored in 2009 and will not be monitored in 2010.

The results for monitoring of total nitrogen, nitrates and total phosphorus were compiled for Wilmot and Lawrencetown from 2006 to 2009 (Figures 13, 14 and 15 respectively). Nutrients were not monitored at Lawrencetown for 2009, therefore the charts only display data from 2006 to 2008.

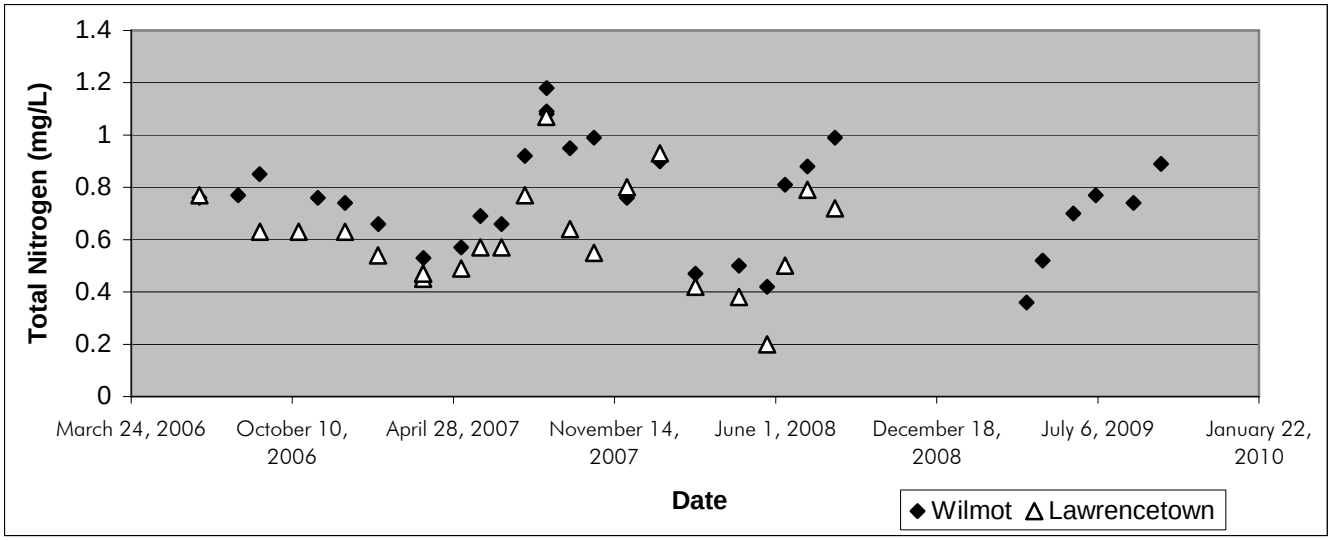

Figure 13. Total nitrogen results from 2006 to 2008 Lawrencetown and 2006-2009 for Wilmot.

The total nitrogen results for Wilmot and Lawrencetown are very similar and display the same spikes, although the Wilmot reading is almost always higher than the Lawrencetown reading (Figure 13). The lowest result was 0.2 mg/L and occurred at Lawrencetown on May 21st, 2008 and the highest was 1.18 mg/L, occurring at Wilmot on August 21st, 2007. All of the results fall into a range described by Dodds and Welch (2000) that potentially causes adverse ecological effects. The 2009 results show a similar pattern of increasing throughout the season as is displayed in both 2007 and 2008. This increase may be due to the use of nitrogen-containing fertilizers in the springtime on surrounding agricultural operations. It is also possible that groundwater inputs influence this increase. Groundwater in the Wilmot area has been shown in the past to have elevated nitrate levels (Nova Scotia Environment, 2009)

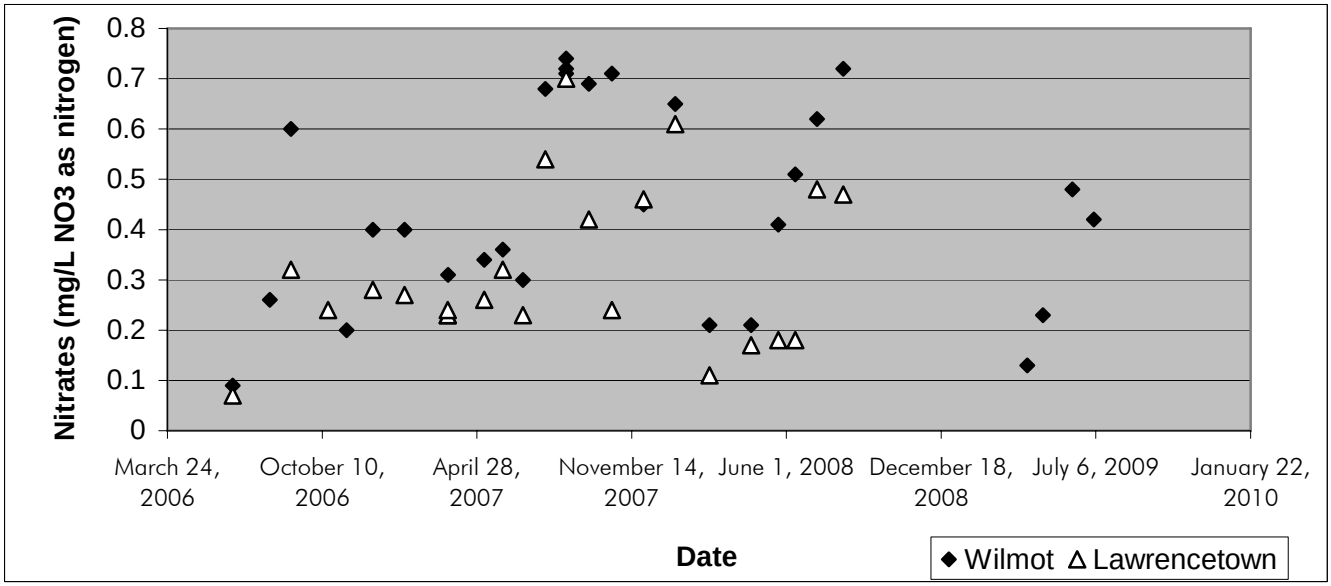

Figure 14. Nitrate results from 2006 to 2008 Lawrencetown and 2006-2009 for Wilmot.

The results for nitrates display most of the same spikes as the total nitrogen graph to different magnitudes (Figure 14). The lowest result was 0.07 mg/L NO_3 as N and occurred at Lawrencetown on June 16th, 2006 and the highest was 0.74

mg/L NO_3 as N, occurring at Wilmot on August 21st, 2007. These levels are far below the CCME guideline of 2.9 mg/L NO_3 as N. Only 4 dissolved nitrogen readings taken in 2009 were available at time of reporting.

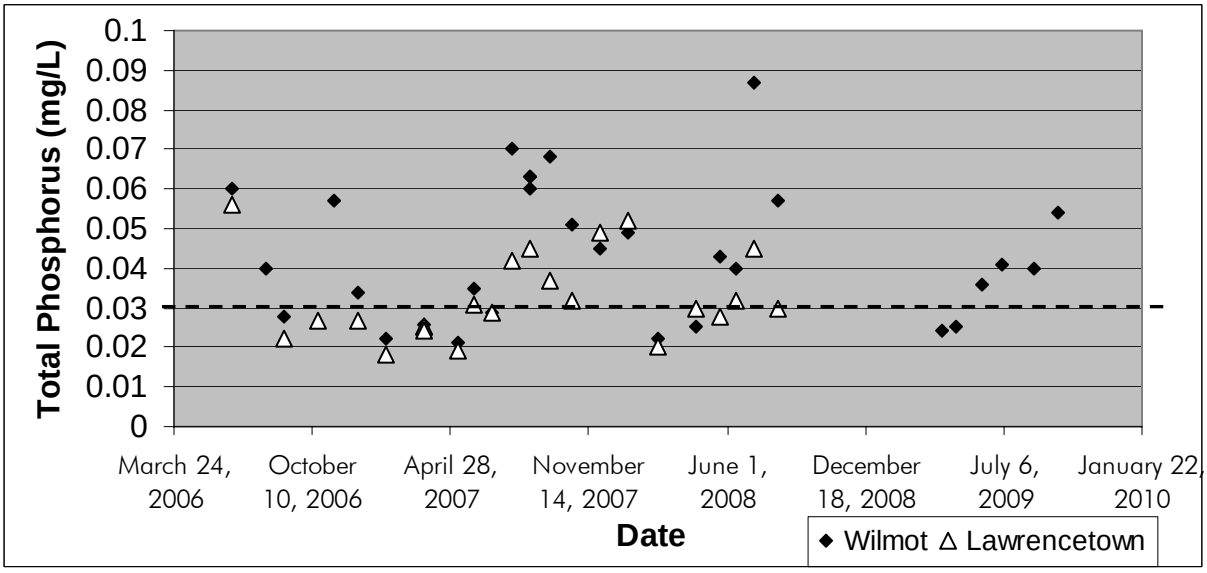

Figure 15. Total phosphorus results from 2006 to 2009 for Wilmot and from 2006 to 2008 for Lawrencetown. The dashed line represents the phosphorus guideline of 0.030 mg/L (Mackie, 2004).

The lowest total phosphorus result was 0.018 mg/L and occurred at Lawrencetown on January 24th, 2007 and the highest was 0.087 mg/L, occurring at Wilmot on July 10th, 2008 (Figure 15). Unlike nitrogen, phosphorus has a better-defined upper limit of 0.030 mg/L. Thirty-one out of fifty-three samples (58%) were above this limit. In 2009, 4 of the 6 (66%) total phosphorus samples were above the desired threshold at Wilmot. The data for Wilmot and Lawrencetown display similar peaks in the data.

In the past, large algal blooms have occurred on the Annapolis River. On July 27th, 2008, the River Guardian volunteer for Bridgetown noted a green colour to the water. On August 1st, 2008, CARP staff observed a dark green colour to the water at this location only. This colour seems to be indicative of an algal bloom and may have been a result of excess levels of nitrogen and/or phosphorus. On the next collection day, August 10th, 2008, the green colour was no longer observable. No instances of an algal bloom were noted in 2009 although the river is not regularly monitored for this phenomenon.

Beginning in May 2008 and continuing into 2009, the South Annapolis River was monitored for nutrients as well. The site was at Millville, near the bridge on Victoria Road, and was chosen as a baseline, relatively unimpacted site. As a result, most of the results for nutrients were lower than the results for either Lawrencetown or Wilmot (Figure 16).

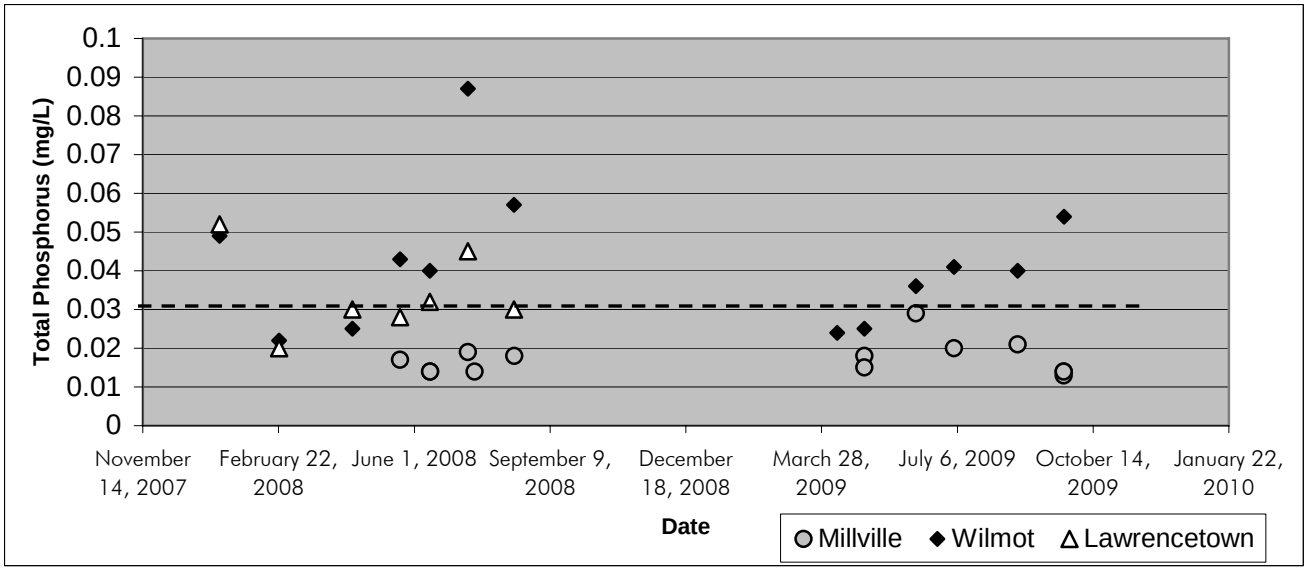

Figure 16. Total phosphorus levels for Lawrencetown in 2008 and for Wilmot and Millville in 2008 and 2009. The dashed line represents the phosphorus guideline of 0.030 mg/L (Mackie, 2004).

