

Annapolis River 2006 Annual Water Quality Monitoring Report

including results from the

Annapolis River Guardians Volunteer Water Quality Monitoring Program



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Contents

Acknowledgements	2
Executive Summary	3
Introduction	4
History.....	4
Program Objectives.....	4
Overview of 2006 Monitoring Season	4
2006 Monitoring Results.....	6
E.coli Bacteria	6
Dissolved Oxygen	16
Temperature	19
pH and Conductivity	22
Conclusions	24
Recommendations	29
References	30
Appendices	32
Appendix A – Parameters Tested and Methodologies.....	32
Appendix B – Sites Monitored	34
Appendix C – Quality Assurance / Quality Control Data	35

This report is available electronically at www.annapolisriver.ca

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Executive Summary

In 2006, the Annapolis River Guardians completed their 15th 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. A number of parameters were measured, including 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 2006 were significantly higher than those observed in 2005. E.coli counts greater than 1000 cfu/100 ml were observed at all sites at least once in 2006. Elevated E.coli levels occurred predominantly during the months of June, July and October, which had precipitation levels significantly above the monthly averages. On each of the sample days where E.coli levels exceeded 1500 cfu/100 ml, daily precipitation of more than 10 mm occurred in the preceding two days. Of the 119 E.coli bacteria samples collected and analyzed, 46% (55) exceeded the contact water recreation guideline of 200 cfu/100ml. In 2004, the last year with very high fecal bacteria counts, 37% exceeded this threshold. In 2006, water samples were collected at an additional monitoring station in the Aylesford area at Aylesford Road. The comparison of monitoring results from this and the nearby, existing Aylesford station, has provided a number of useful insights into the nature of fecal contamination in this area.

Over 15 years of monitoring, mean dissolved oxygen saturation (DOSAT) levels have remained in the range of 80-94%. In 2006, the mean DOSAT level was 80.5%. The mean 2006 DOSAT for all sites was below the 1992-2005 averages for the sites. As a result of the regular monitoring provided by the Annapolis River Guardian program, low DO levels were observed in the lower river. This prompted a more in-depth examination, which is reported separately.

The mean summer water temperature for the Annapolis River during 2006 was 19.1°C or 0.9°C cooler than for the same period in 2005. As in previous years, water temperatures during 2006 continued to reach levels stressful to aquatic life regularly during the summer months.

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 6.9. Although slightly lower pH values were observed in 2005, the 2006 results are more typical of those seen in 2003 and 2004.

A limited number of nutrient samples (n=15) were collected from the lower Annapolis River in 2006 in the area of Bridgetown. Median concentrations of dissolved inorganic nitrogen (1.07 mg/L) and phosphate (0.029 mg/L) were observed. Nutrient concentrations in this magnitude are sufficient to pose a risk of eutrophication.

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 +/- 0.60mg/L, compared with 0.32 mg/L recorded in 2005. Field and travel blank samples analysed for fecal coliforms consistently produced plates with 0 cfu/100ml. E.coli split samples had a Relative Percent Difference of 16%.

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 on-going 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. 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 ten years, the core program 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 2006 Monitoring Season

The 2006 monitoring season commenced on April 30 and concluded on November 14. Samples were collected fortnightly, with a total of approximately 120 sampling events during the season. Samples were analysed for a variety of parameters, including E.coli bacteria, dissolved oxygen, temperature, pH and conductivity. Further information on the sampling procedures can be found in Appendix A.

Eight stations were sampled along the Annapolis River. Further information on these sampling locations is contained in Appendix B. Figure 1 shows the Annapolis Watershed and the 2006 monitoring sites. The data collected by the volunteers is stored in an in-house Microsoft Access database, as well as a publicly accessible web-based, searchable, database at www.fundybay.com.

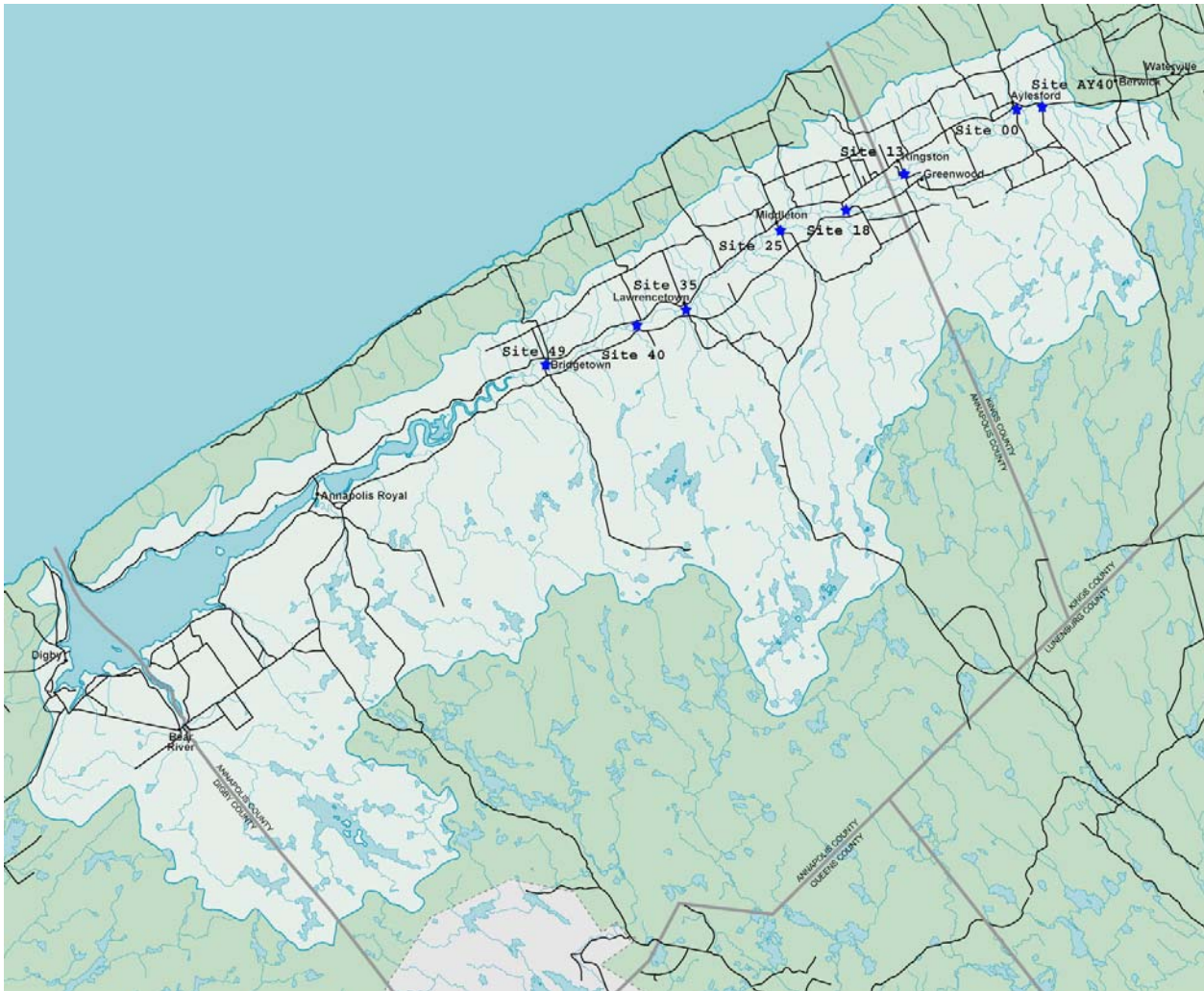


Figure 1. Annapolis Watershed with 2006 Monitoring Sites.

The 2006 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.

2006 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. Fecal matter of warm-blooded animals is the predominant source of E.coli bacteria. Because they occupy the same ecological niche as many human pathogens, E.coli are used as indicators for the possible presence of other potentially dangerous pathogens. E.coli have been identified in the past as a major cause of concern in the Annapolis River watershed (Pittman *et al* 2001). The potential sources of fecal contamination in the watershed include central sewage treatment plants, malfunctioning on-site septic systems, aquatic wildlife (i.e. beavers, muskrats, waterfowl), domestic animals, and livestock.

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 are 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)
- In situ Freshwater Sediment: 57 days (Davies *et al* 1995)

Spatial and temporal trends in E.coli data over the last fifteen years are analyzed below. Over the period of 1992 to 2006, 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.

While the core River Guardian monitoring program has been maintained over the period of 1992 to 2006, a number of modifications have been made. For example, in 1996, the collection of E.coli samples was standardised 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 2006).

Canadian Water Quality Guidelines

Various governmental 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). CARP has summarized the guidelines for fecal coliform contamination into a concise table for public awareness purposes, shown in Table 1.

Table 1. Summary of Water Quality Guidelines for Fecal Coliforms.

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

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 2005 and 2006 E.coli results (see Figure 2). 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. The three water quality guidelines for E.coli discussed in the report are shown as dotted lines at 50, 100, and 200 cfu/100ml. It is important to note that the y-axis of the graph is plotted using a logarithmic scale (Log E.coli).

