Assessing Risk Associated with Waterborne Parasites in Calgary's Drinking Water

by

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Abstract

Giardia and *Cryptosporidium* are waterborne pathogens that are raising public health concern worldwide. Outbreaks caused by *Giardia* or *Cryptosporidium* have been reported even after drinking water facilities have met regulatory compliance. The goal of this thesis was to examine vulnerability of the City of Calgary's drinking water to parasite contamination and assess the risks posed by these parasites based on three different risk frameworks: 1) Alberta Environment and Sustainable Resources Development (AESRD) regulatory approval requirements, 2) United States Environmental Protection Agency's (U.S. EPA) Long-term 2 Enhanced Surface Water Treatment Rule (LT2 Rule), and Health Canada's Quantitative Microbial Risk Assessment models [HC QMRA]). Parasite monitoring data was collected from 2003 to 2011 at the Glenmore and Bearspaw water treatment plants (WTPs) in the City of Calgary (428 and 408 data points, respectively). Drinking water quality met all regulatory requirements for parasite risks regardless of the risk models used. However, the overall level of risk varied depending on the models used and the assumptions in certain models (i.e., HC QMRA), and in particular the risks associated with *Giardia*. AESRD's regulation requires that, for example, the Glenmore WTP should provide 5-$\log_{10}$ reduction against *Giardia* based on the current concentrations of parasites in source water. The Health Canada QMRA model suggested that the Glenmore WTP could handle 114,000 *Giardia* cysts/100 L. However, each of the risk frameworks lacked resolution for identifying potential periods of peak risk. An association between *Giardia* concentration and season was observed in source water for the Elbow River (winter/spring) and Bow River

(winter/spring [2003-2007] and summer in the Bow River [2008-2011]). Environmental factors such as rain and snowmelt run-off were shown to correlate with *Giardia* occurrence and could be used to predict peak occurrence/risk periods associated with source water contamination with this parasite.

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List of Symbols, Abbreviations and Nomenclature

R^2	Regression coefficient
ID_{50}	a dose of microorganisms required to produce infection in 50 % of exposed subjects
AESRD	Environment and Sustainable Resources Development
CCME	Canadian Council Ministers of Environment
CI	Confidence interval
cm	centimetre
Ct	Concentration (mg/L) and contact time (h)
DALY	Disability Adjusted Life Years
DBP	Disinfection by-product
df	Degree of freedom
HC QMRA	Health Canada Quantitative Microbial Risk Assessment
HIV	Human Immune-deficiency virus
ICR	Information Collection Rule
KWR	KWR Watercycle Research Institute, Nieuwegein, The Netherlands
L	Litres
LT2 Rule	Long Term 2 Enhanced Surface Water Treatment Rule
Max-RAA	Maximum running annual average
mm	millimetre
MSR	Matrix spike recovery
NTU	Nephelometric turbidity unit
OPR	On-going precision recovery
PWS	Public water system
QC	Quality control
r	Pearson regression coefficient
RIVM	National Institute of Public Health and Environment (The Netherlands)
RR60DRA	Rain run-off 60 days running average

STD	Standard deviation
SMR60DRA	Snowmelt run-off 60 days running average
STATA™	Statistical software (STATA Corp LP™)
t	t-statistic
U.S. EPA	United States Environmental Protection Agency
US	United States
UV	Ultra violet light
WHO	World Health Organization
WSP	Water safety plan
WTP	Water treatment plant

Chapter 1 : Literature review

1.1 Introduction

Water is essential for life. History has documented the deadly consequences of waterborne diseases outbreaks. *Vibrio cholerae, Shigella* spp., *Salmonella enterica*, and various other waterborne pathogens have claimed millions of human lives in the past (Ferrie, 2012; Davey Smith, 2002; Lee *et al.*, 2013). In modern times, mortality due to waterborne diseases claims some 20 million lives annually in the developing countries (WHO, 2009) and despite improved sanitation and drinking water treatment in developed countries, morbidity due waterborne infections is still prominent and costly (Hrudey & Hrudey, 2007; Craun *et al.*, 2006; Lippy & Waltrip, 1984; Smith *et al.,* 2006; Corso *et al.,* 2003). For instance, a large outbreak of cryptosporidiosis was recorded in Milwaukee in 1993 where approximately 400,000 people were infected with the parasite, *Cryptosporidium hominis* and was reported to have resulted in over 4,000 hospitalizations and over 40 deaths (Mackenzie *et al.*, 1994; Vakil *et al.*, 1996).

Water treatment is considered the fourth greatest engineering achievement of the 20th century (National Academy of Engineering 2000). Municipal water treatment was considered a breakthrough to achieving higher standards of microbiological safety and an effective measure for drinking water supply for urban type settlements. Municipal water treatment has dramatically decreased waterborne disease occurrence due to improving drinking water sanitation (Water Quality and Health Council, 2014).

Supplying safe drinking water is a vital component for a prosperous modern society. At the same time industrial-level water treatment centralizes the supply of drinking water, and consequently, failures in treatment can exacerbate the sudden spread of infection if the water is contaminated with microbial hazards. Health of an entire community may be threatened as in the case of the Milwaukee outbreak highlighted above.

1.2 Environmental health risk assessment

Intuitive risk assessment has been fundamental for human survival and evolution. Individuals who recognized risk were able to survive and reproduce whereas those who could not were more likely to perish from environmental hazards (Thomas & Hrudey, 1997). In considering risk, particularly in the context of prediction or expectation, Thomas & Hrudey (1997) argue that there are certain elements that comprise the basic risk paradigm:

- a source of danger must exist
- an uncertainty of occurrence and outcome associated with this danger
- possible adverse health outcome
- a target for the adverse health outcome
- a time frame, and
- an evaluation of the importance of the risk for people affected by it.

Risk assessment provides a systematic approach for characterizing the nature and magnitude of the risks associated with environmental health hazards (Merkhoher, 1993; Commonwealth of Australia, 2002). Accordingly, most environmental risk assessments

(including microbial risk assessments), follow a general framework of which the following 5 elements are critical:

- **Hazard Identification** – characterizing the physical, chemical or biological hazards within an environment and determining the nature of the adverse health effects that might be caused by the agent and/or how quickly the problem might be experienced (Health Canada, 1999).
- **Exposure Assessment** - determining the frequency, magnitude, extent, duration and character of exposures to a hazard. Estimates can be made for former, existing and preferably future exposures. Exposed populations (particularly vulnerable groups within population) and potential exposure pathways should be identified. Environmental monitoring and predictive models can be used to estimate the amount of exposure at distinct points on the exposure pathways (Singer, 1994; Commonwealth of Australia, 2002).
- **Dose-response Assessment** - evaluates qualitative and quantitative information to estimate the incidence of adverse effects occurring in humans at different levels of exposure (U.S. EPA, 1989a). Microbiological dose-response assessment is challenged with the difficulty of considering biological variation in infectivity and virulence of the pathogen and status of the host. For example, the chance of acquiring an infection is influenced by factors such as host immune status and/or virulence of the specific type of infecting microbe (Commonwealth of Australia, 2002).

- **Risk Characterization -** incorporates the prior three steps into a logic-based framework in order to the determine plausibility and potential severity/incidence of adverse effects, including uncertainties and assumptions that may affect risk outcomes/interpretation and consequently the risk management actions that may be taken.
- **Risk Management –** the process of establishing risk mitigating measures to prevent or minimize adverse health effects associated with exposure to the hazard, including the development of science-based regulations, and incorporating estimates of uncertainty (Kolluru & Stricoff, 1996).

The level of risk can be described either quantitatively (using point estimates or probability density distribution) or qualitatively (i.e., using evaluative risk categories such as “high”, “medium” or “low”). Providing accurate quantitative estimates of risk from low levels of exposure to any environmental hazard is a very difficult task using current risk assessment methods (Commonwealth of Australia, 2002). Every opportunity should be used to obtain the most precise and accurate quantification estimates during exposure assessment, however, the consequences (e.g. uncertainties) of testing limitations should be stated explicitly. For example, in the context of quantitative microbial risk assessment (QMRA) it is impossible to give certain numerical value to the absolute concentrations of pathogens in every liter of drinking water consumed by every person in a population. Consequently, risk assessment is based on probabilities rather than absolutes, and an understanding of the distribution of risk is also

important and should be reflected in the decision making process. A distinction should be made between variability and uncertainty (Schmidt & Emelko, 2011). Variability can exist as differences in temporal and/or spatial concentration (Emelko *et al.*, 2010). Statistical methods can be used to deal with variability. However, statistics can be less helpful with the true uncertainty, such as inadequate knowledge, systematic errors (e.g. deficiency of analytical methods), model uncertainty, and decision-rule uncertainty. Systematic errors, if identified, can be characterized by their direction (e.g., overestimation/ underestimation). For example, analytical methods often underestimate concentrations of both *Giardia* and *Cryptosporidium* in water because of poor (oo)cyst recovery if recovery correction is not applied (Schmidt & Emelko, 2011; Krometis *et al.*, 2009). Uncertainty often arises because of lack of data or bias in study design. Monte Carlo simulation has increasingly been used to address variability and/or uncertainty in many microbial risk assessment approaches (Signor & Ashbolt, 2006; Haas *et al.*, 1993, 1999; Smeets *et al.*, 2007; Jaidi *et al.*, 2009; Cummins *et al.*, 2010; Medema *et al.*, 2003; Pouillot *et al.*, 2004; Gale, 1998; Teunis & Havelaar, 1999; Masago *et al.*, 2002). Uncertainty is introduced by any factor that deviates or affects our estimates about risk. Consequently, there is room for inappropriate management actions if this deviation is large (Schmidt *et al.*, 2013).

Risk assessment has become a tool in the decision-making process, and its importance is increasing as it has become evident that situations cannot be judged simply as either “safe” or “unsafe” (Payment & Pintar, 2006; Commonwealth of Australia, 2002). Risk

assessment is intended to provide a credible, objective, realistic and balanced analysis (U.S. EPA, 1992), ensuring complete information to risk managers, specifically policymakers and regulators, so that the best decisions are made (Paustenbach, 1989).

1.3 Application of QMRA to protection of drinking water

Risk-based approaches are essential for the effective management of drinking water systems (CCME, 2004). The consumption of contaminated water is considered the most relevant source of exposure to waterborne pathogens, and accordingly, it draws major attention in water-related health risk assessments (Ashbolt, 2004). Organisms of particular waterborne risk include bacteria, protozoa, toxic blue-green algae (cyanobacteria), viruses and helminths. These microbiological hazards generally pose an acute risk and even sporadic violations of guideline levels can potentially pose an increased danger for waterborne disease transmission (Commonwealth of Australia, 2002); hence current compliance monitoring may well miss such events (e.g. Signor & Ashbolt, 2006). Real time microbiological monitoring currently remains impossible, thus, drinking water process controls (i.e., turbidity, chlorine contact times [Ct]) are often used to monitor water treatment performance as surrogates for acceptable microbiological control (Hijnen & Medema, 2010). These treatment-based standards of performance have, historically, been the frameworks used in the derivation of drinking water regulations nationally and internationally and are grounded upon water treatment technology performance against pathogens, and for which implementation of the technology is amenable to small or large-scale drinking water treatment systems. More recently,

countries such as Australia, the Netherlands, the United States of America (U.S.) and Canada, as well as agencies such as the World Health Organization (WHO), have evoked the use of health-based microbial risk assessments in derivation of regulatory compliance targets in the drinking water industry (NHMRCeNRMMC, 2011; VROM-Inspectorate, 2005; U.S. EPA, 2006; Health Canada, 2012; WHO, 2011). Health-based microbial risk assessments encompass treatment-based performance measures but also account for other key parameters of risk such as pathogen (or surrogate) occurrence in water, the likelihood of illness given the level of exposure, and an estimation of health impact associated with the exposure (i.e., morbidity and mortality as disability adjusted life years [DALY]) (WHO, 2006).

Indicators or surrogate parameters have been used for water quality monitoring to provide information on the likely presence of the agents of health concern (Commonwealth of Australia, 2002). The use of bacterial indicators as a measure of faecal pollution in water was originally based on the simple association that water was an important source of disease, and for which modern approaches to public health protection can be largely attributed to the efforts of John Snow during the cholera outbreaks in London during the 3rd pandemic (*circa* 1854) (Vachon, 2005). In 1892, Schardinger proposed *Escherichia [Bacterium] coli* as a microbial indicator of faecal pollution in water (Kornacki and Johnson, 2001). Consequently, *E. coli* and total coliforms have long been used as indicators of faecal-derived bacterial contamination in water (Edberg *et al.,* 2000). These microbes were chosen because of their commonality to the gut

microbiota of humans and other warm-blooded animals, and because of their relative ease of detection. The presence of indicator organisms in drinking water implies that water quality may have been compromised by faecal contamination, and by association, enteric pathogens may also be present. Interestingly, cases when pathogenic microorganisms are present and the indicator bacteria are absent abound in the literature (Hrudey & Hrudey, 2007). A study by Payment *et al.* (1991) demonstrated that the consumption of municipal drinking water, although causing gastroenteritis, met current guidelines for bacteriological drinking water quality. Additionally, certain total coliform bacteria are free living in the environment, and their ubiquitous distribution precludes the use of these microbes as clearly of faecal origin (WHO, 2004).

The waterborne protozoan parasites, *Cryptosporidium* and *Giardia*, have been recognized as major causative agents of enteric infection and with respect to the aetiology of waterborne diseases worldwide (Hunter *et al.,* 2010). These microorganisms are prevalent in humans, animals and the environment and are able to withstand relatively high doses of chemical disinfectants commonly used in water treatment (i.e., chlorine and monochloramine) (Hassan *et al.*, 2012; Smith *et al.*, 1989; Robertson *et al.*, 1992). Testing for *Cryptosporidium* and *Giardia* as reference protozoa have been included in a multi-barrier approach to ensure supply of safe drinking water from source, through treatment and distribution to customers, and are key pathogens in *quantitative microbial risk assessment* (QMRA) approaches, such as those adopted by Health Canada, as the

primary health-based targets for drinking water treatment in Canada (Health Canada, 2012).

Microbial risks to drinking water exist within the watershed, source water intakes, treatment process, distribution system and ultimately at the point of consumer consumption. Assessing system-level vulnerability of a public water system (PWS) to waterborne pathogens represents a multiple barrier approach to risk reduction - the concept of using more than one treatment process to control contamination so as to provide improved overall process reliability, redundancy, performance and water quality (Commonwealth of Australia, 2002). Hence, QMRA is increasingly being used to inform the management of drinking water systems regarding their vulnerability to microbial threats, which in Alberta occurs at a systems-level through drinking water safety plans (AESRD, 2014). Pathogen barriers include the following:

- Protection of a watershed from human pathogens;
- Retention times of protected source waters (i.e., open reservoirs) to facilitate particle removal by sedimentation and sunlight inactivation;
- Physical water treatment plant coagulation and filtration;
- Chemical (i.e., chlorine) or physical (i.e., ultraviolet light) disinfection before finished water enters the distribution system;
- Maintenance of an adequate disinfectant residual within the distribution system as well as physical integrity of the distribution system;

- Verification monitoring of microbiological parameters to check that the barriers are being maintained.

QMRA provides an estimation of the potential burden of illness associated with exposure to a particular microbe (or microbes), and how much uncertainty and variability exists within these estimates (Smeets *et al.*, 2010). Hence, QMRA can be used as a tool to aid in determining whether treatment is meeting a health-based target and to what degree of confidence there is in the estimate.

The benefit of using QMRA approach is that assessments can be performed by each water system to provide site-specific information related to:

- how changes in source water quality could influence microbiological risk from water that being produced;
- the appropriateness of existing (treatment) barriers, given site-specific variations;
- investigating potential improvements in microbiological drinking water quality with additional treatment barriers or upgrading existing treatment barriers/performance;
- help establish target limits for critical control points in the whole drinking water treatment system (Health Canada, 2010).

Concentrations of pathogens in source water and potential health risks are dynamic and change in real time. Current verification (compliance) test results for indicator bacteria may take up to a 72-hour from the time of sampling (Signor & Ashbolt, 2006). Management actions may well further delay the release of any notice, and human bias and errors can play a significant role in delivering

the final action (Hrudey, 2004). Of increasing recognition as the cause of waterborne outbreaks are drinking water distribution system intrusions (Craun *et al.*, 2010), and the growth of environmental pathogens in premise plumbing systems (Collier *et al.*, 2012). QMRA has also been applied to these distribution system issues to aid in their management (Teunis *et al.*, 2010; Schoen & Ashbolt, 2011).

In Canada, risks from microbiological hazards in drinking water are commonly regulated in two different forms: treatment and/or health-based standards. The 3-$\log_{10}$ *Giardia* removal requirements for system that use filtration for drinking water treatment (U.S. EPA, 1998) represents an example of a treatment based standard. The problem with treatment-based specifications is that they do not account for the (expected) wide range of source water pathogen concentrations in water and the variable treatment performance *in situ*, which may lead to pathogens in drinking water well above the theoretically safe level during such events (Davies & Mazumder, 2003). For example, a required 3-$\log_{10}$ reduction in *Giardia* cyst occurrence does not guarantee safety for consumption particularly if the concentration of cysts in the source water being filtered is several orders of magnitude greater than the treatment requirement. QMRA may provide a better estimate of health-based outcomes associated with complex systems such as drinking water production where the quality of source water is as important as the treatment process itself in the context of risk (Petterson *et al.*, 2007; Smeets *et al.*, 2010). Other treatment-based surrogates (such as turbidity, disinfectant concentration-time) can be used to check for

unit operation performance in near real-time (Betancourt & Rose, 2004; Alegre, 2006; Jofre *et al.*, 1995).

1.4 The QMRA process

QMRA uses mathematical modelling and relevant information (e.g., dose-response data) from selected reference pathogens to derive disease burden estimates in a population. It follows a common approach as classical risk assessment with inclusion of the four major components: hazard identification, exposure assessment, dose–response, and risk characterization (ILSI, 2000), although sometimes these components are expressed in slightly different terms (U.S. EPA; USDA/FSIS, 2012), for example, risk management is added as the fifth component. Each of these elements is discussed in detail below within the context of its application to QMRA approaches in drinking water.

1.4.1 Hazard identification

A wide array of microbial threats to drinking water exist including enteric viruses, bacteria, protozoa, helminths and even toxic microbial metabolites (i.e., those produced by cyanobacteria). For the purposes of this thesis, emphasis is placed on the protozoan parasites, *Giardia* spp. and *Cryptosporidium* spp. in the context of drinking water. *Giardia* and *Cryptosporidium* are enteric parasitic protozoa whose characteristics are suitable for use as reference protozoan pathogens, due to their high prevalence in surface waters, potential to cause widespread disease, resistance to chemical disinfection, and availability of a dose–response models for each organism (Health Canada, 2012). It is assumed that by controlling these reference protozoa the treatment processes should control other

waterborne protozoa and, potentially, other pathogen classes of concern (Health Canada, 2012).

Life cycles of both parasitic protozoa include an active stage inside the host and an environmentally-resistant stage, the (oo)cyst, that is excreted with faeces. From the risk assessment point of view, (oo)cysts are of primary interest as they may remain infectious for months in the environment (Robertson *et al.*, 1992; Johnson *et al.*, 1997; Fayer *et al.*, 1997). Uptake by a host results in excystation and the life cycle starts again and multiplication occurs, using resources of the host.

Giardia. Cysts are ovoid (8–14 um long by 7–10 um wide), with two or four nuclei. Environmentally stable cysts are passed out in the faeces, often in large numbers necessary to infect the next host. A complete life cycle description can be found in a review paper by Adam (2001). The current taxonomy of the genus *Giardia* is based on the species definition proposed by Filice (1952), who defined three species: *G. duodenalis*, *G. muris* and *G. agilis*. In the context of *G. duodenalis*, the International Committee for Zoological Nomenclature (ICZN) recognizes the official name to be *G. intestinalis* (syn. *G. duodenalis*, *G. lamblia*). *Giardia intestinalis* is the parasite of concern to human health. Of the eight genetic assemblages of *G. intestinalis* (termed A-H) only assemblages A and B are known to cause human disease. More recently, it has been proposed that the species *Giardia intestinalis* should be divided into six separate species based on host specificity/preference (Thompson, 2004; Thompson & Monis, 2004). *Giardia intestinalis* (assemblage A) has been reported to cause

waterborne outbreaks (van Keulen *et al.*, 2002), and this assemblage is usually associated with humans and livestock (Caccio *et al.*, 2005).

Cryptosporidium. This small, obligate, intracellular protozoan parasite infects mammals, birds, reptiles and fish. Environmentally robust spherical oocysts (4-6 μm dia) are shed in the faeces by the infected host into the environment (Fayer *et al.*, 2000). Each oocyst (containing four sporozoites) can survive adverse conditions in the environment for months until ingested by a new suitable host (Robertson *et al.*, 1992; Johnson *et al.*, 1997; Fayer *et al.*, 1997).

Currently, 25 species and close to 50 genotypes of *Cryptosporidium* have been identified (Ruecker, 2013). Across studied countries, nearly 90 % of human cryptosporidiosis was attributed to two species: *C. parvum* and *C. hominis* (Xiao, 2010). The other 10 % of cryptosporidiosis cases in humans were caused by *C. meleagridis, C. canis, C. felis, C. ubiquitum, C. cuniculus* (Xiao, 2010; Xiao *et al.*, 2004; Ryan & Power, 2012; Chalmers *et al.*, 2011). Only *C. hominis* (Peng & Xiao, 1997), *C. parvum* (Peng & Xiao, 1997) sourced from young cattle (Fayer *et al.*, 2000) and sheep (McLauchelin *et al.*, 2000), and *C. cuniculus* from rabbits (Chalmers *et al.*, 2011) were identified as causing drinking water outbreaks in humans.

1.4.2 Exposure assessment

Host sources of faecal pollution and routes of transporting of parasite in the environment greatly affect the probability of contracting infection. Exposure to *Giardia* and/or *Cryptosporidium* (oo)cysts occurs when people ingest contaminated water.

Exposure assessment is a process of estimation of the amount of the contaminant ingested [(oo)cysts] in a specified volume of water

at the time of ingestion (Ott *et al.*, 2006). The consumption of drinking water is the principal route of exposure examined in this thesis. The concentration of parasites and the volume of water ingested are necessary to be measured or estimated for QMRA. In this case, an exposure is a single dose of the pathogen ingested by a consumer at one time (Health Canada, 2010). Empirical measurements of exposure are preferable to estimates, as this will result in the highest-quality exposure assessment (Health Canada, 2012). Seasonal variation and peak events such as storms should be incorporated in the measurements and estimates to understand variation in the exposure levels (Medema, 2013; Dahlgren *et al.,* 1999). Other considerations in the context of estimating pathogen occurrence for QMRA include recovery efficiency and robustness of the analytical method as a means of empirically obtaining a true occurrence of (oo)cysts concentration in water (Schijven *et al.*, 2011; Schmidt *et al.*, 2010). Other considerations include whether the parasites are viable and infectious to humans. Health Canada (2010) suggests that every (oo)cyst should be considered viable and infectious and still able to cause illness. However, it is known that not all (oo)cysts in water are viable and infectious, and their infectivity can vary significantly (Chalmers & Davies, 2010). Coupled with the emerging concept of host-specificity in the genus *Cryptosporidium*, even relatively high concentrations of parasites in water may not pose an acute human health risk (Ruecker *et al.,* 2007).

The major factors that may affect exposure of people to water borne transmission of *Giardia* and *Cryptosporidium* relate to drinking water consumption patterns, occurrence of the parasites in

environmental water used for drinking water sources, analytical methods for parasite detection in source water, and removal or/and inactivation during water treatment.

1.4.2.1 Consumption of water

An average consumption of 1 L of water per person per day of unboiled treated drinking water was considered for the estimation of exposure for the current risk assessment recommended by Health Canada (2010) and WHO (WHO, 2008; 2011), and which is similar to other developed nations (Mons *et al.*, 2007). However, it is important to consider quantity of only unboiled tap water consumed, because boiling of water will inactivate pathogens. Data based on the volume of cold tap water consumed likely indicates a more realistic estimate of exposure based on consumptive volume (Table 1.1).

Table 1.1 Daily mean unboiled tap water consumption in litres reported in different countries

Country	Volume of water (L)	Reference
Canada	0.386	Levallois *et al.*, 1998
USA	0.506	U.S. EPA, 2000
Sweden	0.86	Westrell *et al.*, 2006
France winter	0.77	Gofti Laroche *et al.*, 2001
France spring	0.90	Gofti Laroche *et al.*, 2001
England and Wales	0.19	FWR, 1996
The Netherlands	0.153*	Teunis *et al.*, 1999

*-median value

1.4.2.2 Parasite occurrence

Giardia and *Cryptosporidium* are prevalent in environmental waters across North America, however, in varied concentrations (Gammie *et al.*, 2000). Knowledge on pathogen occurrence should be formed on historical data and current research. Concentrations of *Giardia* and *Cryptosporidium* in surface waters across Canada usually range from 2 to 200 cysts/100 L and from 1 to 100 oocysts/100 L,

respectively (Health Canada, 2012). However, sampling and methods analysis employed in studies across Canada varied, therefore, it may not be appropriate to consider these general estimates. A study of parasite occurrence in Alberta conducted in 2000 showed that annual geometric mean concentrations of *Giardia* range between 8-193 cysts/100 L (Gammie *et al.*, 2000). A maximum recorded concentration of 2,500 cysts/100 L was observed and which was associated with heavy spring run-off (Gammie *et al.*, 2000). Annual geometric mean concentrations of *Cryptosporidium* range between 6-83 oocysts/100 L. A maximum recorded concentration of 10,300 oocysts/100 L was observed and which was also associated with heavy spring run-off (Gammie *et al.*, 2000). Data collected by EPCOR (Edmonton) in 2005 on parasites indicate an annual geometric mean of 98 cysts/100 L, and a maximum recorded concentration as high as 8,700 cysts/100 L for *Giardia*, and an annual geometric mean concentration 9 oocysts/100 L with maximum recorded concentration 69 oocysts/100 L for *Cryptosporidium*. In British Columbia, a geometric mean of 60 cysts/100 L at a range of concentrations 4.6-1,880 cysts/100 L was for *Giardia* and a geometric mean 3.5 oocysts/100 L at a range of concentrations 1.7-44 oocysts/100 L was for *Cryptosporidium* (Ong *et al.*, 1996). In Ontario in 2006, separate studies found that a median concentration for *Giardia* was 71 cysts/100 L, with maximum concentration reported of 486 cysts/100 L in the first study (Van Dyke *et al.*, 2006), and arithmetic means of 3.6 and 3.9 cysts/100 L (at two intakes) that were in a range <2.5-20 cysts/100 L (Douglas *et al.*, 2006) in the second study. The same studies for *Cryptosporidium* found that a

median concentration of 15 oocyst/100 L, with a maximum concentration of 186 oocysts/100 L in the first study, and arithmetic means of 3.4 and 5.7 oocysts/100 L (at two intakes) that were in a range <2.5-95 oocysts/100 L in the second study. In Quebec, Payment *et al.*, (2000) reported a geometric mean of 200 cysts/100 L for *Giardia* and geometric mean of 14 oocysts/100 L for *Cryptosporidium*. A recent international review conducted by Dechesne and Soyeux (2007) found that *Giardia* concentrations across North America and Europe ranged from 0.02 to 100 cysts/L, and *Cryptosporidium* concentrations range 0.006 to 250 oocysts/L.

Occurrence of *Cryptosporidium* and *Giardia* in source waters depends largely on factors affecting the mobilization of faeces into water sources. Certain factors such rain run-off and snowmelt can act as important mobilization forces of faeces deposited on land (Davies *et al.*, 2004; Miller *et al.*, 2008; Lewis *et al.*, 2009). Direct deposition of faeces (i.e., cattle in a stream) or wastes (i.e., wastewater discharge) to river systems can also occur, and consequently mobilization is directly through river flows. These factors can result in large variation on the spatiotemporal occurrence of parasites in source water.

The intensity of rain and amount of precipitation are substantial determinants for efficiency of parasite mobilization (e.g. Brookes *et al.*, 2005; Ferguson *et al.*, 2005; 2007; Muirhead at al., 2006). The amount of rain should be sufficient to moisten, for example, deposited faeces on ground, and the amount of run-off must be sufficient to suspend the faecal particles in water to carry them to receiving water bodies. Parasite concentrations flowing into surface

water bodies gradually decreases when the depositions of faeces on the ground becomes depleted particularly during periods of extensive rainfall, a phenomenon known as 'first-flush' (Trask *et al.*, 2004). Thereafter, an influx of parasite into water bodies would be possible by next flush at the cost of further transporting (oo)cyst that were retained on the soil surface and near surface (Davies *et al.*, 2004), unless they were attached to more dense soil particles (Tyrrel & Quinton, 2003). Moreover, a large volume of water from rain can dilute contaminants in source water. Therefore, rain run-off can mobilize the parasite into surface water, cause a temporal increase in their concentration in surface water, but upon persistent run-off conditions result in dilution or depletion of parasites entering the source water (Trask *et al.*, 2004). Excessive rain can occasionally flood sewers, which can result in a profound contamination of source water. These types of processes have been modelled to better understand parasite occurrence in water systems (Ferguson *et al.* 2005; 2010).

Snowmelt run-off accommodates a similar principle of mobilization of the parasite contaminants except that temperature fluctuations around the melt temperature play an important role in mobilization. The infamous Milwaukee outbreak in 1993 followed after heavy snowmelt run-off (MacKenzie *et al.*, 1994; Fox & Lytle, 1996), that was thought to have resulted from unusual hydrodynamics bringing a sewage outfall effluent to the plant's water intake, given the human-specific *C. hominis* etiologic agent (Zhou *et al.*, 2003). In the context of risk assessment, the spatiotemporal variability of parasite occurrence associated with mobilization of contaminant sources

translates into spatiotemporal variability in risks associated with drinking water.

1.4.2.3 Analytical methods for detection and source water monitoring

In North America, the most widely used method for detection of *Giardia* and *Cryptosporidium* in water is the U.S. EPA Method 1623 (U.S. EPA, 2005; 2006). This method is comprised of four steps: 1) sample collection, 2) sample filtration and elution, 3) sample concentration and separation (purification) and 4) (oo)cyst detection.

Unfortunately, Method 1623 inherited many deficiencies from the ancestral method of U.S. EPA Method 1622 (McCuin & Clancy, 2003) and the original Information Collection Rule (ICR) Method (Sinclair, 2000; McCuin & Clancy, 2003). Detection of the pathogenic protozoa is complicated by the necessity to filter a large volume of water and the impaired recovery efficiency of the method. For the test, a grab sample of 100 L of the source water is usually required. Depending on source water quality, filtration of this large volume can be difficult. Feng *et al.* (2003) reported the greatest loss of (oo)cysts during filtration. Large (oo)cyst losses occur during concentration and separation procedures (LeCevallier *et al.*, 1995). Overall losses can result in 48.4±11.8 % oocysts and 57.1±10.9 % cysts for previously spiked tap water (Health Canada, 2010). Recoveries from raw water samples can be significantly less and can be in the range from 19.5 % to 54.5 % for oocysts and from 46.7 % to 70 % for cysts, as an example for Method 1622 (McCuin & Clancy, 2003). High turbidity usually impairs recovery efficiency. However, Feng *et al.* (2003) observed that a moderate degree of turbidity (e.g. 5 NTU) enhanced

recovery compared to less turbid water. The nature of turbidity is likely as important as the turbidity measurement of the water sample itself (DiGiorgio *et al.*, 2002). Additionally, Method 1623 does not differentiate between different species or strains (i.e., genotypes/genetic assemblages) of *Giardia* or *Cryptosporidium*. Viability of (oo)cysts is also not known with the test result (LeChevallier *et al.*, 2003). Thus, infectivity due to (oo)cyst host specificity and viability is usually unknown (Allen *et al.*, 2000; Connell *et al.*, 2000; Simmons *et al.*, 2001). At present, most regulations or approaches to QMRA assume that any (oo)cyst found in water is potentially infectious to humans, irrespective of whether the (oo)cyst is viable or infectious for humans.

Monitoring for bacterial indicators of water quality, such as total coliforms and *E. coli*, as well as physical parameters of water quality such as turbidity cannot guarantee microbiological safety against all pathogens. Health Canada recommends monitoring for both *Cryptosporidium* and *Giardia* in source water supplies used for drinking (Health Canada, 2012). Since promulgation of the Long Term-2 Enhanced Surface Water Treatment Rule (LT2 Rule) in 2006, U.S. EPA requires that surface source waters that are used for public purposes be monitored for *Cryptosporidium* to estimate oocysts concentrations in source water and to guide water treatment measures.

Routine testing for *Giardia* and *Cryptosporidium* is challenging because it is expensive and it requires highly trained personal and a sufficiently equipped laboratory to perform the testing (LeChevallier *et al.*, 2003). Small PWSs could experience a financial burden in an

effort of complying with the regulatory requirements. According to the U.S. EPA and under the LT2 Rule, PWS that serve a population of more than 10,000 people can choose a few options to conduct water testing for *Cryptosporidium*, but with at least 24 samples collected monthly, if the plant chooses monitoring instead of just providing 5.5-$\log_{10}$ treatment reduction (U.S. EPA, 2006). In this case, the PWS has to use a maximum running annual average (Max-RAA) for estimating *Cryptosporidium* concentration in source water, arguing that this would achieve a low false negative rate. For a more extensive monitoring program option of 48 samples over two years can be done, a PWS to applying an arithmetic mean for estimating *Cryptosporidium* concentration that would reduce a false positive rate in addition to reducing a false negative rate similar to Max-RAA. A geometric mean can also be applied for estimating parasite concentration in water as it is an unbiased estimator of the population median of sample sizes of 4 to 100 (Parkin & Robinson, 1993). An arithmetic mean compared to the geometric mean is considered to be more sensitive for outliers. Outliers may indicate peak parasite concentration periods (Schijven *et al.*, 2011; Parkhurst, 1998). Health Canada does not clarify the frequency of testing required and a measure of central tendency that would be appropriate. The challenging task is to match a bearable financial burden with an extent of monitoring that would be sufficient to provide realistic concentration estimates for parasite occurrence.

1.4.2.4 Treatment technologies

A WTP that uses full scale conventional filtration (i.e., chemically assisted coagulation, flocculation, sedimentation, and filtration) should be able to achieve sufficient removal of protozoa

(oo)cyst from treated water (Hijnen & Medema, 2010; Ireland Environmental Protection Agency, 1995). It has become a common practice to assume that the conventional filtration process achieve 3-$\log_{10}$ removal of both *Giardia* (Nieminski & Ongerth, 1995; McTigue *et al.*, 1998; Schuler & Ghosh, 1990; Schuler *et al.*, 1991) and *Cryptosporidium* (U.S. EPA, 2006). However, the efficiency of conventional filtration process can vary according to site specific conditions (Hijnen *et al.*, 2004). Conventional filtration is also effective for controlling particulate and organic matter (Hijnen & Medema, 2010).

Chemical disinfection against *Giardia* is effective but also complicated because the cold water conditions that are observed during the larger part of the year in Canada makes inactivation less effective (Health Canada, 2012). Chlorine is considered ineffective against *Cryptosporidium* oocysts in the context of drinking water treatment, due to the extremely high concentrations and contact times needed for any reasonable level of inactivation to be achieved (Korich *et al.*, 1990; Venczel *et al.*, 1997; Gyürék *et al.*, 1997). Other chemicals disinfectants that have commonly been used are chloramine, chlorine dioxide and ozone (Korich *et al.*, 1990). The effectiveness of chloramine for (oo)cyst inactivation is comparable to chlorine effect, thus, it is very weak, especially, against *Cryptosporidium* (Chauret *et al.*, 1998). Chlorine dioxide and ozone are more effective against protozoan (oo)cyst (Huber *et al.*, 2005; Peeters *et al.*, 1989; Betancourt & Rose, 2004; Lazarova *et al.*, 1999), however, their use requires more specialized plant operations and is expensive compared to chlorination (Hey *et al.*, 2012).

Ultraviolet light (UV) is considered an acceptable method to inactivate protozoa and bacteria; however, UV is less effective against adenoviruses unless polychromatic UV light (medium-pressure) is used (Shin *et al.*, 2009), thus the water would still require full-scale chemical disinfection or use of medium-pressure UV treatment. The LT2 Rule requires UV doses of 11 and 12 mJ/cm² to receive a 3-$\log_{10}$ credit for *Giardia* and *Cryptosporidium,* respectively (U.S. EPA, 2006). Source water parameters can significantly influence chemical and UV disinfection, for example, in the presence of natural particulate matter (Amoah *et al.*, 2005; Craik *et al.*, 2001). The U.S. EPA has offered additional options from a "microbial toolbox" for suitable water treatment conditions to receive a treatment log-credit, for example membrane filtration (U.S. EPA, 2006).

1.4.3 Dose-response assessment

Exposure to *Giardia* or *Cryptosporidium* (oo)cysts is associated with a potential human health effect. Theoretically, ingestion of a single (oo)cyst may potentially cause infection, the so-called single-hit model (Haas, 1983). Nevertheless, many factors affect microbial pathogenicity and virulence. Dose-response models have been derived from trials on human volunteers. Rendtorff (1978) reported that a dose of 19 cysts was sufficient to develop illness in 50 % of humans (i.e., ID_{50}). A subsequent study by Hibler *et al.* (1987) characterized an ID_{50} of *Giardia lamblia* at around 50 cysts (Hibler *et al.*, 1987). In yet another study the ID_{50} of *Giardia* was extrapolated from dose-response curves and found to be around 35 cysts (Rose & Gerba, 1991).

Similarly, dose-response models have been generated for ingestion of *Cryptosporidium* oocysts (DuPont et.al., 1995; Chappell *et al.*, 1999). The ability of *Cryptosporidium* to develop illness varies greatly from strain to strain. The TAMU strain of *C. parvum* demonstrated a very low infectious dose of ID_{50} of 9 oocysts with illness attack rate of 86 %, however at the same time for ID_{50} of UCP strain of *C. parvum* (isolated from a cow) required 1042 oocysts (Okhuysen *et al.*, 1999; Messner *et al.*, 2001).

Mathematical models have been widely used for estimation of disease burden in response to the assumed exposure (Haas *et al.*, 1999). During the exposure, the exposed individuals ingest a discrete number of parasites. Parasites in drinking water are distributed randomly, therefore the chance of ingesting a discrete number of parasites can be explained by a Poisson distribution (Petterson *et al.*, 2006). Exponential and beta-Poisson models assume that a discrete number of parasites arranged around a Poisson distribution occurs between individuals with a constant arithmetic mean (FAO/WHO, 2009). The beta-Poisson model has an advantage as it is accommodating variances in host susceptibility to the pathogen and its ability to start infection (Petterson *et al.*, 2006), but these parameters remain unknown. Thus, the exponential model (Haas, 2002) best explains *Giardia* and *Cryptosporidium* dose-response data. The beta-Poisson model has usually been used to explain dose-response data for *Campylobacter* and enteric viruses (Haas *et al.*, 1999).

Probability of infection is calculated in relationship to the ability of specific pathogen to start infection and a dose of pathogen

ingested per day (Haas *et al.*, 1999). The dose-response models and parameters are obtained from the scientific literature. The dose-response equations estimate the risk of infection for each dose of pathogens ingested. Because there are a range of possible doses ingested based on Poisson probability distribution rather than constant values of doses, the risk of infection is considered to be (P_{INF} dose) for each potential dose ingested and sums the weighted probabilities (P_{INF} for 0 pathogens ingested + P_{INF} for ingesting 1 pathogen + P_{INF} for ingesting 2 pathogens + etc.). The sum of these products gives the daily weighted probability of infection for an individual.

Not every infected consumer becomes ill, but some may become asymptomatic carriers of the infection (Ajjampur *et al.*, 2010; Houpt *et al.*, 2005). The probability of developing cryptosporidiosis from consumption of a sufficient dose to cause infection by the parasite is estimated to be 0.70 (Casman *et al.*, 2000), and for *Giardia* is 0.24 (Macler & Regli, 1993). Probability of illness is calculated by multiplying the probability of contracting an infection per year by the product of proportion of the population susceptible to infection (assumed to be 100 %) and the probability that the infection would result in illness (Medema, 2013).

1.4.4 Risk characterization

Risk characterization brings together probable exposure estimates, dose-response relationship, and pathogen reduction through treatment barriers, and consumption pattern to estimate the burden of disease (Haas *et al.*, 1999). If the estimated burden of disease does not meet specified health target, the QMRA can be used

to calculate the necessary level of treatment to achieve the required level of protection (Medema, 2013).