Samples collected from Millville did not exhibit as much variability as those collected from the two sampling stations on the main Annapolis River.

Table 12. Average results for each location and nutrient. Lawrencetown data are from 2006 to 2008, Wilmot data are from 2006 to 2009 and Millville data are from 2008 to 2009.

Location	Total N (mg/L)	Nitrates (mg/L NO_3 as nitrogen)	Total P (mg/L)
Wilmot	0.76	0.45	0.044
Lawrencetown	0.61	0.32	0.032
Millville	0.33	0.09	0.017

For each nutrient, the average is highest at Wilmot and lowest at Millville (Table 12). The Millville site on the South Annapolis River tributary was chosen as a relatively unimpacted site for reference, and to possibly establish baseline nutrient levels, therefore the low averages are expected. The Wilmot site is downstream of the Kingston and Aylesford sites, which have recently displayed elevated *E. coli* levels. The Lawrencetown site is downstream of the Wilmot site, but its nutrient averages are less than those of the Wilmot sites. This is possibly due to dilution of the nutrients between the two sites; there are several large tributaries between the Wilmot and Lawrencetown sites, including the Nictaux River and the Black River.

Recommendations

- Work in collaboration with Environment Canada to ensure the continued collection of nitrogen and phosphorus samples at Millville and Wilmot.
- Examine flow rates in the Annapolis River near the nutrient sample collection points, as flow has a great influence on nutrient concentrations.
- Conduct analyses for traceable compounds found in fertilizers and wastewater treatment discharges to determine sources of nutrient inputs.

Total Suspended Solids and Turbidity

Introduction

Total suspended solids (TSS) and turbidity are both terms that describe the amount of suspended particulate matter in water, although they are measured in different ways. TSS describes the physical mass of the particulate matter, while turbidity refers to the extent that light will penetrate the sample. Highly turbid waters have poor light penetration, which can hinder the growth of aquatic plants and in turn can affect the health of aquatic animals.

Throughout 2008 and 2009, CARP and Environment Canada worked together in order to establish baseline levels of TSS and turbidity for the Annapolis River, which can then be used in determining a water quality objective for these parameters. This water quality objective could then be used in the calculation of a water quality index for the Annapolis River, which would be useful for annual reporting. The monitoring was also conducted to help determine the relationship between TSS and Turbidity. The two measurements are related, but this relationship is unique for every waterway and must be determined. In order to develop this relationship, for the duration of the 2008 and 2009 sampling season, TSS and turbidity samples were taken simultaneously for each station along the Annapolis River.

TSS was measured by the River Guardian program from the period of 1992 to 2001. Although it was recognized that TSS was an important parameter for the Annapolis River, the variable was discontinued in 2003. It was felt that the procedure was time-consuming, failed to record the inherent variability of the parameter and was producing unreliable results (Dill, 2003). The revised protocol used in 2008 and 2009 required biweekly sample collection. As well, samples were gathered after events of significant rainfall or snowmelt. These event readings were taken by either CARP staff or volunteers. At first, event samples were gathered after rainfall amounts of at least 5 – 10 mm, but it was found that this amount of rainfall had very little effect on the TSS and turbidity readings. The collection protocol was subsequently revised, with samples only being collected for rainfall amounts of at least 20 – 30 mm.

Monitoring results

In addition to the regular eight sampling sites, two more sites were sampled for TSS and turbidity. The first site is on the main stem of the Annapolis River, at the bridge on Bayard Road in Wilmot. This is the site of an Environment Canada gauging station that can also measure turbidity. The other site is the baseline reference site, located in Millville on the South Annapolis River. Samples at these sites were gathered by CARP staff.

Turbidity and TSS data collected in 2008 and 2009 from May through to the beginning of December for all of the sampling locations along the Annapolis River were compiled (Figures 17 and 18). There are several spikes in the data, most notably at the beginning of June, early September, and the end of November. Each event corresponds to high-flow events caused by very significant precipitation. At the beginning of June, there was a rain event of approximately 40 mm over a 2-day period, and on September 6th and 7th, the Annapolis River watershed was hit by the tail end of Hurricane Hanna, depositing approximately 80 mm of rain. The November and December spikes were produced by large snowfalls followed by some rainfall, melting most of the snow. For most of the summer, the turbidity and TSS levels remained around baseline, as there was there were not enough large precipitation events that would potentially have resulted in elevated levels of TSS and turbidity.

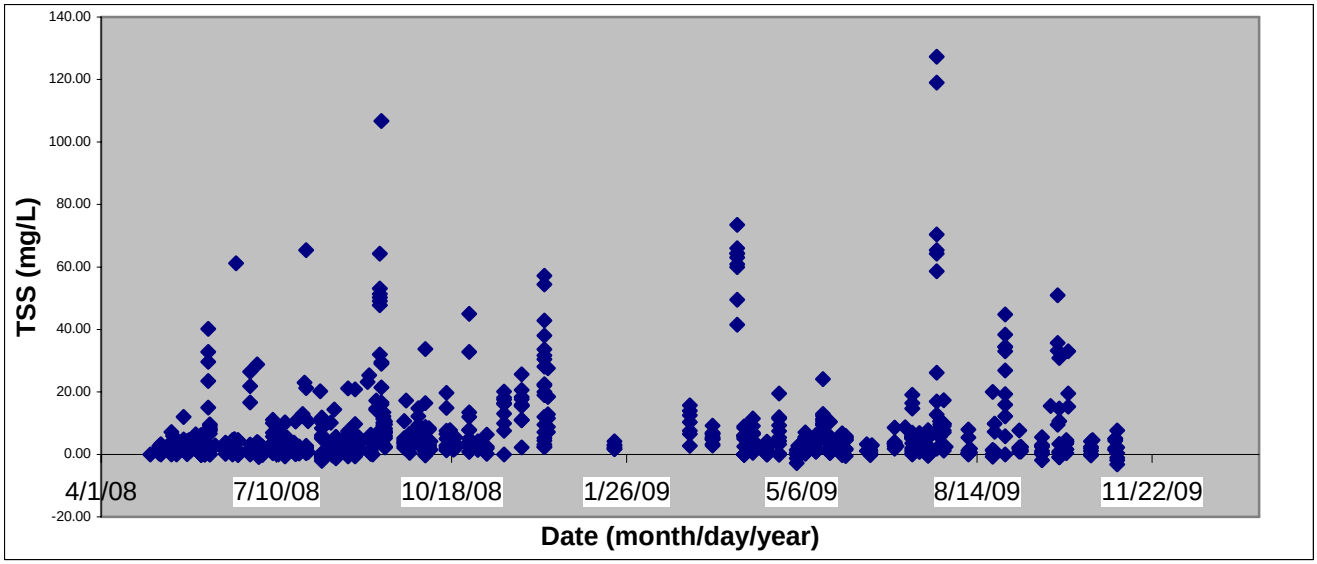

Figure 17. 2008 turbidity results in NTU by date at all sampling locations.

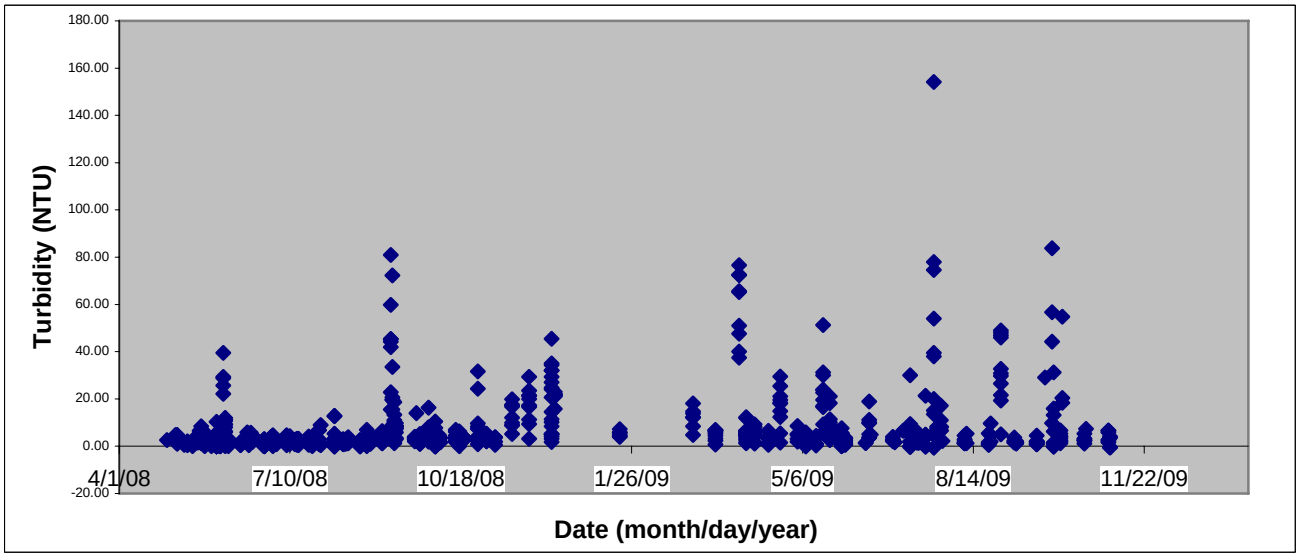

Figure 18. 2008 TSS results in mg/L by date.

All of the data for the turbidity and TSS sample grabs for both 2008 and 2009 were compiled in box and whisker plots and organized by station to show the variability of the parameters between stations (Figures 19 and 20). In addition to the regular 8 monitoring stations, the reference site in Millville was added as well as a sampling site on Bayard Road in Wilmot at which an Environment Canada water quality monitoring station is located. The results have a large range and are shown in a logarithmic scale.

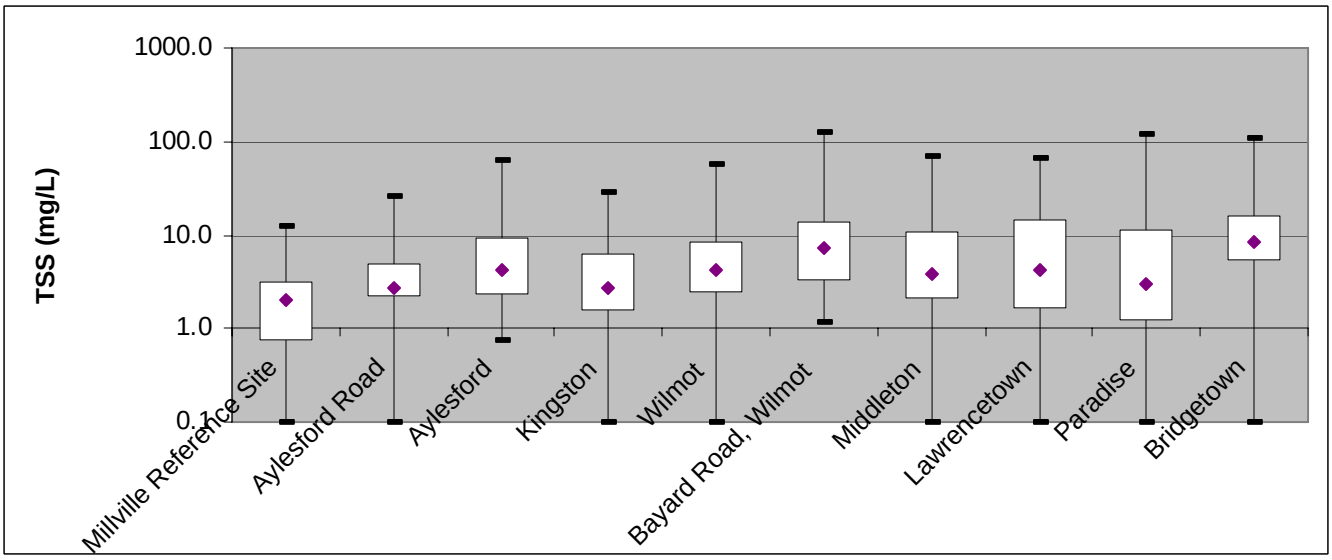

Figure 19. Turbidity results from 2008 and 2009 by location.