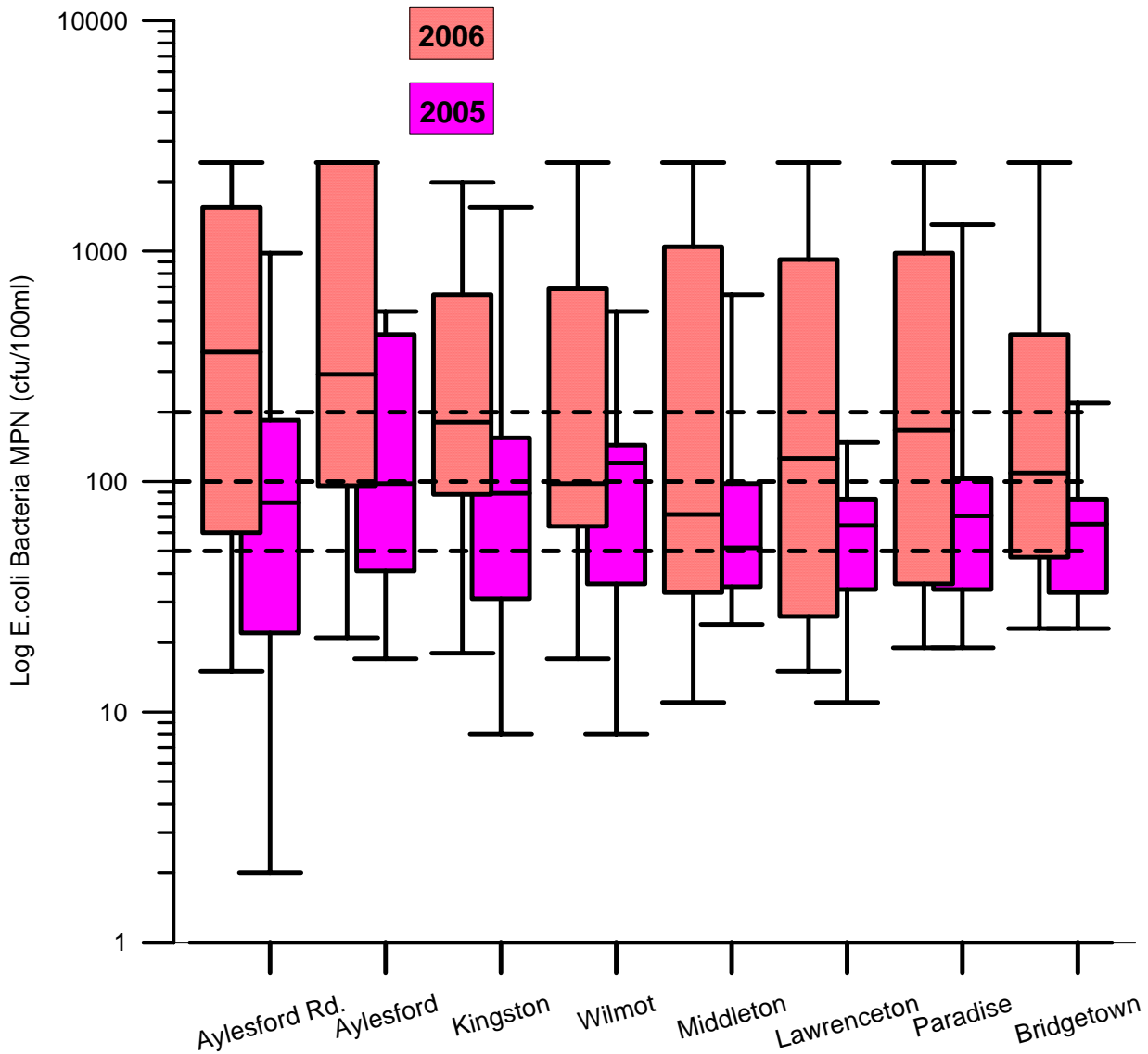


Figure 2. Box and Whisker Plot of E.coli Bacteria Results for 2005 and 2006.

From Figure 2, it is evident that there has been a significant increase in E.coli bacteria counts between 2005 and 2006. E.coli bacteria levels are seen to be highly variable across most monitoring sites. The median bacteria level increased for all sites, with the exception of Wilmot. The E.coli ranges shown in Figure 2 are artificially capped at 2420 MPN cfu/100 ml, as this is the maximum value possible with the IDEXX Colilert testing system. Another interesting observation from Figure 2 is that the 2006 E.coli results had a much larger range. The reason for this increased variability is not known.

Figure 3 presents all E.coli data collected in 2006 on the Annapolis River. While most results were less than 500 cfu/100 ml, on six distinct occasions, significantly higher E.coli values (> 1500 cfu/100 ml) were observed. On each of these occasions (June 11, June 25, July 23, August 7, October 1 & 29) there was a daily total precipitation of greater than 10 mm within two days of the samples being collected. The heaviest precipitation occurred on July 21, when 43.6

mm of rain fell. On July 23, all eight E.coli samples exceeded 1500 cfu/100 ml. From Table 2, June, July and October can be seen to be very wet months.

The response of E.coli levels to precipitation appeared to depend in part on the where the samples were collected. E.coli levels at Aylesford (Site 00) exceeded 1000 cfu/100 ml on five occasions, while Middleton (Site 25) and Aylesford Road (Site AY40) exceeded 1000 cfu/100 ml on four occasions each. Wilmot (Site 18), Lawrencetown (Site 35) and Bridgetown (Site 49) exceeded 1000 cfu/100 ml only on two occasions.

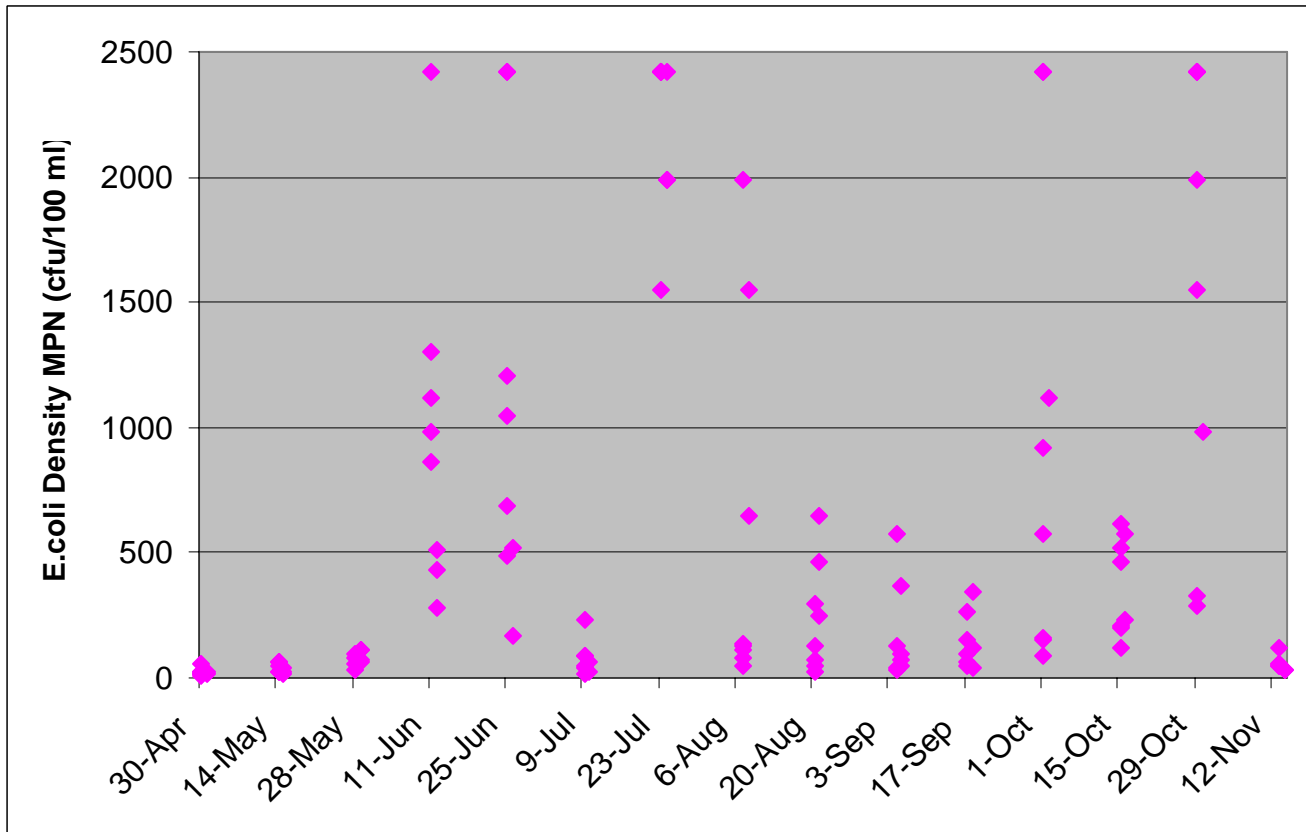


Figure 3. E.coli data collected in 2006.

It is known that E.coli levels in surface water are responsive to precipitation events. As is shown in Table 2, precipitation in June and July 2006 significantly exceeded the long-term averages for the area.

Table 2. Monthly Precipitation Data for Greenwood.

	April	May	June	July	Aug.	Sept.	Oct.	Nov.
1971 to 1990 Average	77.4	84.6	77.5	93.5	79.4	97.4	98.3	110.6
2006 Monthly Totals	100.2	98.8	217.4	150.7	78.8	50.2	130.0	102.6

In order to further explore the relationship further between precipitation and E.coli densities, field data sheet records for the period of 1993 to 2006 were examined with the E.coli densities collected on that day. When the Annapolis River Guardians complete their field data sheets, the level of recent precipitation in the past three days is recorded as: none,

drizzle, moderate or heavy. While these observations are somewhat subjective, they do give an indication of the extent of recent precipitation. The results of 1632 observations for the 1993 to 2006 period are presented in Figure 4.

From these results, it is evident that the E.coli range is consistent across the four precipitation categories. Only where Heavy precipitation was recorded in the past 3 days is the median E.coli density significantly higher. This data would seem to suggest that while precipitation events have an impact on E.coli levels, they are only one of a number of factors. These other factors may include, but not be limited to, periodic upstream discharges, water temperature and the historic pattern of precipitation over the past weeks to months.