1.4.4.1 Health effect

Because of low likelihood of people to develop illness from *Giardia* infections, the majority of cases result in transient infection without signs of illness or in an asymptomatic carrier state (Prado *et al.*, 2005). Symptomatic *Giardia*sis manifests as nausea, diarrhoea (usually sudden and explosive), anorexia, uneasiness in the upper intestine, malaise, and occasionally low-grade fever (Wolfe, 1992). Infections usually resolve spontaneously (Wolfe, 1992).

For *Cryptosporidium*, if an infection was contracted, symptoms of illness most likely would develop. The most common symptom is diarrhoea, characterized by very watery non-bloody stools; other symptoms such as cramping, nausea, vomiting, low-grade fever, anorexia and dehydration are usually observed (Guerrant, 1997).

1.4.4.2 Gauging risk

Burden of disease estimates calculated during a risk assessment can be compared with the reference health target level of risk 10^{-6} Disability Adjusted Life Years (DALY) (WHO, 2008). WHO's *Guidelines for Drinking-water Quality* (WHO, 2008) use DALYs as a unit of measure of risk.

The basic principle of DALY is to calculate a value that considers both the probability of experiencing an illness or injury and an assumption of the impact of the associated adverse health effects (Murray & Lopez, 1996; Havelaar & Melse, 2003). The DALY combine Life-Years-Lost (LYL) as the estimate of a not fulfilled full life span, severity of illness reflected by years lived with disability (YLD), and

adjusts it to an affected population (Havelaar & Melse, 2002). The LYL is calculated multiplying the severity of disease weight by a difference between life expectancy and age at death. The YLD is a sum of health outcomes contributing to morbidity. DALYs are calculated separately for each pathogen. The value of 1.7 DALYs per 1,000 cases of illness is used for both parasites *Giardia* and *Cryptosporidium*, however, this assumption may not truly reflect reality. The health burden is generally different when comparing *Giardia*sis and cryptosporidiosis, as well as being different depending on the species and genotypes of each parasite that are associated with an infection (Xiao *et al.*, 2000). Accordingly, the final step for a risk assessment is obtaining a disease burden estimate by multiplying probability of illness and health burden (1.7 DALY per 1,000 cases) (Health Canada, 2012). The complete derivation process of health burden for *Giardia* and *Cryptosporidium* can be found in the Health Canada document "Enteric Protozoa: *Giardia* and *Cryptosporidium*" (Health Canada, 2012).

For example, the estimation of the health risk of 2.6×10^{-5} DALY per person per year by Health Canada (2004) would correspond to concentration 1 cyst in 60,000 L of drinking water. One DALY can be seen as a health burden as a result if one person in a million population would experience mild enteric symptoms (e.g., diarrhea, ets.) during one year. However, it may make more sense to target daily risk to better manage potential outbreaks (Signor & Ashbolt, 2009).

The fraction of parasites that survive and initiate illness derive from dose-response data based on studies of healthy volunteers and might not adequately represent health risk from the parasite for the

sensitive part of the population (HIV-positive, elderly, and young children) (Health Canada, 2012). Therefore, using weighted combined estimates of risk in DALY for the sensitive subgroups with the general public may be a more appropriate way for addressing health risk in a population because a portion of disease burden attributed to sensitive subgroups can be large (Perz *et al.*, 1998).

1.4.5 Strategies for risk management

Mitigation of health risk due to exposure to waterborne pathogens is very dependent on water treatment measures that are being used for production of drinking water. All types of surface waters and ground water under direct influence of surface water have to be filtered and disinfected (U.S. EPA, 2006). Full-scale conventional filtration (chemically assisted coagulation, flocculation, sedimentation and filtration) is the most effective physical barrier against waterborne pathogens (Hijnen & Medema, 2010). The U.S. EPA LT2 Rule (2006) grants a 3-$\log_{10}$ reduction credit to water treatment plants that employ a full-scale conventional filtration process. Up to additional 1-$\log_{10}$ credit can be received for the proper combined and individual filter performance that meets necessary criteria (U.S. EPA, 2006). Health Canada (2010) indicates that to reach health target of 10^{-6} DALY per person per year, a 3-$\log_{10}$ reduction is appropriate to deal with up to 13 oocysts and/or 34 cysts per 100 L of *Cryptosporidium* and/or *Giardia* in source water, respectively.

To establish the risk-targeted treatment requirements for *Cryptosporidium,* the U.S. EPA (2006) addresses several important questions:

- What is the risk associated with *Cryptosporidium* in a drinking water source?
- How efficient is filtration against *Cryptosporidium* at a particular plant?
- What degree of additional treatment is needed for higher source water *Cryptosporidium* concentrations?

Similar questions may be applied with respect to *Giardia*.

Regulatory agencies typically include an additional level of safety in water treatment regulations. Earlier regulations formulated by the U.S. EPA stipulated that at least a 3-$\log_{10}$ reduction for *Giardia lamblia* (U.S. EPA, 1989b) and a 2-$\log_{10}$ reduction for *Cryptosporidium* (U.S. EPA, 1998) should be provided for a system that filters. In the most recent derivation of regulations, the LT2 Rule required *Cryptosporidium* monitoring to determine bin concentration and corresponding removal and inactivation that would be necessary (U.S. EPA, 2006) in order to achieve a target health outcome of less than one case of illness per 10,000 population annually (Table 1.2). U.S. EPA requires monitoring for *Cryptosporidium*, but not for *Giardia*. In addition, the U.S. EPA requires a minimum 3-$\log_{10}$ treatment reduction for *Cryptosporidium* for all PWS systems, even if monitoring demonstrates that no parasites are present. As parasite concentrations increase, additional treatment barriers are required.

Table 1.2 U.S. EPA (LT2 Rule). *Cryptosporidium* concentration classifica-tion and treatment standards for filtered PWSs (Adapted from LT2 Rule, U.S. EPA, 2006).

PWS	*Cryptosporidium* concentration in source water	The bin classification	Treatment that is necessary
required to monitor for *Cryptosporidium**	Less than 0.075 oocysts/L	Bin 1	3-$\log_{10}$
	0.075 oocysts/L or higher, but less than 1.0 oocysts/L	Bin 2	4-$\log_{10}$
	1.0 oocysts/l or higher, but less than 3.0oocysts/L	Bin 3	5-$\log_{10}$
	3.0 oocysts/L or higher	Bin 4	5.5-$\log_{10}$

*Filtered PWS serving fewer than 10,000 people are not required to monitor for *Cryptosporidium* if they monitor for *E. coli* less than or equal to 10/100 mL for lake/reservoir sources or 50/100 mL for flowing stream sources or do not exceed an alternative State-approved indicator trigger.

Alberta Environment and Sustainable Resource Development (AESRD) uses similar approaches to the U.S.EPA's LT2 Rule for managing risks against *Cryptosporidium*. However, unlike the U.S. EPA, AESRD requires monitoring for *Giardia* in some PWS. Consequently, treatment requirements can be driven by either *Cryptosporidium* or *Giardia* parasite concentrations found in the source waters. In addition, AESRD maintains an exceptionally strict treatment requirement in regards to *Giardia* occurrence. For instance, if *Giardia* concentration exceeds a single cyst in 100 L of source water based on running annual average, the rule requires 4-$\log_{10}$ reduction, whereas for *Cryptosporidium* the same 4-$\log_{10}$ reduction is enacted if a concentration > 7.5 oocysts/100 L was

observed (Table 1.3). Despite the fact that *Giardia*sis is considered a less severe disease than cryptosporidiosis, a clear discordance in tolerable levels of parasites exists.

Table 1.3 Alberta Environment protozoa requirements for filtered PWSs (Alberta Environment, 2006).

Raw Water *Giardia* Levels (cysts/100 L)[a,b]	Raw Water *Cryptosporidium* Level (oocysts/100 L) [a,b]	log_{10} Reduction
< 1	< 7.5	3.0-log_{10}
> 1 and < 10	> 7.5 and < 100	4.0-log_{10}
> 10 and < 100	> 100 and < 300	5.0-log_{10}
> 100	> 300	5.5-log_{10}

[a] For communities with population large than 10,000, the levels are based on running annual average of monthly samples over a two year period.
[b]For communities with population less than 10,000 that are triggered based on *E. coli* sampling, the levels are based on running annual average of quarterly samples over a two-year period.

In Canada, treatment requirements vary across the country in regards to enteric protozoa. Health Canada introduced treatment *Guidelines* in October 1995 stipulating removal or/and inactivation against *Giardia* and *Cryptosporidium* of 3-log_{10} and 2-log_{10} reduction, respectively (Health Canada, 2003), and in line with U.S. EPA requirements at the time. In 2012, new guidelines were adopted by Health Canada, and rooted in a Quantitative Microbial Risk Assessment framework (Health Canada, 2012).

The multi-barrier approach that includes watershed protection, optimized filtration and disinfection, well-maintained distribution system and routine water quality monitoring, is the best approach to reduce presence of waterborne pathogens in drinking water (Health Canada, 2010). The best way to build understanding of source water quality is to conduct routine analysis for *Giardia* and *Cryptosporidium*. Special attention is required during extreme weather

condition, for example, high precipitation, or spills of wastewaters into the watershed. Once the source water quality has been characterized, optimal pathogen removal and inactivation strategies should be developed. The treatment system should be assessed against probable malfunction for each barrier. Mechanisms of early warning should be implemented and appropriate response measures should be ready for action to prevent production of contaminated water. The combination of physical removal and disinfection is the most effective against protozoa. Other treatment requirements such as the maximum turbidity level, reduction of disinfection by products (DBP), and maintenance of residual chlorine concentration in a distribution system should be controlled also (U.S. EPA, 2006).

It should be stated clearly that simply following the guidelines for water treatment does not guarantee the production of safe drinking water from every raw water source (Health Canada, 2006). In some instances, complex treatment and water protection should be used to satisfy quality standards, as advocated in drinking water safety plan approaches that have been implemented in Alberta and other provinces across Canada (FitzGibbon & Plummer, 2004).

1.5 International approaches

A few nations have introduced QMRA into their state's regulation requirements. Since 2001, the Dutch Drinking Water Act requires all PWSs to conduct risk assessment in compliance that the produced drinking water is meeting a specified health target (Anonymous, 2001). The Dutch Inspectorate together with water utilities, RIVM (National Institute of Public Health and the Environment, scientific advisor of Inspectorate), and KWR (the

scientific research institute of the water utilities) developed Inspectorate Guideline 5318 (VROM-Inspectorate, 2005) that stipulated specific directives on how to perform risk assessments using QMRA. Every water utility has to perform a monitoring campaign every three years and for three years. Duration of a campaign can be shortened if a utility intensifies sampling. In total, PWSs must collect at least 26 regular samples (every two weeks) and 9 incidental samples during expected peak events, when high pathogen concentration is assumed to occur, for example during rainfall (Kistemann *et al.*, 2002; Kay *et al.*, 2007). Data must include *Cryptosporidium, Giardia, Campylobacter*, and enterovirus results. Recovery efficiency is estimated using organism spiking and recovering techniques. If recovery data is lacking, recovery is assumed equal to 100 %. Treatment efficiency is estimated based on pilot testing, because it can be very location specific (Schijven *et al.*, 2011). A pattern of consumption of unboiled tap water is assumed corresponding to a mean ranging from 0.25 to 2L per person per day (Teunis *et al.*, 1997). All calculations are performed stochastically addressing variations in pathogen concentration, treatment reduction, and consumption pattern using *QMRAspot* software (Schijven *et al.*, 2011). The health-based target is set at less than one case of illness per 10,000 individuals per year. The combination of using QMRA with Water Safety Plan (WSP) is gaining popularity in the Netherlands (Bichai & Smeets, 2013). While WSPs help to identify periods of peak risk, QMRA can be used to provide quantitative estimates of risks.

In England and Wales, it is a legal requirement for every drinking water utility to conduct a risk assessment of it's water

supply (UK Drinking Water Inspectorate, 2005). This risk-based approach is grounded in the obligation to develop and implement "Distribution Operation and Maintenance Strategies" for the proactive management of drinking water distribution system (UK Drinking Water Inspectorate, 2002). If drinking water from a particular water utility is deemed to be at risk due to contamination of *Cryptosporidium*, the utility has to, ideally, conduct continuous monitoring. However, routine parasite monitoring is not sensitive to predict or detect periods of contamination events, thus, ineffective for public health protection (Clancy & Hunter, 2004). In UK and Wales, it is a criminal offence to have concentration of *Cryptosporidium* of more than 1 oocyst in 10 L of finished drinking water over 24 hours. Contrastingly, a risk from *Giardia* is not addressed by the regulator (Clancy & Hunter, 2004).

In Australia, through enactment of the Guidelines for Water Recycling (Phase 2 – Augmentation of drinking water supply), QMRA was legislated for using for the drinking water industry purposes (NRMMC-EPHC-NHMRC, 2008), for waters from recycled water sources only. Australian Guideline for Water Recycling use the same index pathogens as the Dutch's, except that rotavirus/adenovirus is used instead of enterovirus. In addition, the health target is set at 10^{-6} DALY. Since water from recycling schemes has been used, exposure varies according to the different uses of the produced water (Bichai & Smeets, 2013). The 'ReQuality' Software provides QMRA calculations (point estimates) relying on the log-removal data taken from scientific literature. Currently, QMRA focuses at the point where the produced water leaves a treatment plant. Australia developed their

approach using WHO (2004) methodologies. Particularly, the health target 10^{-6} DALY was inherited. The WHO used a deterministic approach when dealing with estimates as opposed to stochastic calculations.

1.6 Overview of Health Canada QMRA model

By comparison, to the WHO approach, Health Canada included the same deterministic approaches as the basis for developing their own QMRA model. The version (revision April, 2008) of Health Canada's QMRA model (version 1.0 HC QMRA model for purpose of this dissertation) used point estimates throughout all calculations, including pathogen concentrations in source water, a log-reduction during treatment, a standard volume of drinking water consumption, and an estimate of burden of disease (Douglas, 2008). The revision of July, 2011 of the Health Canada QMRA model (version 2.0 HC QMRA model for purpose of this dissertation) addressed variability in estimates stochastically for pathogen concentrations in source water (assuming log-normal distribution) and estimates of the burden of disease, however, estimates of water consumption and a log-treatment reduction remained fixed (McFadyen *et al.*, 2011). At the request of the City of Calgary, health risk estimates were provided in the form of point estimates for the purposes of the current risk assessment.

Source water concentration estimates for a given set of reference pathogens (*Cryptosporidium, Giardia*, rotavirus, diarrheogenic *E. coli* and *E. coli* O157:H7 are used), are entered by the user as inputs. Treatment efficiency is often determined by studies addressing pathogen removal and inactivation. Values of the final concentration of pathogens in the treated drinking water are derived

based on original pathogen concentrations in source water and $\log_{10}$ treatment reductions in pathogen numbers as a result of the water treatment process. Applying ingestion and dose-response parameters specific to each reference pathogen, the model estimates the number of waterborne illness and quantifies burden of disease within the population using DALY.

Parameters of water treatment capabilities based on conventional filtration can be inferred from the literature that compile information from studies used to provide estimates for the whole industry. Also, WTP can conduct a pilot study to estimate treatment performance that the facility can achieve. Values for pH, water temperature (°C), contact time (minutes), and residual disinfectant concentration (mg/L) can be entered by the user for the source water treatment conditions during disinfection. The software uses these data to automatically calculate Contact Time (Ct) of chemical inactivation. Mathematical modelling of water disinfection can be found in the edited book by Block (2001) or in Gyürék & Finch, (1998).

At present time, two Health Canada QMRA models are available (HC QMRA version1.0 and HC QMRA version 2.0). At the beginning of the current research project (Sep. 2011), the first version of Health Canada QMRA Model was used for the analysis. Later, the second version was also used for the analysis. The format of water disinfection parameters (Ct) that was provided by the water treatment plants was not suitable for use in version 2.0 of the model; therefore, health risk from *Giardia* was estimated using solely version 1.0 of the model. Health risk from *Cryptosporidium* was estimated using both

versions of the model. The important difference between the models for analysing health risk from *Cryptosporidium* was grounded in the underlying assumption of a degree of physical oocyst removal that the conventional filtration can achieve. For the version 1.0 this estimate was 3-$\log_{10}$ based on the U.S EPA's estimation (U.S. EPA, 2006), however for the version 2.0, Health Canada approved using 4.3-$\log_{10}$ removal estimate based on meta-analysis of pilot studies of efficiency of conventional filtration process against *Cryptosporidium* (Hijnen & Medema, 2007).

The models assume that the amount of pathogen theoretically ingested by each person based on a consumption of 1L of unboiled tap water per person per day. Using a Poisson distribution, the probability of ingesting 1,2,3,4, etc. pathogens is calculated using the mean concentrations in the treated drinking water (Health Canada, 2011). This modelled the condition as if 1L of tap water per day was randomly sampled.

In the model (version 2.0), the user can manually adjust percentage of viable and human infectious (oo)cysts if such information is available. The City of Calgary recently conducted a study in the watersheds of both the Elbow and Bow Rivers for estimating the fraction of human infectious *Cryptosporidium.* From 298 samples processed over 3 years and tested for *Cryptosporidium* species and genotypes, high risk species (*C. hominis*, *C. parvum*) were never observed (unpublished data). The output of both models is the estimate of the overall burden of the disease. The output is given in DALYs.

1.7 Proposed research

Giardia and *Cryptosporidium* are waterborne pathogens of public health concern. Their prevalence in environmental waters, the difficulty of their removal and inactivation during water treatment, and their ability to cause illness make these parasites suitable reference organisms for QMRA assessments for drinking water quality. Increased anthropogenic influences can exacerbate microbiological contamination in the environment and environmental factors can facilitate the influx of the pathogens into receiving water bodies from animal and/or human sources of pollution. Understanding sources of the pathogens and contribution of environmental conditions to the microbial occurrence would provide valuable information for estimation human health risk and guiding mitigating actions related to treating of water for drinking purposes. Currently, treatment-based and health-based approaches dominate regulatory frameworks focused on drinking water quality, but it is hypothesized that these approaches can led to disparate outcomes associated with understanding risks to drinking water supplies posed by these parasites. Moreover, it is hypothesized that environmental factors, particularly those that mobilize parasite sources (i.e., precipitation from snowmelt and rainfall separately) play a critical role in the spatiotemporal variability of parasite occurrence in source water and consequently the spatiotemporal risks and vulnerability of treated drinking water to parasite contamination.

The goal of this thesis was to evaluate health risks associated with *Cryptosporidium* and *Giardia* contamination in drinking water supplies at the City of Calgary using Health Canada's QMRA models,

and comparatively evaluate these QMRA outcomes to existing provincial operating approval requirements and other North American risk assessment standards (i.e., U.S. EPA LT2 Rule). The objectives of the proposed research are:

1. Assess risks associated with *Cryptosporidium* and *Giardia* in drinking water produced from the Elbow and Bow Rivers by:
    a. comparing and contrasting risk assessment outcomes based on approaches used by AESRD, U.S. EPA, and Health Canada (Chapter 3).
    b. characterize the impact that scientific assumptions have on risk assessment outcomes, with special emphasis on how they affect Health Canada's QMRA model (Chapter 4).
2. Using QMRA, evaluate the existing capability of the Bearspaw and Glenmore Water Treatment Plants to provide safe water under treatment barrier malfunction and/or unfavourable weather events that may result in sporadic periods of high risk (Chapter 4).
3. Examine the association between environmental risk factors and the parasite occurrence in order to address the spatiotemporal variability in risk that may be associated with these parasites in the City of Calgary's source waters (Chapter 5).
4. Report on limitations of QMRA and recommend solutions or improvements in QMRA approaches for better understanding and characterizing risks associated with *Cryptosporidium* and *Giardia* in drinking water supplies (Chapter 6).

Chapter 2 : Methods and materials

2.1 Research Methods - Objective 1: Construct a database of systematic physico-chemical, environmental indicator data, along with the pathogen occurrence data collected over time from the Bow and Elbow Rivers watersheds

2.1.1 Specific Aim A1: Collection of parasite occurrence data in the Elbow and Bow River

The Bearspaw WTP (Bow River), and the Glenmore WTP (Elbow River), provide approximately 60 % and 40 % of the drinking water supply, respectively, for the city (City of Calgary, 2011). The supplies from both plants are interconnected through transmission mains to stabilize water supply at all times.

The Elbow River collects water from an area of 1,210 square kilometres. The Elbow River is 120 kilometres long and passes through four sub-climates before it enters the Glenmore Reservoir on the east-south side of the City of Calgary (City of Calgary, 2011). The Bow River collects water from an area of 7,770 square kilometres. The Bow River originates from the Bow Glacier north of Lake Louise, Alberta. It enters the City of Calgary from the northwest side of the city. The Bearspaw WTP is located on the river upstream of the city.

The Elbow and Bow River watersheds constitute together the Bow River Basin. The basin has experienced the most significant anthropogenic impact compared with other river basins in Alberta in the last ten years (City of Calgary, 2011). Population has grown by over a quarter of a million people in the basin. Human management highly

affects the natural river flow. Between 2005 and 2009, Calgary regional watersheds have shown a range of good to poor ratings according to the Canadian Council of Ministers of the Environment's (CCME) Water Quality Index (City of Calgary, 2011).

The climate in the basin is characterized by long, cold winters and short, warm summers. Dry westerly Chinook winds can result in as much as a 30 °Celsius change in temperature, and a 40 % change in humidity within a few hours at mid-winter time (BRBC, 2010). Snow accounts for approximately half of annual precipitation in the basin that ranges from 500 to 700 millimetres (BRBC, 2010).

In total, 428 and 408 samples were tested for *Giardia* and *Cryptosporidium* for the Glenmore and Bearspaw WTPs, respectively, in the period from May 2003 to December 2011 (Table 2.1). All parasite testing was performed at the City of Calgary water quality laboratories. The water samples were collected weekly at source water intakes of both WTPs. Method 1623 (U.S. EPA, 2005) was used for the analysis of parasites.

Quality control (QC) criteria were set for parasite testing data and data points were omitted for analysis in any of the following cases:

- No on-going precision recovery (OPR) data was provided for the weekly sample results;
- The OPR was below the acceptable criterion, as defined by US EPA (2005, 2009) – i.e., <22 % recovery for *Cryptosporidium* and < 14 % for *Giardia*;

- The matrix spike recovery (MSR) was below the acceptable criterion, as defined by US EPA (2005) – i.e., <13 % for *Cryptosporidium* and <15 % for *Giardia*;
- No (oo)cysts concentration was provided for a given date.

For the Glenmore WTP, 66 tests for *Giardia* and 94 tests for *Cryptosporidium* did not pass QC criteria (Table 2.1). For the Bearspaw WTP, 76 and 92 tests for *Giardia* and *Cryptosporidium*, respectively, failed QC also. Therefore, 362 *Giardia* and 332 *Cryptosporidium* water samples were available for the analysis for the Glenmore WTP. For the Bearspaw WTP, 334 *Giardia* and 316 *Cryptosporidium* water samples were available for the analysis, respectively.

Table 2.1 Sample data for the Glenmore and Bearspaw WTPs.

WTP	Parasite	Samples tested	Samples failed QC	Samples available for analysis
Glenmore WTP	*Giardia*	428	66	362
	Cryptosporidium		94	332
Bearspaw WTP	*Giardia*	408	76	334
	Cryptosporidium		92	316

The cleansed dataset, as described above, was used for evaluating vulnerability of drinking water treatment plants to parasite contamination based on risk assessment approaches used by; i) AESRD - as described in the operational approval requirements for the City of Calgary), ii) U.S. EPA - according to the LT2 Rule, iii) Health Canada -

according to QMRA approaches referenced in *the Guidelines for Canadian Drinking Water Quality*, *Guideline Technical Document - Enteric Protozoa*: *Giardia and Cryptosporidium* (Health Canada, 2012). Additionally, the data was also used for examining of the association between environmental risk factors and the parasite occurrence. In the context of Health Canada, a QMRA mathematical model developed by this federal ministry was kindly provided, courtesy of Stéphanie McFadyen (Head, Microbiological Assessment Section), and used in the evaluation of parasite risks. Additional adjustment of the data was performed prior to applying it to the Health Canada QMRA model, based on recommendations and discussions with Health Canada:

- Zero values (i.e., non-detects) were replaced with a value of 0.5, in an effort to; a) avoid underestimating the "true" concentration, thus, ensuring a more conservative approach to risk assessment, and b) that the mathematical model was based on a log-normal distribution, therefore requiring integer values;
- In the cases where a matrix spike recovery percent was not available, the arithmetic mean value of the available matrix spike recovery percentages (for all years) was used;
- Parasite concentrations were *corrected* to matrix spike recovery in an effort for counterbalancing loss of (oo)cysts during testing. Concentrations were corrected to matrix spike recoveries with a range of recoveries for the Glenmore WTP: arithmetic mean recovery 45 % (STD=15; min.=15; max.=80) for *Giardia*, arithmetic mean recovery 44 % (STD=16; min.=13; max.=86) for

Cryptosporidium; and for the Bearspaw WTP: arithmetic mean recovery 40 % (STD=15; min.=15; max.=88) for *Giardia*, arithmetic mean recovery 45 % (STD=17; min.=13; max.=85) for *Cryptosporidium*.

These adjustments were specific to the Health Canada model and are not reflected in the other risk assessment approaches (i.e., AESRD or U.S. EPA). In fact, this correction adjustment is not recommended by U.S. EPA under LT2 Rule, based on the argument that approximately 40 % of *Cryptosporidium* oocyst counted on slides appear to be intact and viable, and this fraction can be cancelled out by the approximately 40 % loss in recovery of parasites using Method 1623 (U.S. EPA, 2006).

2.1.2 Specific Aim A2: Collection of meteorological data

Weather monitoring data was obtained from Environment Canada's National Climate Data and Information Archive website (Environment Canada, 2014). Data was recorded at the Calgary International Airport (Latitude: 51°06'50.000" N, Longitude: 114°01'13.000" W, Elevation: 1,084.10 m; Climate ID: 3031093; WMO ID: 71877; TC ID: YYC). The data comprised air temperature (°C [max, min, mean]), snow on ground (cm), total snow (cm) and total rain (mm). All weather data was recorded daily. Corresponding meteorological data was integrated with the database on the parasite monitoring built in EXCEL™ and STATA™ data files.

2.1.3 Specific Aim A3: Derivation of snowmelt and rain run-off calculations

For calculating snowmelt, the method of the U.S. Corps of Engineers was used (U.S. Army Corps of Engineers, 1998). This method recognizes complexity of the snowmelt calculation and attempts to simplify this. The method employs the concept of an "index," where a known variable is used to explain snowmelt in a statistical rather than in a physical sense. Air temperature is an essential variable used for deriving snowmelt estimates. Air temperature is commonly available in historical and real-time databases. The temperature index method can be applied in snowmelt modeling and river forecasting.

The basic equation for the temperature index solution is:

$$M_s = C_m\,(T_a - T_b)$$

where:

M_s = snowmelt, cm. per period s (daily)

C_m = melt-rate coefficient that is often variable, m is centimetre/(degree/period)

T_a = air temperature, °C

T_b = base air temperature, °C.

In the above equation, the melt-rate coefficient (C_m) typically varies between 1.8 and 3.7 cm/°C increase. An arbitrary midpoint value of 2.8 was applied for the current research. The base temperature is typically a value near 0 °C. The maximum daily temperature is used as the index because it is an indicator of cloud cover in the basin (U.S. Army Corps of

Engineers, 1998). The outcome of the explained calculation was an estimate of a layer of snow that was melted.

For inferring an amount of water from snowmelt, it was assumed that a snow to water proportion was 10 to 1. The 10-to-1 rule appears to originate from the results of a nineteenth-century Canadian study. Potter (1965, p. 1) quotes from this study: "A long series of experiments conducted by General Sir H. Lefroy, formerly Director of the Toronto Observatory, led to the conclusion that this relation [10 to 1] is true on the average. It is not affirmed that it holds true in every case, as snow varies in density. . . ." The 10-to-1 rule has persisted, however, despite the almost immediate warnings concerning its accuracy. For the purposes of this thesis, the calculation was enacted only if at least 1 cm of a snow layer on ground was present.

For simplicity, rain run-off (daily measurement) was considered only if the cumulative daily rain was greater than 1 mm.

The 60-day running average of rain run-off (RR60DRA) was used to analyse for relationships between parasite concentration in source water and rain run-off, and to represent source water contamination by (oo)cysts that occurred upstream as the result of rain run-off at any day during the monitoring campaign. A 60-day running average of snowmelt run-off (SMR60DRA) was also used as a predictor of parasites contamination of the source water due to snowmelt run-off.

2.1.4 Specific Aim A4: Database construction and development

Critical to the data collection and analysis, was the development and maintenance of two comprehensive databases to house all relevant

information. All water and weather related variables were compiled into both a Microsoft EXCEL™ database and in STATA™ statistical software database (STATA Corp LP™). One database contained the data of all environmental factors along with corresponding units, their spatial locations, and the parasite occurrence data. Separately, another database was created that contained only the parasite occurrence data and was used for transitional derivation of inputs for the Health Canada QMRA Models. These databases featured extensive data storage capabilities with high flexibility for data analyses.

For the purpose of the analysis, the databases were developed to store the data in two ways. One database was specifically built using combinations of water collection sites and parasite occurrence data with integration of the Environment Canada weather monitoring data. Additional data storage was developed to adapt the parasite occurrence data with the Health Canada QMRA Model. Arranging the database according to these inputs simplified data analysis in the EXCEL™ application and STATA™ statistical software. The details of the databases as follows:

WTP-Collection Site specific database. The EXCEL™ and STATA™ database each was arranged into two worksheets based on the collection site. The data on weather monitoring in proximity to the WTPs was obtained from Environment Canada and was integrated into the database (Air Temperature [°C], Snow on Ground [cm], Total Snow [cm], and Rain [mm]). Water quality information was provided by the City of Calgary

and was integrated into the database for each of the WTPs (Water Turbidity [NTU], Water Temperature [°C]).

The EXCEL™ spreadsheet database was arranged according to the combinations of the site specific and parasite specific information and provided data transition to the Health Canada QMRA Model.

2.2 Objective B: Estimate human health risk using Health Canada QMRA model

To infer a burden of disease to a human population that was exposed to parasites in drinking water, a probabilistic HC QMRA model was used. An estimate of parasite concentration per 100 L of source water was used for the input to the HC QMRA model. The $\log_{10}$ reduction of parasite was set by the user for the HC QMRA model (version 1.0). For the HC QMRA model (version 2.0), the $\log_{10}$ reduction is predetermined by the Health Canada depending on treatment barriers employed as explained in the Literature Review. In the HC QMRA model (version 2.0), the user can set a fraction of infectious organisms. The output was given as an estimate of burden of disease using DALY per person per year. A human health risk was evaluated on the base of the estimate of burden of disease per person per year. The HC QMRA model (version 2.0) is able to provide health risk estimates stochastically; however, upon request of the City of Calgary, the deterministic approach was used, particularly, as mean point estimates of health risk.

2.3 Objective C: Analysis of relationship between parasite concentration in source water with environmental and physical parameters

The Microsoft EXCEL™ spreadsheet database enabled automated statistical analysis between pathogen concentration and environmental factors, physico-chemical water quality variables (i.e., Pearson Correlations, generation of scatter plots, building graphs and diagrams) and allowed for ease of use and integration with STATA™ statistical software for more sophisticated analysis and modeling.

For the purpose of the analysis of relationship between concentration of parasites in source water with environmental factors and source water physical parameters, the recovered parasite concentrations were used. Substitutions for concentrations below detections were not applied. In addition, natural log-transformation of parasite concentrations was performed for the purposes of correlation and multiple regression analysis. To deal with zero values of concentrations during log-transformation, every data point was corrected by adding a value of one.

Arithmetic mean values were used as a measure of central tendency for estimating the average parasite concentrations in source water entering the treatment facilities. According to the U.S. EPA LT2 Rule, Public Water Systems (PWS) that choose to sample at least twice per month over two years (48 samples total) must use the mean of all 48 samples. The U.S. EPA argues that this approach achieves a low false negative rate similar to Maximum Running Annual Average (Max-RAA)

for 24 samples, and, additionally reduces the false positive rate (the likelihood that the total concentration estimate would be overestimated) (U.S. EPA, 2006).

The arithmetic mean is the sum of observations divided by the number of observations (Weisstein, 2013).

$$\bar{x} \equiv \frac{1}{N}\sum_{i=1}^{N} x_i$$

where:

N – is the number of observations

x_i – are measured values

The averaging of point concentrations over a particular period of monitoring was applied in view of the low confidence that one "grab" sample can represent a realistic concentration of parasite in source water. The averaging is also applied in the effort to normalize the variation of concentrations of microorganisms that can commonly be observed in environmental waters providing an aggregated concentration estimate that, as it is believed can provide a statistically supported inference about a true concentration. Simple arithmetic mean or running average were applied depending on circumstances of their application. The Health Canada does not specify which measure of central tendency of parasite concentration in source water should be used.

Scatter plots were used to visualize possible relationships of dependent variables (i.e., point concentration of *Giardia* or *Cryptosporidium* (oo)cysts) with independent variables or what were considered in this research as potential predictors of parasite concentration (source water temperature, source water turbidity,

RR60DRA, SMR60DRA). Parasite monitoring data was plotted against predictors based on the following assumptions and scenarios:

a) Aggregate Data – the entire monitoring period (May 2003 – Dec 2011) was used for assessing correlations between parasite concentration at each of the sites (Bearspaw WTP and Glenmore WTP) with a suite of environmental factors, such as source water temperature, source water turbidity, RR60DRA, SMR60DRA.
b) *Annualized Data* – individual annualized periods (2003, 2004, or 2005, etc.) were used for assessing correlations between parasite concentration at each of the sites (Bearspaw WTP and Glenmore WTP) with a suite of environmental factors, such as source water temperature, source water turbidity, RR60DRA, SMR60DRA. This was done in order to assess the robustness of any associations observed in one year to be generalizable to all years. For example, an increase of rainfall in a given "wet" year may hypothetically result in an increase in parasite concentrations.

For certain comparisons, the t-test statistic was used to compare the means of two independent groups with the null hypothesis stating no difference between means in the two groups. For example, the t-test was performed when assessing *Giardia* concentration with source water temperature and in which parasite concentration data was divided to two groups using a boundary 5 °C of water temperature. The t-test statistic was calculated as follow:

$$t = \frac{\bar{x} - \bar{y}}{s\sqrt{\frac{1}{n} + \frac{1}{m}}}$$

Where:

$\bar{x}$ is arithmetic mean in the first group x_i=1,n

$\bar{y}$ is arithmetic mean in the second group y_j=1,m

and **s** is the pooled sample standard deviation (Helsel & Hirsch, 1992).

2.3.1 Correlation analysis

Correlation analysis is used to assess whether there is an association between an explanatory variable (i.e., environmental factor) and a response variable (i.e., concentration of parasite in water). Source water temperature, source water turbidity, RR60DRA and SMR60DRA were used as the explanatory variables (predictor) for parasite occurrence (i.e. concentrations) in source water (response variable). Correlations between parasite occurrence and explanatory variables were assessed based on the Pearson correlation coefficient, as indicated by the symbol "r". The correlation coefficient lies between -1.00 and +1.00. A measure of +/- 1.00 represents a perfect positive or negative correlation (Helsel & Hirsch, 1992). A value of zero indicates no relationship between variables. The underlying assumptions of the test are that linear relationship between the variables exist, have normally distributed residuals of the data, an equal variance of the residuals, and independence of observations. The Pearson Correlation is mathematically expressed by the following equation:

$$r = \frac{\sum_{i-1}^{n}(Xi - \bar{X})(Yi - \bar{Y})}{(n-1)SxSy}$$

where:

- $X_{i,}\ Y_i$ are the measured variables;
- $\bar{X}, \bar{Y}$, represent their respective means; and
- S_x and S_y represent their respective standard deviations.

(Helsel & Hirsch, 1992)

2.3.2 Trend line analysis

Trend line analysis is a simple technique used for connecting data points into trends to demonstrate behaviour of a studied effect in time. Trend line accommodates the method of moving average. The moving average (trend line) smoothes fluctuations of the data points to show if the data has patterns or trends in time more clearly. A moving average trend line uses a specific number of data points (set by the 'Period' option), averages them, and uses the average value as a point in the trend line. For the trend line analysis of *Giardia* association with environmental factors, a '60 Day Running Average' of the parasite and a '60 Days Running Average' of either rain or snowmelt run-off was used. Correspondence of the trend line that was representing concentration of *Giardia* cysts in source water to the trend line that was representing either rain run-off or snowmelt run-off would indicate that a change in the latter has triggered a change in the former.

2.3.3 Multiple linear regression analysis

Multiple linear regression can be used to model and analyze continuous data containing response (parasite) and explanatory (environmental data) variables. Data on environmental factors X1, X2, …, Xp (RR60DRA, SMR60DRA, water temperature) were used to model a

linear relationship with parasite concentration (Y) in the source water . When predictor variables had zero values, the estimated concentration of parasite is β_0, as described in the equation below. The coefficient β_j is a parameter (numerical value) provided by the model that indicates how much concentration of parasite changes with one unit change of a given variable-predictor adjusting for other explanatory variables.

$$y_i = \beta_0 + \sum_{j=1}^{p} \beta_j \; x_{ij} \; \varepsilon_i$$

Where:

- y_i is the value observed for the dependent variable for observation i,
- x_{ij} is the value taken by variable j for observation i, and
- ε_i is the error of the model.

STATA™ (statistical software) was used to display a number of summary statistics such as the following: parameters of the model, p-values with t-values indicating predictive power, standard error, and confidence interval for each environmental factor-predictor being in the set of used predictors. Output of the F-test (test of significance) and R^2 was used to indicate goodness of the model fit. The R^2, the determination coefficient for the model, ranges in value between zero and one. It is calculated as followed:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} w_i (y_i - \hat{y}_i)^2}{\sum_{i=1}^{n} w_i (y_i - \bar{y}_i)^2}, \text{ where } \bar{y} = \frac{1}{n} \sum_{i=1}^{n} w_i y_i$$

Where:

- y_i is the value observed for the dependent variable for observation i,

- n is the number of observations,
- w_i is the error weight (Helsel & Hirsch, 1992).

The R^2 is interpreted as the proportion of variation of the dependent variable (parasite concentration) that can be explained by the model. When R^2 is equal to one, it is to be said that the predictor variables fully explain variation of the variable of interest. Using multiple predictor variables requires taking into account the influence of a number of predictors, therefore, adjusted R^2 to be used as the best estimate of the goodness of fit.

For the evaluation of a linear model, an interpretation was performed as follows: as closer adjusted R^2 was to one, the better the set of environmental factors was able to predict concentration of parasite at a particular time. When the confidence interval around the standardized coefficients (parameters) includes zero, the predictive potential of the predictive variable is not statistically significant.

The four assumptions of multiple linear regression are: the relationship is linear, the residuals have same variance, the residuals are independent of each other, and the residuals are normally distributed (Helsel & Hirsch, 1992). The linearity assumption is one of the strongest assumptions. It is evaluated using scatterplot with lowess line (in STATA™) and regression line. According to this, the lowess line should be as close as possible to the regression line. The equal variance assumptions is evaluated using scatterplot of the residuals, which should be distributed homogenously. The Breusch-Pagan / Cook-Weisberg test can be used also for checking the equal variance

assumption. The null hypothesis of the test indicates that the variance of the residuals is the same for all values of the independent variable, and failure to reject the null hypothesis satisfies the assumption (Zeileis & Hothorn, 2002). Log-transformation of the response variable is intended to "normalize" the data and to decrease influence of outliers (Rózsa *et al.*, 2000). Also, it was assumed that the samples were taken independent from each other during the sampling campaign.

In the multiple linear regression analysis, the response variable *Giardia* concentration was modeled using the following explanatory variables: a) RR60DRA, b) SMR60DRA, and c) water temperature (°C). The significance of models for each predictor-variable was evaluated based on the above goodness of fit statistics and the most significant predictor-variables were selected for further analysis.