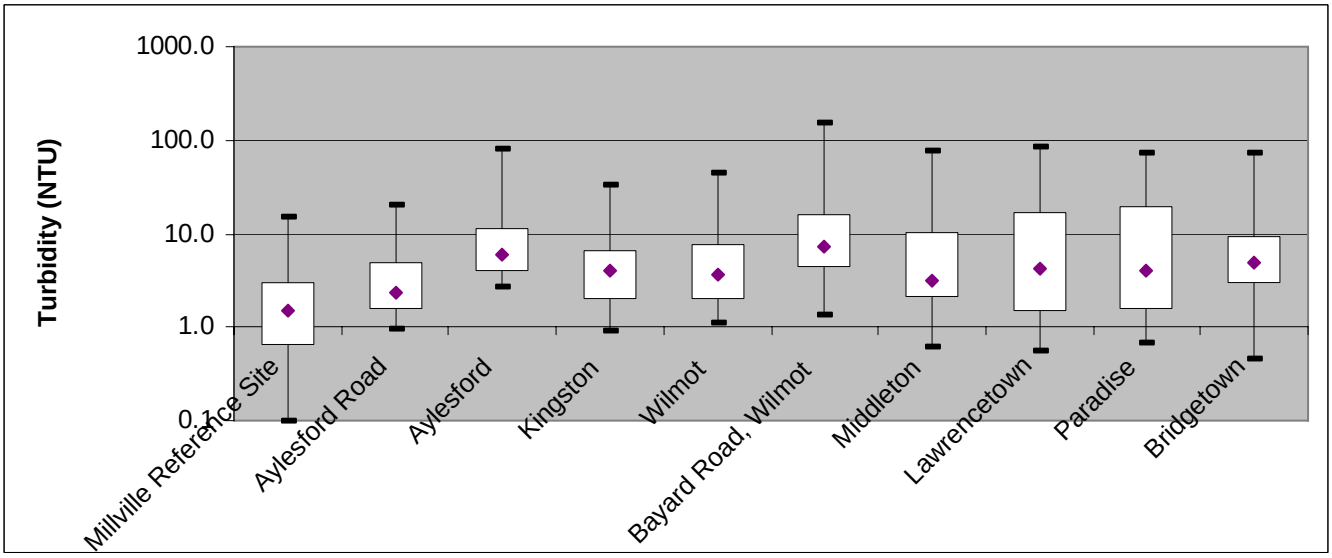

Figure 20. TSS results from 2008 and 2009 by location.

Note that the Bridgetown location shows high TSS readings when compared with the other sites, which is not reflected in the turbidity results. The Bridgetown location is the only monitored location that sometimes has salt water due to tidal influence, which may be a possible explanation for this discrepancy.

One of the purposes of measuring these two parameters was to establish a relationship between TSS and turbidity (Figure 21). Upon visual examination, it seems as though these two variables are directly correlated, although further analysis is required to determine exactly what that relationship is.

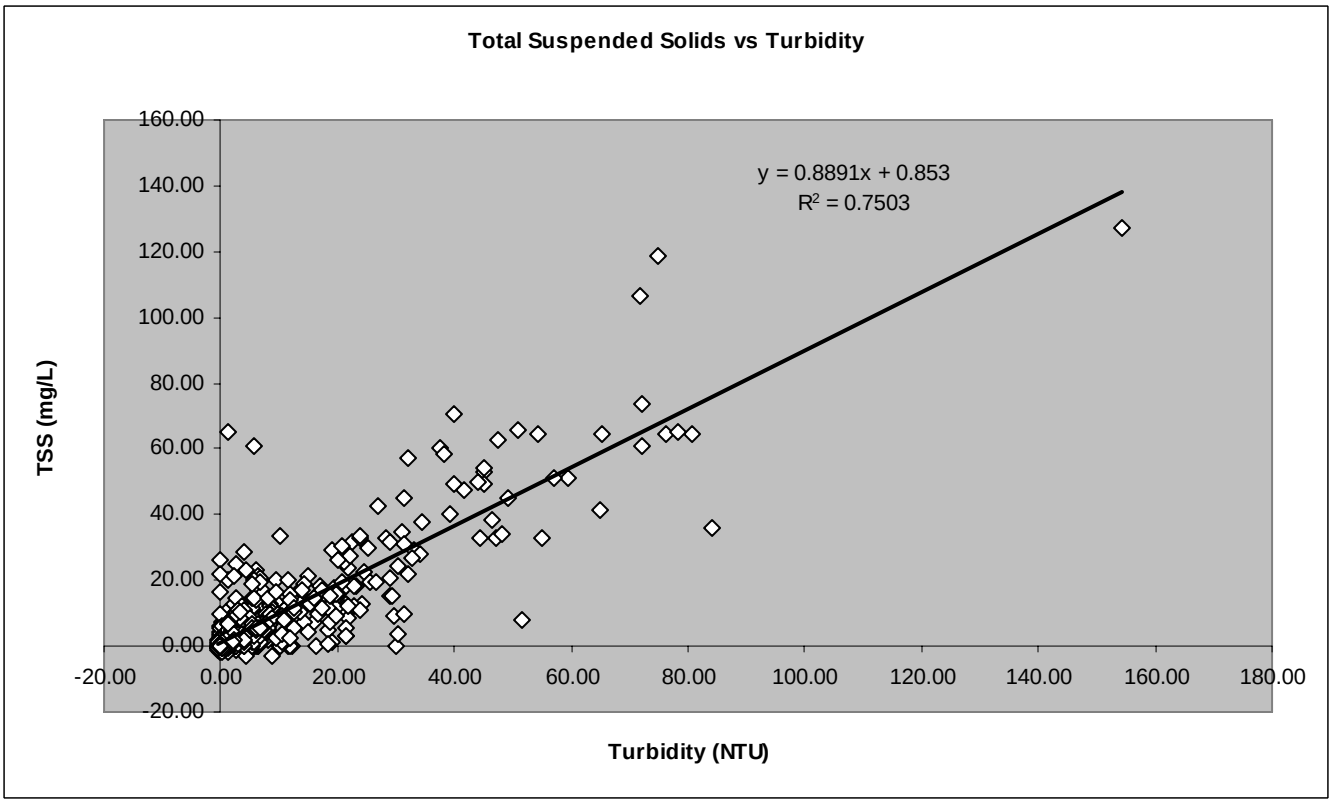

Figure 21. TSS in mg/L vs. turbidity in NTU for all sampled locations along the Annapolis River with the best-fit straight line and equation.

Although the best-fit straight line and equation are included in the chart above, this is only a preliminary estimate of what the relationship between TSS and turbidity is. A more rigourous analysis will be conducted on this data and will be presented in a TSS and turbidity report that is currently in preparation by CARP and Environment Canada.

From 1992 to 2002, TSS data was collected by River Guardians volunteers. This data was compared to the TSS data from 2008 and 2009 gathered during routine biweekly collections (Figure 22). The medians of the two data sets are similar, but the spread of the original data is larger and has a greater number of small values (between -15 and 1 mg/L). This might be due to the size of the original data set, which contains 9 years of data, whereas the 2008/2009 data set is only for 2 years. Note that the scale of the y-axis is logarithmic, therefore, negative values cannot be shown. However the minimum values for both sets of data are negative: -78.0 mg/L for the historical data and –3.21 mg/L for the 2008/2009 data.

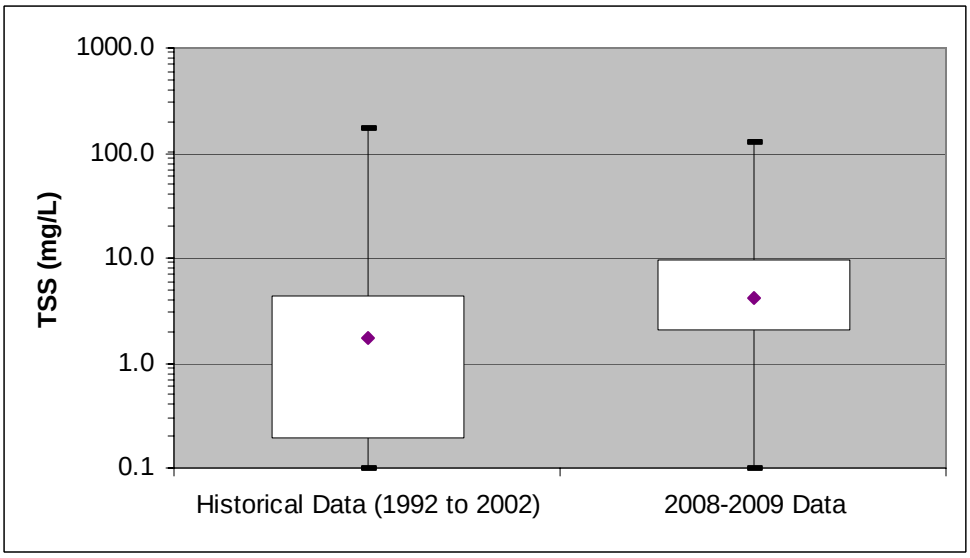

Figure 22. Comparison of the historical River Guardians TSS data (1992 – 2002) and the 2008/2009 TSS data collected as part of the TSS/Turbidity project.

The data collected within this period may not be usable. With regards to this data, Dill (2003) stated:

> The current [TSS] data in the River Guardians database is flawed by the fact that 15% of the samples have a negative value for [suspended particulate matter], which is not possible. The problem of negative values has occurred as recently as 2001 and is distributed through most of the years.

The data taken in 2008 and 2009 also contained negative values. Before correction, approximately 15% of the data was negative. However, as part of the project's Quality Assurance/Quality Control (QA/QC) plan, blank samples were also processed. Many of the blank samples produced negative numbers as well, and using these results as a correction factor, the sample data was adjusted. The result was that only approximately 4% of the corrected data was negative. The absence of similar QA/QC data for the original data set makes it difficult to work with the results. In addition, although some of the 2008/2009 data was negative, the 1992 to 2002 data tended to be negative to a much greater degree (as much as –78.0).

Recommendations

- Complete the analysis of the TSS and turbidity data.
- Once baseline parameters and a relationship between TSS and turbidity have been developed, add turbidity to the regular monitoring procedure. This procedure can employ the use of the turbidity probe on the Hydrolab Quanta.
- Investigate possible correlations between TSS/Turbidity data, *E. coli* readings and rainfall amounts.

Trend Analysis

Purpose

A trend analysis has been done for several of the water quality monitoring parameters since 2006. The results of this analysis were included as part of the annual River Guardians Report Card. These trend calculations were simple three-year rolling average comparisons, and a trend was indicated for a certain parameter if it had changed by a given percentage, which varied according to the parameter. If a trend was found, it was reported as either increasing or decreasing, otherwise the parameter was reported to have no trend indicated.

In 2008, new methods of performing trend analyses were researched in an effort to increase the statistical validity of the results. Literature sources consulted included: Australian and New Zealand Environment and Conservation Council (2000), Helsel and Hirsch (2002), Hirsch, Alexander and Smith (1991) and Cooke (2006). Bacteria count, DO and temperature data have been collected since the inception of the River Guardians program in 1992, pH has been collected since 2003 and nutrients have been monitored by Environment Canada since 2006.

Background Information

There are several different ways of reporting trends in a series of data, depending on the nature of the data set. Many of the statistical methods fall under two broad categories, parametric and non-parametric. Parametric methods are used for normally distributed data, while non-parametric methods are suited for non-normally distributed data. Methods of each type were attempted for the trend analysis of the water quality data.

The parameters that were assessed using these two methods were bacteria counts, DOSAT, temperature and pH. DOSAT was used over DO because DO values are dependent on temperature, therefore, temperature trends might cause DO trends to be masked or indicated when they are not appropriate. Nutrient trends were also analyzed for Wilmot and Lawrencetown using parametric methods.

The procedure used for the non-parametric analysis was based on a procedure provided by D. Parent of Environment Canada and used by Glozier, Crosley, Mottle and Donald (2004). This procedure involved:

- separation of the data by station for each parameter
- a visual assessment of the data time series, which includes dividing the data into season according to the box-plot
- checking outliers for errors in measurement
- the Kruskal-Wallis test for seasonality
- either the Seasonal Kendall test or the Mann-Kendall test depending on whether the data displayed seasonality.