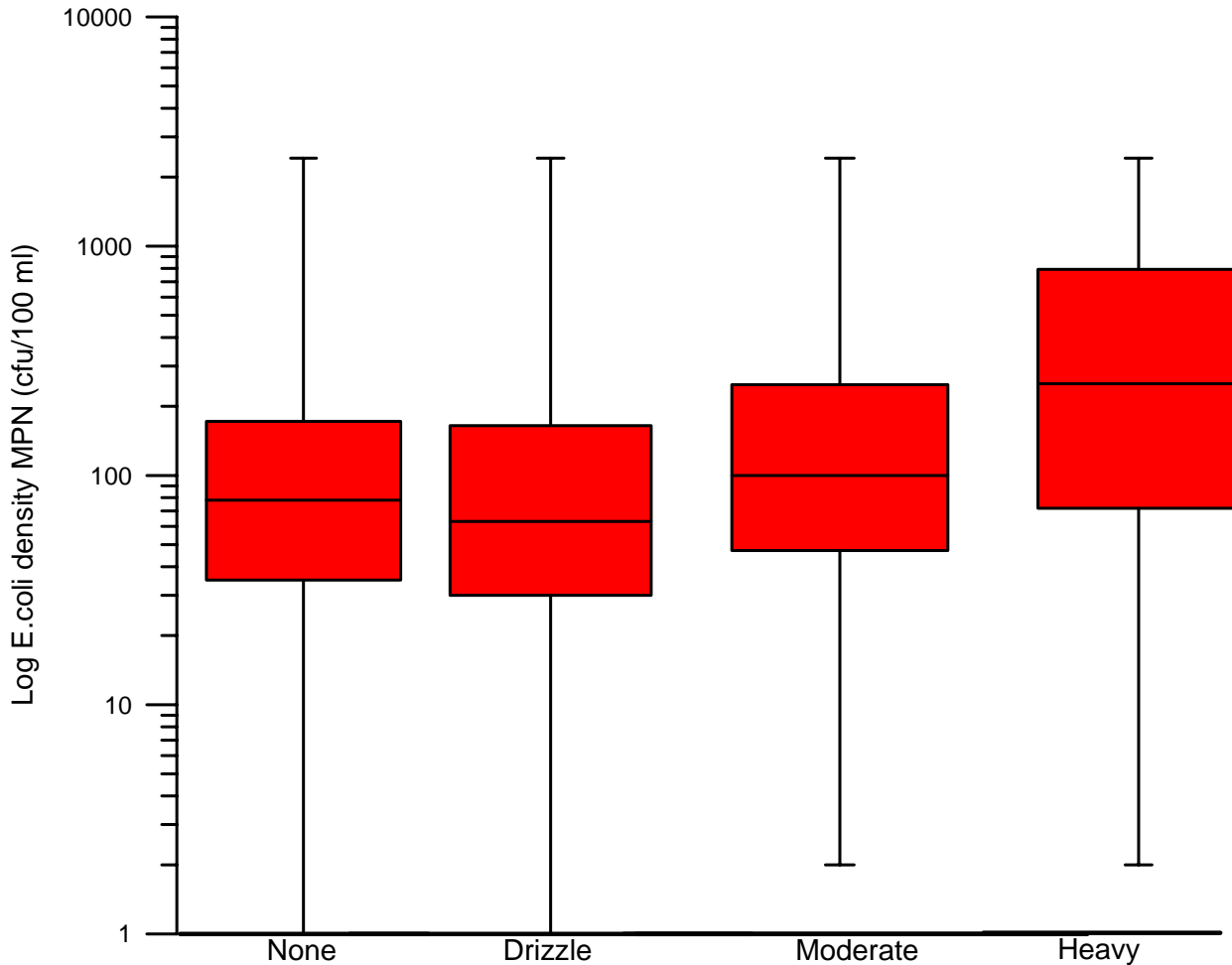


Figure 4. 1993 to 2006 E.coli density with recent precipitation.

Table 3 presents the proportion of E.coli bacteria samples exceeding 50 cfu/100 ml, the water quality guideline for livestock watering. For example, at Aylesford in 2006, 0.93 or 93% of water samples collected had E.coli bacteria counts in excess of 50 cfu/100ml, as compared to 61% in 2005. From the data presented in Table 3, it is evident that all sites had an increase in the number of samples that exceeded 50 cfu/100 ml during 2006. Upon examining the data, it appears as though Lawrencetown had a significant improvement in bacterial water quality, occurring between 1996 and 1998. The cause of this improvement is not known.

Table 3. Proportion of Fecal Coliform Samples Exceeding 50 cfu/100 ml.

	Aylesford Road	Aylesford	Kingston	Wilmot	Middleton	Lawrencetown	Paradise	Bridgetown
1992		1.00	0.33	1.00	1.00	1.00	1.00	
1993		0.91	0.79	0.81	0.86	0.93	0.86	
1994		0.83	0.73	0.88	0.91	0.81	0.86	0.92
1995		0.40	0.14			0.80	0.50	0.71
1996		0.50	0.80		0.75	0.93	0.75	0.80
1997		0.86	0.81	0.81	0.88	0.71	0.50	0.65
1998		0.92	0.75	0.40	0.50	0.55	0.60	0.75
1999		0.86	0.67	0.71	0.55	0.33	0.43	0.65
2000		0.60	0.53	0.45	0.46	0.50	0.57	0.36
2001		0.67	0.83	0.83	0.54	0.33	0.55	0.20
2002		1.00	0.53	0.64	0.38	0.38	0.20	0.60
2003		1.00	0.90	1.00	0.56	0.50	0.50	0.55
2004		0.94	0.93	1.00	0.85	0.79	0.86	0.86
2005	0.53	0.61	0.53	0.63	0.47	0.53	0.61	0.56
2006	0.80	0.93	0.93	0.80	0.60	0.60	0.67	0.73

Table 4 presents the proportion of samples exceeding the water quality guideline for food crop irrigation (100 cfu/100 ml). Again in 2006, it is evident that there was an increase in the number of E.coli bacteria samples exceeding 100 cfu/100 ml for seven of the eight monitoring sites, when compared with 2005. The 2005 and 2006 exceedences of this threshold are also compared in Figure 4.

Table 4. Proportion of Fecal Bacteria Samples Exceeding 100 cfu/100 ml.

	Aylesford Road	Aylesford	Kingston	Wilmot	Middleton	Lawrencetown	Paradise	Bridgetown
1992		1.00	0.00	0.67	0.67	0.67	1.00	
1993		0.82	0.57	0.69	0.71	0.79	0.71	
1994		0.67	0.55	0.88	0.82	0.75	0.57	0.69
1995		0.40	0.14			0.80	0.50	0.57
1996		0.50	0.50		0.63	0.79	0.56	0.60
1997		0.71	0.44	0.69	0.63	0.36	0.14	0.53
1998		0.83	0.50	0.10	0.50	0.27	0.40	0.25
1999		0.71	0.53	0.43	0.45	0.00	0.29	0.41
2000		0.60	0.40	0.27	0.23	0.33	0.43	0.07
2001		0.56	0.42	0.50	0.31	0.08	0.45	0.13
2002		1.00	0.33	0.29	0.06	0.38	0.10	0.27
2003		0.70	0.80	0.45	0.33	0.29	0.25	0.36
2004		0.94	0.79	0.79	0.62	0.50	0.64	0.79
2005	0.42	0.50	0.47	0.58	0.21	0.21	0.28	0.11
2006	0.73	0.73	0.64	0.47	0.40	0.53	0.60	0.53

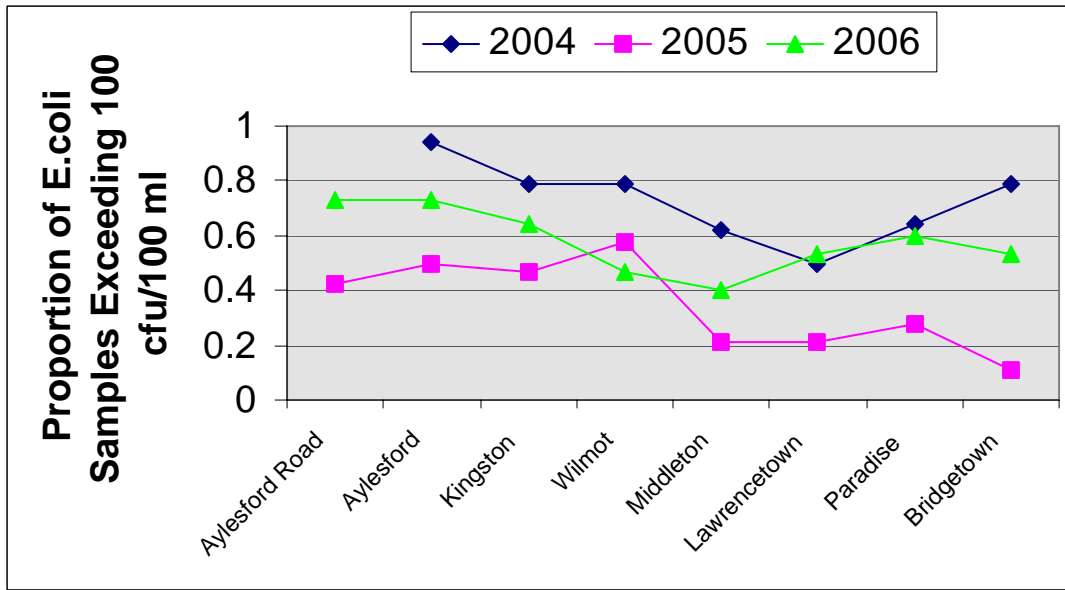


Figure 5. Proportion of Fecal Bacteria Samples Exceeding 100 cfu/100ml during 2004, 2005 and 2006.

As is evident in Figure 5, all monitoring sites except Wilmot exhibited an increase in the number of E.coli samples exceeding 100 cfu/100ml in 2006 when compared to 2005. Table 5 presents the proportion of E.coli bacteria samples exceeding 200 cfu/100 ml, the water quality guideline for contact water recreation. The table shows a general increase with respect to this threshold. Figure 6 presents the proportion of E.coli bacteria samples collected at Aylesford that exceed 200 cfu/100 ml. Over 15 years of monitoring, the data from this site is observed to be highly variable, with the 2006 results approaching those observed in 2004.

Table 5. Proportion of E.coli Bacteria Samples Exceeding 200 cfu/100 ml.

	Aylesford Road	Aylesford	Kingston	Wilmot	Middleton	Lawrencetown	Paradise	Bridgetown
1992		0.50	0.00	0.67	0.67	0.33	0.33	
1993		0.55	0.21	0.50	0.29	0.57	0.36	
1994		0.50	0.55	0.56	0.55	0.31	0.57	0.46
1995		0.20	0.14			0.40	0.33	0.29
1996		0.50	0.40		0.38	0.43	0.44	0.40
1997		0.43	0.13	0.19	0.13	0.07	0.07	0.06
1998		0.58	0.13	0.00	0.25	0.09	0.20	0.08
1999		0.43	0.33	0.29	0.18	0.00	0.14	0.18
2000		0.40	0.07	0.18	0.15	0.25	0.43	0.00
2001		0.22	0.25	0.33	0.15	0.08	0.09	0.13
2002		0.50	0.13	0.14	0.00	0.00	0.00	0.13
2003		0.10	0.40	0.27	0.22	0.21	0.08	0.27
2004		0.71	0.36	0.21	0.23	0.29	0.43	0.29
2005	0.21	0.44	0.21	0.05	0.11	0.00	0.06	0.06
2006	0.67	0.67	0.50	0.33	0.33	0.40	0.47	0.33