Chapter 3 : Comparative risk assessment of the vulnerability of the City of Calgary's drinking water to contamination with *Cryptosporidium* and *Giardia*

3.1 Introduction

As outlined in Chapter 1 of this thesis, provincial standards for drinking water treatment in Alberta are grounded in both treatment-based (i.e., *Giardia*) and health-based (*Cryptosporidium*) standards. In the U.S., water treatment requirements are primarily focused on human health risks posed by *Cryptosporidium*, and supported by source water monitoring programs such as the LT2 Rule. The U.S. EPA does not require monitoring for *Giardia* since monitoring for *Cryptosporidium* as a reference protozoan is believed to be sufficient to draw conclusions about water treatment measures that would be protective against pathogenic bacteria, viruses, and other protozoa. Consequently, a 3-$\log_{10}$ removal or inactivation level has been enacted against *Giardia* in the U.S. (U.S. EPA, 2006). This health risk-based approach for *Cryptosporidium* developed by the U.S. EPA has been essentially adopted by AESRD, but monitoring for *Giardia* is still required by the City of Calgary under AESRD's drinking water approval requirements. Risk assessments associated with *Giardia* are largely qualitative and focus primarily on incremental achievable treatment standards for control (i.e., a $\log_{10}$ increase in annual running average of parasite occurrence requires a concomitant $\log_{10}$ increase in treatment). Consequently, these different approaches to risk assessment

have led to some disparity in the tolerance levels associated with each of these parasites in drinking water systems. For example, under AESRD's regulatory approval requirements for the City of Calgary, < 1 *Giardia* cyst/100 L of water requires a treatment level of 3-$\log_{10}$ inactivation to be adopted, whereas this same level of treatment is required when *Cryptosporidium* oocyst concentrations are < 7.5 oocysts/100 L of water (See Table 1.3 in Chapter 1). Consequently, under AESRD's current standards there is less *tolerance* for the occurrence of *Giardia* when compared to *Cryptosporidium* within source waters used for drinking, even though *Cryptosporidium* may be harder to inactivate and remove during the water treatment process. AESRD's numerical criteria for parasite occurrence are based on an annual running average with a minimum monthly frequency of sampling and collected over a minimum of two years. The U.S. EPA numerical criteria are also based on a maximum annual running average for utilities taking 24 samples, but for utilities willing to sample on a more frequent basis and for longer periods of time, the arithmetic mean across the entire dataset but not less than 48 samples over two year period, can be used as the most accurate estimate of central tendency (U.S. EPA, 2006) of parasite concentration.

U.S. EPA and AESRD specify a level of treatment corresponding to a particular concentration of parasite in source water, arguing that this would result, on average, in a health burden that would not exceed 1/10,000 person cases of illness annually (U.S. EPA 2006). Health Canada has adopted the approach used by the World Health Organization (WHO), an approach that accommodates a metric of health

risk know as a disability adjusted life year (DALY), targeting 10^{-6} DALY per person annually as the acceptable threshold of health burden (Health Canada, 2012). Consequently, these agencies have different methodologies in estimating the health risk endpoint.

This chapter attempts to examine the vulnerability of the City of Calgary's WTPs to *Cryptosporidium* and *Giardia* contamination and assess the consequential microbial risks based on the risk assessment approaches used by the U.S. EPA, AESRD, and Health Canada.

3.2 Results and Discussion

3.2.1 Overview of parasite occurrence and prevalence

Parasite prevalence, for the purpose of this thesis, was defined as the proportion of water samples testing positive for *Cryptosporidium* and/or *Giardia*. Prevalence of *Giardia,* was in general, much higher than prevalence of *Cryptosporidium* in both water treatment plants [WTP] (Figure 3.1), with an overall prevalence (i.e., across all years) of 71 % and 85 % in the Glenmore and Bearspaw source waters, respectively (Tables 3.1; 3.2). For the Glenmore WTP (i.e., Elbow River), the overall mean *Giardia* cyst concentration across all years was 23 cysts/100 L (STD=37), with a maximum concentration 340 cysts/100 L recorded on February 01, 2004. For the Bearspaw WTP (i.e., Bow River), the overall mean concentration across all years was 31 cysts/100 L (STD=40) with the maximum concentration of 330 cysts/100 L recorded on October 17, 2011. The highest mean *Giardia* concentration in any given year was 56 cysts/100 L (STD=74) observed in 2003. The distribution of *Giardia* concentrations was strongly skewed to the right for both the Glenmore

and Bearspaw WTPs (Figures 3.2 and 3.3), indicating a log-normal distribution of the parasite in water samples, consistent with the general notion of how microorganism are usually distributed in the environment (Haas, 1983; Hirano *et al.* 1982, Loper *et al.* 1984; Biondini, 1976; Limpert *et al.*, 2001).

Prevalence for *Cryptosporidium* was 16 % and 25 % for water samples collected at the Glenmore and Bearspaw WTPs, respectively (Tables 3.1 and 3.2) (Figures 3.2 and 3.3). For the Elbow River at Glenmore, the overall mean concentration across all samples was 1.2 oocysts/100 L (STD=3.7) with a maximum concentration of 31 oocysts/100 L recorded on January 20, 2008 (Table 3.1). For the Bow River at the Bearspaw WTP, the overall mean concentration of *Cryptosporidium* was approximately twice as high as that observed for the Elbow River (2.2 oocysts/100 L; STD=6.9) with a maximum concentration of 40 oocysts/100 L recorded on October 11 and 28, 2008 (Table 3.2). Similar to that observed for *Giardia*, the given distribution of *Cryptosporidium* concentrations were strongly skewed to the right for both sites, indicating a log-normal distribution of *Cryptosporidium* oocysts in source water.

Parasite concentrations fluctuated at both sites during every year and across separate years for *Giardia* but were relatively stable for *Cryptosporidium* (Tables 3.1; 3.2). For the Glenmore WTP, higher concentrations of *Giardia* were observed during winter-spring seasons and lower concentrations were observed during the summer-fall seasons, displaying a “cyclic” pattern of prevalence across the 8-year sampling

period (Figure 3.4). The highest *Giardia* cyst concentrations were recorded during the winter season of the years 2003-2004 and during the winter-spring season of the year 2010. For the Bow River at the Bearspaw WTP, similar seasonal fluctuations of *Giardia* concentrations were observed from 2003-2007, with increasing parasite concentrations occurring in the winter-spring season and with decreased parasite concentrations observed during the summer-fall season (Figure 3.5). However, after 2007, an apparent gradual increase in *Giardia* cyst concentrations was observed at the Bearspaw WTP (Figure 3.5), and with the highest observed cyst concentrations occurring in the fall of the year 2011. Interestingly, this dominant winter/spring pattern of *Giardia* occurrence that was observed between 2003 and 2007 at the Bearspaw WTP shifted towards a summer/fall pattern of predominance as of 2008 and remained this way until the end date of the monitoring period (i.e., 2011). *Cryptosporidium* was, on occasion, detected in the early fall (Figures 3.4; 3.5), but long-term monitoring revealed neither a stable pattern nor a consistent trend in the occurrence of the parasite for any season (Figures 3.4; 3.5), and with the vast majority of water samples having no detectable *Cryptosporidium* (Figures 3.2; 3.3).

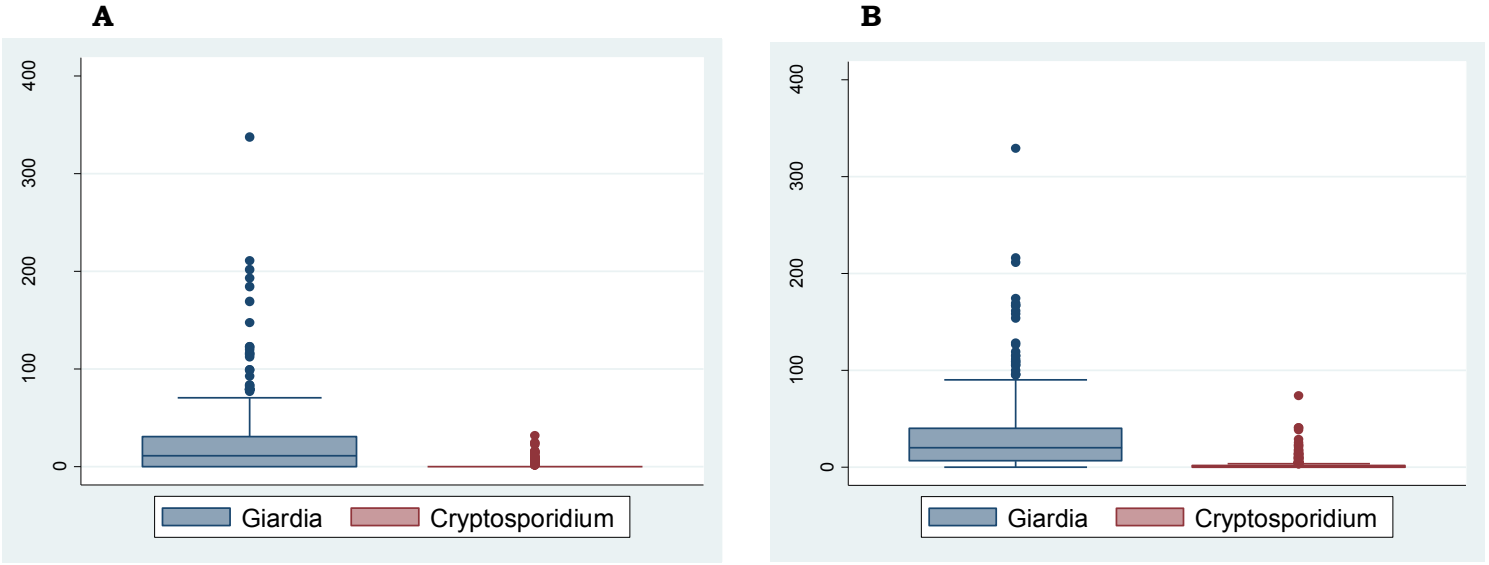


Figure 3.1 Boxplots depicting overall parasite concentrations from Glenmore WTP (Panel A) and Bearspaw WTP (Panel B). Data represents the aggregate across the 2003-2011 sampling campaign. Boxplots reflect median±25 -75th percentiles (colored boxes) with whiskers representing1.5*interquartile range. Interquartile range is a distance between 25 th - 75th percentiles. Concentrations were corrected to matrix spike recoveries.

Table 3.1 Descriptive statistics related to parasite occurrence (*Giardia* and *Cryptosporidium*) within water samples collected from the Glenmore Reservoir at the Glenmore WTP.

Giardia						
Monitoring period (years)	Observations (N)	Mean (cysts/ 100 L)*	Std. Dev.	Minimum (cysts/ 100 L)	Maximum (cysts/ 100 L)	Prevalence (%)
2003-2011	362	23	37	0	340	71
2003	42	56	74	0	340	73
2004	38	20	19	0	83	86
2005	41	12	18	0	79	63
2006	41	10	16	0	55	51
2007	43	9.5	16	0	63	48
2008	41	18	27	0	99	46
2009	43	26	37	0	170	79
2010	73	30	29	1.7	150	100
Cryptosporidium						
Monitoring period (years)	Observations (N)	Mean (oocysts/ 100 L)*	Std. Dev.	Minimum (oocysts/ 100 L)	Maximum (oocysts/ 100 L)	Prevalence (%)
2003-2011	332	1.1	3.7	0	32	16
2003	39	2.4	4.8	0	22	25
2004	32	0.4	1.7	0	9	6
2005	37	0.9	2.2	0	11	21
2006	34	0	0	0	0	0
2007	41	1.7	5.2	0	31	19
2008	37	1.6	5.3	0	24	10
2009	39	0.2	0.9	0	5	7
2010	73	1.4	3.8	0	23	26

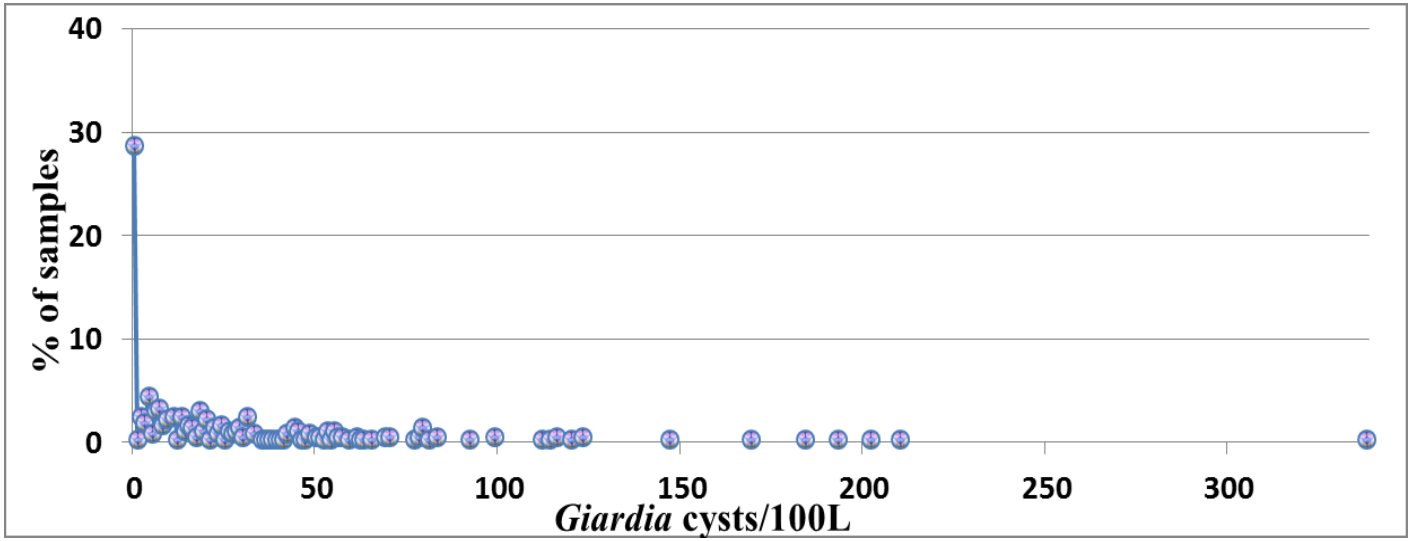
*Concentrations were corrected to matrix spike recoveries. A range of recoveries for *Giardia*: arithmetic mean recovery 45 % (STD=15; min. =15; max. =80); for *Cryptosporidium*: arithmetic mean recovery 44 % (STD=16; min. =13; max. =86).

Table 3.2 Descriptive statistics related to parasite occurrence (*Giardia* and *Cryptosporidium*) within water samples collected from the Bow River at the Bearspaw WTP.

Giardia						
Monitoring period (years)	Observations (N)	Mean (cysts/ 100 L)*	Std. Dev.	Minimum (cysts/ 100 L)	Maximum (cysts/ 100 L)	Prevalence (%)
2003-2011	334	31	40	0	330	85
2003	42	19	21	0	95	83
2004	38	21	19	3	100	79
2005	38	21	29	0	100	76
2006	30	10	9.0	0	31	73
2007	33	3.9	5.7	0	21	42
2008	46	33	38	0	210	76
2009	41	44	40	5	220	100
2010	66	65	56	0	330	98
Cryptosporidium						
Monitoring period (years)	Observations (N)	Mean (oocysts /100 L)*	Std. Dev.	Minimum (oocysts/ 100 L)	Maximum (oocysts/ 100 L)	Prevalence (%)
2003-2011	316	2.2	6.9	0	73	25
2003	41	0.9	2.7	0	13	14
2004	32	0.9	1.4	0	4	37
2005	35	1.3	4.1	0	22	17
2006	22	0.2	1.1	0	5	4
2007	34	0.8	2.2	0	10	14
2008	43	3.2	10	0	40	11
2009	37	0.8	1.8	0	9	24
2010	72	5.4	10	0	73	51

*Concentrations were corrected to matrix spike recoveries. A range of recoveries for *Giardia*: arithmetic mean recovery 40 % (STD=15; min. =15; max. =88); for *Cryptosporidium*: arithmetic mean recovery 45 % (STD=17; min. =13; max. =85).

A



B

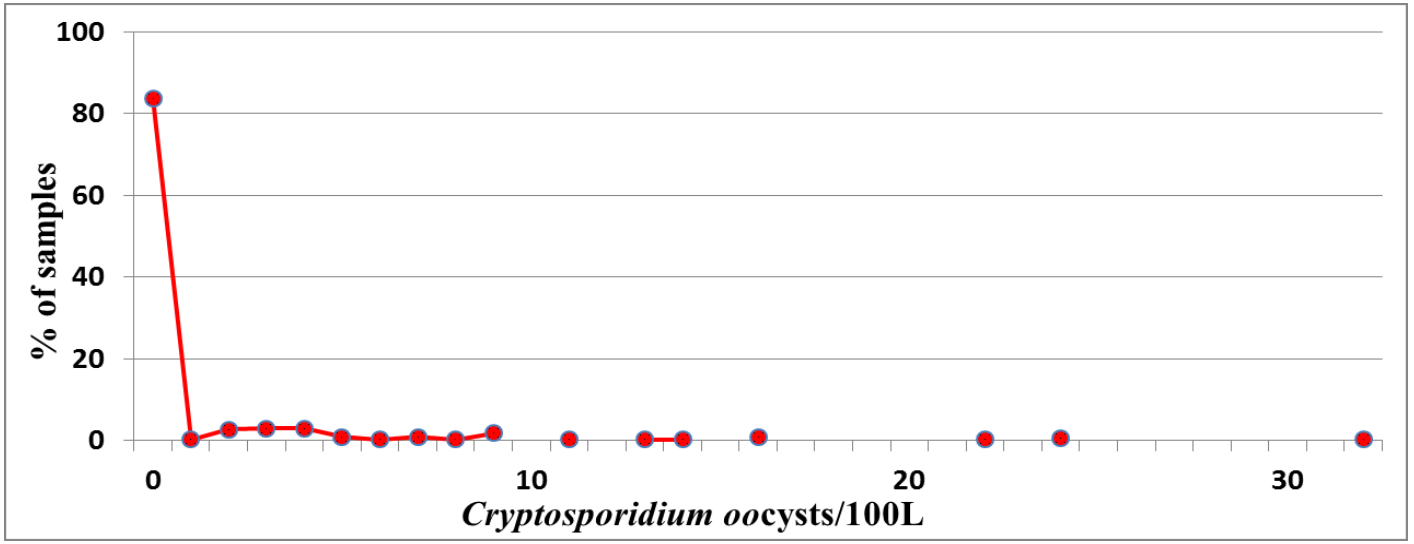
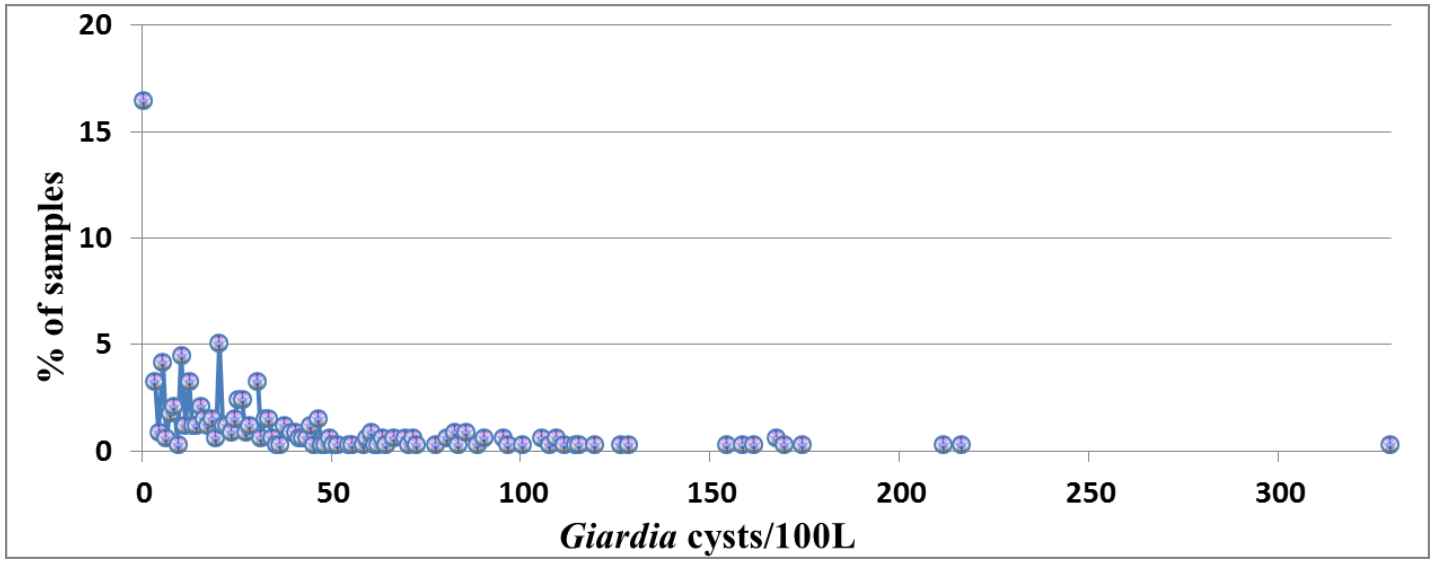


Figure 3.2 Frequency distribution of *Giardia* cyst (Panel A) and *Cryptosporidium* oocyst (Panel B) concentrations in water samples collected from the Glenmore Reservoir at the Glenmore WTP (2003-2011). Concentrations were corrected to matrix spike recoveries.

A



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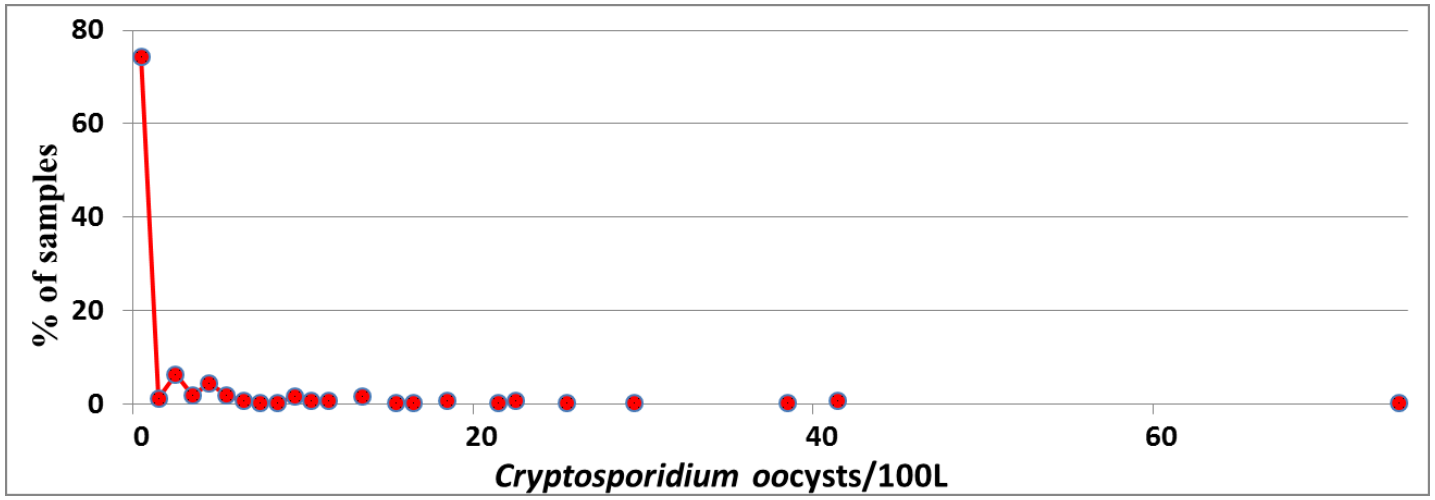

Figure 3.3 Frequency distribution of *Giardia* cyst (Panel A) and *Cryptosporidium* oocyst (Panel B) concentrations in water samples collected from the Bow River at the Bearspaw WTP (2003-2011). Concentrations were corrected to matrix spike recoveries.

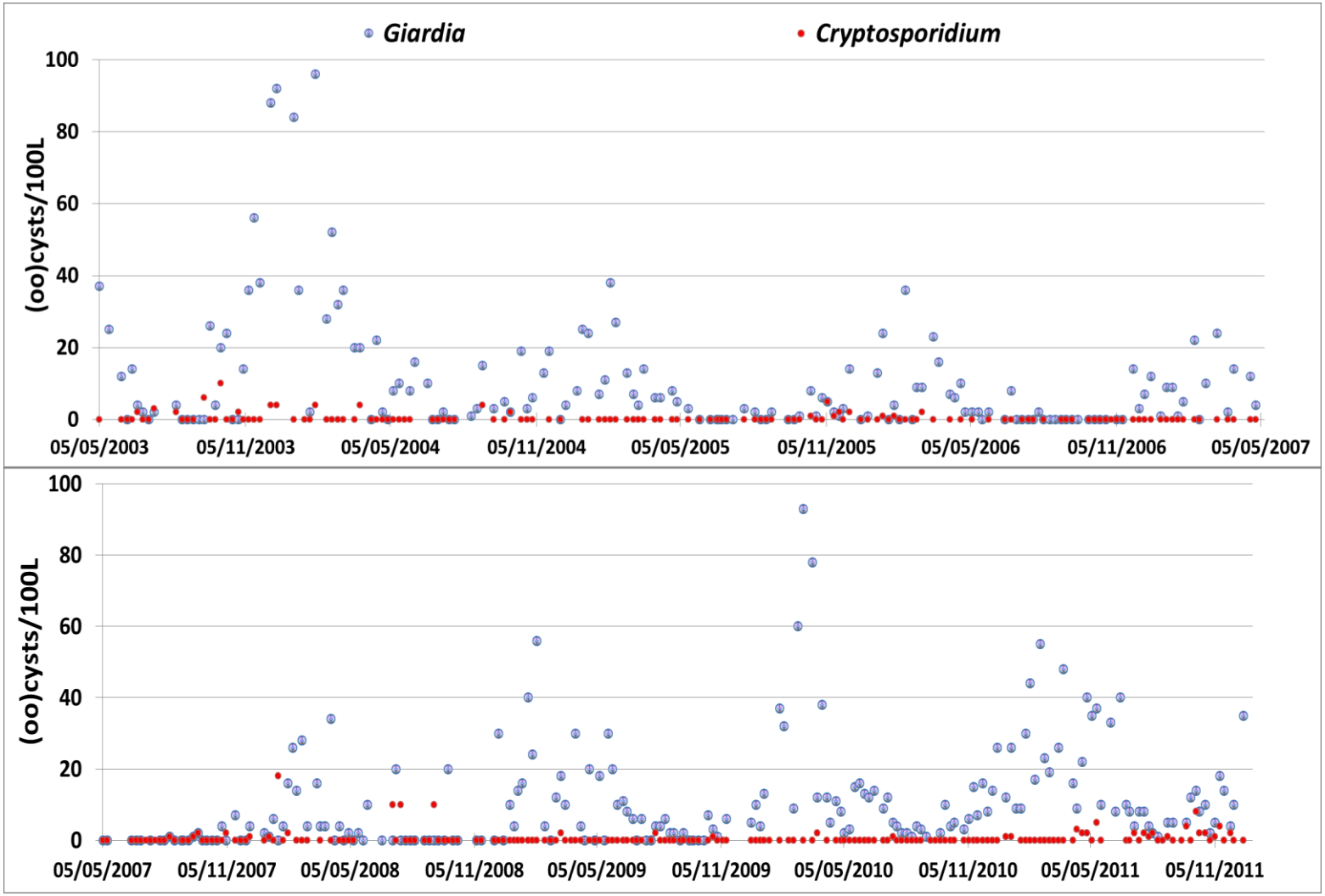

Figure 3.4 Scattergram depicting parasite concentrations recorded at the Glenmore WTP and plotted with respect to the date of testing. The graphs reflect the years 2003-2007 (top panel) and 2007-2011 years (bottom panel). Dates on the X-axis are entered as 'dd/mm/year'. Presented parasite concentrations on this diagram were not corrected to matrix spike recoveries.

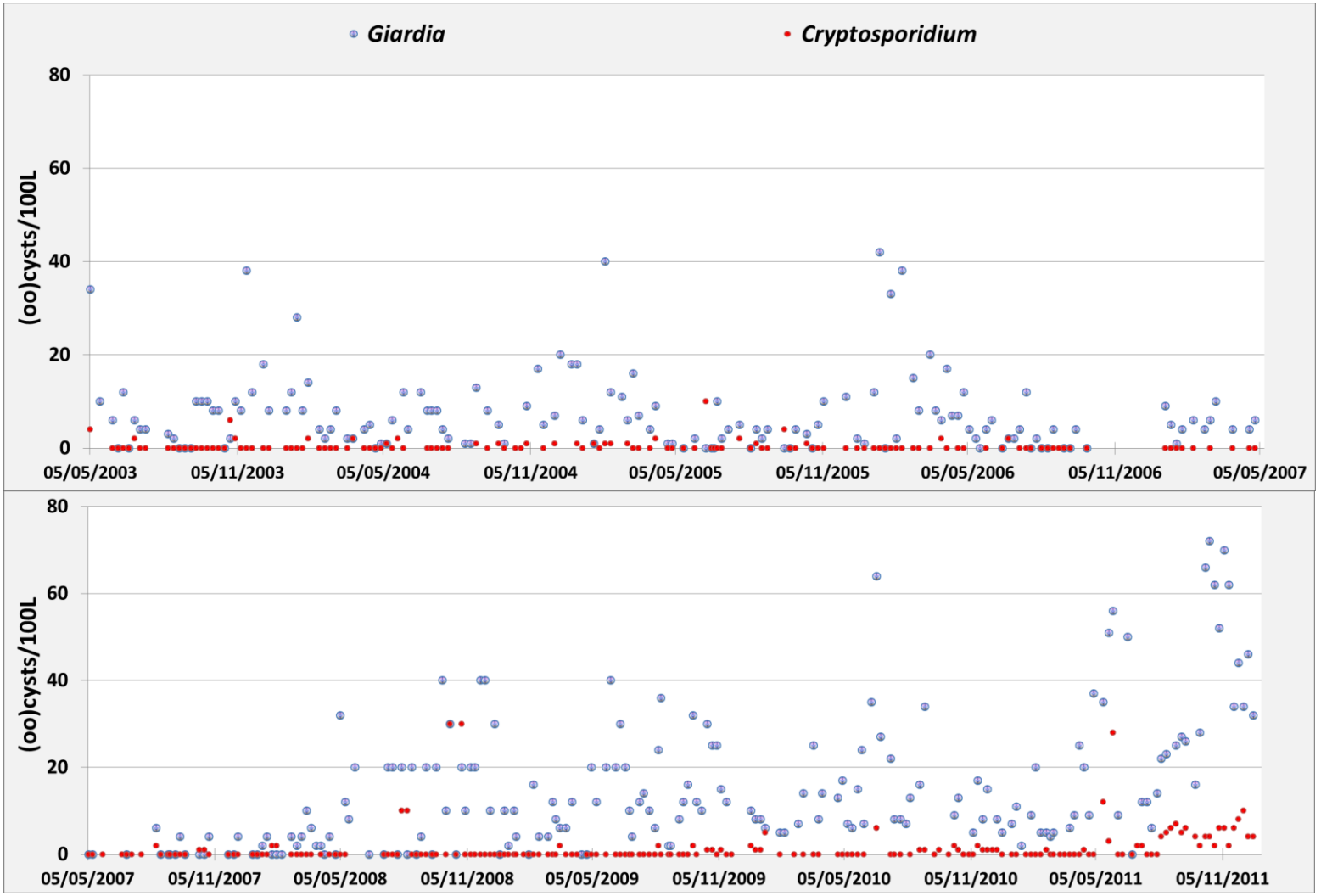

Figure 3.5 Scattergram depicting parasite concentrations recorded at the Bearspaw WTP and plotted with respect to the date of testing. The graphs reflect the years 2003-2007 (top panel) and 2007-2011 years (bottom panel). Dates on the X-axis are entered as 'dd/mm/year'. Presented parasite concentrations on this diagram were not corrected to matrix spike recoveries.

3.2.2 Evaluating health risks associated with parasite occurrence based on existing regulatory standards or guidelines

3.2.2.1 Evaluating water treatment plant vulnerability to waterborne parasites based on AESRD's requirements

Giardia occurrence and risks at both the Glenmore and Bearspaw WTPs, in the context of AESRD's drinking water approval requirements for the City of Calgary (i.e., annual running average), was evaluated. Although monitoring was done over nine years, a running annual average was calculated across the composite seven years in order to ascertain how the annual running average fluctuated during this time period, and for the purposes of understanding how robust the annual average is as a measure of central tendency over the course of longer monitoring periods. Analysis of parasite occurrence data demonstrated that both WTPs would be required to achieve a 5-$\log_{10}$ reduction in *Giardia* concentrations for finished drinking water quality (Figures 3.6 and 3.8, respectively), as specified by an annual running average over a two year period exceeding 10 cysts/100 L of water for *Giardia*, but only based on annual running averages observed before 2005 and after 2010. The data suggest that in the event that the City of Calgary implemented a 2-year monitoring program for parasites between May 2005 to November 2010 (as specified in U.S. EPA LT2 Rule and in AESRD regulations), an annual running average of <10 cysts/100 L would have been observed and would require treatment for only 4-$\log_{10}$ against *Giardia*. Although not shown, concentrations above 10 cysts/100 L were recorded 51 % of the

time with respect to all samples for the Glenmore WTP and 65 % of the time for the Bearspaw WTP (Figures 3.2; 3.3). These data suggest that parasite monitoring programs implemented over a two year period may underestimate parasite concentration and that longer monitoring periods may be more accurate in evaluating and accounting for true prevalence and temporal fluctuations in parasite concentration.

Similarly, an evaluation of *Cryptosporidium* occurrence at both the Glenmore and Bearspaw WTPs, and in the context of AESRD drinking water approval requirements for the City of Calgary, demonstrated that both of the WTPs fall within the requirement to achieve a 3-$\log_{10}$ reduction in *Cryptosporidium* concentrations for finished drinking water quality (Figures 3.7 and 3.9). In all water samples tested for *Cryptosporidium*, although not shown, only 5.1 % and 8.9 % of samples for the Glenmore and Bearspaw WTPs, respectively, had *Cryptosporidium* concentration above 7 oocysts/100 L (Figure 3.2; 3.3). It is important that both U.S. EPA and AESRD's latest regulations designate an equivalency mean concentration of 7.5 oocysts/100 L (i.e., for U.S. EPA the value is set at 0.075 oocyst/L) as a threshold for enacting 4-$\log_{10}$ oocysts reduction, based on the correspondingly applicable average concentration estimate to achieve a < 1 infection per 10,000 risk.

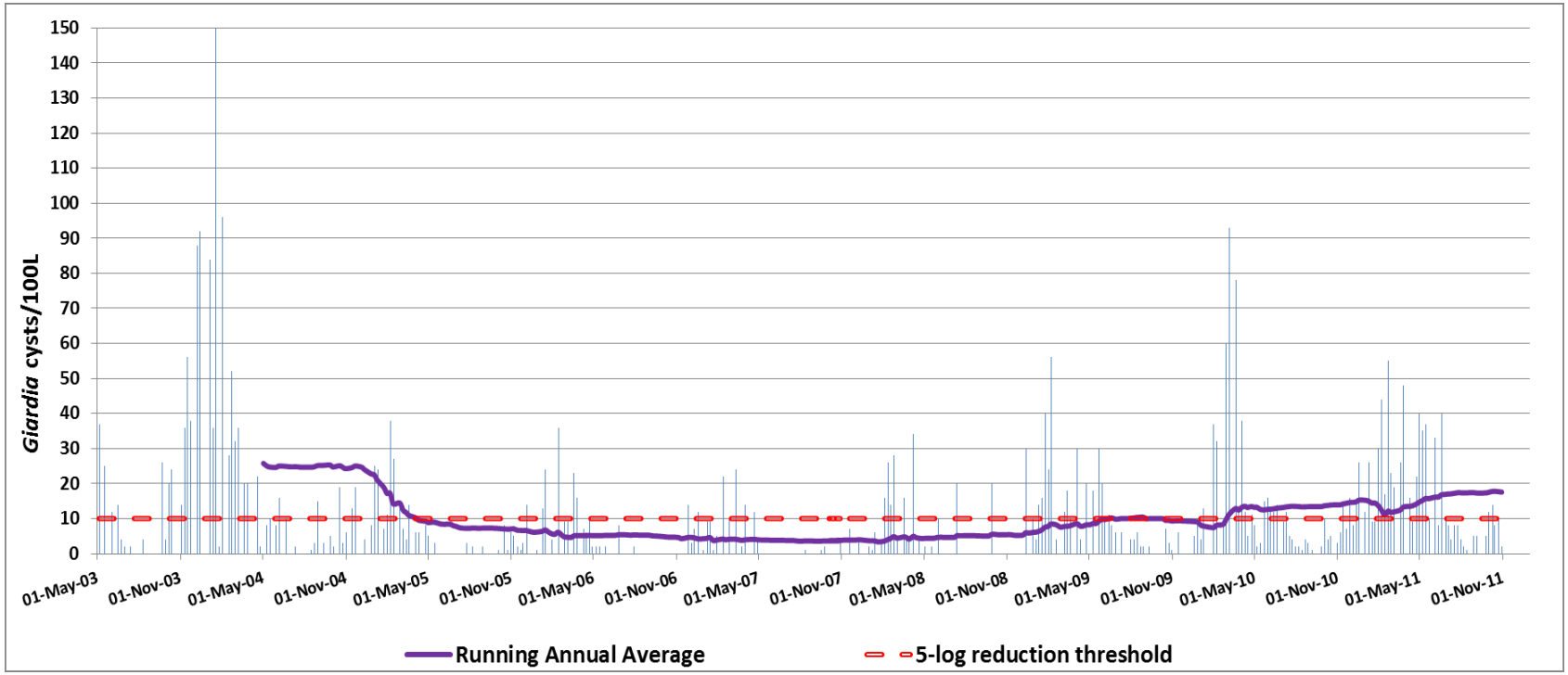

Figure 3.6 *Giardia* cyst occurrence at the Glenmore WTP based on a running annual average (purple line) and in conjunction with AESRD's treatment requirements for the City of Calgary. The dashed line represents AESRD's 5-$\log_{10}$ implementation threshold for treatment control of *Giardia*.

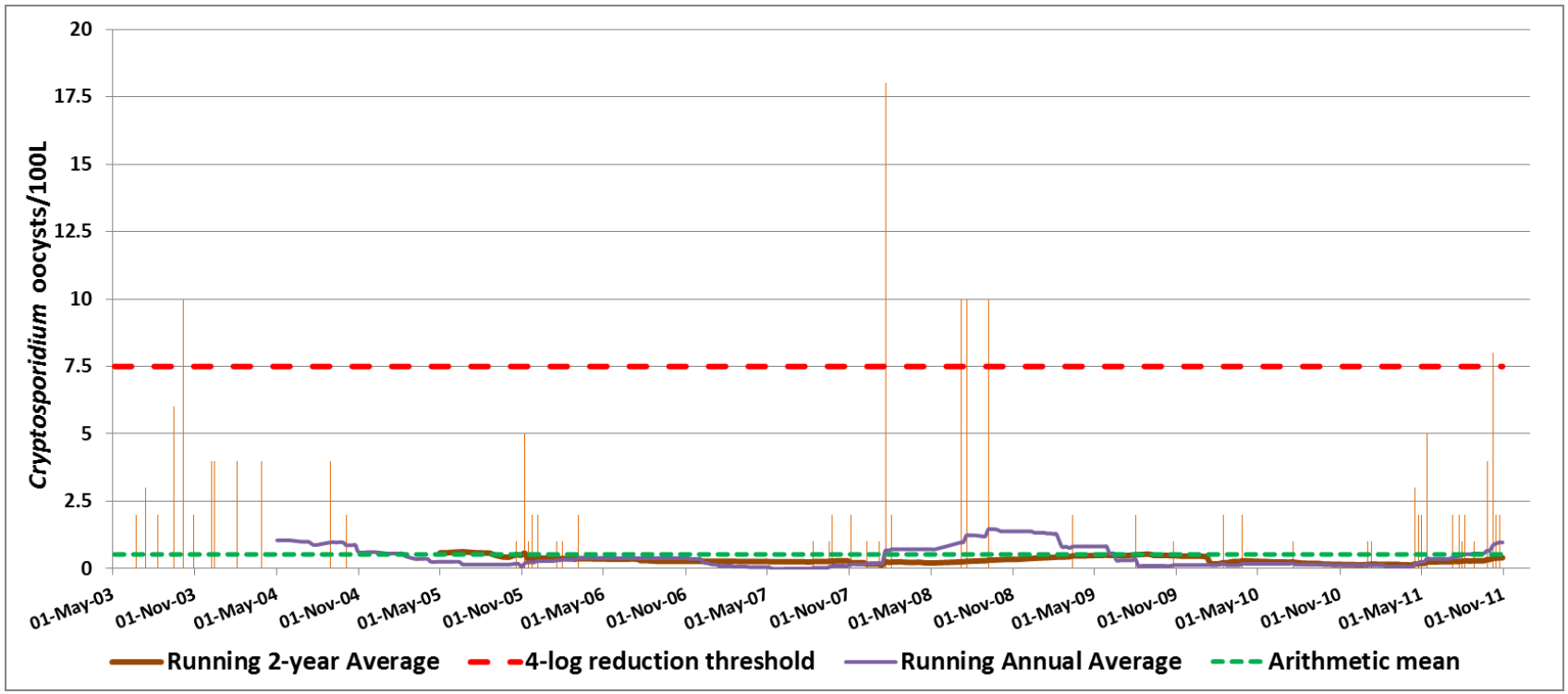

Figure 3.7 *Cryptosporidium* oocyst occurrence at the Glenmore WTP based on a running annual average (purple line) and in conjunction with AESRD's treatment requirements for the City of Calgary. The dashed line represents AESRD's 4-$\log_{10}$ implementation threshold for treatment control of *Cryptosporidium*.