The Kruskal-Wallis test was performed using Systat 8.0 and the Kendall tests were performed using a free DOS-based computer program for the Kendall family of trend tests developed by the United States Geological Survey. The program is available at http://pubs.usgs.gov/sir/2005/5275/downloads/ (Helsel, Mueller, Slack, 2006)

The parametric procedures that were performed on the data were suggested by Drs. Y. Zhang and M. Brylinsky of Acadia University (pers. comm, December 2008). This procedure involved:

- separation of the data by station for each parameter
- a visual assessment for correlations between locations using scatterplot matrices

- a check for autocorrelation for each parameter and location
- an assessment for normality using the Shapiro-Wilks test
- transformations of the data if the parameter was found to be non-normal
- a linear regression of the data to determine whether a trend was present.

Systat 8.0 was used to produce scatterplot matrices and autocorrelation plots; the Analyse-It add-on for Microsoft Excel was used to perform the Shapiro-Wilks test and regression analyses.

Methodology

Before any trend analyses were performed, outlier tests were conducted. The mean and standard deviation of a particular data set were calculated and each value was compared to the mean. If any value differed from the mean by more than twice the standard deviation, it was considered an outlier and was checked against the original data sheets. If there was reason to suspect the data point of being invalid, the data was not included in the trend analysis. If no notes or calculation errors were made on the original data sheet, the outlier value was retained in the data set. The analysis for the temperature data was performed only on data from the summer months (July, August and September), as elevated water temperatures that occur in the summer months are the principal concern. The outlier analysis was not performed on the bacteria data, as the nature of the data is not conducive to outlier analysis. The data is highly variable with a wide range of 0 to 2419 cfu/100 mL and is capped at 2419 cfu/100 mL. The cap of 2419 cfu/100 mL is due to method limitations; the IDEXX Colilert testing method will not produce a reading greater than this number. Some of the earlier data was analyzed using a different method that was not capped, so any data point above the 2419 cfu/100 mL threshold was artificially capped at 2419 cfu/100 mL for consistency purposes.

Non-Parametric Analysis

A box and whisker plot was made for each parameter, with the data grouped by month. For the temperature, bacteria and pH data, months of January through March were excluded, as very little data was recorded for those months. The box plots were then visually assessed for similarities across months. Adjacent months with similar medians and ranges were grouped together as a season (Figure 23).

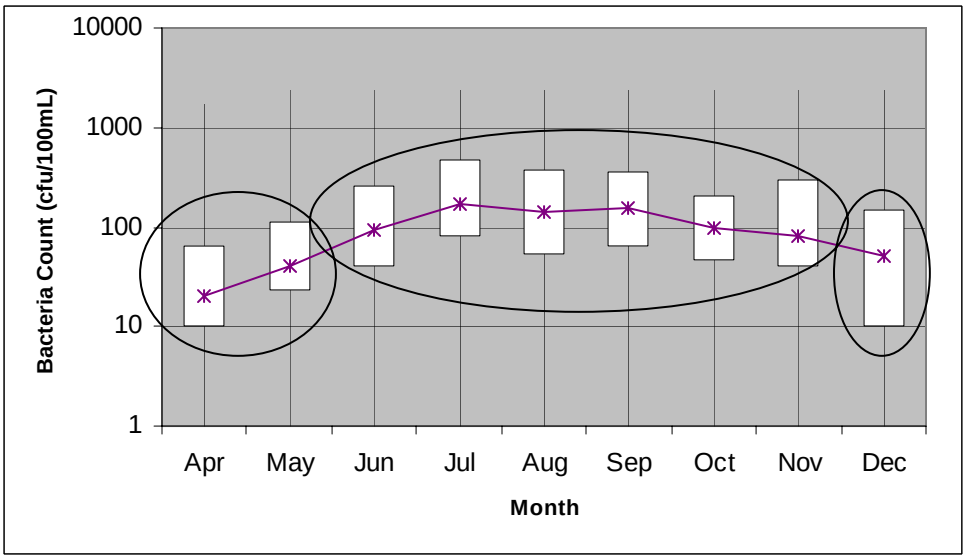

Figure 23. Bacteria count data for all years grouped by month. The circles indicate the seasons that were determined from this plot. There was very little data for the January to March period; these months were not used in the analysis. A 'dummy season' containing no data was used in the analysis to represent the January to March period.

Three seasons were indicated by the bacteria count box plot shown above and a fourth season was included in the analysis to represent the January to March months. The bacteria data was grouped according to these seasons and the Kruskal-Wallis for seasonality test was performed. A similar box plot was constructed for each other parameter using the same procedure. Bacteria count data were indicated as being seasonal, while the pH, DOSAT and summer temperature data were not. Based on this, the Seasonal Kendall test was performed on the bacteria count data and the Mann-Kendall test was performed on pH, DOSAT and temperature data. These tests produce a linear trend equation and a probability statistic (p value), which indicates whether or not the trend is statistically significant. A trend was considered significant if the p value was less than 0.05. Non-parametric analyses were not performed on the nutrient data as there was not enough data to assess the seasonality of the data set.

Parametric Analysis

The data was grouped by parameter and location, and the Shapiro-Wilks test was performed on each data set. The Shapiro-Wilks test is a test for non-normality and produces a histogram of the data overlaid with a normal distribution curve as well as some significance and probability statistics. For this procedure, the histogram and normal curve are examined to determine whether the data visually resembles a normal distribution. If the data does not resemble a normal distribution (in this case, the *E. coli* data did not), the data set can be transformed until it resembles a normal distribution. CARP's *E. coli* data distribution resembled a logarithmic distribution, so the data was transformed by taking the base-10 logarithm of the bacteria results. The logarithmic transformation produced a normally distributed data set (Figure 24).

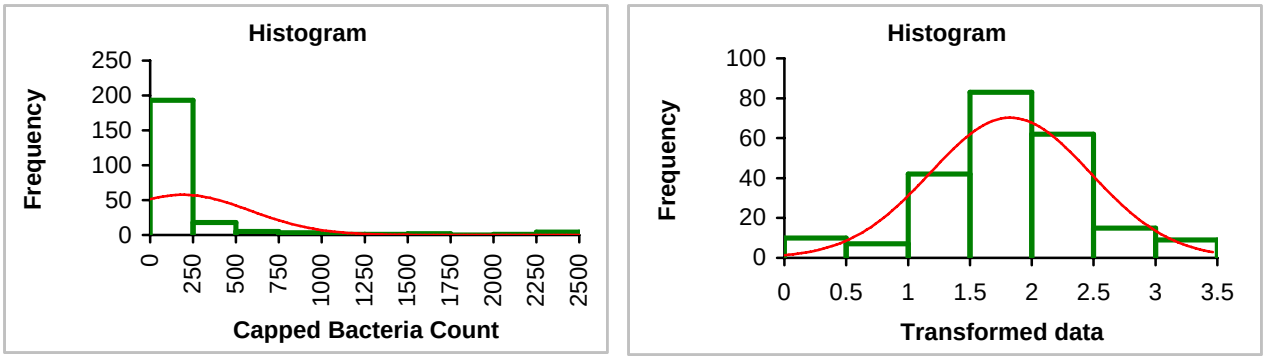

Figure 24. Bridgetown bacteria count data distribution before transformation (left) and after transformation (right).

The transformed data much more closely resembles a normal distribution and can be used for the regression analysis. The data for DO, temperature and pH did not require transformation to resemble a normal distribution. After the trend analysis procedure below was completed, the bacteria data trend results had to be transformed back using an inverse logarithmic function.

After normality was established for each parameter, a linear regression was performed on its data set. This produced a linear slope of the trend, as well as a confidence interval, prediction interval, probability value and residual histogram. The trend slope provides the rate of change of the variable by year, the confidence interval and probability value allow for the determination of statistical significance of the trend and the residual plot and histogram indicate whether the data set varies in a non-linear fashion, which would indicate that the linear regression calculation is not appropriate for the data set. For the determination of statistical significance, three tests were performed. If any of these tests were failed, the trend was not considered significant. The three tests included:

- verification of the slope's p value. If the value was less than 0.05, this test was passed.
- examination of the confidence intervals of the regression plot. If the confidence interval range at the beginning of the data set did not overlap with the range at the end of the data set, this test was passed (Figure 25).
- examination of the residual plot and distribution. If the residual scatterplot was randomly distributed and the associated histogram resembled a normal distribution, this test was passed. Non-linear correlation of the data would be indicated if this test was failed (Figure 26).

As an example, the DOSAT data for the Kingston location is displayed below. The p value for the slope produced by the regression analysis was 0.0003. This value is less than the 0.05 threshold, therefore, the data passed this significance test. Figures 25 and 26 below show that the Kingston data set passed the other two significance tests as well, therefore the trend slope of –0.6 %/year was accepted as significant. This indicates that dissolved oxygen levels are decreasing at the Kingston location.

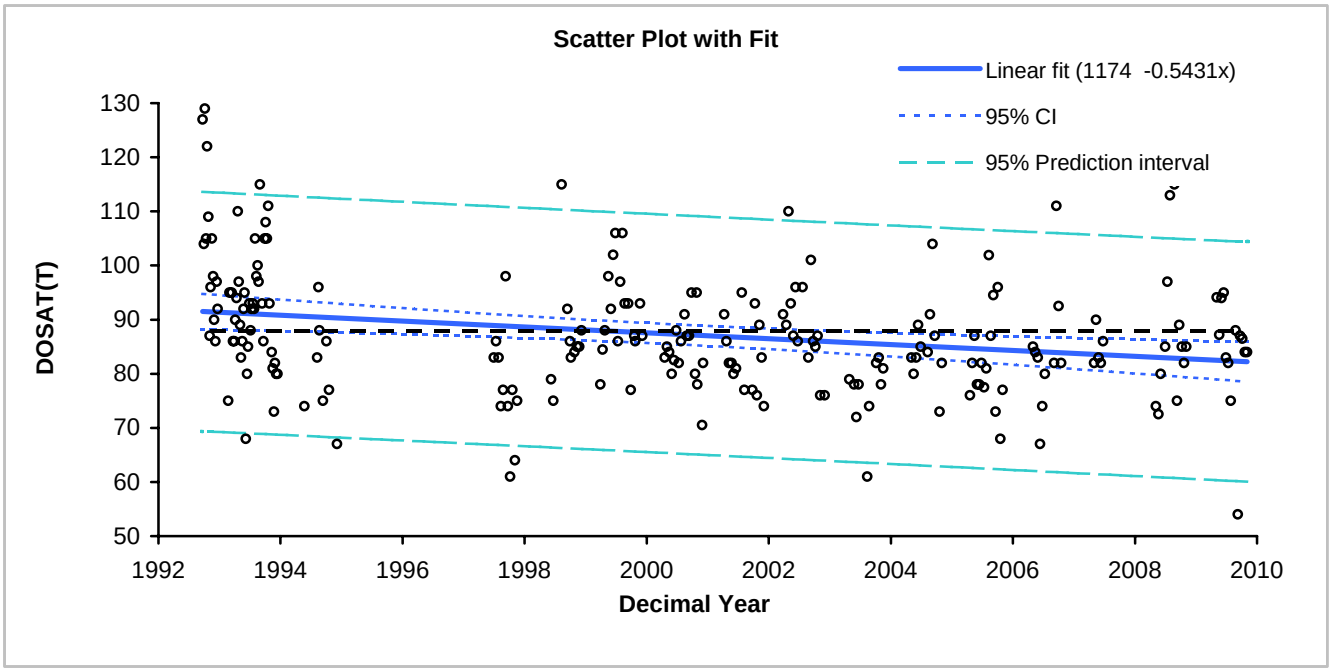

Figure 25. Linear regression for DOSAT data at the Kingston location. The thick dashed line is for the purpose of comparing the confidence interval range at the beginning of the data set. If this horizontal line had remained within the confidence interval range for the entire domain of the data set, a trend could not be concluded. This did not occur for the Kingston DO data set; this data set passes this significance test.