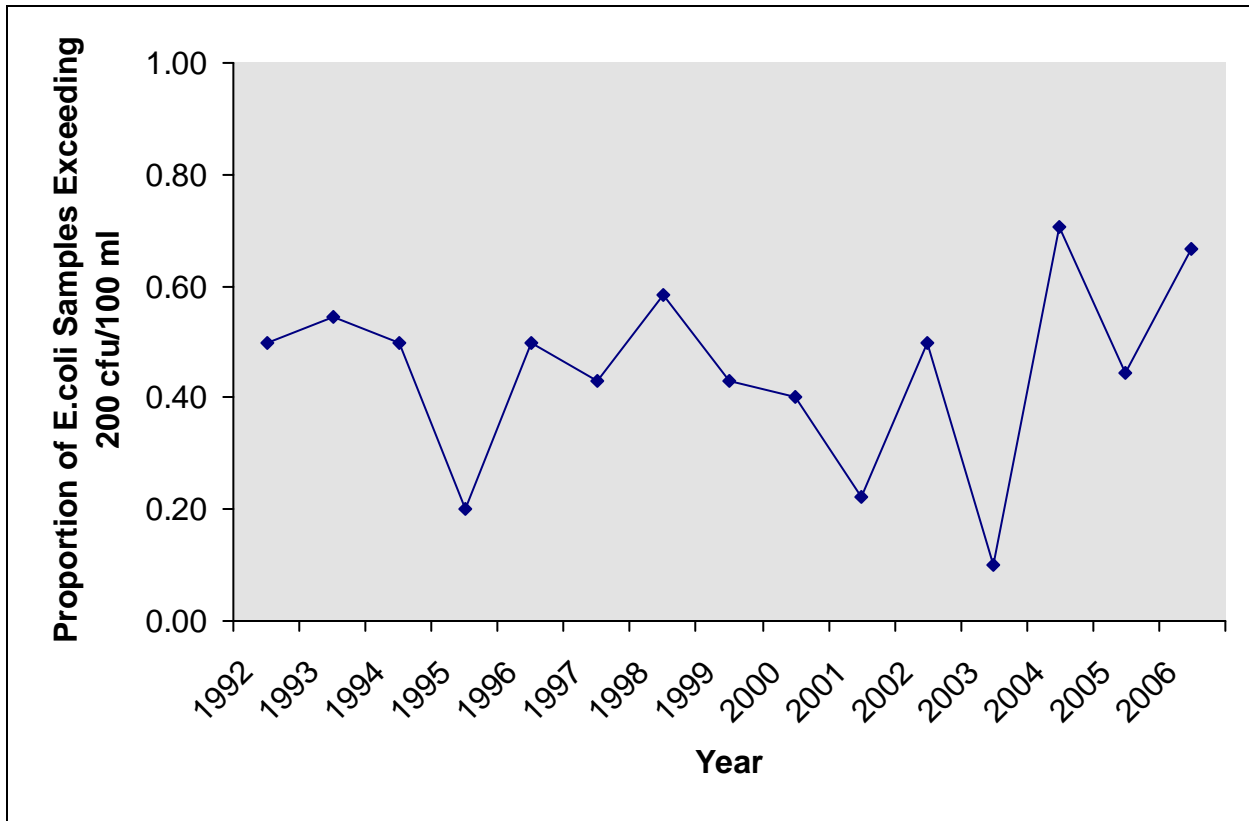


Figure 6. Proportion of E.coli Bacteria Samples Collected at Aylesford Exceeding 200 cfu/100 ml.

During the summer of 2003, CARP staff undertook a survey of tributaries to the Annapolis River in the Aylesford area, as part of the larger Aylesford East Project, to identify watercourses with impaired water quality (Sharpe and Sullivan 2004). A number of tributaries were identified as having elevated fecal coliform and nutrient levels, including Patterson, Parker and Skinner Brooks. These tributaries originate on the North Mountain and enter the Annapolis River approximately 2km above the Aylesford River Guardian sample site (Site 00 – Aylesford).

During the 2005 and 2006 monitoring season, an additional monitoring site was added (Site AY40) at Aylesford Road, approximately 2.5 km upstream of the Site 00 Aylesford location. The purpose of this was to collect regular samples above and below the mouth of the above brooks, to assess if there was a significant difference in fecal bacteria results. Figure 7 presents the monitoring results for the AY40 (Aylesford Road) and 00 (Aylesford) locations. Table 6 presents the respective geometric means for these two sample stations.

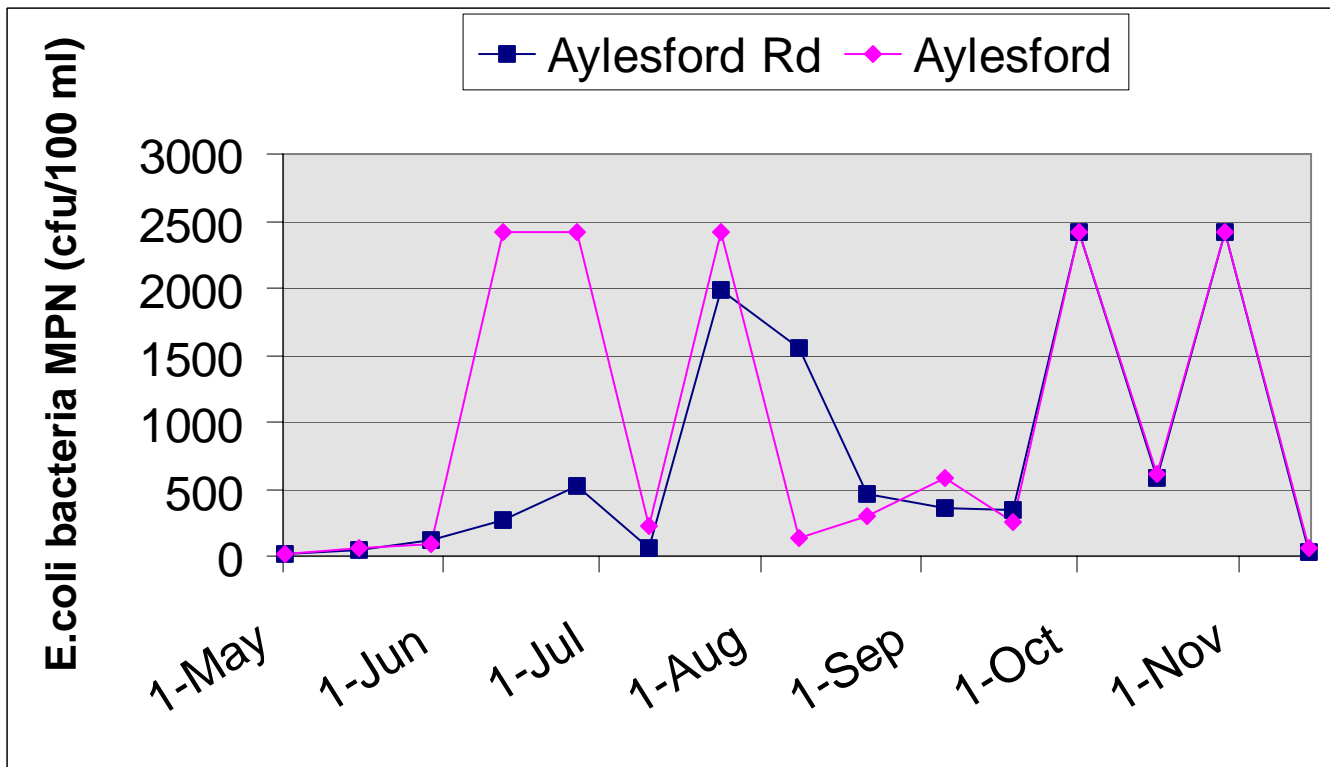


Figure 7. Fecal bacteria densities for Aylesford Road and Aylesford monitoring sites.

The E.coli bacteria levels for the two monitoring sites are very similar from April to early June, after which the downstream Aylesford site (00) exhibited significantly higher bacteria levels during the remainder of June. From July to November, the two sites had comparable bacteria levels. June 2006 had a total monthly precipitation of 217 mm, compared to the 1971 to 2000 average of 78 mm. During 2006, the higher downstream E.coli values occurred during a period of higher than average precipitation and river discharge. During 2005, the higher downstream E.coli values occurred during the summer months with limited rainfall.

Taking the data for an entire season for each monitoring station, the high variability of E.coli samples results in large values for standard error, with the conclusion that the geometric means may not be statistically different. The data in Table 6 must be considered together though with temporal trends presented in Figure 7.

Table 6. Geometric Means and Standard Error Aylesford Road and Aylesford Sample Stations (E.coli reported as MPN cfu/100 ml).

Year	Aylesford Road (upstream)		Aylesford (downstream)	
	Geometric Mean	Standard Error	Geometric Mean	Standard Error
2005	88	67	183	53
2006	294	228	382	279

The monitoring results for 2005 and 2006, coupled with the findings noted above from the 2003 Aylesford East survey, indicate a source (or sources) of E.coli contamination entering the Annapolis River between Aylesford Road and Victoria Road in Aylesford.

Patterson Brook was identified by MacMillan *et al* (2003) as one of the few streams in the Annapolis watershed with consistently cool water temperatures throughout the summer months. The stream was also found to contain high populations of brook trout (MacMillan and Crandlemere 2003). Patterson Brook may therefore have the appropriate combination of cool water and necessary habitat to serve as nursery and/or summer refuge for brook trout. For these reasons, any contamination present should be investigated and remediated where possible.

Recommendations

- Continue regular River Guardian E.coli monitoring at the eight main river sample locations.
- Conduct simultaneous monitoring at Sites 00 and AY40, together with intervening tributary streams.
- Conduct a foot survey of the Annapolis River between these two sites, and the intervening tributary streams, to identify possible contamination sources.
- Review current and historic air photos of this area to identify land use changes and possible sources of contamination.

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, just as terrestrial organisms need oxygen 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 and other highly organic inputs, lower rates of photosynthesis, and diffusion from the atmosphere due to ice cover can lead to decreased oxygen levels.

As the temperature of water decreases, a greater concentration of oxygen is able to dissolve in the water. 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. As water temperature decreases, more oxygen can be dissolved. 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, including fish.

Monitoring Results

a) How have dissolved oxygen levels changed over 15 years of monitoring on the main Annapolis River?