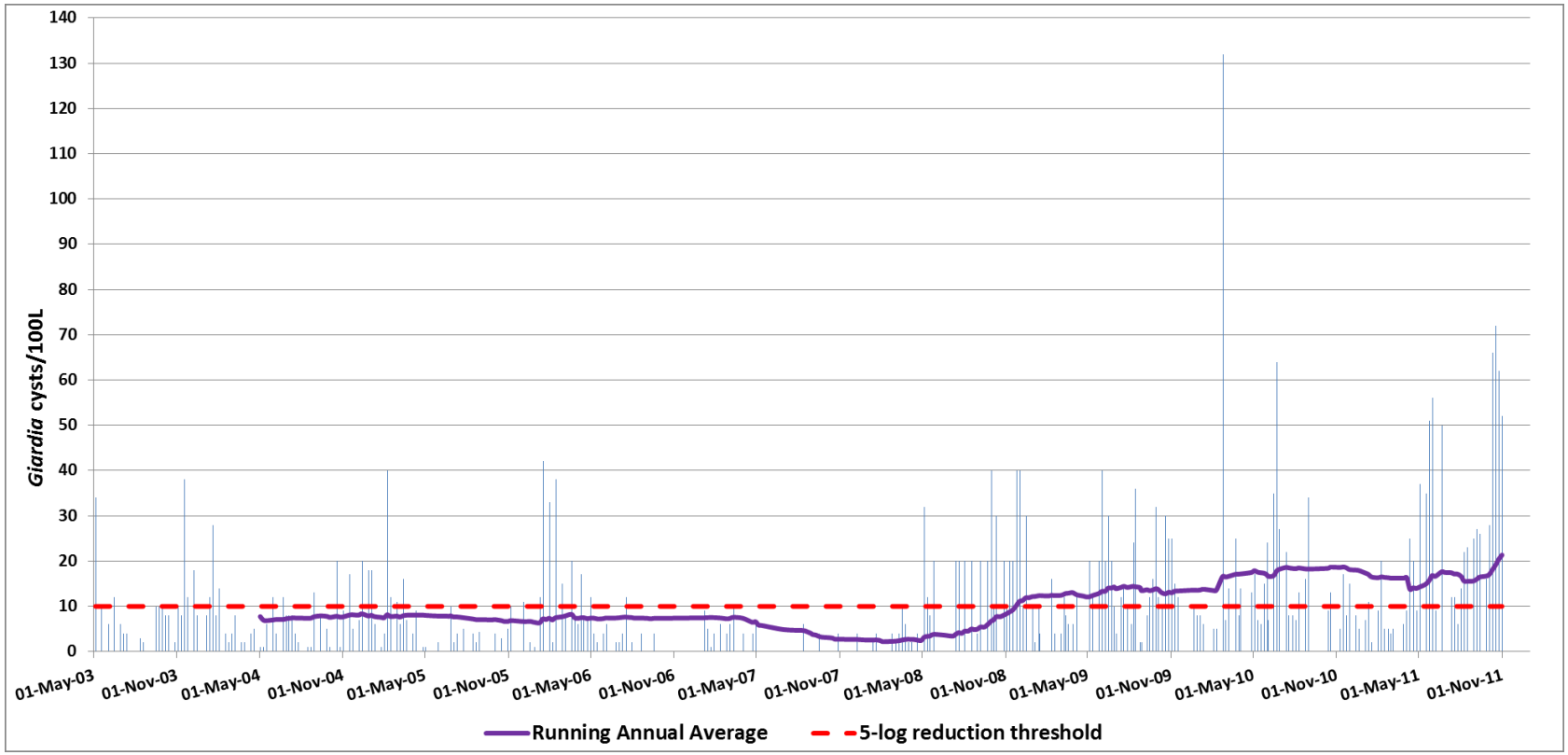

Figure 3.8 *Giardia* cyst occurrence at the Bearspaw WTP based on a running annual average (purple line) and in conjunction with AESRD's treatment requirements for the City of Calgary. The red dashed line represents AESRD's 5-$\log_{10}$ implementation threshold for treatment control of *Giardia*.

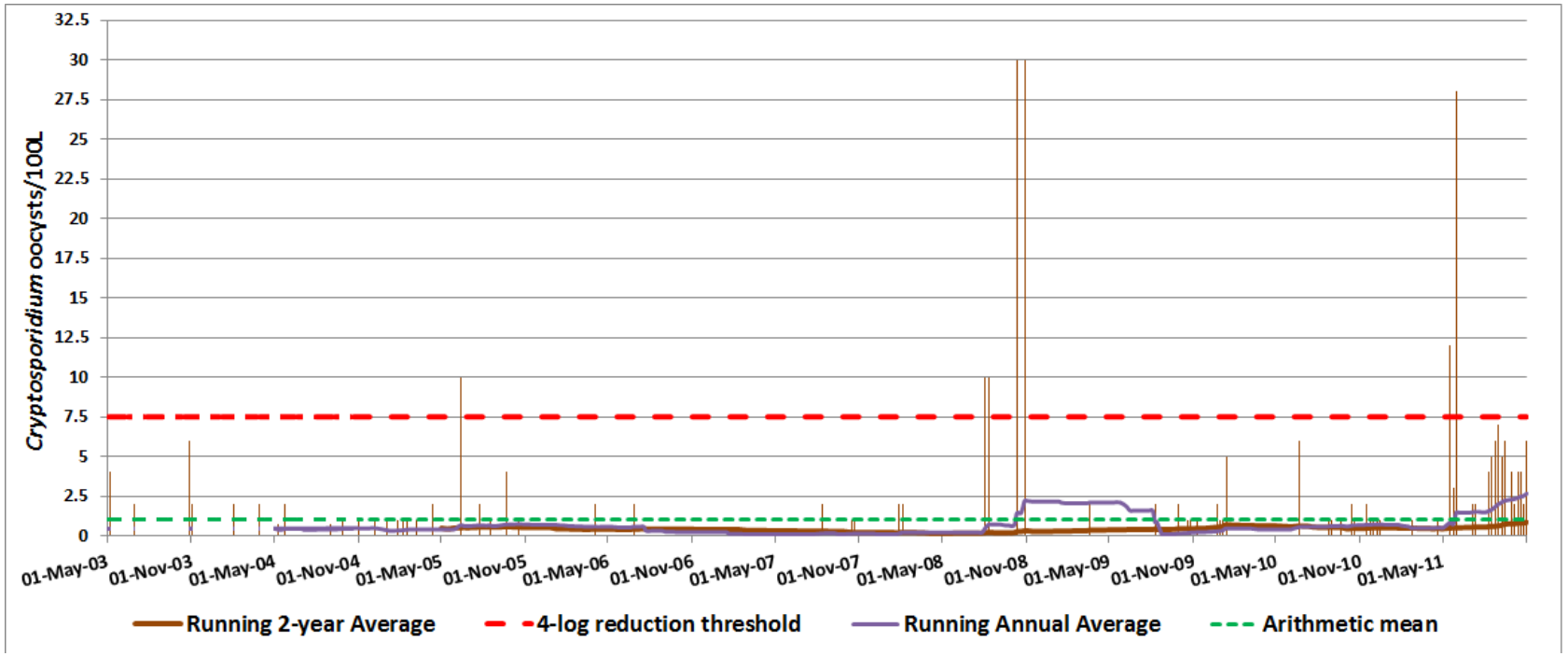

Figure 3.9 *Cryptosporidium* oocyst occurrence at the Glenmore WTP based on a running annual average (purple line) and in conjunction with AESRD's treatment requirements for the City of Calgary. The red dashed line represents AESRD's 4-$\log_{10}$ implementation threshold for treatment control of *Cryptosporidium*.

3.2.2.2 Evaluating water treatment plant vulnerability to waterborne parasites based on the U.S. EPA's LT2 Rule risk framework

The U.S. EPA's analysis of ICR data (U.S. EPA, 1996a), and as reflected within the LT2 Rule, requires that the arithmetic mean be used as a measure of central tendency to estimate parasite concentration. Under the LT2 Rule (U.S. EPA, 2006), WTPs serving >10,000 people (such as the City of Calgary) are required to monitor *Cryptosporidium* on a monthly basis over the course of 2 years (24 minimum sample size but <48 in total), with the highest arithmetic mean of all sample concentrations in any 12 consecutive months used in the bin categorization for the utility (i.e., annual running average). For water utilities in which 48 samples or more are analyzed over two years (i.e., weekly/bi-weekly), the *Cryptosporidium* bin concentration is calculated based on the arithmetic mean of all sample concentrations. The LT2 Rule stipulates that a minimum of two years of monitoring is required for these utilities.

Since the City of Calgary's data comprised nine years of continuous monitoring, the data was subjected to analysis by examining: a) an annual running average across all nine years, and b) a 2-year running average across all nine years. The rationale for this approach was to determine the variability in mean parasite concentrations across all incremental 2-year monitoring periods throughout the nine years of data, and consequently the stability in bin categorization across all nine years. In addition, and according to the LT2 Rule, for utilities monitoring more than 48 samples, an arithmetic mean of parasite occurrence across all nine years of

sampling was also used for bin categorization, and compared to other previous mean values.

Mean *Cryptosporidium* concentrations did not exceed 0.025 oocysts/L in any 2-year monitoring period (annual running average and annual 2-year running average) at either the Glenmore WTP or the Bearspaw WTP, meeting the lowest bin category (i.e., Bin 1) and consequently requiring both WTPs to meet only a 3-$\log_{10}$ treatment requirement for *Cryptosporidium* removal/inactivation (i.e., that achieved by conventional filtration alone) (Figures 3.7 and 3.9). The overall mean concentration of parasites across all nine years was 1.1 (STD=3.7) oocysts/100 L and 2.2 (STD=6.9) oocysts/100 L for the Glenmore and Bearspaw WTPs, respectively (Tables 3.1 and 3.2), and adhered to the Bin 1 categorization of treatment risk under the LT2 Rule.

3.2.2.3 Evaluating water treatment plant vulnerability to waterborne parasites based on Health Canada's risk framework

Unlike the U.S. EPA's LT2 Rule risk assessment framework that focuses on *Cryptosporidium* only, Health Canada stipulates an acceptable health risk target for both *Cryptosporidium* and *Giardia,* targeting a value of 10^{-6} DALY per person per year. The estimated health risk, across all years, was well below this target for either of the parasites at both the Glenmore and Bearspaw WTPs (Figures 3.10; 3.11). Interestingly, although *Giardia* was quite prevalent in both rivers, the health risk estimates for this parasite was, in most cases, well below 10^{-9} DALY per person per year in any given year for the Glenmore or Bearspaw WTPs (Figures 3.10 and 3.11). Meanwhile, the overall estimated $\log_{10}$ treatment reduction against *Giardia* was above

6-$\log_{10}$ for both plants. For the Bearspaw WTP, health risk estimates for *Giardia* were a little higher than for the Glenmore WTP but still very low, with the highest values observed as 1.2×10^{-9} and 1.2×10^{-9} DALY per person per year in the years 2009 and 2011, respectively (Figure 3.11).

Unlike the risk framework proposed by AESRD, the estimated health risk from *Cryptosporidium* was higher than the health risk posed by *Giardia* within both river water sources, despite the low prevalence and the very low concentrations of *Cryptosporidium* oocysts observed in these source waters. Less than ten samples returned concentrations higher than 10 oocysts/100 L over nine years of monitoring (Figure 3.2). The DALY risk estimates provided above for *Giardia* were based on Health Canada Quantitative Risk Assessment (HC QMRA) model version 1.0, in which 3-$\log_{10}$ was used as the treatment estimates for conventional filtration, and with these treatment estimates similar to those used under the AESRD approvals program and the U.S.EPA LT2 Rule. However, under the HC QMRA model version 2.0, estimated $\log_{10}$ reduction against *Cryptosporidium* were increased from 3-$\log_{10}$ to 4.3-$\log_{10}$ for conventional filtration and based on the operational criteria achieved by both plants. For the Glenmore WTP, the highest estimates of risk from *Cryptosporidium* were in 2003 and 2011 evaluated at, 2.6×10^{-7} and 3.3×10^{-7} DALY per person per year respectively using the HC QMRA model version 1.0, and 1.1×10^{-8} and 1.5×10^{-8} DALY per person per year using the HC QMRA model version 2.0 (Figure 3.10). For the Bearspaw WTP, the health risk estimates for *Cryptosporidium* were comparable to the health risk estimates for the Glenmore WTP and were $9.5\times10^{-7}$and

4.6×10^{-8} DALY per person per year in 2011 (the highest observed mean concentration in the given years) when using versions 1.0 and 2.0 of the HC QMRA models, respectively (Figure 3.11).

The difference in risk estimates observed between the two parasites, as characterized under Health Canada's model rests on the fact that chlorine is ineffective against *Cryptosporidium* whereas it is reasonably effective against *Giardia*. Efficiency of water treatment against *Cryptosporidium* is purely dependent on removal of oocysts through conventional filtration. Modelling *Giardia's* health risk, the average $\log_{10}$ inactivation by chlorine was calculated for every separate year, and was combined with the 3.5-$\log_{10}$ removal credit received for conventional filtration. The final treatment credits against *Giardia* resulted in a constant 6.5-$\log_{10}$ cysts reduction for the Glenmore WTP and from 6.1 to 6.4-$\log_{10}$ cysts reduction for the Bearspaw WTP. In fact, treatment performance can vary according to the site specific settings and treatment condition (Hijnen & Medema, 2010). The impact of treatment performance on health risk estimates will be discussed further in Chapter 4 of this dissertation.

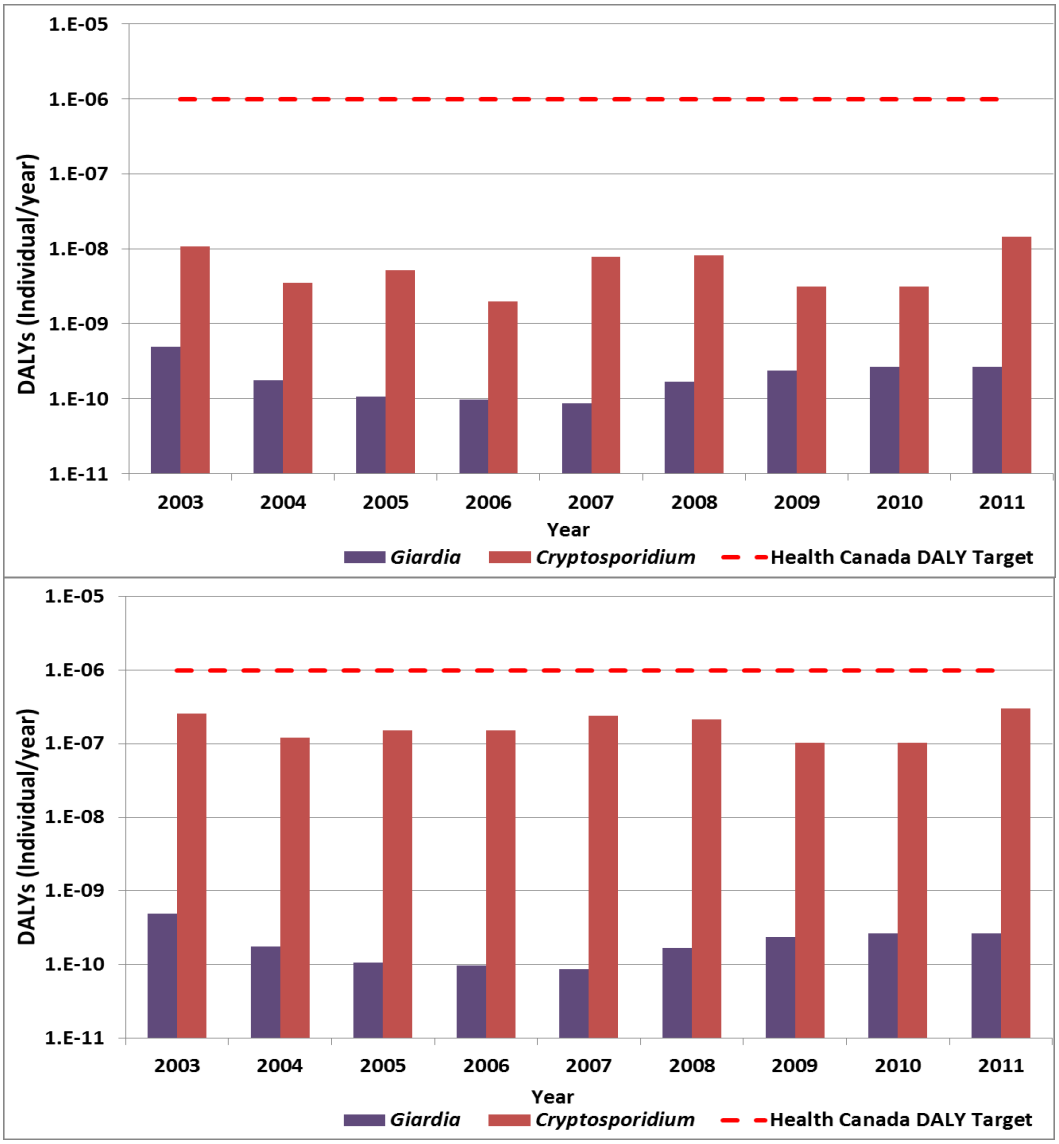

Figure 3.10 Annualized health risk estimates for *Giardia* (both panels using version 1.0 HC QMRA model) and for *Cryptosporidium* (version 1.0 HC QMRA model [lower panel] and version 2.0 HC QMRA model [upper panel]) in drinking water produced at the Glenmore WTP.

Figure 3.11 Annualized health risk estimates for *Giardia* (both panels using version 1.0 HC QMRA model) and for *Cryptosporidium* (version 1.0 HC QMRA model [lower panel] and version 2.0 HC QMRA model [upper panel]) in drinking water produced at the Bearspaw WTP.

For comparative purposes, a reverse QMRA was performed to determine the mean concentration of *Cryptosporidium* and *Giardia* (oo)cysts that would need to be observed within source waters of the Elbow and Bow River in order to violate Health Canada's risk target of 10^{-6} DALYs per person per year, and given the treatment criteria currently available at the Glenmore and Bearspaw WTPs. Outcomes of this analysis are provided in Table 3.3.

The data suggests that the currently available water treatment processes at the Glenmore and Bearspaw WTPs, hypothetically, can cope with much higher concentrations of *Giardia* compared to the concentrations commonly observed in environmental waters (Table 3.3). For example, the highest mean annual *Giardia* concentration of 56 cysts/100 L was observed in 2003 for the Glenmore WTP, but a reverse QMRA analysis on plant performance suggested that the plant could handle *Giardia* mean concentrations up to 114,000 cysts/100 L based on the HC QMRA model 1.0. These high concentrations of *Giardia* have never been reported for environmental waters. For example, during the Sydney drinking water quality crisis in 1998, the highest *Giardia* concentration reported was 7 620 cysts/100 L in source water (Cox *et al.*, 2003). Against *Cryptosporidium*, the protection was not as overwhelming but still sufficient against the concentrations observed during the time of monitoring. The Glenmore WTP could handle mean concentrations of 13 and 256 *Cryptosporidium* oocysts/100 L in the source water according to the HC QMRA model 1.0 and 2.0, respectively. The highest annual average concentration estimate observed during the entire monitoring campaign was 2.4 oocysts/100 L in 2003.

Table 3.3 Estimated tolerable mean concentrations of parasites in source water as determined by reverse QMRA of the HC QMRA model and with respect to treatment criteria currently available at the Glenmore and Bearspaw WTPs*.

WTP	Parasite	Health Canada QMRA Model	Available $\log_{10}$ reduction capability based on HC QMRA model version used	Maximum mean concentration of (oo)cysts tolerated based on reverse QMRA
Glenmore WTP	*Cryptosporidium*	Version 1.0	3	13
		Version 2.0	4.3	256
	Giardia	Version 1.0	6.5	114,000
Bearspaw WTP	*Cryptosporidium*	Version 1.0	3	13
		Version 2.0	4.3	256
	Giardia	Version 1.0	6.1	45,500

*Tolerable concentrations referenced from Figure 3 and Figure 4 in Enteric Protozoa: *Giardia* and *Cryptosporidium*, Health Canada, 2012.

3.2.3 Comparative overview of waterborne parasite risks

Interestingly the three approaches used to assess waterborne parasite risks (AESRD, U.S. EPA, and Health Canada) result in slightly different consequences. In the context of *Cryptosporidium* all three approaches to risk assessment result in very similar outcomes, whereas the greatest disparity in the risk assessment outcomes pertains to risks associated with *Giardia*.

AESRD's risk-based approach to *Cryptosporidium* is modeled after the U.S. EPA LT2 Rule, and consequently the standards required for treatment are similar between these two agencies. For example, a 3-$\log_{10}$ treatment requirement is necessary when *Cryptosporidium* mean oocyst concentrations are less than 7.5 oocysts/100 L (or 0.075 oocysts/L) for both agencies. By comparison, the critical threshold for violation of Health Canada's 10^{-6} DALY per person per year for *Cryptosporidium* approximates to 13 oocysts/100 L for a water utility employing a 3-$\log_{10}$ treatment technology (i.e., conventional filtration for the Glenmore and Bearspaw WTPs). The discrepancy between 13 oocysts/100 L and 7.5 oocysts/100 L observed between Health Canada and AESRD/ U.S. EPA relates to a subtle difference in the reportable parasite numbers used by the agencies in the calculation of the mean. Health Canada stipulates that all raw data should be 'corrected' against controls (i.e., spiked matrix recoveries). Consequently, a laboratory that observes 10 oocysts/100 L of water would use a value of 20 oocysts/100 L in the calculation of a mean if method recovery was 50 % on that day (or within the lot of samples processed). Conversely, the U.S. EPA does not require values to be

corrected against method recoveries, arguing that the impaired recovery would be counterbalanced by the fact that only a fraction of oocysts commonly found in source waters are viable and infectious to humans (U.S. EPA, 2006). An uncorrected concentration of 7.5 oocysts/100 L (i.e., AESRD/U.S. EPA) would translate to a corrected concentration of 19 oocysts/100 L, assuming the average reported recovery for U.S. EPA's Method 1623 is 40 % (U.S. EPA, 2006).

When considering risks related to *Giardia*, and assuming a 3-$\log_{10}$ reduction by conventional filtration, AESRD requires mean concentrations of <1 cyst/100 L, based on running annual average of monthly samples over 2-year period. When this is compared to the Health Canada QMRA models, water having a mean annual parasite concentration of <34 cysts/100 L would fall into a 3-$\log_{10}$ treatment requirement. A 5-$\log_{10}$ treatment reduction requirement would be protective against *Giardia* concentrations up to 100 cysts/100 L according AESRD (250 cysts/100 L after concentration correction assuming 40 % method recovery), however under the HC QMRA model (version 1.0) the same level of water treatment would be sufficient to deal with 3,400 cysts/100 L in source water. Although, AESRD's requirements may be considered more protective toward *Giardia,* the more important question is whether the numbers reflected in AESRD's approval conditions are valid and whether less stringent criteria should be adopted for *Giardia* occurrence. As mentioned previously, the U.S. EPA does not require monitoring for *Giardia* as it is generally considered that treatment requirements centered on *Cryptosporidium* removal by conventional filtration, when coupled with chlorine treatment, are sufficient in dealing with *Giardia* risks

(U.S. EPA, 2006). The QMRA results obtained for *Giardia* from Health Canada's models support the U.S. EPA stance of low risk and vulnerability of systems to *Giardia*, and it can therefore be argued that AESRD's approach may be inappropriate for dealing with *Giardia* risks in treated drinking water systems.

Other discrepancies between the risk assessment approaches for *Cryptosporidium* are also apparent. The Health Canada QMRA model requires using the arithmetic mean as a concentration estimate for the model input but Health Canada does not specify a pre-defined period of monitoring for determining an arithmetic mean (McFadyen *et al.*, 2011). This is in contrast to U.S. EPA that has defined criteria for monitoring and calculating mean parasite values. U.S. EPA's LT2 Rule specifies that the arithmetic mean of 48 samples in total over 24 month period or the Max-RAA of 24 samples over two year period should be used as a measure of central tendency (U.S. EPA, 2006). U.S. EPA argues that the arithmetic mean is preferable because it gives lower rates of false positive and false negative results. The two-year monitoring requirement under the U.S. EPA LT2 Rule is intended to capture any temporal variation observed in parasite occurrence between years. Similarly, AESRD stipulates using a running annual average over two years of *Cryptosporidium* as a measure of central tendency (Alberta Environment, 2006). Unfortunately, all models incorporate, at minimum, annual arithmetic mean values as an estimate of parasite concentration, and consequently are deficient in defining and addressing potential events that may lead to higher periods of risk during certain times of the year. The concept of

spatiotemporal variation in risk is examined and discussed further in Chapter 5 of this dissertation.

In conclusion, applying the AESRD and U.S. EPA regulations to the data of parasite occurrence at the Glenmore and Bearspaw WTPs demonstrated, using the conditions set by the regulatory bodies, a low health risk with respect to *Cryptosporidium* at both sites. The outstanding issue was AESRD's perception of high risk from *Giardia* at both sites if treatment would fail to provide 5.5-$\log_{10}$ cysts reduction. However, the elevated risk associated with *Giardia* appears to exist only because of the strict water treatment requirements imposed by AESRD against this parasite when compared to the health risk estimates derived from the HC QMRA model (version 1.0). Also, the presented understanding of health risk were inferred purely on arithmetic counts of microorganisms without taking into consideration that not all (oo)cysts were viable and infectious, non-detects were corrected using an arbitrary designation, and the genotype of parasite was not accounted for. In the next chapter, these uncertainty factors are discussed in the risk assessment using the Health Canada QMRA model.

Chapter 4 : Evaluating the effect of model assumptions on the estimation of human health risks associated with *Cryptosporidium* and *Giardia* using Health Canada's QMRA model

4.1 Introduction

Inherent to all QMRA models estimating health outcomes are model assumptions. Model assumptions and general parameter estimates can greatly affect modeling outcomes. For example, values represented as non-detects in a dataset signify values in which the concentration of parasites is below the limit of detection for the diagnostic assay, and consequently method sensitivity often plays an important role in the risk assessment process. Related to this are challenges associated with method recovery for parasite detection methods in water and the influences that the matrix composition may have on method recovery and sensitivity. The performance of methods 1622 and 1623 during monitoring of 87 source waters from United States using more than 400 samples demonstrated mean *Cryptosporidium* recoveries of 43 % and mean *Giardia* recoveries of 53 % (Connell *et al.*, 2000). An analysis of U.S. EPA Method 1623 recovery results from seeded filtered tap water demonstrated that the average parasite recoveries for *Cryptosporidium* and *Giardia* are 48.4±11.8 % and 57.1±10.9 %, respectively, and the recovery percentages from raw source water samples ranged, as a minimum from 19.5 to 54.5 % for oocysts and from 46.7 to 70.0 % for cysts (McCuin & Clancy, 2003). This parasite recoveries were received by using a total of 15 blind samples analyzed by using the Filta-Max system (McCuin & Clancy, 2003). Furthermore, when *Cryptosporidium*

oocyst concentrations in water are low, method recoveries are poor and often result in non-detection (Parkhurst *et. al.*, 1998). Physicochemical parameters, such as turbidity, can also affect method recovery (Yakub *et al.*, 2000; Bukhari *et al.*, 1998). At the same time, non-detects may actually represent true negative samples, and consequently the application of a zero value in calculating an arithmetic average of parasite concentration may be warranted. Accordingly, the U.S. EPA has established acceptable parasite recoveries for Method 1623 to be between 22 % to 100 % for OPR results and 13 % to 100 % for MSR result for *Cryptosporidium* and 14 % to 100 % and 15 % to 100 % for OPR results and MSR results for *Giardia*, respectively (U.S. EPA, 2009). However, the U.S. EPA does not recommend *correcting* these parasite concentrations based on method recovery and subsequently use observable parasite numbers in the calculation of the arithmetic mean for bin categorization under the LT2 Rule. The rationale for this is that the average recovery of *Cryptosporidium* oocysts with Method 1623 in a national monitoring program was approximately 40 percent (U.S. EPA, 2006). Regarding the fraction of oocysts that are infectious, LeChevallier *et al.* (2003) tested natural waters for *Cryptosporidium* using both Method 1623 and a cell culture method to test for infectivity. Results suggested that 37 percent of the *Cryptosporidium* oocysts detected by Method 1623 were infectious. Moreover, it is now well established that not all *Cryptosporidium* species or genotypes are infectious to humans (Xiao *et al.*, 2004), and studies have demonstrated that human infectious species are relatively rare in source waters in Canada (Ruecker, 2012; Wilkes *et al.*, 2013). While it is not possible to establish a precise

value for method recovery or determine the fraction of oocysts that are infectious, available data suggest that these parameters may be of similar magnitude. Therefore, U.S. EPA specifies using non-detects as zero values and not correcting values to method recovery. Conversely, Health Canada recommends using parasite values corrected to method recovery, the argument for which is based on the precautionary principle and establishing a safety factor in the calculation of risk. As an example, if 10 *Cryptosporidium* oocysts/100 L are detected in a water sample, and method recovery was 25 % in that sample (based on parasite recoveries from a spiked sample), then the Health Canada QMRA model would use 40 oocysts/100 L of water in calculating an arithmetic mean for QMRA outcomes, whereas the U.S. EPA would use 10 oocyst/100 L of water in calculating the mean for bin categorization. In Alberta, AESRD does not specify the use of a corrected value in calculation of the arithmetic mean (i.e., running average) for reporting results under their criteria.

The varying approaches also affect the way non-detects are dealt with within the dataset itself. Under the U.S. EPA risk model (i.e., LT2 Rule), non-detects are reported as true negatives (i.e., they represent zero values used in the calculation of the mean), whereas under the Health Canada QMRA model, it is recommended that all non-detects be given an arbitrary value (i.e., 0.5 oocysts/100 L) which is then corrected to method recovery results. For example, in a water sample in which no *Cryptosporidium* oocysts are detected, and in which method recovery is 25 %, a value of 2 oocysts/100 L would be used in the calculation of the arithmetic mean for inclusion in Health Canada's QMRA risk estimates.

Other model assumptions include suppositions related to temporal and spatial homogeneity of risk. For example, all risk frameworks evaluated in this thesis (U.S. EPA LT2 Rule, AESRD, and Health Canada's QMRA models) use the arithmetic mean as a measure of central tendency, and are either an annualized value (i.e., for Health Canada) or represented as a running annual average over two years (AESRD and U.S. EPA). None of the models consider temporal variations in health risk associated with parasite occurrence (i.e., periods of time during the year in which risk may be higher than in other periods). U.S. EPA has argued that the required 2-years of monitoring should capture spatiotemporal variability of parasite concentration (U.S. EPA, 2006). However, as discussed in Chapter 3, *Giardia* occurrence is cyclic throughout any given year in both the Elbow and Bow Rivers, with higher numbers occurring in winter/spring in the Elbow River and in the summer/fall in the Bow River. Consequently, distinct peak periods of risk may exist during the year and for which annualized averages may underestimate true risk. This periodicity in risk is discussed in the Chapter 5.

The goal of this Chapter was to evaluate the various Health Canada model assumptions on the estimated human health risk associated with parasites occurrence in source drinking waters in Calgary.

4.2 Results and Discussion

4.2.1 Effect of non-detects and method recovery issues on Health Canada QMRA model outcomes

As outlined in Chapter 3, 83 % of water samples collected at the Glenmore WTP and 74 % of water samples collected at the

Bearspaw WTP resulted in non-detects for *Cryptosporidium* over the nine year monitoring period (Figures 3.2 and 3.3). Similarly, 28 % of samples collected at the Glenmore WTP and 14 % of water samples collected at the Bearspaw WTP resulted in non-detects for *Giardia* over this same nine-year period. In dealing with the issue of non-detects, Health Canada proposed replacing all non-detect values with an arbitrary value of 0.5 (oo)cysts/100 L of water in an effort to decrease underestimation of a "true" concentration of parasites in water (Health Canada, 2011). Due to uncertainty that arises because of accuracy and precision of the U.S EPA's Method 1623, this results in a conservative approach to risk estimation. Average method recovery for *Cryptosporidium* over the course of the monitoring campaign at the City of Calgary was approximately 44 %, and consequently it can be argued that there may be some merit in applying a 50 % value of the lowest detection limit (i.e., 1 (oo)cyst/100 L) for all non-detects in the dataset (i.e., 0.5 (oo)cysts/100 L). In addition, Health Canada's QMRA model is based on a lognormal distribution of parasites, and consequently inputs of zeros (as mean values) cannot be incorporated into the base model. As mentioned previously, all values, including arbitrary values for non-detects, are subsequently corrected based on method recovery from samples spiked with known concentrations of parasites. Arbitrarily applying a value 0.5 to all non-detect values, and subsequently correcting these values based on method recovery, may bias estimates of the average parasite concentrations in a sample, and consequently the risk estimates generated by QMRA models. In addition, overall model sensitivity and resolution may be affected, emphasizing the

importance of understanding basic model assumptions as they relate to QMRA outcomes. This may be particularly relevant in the context of the City of Calgary for which *Cryptosporidium* was not observed in the vast majority of water samples tested.

Figure 4.1 illustrates the effect on model sensitivity and model resolution when applying arbitrary values to non-detects in a sub-sample of the Bearspaw WTP dataset (i.e., 2009 dataset). Arbitrarily applying a value of 0.01 oocysts/100 L of water for all non-detects shifted the QMRA model sensitivity outcomes within the 2009 dataset by ~0.5 $\log_{10}$ DALYs (individual/year) when compared to a situation in which a value of 0.5 oocysts/100 L of water was applied to non-detect values in that same year (compare blue bars in Figure 4.1).

The lower limits of model sensitivity were also affected by the arbitrary designation of non-detect values. To evaluate this, we used a hypothetical situation in which all values in the Bearspaw WTP 2009 dataset were deemed non-detects for *Cryptosporidium* and given a value of either 0.5, 0.1, or 0.01 oocysts/100 L , and these values subsequently corrected based on method recovery. When data was fed into the QMRA model, lower limits of model sensitivity varied by almost 2-$\log_{10}$ DALYs per person per year when comparing situations when non-detects were arbitrarily applied a 0.5 value or a 0.01 value. Consequently, the lowest DALY achievable by Calgary's WTPs under a scenario in which no parasites were observed (i.e., as occurred in 2006 at the Glenmore WTP) was approximately $1x10^{-7}$ DALY per person per year (Figure 4.2).

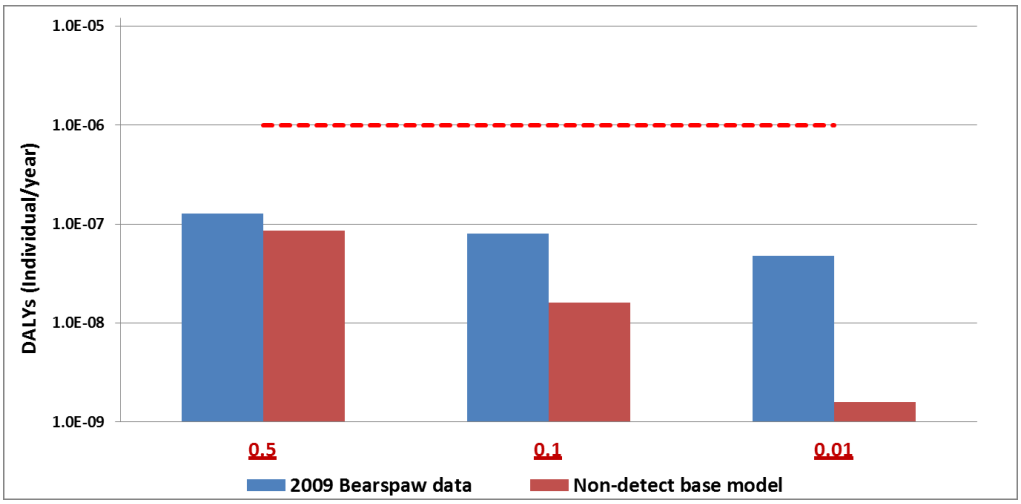

Figure 4.1 A sensitivity and resolution analysis of Health Canada's QMRA model (version 1.0) for assessing *Cryptosporidium* risks based on arbitrary designations of 0.5, 0.1 or 0.01 oocysts/100 L to all non-detect values. Blue bars represent the effect of the listed arbitrary values to non-detects within the 2009 sampling year at the Bearspaw WTP. All values used in the QMRA were also corrected to method recoveries with a mean recovery 52 % (min.=24; max.=74). Red bars represent a hypothetical situation in which no *Cryptosporidium* were detected across the 2009 sample set, and with corrected values also used in the QMRA model. Consequently red bars represent the lower limits of model sensitivity for each arbitrary value designation. The difference between the blue and red bars represents the model resolution between the real dataset and the hypothetical dataset, and the effects that arbitrary non-detect values have on model resolution. The red dashed line is a Health Canada's health target of $\mathbf{10^{-6}}$DALY per person per year.

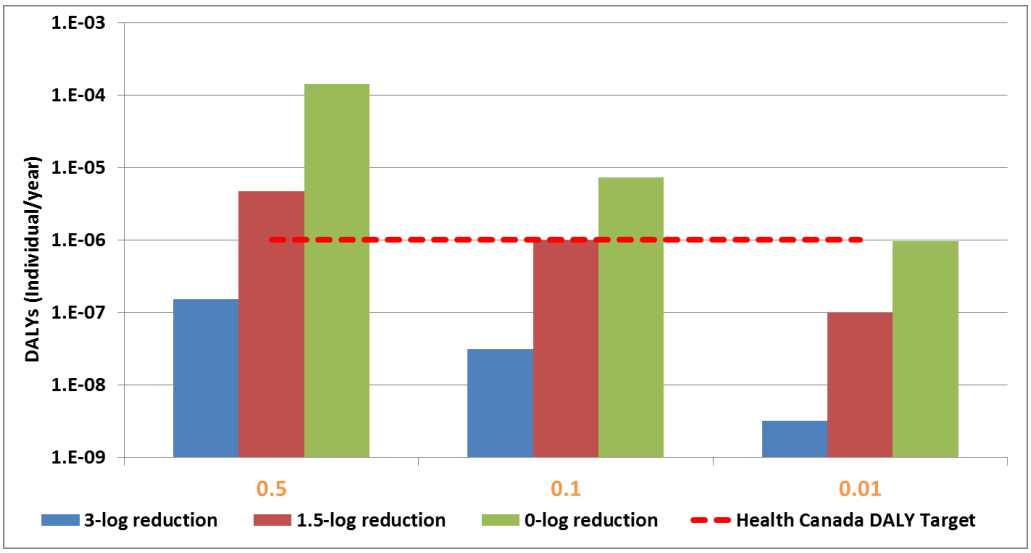

Figure 4.2 Sensitivity of the Health Canada QMRA model (version 1.0) to *Cryptosporidium* risk based on an analysis of the Glenmore 2006 *Cryptosporidium* occurrence data (i.e., a year in which no *Cryptosporidium* was observed) given under varied water treatment performance conditions (colors). All values used in the QMRA were also corrected to method recoveries with a mean recovery 29 % (min.=13; max.=49).