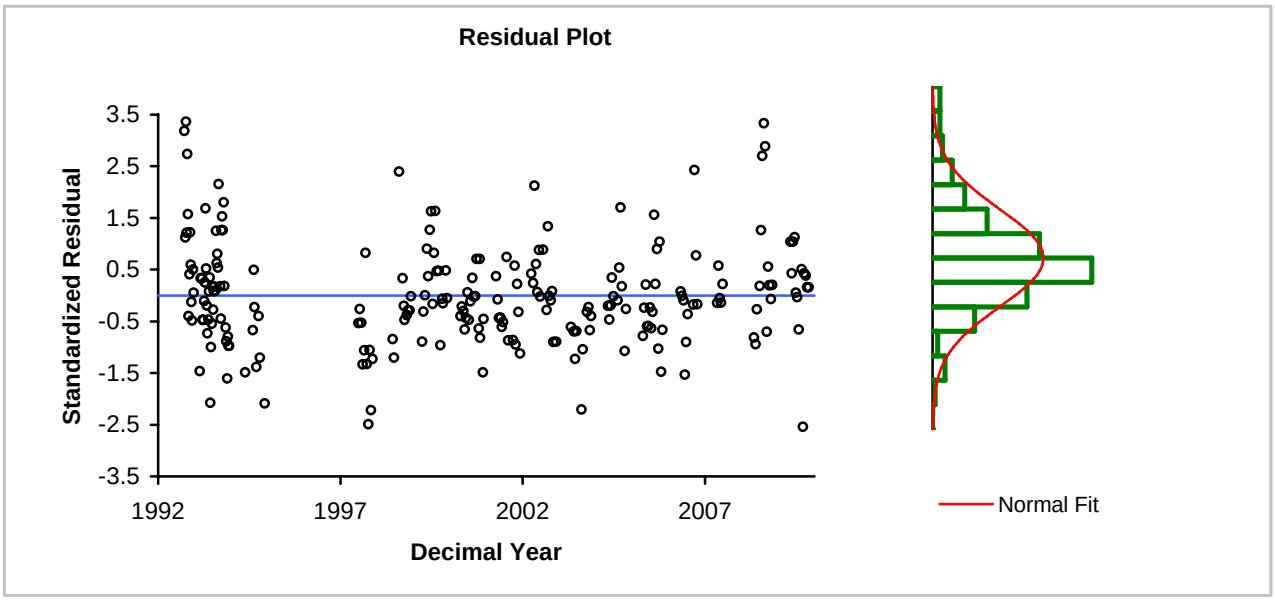

Figure 26. Residuals plot for the DOSAT regression for the Kingston location. The scatterplot does not display significant clustering and appears to be randomly distributed and though the histogram displays a small spike around the centre, it still resembles a normal distribution; therefore this test is passed for the Kingston DO data.

Autocorrelation and Serial Dependence

Autocorrelation is an important consideration for both parametric and non-parametric statistical trend analyses (Helsel and Hirsch, 2005) as its existence invalidates most statistical tests. Autocorrelation refers to serial dependence within a data set, meaning that observation pairs separated by a constant time lag are correlated (Australian and New Zealand Environment and Conservation Council, 2000). One of the assumptions of the linear regression fit is that there must be no correlation between data points (i.e. data points must be independent). In the case of water quality data, the potential existed for data points collected temporally close or along the same stretch of river to be correlated. To assess whether the data was affected by this serial dependence, an autocorrelation plot for each variable at each location was performed, as well as for the entire data set for each parameter (Figure 27).

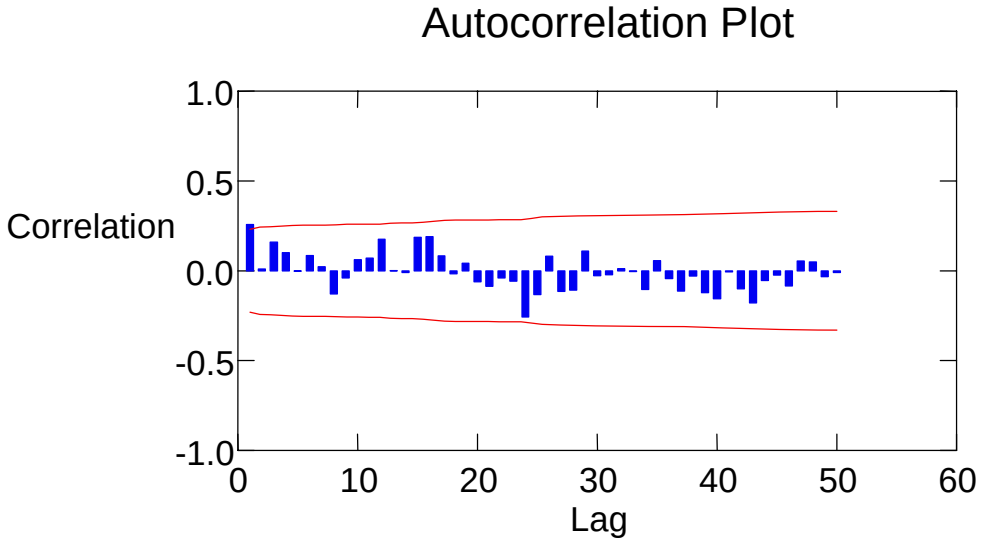

Figure 27. Autocorrelation plot for temperature at the Kingston location.

Significant serial dependence is indicated when the vertical bars extend beyond the 95% confidence curves. In the Kingston plot, most of the bars do not extend beyond the confidence interval, thus serial dependence is not indicated. When an autocorrelation plot was made for all locations, significant serial dependence was displayed; therefore a trend analysis was not performed on the data for all locations (Figure 28).

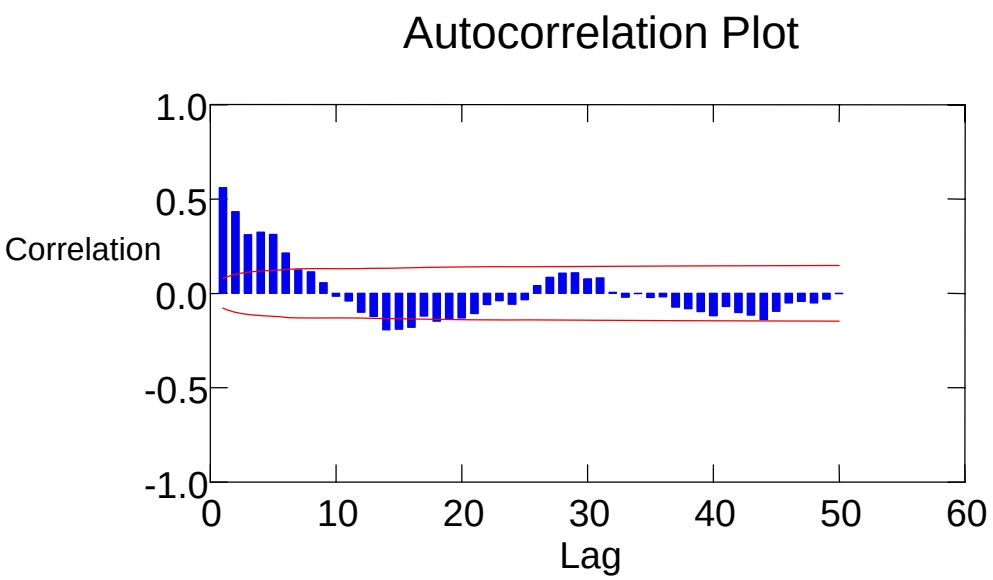

Figure 28. Autocorrelation plot for the entire temperature data set. Several of the bars extend beyond the confidence interval range; therefore significant serial dependence is indicated.

Results

The results for the non-parametric tests (Table 13) and the results for the parametric tests (Table 14) were compiled.

Table 13. Statistically significant trends* and rates of change using non-parametric procedures.

	Bacteria Count	Dissolved Oxygen	pH	Temperature
Aylesford Road	No	No	No	No
Aylesford	No	No	No	No
Kingston	Yes (+3 cfu/100mL/year)	Yes (-0.5 %/year)	No	Yes (+0.14°C/year)
Wilmot	No	Yes (+0.3 %/year)	No	No
Middleton	No	No	No	No
Lawrencetown	Yes (-4 cfu/100mL/year)	No	No	Yes (+0.13°C/year)
Paradise	No	No	No	No
Bridgetown	No	Yes (-0.4 %/year)	No	No

*Statistically significant trends ($p<0.05$) using Seasonal Kendall and Mann-Kendall tests.

Table 14. Statistically significant trends* and rates of change using parametric procedures.

	Bacteria Count	Dissolved Oxygen	pH	Temperature	Total Nitrogen	Total Phosphorus
Aylesford Road	No	No	No	No		
Aylesford	Yes (+9 cfu/100mL/year)	No	No	No		
Kingston	Yes (+9 cfu/100mL/year)	Yes (-0.5 %/year)	No	No		
Wilmot	No	No	No	No	No	No
Middleton	No	No	No	No		
Lawrencetown	No	No	No	No	No	No
Paradise	No	No	No	No		
Bridgetown	No	No	No	No		

*Statistically significant trends ($p<0.05$, residual plot randomly distributed, initial confidence interval range does not overlap with final confidence interval range) using linear regression fit.

Values resulting from these calculations indicate a statistically significant trend as a rate of change, with a positive value as an increasing trend and a negative value as a decreasing trend. The two test types generate slightly different results, but were mostly consistent. Both indicate increasing bacteria trends upriver, at Kingston, with the parametric results also producing an increasing result at Aylesford. The non-parametric tests also produced a decreasing bacteria trend at Lawrencetown. The non-parametric tests produced a result of +9 cfu/100mL/year with a p value of 0.0671 for Aylesford and a result of –2 cfu/100mL/year with a p value of 0.0997 for Paradise. These were not included in the table above because their p values are greater than 0.05, but the results may still be significant. Both methods display a decreasing DO trend upriver, especially at Kingston and the non-parametric tests show a decreasing trend at Lawrencetown. No pH trends were indicated for any location by either method and no temperature trends were found using the parametric methods. The non-parametric tests showed an increasing temperature trend at Kingston and Lawrencetown. No nutrient trends were shown for nitrogen or phosphorus for either Lawrencetown or Wilmot. The Aylesford Road site has only been monitored since 2003, and was only monitored sporadically until 2006, so there is not a large amount of data for that location. The data produced confidence intervals with a wide range; therefore no trends could be concluded. Similarly, nutrient data has only been collected since 2006, usually with 6 – 8 samples at one location per year, which may be why no trends were concluded for these parameters.

When compared to the results of the 2008 trend analysis, the results are fairly consistent. The non-parametric method indicated that all of the same trends were present in 2009 as in 2008 albeit with slightly different magnitudes. The parametric methods no longer indicate a DO trend in Bridgetown nor a temperature trend in Lawrencetown. The temperatures in 2009 were lower for all locations than in 2008, which may indicate why the slight increasing trend at Lawrencetown disappeared in the 2009 analysis. There is no indication as to why the Bridgetown DO trend disappeared. Nutrient trends were not calculated for 2008 and could not be compared to the 2009 calculations.

Because of the presence of the serial dependence, it was not possible to conduct trend analysis for all of the sites as a single data set.

Aylesford *E. coli* Investigation

Introduction

E. coli testing of the Annapolis River has indicated that the Aylesford sampling site (site 00) often has elevated bacteria counts when compared to the other sampling locations. In 2003, the Aylesford Road sampling location (site AY40) was added to the regular monitoring regime to further investigate this phenomenon. The Aylesford Road sampling showed consistently lower *E. coli* results than site 00, which appears to indicate that contamination is entering the river between these two locations.

Three tributaries connect with the main stem of the river between site 00 at Victoria Road and site AY40 at Aylesford Road: Patterson Brook, Parker Brook and Skinner Brook (Figure 29). All these brooks originate on the North Mountain. Parker and Skinner brooks merge shortly before connecting with the Annapolis River. The source of contamination may not just be somewhere along the main stem of the river, but could also be along one of these tributaries.

Possible sources of *E. coli* include:

- poorly maintained or failing domestic septic systems.
- wildlife.
- unrestricted livestock access to watercourses.
- manure runoff from agricultural fields.
- campgrounds.

Methods

In order to investigate the *E. coli* contamination, a sampling regime was set up. Thirteen locations were selected in the Aylesford area, some along the main stem of the river and others on each of the tributaries (Table 15 and Figure 29). Of these locations, only twelve could be sampled at one time as only twelve Petri dishes would fit in the incubator at one time. The locations that were selected generally occurred where a road crossed the river or brook, near a culvert or bridge.

Table 15. Monitoring locations for Aylesford *E. coli* investigation.

Stream Name	Street	Code	Latitude	Longitude	Comments
Annapolis River	Aylesford Road	AY40	45.0283	-64.8102	
Annapolis River	Highway 1	AnnH1	45.0288	-64.8206	No bridge/culvert. Go to river's edge
Annapolis River	Sun Valley Drive	AnnSVD	45.0273	-64.8242	
Annapolis River	Campground	AnnKla	45.0246*	-64.8291*	Access through Klahanie Kamping park.
Annapolis River	Victoria Road	00	45.0266	-64.8357	
Patterson Brook	Brooklyn Street	PatBk	45.0427	-64.8348	
Patterson Brook	Highway 101	Pat101	45.0384	-64.8311	
Patterson Brook	Highway 1	PatH1	45.0284	-64.8230	
Parker Brook	Brooklyn Street	PrkBk	45.0503	-64.8156	
Parker Brook	Highway 101	Prk101	45.0430	-64.8151	
Parker/Skinner Brook	Highway 1	SkH1	45.0296	-64.8123	Parker & Skinner brooks converge; water stagnant
Skinner Brook	Brooklyn Street	SkBk	45.0552	-64.7954	Cattle can wade into stream here
Skinner Brook	Highway 101	Sk101	45.0470	-64.7920	

*Not taken by a GPS; estimated using Google Earth

Figure 29. Locations of the Aylesford sampling sites. The Annapolis River flows from east to west.