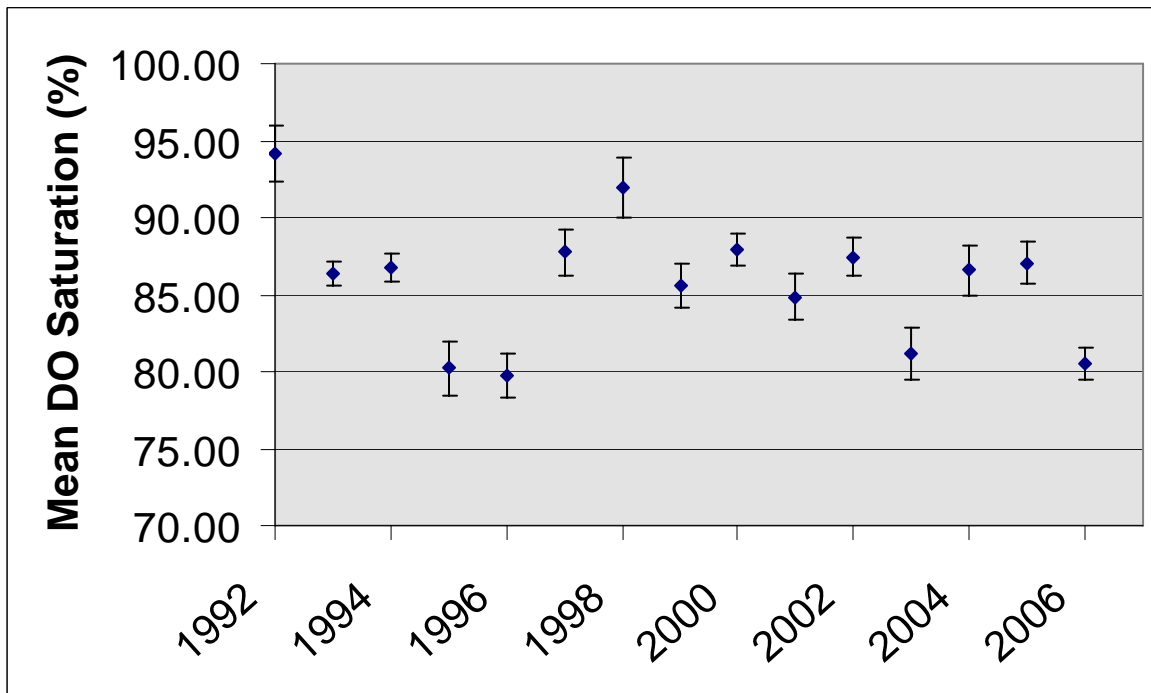


Figure 8. Mean Dissolved Oxygen Saturation (DO SAT) by year, 1992 to 2006 (showing standard error of the mean).

Figure 8 shows that during the period of 1992 to 2006, annual mean dissolved oxygen (percent saturation) levels have varied from a high of 94.2% in 1992, to a low of 79.7% in 1996. For the values recorded during 2006, the mean

issolved oxygen saturation was 80.5%. While this value is lower than that observed in recent years, it is still within the normal range of variability. The standard error of the mean is shown with error bars.

b) How do dissolved oxygen levels differ between each of the main river sampling sites?

Figure 9 presents the 14-year mean dissolved oxygen (percent saturation) values for each of the main river monitoring sites. The standard error of this mean is shown with error bars. This is overlaid with the mean values for the 2006 monitoring season. From these data it is evident that DO values in 2006 were significantly below the long term average for the river. There does not appear to be a significant increasing or decreasing trend for any of the sites. A 14-year mean for Middleton is not available, as dissolved oxygen monitoring was not conducted at this site during 1995, 1996, and 1997. The reason for the lower mean DO levels observed in 2006 is not known.

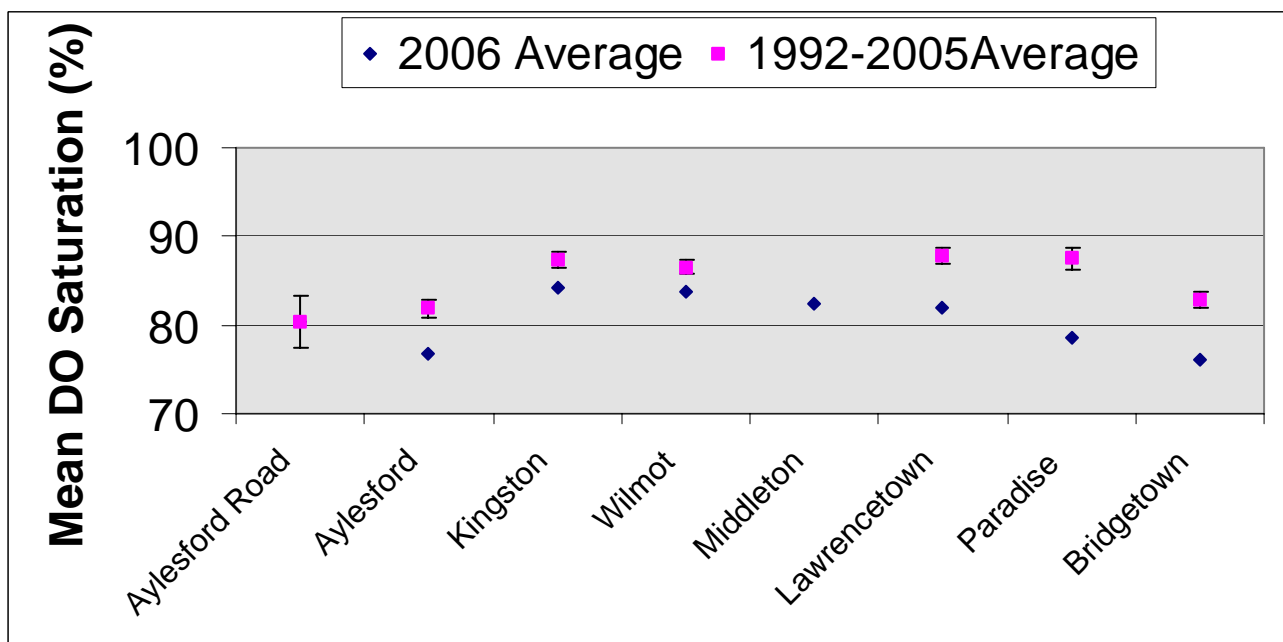


Figure 9. Mean Dissolved Oxygen Saturation (DO SAT) by sampling site, 1992 to 2005 means showing standard error of the mean.

c) Which River Guardian monitoring sites experienced low dissolved oxygen levels in 2006?

As is indicated in Figure 9 and Table 7 above, dissolved oxygen levels were observed below 60% saturation on three occasions, at Bridgetown, Aylesford Road and Aylesford. It is unclear why Paradise had only five samples with dissolved oxygen saturation above 75%. Low DO levels observed at Bridgetown in 2004 and 2005 prompted a more in-depth study of the lower Annapolis River in 2006. The results of this investigation are reported separately (Report on Low Dissolved Oxygen Levels Observed in the Upper Estuary of the Annapolis River, CARP, 2007).

The Canadian Water Quality Guideline for the Protection of Freshwater Aquatic Life for Dissolved Oxygen is 5.5 mg/L (CCME 2002). Only two of the ninety-nine water samples analyzed by the Annapolis River Guardians in 2006 had a dissolved oxygen level below this guideline level (Bridgetown, October 1 2.8 mg/L; Aylesford Road, July 24, 4.4 mg/L).

Table 7. Dissolved Oxygen Percent Saturation (DOSAT) Thresholds for Annapolis River.

Site	Number of Samples Collected in 2006	Number of Samples with DOSAT below 60%	Number of Samples with DOSAT below 75%	Number of Samples with DOSAT above 75%
Aylesford Road	15	1	3	12
Aylesford	14	1	5	9
Kingston	10	0	2	8
Wilmot	13	0	2	11
Middleton	12	0	1	11
Lawrencetown	15	0	0	15
Paradise	8	0	3	5
Bridgetown	12	1	2	10

Recommendations

- Continue regular River Guardian DO monitoring program at eight main river sample locations.

Additional recommendations concerning monitoring oxygen levels in the Annapolis River estuary are contained in the above noted report.

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 with prolonged exposures to temperatures over 24°C (MacMillan *et al* 2003).

Annapolis River Guardian Monitoring Results

The mean summer water temperature for the Annapolis River in 2006 was 19.1°C or 0.9°C cooler than for the same period in 2005. As in previous years, water temperatures during 2006 continued to reach levels stressful to aquatic life regularly during the summer months. Figure 10 presents the mean summer water temperature (July, August, September) by year for all the mainstem monitoring sites. Figure 10 also includes the 1993 to 2006 mean summer water temperature (18.7 °C).

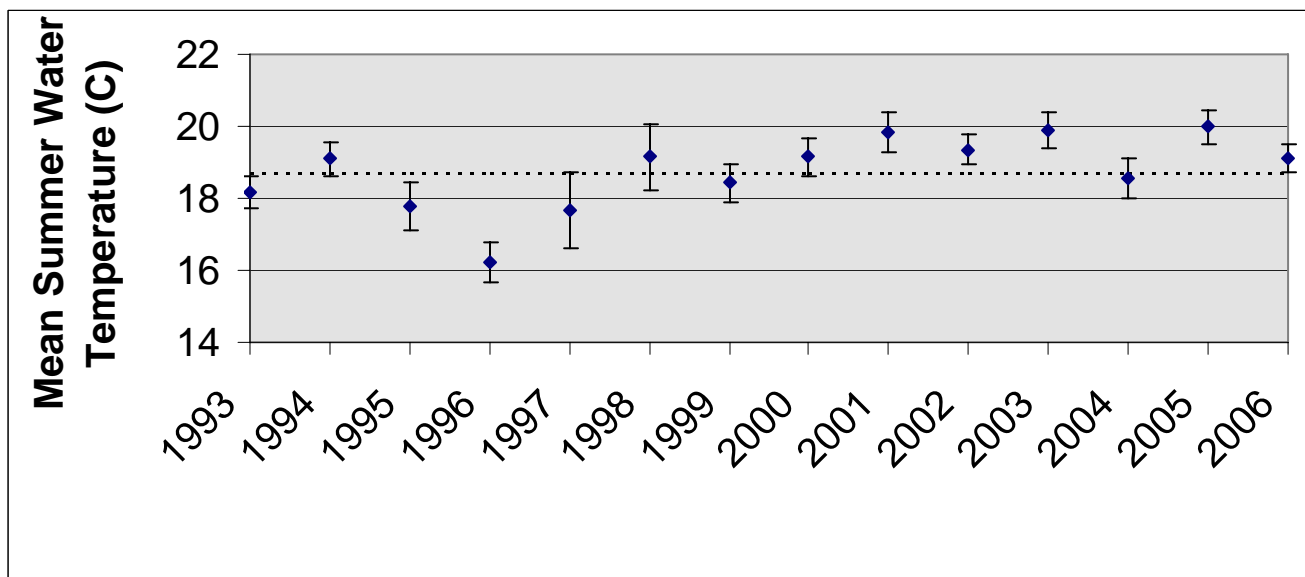


Figure 10. Mean Summer Water Temperature by Year (showing standard error of the mean) with 1993-2006 mean shown as dashed line.