This 'baseline' model sensitivity analysis was important in understanding the overall risks posed by this parasite across the various years, and the implications that may be associated with treatment failure (Figures 4.2 and 4.3). For example, in 2006, no *Cryptosporidium* oocysts were detected in water samples collected at the Glenmore WTP (Table 3.1), suggesting that baseline risk during this year was completely contingent on the designation of arbitrary values for non-detects. Furthermore, health risks associated with a hypothetical treatment failure for *Cryptosporidium* during this time (e.g., 1.5-$\log_{10}$ [mimicking a partial loss in conventional filtration capacity] or 3-$\log_{10}$ [i.e., mimicking a complete loss of conventional filtration]), were completely contingent on the arbitrary designation of non-detect values to the dataset. Interestingly, a designation of 0.01 oocysts/100 L to all non-detects in the 2006 dataset would still result in a DALY value below Health Canada's recommended guideline (i.e., 10^{-6} DALY per person per year), even in the event that a complete loss in conventional filtration capacity was observed at the Glenmore WTP in 2006 (i.e., 3-$\log_{10}$), (Figure 4.2). Conversely, an arbitrary designation of 0.5 oocysts/100 L to this same dataset would result in a violation of the Health Canada target for that same year in the event of a partial or complete failure in conventional filtration. It is recognized that the efficiency of conventional filtration can be temporally variable in the context of parasite removal. A major review of pilot and full-scale water treatment studies by Hijnen & Medema (2007), demonstrated that oocyst removal using coagulation and sedimentation at 25 sites varied from 3.8-$\log_{10}$ down to 0.4-$\log_{10}$. A recent review of conventional filtration removal efficiencies by Health

Canada demonstrated that oocyst removal varied between 0.8-$\log_{10}$ and 5.5-$\log_{10}$ (Health Canada QMRA model version 2 - McFadyen *et al.*, 2011). Although a 3-$\log_{10}$ oocyst removal credit is assumed by AESRD's approval regulations and U.S. EPA's LT2 Rule requirements for conventional filtration true oocyst removal is likely to be temporally influenced. Using the aforementioned example of non-detect base model, the arbitrary designation of a value to non-detection can have a practical implication for understanding of health risk, especially in the locations where source water is usually of good quality, such as the Bow and Elbow Rivers at the City of Calgary in particular years. Over longer monitoring periods, for example, if *Cryptosporidium* data for the Bearspaw WTP of all samples was used, and in which non-detects were replaced with 0.5, 0.1, and 0.01 respectively, the estimates of risk would be less different among the estimates of using different arbitrary values (blue bars) (Figure 4.3). This happened because the records of a few high point concentrations of the parasite in the dataset levelled the risk, making non-detects less influential onto the health risk estimates. The analysis also emphasizes the importance of collecting multi-year monitoring data on parasite occurrence in order to capture periods in which method sensitivity may compromise detection limits.

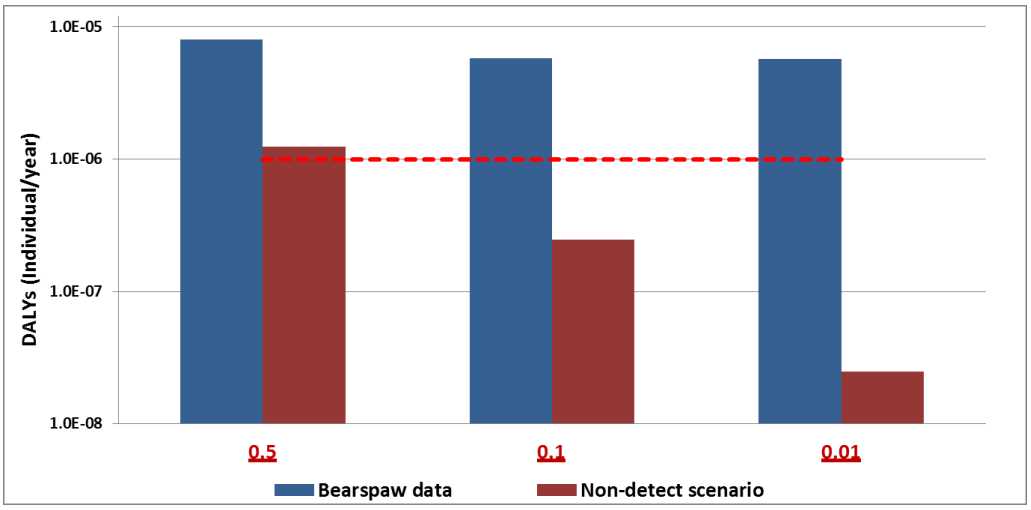

Figure 4.3 Sensitivity of Health Canada's QMRA model (version 1.0) outcomes based on how non-detects are handled under a scenario of treatment deficiency. It was assumed overall 1.5-$\mathbf{log_{10}}$ oocysts reduction. The red bars show the estimates of health risk using a hypothetical non-detect base model when no oocysts were actually present in source water. The blue bars show the health risk when real parasite concentration records were used for the Bearspaw WTP of all samples in period 2003-2011. The red dashed line is the Health Canada's health target $\mathbf{10^{-6}}$ DALY per person per year. The 0.5, 0.1, and 0.01 are the arbitrary values used to replace a zero concentration in the hypothetical and in the real parasites concentration datasets.

4.2.2 Parasite Infectivity

As outlined in Chapter 1 of this dissertation, not all parasites (*Cryptosporidium* or *Giardia*) observed in a water sample may be infectious to humans. In fact, most genotypes/species of *Giardia* and *Cryptosporidium* appear to exhibit some degree of host specificity. Of the six known species of *Giardia*, only certain assemblages of *G. intestinalis* are known to cause infections in humans. Of the seven major genetic assemblages of *G. intestinalis* (A, B, C, D, E, F, G), assemblages A and B are those of concern for human infection. In the context of *Cryptosporidium spp.*, only *C. hominis*, *C. parvum*, and *C. cunniculis* have been reported to cause general community outbreaks in humans associated with drinking water consumption (Chalmers, 2010). Environmental waters analyzed by Method 1623 often contain a mixture of parasites genogroups (Ruecker *et al.*, 2011), providing evidence that the parasites observed within water samples originate from diverse host sources of faecal contamination, a portion of which are not likely to cause infections in humans (Ruecker, 2013). Method 1623 does not differentiate between the various species and genotypes of *Cryptosporidium* or *Giardia*, nor is the method suited for determining whether the parasites observed are viable, which is a pre-requisite for infectivity. It is inherently assumed in the various risk models described in this thesis (AESRD, U.S. EPA and Health Canada) that all parasites are considered infectious to humans. This assumption is also grounded in providing a more conservative estimate of risk, but may dramatically affect overall levels of risk associated with these parasites.

Based on molecular methods recently developed for identifying various *Cryptosporidium* and *Giardia* parasite species and genotypes in water samples analyzed by Method 1623 (Amar *et al.*, 2002; Read *et al.*, 2004; Bertrand *et al.*, 2005; Caccio *et al.*, 2002; Lalle *et al.*, 2005; Ferguson *et al.*, 2006; Xiao & Fayer, 2008), a new version of the Health Canada QMRA Model (version 2.0) was released (i.e., July 2011) and which incorporated mathematical inputs accounting for the percentage of samples containing human infectious parasites. The input value represented the proportion of water samples collected from a specific site that contained human infectious species/genotypes of the parasites.

Recent data collected from source tracking studies in our laboratory demonstrate that the *Cryptosporidium* species/genotypes observed at the Glenmore or Bearspaw WTPs are of little public health concern, with the populations of parasite observed considered non-infectious to humans (unpublished data). Since 2011, the genotyping study ongoing in our laboratory revealed that *C. andersoni* was the only species of the parasite identified in the source waters entering the two drinking water treatment plants. Conversely, *Giardia intestinalis* populations present in the Elbow and Bow Rivers represent assemblages known to be zoonotic in humans (i.e., A and B assemblages, personal communication, Jonathan Slone, MSc Thesis).

This information was subsequently incorporated into the HC-QMRA model (version 2.0) and compared to conservative model parameters in which all parasites were considered infectious to humans (Figures 4.4 and 4.5). Even though no human infectious *Cryptosporidium* was observed in water samples collected at either

site, a refined estimate of 10 % of water samples containing human infectious *Cryptosporidium* was included in the analysis. In order to increase our understanding of overall uncertainty in risk estimates the analysis also examined the arbitrary designation of non-detect values in the context of the percentage of samples containing infectious parasites (Figures 4.4 and 4.5). Revising the model inputs to be 10 % of samples containing human infectious parasites resulted in a reduction of health risk by ~1.0–$\log_{10}$ compared to model outcomes in which all parasites were considered infectious. When the conservative parameters (i.e., all parasites considered infectious and 0.5 oocysts/100 L applied to non-detects) was compared to the most stringent refined parameters (i.e., 10 % of parasite are considered infectious and 0.01 oocysts/100 L is applied to non-detects) an overall difference on ~1.5-$\log_{10}$ in model outcomes was observed (Figure 4.4). The difference between the conservative model outcome and the most stringent refined model outcomes represents the relative risk range for *Cryptosporidium* for Calgary's WTPs (Figure 4.4). When using the most stringent refined estimates, the overall risk for *Cryptosporidium* was more than 3 orders of magnitude (i.e., 3-$\log_{10}$) below the Health Canada DALY Target for either the Glenmore WTP (Figure 4.4) or the Bearspaw WTP (Figure 4.5); i.e. possibly slightly less impacting on uncertainty than the likely range in treatment plant performance variation (Hijnen & Medema, 2007).

In addition to the uncertainty in the QMRA models estimates associated with various *Cryptosporidium* species/genotypes in a water sample, a variety of environmental factors can affect parasite viability (and consequently infectivity). For example, Neumayerová and

Koudela (2008) found that exposure of *Cryptosporidium* oocysts to freezing or heating was crucial to change their infectivity toward mice. No inoculated mice that received oocysts frozen at -5 °C for 3, 5, 7, and 10 days and -20 °C for 1, 3, 5, and 8h become infected. In contrast, *Cryptosporidium muris* oocysts frozen at -5 °C for 1 day remained infective for inoculated mice. Results also indicated that when water containing *Cryptosporidium muris* oocysts was exposed to a temperature of 55 °C or higher for 1 min, the infectivity of oocysts was lost. Although, the parasite's response to the high temperature might not have implication for risk assessment in water sources, vulnerability of the parasite to extended or strong freezing certainly should have implication, particularly in Alberta. In previous study, it was estimated that approximately 37 % of counted oocysts (LeChevallier *et al.*, 2003) are usually intact and viable. The model outcomes provided above do not include caveats for infectivity, other than genotype/species designations.

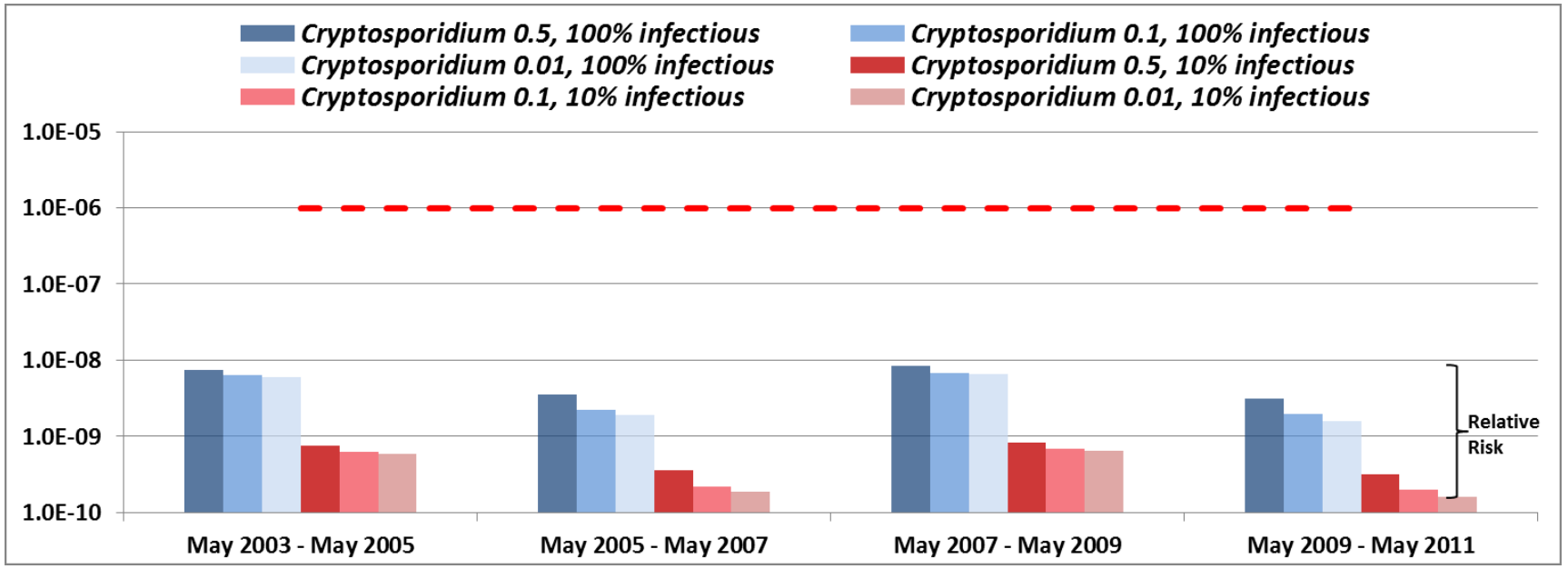

Figure 4.4 Sensitivity of Health Canada QMRA model (version 2.0) as influenced by parasite infectivity assumptions. The arbitrary values 0.5, 0.1, 0.01 for non-detects were used for estimating a relative risk range between the conservative assumption (darkest blue bars) and the most stringent revised assumptions (lightest red bars). *Cryptosporidium* data was used from the sampling campaign in the Glenmore WTP.

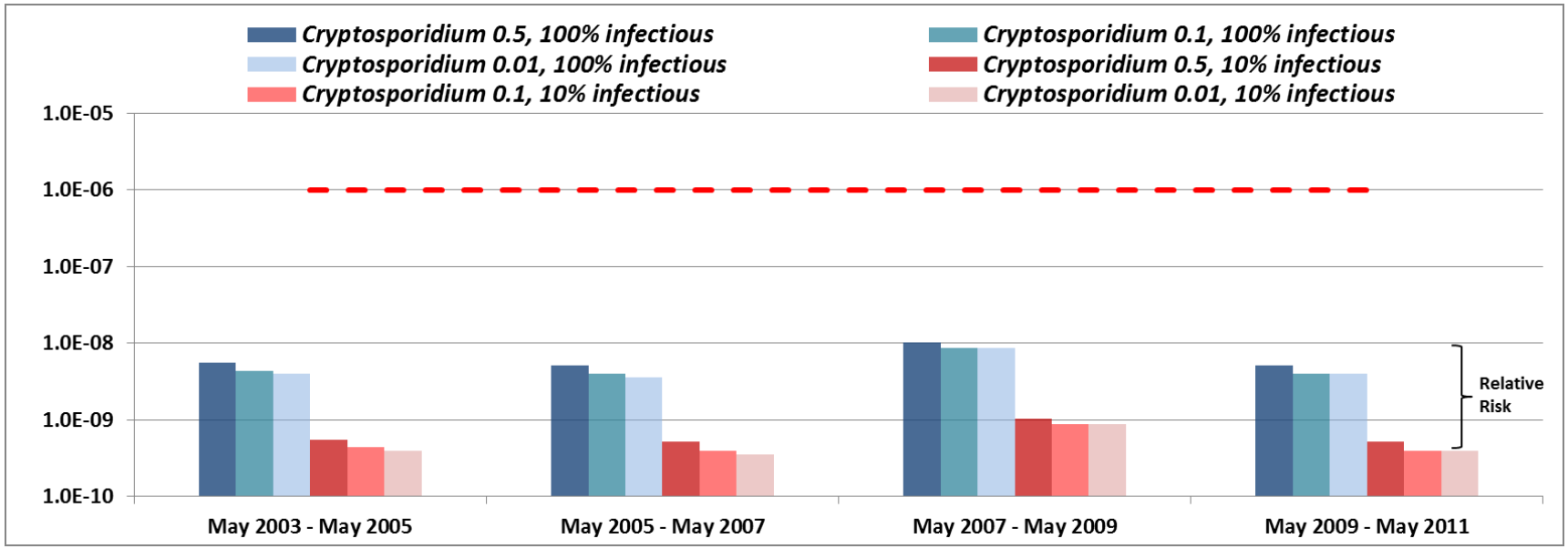

Figure 4.5 Sensitivity of Health Canada QMRA model (version 2.0) as influenced by parasite infectivity assumptions. The arbitrary values 0.5, 0.1, 0.01 for non-detects were used for estimating a relative risk range between the conservative assumption (darkest blue bars) and the most stringent revised assumptions (lightest red bars). *Cryptosporidium* data was used from the sampling campaign in the Bow River at the Bearspaw WTP.

4.2.3 "Problem of means" - using statistical representative values of true concentration estimates

Currently, measures of central tendency have been used for determining concentration estimates for parasites in source water (Parkhurst *et al.*, 1998). U.S. EPA requires using an arithmetic mean or a maximum running annual average depending on the length of a monitoring period (U.S. EPA 2006). AESRD requires applying a running annual average of monthly samples over a two-year period (Alberta Environment, 2006). Health Canada does not stipulate how estimates of parasite occurrence should be done, including the duration of monitoring (e.g., monthly, annualized), but has specified for users of the Health Canada QMRA models that the arithmetic mean concentration ought to be used for inputs.

Several approaches have been proposed for estimating parasite concentrations and for tackling issues such as poor (oo)cyst recovery associated with U.S. EPA Method 1623. These include the following:

1. the "non-zero" method, in which non-detects are treated as non-zero arbitrary values such as 1.0, 0.5, 0.1, or 0.01 (LeChevallier & Norton, 1995; Stern, 1996), and with the resulting values referenced to detection limits (Fout *et al.*, 1996). Thereafter, an average concentration estimate is derived by summing up values of all separate samples and dividing the sum by a total number of samples.
2. "the positives-only" method that uses averaging of only the positive results and ignoring non-detect samples (LeChevallier &

Norton, 1995; Stern, 1996; LeCevallier *et al.*, 1991; Regli *et al.*, 1991);

3. the "percentile" method orders data from smallest to largest determining the concentration that falls at the upper 90th or 95th percentile (LeChevallier & Norton, 1995);
4. effective filtration volume method that uses a sum of (oo)cysts counted divided by a volumetric sum of water analyzed (Parkhurst *et al.*, 1998);
5. a method that uses technic when (oo)cysts counted for each sample divided by an effective volume of the corresponding sample, and then a mean of samples is taken disregard of a difference in the samples effective volume.

Each of the methods has its own advantages and disadvantages. The non-zero method is useful when a majority of samples are non-detects in a dataset, such as the City of Calgary dataset, and consequently this method was used in the current research project. Method 2 described above can be useful as a "precautionary principle" approach for water treatment system that uses a source water that is known to be polluted with human infectious parasites, or when much uncertainty exists on parasite occurrence. Methods 4 and 5 listed above can be advantageous in situation where source water limits filtration capacity (e.g., highly turbid waters). There is no universal solution which method to choose. Each method can be useful and better suited under appropriate conditions.

When parasite point concentrations in source water are used to derive parasite concentration estimates inferred over a large time

interval, the possibility exists that heterogeneous periods of risk may be 'normalized', resulting potentially in overestimating risks during periods when parasite concentrations are truly low, but more importantly, underestimating risks for periods when parasite concentrations may be truly high. The overestimated and the underestimated concentration can greatly deviate from a true concentration of parasite at a particular time in source water. Identifying periods of high parasite concentration in source water is of primary public health interest. The three risk assessment approaches studied (AESRD, U.S. EPA and HC) suffer from the inability of identifying peak periods of risk primarily due to the methodological challenges. Parasite monitoring using U.S. EPA Method 1623 is costly, inefficient (i.e., relatively poor recoveries), and plagued with uncertainty due to "grab" sample analysis (Allen *et al.*, 2000; Signor & Ashbolt, 2006). The implausibility of developing real-time monitoring technology for detecting of parasites is another major obstacle.

Identification of periods of peak contamination of source water would provide QMRA with the data to identify more timely and responsive periods of high risk, thus guiding water treatment measures/management more effectively. Measures of central tendency from "grab" samples remain the most used tool for inferring estimates of pathogen concentration but under conditions of high uncertainty. Temporal and spatial variability of parasite concentration in water exacerbates the challenges in understanding potential periods of peak risk to drinking water. An attempt to elucidate the role of environmental factors in source water contamination with parasites will be presented in the next chapter.

Chapter 5 : Assessing relationships between waterborne parasite occurrence and environmental factors in the Elbow and Bow River watersheds

5.1 Introduction

Understanding the occurrence of waterborne parasites in surface water sources used for drinking water is crucial for planning protective water treatment measures. Parasite presence in environmental waters is associated with environmental features that can mobilize faecal sources of pollution into the receiving waters. Environmental factors such as weather, seasonal changes, habitat alterations, land use patterns, and urban environments can all influence microbial contamination dynamics (U.S. EPA & USDA, 2012).

Understanding environmental processes associated with mobilization and survival of parasites into source water could possibly improve prediction of periods of high health risk and consequently make water treatment decisions better prepared to deal with impending challenges for water treatment. Currently, despite much research effort, the field is still lacking consistency in a systematic pattern of occurrence of both *Cryptosporidium* and *Giardia.* It was noticed that *Giardia* cysts can be resistant to some environmental factors for survival while vulnerable to others (Samuel at al., 2001). For instance, the observation of Jakubowski (1990) suggested that the optimal temperature for *Giardia* survival in water is between 4° C and 8° C and a high concentration of bacteria in a surrounding environment leads to cysts biodegradation. Desiccation and ultraviolet irradiation inactivated cysts within 24 hours (Bingham *et al.*,

1979). *Giardia* cysts undergo degradation within weeks in faeces and soil (Olson *et al.*, 1999b). Other studies have demonstrated association of turbidity with parasites (Selvakumar & Borst, 2006; Ryu *et al.*, 2005) while others failed to demonstrate this (Horman *et al.*, 2004). Data on factors that contribute to *Cryptosporidium* occurrence are not consistent. High concentrations of *Cryptosporidium* in environmental waters occasionally were reported during a record spring run-off (Gammie *et al.*, 2000). Several studies have documented significant increases in pathogen loads in raw waters associated with rainfall and run-off events (Kistemann *et al.*, 2002, Atherholt *et al.*, 1998). Ferguson *et al.* (2007) using watershed modelling demonstrated that slow decay of protozoan pathogens combined with their rapid transport in water during wet weather events results in a cumulative export of *Cryptosporidium* to downstream sub-catchments. Tang *et al.* (2011) concluded that the vast majority of small agricultural catchments are ungauged, therefore, it is difficult to use a process model to predict and understand the mechanisms and activities that regulate the risk of surface water contamination from agricultural areas. Nevertheless, the study suggested that temperature was the most important parameter that affected survival of oocysts during their transport in the study catchments and that the timing of land field fertilization using manure application relative to the occurrence of water run-off event was also critical.

A major drawback of the current risk assessment frameworks described in this thesis (AESRD, U.S. EPA, and Health Canada) pertains to the use of a mean as a measure of central tendency over a long period to describe parasite occurrence in watersheds. As discussed in previous

Chapters, mean values can be annualized or amalgamated across a defined period. Consequently, periods of high parasite occurrence may be melded with periods of low parasite occurrence to arrive at a mean value used in risk calculations. However, the peak periods of parasite occurrence may in fact represent true occurrence patterns and therefore distinct periods of high risk may stand out from periods of low risk. The fact that parasite occurrence is highly variable over time implies that risk is also temporally heterogeneous, and consequently sporadic periods of high risk may exist when sources of faecal pollution in the environment are mobilized and transported to receiving water bodies.

It was hypothesized that environmental factors that could mobilize parasites into waters such as rain and snowmelt (i.e., run-off) may represent more important variables associated with distinct and defined peak risk periods associated with contamination of drinking water sources. The focus of this Chapter was to examine parasite occurrence in the Elbow and Bow River watershed in the context of key environmental features and provide a basis for understanding and defining peak periods of risk associated with waterborne parasites and evaluate these periods of peak occurrence in the context of QMRA.

5.2 Results and Discussion

5.2.1 Analysis of occurrence of *Giardia* in source water depending on water temperature

As described in previous chapters, *Giardia* occurrence in both the Elbow and Bow Rivers, displayed a cyclic seasonal pattern (Figures 3.4; 3.5). Concentrations of *Giardia* were generally higher during the cold

seasons for the Elbow River throughout the nine-year monitoring period and for a portion of the monitoring period in the Bow River (2003-2007). Highest cyst concentrations were recorded when water temperature was below 5 °C (Figure 5.1). This observation was consistent with findings of Jakubowski (1990) indicating the high survival of *Giardia* cysts when water temperature was between 4 °C and 8 °C.

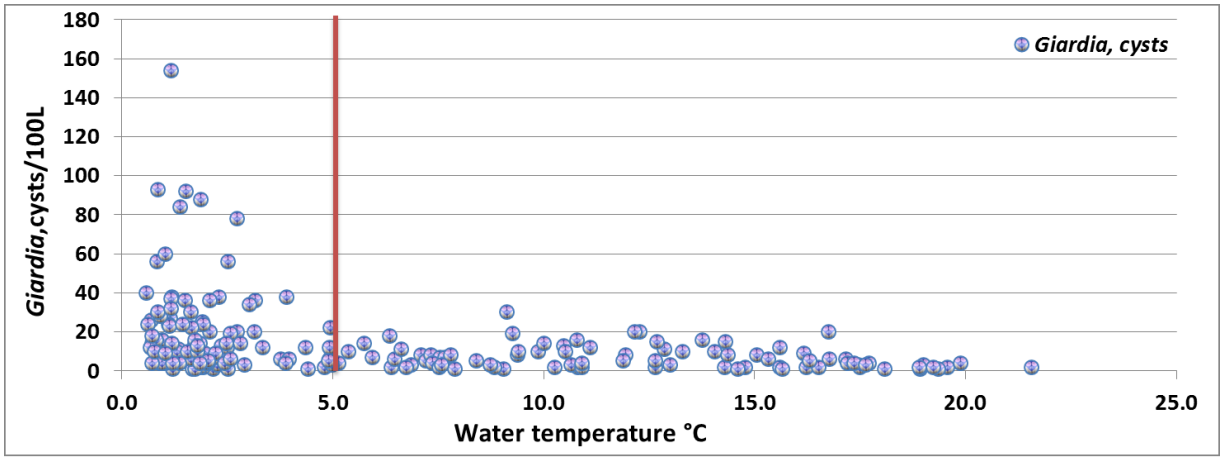

Figure 5.1 Association between *Giardia* cysts concentration with water temperature below 5 °C and above 5 °C (as defined by the red boundary line) at the Glenmore WTP.

In order to better understand the association between water temperature and parasite occurrence, *Giardia* monitoring data collected between 2003 and 2011 was grouped according to source water temperatures recorded on the day of sampling. For simplicity, two groups were created: samples collected when water was ≤ 5 °C (i.e., cold water) and >5 °C (i.e., warm water) (Figure 5.2). A statistical comparison of the two groups was performed using a two sample (independent) t-test statistic. Mean *Giardia* concentrations were 38 cysts/100 L (STD=50; 95 % CI: 29 to 46) and 7.8 cysts/100 L (STD=11; 95 % CI: 5.9 to 9.6) for the cold water and warm water groups, respectively. The mean difference was

29 cysts/100 L (t=6.8; df =151; 95 % CI: 21 to 38), and the difference in mean concentrations of cysts was highly significant (p-value<0.0001). Therefore, *Giardia* cysts concentration in the Glenmore Reservoir were associated with water temperature and, thus, to seasonality.

When *Giardia* occurrence data was amalgamated across the entire nine year (2003-2011) monitoring period for the Bearspaw WTP, and categorized into the two groups (i.e., $\leq$ 5 °C [cold water] and > 5 °C [warm water]) no difference was observed between occurrences of *Giardia* in the groups. The mean *Giardia* concentration was 27 cysts/100 L (STD=33; 95 % CI: 22 to 32) for the cold water group and 35 cysts/100 L (STD=46; 95 % CI: 28 to 43) for the warm water group, and consequently no statistical significance between the two groups was observed (mean difference was -8.2 cysts/100 L; t=-1.8; df=290; 95 % CI: -17 to 0.49; p-value=0.06). The data was further sub-categorized according to distinct time periods based on monitoring data collected between 2003-2007 and from 2008-2011, the rationale of which was based on an observed shift in the predominance of parasite occurrence at the Bearspaw WTP into the summer/fall season after 2007 (See Chapter 3, Figure 3.5). In this case the difference between the groups became statistically significant in both periods, after the adjustment in time. In the period 2003-2007, the mean concentration of *Giardia* was 23 cysts/100 L (STD=24; 95 % CI: 18 to 28) in the cold water group and 11 cysts/100 L (STD=11; 95 % CI: 7.5 to 13) in the warm water group. The mean difference of 13 cysts/100 L between the two groups was statistically significant (t=4.2; df =139; 95 % CI: 6.7 to 18; p-value<0.0001). In the period 2007-2011, the mean concentration of *Giardia* was 33 cysts/100 L

(STD=41; 95 % CI: 25 to 42) in the cold water group and 48 cysts/100 L (STD=52; 95 % CI: 39 to 58) in the warm water group. The mean difference between the groups was -15 cysts/100 L and was statistically significant (t=-2.2; df =181; 95 % CI: -28 to -1.8; p-value<0.03), in the context of higher values in warmer water (Figure 5.3).

The observed dependence on cold water conditions for higher concentrations of *Giardia* at the Glenmore WTP and at the Bearspaw WTP (before 2007) was consistent with the perspective that increased water temperature detrimentally affects cyst survival, as reported in the literature and discussed above. Consequently, cyst survival may be enhanced under low water temperature conditions throughout the watersheds. However, the shift of *Giardia* occurrence observed at the Bearspaw WTP after 2007 potentially indicated that a new source of *Giardia* contamination was introduced in the watershed during warmer periods of the year. Parasite loading during this time period may have masked the previously observed dependence of *Giardia* cyst in cold water, resulting in the higher concentrations of the parasite in warm water after 2007.

The higher concentrations of *Giardia* cysts observed in source water during the cold water season in the Glenmore Reservoir also has implications for parasite inactivation with chlorine disinfection in cold water conditions (Clark *et al.*, 1989). The Ct data for chlorine disinfection from Glenmore WTP indicated that the chlorination could be highly variable during the period 2003 to 2011 (Figure 5.4), and with the lowest recorded Ct values occurring during cold-water conditions. Considering that higher concentrations of *Giardia* cysts occurred during cold-water conditions for

the Elbow River, the overall health risk may be further elevated due to reduced treatment efficiency.

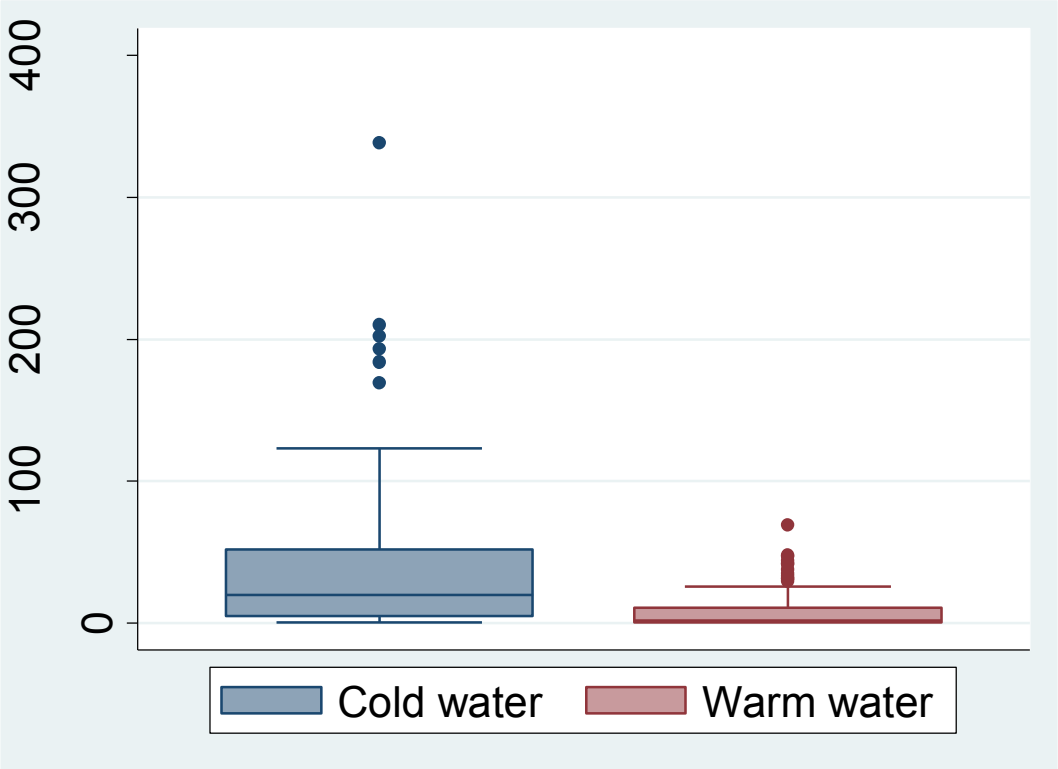

Figure 5.2 Boxplot depicting difference between the two *Giardia* groups depending on water temperature observed at the Glenmore WTP. The boundary was set at 5 °C of water temperature (cold water < 5 °C and warm water > 5 °C) on the day of parasite testing. Data represents the 2003-2011 year sampling campaign. Boxplots reflect median±25-75th percentiles (colored boxes) with whiskers representing1.5*interquartile range. Interquartile range is a difference between the upper and lower quartiles.

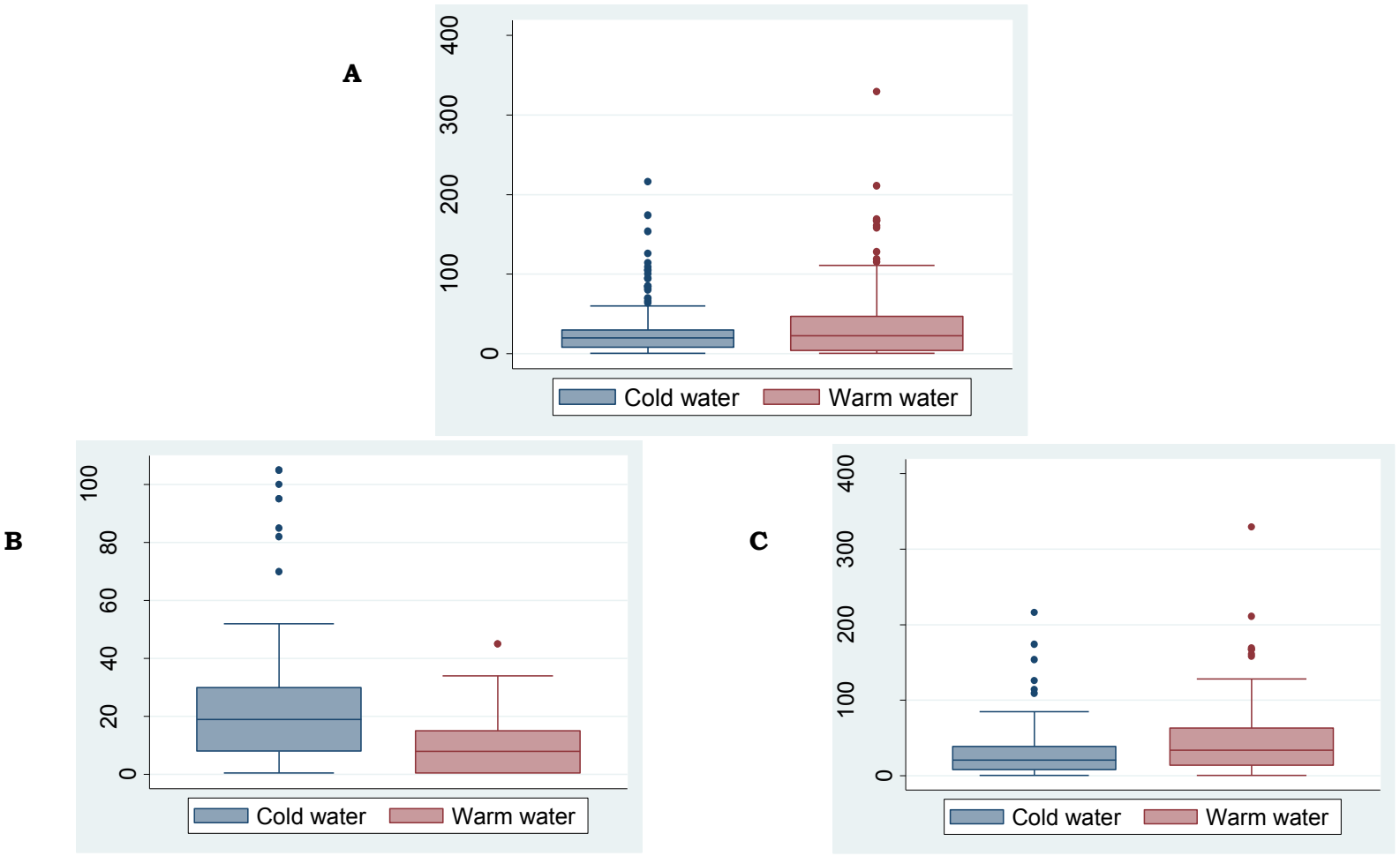

Figure 5.3 Boxplot depicting difference between the *Giardia* occurrences depending on water temperature in the Bow River. The boundary was set at 5 °C of water temperature (cold water < 5 °C and warm water > 5 °C). Data represents the time periods between 2003 and 2011 (Panel A); 2003-2007 (Panel B); and 2007-2011 (Panel C). Boxplots reflect median$\pm$25-75th percentiles (colored boxes) with whiskers representing1.5*interquartile range.

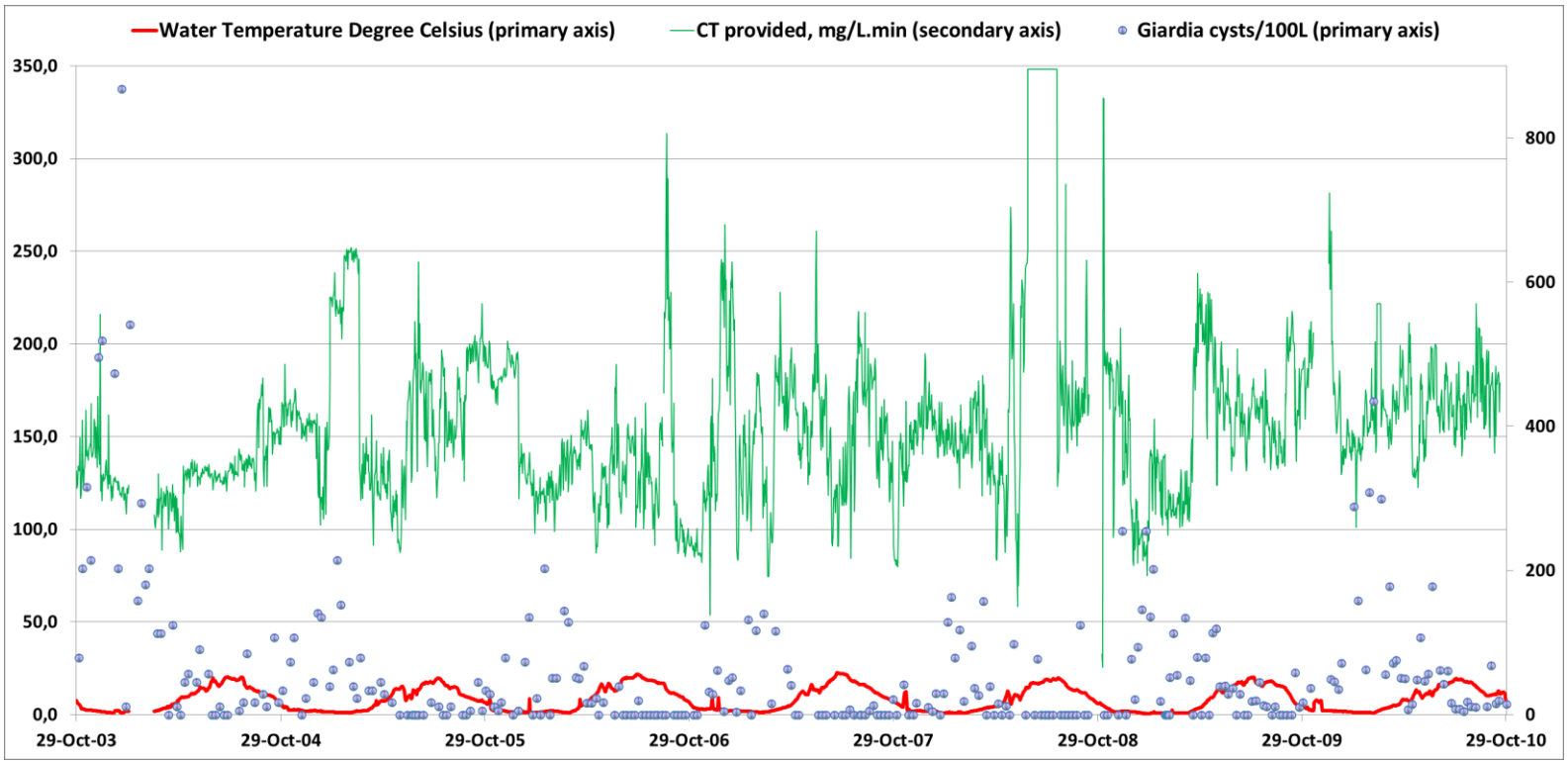

Figure 5.4 Actual chlorination [Ct, green line] that was performed by the Glenmore WTP, *Giardia* concentration [blue dots] and source water temperature [red line] during 2003-2011 years sampling campaign. Concentrations of *Giardia* are recovered.