The Coliscan Easygel method was used to test for *E. coli* (O'Brien, 2006, Micrology Laboratories, 2009). Samples of approximately 100 mL were collected from each location in sterile Whirl-pac bags and stored in a cooler with ice packs. Upon returning to the lab, 3 to 5 mL of each sample were added to a Petri dish containing the Easygel growth medium treatment. The Petri dishes were labelled with the collection date and time, location and the amount of sample used. The dishes were then placed in an incubator at 33°C for a period of approximately 24 hours. After being incubated, the samples were removed and the blue/purple cultures were counted, as these spots represent *E. coli* bacteria. The count was then multiplied to produce a cfu/100mL result. After the samples were counted, they were destroyed by pouring enough bleach onto the plates to cover the surface. The plates and bleach were allowed to sit for a few moments before being sealed in plastic bags for disposal in the trash.

Results

In total, seven sets of *E. coli* samples from the Aylesford area were taken in 2009 (Table 16).

Table 16. Results from Aylesford *E. coli* sampling in cfu/100mL with geometric mean and rainfall data. Samples in bold were analyzed in triplicate. The zeroes in the data were counted as ones for the calculation of the geometric mean. The rainfall data was taken at Greenwood by Environment Canada and represents the three days prior to sampling.

	Sampling sites													Rainfall (mm)
	Annapolis River					Patterson Brook			Parker Brook		Skinner Brook			
Date	AY40	AnnH1	AnnSVD	AnnKla	00	PatBk	Pat101	PatH1	PrkBk	Prk101	SkBk	Sk101	SkH1	Greenwood
21-Jul-09	950	550	550		400	900		100	200		400		150	3.2
29-Jul-09	165	165	231		231	99	1353	264	99		3432		99	0
05-Aug-09	561	528	297		594	165	528	231	0	66	943	231	33	4.6
26-Aug-09		**720**	**429**		**825**	**160**	**165**	**500**	**120**	**165**	**165**	**465**	**40**	31.8
26-Aug-09		**1200**	**396**		**561**	**160**	**99**	**520**	**160**	**132**	**198**	**363**	**200**	
26-Aug-09		**580**	**561**		**660**	**180**	**66**	**720**	**100**	**66**	**363**	**627**	**340**	
03-Sep-09	99	132	66		33	231	165	231	99	99	231	198	66	0
09-Sep-09	99	33	165		66	297	99	561	957	33	132	396	33	0
16-Sep-09	**40**		**80**	**120**	**180**	220	160	240	80	80	2000	140	40	12.4
16-Sep-09	**60**		**80**	**140**	**200**									
16-Sep-09	**0**		**80**	**160**	**120**									
Geometric Mean	82	318	200	139	241	216	194	320	87	82	461	309	79	

The results of the sampling appear to be rather erratic, making it difficult to draw conclusions from the data. In general, elevated readings were consistently shown at all sampling sites on the main stem of the Annapolis River as well as on Skinner Brook at Brooklyn Street and Highway 101 and Patterson Brook at Brooklyn Street and Highway 1. However, Skinner Brook at Highway 1, which is downstream of the Brooklyn Street and Highway 101 sites (as well as the Parker Brook sites), did not display an elevated count. In addition, Patterson Brook at Highway 101, which is between the Brooklyn Street and Highway 1 sites, showed readings that were sometimes much higher than the other Patterson Brook sites and sometimes much lower. There may be a relationship between elevated E.coli counts and periods of high rainfall.

To further investigate these sampling sites, Hydrolab readings were taken at each location on September 16th, 2009 (Table 17).

Table 17. Water quality results for Aylesford sampling locations. Means are calculated for each stream. SkH1 is included in the results twice as it is part of both Parker and Skinner brooks.

Background			Water Chemistry Data							
Site/Sample	Date	Time	Temperature (°C)	Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	pH	Salinity (pss)	DOsat (%)	Turbidity (NTU)	Comments
AY40	16-Sep-09	12:13	13.69	0.094	9.98	7.08	0.05	96.2	0.4	
AnnSVD	16-Sep-09	11:50	13.23	0.149	8.97	7.06	0.07	85.6	11.9	
AnnKla	16-Sep-09	11:30	13.15	0.148	9.09	7.02	0.07	86.6	17.3	
00	16-Sep-09	11:43	13.19	0.153	8.71	7.10	0.07	83.0	5.5	
Mean for Annaolis River			13.32	0.136	9.19	7.07	0.07	87.8	8.9	
PatBk	16-Sep-09	13:15	12.07	0.285	8.93	7.60	0.13	83.3	62.5	
PatH1	16-Sep-09	11:56	12.55	0.299	9.78	7.72	0.14	92.0	9.0	
Pat101	16-Sep-09	12:25	12.45	0.294	10.36	7.71	0.14	97.2	2.4	
Mean for Patterson Brook			12.36	0.292	9.69	7.68	0.14	90.8	24.6	
PrkBk	16-Sep-09	13:08	12.30	0.306	11.20	7.86	0.14	104.7	21.8	
Prk101	16-Sep-09	12:34	12.67	0.307	9.76	7.84	0.14	92.1	0.6	
SkH1	16-Sep-09	12:05	11.19	0.266	4.63	7.15	0.12	42.2	20.8	
Mean for Parker Brook			12.05	0.293	8.53	7.62	0.13	79.7	14.4	
SkBk	16-Sep-09	12:58	12.66	0.503	7.38	7.65	0.24	69.6	71.8	Cows in stream
Sk101	16-Sep-09	12:41	12.87	0.311	10.27	7.77	0.15	97.3	26.4	
SkH1	16-Sep-09	12:05	11.19	0.266	4.63	7.15	0.12	42.2	20.8	
Mean for Skinner Brook			12.24	0.360	7.43	7.52	0.17	69.7	39.6	

When examining these results, they seem to be fairly consistent from site to site. The upstream sites of the tributaries tend to have higher pH levels than those on or close to the Annapolis River, although none of the readings are outside of acceptable limits. Skinner/Parker Brook at Highway 1 (SkH1) displays depressed dissolved oxygen levels and the water is visibly stagnant at this location. This site is a convergence of Parker and Skinner brooks and the water is pumped out and used for irrigation. There are two locations that displayed turbidity spikes: Skinner and Patterson brooks on Brooklyn Street. Skinner Brook on Brooklyn Street is muddy, likely due to the fact that cattle can enter the stream here. Cattle were present when the readings were taken, which may be why the waters were very stirred up and turbid. There was no immediate indication as to why the turbidity would be elevated at Patterson brook on Brooklyn Street, although further upstream, on Highway 221, the stream passes over a dirt road and vehicles may routinely drive through.

Quality Assurance/Quality Control

As the Easygel method had not been used by CARP in the past, a number of QA readings were taken to assess the precision and accuracy of the method. On August 9th, 2009, the River Guardians took duplicate *E. coli* samples of each location. One set of these samples was analysed at the Valley Regional Hospital using the IDEXX Colilert method while the other set was taken to the CARP lab and analysed using the Coliscan Easygel method. These were compared using a Relative Percent Difference (RPD) calculation (Table 18). The formula for this calculation can be found in Appendix C.

Table 18. QA readings comparing the IDEXX Colilert method with the Coliscan Easygel method including percent difference.

Site	Date	Time	Volunteer	Lab Result	EasyGel result	Difference	RPD
49	09-Aug-09	7:00	Ron Jones	84	60	24	33
35	09-Aug-09	11:55	Daren Parks	47	60	-13	24
40	09-Aug-09	12:30	Matthew Guy	54	80	-26	39
25	09-Aug-09	13:55	Claire Diggins	135	60	75	77
13	09-Aug-09	16:00	Robert Garand	131	200	-69	42
18	09-Aug-09	20:00	Chelsea Fougère	387	60	327	146
00	10-Aug-09	11:04	Jeffrey Glenen	517	160	357	105
AY40	10-Aug-09	11:20	Jeffrey Glenen	167	100	67	50
					Average	93	65

The QA results indicate that the two methods do not produce similar results for the same sample, with the smallest percent difference reading being 24%. In some cases, the Easygel method greatly underestimates the IDEXX result and in other cases it overestimates the IDEXX result. The River Guardians results using the IDEXX results have consistently shown bacteria counts at Victoria Road (site 00) that are much higher than those at Aylesford Road (site AY40), which is not reflected by the Coliscan results. CARP's IDEXX results have had relatively low levels of average RPD; the QA results had an RPD of 29% in 2009 and 24% in 2008 (see appendix C). When comparing the Easygel results to the IDEXX results, the RPD is significantly higher, at 65%. Although *E. coli* tests tend to have high variability, this result suggests that the Easygel method cannot be used to precisely gauge bacteria levels.

Other programs have also used Coliscan Easygel to test for bacteria and performed QA analyses. Virginia Save Our Streams compared the Easygel method to the Virginia State Lab method (United States Department of Agriculture, 2004) (Table 19).

Table 19. Readings taken as part of the Reedy Creek Coatlition project (2004).

Given	Easygel	Difference	RPD
2250	2270	-20	1
460	400	60	14
130	200	-70	42
70	30	40	80
130	230	-100	56
210	130	80	47
510	170	340	100
	Average	47	49

CARP found the average RPD when compared to the IDEXX method was 64%; Virginia Save Our Streams found a RPD of 49%. From their RPD result, Reedy Creek Coalition (2004) stated that the Easygel method is more likely to produce false negatives than false positives and that the "[Department of Environmental Quality] has approved Easygel for 'screening' purposes."

Sample readings were taken in triplicate on August 26th, 2009 and September 16th, 2009 (Table 20). The sample was collected in one bag and then split into three different Petri dishes for analysis.

Table 20. QA readings taken on August 26 and September 16 in cfu/100mL. Average and standard deviation measurements are included.

	August 26th, 2009											September 16th, 2009			
Trial	AnnH1	AnnSVD	00	PatBk	Pat101	PatH1	PrkBk	Prk101	SkBk	Sk101	SkH1	AY40	AnnSVD	AnnKla	00
1	720	429	825	160	165	500	120	165	165	465	40	40	80	120	180
2	1200	396	561	160	99	520	160	132	198	363	200	60	80	140	200
3	580	561	660	180	66	720	100	66	363	627	340	0	80	160	120
Average	833	462	682	167	110	580	127	121	242	485	193	33	80	140	167
Std Dev	325	87	133	12	50	122	31	50	106	133	150	31	0	20	42

The triplicate readings taken on August 26th, 2009 show little consistency, with many of the average results not even being certain to the hundreds place according to the standard deviation result. The September 16th readings are slightly more consistent although large variability was still recorded for some locations. A blank sample was processed in triplicate on September 30th, 2009, showing results of 0 cfu/100 mL for each trial.

Past Monitoring

Some limited monitoring was conducted for several similar points in 2007 for *E. coli*. The sites monitored were the same, although they were identified using different site codes. The codes used in 2007 were matched with the site codes used in 2009 (Table 21).

Table 21. Aylesford monitoring site code differences between 2007 and 2009.

2007 site code	2009 site code	Description
AY40	AY40	Annapolis River, Aylesford Road
AY09*	AnnH1	Annapolis River, Highway 1
AY06*	AnnSVD	Annapolis River, Sun Valley Drive
AY03*	AnnKla	Annapolis River, Klahanie Kamping
AY00	00	Annapolis River, Victoria Road
AY15	PatBk	Patterson Brook, Brooklyn Street
AY12*	Pat101	Patterson Brook, Highway 101
AY10	PatH1	Patterson Brook, Highway 1
AY25	PrkBk	Parker Brook, Brooklyn Street
AY22*	Prk101	Parker Brook, Highway 101
AY20	SkH1	Skinner/Parker Brook, Highway 1
AY35	SkBk	Skinner Brook, Brooklyn street
AY32*	Sk101	Skinner Brook, Highway 101

*These sites were not monitored in 2007. Site codes consistent with the naming of the other sites were retroactively given to them.

E. coli data were gathered twice in 2007 from Aylesford tributaries, October 29th and November 11th (Sharpe, 2007) (Table 22).