The data from the 2003 and 2004 River Guardians annual reports suggested a gradual increase in temperature in the lower river sites, particularly in the summer data. Figure 11 presents the mean summer water temperature along the main Annapolis River in 2006, indicating that this spatial trend was evident during 2006. Of the 39 temperature measurements recorded during the months of July, August and September in 2006, approximately half (49%) exceeded 20°C. The maximum temperature observed was 23.4°C, recorded at Bridgetown on July 9, 2006.

It is unclear if the increase in temperature of water in the Annapolis River between Aylesford and Lawrencetown is due to direct warming of water within the main stem, or inputs of water from tributaries. Between Aylesford and Lawrencetown, a number of major tributaries (South Annapolis, Fales and Nictaux) join the Annapolis River, along with many small tributaries. Limited temperature data exists for these tributaries.

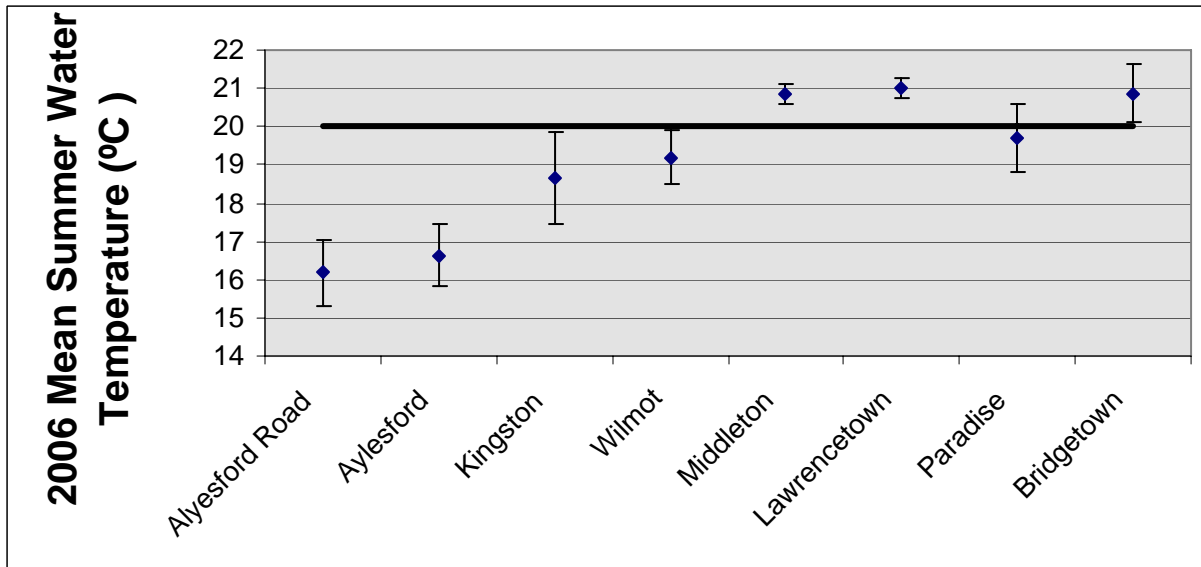


Figure 11. Mean 2006 Summer Water Temperatures by Site, with standard error of the mean and 20 °C threshold.

Thermal Status Monitoring

CARP has an on-going program to investigate the thermal status of tributaries within the Annapolis watershed. The purpose of this monitoring is to identify temperature trends and watercourses that may be suitable for fish habitat improvements. During 2006, the thermal status of two tributaries of the Moose River were examined to assess their suitability for fish habitat improvements. Temperature measurements were made by placing pre-programmed temperature data loggers within the watercourses during summer and autumn. Table 8 describes the location information for these placements.

Table 8. Placement Information for Temperature Data Loggers.

Tributary	Location of Data Logger Placement	UTM Easting	UTM Northing	Instrument Used	Sampling Interval
Moose River — East Branch	Powerlot Road Bridge	0295095 (Zone 20)	4948045	MiniLog-T; Number 4261	1 hr 00 min
Moose River — West Branch	Approx. 200 m below Quarry Road Bridge, at site of former fish hatchery	02996711 (Zone 20)	4945177	MiniLog-T; Number 4264	1 hr 00 min

The framework for assessing water temperature data developed by MacMillan *et al* (2005) was used to assess the temperature monitoring results. MacMillan *et al*. found that the number of trout in a system was directly related to the amount of cool water habitat available in the summer. Summer average water temperatures were used to rank sites into three categories: cool, intermediate and warm. Cool water sites had a summer average temperature of less than 16.5°C. Intermediate sites had a summer average water temperature between 16.5 and 19.0 °C. Warm sites had a summer average temperature greater than 19.0°C. Table 9 summarizes the 2006 results for the East and West Branches of Moose River. The data loggers were deployed on July 19 and retrieved on November 2, 2006.

Table 9. Thermal Status Monitoring Results.

Tributary	Number of Days Cool (<16.5°C)	Number of Days Intermediate (16.5 to 19°C)	Number of Days Warm (>19°C)	20 July to 5 Sept. Daily Average (°C)	Warmest Daily Average (°C)	Number of Days >20°C (daily average)
Moose River – East Branch	21	17	10	16.7	20.2 (July 29)	1
Moose River – West Branch	6	21	21	18.7	21.8 (July 20)	11

Based on the data collected from a temperature data logger that was deployed in West Branch of the Moose River in the summer of 2005, the river was ranked as Warm. While the 2006 deployment did not cover the full recommended period of 15 June to 5 September, the results clearly indicate that the East Branch of the Moose River is the cooler of the two. The average summer water temperature for the West Branch in 2006 (18.7°C) was very similar to that observed in 2005 (19.1°C). These results would indicate that the East Branch is the preferred branch for fish habitat improvements, based on water temperature. Additional monitoring of the headwaters of the West Branch of the Moose River may be warranted to assess if cold water habitat exists.

Recommendations

- Continue regular River Guardian temperature monitoring program at eight main river locations.
- 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.
- Install temperature loggers in candidate streams to assess for fish habitat improvements.
- Temperature data loggers be calibrated immediately prior to deployment and at least once *in situ*. These procedures should be added to the QA/QC Project Plan.

pH and Conductivity

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, zero being the most acidic. Because the scale is logarithmic, 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 negatively 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 and conductivity were measured on the Monday 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

Table 10 shows that pH values all along the Annapolis River are generally good, being only very slightly acidic. In total, 116 individual pH measurements were made during 2006. The pH values are consistently well within the range recommended by the CCME for the protection of freshwater aquatic life. A number of the principal tributaries of the Annapolis River pass through the Torbrook formation that buffers rivers and streams in the watershed from acidification.

Table 10. Mean pH Values at Each River Guardian Monitoring Site, 2006.

Site	Mean pH	Standard Deviation
AY40-Aylesford Road	6.80	0.35
00-Aylesford	6.91	0.26
13-Kingston	6.92	0.20
18-Wilmot	6.93	0.27
25-Middleton	6.81	0.19
35-Lawrencetown	6.68	0.17
40-Paradise	6.83	0.20
49-Bridgetown	6.89	0.27

pH data collected from eight main river sites for 2003 to 2006, using the Quanta Hydrolab meter, are presented below (Figure 12). From the plot, it is apparent that pH levels were lower in 2005. 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 6.95, based on 620 individual measurements. This historic pH is similar to that observed in 2003 and 2004, but appears to differ significantly from that in 2005. pH levels in the Annapolis River in 2006 returned to more typical levels.

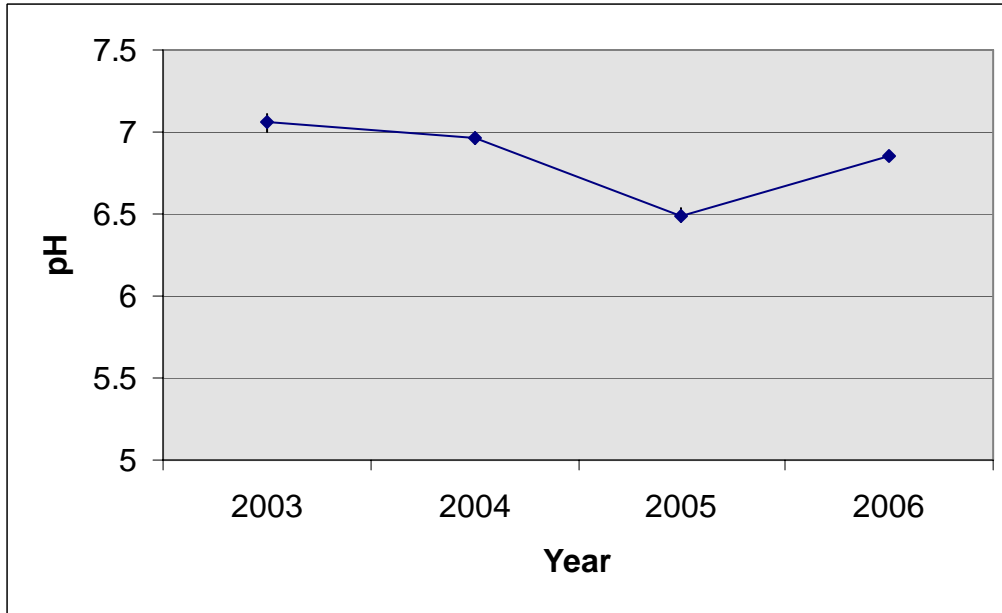


Figure 12. pH measurements from Annapolis River, 2003 to 2006, with standard error of mean.

Recommendations

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

Nutrients (Nitrogen and Phosphorus)

Introduction

Elevated levels of nitrate in aquatic systems can originate from a variety of sources, including domestic on-site and municipal wastewater discharges, the use of chemical fertilizers and manure on urban and agricultural land, industrial discharges, and atmospheric deposition. Nitrogen concentrations in water can be reported in a number of forms, including: nitrate, nitrate + nitrite, ammonia and dissolved inorganic nitrogen (DIN).

As part of the investigation of low dissolved oxygen levels in the lower Annapolis River in 2006, nutrient samples were collected. A total of 15 samples were collected in the Bridgetown area during 2006. These results are presented in Table 11 and Figure 13.

Table 11. Nutrient Analysis Results for lower Annapolis River.