5.2.2 Association between parasite occurrence and environmental factors

Glenmore WTP: The observation that *Giardia* occurrence was cyclic in nature, with the highest concentrations observed during spring and under cold-water conditions, lead to the following hypothesis to potentially explain this pattern. Environmental parameters that may affect the mobilization of parasites from faecal sources during winter/early spring may be related to snowmelt as a mobilization force upon faeces deposited in the environment during this time. Indeed, snowmelt during the winter and early spring may not affect overall water temperatures within a river due to the abundance of ice cover on rivers in Northern latitudes. Moreover, snowmelt water temperatures are often ~ 0 °C. In addition, snowmelt may not affect turbidity in the river due to the inability of snowmelt to infiltrate frozen soils and mobilize particulates within these soils (Seyfried & Murdock, 1997). Consequently, snowmelt may have an association between season (i.e., late winter/early spring) and cold-water temperatures, but may not correlate with other water quality parameters such turbidity, yet may act as a mobilization force on faecal material in the environment. Furthermore, extended periods of colder temperatures (i.e., little or no snowmelt) may result in greater accrual of faeces in the environment (i.e., parasite burden) followed by mobilization of parasites from these sources when weather conditions permit the melting of snow. Consequently, variables such as snowfall/ snow pack (or snow on ground) are also critical since snow is a prerequisite for melt water.

In order to address this hypothesis, snow pack, snowmelt (daily values), rain (daily values), and water temperature data were plotted against parasite numbers and turbidity (Figures 5.5 and 5.6). Periods of high *Giardia* concentration corresponded with periods of snow on ground and snowmelt in the Elbow River across all years (Figure 5.5) and between 2003 and 2007 in the Bow River (Figure. 5.7). Increases in the concentration of *Giardia* parasites were especially noticeable during the winter-spring of 2008, 2009, 2010, and 2011 (Figure 5.5). Concentrations of *Giardia* were always low during the periods when water temperature was higher than 5 °C. Increases of water turbidity were most noticeable in the summer/fall and did not correspond with *Giardia* concentration increases (Figure 5.5). Rain periods were usually accompanied by low parasite concentrations (Figure 5.6). When high concentrations of *Giardia* were recorded, these were most often preceded by snowmelt.

As discussed in Chapter 3, *Cryptosporidium* was not detected in a large portion of the sampling program at the Glenmore WTP, and no pattern of occurrence was discernable throughout the monitoring period. Slight increases were recorded only during the fall of the years 2003, 2008, and 2011. Interestingly, studies on cryptosporidiosis monitoring data in clinical studies suggested that the peaks of the infection in people often occur during the early-summer to early-fall period, reflecting recreational exposures (Deitz & Roberts, 2000; Hlavsa *et al.*, 2005; Yoder *et al.*, 2007; Yoder *et al.*, 2010; Yoder *et al.*, 2012).

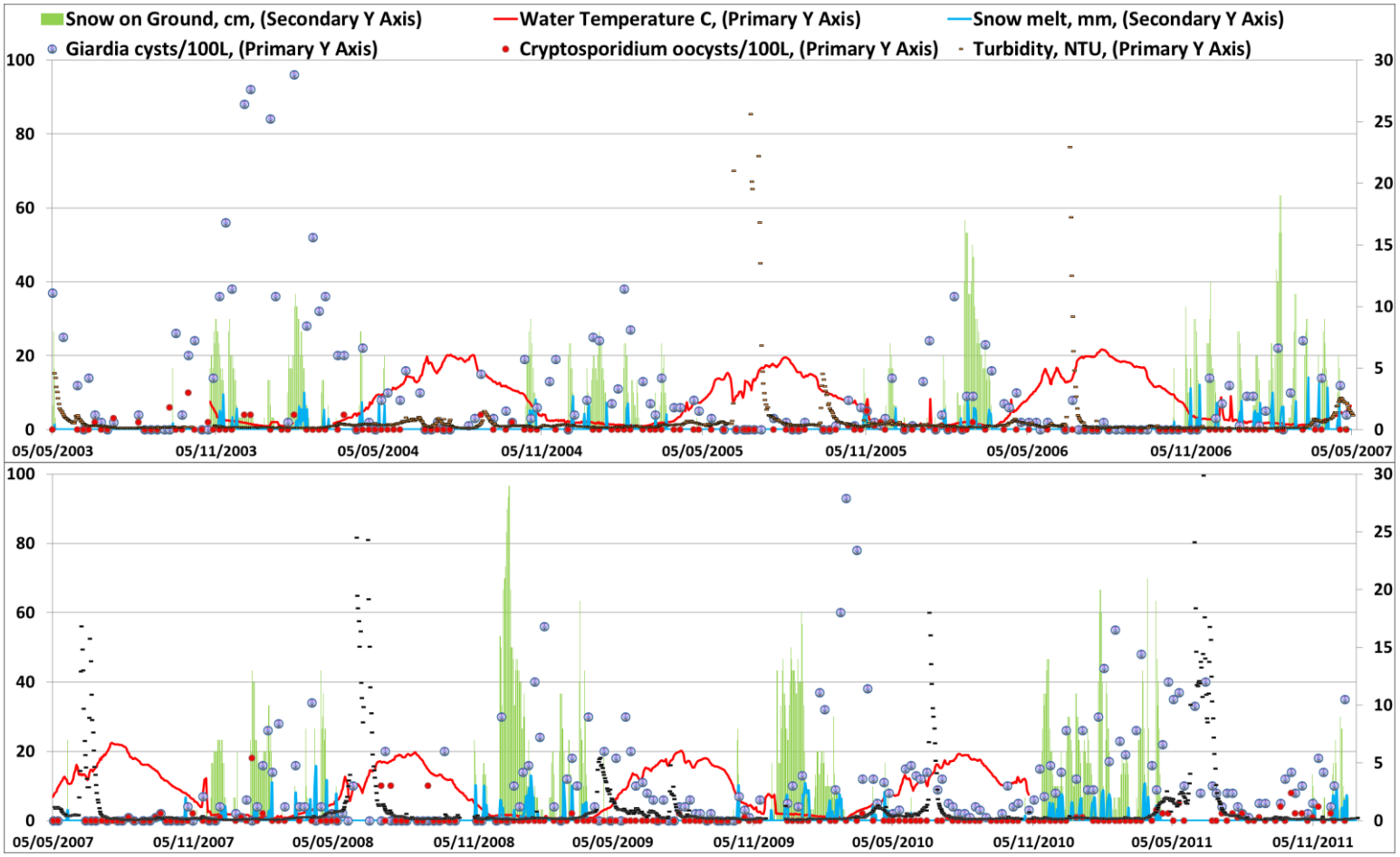

Figure 5.5 Diagram depicting the relationship between the occurrence of waterborne parasites (*Cryptosporidium* [red dots] and *Giardia* [blue dots]) with snow on ground (green bars), snowmelt (daily value [blue bars]), source water temperature (red line) and source water turbidity (black dots) at the Glenmore WTP. The data was plotted with respect to the time of testing. The records reflect 2003-2007 years (top panel) and 2007-2011 years (bottom panel) of the monitoring campaign. Dates on the x-axis are recorded as dd/mm/year.

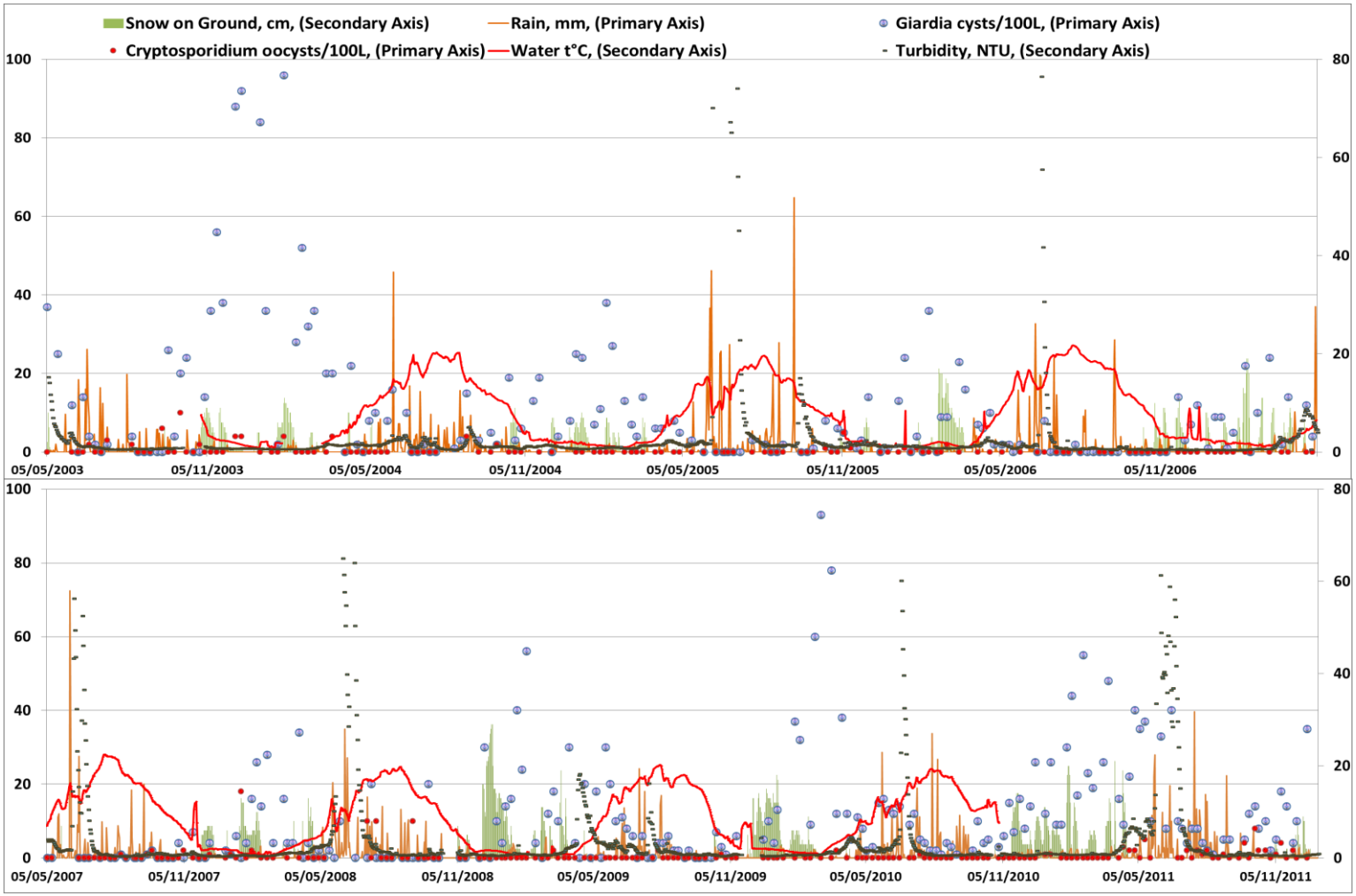

Figure 5.6 Diagram depicting the relationship between the occurrence of waterborne parasites (*Cryptosporidium* [red dots] and *Giardia* [blue dots]) with rain (daily value [orange line]), snow on ground (green bars), source water temperature (red line) and source water turbidity (black dots) at the Glenmore WTP. The data was plotted with respect to the time of testing. The records reflect 2003-2007 years (top panel) and 2007-2011 years (bottom panel) of the monitoring campaign. Dates on the x-axis are recorded as dd/mm/year.

Bearspaw WTP: At the Bearspaw WTP between 2003 and 2007, the pattern of *Giardia* occurrence was very similar to that that was seen at the Glenmore WTP (Figure 5.7). Occurrence of higher cysts concentrations after snowmelt events was the most noticeable. During the 2003-2007 sampling campaign, no increase in cyst concentration was observed after rain, and no influence of turbidity was seen on *Giardia* occurrence (Figure 5.8). However, after 2007, the year during which *Giardia* cysts concentrations were low compared to other years, the summer of 2008 manifested with the increasing trend of higher cyst concentrations during the summer seasons and in the subsequent years. This pattern was seen until the end of monitoring. Also, after 2008 and until the end of monitoring, *Giardia* cyst concentrations during winter-spring seasons were in the comparable range with the concentrations that were observed during winter-spring seasons in the previous years (2007 and earlier).

Regarding *Cryptosporidium* at the Bearspaw WTP, no parasites were observed in most water samples. A sporadic increase was observed in the fall of 2008. Contrastingly, multiple recordings of *Cryptosporidium* oocysts concentrations in the range from one to 10 oocysts/100 L were recorded in the summer-fall season of 2011. The multiple positive concentrations that were recorded in 2011 looked differentially on the background of the great prevalence of non-detects in the previous years.

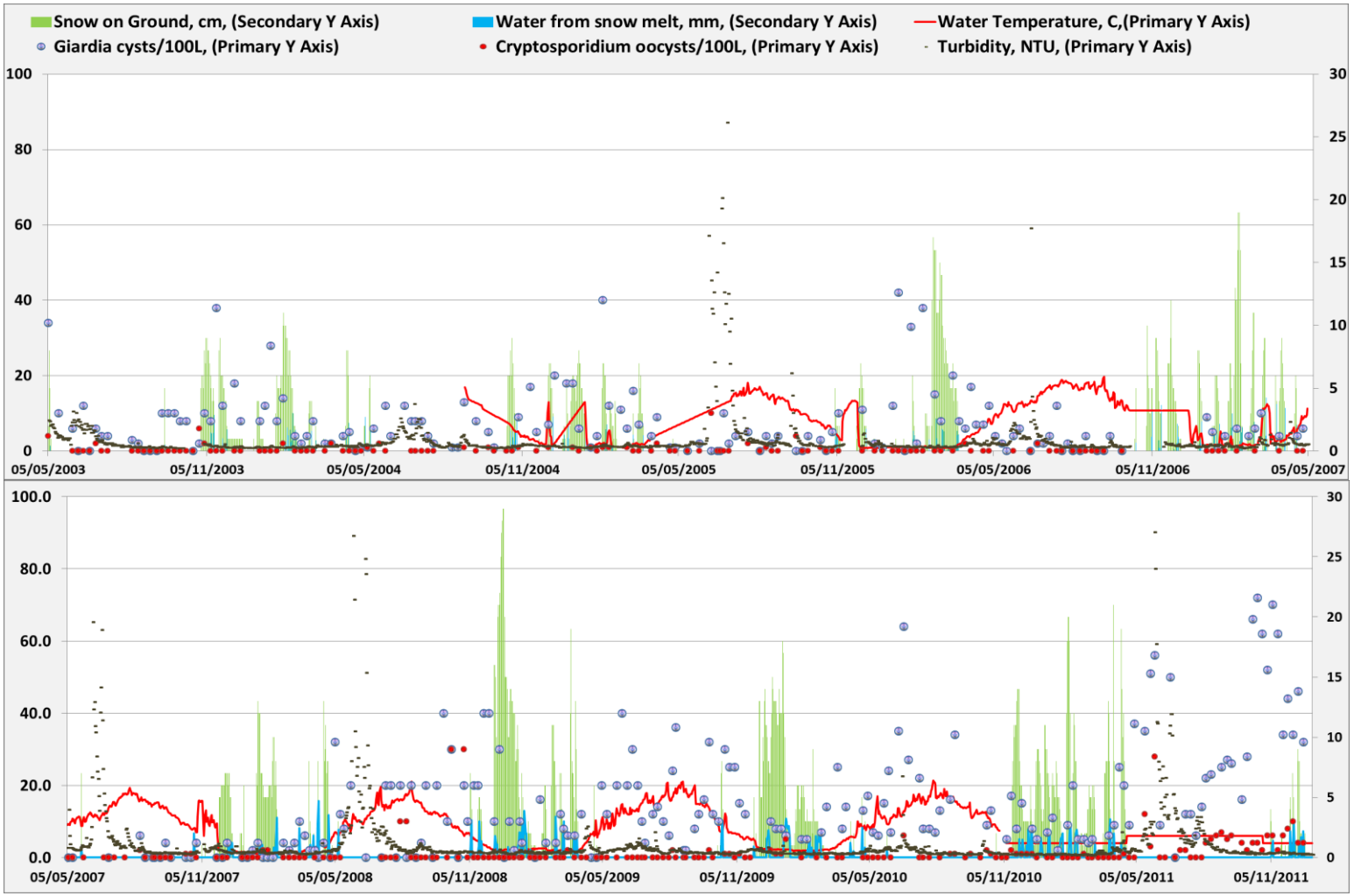

Figure 5.7 Diagram depicting the relationship between the occurrence of waterborne parasites (*Cryptosporidium* [red dots] and *Giardia* [blue dots]) with snow on ground (green bars), snowmelt (daily values [blue bars]), source water temperature (red line) and source water turbidity (black dots) in the Bow River. The data was plotted with respect to the time of testing. The records reflect 2003-2007 years (top panel) and 2007-2011 years (bottom panel) of the monitoring campaign. Dates on the x-axis are recorded as dd/mm/year.

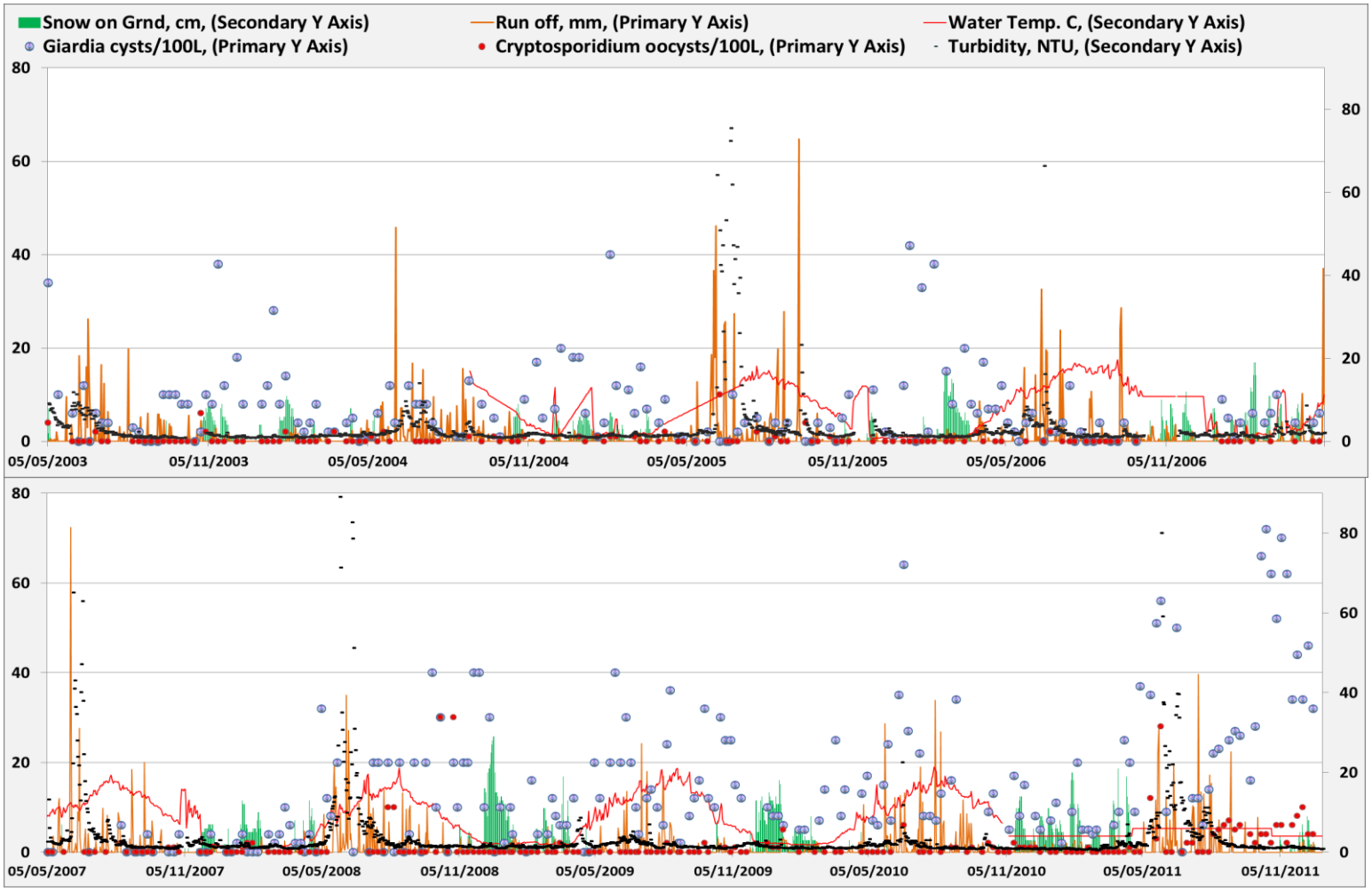

Figure 5.8 Diagram depicting the relationship between the occurrence of waterborne parasites (*Cryptosporidium* [red dots] and *Giardia* [blue dots]) with rain (daily values [orange line]), source water temperature (red line) and source water turbidity (black dots) in the Bow River. The data was plotted with respect to the time of testing. The records reflect 2003-2007 years (top panel) and 2007-2011 years (bottom panel) of the monitoring campaign. Dates on the x-axis are recorded as dd/mm/year.

5.2.3 Correlation between parasite contamination of source water and environmental factors

5.2.3.1 Correlation between *Giardia* concentration at the Glenmore WTP and rain run-off

Correlation analysis demonstrated a negative relationship between rain run-off 60 days running average (RR60DRA) and *Giardia* cyst concentrations for the Glenmore WTP in data aggregated across all years. The Pearson correlation coefficient for the whole period of monitoring was r=-0.46, and was statistically significant (p-value<0.01) (Table 5.1). The correlation coefficient across the entire period was smaller compared to the correlation coefficients in separate years, apparently because of the varying level of *Giardia* contamination of source water and the varying amount of rainfall observed in different years, or other unknown factors. Pearson correlation coefficients in the given years indicated significant negative relationships between *Giardia* contamination of source water and RR60DRA. The coefficients ranged from r=-0.43 (p-value<0.01) in 2008 (the lowest correlation) to r=-0.64 (p-value<0.01) in 2004 (the strongest correlation) (Table 5.1, Figure A-1 [Appendix]). The RR60DRA was considered a good negative predictor of *Giardia* concentration at the Glenmore WTP. Considering that run-off is a very complex process, the associations appeared to be substantial for the understanding of *Giardia* occurrence with respect to rain run-off for this location.

Table 5.1 Relationship between *Giardia* concentration at the Glenmore WTP (Elbow River) and RR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.46	-0.49	-0.64	-0.52	-0.59	-0.55	-0.43	-0.55	-0.55
p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
observations	362	42	38	41	41	43	41	43	73
Normality assumption	+++++	++++	++++	++++	++++	++++	++++	++++	+++++
Linearity assumption	++++	+++	+++	++++	+++	++	+++	++	+++++
Equal variance assumption (Breusch-Pagan / Cook-Weisberg test for heteroskedasticity p-value)	0.19	0.50	0.26	0.06	0.61	0.03	0.59	0.31	0.53

5.2.3.2 Correlation between *Giardia* concentration at the Glenmore WTP and snowmelt run-off

A positive relationship between snowmelt run-off 60 days running average (SMR60DRA) and *Giardia* cysts concentration in source water for the Glenmore WTP was observed. For the entire period of monitoring, Pearson correlation coefficient was r=0.46 (p-value <0.01) (Table 5.2; Figure A-2 [Appendix]). In given years, the values of the Pearson correlation coefficient varied from r=0.36 in 2004 to the high of r=0.71 in 2006. The correlation coefficients were statistically significant using 0.05 α level in 2004 and 2007 and very highly statistically significant using 0.01 α level in the other given years. The SMR60DRA was considered a positive predictor for *Giardia* concentration for the Glenmore WTP.

The City of Calgary is located in the southern Alberta region with winters being both long and cold. Occasionally, winds known as 'chinooks' bring masses of warm air to this region and snowmelt occurs. During a particular winter, many such cycles of rapid air temperature change can occur. Therefore, the City of Calgary is located in a unique geographic region, where the rapid weather change during winter/spring may explain the increased parasite concentration in the surface waters during this time.

Table 5.2 Relationship between *Giardia* concentration at the Glenmore WTP (Elbow River) and SMR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	0.46	0.53	0.39	0.53	0.71	0.36	0.61	0.69	0.58
p-value	<0.01	<0.01	0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
observations	362	42	38	41	41	43	41	43	73
Normality assumption	+++++	++++	++++	++++	++++	++++	++++	++++	+++++
Linearity assumption	++++	+++	+++	+++++	++++	++	++++	++++	++++
Equal variance assumption (p-value)	0.45	0.34	0.28	0.95	0.07	<0.01	0.86	0.15	0.21

5.2.3.3 Correlation between *Giardia* concentration at the Glenmore WTP and source water temperature

Correlation analysis between *Giardia* cysts concentration in the source water and water temperature at the Glenmore WTP demonstrated a negative correlation. Increased concentrations of parasite were observed during the periods when water temperature was low. Pearson correlation coefficient was r=-0.51 for the entire period of monitoring, and it was very highly statistically significant (p-value <0.01) (Table 5.3; Figure A-3 [Appendix]). Negative correlations were observed in every given year. The lowest correlation occurred in 2005 (r=-0.49; p-value <0.01) and the highest correlation was in 2009 (r=-0.64; p-value <0.01). The monitoring for water temperature started on October 29, 2003, which resulted in a small sample size for the first year of monitoring, and the samples that were available for this year mostly covered the cold-water season. This most likely explains why the correlation coefficient at this particular year numerically was higher than in other given years. The year 2010 was withdrawn from the analysis because water temperature data was not provided after October 30, 2010, thus data on water temperature was incomplete for correlation analysis in this particular year. In each of the given years, Pearson correlation coefficients were higher compared to the coefficient in the entire period of monitoring, most likely due to year to year variation in water temperature, changing parasite prevalence in given years, and different mean parasite concentration in given years. Therefore, adjusting of the data for the analysis by years had proven useful for examining shorter periods for the purpose of a risk assessment.

Table 5.3 Relationship between *Giardia* concentration at the Glenmore WTP (Elbow River) and source water temperature.

	Period of monitoring								
	All years of monitoring	**2003***	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010***
Pearson coefficient(r)	-0.51	-0.69	-0.60	-0.49	-0.61	-0.59	-0.54	-0.64	-0.29
p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
observations	285	16	38	41	41	43	41	43	22
Normality assumption	+++++	+	++++	++++	++++	++++	++++	++++	++++
Linearity assumption	++++	++++	+++	++++	++++	++++	++++	++++	+
Equal variance assumption (p-value)	0.01	0.83	0.51	0.15	0.11	0.02	0.23	0.31	0.18

*- estimates are biased because of not complete data

5.2.3.4 Correlation between *Giardia* concentration at the Glenmore WTP and turbidity

Correlation between *Giardia* cysts concentration in source water and source water turbidity revealed no relationship in the aggregated data across all years and in most of the given years for the Glenmore WTP. Only in 2010, the association considered moderate ($r=0.32$) and highly statistically significant (p-value <0.01), but appeared to represent an exception, because there was no relationship observed in the other separate years or in the entire period of monitoring campaign (Table 5.4; Figure A-4 [Appendix]).

Looking at Figure 5.9, the disconnectedness between source water turbidity and *Giardia* occurrence becomes apparent. The period of high turbidity was observed when water temperature was between 9 °C and 17 °C; this was usually during late spring and early summer. Prevalence of the parasite appeared to be low at this period, and it was repetitive in every given year.

It can be hypothesised that the chaotic turbulence in the water flow during the high water discharge in the river produced high turbidity due to the silt particles suspended in the water column. The nature of suspended mineral particles and solution chemistry could play a substantial role for parasites attaching to the mineral material, and because of low buoyancy of the mineral material, parasites could be trapped into sediments (Scholl *et al.*, 1990; Atwill *et al.*, 2002; Davies *et al.*, 2004), however, some parasites can be re-suspended during next flush of run-off (Davies *et al.*, 2004). Therefore, the highly turbid water at those periods may have confounded the relationship of *Giardia* concentration with SMR60DRA. At the same time, the high

turbidity of the source water may have nothing in common with parasite contamination sources, because turbidity could have been of mineral origin.

Table 5.4 Relationship between *Giardia* concentration at the Glenmore WTP (Elbow River) and source water turbidity.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.03	0.12	-0.05	-0.27	0.25	-0.22	-0.18	-0.21	0.32
p-value	0.57	0.47	0.78	0.10	0.11	0.15	0.26	0.18	<0.01
observations	351	38	37	37	40	43	40	43	73
Normality assumption	+++++	++++	++++	++++	++++	++++	++++	++++	+++++
Linearity assumption	_	_	++	_	_	_	++	++	+++
Equal variance assumption (p-value)	0.27	0.32	0.72	0.55	0.35	0.37	0.36	0.44	0.33

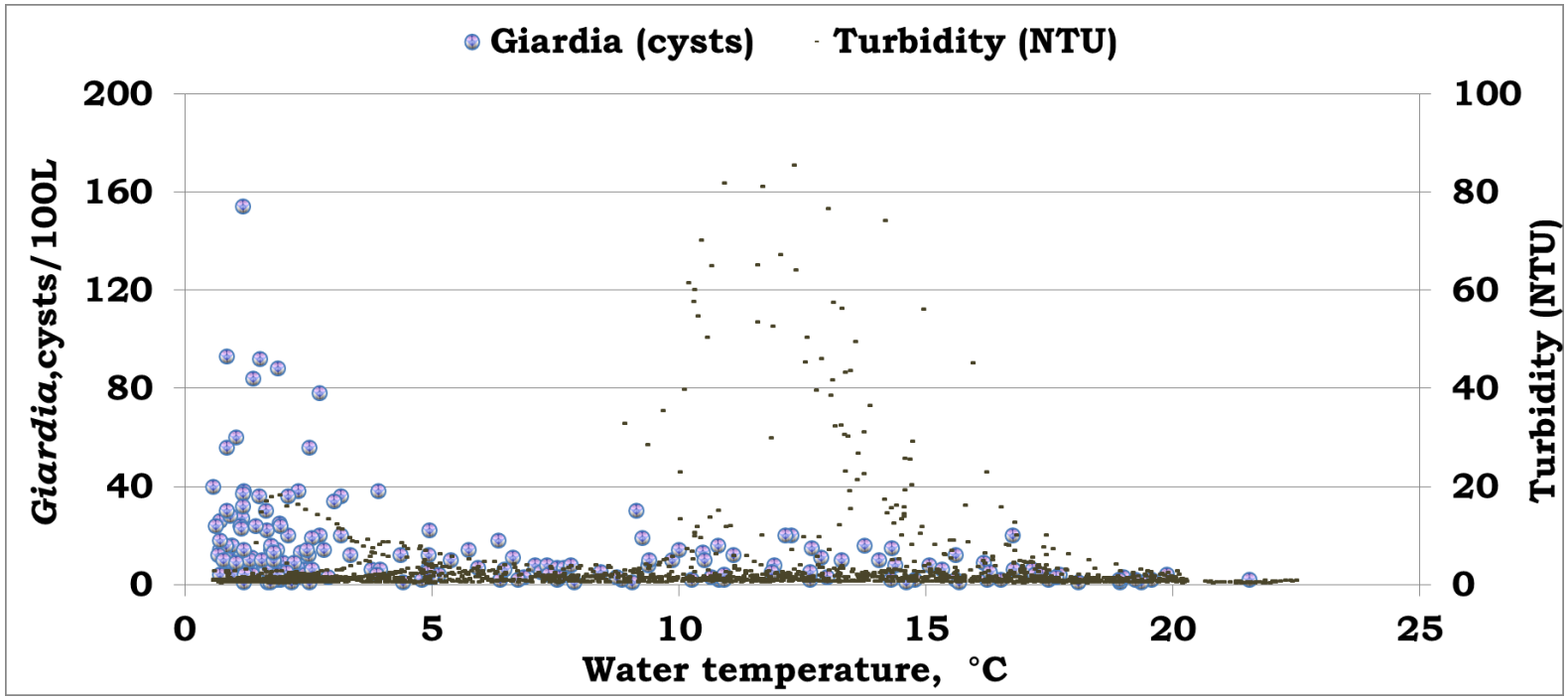

Figure 5.9 Occurrence of *Giardia* and source water turbidity in relation to water temperature. Water temperature was used for this graph as a factor of seasonality. Data is reflective of the sampling campaign 2003-2011 for the Glenmore WTP.

5.2.3.5 Correlation between *Cryptosporidium* concentration at the Glenmore WTP and rain run-off

No correlation between *Cryptosporidium* concentration and RR60DRA was observed at the Glenmore WTP during the entire period of monitoring 2003-2011. Pearson correlation coefficient was low and it was not statistically significant (r=0.05; p-value=0.33) (Table 5.5; Figure A-5 [Appendix]). Pearson correlation coefficients were low and not statistically significant in any of the given years also, and the direction of correlations varied across years. The exception was only the year 2008 when the relationship appeared to be positive (r=0.47), and statistically significant (p-value<0.01).

Cryptosporidium was detected rarely in source water, and when *Cryptosporidium* was detected the concentrations were low. A 16 % prevalence of *Cryptosporidium* at the Glenmore WTP was observed during 2003-2011(Table 3.1), and rare positive concentrations in given years. No *Cryptosporidium* oocysts were detected during the monitoring year 2006.

Table 5.5 Relationship between *Cryptosporidium* concentration at the Glenmore WTP (Elbow River) and RR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	0.05	0.13	0.28	-0.24	.	-0.17	0.47	-0.01	<0.01
p-value	0.33	0.42	0.12	0.14	.	0.27	<0.01	0.96	0.99
observations	332	39	32	37	34	41	37	39	73
Normality assumption	++++	+++	+++	+++		++++	+++	+++	++++
Linearity assumption	++++	++++	+++	++++		++++	+++	++++	++++
Equal variance assumption (p-value)	0.04	0.96	<0.01	0.09		0.04	<0.01	0.69	0.72

5.2.3.6 Correlation between *Cryptosporidium* concentration at the Glenmore WTP and snowmelt run-off

Correlation analysis between the concentration of *Cryptosporidium* oocysts in source water and SMR60DRA faced similar difficulties as the correlation analysis of association of *Cryptosporidium* concentration with RR60DRA, due to the rarity of *Cryptosporidium* occurrence in source water. An analysis for the whole period of monitoring and for the year 2004 showed weak negative correlations r=-0.11 and r=-0.37, respectively. Pearson correlation coefficients were statistically significant at α level 0.05 (p-value=0.04 and p-value=0.03, respectively) (Table 5.6; Figure A-6 [Appendix]). Pearson correlation coefficients in 2003, 2007, 2008, and 2010 indicated negative correlation, but the Pearson coefficients were not statistically significant.

Table 5.6 Relationship between *Cryptosporidium* concentration at the Glenmore WTP (Elbow River) and SMR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.11	-0.12	-0.38	0.14	.	-0.12	-0.17	0.01	-0.13
p-value	0.04	0.47	0.03	0.39	.	0.46	0.30	0.94	0.28
observations	332	39	32	37	34	41	37	39	73
Normality assumption	++++	+++	+++	+++		+++	+++	+++	++++
Linearity assumption	+++	++	++	++		+++	+++	+++	+++
Equal variance assumption (p-value)	<0.01	0.55	<0.01	0.06		0.48	0.01	0.46	0.11

5.2.3.7 Correlation between *Cryptosporidium* concentration at the Glenmore WTP and source water temperature

Due to the high prevalence of non-detections for *Cryptosporidium* (83 % of total number of samples through all years of monitoring) and very low concentrations when oocysts were detected, it was not feasible to determine if there was association between *Cryptosporidium* concentration in the source water and source water temperature in any given year in the Glenmore Reservoir. The correlation for the whole period of monitoring was very weak and statistically insignificant (r= -0.03; p-value=0.58) (Table 5.7; Figure A-7 [Appendix]), and consequently source water temperature had no predictive value for *Cryptosporidium* concentration in the Glenmore Reservoir.

Table 5.7 Relationship between *Cryptosporidium* concentration at the Glenmore WTP (Elbow River) and water temperature.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.03	-0.31	0.22	-0.29	.	-0.15	0.32	-0.01	0.17
p-value	0.58	0.26	0.23	0.07	.	0.33	0.05	0.99	0.42
observations	258	15	32	37	34	41	37	39	23
Normality assumption	+++++	+++	+++	+++		+++	+++	+++	+++++
Linearity assumption	++++	+	++	++++		++++	++	++	++
Equal variance assumption (p-value)	0.79	0.12	<0.01	0.07		0.05	<0.01	0.73	<0.01

5.2.3.8 Correlation between *Cryptosporidium* concentration at the Glenmore WTP and turbidity

Correlation between *Cryptosporidium* oocysts concentration in source water and turbidity was not attempted over individual years given the low prevalence of the parasites in source water. For the entire period of monitoring 2003-2011, the correlation analysis failed to reject null hypothesis that such relationship did not exist (r= -0.03; p-value=0.57) (Table 5.8; Figure A-8 [Appendix]).

The most turbid water in Glenmore Reservoir was observed when water temperature was between 9 °C and 17 °C (Figure 5.10). The changing of water temperature corresponded to the seasonal changes, and water turbidity usually increased in periods of late May to early July every year. *Cryptosporidium* oocyst concentration in source water usually did not increase in these periods (Figure 5.10).

Table 5.8 Relationship between *Cryptosporidium* concentration at the Glenmore WTP (Elbow River) and turbidity.

	Period of monitoring								
	All years of monitoring	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.03	-0.12	-0.07	-0.10	.	-0.14	0.09	-0.09	0.03
p-value	0.57	0.48	0.72	0.55	.	0.39	0.60	0.60	0.82
observations	322	35	31	33	33	41	37	39	73
Normality assumption	+++++	+++	+++	+++		+++	+++	+++	+++++
Linearity assumption	+	+	+	+		+	+	+	+
Equal variance assumption (p-value)	0.42	0.38	0.14	0.44		0.23	0.20	0.23	0.54

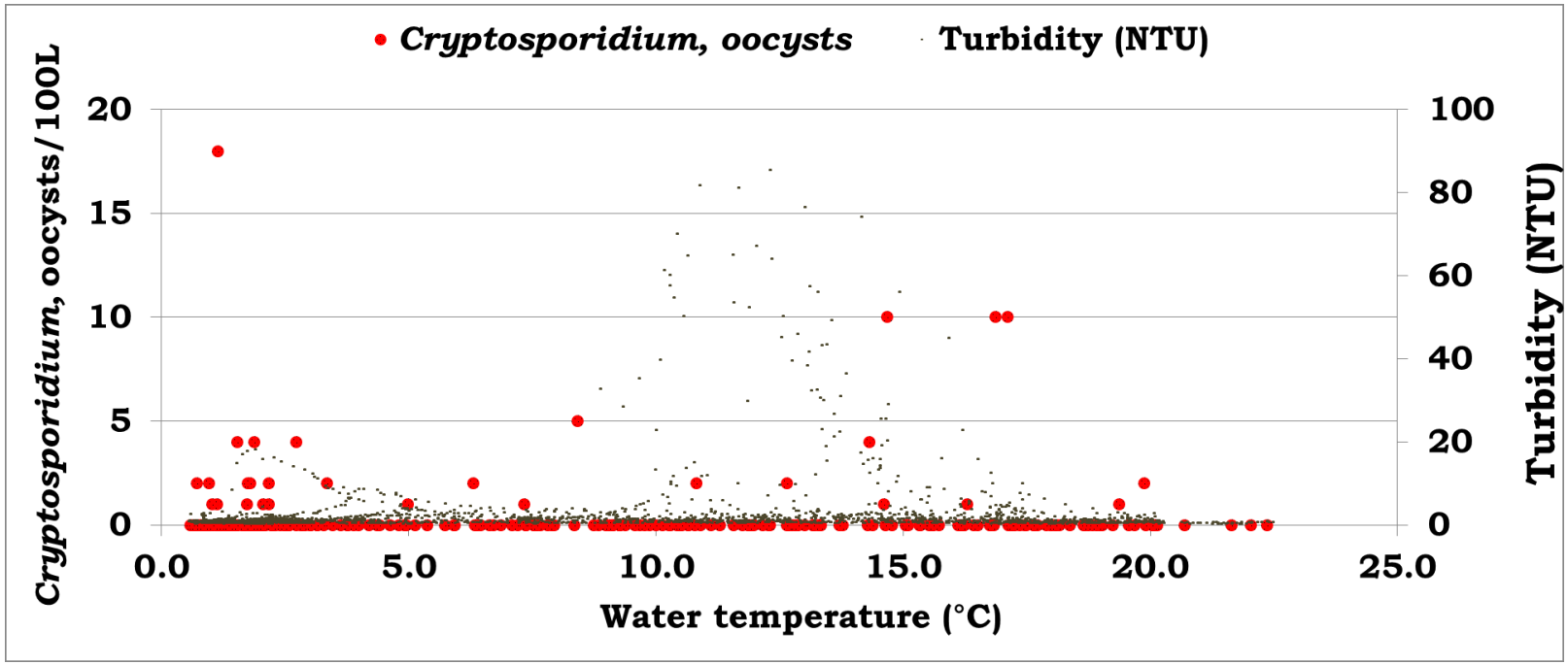

Figure 5.10 Occurrence of *Cryptosporidium* and turbidity in relation to water temperature. Water temperature was used as a factor of seasonality for this graph. Data is reflective of the sampling campaign from 2003-2011 for the Glenmore WTP.