Table 22. *E. coli* results (cfu/100mL) at Patterson, Parker and Skinner Brooks taken in 2007

Site	Oct 29, 2007	Nov 11, 2007
AY15 (PatBk)	34	914
AY10 (PatH1)	1203	2419
AY25 (PrkBk)	291	166
AY35 (SkBk)	649	461
AY20 (SkH1)	219	770

Discussion

The erratic nature of the results makes it difficult to draw conclusions from this sample set. Based on the QA readings, it seems that the Coliscan Easygel method is neither accurate nor reliable, especially when *E. coli* levels are elevated.

When examining the geometric means of the results, a low result is shown for Aylesford Road (AY40) and a higher result is shown for Victoria Road (00) which is generally reflected in the River Guardians results. High geometric means are also shown for the Annapolis River at Sun Valley Drive and off of Highway 1, which suggests that the majority of contamination is coming from above these points. Skinner and Parker brooks merge with the Annapolis River above these stations. Elevated bacteria counts were reported for some sections of Skinner Brook, especially near Brooklyn Street, although high counts were rarely reported for the Skinner Brook station nearest the Annapolis River. Elevated geometric means were also found for all stations along Patterson Brook, which joins the Annapolis River between the Sun Valley Drive station and Highway 1 station. The 2007 data suggests that all three tributaries may be contributing to the elevated bacteria counts, especially Patterson Brook.

Macmillan (2005) identified Patterson Brook as one of the few streams in the Annapolis watershed that was consistently cool enough to support populations of fish. Populations of brook trout have been found in Patterson brook, making it very important that *E. coli* sources for this stream be investigated and remediated.

As shown on the map in Figure 29, each of the tributary streams has been significantly altered for agriculture purposes. Cattle have access to Skinner Brook at site SkBk and it is possible that there are other locations between sampling sites to which they may also have access. The water is relatively clear on the Annapolis River at Aylesford Road, but the sites downstream tend to be murkier. This may be due to anthropogenic influences or this area of the stream may be naturally siltier.

Recommendations

- Resume sampling these locations for *E. coli* next season using a different analysis method.
- Review current and historic air photos of this area to identify land use changes and possible sources of contamination.
- Conduct a foot survey along the Annapolis River between Victoria Road and Aylesford Road as well as along Patterson, Parker and Skinner brooks to identify possible contamination sources.
- Work with landowners on the Skinner Brook catchment to exclude livestock from watercourses.

Recommendations

Recommendations for the River Guardians Program

- Continue regular River Guardian *E. coli* monitoring at the eight main river sample locations.
- Continue regular River Guardian DO monitoring program at the eight main river sample locations.
- Undertake periodic DO monitoring of the Annapolis River estuary in the late summer and early autumn. These times are most likely to display depressed levels of DO. Depth profiling should be included as part of this monitoring.
- Investigate atmospheric pressure readings to determine whether or not they vary enough to affect dissolved oxygen readings.
- Continue regular River Guardian temperature monitoring program at the eight main river locations.
- Install temperature loggers in candidate streams to assess for fish habitat improvements.
- Temperature data loggers should be calibrated immediately prior to deployment and at least once *in situ*. These procedures should be added to the QA/QC Project Plan.
- Investigate the temperature increase on the Annapolis River between Aylesford and Lawrencetown. This may include collection of thermal status data on tributaries to the Annapolis River.
- Regular pH monitoring should be continued at the eight Annapolis River Guardian monitoring locations.
- Work in collaboration with Environment Canada to ensure the continued collection of nitrogen and phosphorus samples at Millville and Wilmot.
- Examine flow rates in the Annapolis River near the nutrient sample collection points, as flow has a great influence on nutrient concentrations.
- Conduct analyses for traceable compounds found in fertilizers and wastewater treatment discharges to determine sources of nutrient inputs.
- Complete the analysis of the TSS and turbidity data.
- Once baseline parameters and a relationship between TSS and turbidity have been developed, add turbidity to the regular monitoring procedure. This procedure can employ the use of the turbidity probe on the Hydrolab Quanta.
- Investigate possible correlations between TSS/Turbidity data, *E. coli* readings and rainfall amounts.
- Resume sampling locations in Aylesford for *E. coli* next season using an analysis method other than Coliscan Easygel.
- Review current and historic air photos of the Aylesford area to identify land use changes and possible sources of contamination.
- Conduct a foot survey along the Annapolis River between Victoria Road and Aylesford Road as well as along Patterson, Parker and Skinner brooks to identify possible contamination sources.
- Work with landowners on the Skinner Brook catchment to exclude livestock from watercourses.

Recommendations for CARP

- Complete the Quality Assurance Project Plan for all of CARP's Water Quality monitoring programs.

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Appendices

Appendix A – Parameters Tested and Methodologies

Parameters Analyzed in 2009	Additional Parameters Analyzed in Previous Years of the Program
E. coli bacteria densities	Salinity
Dissolved Oxygen	Chlorophyll a
Temperature (Water and Air)	Nitrate-N, Chloride, Sulphate, Total Phosphate
Weather conditions	Colour
pH, Conductivity, Total Dissolved Solids	Transparency
Nitrate, Nitrite, Ammonia, Phosphate	
Total Suspended Solids (TSS)	
Turbidity	

Water Collection for Fecal Bacteria Analysis

Following the contamination of some sampling equipment in 2003, a new collection procedure for fecal coliform samples was developed and used during the 2004 through 2009 seasons. The sampling units (Figure A1) allow for representative sampling from mid-span of bridges at the sampling sites.

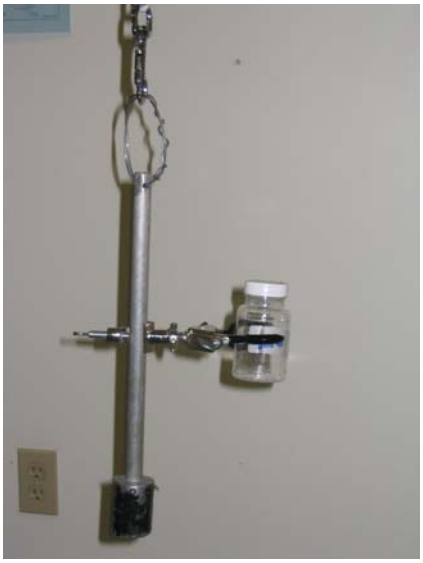

Figure A1. Collection unit used for fecal coliform samples in 2009.

The open sample bottle is secured in the clamp, and lowered from the mid-span of the bridge into the river, to a depth of 1 meter. Samples are collected on the upstream side of bridges, where a safe pedestrian walkway exists. After collection, water samples are refrigerated until delivery to the lab, typically within 24 hours of collection.

Enumeration of Fecal Bacteria

Prior to the 2005 season, bacterial samples collected by Clean Annapolis River Project's Annapolis River Guardians program were tested for Fecal Coliforms (FC) using the membrane filtration method. During the winter of 2005, the

program's Science Advisory Committee suggested that the program switch to testing for *E. coli* (EC) using the Most Probable Number method, to bring testing more in line with national guidelines. In order to ensure the continuity of the dataset, a period of duplicate analysis with the two methods was conducted. Duplicate samples were analysed using both methods over a two-month period (four biweekly sample events at eight locations along the river). Analysis of the paired results indicated no significant difference between the two testing methods. Further information on the comparison of the two testing methodologies is presented in the 2005 Annapolis River Guardian Report, Appendix C, which is available at the CARP office.

All fecal bacteria samples were submitted to the Valley Regional Hospital Microbiology Laboratory in Kentville, Nova Scotia. The Valley Regional lab is recommended by Nova Scotia Environment to perform water quality analysis. From 1997 to 2003 and again since 2005, fecal bacteria densities were determined using the IDEXX Colilert procedure, to give a Most Probable Number of *E. coli* bacteria present. For the 2004 sample season, fecal coliform analysis was performed using the membrane filtration method.

Dissolved Oxygen Content

Dissolved oxygen samples are collected from the mid-span of bridges using a horizontal van Dorn sampler, at a depth of 1 meter. Dissolved oxygen in mg/L is determined using the modified Winkler titration using pre-packaged Hach reagents. The Winkler titration procedure is a widely recognized standard for determining dissolved oxygen. The procedure is reported to have an accuracy of at least +/- 1 mg/L. Dissolved oxygen as percent saturation is determined using Rawson's nomogram. Further information on the collection and analysis procedure for dissolved oxygen can be found in the Annapolis River Guardians Procedure Manual, which is available at the CARP office.

Temperature

The Annapolis River Guardians used a combination of glass/alcohol and digital thermometers during 2009. Prior to the start of the season, all thermometers were compared with the temperature reading from CARP's HydroLab Quanta water meter. This unit had recently been serviced and calibrated, with a reported accuracy of +/- 0.10 ^{o}C. From this comparison, a correction factor was determined for each River Guardian thermometer. These correction factors were applied to all River Guardian temperature measurements.

pH and Conductivity

Water chemistry data, including pH and conductivity, was collected using CARP's portable HydroLab Quanta water quality monitoring meter. Data was collected on a fortnightly basis by CARP staff, typically the day following the volunteers' sampling day, at a set location on the riverbank at each River Guardian site. The meter was placed in the river approximately 1 to 2 meters away from the bank, and allowed to stabilize, usually for two to three minutes before a reading was taken. Once stabilized, the values were stored in the meter's memory and recorded on the data sheets upon return at the CARP office. The data is stored using an in-house Microsoft Access database. Approximately every two to three weeks, the multi-sensor water meter was calibrated for pH, conductivity and dissolved oxygen according to the directions in the Operating Manual (Hydrolab Corporation 2002).

Procedures for Investigation of Low Dissolved Oxygen in Lower River

At several points through the 2009 sampling season, from mid-August to early October, DO readings and nutrient samples were gathered in the estuarine section of the river. The DO readings were taken using the Hydrolab Quanta multi-probe unit from a boat at eight locations along the river. The Hydrolab also records temperature, conductivity, pH and salinity. Two measurements were performed for each location, one at a depth of approximately 0.5 m (above the

halocline) and one at approximately 3.5 m (below the halocline). Nutrient samples (silicate, nitrate, nitrite, ammonia, phosphate) were gathered in 30 mL sample bottles and field-filtered using Millipore glass fibre filters (Cat. No. APFC02500). All samples were handled in accordance with the protocols of the Bedford Institute of Oceanography (BIO), where the final analysis was conducted. The nutrient analysis was performed using Colorimetric Segmented Flow with a Technicon II.

Procedures for TSS/Turbidity collection and processing

Samples were taken using either van Dorn Samplers or by hand from the shore near the bridge. If a van Dorn sampler was used, it was used mid-span of the bridge at a depth of approximately 30 to 60 cm. If taken from the shore, the bottle was dunked in an area where the water's flow was constant and at a depth the length of a forearm, approximately 30 to 40 cm. Occasionally, an extendible rod with the bottle attached to the end was used from the shore. The collection method was not recorded for particular samples, although any sample collected by a River Guardian was collected using a van Dorn Sampler. A collection of approximately 1 litre of water was attempted for each collection, but limited quantities of sample bottles sometimes forced a collection of only 500 millilitres. Lab Turbidity was assessed at Acadia University using a 2100P Hach Turbidimeter.

TSS data was collected through filtration. Filters were stored in a desiccator for at least 24 hours and were then weighed in a weighing boat on an analytical balance. The weight of the filter paper and the weighing boat together were recorded on the weighing boat. The filters used were Ahlstrom brand, grade 161, 4.7 cm in diameter, or Whatman brand, grade 934AH, 4.7 cm in diameter. The water sample was passed through one of the pre-weighed filters using a suction filtration procedure. The filter paper was carefully placed back in its weighing boat and dried in an oven at ~90 degrees Celsius before being stored back in the desiccator. After remaining in the desiccator for approximately 24 hours, the filters and boats were removed and reweighed. The new weight was subtracted from the original weight of the filter and boat, and this number was divided by the sample volume to give a g/L TSS reading. The balance used was an A&D Electronic Balance ER-120A.

Aylesford *E. coli* sampling – Coliscan Easygel procedure

Unlike the River Guardians routine *E. coli* analysis for the biweekly sampling, the Aylesford bacteria samples were processed in-house. The procedure uses a product known as Coliscan Easygel, which is produced by Micrology Laboratories.

Water samples were collected by standing on the shore and using a grab sampler and a Whirl-Pac bag. The Whirl-Pac bags are sterile and the inside of the bag cannot be touched or it will be contaminated and useless. The bag was dipped into the water using the sampler, and then sealed and placed in a cooler with ice packs. Approximately 100 mL were gathered using a sampler and Whirl-Pac bags (Figure A2).