	Nitrate (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)	DIN (mg/L)	Phosphate (mg/L)
Mean	1.13	0.01	0.063	1.21	0.029
Median	1.04	0.01	0.023	1.07	0.029
Minimum	0.81	0.01	0.000	0.88	0.010
Maximum	1.70	0.02	0.283	1.73	0.068

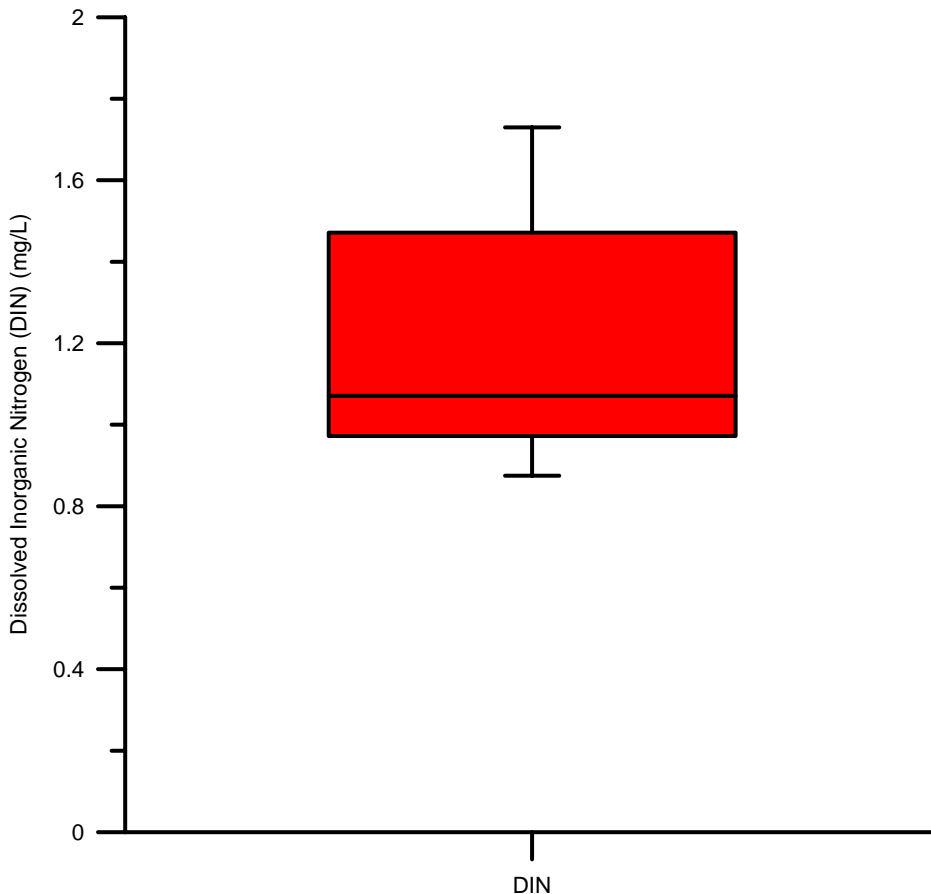


Figure 13. Dissolved Inorganic Nitrogen – Lower Annapolis River (n = 15).

In a survey of 11 mainland Nova Scotia Rivers, Dalziel *et al* (1998) found the Annapolis River to have the highest silica levels, as well as elevated nitrate concentrations. These and other historic nitrogen monitoring results for the Annapolis River are shown in Table 12.

Table 12. Historic Nitrogen Levels in the Annapolis River.

Period	Number of Samples		Total Nitrogen	Nitrate + Nitrite-N	Ammonia	Source
			(mg/L)	(mg/L)	(mg/L)	
1992 to 1996	14	Mean		0.519	0.030	Dalziel et al, 1998 ¹
		Max		1.466	0.060	
		Min		0.151	0.012	
2004	2	Mean	0.74			CABIN, 2004 ²
2005	6	Mean	0.60			CABIN, 2005 ³

Dodds and Welch (2000) have suggested Total Nitrogen limits between 0.25 and 3.0 mg/L and Dissolved Inorganic Nitrogen limits between 0.02 and 1.0 mg/L in order to limit excessive chlorophyll, periphyton and macrophyte growth in fresh water systems. The nitrogen levels observed in the Annapolis River in 2006 are on the upper end of these ranges. From these results, the DIN to Phosphate ratio is 72, indicating that phosphorus may be limited.

Phosphorus is an essential nutrient required by plants and animals, with phosphorus-containing organic compounds found in all living matter. Orthophosphate (PO_4^{3-}) is the only form readily used as a nutrient by plants and organisms. Because of its low dissolved concentrations, phosphorus can be the limiting nutrient in fresh waters. Elevated phosphorus levels in surface waters can lead to algal blooms (Ironside 2001).

While phosphorus is a naturally occurring element in rocks and soils, anthropogenic sources are the predominant cause of elevated concentrations leading to impaired water quality. Anthropogenic sources include human and animal waste, atmospheric inputs, industrial waste and artificial fertilizers. A total phosphorus concentration of 0.030 mg/L is a recommended water quality guideline to avoid excessive plant growth in rivers and streams (OMEE 1994). Total phosphorus levels in excess of 0.030 mg/L are an indicator of eutrophic surface waters (Mackie 2001). Dodds and Welch (2000) have suggested Total Phosphorus limits of 0.002 to 0.07 mg/L in order to limit excessive chlorophyll, periphyton and macrophyte growth in fresh water systems. The results from phosphate monitoring on the lower Annapolis River in 2006 are presented in Figure 14. Historic phosphorus monitoring results for the Annapolis River are shown in Table 13.

¹ Dalziel, J.A., P.A. Yeats and B.P. Amirault. 1998. Inorganic Chemical Analysis of Major Rivers Flowing Into The Bay Of Fundy, Scotian Shelf and Bras D'Or Lakes. Canadian Technical Report of Fisheries and Aquatic Sciences 2226. Science Branch, Department of Fisheries and Oceans, Dartmouth. Samples collected at Paradise.

² CABIN, 2004 – Canadian Aquatic Biomonitoring Network protocol. Samples collected by CARP staff at Aylesford and Kingston on Annapolis River (18/10/04 and 20/10/04, respectively).

³ CABIN, 2005 – Canadian Aquatic Biomonitoring Network protocol. Samples collected by CARP staff at Aylesford, Kingston and Paradise on Annapolis River (Samples collected 13/9/05 and 14/9/05).

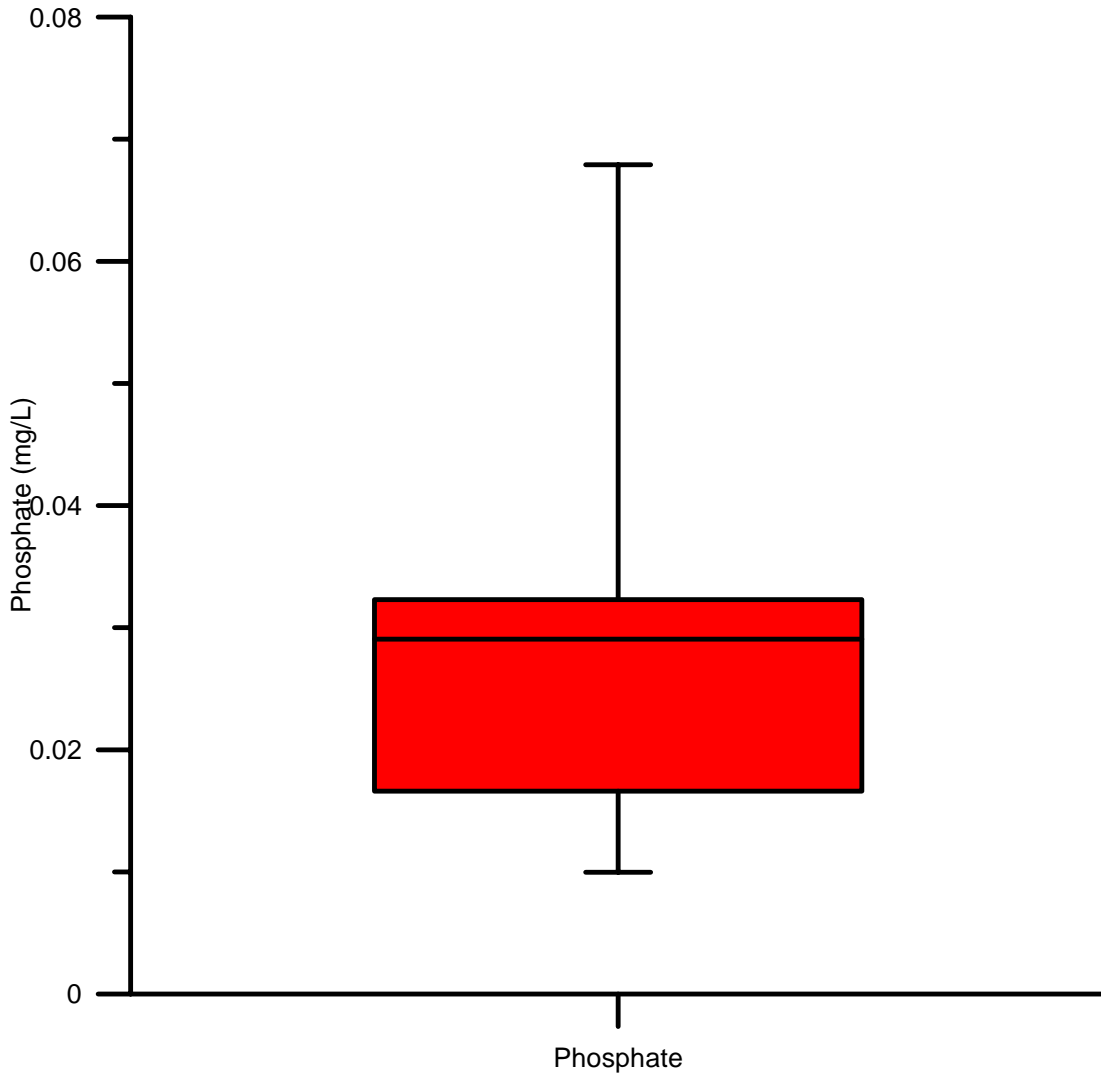


Figure 14. Phosphate results for Lower Annapolis River (n = 15).

Table 13. Historic Phosphorus Levels in the Annapolis River.