5.2.3.9 Correlation between *Giardia* concentration at the Bearspaw WTP and rain run-off

Increasing run-off from rain (RR60DRA) tended to result in low *Giardia* cyst concentration in source water. The relationship was not stable in given years during the monitoring campaign. The relationship was weak and statistically significant across the aggregated dataset (i.e., 2003-2011 [r= -0.16; p-value <0.01]), and it was moderate and statistically significant in 2005 (r=-0.45; p-value <0.01). Weak correlations that were statistically insignificant occurred throughout other given years of monitoring (Table 5.9; Figure A-9 [Appendix]). Despite weak correlation in general, the important outcome of the correlation analysis was that the relationship was negative, and this was consistent with the direction of the association between *Giardia* concentration and RR60DRA for the Glenmore WTP.

The Glenmore WTP on the Elbow River and the Bearspaw WTP on the Bow Rivers share many similarities, but the major difference is that the Bearspaw WTP draws water directly from the Bow River, whereas the Glenmore WTP draws water from a reservoir. The absence of a water reservoir at the water intake at the Bearspaw WTP makes source water subject to conditions that change quickly.

Table 5.9 Relationship between *Giardia* concentration at the Bearspaw WTP (Bow River) with RR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.16	-0.24	-0.17	-0.45	-0.34	-0.24	-0.15	-0.28	-0.05
p-value	<0.01	0.12	0.29	<0.01	0.06	0.18	0.32	0.07	0.71
observations	334	42	38	38	30	33	46	41	66
Normality assumption	+++++	++++	++++	++++	+++	+++	++++	++++	++++
Linearity assumption	++++	+++	++++	++++	+++	+++	+++	+++	+++
Equal variance assumption (p-value)	0.03	0.71	0.58	0.97	0.14	0.78	0.10	0.28	0.39

5.2.3.10 Correlation between *Giardia* concentrations at the Bearspaw WTP and snowmelt run-off

No correlation was observed between *Giardia* cyst concentration and SMR60DRA for the entire period of monitoring (r=0.09; p-value=0.08). A trend of moderate, positive, and highly statistically significant correlations in 2004, 2005, and 2006, changed to an increasing trend of very weak, negative, statistically insignificant correlation in 2008 and 2009, and a statistically significant positive relationship in 2010 (Table 5.10; Figure A-10 [Appendix]). Therefore, the relationship reversed between the early years (prior to 2007) and the latter years (after 2008) of monitoring.

Table 5.10 Relationship between *Giardia* concentration at the Bearspaw WTP (Bow River) and SMR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	0.1	0.22	0.43	0.51	0.46	0.09	-0.02	-0.04	-0.27
p-value	0.08	0.16	<0.01	<0.01	0.01	0.61	0.90	0.80	0.03
observations	334	42	38	38	30	33	46	41	66
Normality assumption	+++++	++++	++++	++++	+++	+++	++++	++++	++++
Linearity assumption	++++	+++	+++	+++	++++	+	++++	+++++	++++
Equal variance assumption (p-value)	<0.01	0.34	0.45	0.10	0.02	0.71	0.14	0.70	0.22

5.2.3.11 Correlation between *Giardia* concentration at the Bearspaw WTP and source water temperature

Pearson correlation coefficient for the entire period of monitoring indicated a weak negative correlation r= -0.14, and the coefficient was statistically significant (p-value=0.02) (Table 5.11; Figure A-11 [Appendix]). Correlations were negative also in the given years. Correlation was moderate and Pearson correlation coefficients were very statistically significant in 2005 and 2006, r=-0.44 (p-value <0.01) and r=-0.48 (p-value <0.01), respectively. In 2007 and after this year, the relationship was weak and statistically insignificant. Water temperature data was lacking or incomplete in 2003, 2004, and 2010, therefore, these years were withdrawn from the analysis.

The observation of the negative correlation between water temperature and *Giardia* concentration is interesting in light of the correlation between *Giardia* concentration and snowmelt run-off, and the subsequent shifts to the pattern of higher *Giardia* concentrations observed in periods of warm water in the Bow River after 2007. *Giardia* concentration associated positively with snowmelt run-off before 2007, but after this year, the relationship changed to the negative and *Giardia* concentration began to correlate with warm water temperature. Remarkably, the patterns of association between *Giardia* concentration and water temperature for the Bow River in 2005 and 2006 were similar to the observed pattern in the Elbow River. Consequently, it would appear that this 'correlative state' in both watersheds preceded an 'environmental shift' in the Bow River with regards to new *Giardia* source contamination events or mobilization patterns towards a spring/summer dominance.

Table 5.11 Relationship between *Giardia* concentration at the Bearspaw WTP (Bow River) and water temperature.

	Period of monitoring								
	Aggregated across all years	**2003***	**2004***	**2005**	**2006**	**2007**	**2008**	**2009**	**2010****
Pearson coefficient(r)	-0.14	.	-0.13	-0.44	-0.48	-0.28	-0.13	-0.16	0.11
p-value	0.02	.	0.52	<0.01	<0.01	0.11	0.37	0.30	0.38
observations	279	0	25	38	30	33	46	41	66
Normality assumption	+++++		++	++++	+++	+++	++++	++++	++++
Linearity assumption	+++++		++++	+++	++++	+++	++++	+++	++++
Equal variance assumption (p-value)	0.04		0.76	0.91	0.04	0.91	0.07	0.46	0.24

*Monitoring of water temperature started 30/08/2004
**The last day of water temperature monitoring 30/10/2010

5.2.3.12 Correlation between *Giardia* concentration at the Bearspaw WTP and turbidity

High turbidity in the Bow River was observed during periods of water temperatures between 10 °C and 15 °C (Figure 5.11). There was no correlation between *Giardia* cyst concentrations in source water with turbidity. No trend for Pearson correlation coefficients was seen across given years, and no statistical significance was observed, either in the entire period of monitoring (2003-2011) nor in the separate years except the year 2010, when a Pearson correlation coefficient was 0.24 and it was statistically significant at α 0.05 level (Table 5.12; Figure A-12 [Appendix]). The moderate, statistically significant relationship in the last monitoring year could have relevance to the shift of *Giardia* occurrence in the Bow River to the spring/summer pattern of occurrence after 2007. This statistically significant and moderate relationship stands out from the commonly observed pattern of no correlation between *Giardia* concentration and source water turbidity in other years of monitoring in the Bow River at the Bearspaw WTP. The lack of correlations in earlier years was consistent with the results of correlation analysis between *Giardia* concentration and source water turbidity for the Elbow River. The consistency of the results was important to draw a conclusion that turbidity was not a predictor for *Giardia* occurrence in the Elbow River in the entire period of monitoring, and it was not a predictor for *Giardia* in the Bow River before 2010, but an apparent environmental shift may have had an effect on the relationship of *Giardia*

concentration in source water and source water turbidity in the Bow River at the Bearspaw WTP.

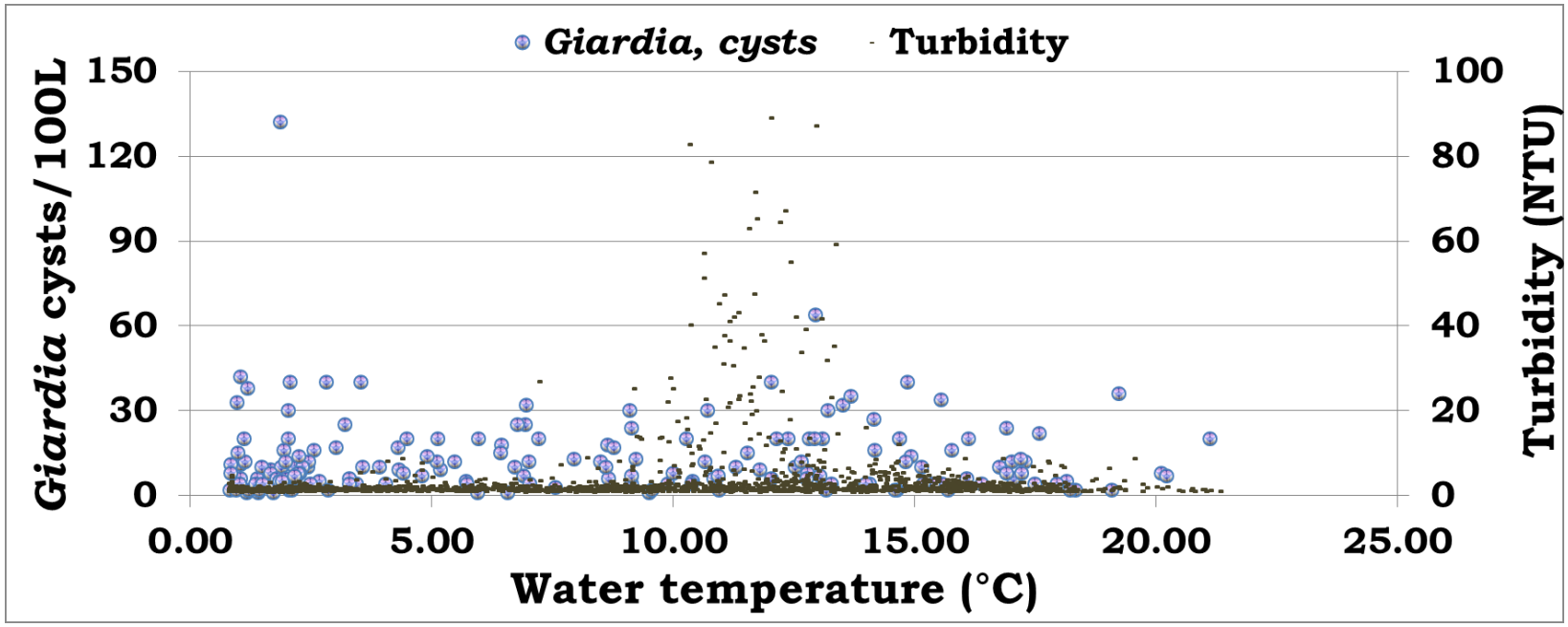

Figure 5.11 Occurrence of *Giardia* in source water and source water turbidity in relation to water temperature. Data is reflective of the entire sampling campaign 2003-2011 in the Bow River at the Bearspaw WTP.

Table 5.12 Relationship between *Giardia* concentration at the Bearspaw WTP (Bow River) and source water turbidity.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.01	-0.01	0.17	-0.15	-0.24	-0.16	-0.17	-0.02	0.24
p-value	0.79	0.96	0.29	0.38	0.19	0.38	0.25	0.88	<0.05
observations	328	39	38	36	30	32	46	41	66
Normality assumption	+++++	++++	++++	++++	+++	+++	++++	++++	++++
Linearity assumption	+	+	+	+	+	+	+	+	+
Equal variance assumption (p-value)	<0.01	0.05	0.10	0.95	0.45	0.41	0.83	0.52	0.36

5.2.3.13 Correlation between *Cryptosporidium* concentration at the Bearspaw WTP and rain run-off

Cryptosporidium in the Bow River was rare during the nine years of monitoring. More than 74 % of grab samples resulted in non-detects in the entire period of monitoring 2003-2011 (see Chapter 3, Table 3.2). The low prevalence of *Cryptosporidium* in given years made it infeasible to determine correlation in given years. Nevertheless, the analysis was possible for the entire period of monitoring and revealed a weak positive statistically significant correlation between *Cryptosporidium* and RR60DRA in this period (r=0.12) (p-value=0.03) (Table 5.13; Figure A-13 [Appendix]).

Table 5.13 Relationship between *Cryptosporidium* concentration at the Bearspaw WTP (Bow River) and RR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	0.12	-0.01	-0.28	0.32	0.24	-0.03	0.22	-0.16	0.16
p-value	0.03	0.94	0.12	0.06	0.29	0.86	0.16	0.33	0.19
observations	316	41	32	35	22	34	43	37	72
Normality assumption	++++	+++	+++	+++	++	+++	+++	+++	++++
Linearity assumption	++++	+++	+++	+++	+++	+++	+++	+++	++++
Equal variance assumption (p-value)	<0.01	0.92	0.23	<0.01	<0.01	0.57	<0.01	0.43	0.30

5.2.3.14 Correlation between *Cryptosporidium* concentration at the Bearspaw WTP and snowmelt run-off

Due to the low prevalence of *Cryptosporidium* oocysts in the source water of the Bow River, no correlation between *Cryptosporidium* concentration in source water and SMR60DRA in given years was observed, except the year 2010. In 2010, the prevalence of *Cryptosporidium* was higher than in any other given year (51 %) (see Chapter 3, Table 3.2). The analysis for this year resulted in the moderate negative correlation between *Cryptosporidium* and SMR60DRA. The Pearson correlation coefficient (r=-0.35) was statistically significant (p-value <0.01) (Table 5.14). For the whole period of monitoring the Pearson correlation coefficient indicated a weak negative statistically significant relationship between the variables (r=-0.16; p-value <0.01) (Table 5.14; Figure A-14 [Appendix]).

Table 5.14 Relationship between *Cryptosporidium* concentration at the Bearspaw WTP (Bow River) and SMR60DRA.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.16	0.06	0.32	-0.04	-0.18	-0.14	-0.29	0.05	-0.35
p-value	<0.01	0.71	0.07	0.81	0.43	0.44	0.06	0.76	<0.01
observations	316	41	32	35	22	34	43	37	72
Normality assumption	++++	+++	+++	+++	++	+++	+++	+++	++++
Linearity assumption	++++	++	++	++	++++	+++	++	+++	++++
Equal variance assumption (p-value)	<0.01	0.72	0.48	0.67	<0.01	0.35	<0.01	0.22	0.06

5.2.3.15 Correlation between *Cryptosporidium* concentration at the Bearspaw WTP and source water temperature

Water temperature data for the Bearspaw WTP was available starting in September 2004 and ending in October 2010. Water temperature data was not complete for both 2004 and 2010, and it was missing for 2003. Correlation analysis was performed for the years in which water temperature data was available. For the entire period of monitoring and in any given year, water temperature had no association with *Cryptosporidium* concentration in the Bow River at the Bearspaw WTP (Table 5.15; Figure A-15 [Appendix]).

Table 5.15 Relationship between *Cryptosporidium* concentration at the Bearspaw WTP (Bow River) and water temperature.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	-0.05	.	-0.01	0.29	0.12	-0.05	0.22	-0.31	-0.17
p-value	0.38	.	0.95	0.08	0.58	0.79	0.16	0.06	0.14
observations	264	0	22	35	22	34	43	37	71
Normality assumption	+++++		++	+++	++	+++	+++	+++	++++
Linearity assumption	++++		+++	+++++	++++	+++	++++	++++	+++
Equal variance assumption (p-value)	0.43		0.88	0.02	0.06	0.53	<0.01	0.04	0.21

5.2.3.16 Correlation between *Cryptosporidium* concentration at the Bearspaw WTP and turbidity

Correlation analysis of association between *Cryptosporidium* oocysts concentration in source water and source water turbidity in the Bow River at the Bearspaw WTP showed a weak positive relationship between the variables (r=0.17) for the entire period of monitoring (Table 5.16; Figure A-16 [Appendix]). A Pearson correlation coefficient was very statistically significant (p-value <0.01). A Pearson correlation coefficient for 2010 was the highest (r=0.28) among the coefficients in other given years and the only one that was statistically significant (p-value=0.02). Among the given years, only the data of 2010 had sufficient prevalence of *Cryptosporidium* in source water for the Pearson correlation test.

Interestingly, the results of analysis of the associations between *Cryptosporidium* and, separately, for *Giardia* concentrations in source water with water turbidity at the Bearspaw WTP were similar in 2010 and in the entire period of monitoring. The associations with turbidity were weak and statistically significant for both parasites. As it was mentioned previously, a new source of parasite contamination of the source water may have been introduced to the Bow River watershed, and both parasite sources may be mutually related to turbidity. Analysis of *Giardia* occurrence indicated that potentially a new source of contamination was introduced in 2007-2008, and after this year *Giardia* contamination in the river gradually increased.

The U.S. EPA's requirement for monitoring water turbidity while controlling risk from parasites is focused on water treatment performance (U.S. EPA, 2006); it does not imply an association of source water turbidity with the parasite. Turbidity was noticed to be correlative with parasites in environmental waters. LeChevallier & Norton (1992) was able to demonstrate correlation of *Cryptosporidium* with water turbidity at three different sites. Water turbidity is a very general term of water quality. Water sources can be turbid for many reasons; however, this does not imply that parasites are necessarily present. For example, if water is turbid due to pollution from livestock, then associations with parasites may be observed; however, if the water is turbid because of suspended mineral matter or decay of vegetation occurring in the water, this may not coincide with parasite occurrence in the water. Therefore, water turbidity does not necessarily have a universal relationship with parasite occurrence.

Table 5.16 Relationship of *Cryptosporidium* concentration at the Bearspaw WTP (Bow River) and turbidity.

	Period of monitoring								
	Aggregated across all years	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Pearson coefficient(r)	0.17	0.24	-0.19	0.30	-0.01	-0.14	-0.08	-0.26	0.28
p-value	<0.01	0.14	0.29	0.08	0.96	0.44	0.59	0.12	0.02
observations	309	38	32	33	22	33	42	37	72
Normality assumption	++++	++	+++	+++	++	++	+++	++	++++
Linearity assumption	+++	+	+	+++	+	+	+	+	++++
Equal variance assumption (p-value)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02

5.2.4 Trend line analysis of parasite occurrence

During the correlation analysis, it was demonstrated that *Giardia* concentrations correlated with SMR60DRA for the entire monitoring period for the Glenmore WTP and until 2007 for the Bearspaw WTP. The 60 days running average of snowmelt run-off was the most suitable yardstick that revealed this association by representing the mobilization of the parasite from land fields and the following period of wash out in the river.

For the Glenmore WTP, a trend line analysis demonstrated correspondence of the trend line of 60 days running average of *Giardia* cysts concentration in the source water to snowmelt run-off events that were depicted using the 60 days running average snowmelt run-off trend line. The *Giardia* concentration trend line ascended every time after snowmelt run-off was recorded (Figure 5.12).

For the Bearspaw WTP, the correspondence of the *Giardia* concentration 60 days running average trend line to the snowmelt run-off 60 days running average trend line was very similar to the explained above for the Glenmore WTP. However, this pattern changed after 2007. As it was pointed out in the Chapter 3, the increased cysts concentrations were recorded for the Bearspaw WTP in the summer of 2008. This resulted in the disruption of the similarity of both trend lines after 2007 (Figure 5.14). The disruption was observed until the monitoring ended. Additionally, the parasite concentration trend line exhibited the stable trend to the parasite concentration increase after 2008 for the Bearspaw WTP.

Analysing the effect of rain run-off on *Giardia* concentration, it was noticeable that the peaks of both the 60 days running average rain run-off trend line and the 60 days running average *Giardia* cysts concentration trend line were out of synchronicity with each other in every given year, and never had similar patterns at the Glenmore WTP during the entire period of monitoring (Figure 5.13) and before the summer of 2008 at the Bearspaw WTP (Figure 5.15). Repeatedly before 2008, and at the beginning of the rain period in the late spring in the given years, rain appeared to trigger a sharp decline in the average trend line of *Giardia* concentration. However, after 2008, the increasing concentrations of *Giardia* were recorded after rain run-off events. Subsequent to 2008, the similarity of patterns of *Giardia* concentration and the rain run-off 60 days running average, prevailed (Figure 5.15).

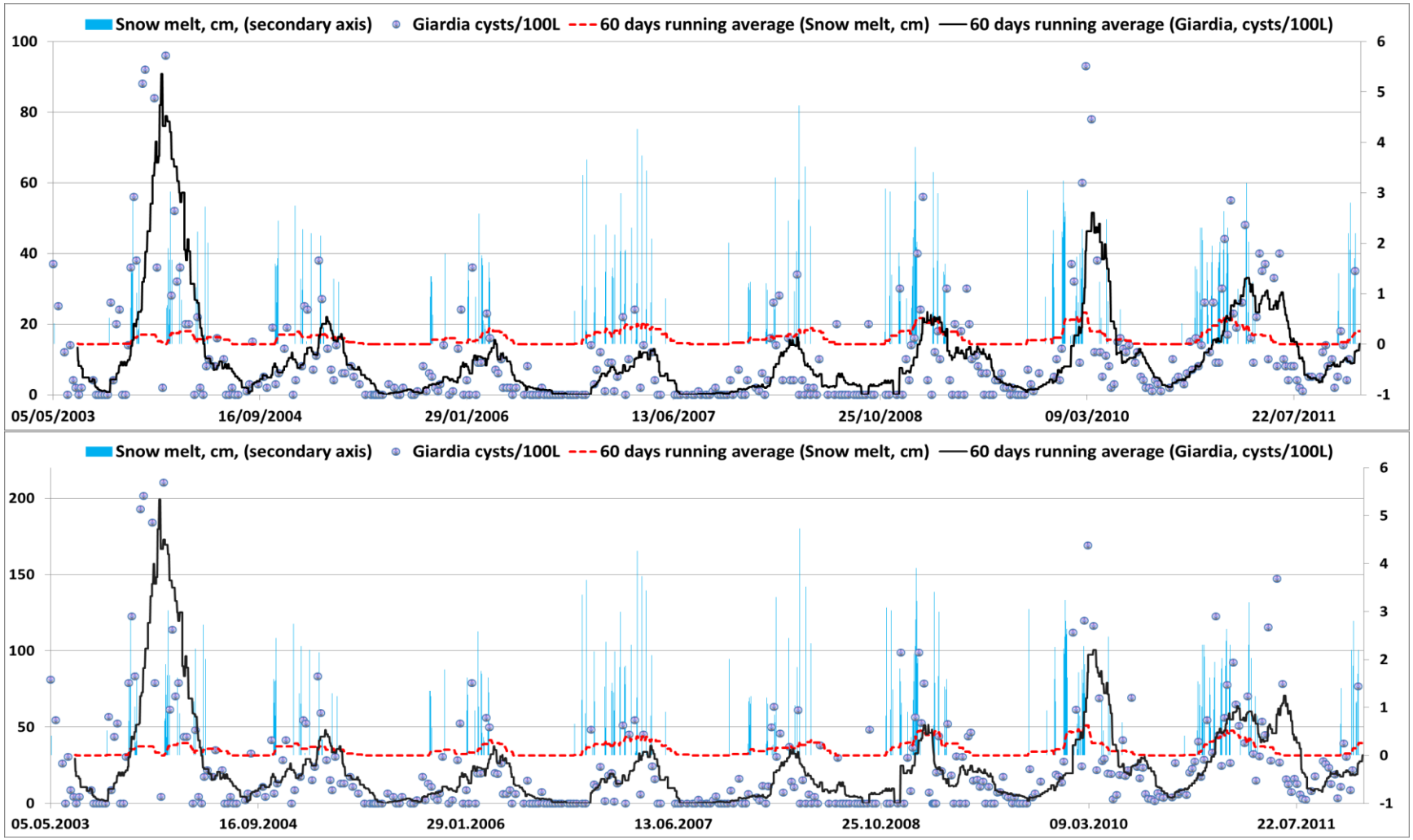

Figure 5.12 Examining correspondence of the trend line of *Giardia* cysts concentration 60 days running average to the trend line of snowmelt run-off 60 days running average. Data is reflective the sampling campaign at the Glenmore WTP (Elbow River) in 2003-2011. Concentrations of *Giardia* are unrecovered (upper panel) and recovered (lower panel).

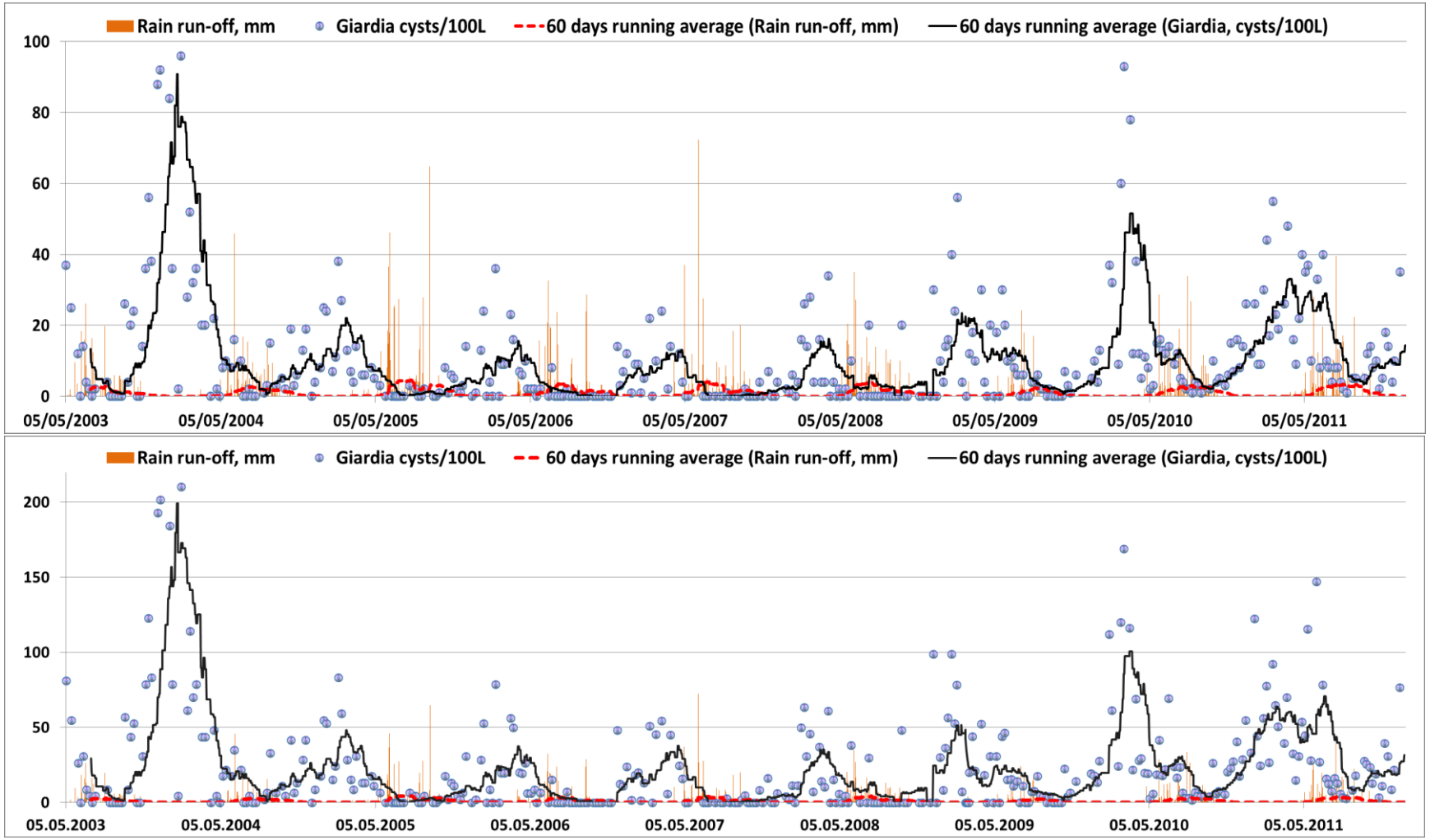

Figure 5.13 Examining correspondence of the trend line of *Giardia* cysts concentration 60 days running average to the trend line of rain run-off 60 days running average. Data is reflective the sampling campaign at the Glenmore WTP (Elbow River) in 2003-2011. Concentrations of *Giardia* are unrecovered (upper panel) and recovered (lower panel).

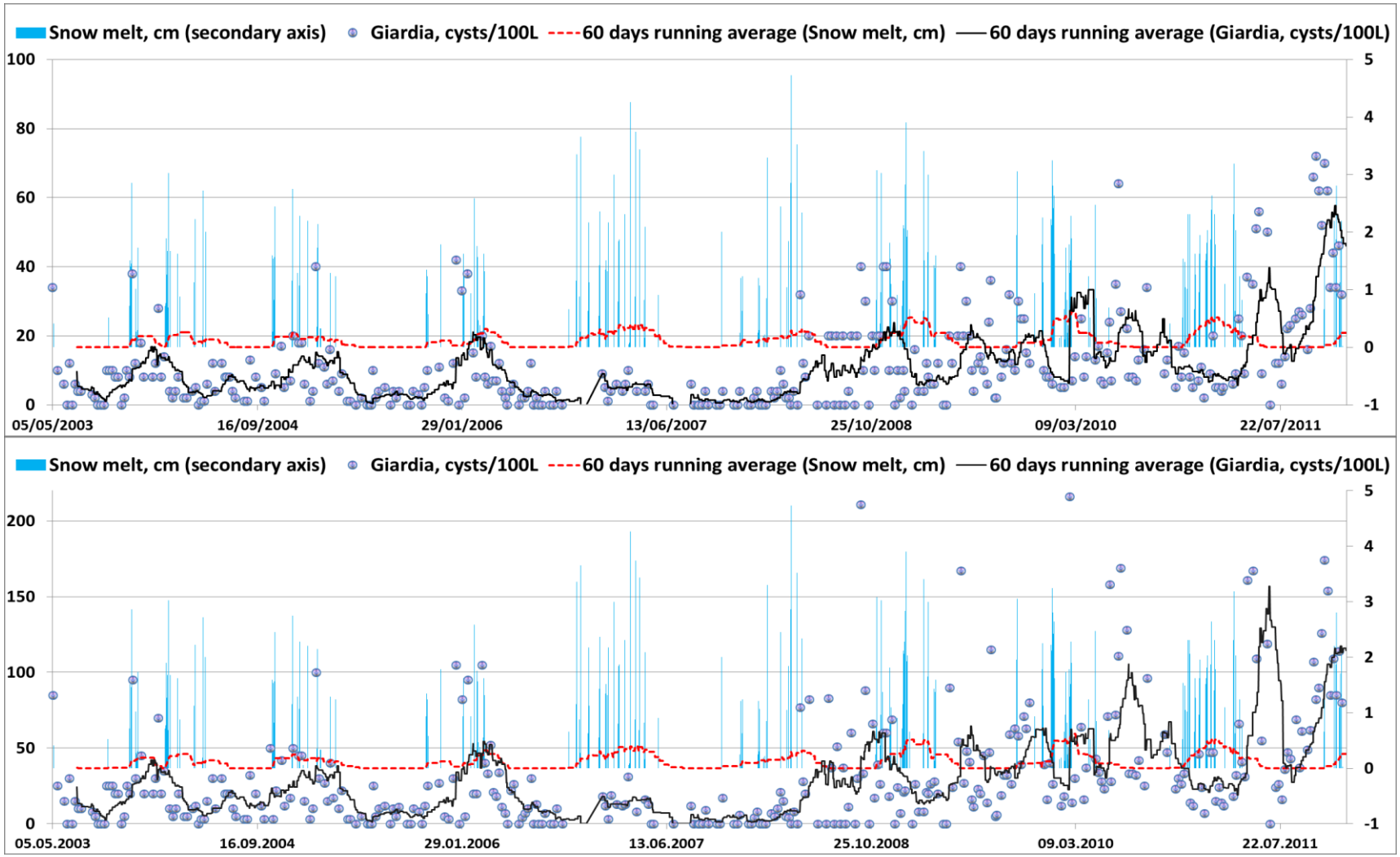

Figure 5.14 Examining correspondence of the trend line of *Giardia* cysts concentration 60 days running average to the trend line of snowmelt run-off 60 days running average. Data is reflective the sampling campaign at the Bearspaw WTP (Bow River) in 2003-2011. Concentrations of *Giardia* are unrecovered (upper panel) and recovered (lower panel).

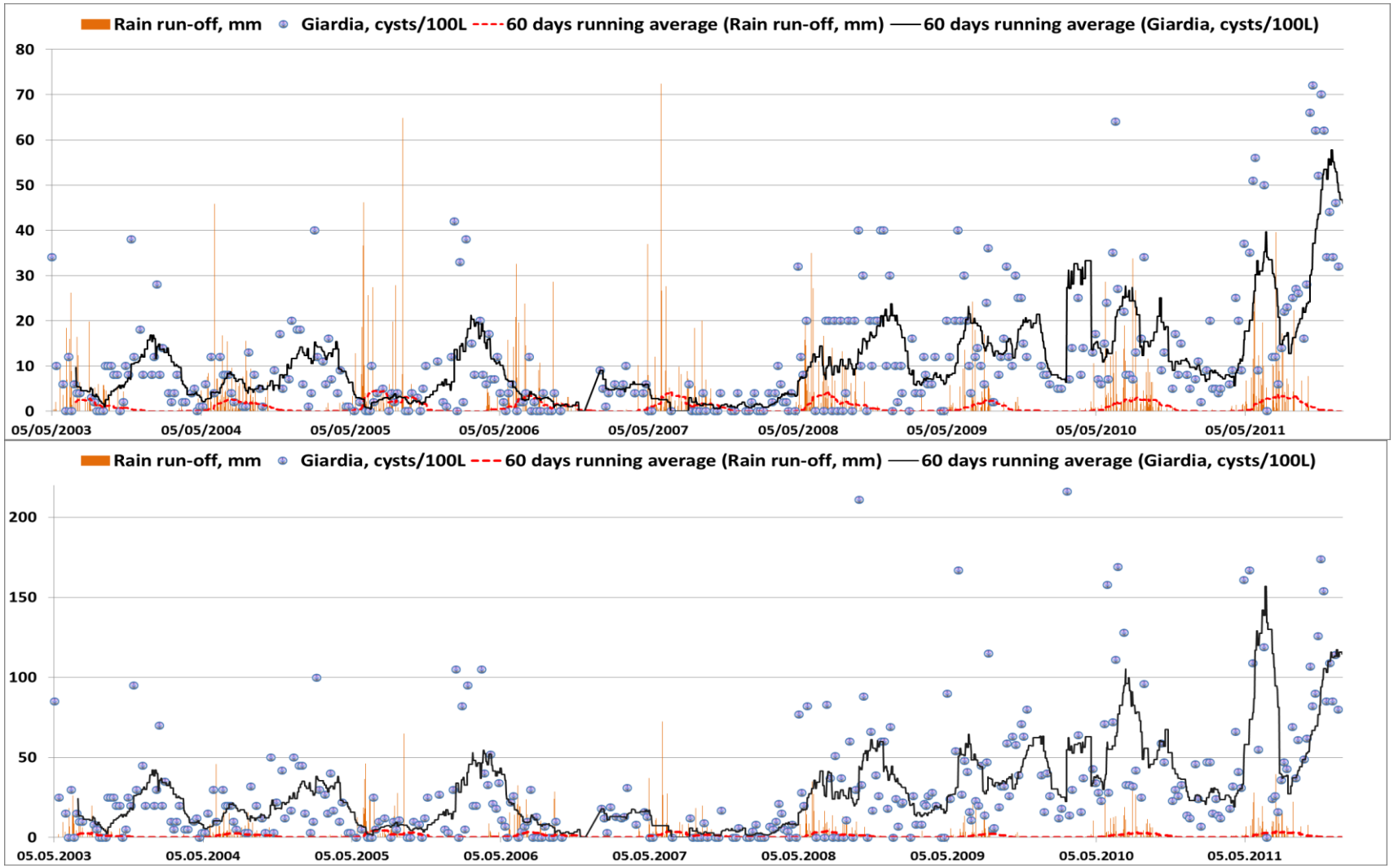

Figure 5.15 Examining correspondence of the trend line of *Giardia* cysts concentration 60 days running average to the trend line of rain run-off 60 days running average. Data is reflective the sampling campaign at the Bearspaw WTP (Bow River) in 2003-2011. Concentrations of *Giardia* are unrecovered (upper panel) and recovered (lower panel).

5.2.5 Predicting of *Giardia* concentration in the source water for the Glenmore WTP

The SMR60DRA and RA60DRA were used for predicting *Giardia* concentration in source water through using multiple linear regression analysis. The combined predictive potential was marginal (R^2=0.27, p-value <0.0001) (Table 5.17). Regression coefficients of both predictors RR60DRA and SMR60DRA were statistically significant. The large sample size supported a normality assumption. The linearity and homoscedasticity assumptions were not violated, based on a scattergram assessment (Figure 5.16) and a heteroscedasticity check by using the Breusch-Pagan / Cook-Weisberg test indicated that the null hypothesis of homoscedasticity was not rejected (p-value = 0.76). The lowess line did not deviate strongly from a zero line, which supported the validity of the linearity assumption (Figure 5.16).

Table 5.17 Multiple linear regression model output between concentration of *Giardia* cysts /100 L and the predictors of RR60DRA and SMR60DRA ***.

Predictor	R^2	p-value	t-value	Coefficient	95 % Conf. Interval	Breusch-Pagan / Cook-Weisberg test for heteroskedasticity p-value
Water T	0.26	<0.0001	-9.88	-0.125	-0.149 to -0.099	>0.01
RR60DRA	0.22	<0.0001	-10.00	-0.666	-0.797 to -0.535	0.19
SMR60DRA	0.21	<0.0001	9.8	5.518	4.410 to 6.625	0.59
RR60DRA & SMR60DRA *	0.27**	<0.001 <0.001	-5.40 5.06	-0.429 3.394	-0.586 to -0.273 2.076 to 4.712	0.76

* RR60DRA and SMR60DRA are used together predictors in multiple linear regression
** Adjusted R^2
***The applied data reflected sampling campaign 2003-2011 years at the Glenmore Reservoir (Elbow River).

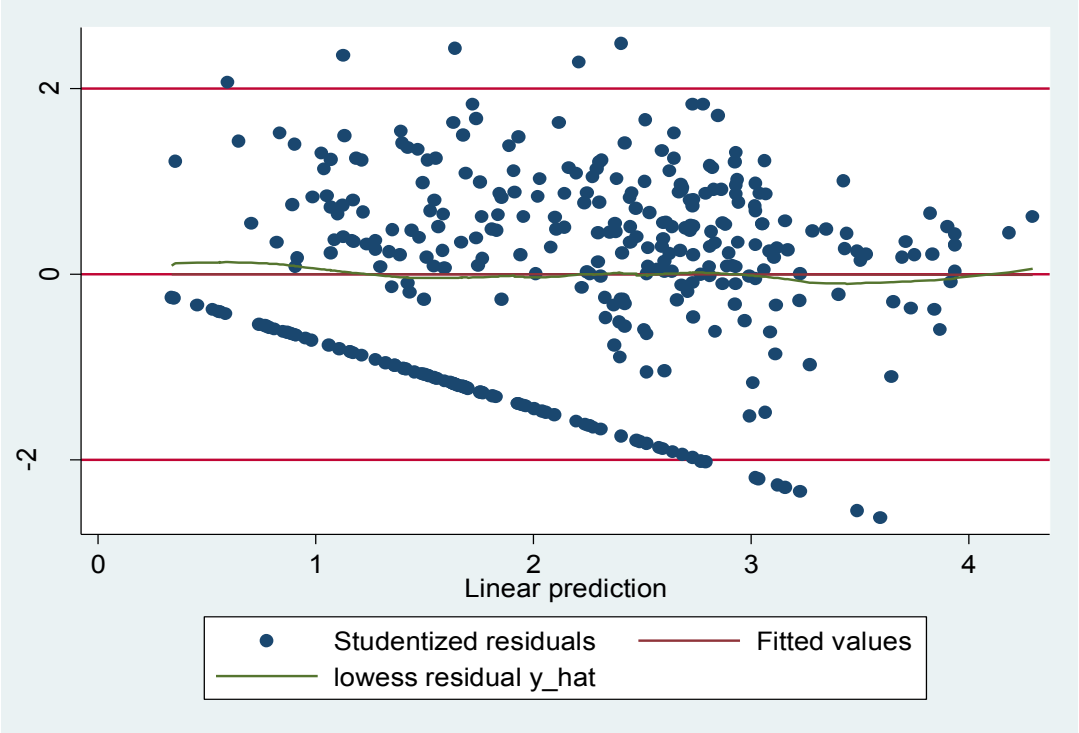

Figure 5.16 Scatter plot for linearity and homoscedasticity check. The lowess (green line) does not deviate from a zero line. Studentized residuals are scattered almost equally. Equal varience is influenced by zero concentration values, the studentized residuals of which are lined up.