Figure A2. Homemade grab sampler with a full Whirl-Pac bag.

Once they were brought to the lab, the samples were plated. The samples were thoroughly mixed and 1 to 5 mL were added to a Coliscan Easygel bottle using disposable sterile pipets. The volume of water used was recorded and varied depending on the desired sensitivity of the test. The bottle was sealed and mixed before being poured into one of the treated Petri dishes. The Petri dishes were then placed in an incubator set at 35 degrees Celsius and left for at least 24 hours. After a sufficient amount of time had passed, the samples were removed and the *E. coli* cultures were enumerated. The samples were then disposed of using bleach. Enough bleach to cover the surface of the gel was poured into the Petri dish and allowed to sit for five minutes. The dishes were then sealed in plastic bags and disposed of. The full procedure can be found at http://www.micrologylabs.com/Home/Our_Methods and a visual guide for colony enumeration can be found at http://web.centre.edu/miles/che250/cs%20ez%20color%20guide.pdf.

Appendix B – Sites Monitored

Water samples were collected during 2009 by the Annapolis River Guardians program at several different locations (Table B1). Coordinates are reported in latitude and longitude, as recorded on a hand-held GPS unit.

Table B1. Coordinates and descriptions for Annapolis River Guardian and TSS/turbidity sample locations.

Site Code	Latitude	Longitude	Site Name	Site Name (Long with Reference Points)
AY40	N45 01.699	W64 48.617	Aylesford Road	Bridge at Aylesford Rd, near Hwy 1
Ref	N45 00.122	W64 49.381	Millville	Bridge on Victoria Rd, South Annapolis River
00	N45 01.606	W64 50.148	Aylesford	Bridge on Victoria Rd, near Hwy 1
13	N44 58.713	W64 56.663	Kingston	Bridge on Bridge St. near Stronach Park
18	N44 57.199	W65 00.096	Wilmot	Bridge on Old Mill Road
NS01	N44 56.942	W65 01.769	Wilmot	Bridge on Bayard Road
25	N44 56.213	W65 03.969	Middleton	Bridge on Hwy 10, near Riverside Park
35	N44 52.850	W65 09.476	Lawrencetown	Bridge on Lawrencetown Lane
40	N44 52.045	W65 12.384	Paradise	Bridge on Paradise Lane
49	N44 50.335	W65 17.492	Bridgetown	Bridge on Queen Street

The NS01 and Ref sites were sampled for turbidity and TSS only.

Appendix C – Quality Assurance / Quality Control Data

Introduction

Following a contamination event in 2003, CARP initiated a number of procedures to ensure the quality of data collected. In addition to instituting a new collection procedure for fecal bacteria, CARP has put in place a program of regular quality control checks on sampling equipment and methods. Further information on the quality assurance/quality control (QA/QC) program can be found in CARP's draft QA/QC Project Plan (Sharpe and Sullivan 2006). An important initial step in the QA/QC program is the training of volunteers. Training with new volunteers was conducted in the field. During the 2009 season, CARP staff conducted visits with each of the Annapolis River Guardian volunteers on collection days in order to both collect a series of blank and split samples, as well as to ensure the consistency in collection procedures. In total, twenty-one QA/QC samples were collected during the 2009 season. These were, in summary:

- 6 Dissolved oxygen split samples
- 7 *E. coli* travel blanks
- 6 *E. coli* duplicate samples
- 6 split turbidity/TSS samples

Background

For the purposes of CARP's water quality monitoring programs, a blank sample is a sample that is known not to contain any of the substance in question. For CARP's monitoring of *E. coli* bacteria, either distilled or un-chlorinated tap water is added to the sample bottle. There are two types of blank samples that are collected for QA/QC analysis:

- Travel blanks are obtained by filling the sample bottle with distilled/tap water before the start of a sampling day, and placing them in the same cooler among other surface water samples. Travel blanks are used to ensure there is no cross-contamination between samples while they are being transported in the same cooler. They should always produce plates with no fecal bacteria growth.
- Field blanks are obtained by performing the entire sampling protocol (i.e.: attaching the bottle to the clamp, and lowering the apparatus to the water surface) but NOT submerging the bottle. The bottle is instead lifted up empty and filled with distilled/tap water on the bridge. This type of blank sample is used to test the sampling procedure and should also always produce plates with no fecal bacteria growth. A positive result on a field blank would lead to further investigations to determine the source of contamination (for example: operator, equipment, distilled water, etc). No field blanks were collected for *E. coli* in 2008 or 2009.

Split samples are used to measure both precision and accuracy. Precision is expressed as the degree of agreement among repeated measurement of the same parameter and provides information on the reproducibility and consistency of the methods used. Accuracy, on the other hand, consists of how close a measurement is to the "true" value.

A split sample is a single sample volume that is divided in two samples that are analysed separately. Split samples can provide information on the precision of the lab method (i.e.: the precision of Valley Regional Hospital's *E. coli* analysis). Split samples can also provide information on the accuracy of the method used (i.e.: the accuracy of volunteers at the Winkler titration).

The degree of variability between two split samples can be evaluated by calculating their relative percent difference (RPD). The RPD is expressed as the absolute difference of the two measurements multiplied by 100 and divided by the average of the two values:

$$RPD = \frac{|X_1 - X_2| \times 100}{(X_1 + X_2)/2}$$

When more than two samples are to be compared, the degree of variability is estimated by calculating their Relative Standard Deviation (RSD). Both the RPD and the RSD are expressions of precision, the smaller the value, the greater the precision.

$$RSD = \frac{s}{X_m} \times 100$$

s = standard deviation
X_m = mean of duplicate samples

Accuracy is estimated by taking the absolute difference between the "true" value and the "test" value. When there are multiple measurements, the true value is subtracted from the average of the test measurements. The result is compared to acceptable accuracy standards for each individual method.

Accuracy = Test/Average value – True Value

Dissolved Oxygen

Dissolved oxygen split samples were taken in 2009 using a single volume of water from a van Dorn sampler. The accuracy of volunteer DO measurements was assessed through the collection of seven split samples. The Winkler titration (described in Appendix A) is widely recognized has a standard for determining dissolved oxygen and is reported to have an accuracy of at least +/- 1 mg/L. Results from the split samples (Table C1) indicate that the volunteers attained an average accuracy of +/- 0.38 mg/L (RPD = 4.2%). For comparison purposes, the average DO accuracy for 2008 was +/- 0.094 mg/L (RPD = 5.4%).

Table C1. Volunteers' level of accuracy at measuring dissolved oxygen using the Winkler titration.

Site	Date	Volunteer result	QA/QC result	Accuracy	Percent difference
49	18-May-09	8.8	8.89	0.09	1.02
40	18-May-09	8.22	8.94	0.72	8.39
35	18-May-09	8.65	9.14	0.49	5.51
18	31-May-09	8.4	8.64	0.24	2.82
00	31-May-09	9.70	9.77	0.07	0.72
13	20-Sep-09	9.8	10.49	0.69	6.80
			Mean	0.3833	4.2092

E. coli Bacteria

Throughout the sampling season, a series of blank samples were submitted blind for analysis to the microbiology laboratory at Valley Regional Hospital. The seven travel blanks analysed all had coliform counts of 0 cfu/100ml, which indicates that no cross-contamination was occurring during transportation of the samples. Although no field blanks were collected in 2008 or 2009, the two field blanks collected in 2007 showed no *E. coli* growth, indicating that the fecal bacteria sample collection procedure was not contaminating the samples. The sample collection procedure has not changed between the two years.

Throughout the 2009 sampling season, a total of six split samples were collected during the sampling visits with the volunteers. These samples were submitted to the Valley Regional Hospital Microbiology Laboratory under fictitious sample identification numbers. The purpose of this was to assess the reproducibility of the *E. coli* MPN analysis method used. The mean RPD for these split samples was found to be 28.8%. The mean RPD for the 2007 and 2008 seasons was 42.5% and 23.3%, respectively (Table C2).

All analysis methods have inherent variability; this is particularly the case with IDEXX, as the Most Probable Number result is statistically derived (Table C3). The variability values are taken from the IDEXX Quanti-Tray®/2000 MPN Table (per 100mL) with 95% Confidence Limits (no date). For each volunteer result, the 95% confidence range was found and compared to the confidence range of the QA result. If these ranges overlapped, then the variability between the two results can be explained by the inherent variability of the procedure. None of the volunteer results had a value whose confidence range that did not overlap with that of the QA result, although the difference between the test and lab samples for the Lawrencetown QA were pronounced.

The 2008 RPD mean is much lower than the 2007 value, which seems to indicate that the test procedure is being carried out with greater consistency that year. The 2009 RPD mean is close to the 2008 mean; the testing precision has not changed much since the previous year. The test performed is the Colilert Most Probable Number analysis, and it is performed at the Microbiology Laboratory at Valley Regional Hospital.

Table C2. Relative percent difference in duplicate samples analysed for fecal coliforms.

Site	Date	Volunteer result	QA/QC result	Accuracy	Percent difference
49	18-May-09	727	980	253	29.64
40	18-May-09	1203	1414	211	16.13
35	18-May-09	866	1553	687	56.80
18	31-May-09	88	64	-24	31.58
00	31-May-09	250	194	-56	25.23
13	20-Sep-09	105	120	15	13.33
			Mean	181	28.784

Table C3. Confidence interval limits for IDEXX Colilert Most Probable Number procedure.

	95% Confidence	
MPN	Lower Limit	Upper Limit
0	0	3.7
10	5	18
50	36	69
100	81	121
150	124	181
200	166	242
500	405	619
1000	740	1320
1500	1010	2350
2000	1220	3300
>2419	1440	infinite

Turbidity/TSS QA/QC

In order to perform QA measurements for TSS and turbidity, split samples were taken from van Dorn samplers and these duplicates were analyzed (Tables C4 and C5).

Table C4. Relative percent difference in duplicate samples analysed for total suspended solids.

Site	Date	Volunteer result	QA/QC result	Accuracy	Percent difference
49	18-May-09	7.40	7.01	-0.39	5.41
40	18-May-09	5.29	10.41	5.12	65.22
35	18-May-09	9.87	7.37	-2.5	29.00
18	31-May-09	2.17	2.89	0.72	28.46
00	31-May-09	2.94	3.41	0.47	14.80
13	20-Sep-09	-2.22	-1.37	0.85	47.35
			Mean	0.7117	31.709

Table C5. Relative percent difference in duplicate samples analysed for turbidity.

Site	Date	Volunteer result	QA/QC result	Accuracy	Percent difference
49	18-May-09	32.10	20.40	-11.7	44.57
40	18-May-09	52.10	24.80	-27.3	71.00
35	18-May-09	23.50	17.40	-6.1	29.83
18	31-May-09	3.16	2.86	-0.3	9.97
00	31-May-09	4.36	4.15	-0.21	4.94
13	20-Sep-09	2.98	2.89	-0.09	3.07
			Mean	-7.617	27.228

The TSS results in Table C4 have a large variety of percent difference results. Some of the QA sampling was done at low-flow events, while the sampling on May 18th coincided with a higher-flow event. Many of the values recorded during low flow events were returned at negative numbers due to random procedural error such as on Sept 20, 2009. A. Cook of Environment Canada suggested that negative TSS readings could be related to not pre-washing the filters (pers. comm., February 2010). Filters can contain soluble materials that are washed out during filtration, which would contribute to the loss of mass of the filter. Also, TSS and turbidity sampling both have a high degree of inherent variability, especially at higher numbers.

In addition to these QA/QC samples taken with the volunteers, regular blank, split, duplicate and triplicate samples were collected. Generally, for TSS and turbidity, when blanks were collected, duplicates and triplicates were collected of them as well. Split and duplicate results tended to be very close to each other; the standard deviations for TSS duplicates tended to be between 0.1 mg/L and 6 mg/L while the standard deviations for turbidity samples tended to fall between 0.01 NTU and 1.0 NTU, with a few exceptions for both parameters. During 2008 and 2009, 196 QA samples were taken for TSS and turbidity.

From 2008 to 2009, 28 blank samples were taken in total. For the TSS and turbidity samples, the type of weighing boat used had a large effect on the results, which is reflected in the blank sample results (Table C6). No weighing boats were used for the first month of sampling in 2008. After that, plastic boats were used until spring of 2009, after which aluminium boats were used. These procedural blanks produced variable results, which may be related to soluble materials in the filter paper.

Table C6. Average results for blank TSS and turbidity samples, organized by weigh boat type.

Boat type	TSS (mg/L)	Lab turbidity (NTU)
None	-0.80	0.26
Plastic	0.04	0.22
Aluminium	-2.47	0.85