Period	Number of Samples		Phosphate-P (mg/L)	Total Phosphorus (mg/L)	Source
1992 to 1996	14	Mean	0.017		Dalziel et al, 1998 ¹
		Max	0.032		
		Min	0.007		
2004	2	Mean		0.055	CABIN, 2004 ²
2005	6	Mean		0.087	CABIN, 2005 ⁴
		Max		0.142	
		Min		0.043	

Of the 15 phosphorus samples collected in 2006, 8 (53%) were at or above the 0.030 mg/L guideline level. This compares with 10 samples (67%) exceeding the limit in 2005.

Recommendations

- Conduct regular nutrient monitoring along the Annapolis River for nitrogen and phosphorus.

Conclusions

E.coli bacteria levels along the Annapolis River during 2006 were significantly higher than those observed in 2005. E.coli counts greater than 1000 cfu/100 ml were observed at all sites at least once in 2006. Elevated E.coli levels occurred predominantly during the months of June, July and October, which had precipitation levels significantly above the monthly averages. Of the 119 E.coli bacteria samples collected and analyzed, 46% (55) exceeded the contact water recreation guideline of 200 cfu/100ml. In 2004, 37% exceeded this threshold.

Over 15 years of monitoring, mean dissolved oxygen saturation (DOSAT) levels have remained in the range of 80-94%. In 2006, the mean DOSAT level was 80.5%. The mean 2006 DOSAT for all sites was below the 1992-2005 averages for the sites. As a result of the regular monitoring provided by the Annapolis River Guardian program, low DO levels were observed in the lower river. This prompted a more in-depth examination, which is reported separately.

The mean summer water temperature for the Annapolis River during 2006 was 19.1°C or 0.9°C cooler than for the same period in 2005. As in previous years, water temperatures during 2006 continued to reach levels stressful to aquatic life regularly during the summer months. Deployment of temperature data loggers in the East and West Branches of Moose River confirmed 2005 results that the West Branch may not be suitable for cold water fish habitat improvements, due to elevated summer temperatures.

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 6.81 and 6.93. Although slightly lower pH values were observed in 2005, the 2006 results are more typical of those seen in 2003 and 2004.

A limited number of nutrient samples (n = 15) were collected from the lower Annapolis River in 2006 in the area of Bridgetown. Median concentrations of dissolved inorganic nitrogen (1.07 mg/L) and phosphate (0.029 mg/L) were observed. Nutrient concentrations in this magnitude are sufficient to pose a risk of eutrophication.

Recommendations

Recommendations for the River Guardians Program

- Continue regular River Guardian E.coli monitoring at the eight main river sample locations.
- Conduct simultaneous monitoring at Sites 00 and AY40, together with intervening tributary streams.
- Conduct a foot survey of the Annapolis River between these two sites, and the intervening tributary streams, to identify possible contamination sources.
- Review current and historic air photos of this area to identify land use changes and possible sources of contamination.
- Continue regular River Guardian DO monitoring program at eight main river sample locations.
- 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.
- Continue regular River Guardian temperature monitoring program at eight main river locations.
- Install temperature loggers in candidate streams to assess for fish habitat improvements.
- Temperature data loggers be calibrated immediately prior to deployment and at least once *in situ*. These procedures be added to the QA/QC Project Plan.
- Regular pH monitoring should be continued at the eight Annapolis River Guardian monitoring locations.

Recommendations for CARP

- Continue supporting Microbial Source Tracking (MST) methods in order to more effectively identify and remediate the sources of fecal contamination in the Annapolis River.
- Complete the Quality Assurance Project Plan for all of CARP's Water Quality monitoring programs.
- Complete the benthic invertebrate survey of the mainstem Annapolis River to help identify some of the major sources of contamination.

Additional Recommendations (carried forward from previous years)

- Examine the relation between observed increases in water temperature to air temperature data to determine if increases are due to climate trends, riparian changes, or other factors that may be influencing flow patterns.
- Examine in further detail the water temperature data to determine whether any statistically significant trends are occurring.
- Given the high contributions of fecal coliforms observed in 2004 from the Fales River, conduct further investigations on this tributary (i.e.: monitoring upstream/downstream of suspected point sources) to gain a better understanding of the sources of fecal coliforms.
- Examine the relationship between fecal coliform levels at each site over 5-10 years and the precipitation data in order to better understand the influence from different sources (i.e.: surface vs. on-site sources).

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Appendices

Appendix A – Parameters Tested and Methodologies

Parameters Analyzed in 2006	Additional Parameters Analyzed in Previous Years of the Program
E.coli bacteria densities	Salinity
Dissolved Oxygen	Chlorophyll a
Temperature (Water and Air)	Total Suspended Solids (TSS)
Weather conditions	Colour
pH, Conductivity, Total Dissolved Solids	Transparency
Nitrate, Nitrite, Ammonia, Phosphate	Nitrate-N, Chloride, Sulphate, Total Phosphate

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, 2005 and 2006 seasons. The sample collection unit is shown in Figure A1.



Figure A1. Collection Unit Used for Fecal Coliform Samples in 2006.

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 the two 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.

All fecal bacteria samples were submitted to the Synova Diagnostics laboratory in Lawrencetown, Nova Scotia. The Synova lab is accredited by the Canadian Association for Environmental Analytical Laboratories (CAEAL) to perform the Most Probable Number (MPN) (*E.coli*) procedure. From 1997 to 2003 and again in 2005, fecal bacteria densities were determined using the IDEXX Colilert procedure, to give a Most Probable Number of *E. coli* bacteria present.

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.

Temperature

The Annapolis River Guardians used a combination of glass/alcohol and digital thermometers during 2006. 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 °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, Conductivity

Water chemistry data including pH and conductivity was collected using CARP's portable HydroLab Quanta Water Quality Monitoring System. Data was collected on a fortnightly basis by CARP staff, typically the Monday 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 two to three minutes. 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 was also stored in 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).

Appendix B – Sites Monitored

Water samples were collected during 2006 by the Annapolis River Guardians program at the following sites. Coordinates are reported in Universe Transverse Mercator (Zone 20) and Latitude/Longitude, as recorded on a hand-held GPS unit.

<u>SITE</u>	<u>LOCATION</u>	<u>Easting</u>	<u>Northing</u>
AY40	Aylesford, Aylesford Road, bridge	357328.48	4987755.13
00	Aylesford, Victoria Road, bridge at the Post Office	353313.34	4985418.70
13	Kingston, Bridge Street, bridge	346748.46	4982480.39
18	Wilmot, Old Mill Road, bridge	342100*	4979500*
25	Middleton, Highway 10, bridge	336981.58	4978044.59
35	Lawrencetown, Lawrencetown Lane, bridge	329581.15	4971984.70
40	Paradise, Paradise Lane, bridge	325738.51	4970620.51
49	Bridgetown, Queen Street, bridge	318900.00	4967621.30

* coordinates determined from 1:50,000 map sheet

Appendix C – Quality Assurance / Quality Control Data

Introduction

Following a contamination event in 2003, the Clean Annapolis River Project 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. A refresher session was held for all volunteers on April 22, 2006 at Middleton High School. Dr. Mike Brylinsky, Acadia University, and CARP staff conducted the session. During the 2005 season, CARP staff conducted visits with all seven 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-nine QA/QC samples were collected during the 2006 season. These were, in summary:

- 8 Dissolved Oxygen split samples
- 7 E.coli travel blanks
- 5 E.coli field blanks
- 9 E.coli split samples

Background

For the purposes of CARP's water quality monitoring programs, a blank sample is one filled with water 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. Over the 2006 season, two different types of blanks were collected: travel blanks and field blanks.

- 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 and 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 (ie: operator, equipment, distilled water, etc).

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 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 Synova'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.

$$Accuracy = \text{Test/Average value} - \text{True Value}$$

Dissolved Oxygen

Dissolved oxygen split samples were taken in 2006 using a single volume of water from a Van Dorn sampler. The accuracy of volunteer DO measurements was assessed through the collection of eight split samples, one from each of the volunteers. The Winkler Titration 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 shown below in Table C1, show that the volunteers attain an average accuracy of +/- 0.60 mg/L (RPD = 6%). For comparison purposes, the average DO accuracy during 2005 was +/- 0.32 mg/L.

Table C1. Volunteers’ level of accuracy at Measuring Dissolved Oxygen Using the Winkler Titration.

Site	Date	Volunteer Result (mg/L)	True Result* (mg/L)	Accuracy +/- (mg/L)	Relative Percent Difference
49	23-Jul-06	7.20	7.39	0.2	2.6
40	23-Jul-06	6.90	7.10	0.2	2.9
00	05-Sep-06	7.56	8.04	0.5	6.2
13	01-Oct-06	10.20	10.82	0.6	5.9
00	01-Oct-06	8.06	8.93	0.9	10.2
18	01-Oct-06	10.0	11.09	1.1	10.3
25	29-Oct-06	9.80	10.53	0.7	7.2
35	29-Oct-06	9.86	10.39	0.5	5.2
			Mean	0.6	6.3

* The ‘True’ DO value was determined by calculating an average of three Winkler titrations, performed by CARP staff.

E.coli Bacteria

Throughout the sampling season, a series of blank samples were submitted blind for analysis at the Synova laboratory. The seven travel blanks analysed had coliform counts of 0 cfu/100ml, indicating that there is no cross contamination between samples while they are being transported. Five field blanks collected also showed no E.coli growth, indicating that the fecal bacteria sample collection procedure is not contaminating the samples.

Throughout the 2006 sampling season, a total of nine split samples were collected during the sampling visits with the volunteers. These samples were submitted to the Synova laboratory under a fictitious sample identification number. The purpose of this was to assess the reproducibility of the E.coli MPN analysis method used at Synova. The results of this are presented in Table C2. The mean RPD for these split samples was found to be 15.5%. The mean RPD for the 2005 season was 14.2%.

Table E2. Relative Percent Difference in Duplicate Samples Analysed for Fecal Coliforms.

Site	Date	Volunteer Result E.coli MPN (cfu/100 ml)	QA/QC Result E.coli MPN (cfu/100 ml)	Relative Percent Difference (RPD)
AY40	05-Sep-06	365	365	0.0
13	01-Oct-06	162	225	32.6
00	01-Oct-06	>2420	>2420	N/A
18	01-Oct-06	155	140	10.2
25	29-Oct-06	1553	1733	11.0
35	29-Oct-06	1986	>2420	N/A
25	05-Jun-26	192	167	13.9
49	23-Jul-06	1553	1203	25.4
40	23-Jul-06	2420	>2420	N/A
			Mean	15.5