Concentrations of *Giardia* cysts were predicted in source water at the Glenmore WTP at any day using the following multiple linear regression model:

$$Giardia\ (\frac{\text{cysts}}{100\ \text{L}}) = 2.718^{(2.209-0.4295*\text{Rain}+3.394*\text{SnowMelt})-1}$$

Where:

- Rain represents the RR60DRA (mm), and
- Snowmelt represents the SMR60DRA (cm).

The average estimated concentration of *Giardia* cysts were predicted in source water at the Glenmore WTP at any day, given the RR60DRA and SMR60DRA for this day. The upper and the lower bound estimates of a predicted concentration of *Giardia* in source water can be calculated by plugging estimates of RR60DRA and SMR60DRA at a particular day into the following equations, respectively:

$$Lower\ bound = 2.718^{(2.209-0.586*Rain+2.076*SnowMelt)-1}$$

$$Upper\ bound = 2.718^{(2.209-0.273*Rain+4.712*SnowMelt)-1}$$

When the predicted concentrations of *Giardia* were plotted against actual data points the model accurately predicted the cyclic nature of occurrence of *Giardia* (Figure 5.17). Both, the 24-month arithmetic mean and an annual running average were unable to demonstrate the cyclic nature of *Giardia* occurrence (Figures 3.6 and 3.8). However, both the 60 days running average of *Giardia* occurrence (Figure 5.12) and the predictive model resolved the cyclic periodicity of risk. For example, when using Health Canada's methodology for estimating annual parasite occurrence in any given

year (e.g., 20 cysts/100 L in 2004) concentrations estimates from the predictive model were in the range from 15 to 25 cysts/100 L for this time period. However, during the following summer, the predictive model would not be prone to overestimate concentration of parasite (i.e.: when data point concentrations were low) when compared to using annual arithmetic mean that Health Canada recommends.

The predictive model was able to discern periods of peak risk addressing variations in parasite concentrations over time. For example, consider the period of high *Giardia* concentrations starting from November 2005 to the end of March 2006. The arithmetic mean for this period was 20 cysts/100 L (STD=24), and the range of parasite concentrations was from 0 to 79 cysts/100 L. The annual arithmetic mean from May 2005 to May 2006 (encompassing the 6 month time frame noted above) was 12 cysts/100 L (STD=18) using the Health Canada QMRA model approach for estimating concentration. The predictive model provides concentration estimates daily in a range from 5 to 26 cysts/100 L. The arithmetic mean of the predicted estimates was 12 cysts/100 L (STD=5). When examining a shorter period (i.e., one month [March 2006]) in which only three samples can be used to derive a mean (i.e., 42 cysts/100 L [STD=19]), Health Canada's approach recommends that one still use the annual arithmetic mean of 12 cysts/100 L (STD=18) for evaluating risk. The predictive model proposes to use a range of concentration estimates from 13 to 26 cysts/100 L and with a mean of 20 cysts/100 L (STD=4). The predictive model is able to better address risk associated with peak parasite occurrence by providing estimates of parasite concentration that are approximately double the annual arithmetic

mean, while adhering to the principles of biological plausibility. Similarly, the predictive model offers lower concentration estimates of parasite occurrence compared to an annual mean, with short periods of low risk associated with environmental conditions that negatively affect *Giardia* concentrations in source water. The validity of the relationship of the actual peak periods of *Giardia* occurrence (data points) to the predicted periods of peak concentration is supported by the fit of the model. Compared to other options discussed here, the predicted concentrations of *Giardia* can be used as input values of *Giardia* concentration for the Health Canada QMRA model for estimating health risk from *Giardia* daily for the Glenmore WTP.

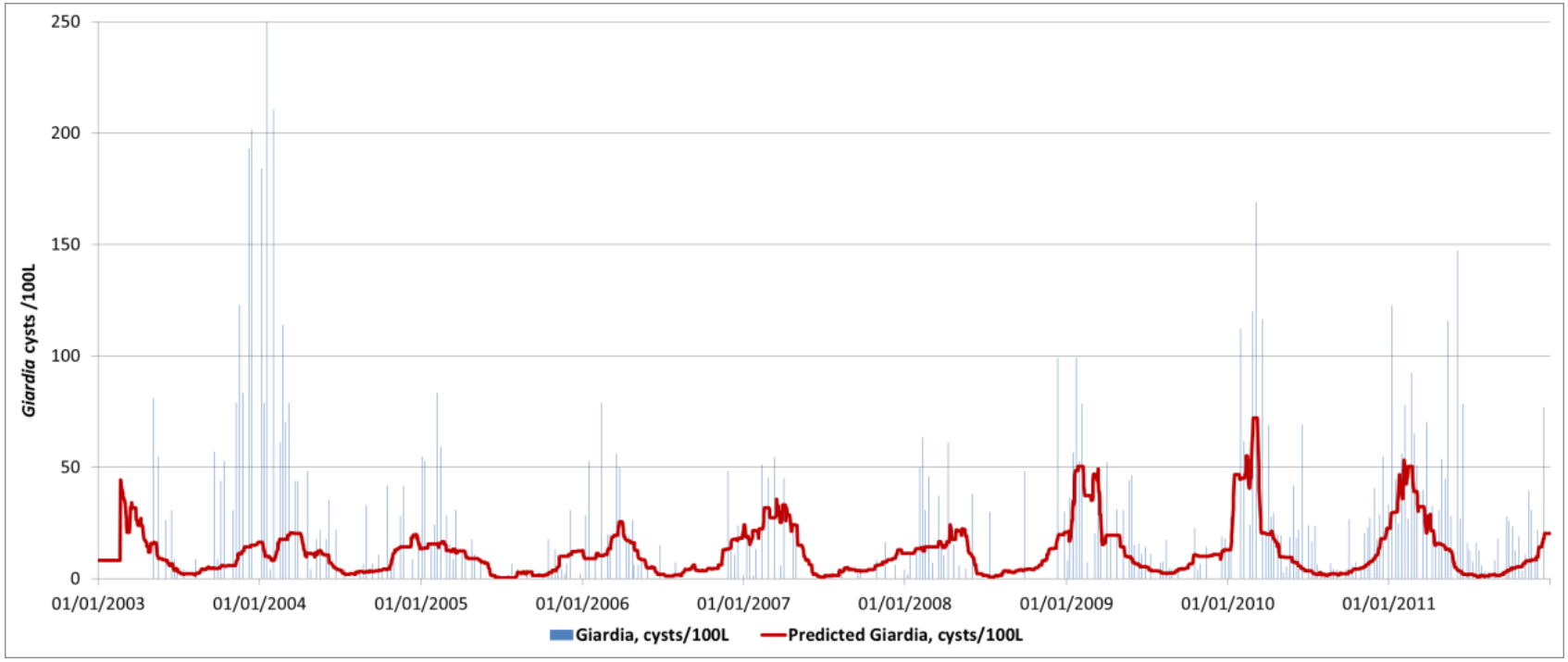

Figure 5.17 Forecasting of *Giardia* cysts concentration/100 L (red line) using predictors RR60DRA and SMR60DRA. *Giardia* point concentrations (blue bars) were recorded during sampling campaign 2003-2011 at the Glenmore WTP (Elbow River).

Giardia concentration correlated not only with RR60DRA and SMR60DRA but also with source water temperature in the Glenmore Reservoir at the Glenmore WTP. Both rain and snowmelt run-off can mobilize *Giardia* cysts to surface waters (Atherholt *et al.*, 1998; Ferguson *et al.*, 2005; 2007; Davies *et al.*, 2004). In addition, *Giardia* is able to survive better in cold water (Cole *et al.*, 1989; Bingham *et al.*, 1979; Wickramanayake *et al.*, 1985). When the *Giardia* concentration was regressed against water temperature, this resulted in a larger portion of variation of *Giardia* concentration that was explained by the model, compared with the portion of variation when the *Giardia* concentration was regressed against either RR60DRA or SMR60DRA separate (Table 5.17). Water temperature would only be important for *Giardia* survival if it was assumed that *Giardia* was mobilized by snowmelt run-off to surface water previously. Mobilization of *Giardia* by SMR60DRA would have a greater predictive importance than water temperature, despite that the SMR60DRA as a predictor had a smaller R^2 compared with the R^2 of water temperature as a predictor (Table 5.17). The R^2 did not increase and regression coefficient of water temperature was not significant when water temperature was included in the model together with SMR60DRA and RR60DRA.

It is also important to consider the uncertainty derived from distal (i.e., snowmelt run-off) compared to proximate variables (i.e., water temperature data). The latter was measured at the WTP, and thus can be considered a more proximate variable for *Giardia*

occurrence within any given sample. Data on snowmelt can be considered a distal variable due to collection of this data at a site remote from the WTP location (i.e., Calgary's International Airport). Improved certainty of environmental data would almost certainly improve the model fit and the accuracy of predicted parasite concentrations.

The R^2 =0.27 value observed for the regression relationship between RR60DRA and SMR60DRA and *Giardia* occurrence was not high; however, RR60DRA and SMR60DRA are not the only risk factors that would explain variation of *Giardia* concentration in the Elbow River. A great complexity of factors can influence parasite occurrence. Prevalence of host species for the parasite in the watershed, temporal immune status of individuals in the host population, environmental factors facilitating parasite degradation in the environment such as freezing (Peng *et al.*, 2008), predation, and desiccation (King & Monis, 2007), as well as exposure to UV radiation (Hijnen *et al.*, 2006), and biodegradation (Peng *et al.*, 2008; Jakubowski, 1990) represent some of the most likely parameters affecting *Giardia* occurrence. Data about direct drainage of human sewage into the source water and/or a prevalence of animals and waterfowl living in the watershed can also be considered for improving model estimates.

Chapter 6 :

General Discussion of Results

6.1. Most Significant Findings

The most significant findings of this thesis were:

I. The Bearspaw and Glenmore WTPs were able to sufficiently reduce risks associated with both *Giardia* and *Cryptosporidium* as evaluated through AESRD, U.S. EPA, and Health Canada's risk assessment frameworks.

II. A major discrepancy in *Giardia* risks was observed when comparing AESRD and Health Canada frameworks.

III. Health risk estimates are very dependent on underlying assumptions incorporated into risk assessment frameworks.

IV. Peak periods of risk were observed for *Giardia* and were associated with rain run-off, snowmelt run-off, and water temperature.

V. Temporal variations in peak risk from *Giardia* were observed in the Elbow and Bow Rivers, but patterns were different.

VI. Modelling of *Giardia* occurrence can add predictability for controlling risk, and can be integrated into a QMRA framework to identify critical concentrations requiring management action.

A detailed discussion on the importance of each of these findings is provided below. Recommendations and limitations of the study are also discussed at the end of this chapter.

6.1.1 The Bearspaw and Glenmore WTPs were able to sufficiently deal with both *Giardia* and *Cryptosporidium* attributed risks in the source water based on AESRD, U.S. EPA, and Health Canada current risk assessment frameworks

The water treatment process at both the Glenmore and Bearspaw WTPs relies on conventional filtration and disinfection by chlorine. Prior to the commencement of this thesis the primary concern related to risk associated with *Giardia*, based on the City's current operating approval requirements as determined by AESRD. The City of Calgary was considering the need for additional treatment barriers (i.e., UV inactivation) to control parasite risks, particularly during cold weather conditions.

Parasite monitoring showed that *Cryptosporidium* in the source waters of the Elbow and Bow Rivers was rare. Prevalence of *Cryptosporidium* oocysts in source water was 16 % for the Elbow River and 25 % for the Bow River. Prevalence of *Giardia* cysts in source water was 71 % and 85 % for the Elbow and Bow Rivers, respectively. Mean concentration estimates for *Giardia* were much higher than for *Cryptosporidium* in source water of both rivers. *Giardia*'s mean concentration was 24 (STD=37) cysts/100 L compared to the mean of 1.1 (STD=3.7) oocysts/100 L for *Cryptosporidium* for the Glenmore WTP, and the *Giardia*'s mean concentration was 32 (STD=40) cysts/100 L compared to the mean of 2.2 (STD=6.9) oocysts/100 L for the Bearspaw WTP, respectively.

Risk assessment using AESRD's regulatory approach indicated that 5-$\log_{10}$ reduction was required against *Giardia* at certain periods for both WTPs, and 3-$\log_{10}$ reduction was necessary against

Cryptosporidium. Application of the U.S. EPA's LT2 Rule (U.S. EPA, 2006) risk assessment framework to parasite monitoring data supported the conclusion that a 3-$\log_{10}$ reduction was sufficient against controlling risks associated with *Cryptosporidium* for both WTPs.

Risk assessment of the drinking water produced by the Glenmore and Bearspaw WTPs, using the Health Canada QMRA model, validated capability of the facilities to reduce risk from both parasites to below of the Health Canada health risk target 10^{-6} DALY per person per year. For the Glenmore WTP, the health risk estimates for *Giardia* were below 10^{-9} DALY per person per year (according to version 1.0 of HC QMRA model) in the given years of 2003 to 2011, and health risk estimates for *Cryptosporidium* were below 10^{-7} DALY per person per year in these same years (version 2.0 of HC QMRA model). For the Bearspaw WTP, health risk estimates for *Giardia* were below 10^{-8} DALY per person per year in given years 2003 to 2011 (version 1.0 HC QMRA model), and the health risk estimates for *Cryptosporidium* were below 10^{-8} DALY per person per year in same years (version 2.0 HC QMRA model). Overall, the estimates of health risk were well below Health Canada's target of 10^{-6} DALY per person per year. It was assumed that the Glenmore WTP, and in context of using HC QMRA model (version 2.0), was able to remove, on average, 4.3-$\log_{10}$ of *Cryptosporidium* oocysts using full-scale conventional filtration process (Hijnen & Medema, 2007), and remove and inactivate

6.5-$\log_{10}$ of *Giardia* cysts from the source water (HC QMRA model [version 1.0]). The Bearspaw WTP was able to remove 4.3-$\log_{10}$ of

Cryptosporidium oocysts, and remove and inactivate 6.1 - 6.4-$\log_{10}$ of *Giardia* cysts in the source water using the version 1.0 HC QMRA Model.

Overall, the risk assessment demonstrated that the applied water treatment technology is capable of providing effective reduction for both parasites from the assessed water sources, if no treatment failures ensued. Incorporation of a reverse QMRA for both the Glenmore and Bearspaw WTPs demonstrated that both plants were capable of handling up to 45,500 cysts/100 L concentration of *Giardia* in the source water if both the conventional filtration and the chlorination barriers were operated reliably. This value was based on conservative HC QMRA model assumptions. Similarly, the plants would be capable of handling *Cryptosporidium* concentrations in the source water of up to 13 oocysts/100 L.

The designation of a tolerable level of health risk from microbial hazards in drinking water is still a subject of discussions among scientists. Mara (2011) has argued that the regulatory administering of the health risk target of 10^{-6} DALY is excessively over protective for developing counties, and imposing it toward drinking water production elicits excessive expenditures and greatly impairs cost-effectiveness. Health Canada adopted the health-based target of 10^{-6} DALY per person per year from the WHO. The WHO (2006) set the annual reference level of DALYs per person per year at 10^{-6} for pathogen exposures from drinking and reuse waters, but are currently questioning whether to revise it to 10^{-5} or even 10^{-4} DALYs per person per year as a part of the rolling revision of its drinking water guidelines (WHO, 2008). The WHO's argument has been that the

reference level of a tolerable disease burden of 10^{-6}DALY per person per year may not be achievable or realistic in some locations and circumstances; and leave it open to the regulated community to set their preferred target. Where the overall burden of disease from microbial exposures through a combination of exposure routes (water, direct personal contact, food, recreational activities, etc.) is very high, setting an annual 10^{-6} DALY per person per year health target against waterborne exposure alone would likely make little difference on the overall disease burden. Reducing the overall level of risk from multiple exposure sources, so call harmonization across risk pathways, should be the public health objective (Prüss *et al.*, 2002; Prüss & Corvalan, 2006). Setting a less stringent level of acceptable risk, such as an annual 10^{-5} or 10^{-4} DALY per person per year from drinking water exposure may be more realistic, and still sufficient in terms of high standards of drinking water safety, particularly given that recreational water exposures are considered acceptable at much high risk levels (>1 %) (WHO, 2003; U.S. EPA 2012). Also, other yet to be assessed environmental pathogens, such as *Legionella* and non-tuberculous mycobacteria may represent much higher health burdens (Collier *et al.*, 2012) and should possibly be the target of future expenditures associated with drinking water use. However, it would be good practice to use the one common health target across all exposure pathways from a community's prioritization perspective.

6.1.2 A major discrepancy in *Giardia* risks was observed when comparing AESRD and Health Canada frameworks

A discrepancy was noticed between the AESRD regulation and the HC QMRA model with respect to *Giardia*. According to AESRD

requirements, parasite data suggested that *Giardia* was viewed as a primary hazard for both WTPs; however, the analysis using HC QMRA model demonstrated the opposite results. The attributed health risk from *Cryptosporidium* was larger than the attributed risk from *Giardia* by orders of magnitude according to HC QMRA model. Additionally, a review of the scientific literature failed to find any data that would support the AESRD's provision of less tolerance toward *Giardia* than toward *Cryptosporidium* in source water.

The health risk from *Cryptosporidium* was similar in all corresponding periods based on AESRD and U.S. EPA regulations, and with no critical difference with the Health Canada QMRA model. Therefore, consistency in estimating health risk from *Cryptosporidium* was similar using all three mentioned risk assessment frameworks. The U.S. EPA excluded monitoring for *Giardia* in the LT2 Rule, arguing that controlling for *Cryptosporidium* would assure control of other waterborne pathogenic protozoa (U.S. EPA, 2006). The U.S. EPA implies that *Cryptosporidium* is a waterborne pathogen of greater public health significance compared to *Giardia* in terms of pathogenicity, resistance to water treatment, and widespread occurrence in surface waters. Conversely, AESRD incorporates *Giardia* monitoring and treatment requirements in regulatory approvals for the City of Calgary, with a predominance of *Giardia* occurrence in these river systems as a driver of risk for both WTPs. Consequently, both WTPs require a 5-$\log_{10}$ treatment reduction when concentrations of *Giardia* in source water are between 10 to

100 cysts/100 L based on running annual average concentration over a two-year period. The same 5-$\log_{10}$ level reduction corresponds to the *Cryptosporidium* removal requirement in a range of 100 to 300 oocysts/100 L according to the AESRD and U.S. EPA regulations. By comparison, and applying a reverse QMRA approach to the Health Canada QMRA model (version 1.0), the data suggests that the Glenmore WTP can handle 114,000 *Giardia* cysts/100 L and the Bearspaw WTP can handle 45,500 cysts/100 L under conservative assumptions and when employing a 5-$\log_{10}$ treatment reduction scheme. Reverse QMRA also demonstrated that both plants can handle only 13 *Cryptosporidium* oocysts/100 L (Table 3.3). This large discrepancy in the tolerable levels of *Giardia* cysts in source water between AESRD's current operating approval requirements and HC QMRA models raises concerns with respect to which model is correct and should be used for regulatory compliance purposes. In the context of providing an additional barrier to control parasite risk, AESRD's requirements may be overprotective and lead to excessive expenditures (i.e., several millions of dollars to install UV) with no appreciable health benefit. On the other hand, the observation that the Glenmore WTP can handle an annual running average of 114,000 *Giardia* cysts/100 L based on reverse QMRA (and while ensuring all operations are meeting treatment targets [i.e., chlorine CT residuals]) suggest that relatively poor source water quality may be used without breaching tolerable/acceptable *Giardia* risks. For example, concentrations of 5,000 to 50,000 *Giardia* cysts/100 L are often observed in secondary treated domestic wastewater (Medema *et al.*, 2003). The fact that the risks are exceptionally low for *Giardia* based

on the HC QMRA model aligns with the U.S. EPA's current stance that *Giardia* risk are effectively managed through managing *Cryptosporidium* risks. AESRD's claim to treat water against *Giardia* at the source water concentrations observed at the City of Calgary's WTPs is evidently lacking support. It is recommended that AESRD consider revising *Giardia* treatment requirements in Alberta or provide a rational for retaining the existing standards.

6.1.3 Health risk estimates are dependent on underlying assumptions incorporated into risk assessment frameworks

In Chapter 4 it was shown that the underlying assumptions (arbitrary value for non-detects, fraction of human infectious (oo)cysts, method of derivation of concentration estimates) have profound influence on output of the HC QMRA model. The underlying assumptions are largely the source for systematic error in the risk assessment process; the type of uncertainty that is difficult to amend using statistical methods, however, the uncertainty can be estimated. Consequently, wrongly addressing or failing to address uncertainty can bias a decision maker to make the decisions that would not be the best solutions in an actual setting. For example, assuming a matrix spike recovery of 15 % for *Cryptosporidium*, correcting the non-detects with 0.5 value would result in a concentration of 3.3 oocysts/100 L for that sample, a concentration which itself is a quarter of the upper limit of the concentration when a 3-$\log_{10}$ treatment reduction would be required under the Health Canada version 1.0 QMRA model. Aggregating these corrected data points with other data points over a particular period to receive an average concentration estimate, can inflate risk. This can result in

misguidance for managers to take actions and result in unnecessary expenditure.

Only a fraction of parasites found in water will contain infectious and viable (oo)cysts. A study of tracking sources of *Cryptosporidium* in the Bow River demonstrated that most oocysts were not infectious for humans (unpublished data). Acknowledging this finding, the risk estimate would drop dramatically. For example, if the fraction of infectious oocysts is 10 %, it would elicit a drop in health risk for an entire $\log_{10}$ compared to a health risk from the same concentration of *Cryptosporidium* when all oocysts are considered infectious.

The Health Canada QMRA model (version 2.0) assumes that the parasites are distributed log-normally in source water, however, it may not be the case for every source water. Fitting and choosing a probability distribution with the actual parasite monitoring data would be preferable. Englehardt et al. (2012) pointed out that pathogens in source waters can have discrete Weibull or closely related discrete growth distributions, which are highly skewed. However, such option of choosing a distribution is lacking in the Health Canada QMRA model. Assuming that the parasites are log-normally distributed in water when they are not can result in an incorrect range of health risk estimates.

Analysis of data in this thesis demonstrated that the health risk estimates were very dependent on the assumption of the $\log_{10}$ removal that conventional filtration can provide. The underlying estimates of the efficiency of conventional filtration against *Cryptosporidium* used for version 1.0 and version 2.0 HC QMRA model

were 3-$\log_{10}$ and 4.3-$\log_{10}$ removal, respectively. These $\log_{10}$ removals can be translated into the capability to remove 13 and 256 oocyst/100 L, respectively. Removal efficiency of a full-scale conventional filtration process can vary from 0.8 to 5.5 $\log_{10}$ (Hijnen & Medema, 2007). The technologies that are used in the conventional filtration process should be applied and maintained properly and the process itself has to be operated in respect to the highest standards. Control measures and documenting should be established also. In some cases, in addition to monitoring source water concentration of pathogens, WTP designs need to be considered in the risk assessment process. For example, recycling water after filter backwash onto the filter beds may increase risks associated with both parasites due to accumulation of parasites within the filter bed. In the study of States *et al.* (1997), recycling of backwash water was considered a potential factor for treatment failure. In a more recent study Cornwell *et al.*, (2003) concluded that conventional filtration can successfully treat spent filter backwash water for *Cryptosporidium* when backwash water is recycled continuously or intermittently even without additional treatment before recycling. It should be stated clearly that recycling of backwash water without posing an additional risk strongly depends on the overall reliability, degeneracy, and even redundancy of the water treatment process, and especially on how conventional filtration steps are attuned to remove these parasites. There is no guarantee that conventional filtration at a specific location can achieve the $\log_{10}$ removals in the higher range of its estimates until it is validated by a treatment performance study. Improvements in operating conventional filtration process could increase efficiency of

water treatment through achieving higher estimates of parasite removal. For example, Edzwald *et al.* (2000) was able to demonstrate that electropositive coating of particles of filtration media for changing zeta potential improved removal of *Cryptosporidium* by 2.9 fold. Dissolved air flotation (DAF) can increase oocysts removal one or two orders of magnitude compared to sedimentation under a variety of conditions (Plummer *et al.*, 1995). Optimal and enhanced coagulation improve removal of *Cryptosporidium* by 1.5-$\log_{10}$ on average compared to suboptimal coagulation (Dugan *et al.*, 2001). Optimization of conventional filtration is the most sensitive factor for reducing health risk due to *Cryptosporidium*.

During 2006 at the Glenmore WTP, all 34 samples returned no detection of *Cryptosporidium*, and 41 samples revealed that *Giardia* prevalence was 51 % with a mean concentration at 11 (STD=16) cysts/100 L. The estimated attributed health risk from *Cryptosporidium* was more than an entire $\log_{10}$ higher than the estimated attributed health risk from *Giardia* in this year. The non-detection of *Cryptosporidium* versus the detection of *Giardia* resulted in the higher health risk estimates for the non-detected organism. This discrepancy arose because of using an arbitrary value of 0.5 (oo)cysts/100 L instead of zeros. This is an example of enacting the precautionary principle in a health risk assessment. This indicates that a large portion of uncertainty exists in health risk assessment of exposure to *Cryptosporidium*, and in particular, how non-detects are handled.

Commonly used parasite monitoring methods lack resolution in tracking temporal variation of parasite concentrations. As a result, the

ability of parasite monitoring to inform WTP personal about periods of peak risk, and consequently enabling additional protective measures, is questionable (Signor & Ashbolt, 2006). Similarly, risk can be overestimated when the risk is truly low. The shortcomings of available analytical methods of detection cannot be corrected currently. Because of this, results of parasite monitoring include a large portion of uncertainty. Tracking temporal variation of parasite concentration is a particular challenge. It is dubious whether statistical methods can reliably resolve the problems inherent to monitoring challenges. Statistical methods are applicable to analyse a population of interest given population parameters. In the real environmental settings, risk assessors have to deal with an unknown number of parasite populations. A parasite population should be defined with respect to a route of dissemination in the environment (i.e., mobilization by rain run-off or direct wastewater discharge), by which the contaminant enters to source water. The currently applied parasite monitoring frameworks view a parasite population based on a set of samples taken directly from source water, which might represent a single source population or even a mix of populations from multiple sources on a smaller scale. Currently, differences in parasite concentrations of samples taken during monitoring campaign represent spatial and temporal variability of parasite concentration, which implies dealing with only one population. This spatial and temporal variability of concentration may actually represent a difference among concentrations of parasite from distinct source populations. There is no confidence that a subsequent sample during a monitoring campaign can represent the same population of parasite

that was observed previously. Using data from different populations assuming that this is one population violates the basic principles of statistical analysis; therefore, it generates conceptual uncertainty.

The theory behind current methods of averaging parasite concentrations assumes that temporal variability of parasite concentrations in environmental waters occurs in a continual succession. This means that a concentration of parasite in water on one particular day depends on the concentration of parasites in water in the same water body in previous time of sampling. However, a previous state of an environmental system is not a prerequisite for its next state. Measures of central tendency are applicable in systems that have a single stability domain and are relatively homeostatic (Green, 2003). Some environmental systems, alternatively, can be seen as complex, nonlinear, and chaotic in nature. These systems experience sudden transitions from one state to another, a behaviour sometimes characterised in terms of chaos theory (Stewart, 1990). Concentrations of parasite, inferred based on grab sampling during monitoring, do not follow the property to be pooled toward a central tendency. Additionally, extreme observations of parasite concentration in a monitoring dataset can be seen as outliers or potentially as representing true periods of peak parasite concentration. For example, the Netherland's QMRAspot model defines peak events as when the concentration of parasite is above the 95th percentile (Schijven *et al.*, 2011). Distinct periods of peak parasite occurrence have to be addressed independently from other periods and should not be grouped together with other periods using measures of central tendency.

Certain processes during environmental events in a system can be seen as the drivers of parasite occurrence. Sometimes, this implies possibility of a relationship rather than a relationship. Therefore, prediction of parasite concentration in surface water may be described better by environmental events and their processes, rather than by routine parasite monitoring. Monitoring programs may be prone to miss peak events by chance, but long-term continuous monitoring program may provide sufficient information about pathogen occurrence patterns (Kistemann *et al.*, 2002; Kay *et al.*, 2007).

6.1.4 Peak periods of risk were observed for *Giardia* and were associated with rain run-off, snowmelt run-off, and water temperature

A negative and statistically significant association was observed between rain run-off and *Giardia* concentration at the Glenmore WTP during the entire period of monitoring and in any given years as well. For the Bearspaw WTP, the negative association between rain run-off and *Giardia* concentration was weak and statistically significant in the aggregate dataset as well as in the year 2005. The association was weak and statistically insignificant in other given years. In addition, a positive and significant association between *Giardia* occurrence and snowmelt was observed in 2004, 2005 ($r=0.43, 0.51$; $p<0.01$, respectively), and 2006 ($r=0.46$; $p=0.01$) for the Bearspaw WTP, but not for the aggregated dataset for the entire period 2003-2011 at the Bearspaw WTP. It can be hypothesised that an ecological disturbance took place in the watershed of the Bow River in 2006-2008 and distorted the relationship between snow run-off and *Giardia* observed prior to this date. The characteristic of rapid water flow in the Bow

River could obscure this association for the Bearspaw WTP. *Cryptosporidium* weakly and statistically significantly associated with rain run-off in the entire period of monitoring for the Bearspaw WTP, but not for the Glenmore WTP.

It is commonly acknowledged that rainfall events can result in an increase of parasite concentration in surface waters (Atherholt *et al.*, 1998; Ferguson *et al.*, 2005; 2007; Davies *et al.*, 2004). Bare land surface (Ferguson *et al.*, 2007) and slope (Tale *et al.*, 2000) are the exacerbating factors for parasite mobilisation during rainfall. Atwill *et al.* (2002) suggested that soils with higher bulk densities are less effective at removing *Cryptosporidium* oocysts than the soils with lower bulk densities. Davies *et al.* (2004) concluded that maintaining a vegetative cover, particularly riparian buffers, is of critical importance for managing transport of *Cryptosporidium*.

Very surprising was that rain run-off negatively correlated with *Giardia* at the Glenmore WTP, given that rain run-off could mobilize *Giardia*. It is possible that the interaction of *Giardia* cysts with certain soil properties was detrimental for cyst transport. A similar process of *Giardia* sedimentation during rain run-off could have been important for the Bearspaw WTP. However, the associations were weaker and less consistent in different years compared to the Glenmore WTP.

The retention time of a contaminant in water in a river can be dependent on velocity of water in the river. As river flow volumes and velocity increase there is greater possibility that a volume of water that has high concentration of parasite descends downstream quicker and water with less concentration of parasite will replace the highly contaminated water in a section of interest in the river. More frequent

sampling may assist in tracking the occurrence of parasites in fast flowing rivers.

Association of *Giardia* with snowmelt run-off was positive and statistically significant for the Glenmore WTP ($r=0.46$; $p<0.01$). In addition, the association was positive and statistically significant in 2004, 2005 ($r=0.43$, 0.51; $p<0.01$, respectively), and 2006 ($r=0.46$; $p=0.01$) for the Bearspaw WTP, but not for the aggregated dataset for the entire period 2003-2011 at the Bearspaw WTP. In 2010, the association become negative and statistically significant. The data suggests that a disturbance of the ecosystem may have distorted the association after 2008. Although *Cryptosporidium* was weakly, negatively, significantly correlated with snowmelt run-off, in the aggregated dataset the validity of the relationship is questionable given the predominance of non-detects observed throughout the dataset of any given year. An absence in a measurable presence of one variable makes it difficult to compare to another measured variable or the relationship between those two.

Possible other sources of contamination of surface water could confound the association between *Giardia* with rain or snowmelt run-off such as contamination of source water by leaking sewage into source water (irrespective of rain), and/or contamination by animals and birds defecating directly to the source water. However, adjusting for these variables was not feasible in this study. Monitoring for these important factors should be included in a watershed control program and become a part of WSPs recommended by AESRD.

Cold water associated with *Giardia* for both WTPs. This observation was consistent with findings of other researchers that

indicated that temperature above 10 °C increases rates of cysts die off (Cole *et al.*, 1989; Bingham *et al.*, 1979; Wickramanayake *et al.*, 1985), and high temperature detrimental for cysts infectivity (Labatiuk *et al.*, 1992). Bingham *et al.* (1979) found that freezing and thawing resulted in great loss of cysts viability, although a small portion (~1 %) remained viable. The hypothesised ecological disturbance may have confounded a relationship of *Giardia* concentration and water temperature for the Bearspaw WTP by decreasing and distorting the estimates of the relationship.

The data also showed that *Cryptosporidium* occurrence was irrespective of source water temperature at both WTPs. It is well known that the increased temperature is detrimental for oocysts survival (Medema *et al.*, 1997; Robertson *et al.*, 1992) and infectivity (Fayer, 1994; King *et al.*, 2005) due to fast depletion of amylopectin reserves (Fayer *et al.*, 1998). Freezing significantly decreases oocysts survival, especially because of thaw-freeze cycles (Walker *et al.*, 2001). However, Fayer and Nerad (1996) found that oocysts may survive for weeks or month at surface soil temperature just below freezing and insulated by a cover of snow. Robertson *et al.* (1992) concluded that it might be unwise to assume that oocysts that have been frozen are incapable to start infection. The same could be true for *Giardia* infectivity after exposure to the temperature below zero in the environment (Fayer & Nerad, 1996).

Source water turbidity weakly and statistically significantly associated with *Cryptosporidium* for the Bearspaw WTP in the entire period of monitoring and in 2010, and turbidity association with *Giardia* was observed only in 2010. For the Glenmore WTP,

association of *Giardia* with source water turbidity was recorded only in 2010, but no association was observed between *Cryptosporidium* and source water turbidity. Interestingly, *Giardia* associated with turbidity at both WTPs only in 2010, which could suggest a common nonpoint source of parasite pollution in this year in both watersheds.

Relationship between parasites and water turbidity is a contradictory subject in the literature. Some authors observed a relationship (Selvakumar & Borst, 2006; Ryu *et al.,* 2005), while others did not (Horman *et al.,* 2004). The data presented in this thesis demonstrated that the association of *Giardia* concentration and water turbidity was weak, positive, and statistically significant at both WTPs in 2010. *Cryptosporidium* concentration weakly associated with water turbidity in 2010 and in the entire period at the Bearspaw WTP, but no statistically significant association was observed for the Glenmore WTP. The long-term dataset provided by the City of Calgary sheds some light on this contradictory relationship. The data suggests that relationships between turbidity and parasite occurrence may exist in one year but not others, challenging an assumption that turbidity can be used as a proxy for event-based monitoring (i.e., remote sensing). Peak period parasite challenges for WTPs can occur when turbidity is low.

The correlation observed between *Giardia* concentration with snowmelt run-off may be under estimated due to uncertainty in measurement methods. The goal of the correlation analysis was to explore the effect of snowmelt on *Giardia* concentration and to accommodate the event base principle of snowmelt run-off effect for *Giardia* occurrence. Accounting for snowmelt is very complicated,

thus a very simplistic way of calculating this value was used in the thesis. Undoubtedly, if precision and accuracy of snowmelt estimates were improved (i.e., watershed measurements instead of closest meteorological station [i.e., Calgary airport]), the estimates of the association may be stronger and statistical significance would likely be improved also.

The opposing directions between correlations for rain (i.e., negative) and snowmelt run-off (i.e., positive) with concentration of *Giardia* cysts in source water for the Glenmore WTP may be explained by noting the fundamental difference in these processes. Run-off over frozen surfaces may be effective for mobilising *Giardia* cysts but not particulates, compared to rain run-off when cysts have a greater chance to be attached to inorganic particles and because of lesser buoyancy of these particles. In some cases, *Giardia* cysts can be trapped. Ferguson *et al.*, (2007) found that efficiency of mobilisation of *Cryptosporidium* due to rain run-off decreased significantly over a distance of soil surface, possibly, because of a larger size of the parasite comparing to bacteria and viruses, which were prone to travel easier. The same principle can be extrapolated for *Giardia* as well. Ferguson *et al.* (2007) identified that soil matrix plays a substantial role for impeding a distance for oocysts travel during run-off. In addition, rain usually occurs during warmer seasons when faeces are prone to desiccation and biodegradation (by bacteria thriving in warm environments). A number of faecal bacteria have been shown to replicate in first few days after faecal pats are deposited (Wang *et al.*, 2004). Desiccation and temperature above 20 °C is known to be detrimental for cysts survival (Bingham *et al.*, 1979). During a cold

season, conditions are different; cold water between 4 °C to 8 °C is optimal for cysts survival (Jakubowski, 1990), cysts are less exposed to sun light during short winter days, fewer bacteria, and less desiccation. Muirhead at al., (2006) found a significant decrease in the number of *E. coli* in faeces and in run-off during winter.

It is important to distinguish between the differences of how cold water may play a role in increasing cyst occurrence compared to rain and snowmelt run-off. Snowmelt and rain run-off act as mobilisation factors that move parasite to waterways (Atherholt *et al.*, 1998), whereas cold water can subsequently enhance the preservation of these cysts once they get into the source water. Warm water conditions may facilitate a decrease in cyst occurrence. It is unknown whether rain run-off actually impairs *Giardia* cyst occurrence during warm times of the year (i.e., increased sedimentation as described above), or whether warm environments facilitate cyst degradation (e.g., cyst desiccation) (Bingham *et al.*, 1979), UV damage (Hijnen, Beerendonk, & Medema, 2006), or biodegradation (Jakubowski, 1990). In addition, all these factors may work together to create a highly dynamic effect on parasite mobilization and survival. The shift in parasite occurrence towards warmer seasons, observed in the data collected from the Bearspaw WTP after 2007, suggests that environmental sources and the burden of parasites in these sources may also affect relationships with environmental factors.

The correlation analysis was performed using the large sample size for the aggregated data across years and log-transformed parasite concentrations. These measures were necessary to meet assumptions for the validity of correlation analysis. In some instances, correlation

assumptions in the given years were not met or were marginal as this was indicated in the relevant tables. In those cases, the results can be considered with skepticism, especially if the linearity assumption was affected. Although for this dissertation a large sample size was used for the analysis and improves normality of the data, other researchers may choose to rely on non-parametric statistical methods for small sample sizes.

Land use practices, such as agricultural development can affect parasite occurrence in water by altering wildlife diversity (i.e., parasite hosts), overgrazing of land fields leading to erosion, direct access of domestic animals to water sources, and saturation of the area with manure that can contain abundant levels of parasites (LeChevallier *et al.*, 1991; Slifko *et al.*, 2000). For example, Wilkes *et al.* (2013) was able to demonstrate increasing relative risk from *Giardia* moving from restricted cattle access pasture to unrestricted cattle access pasture in a riparian zone of a creek. Fencing provided improved risk protection against *Giardia*. Similarly, in this study, increasing relative risk was observed from *Cryptosporidium* due to unrestricted cattle access pasture in a riparian zone of a creek (especially *Cryptosporidium parvum* which is normally associated with cattle and potentially zoonotic).

6.1.5 Temporal variations in peak risk from *Giardia* were observed in the Elbow and Bow Rivers, but patterns were different

An important finding of this study was the observation that *Giardia* contamination was cyclic in nature, a phenomenon supported by correlation analysis and biological plausibility, and suggesting that

parasite risks are not homogeneous throughout the year in these watershed. Given rain and snowmelt run-off (the predictors of *Giardia* in Elbow River), concentrations were changing from year to year and even during given years in response to a change of the predictors.

Risk analysis using AESRD and Health Canada frameworks were insensitive for discerning a change of risk from *Giardia* over time. Both methodologies were bias in portraying the risk as homogeneous. A discrepancy in health risk assessments between AESRD and Health Canada approaches in context of the level of treatment necessary to achieve acceptable levels of risk for *Giardia* (and *Cryptosporidium* for that matter) is further complicated by their approaches in assuming parasite occurrence as homogeneous across time (i.e., of averaging concentration across a long period).

The rational of unilaterally using *Cryptosporidium* as a reference protozoa and ignoring *Giardia* as a reference protozoa can be questioned with respect to watersheds where *Cryptosporidium* is rare but *Giardia* is prevalent, and where chlorine may not be used for water disinfection. The monitoring of parasites in the Elbow and Bow Rivers demonstrated that in many instances, while *Cryptosporidium* was rare, health risk from *Giardia* could have been missed if *Cryptosporidium* was the only parasite being monitored. For improving our understanding of risk, Signor & Ashbolt (2009) suggest targeting daily risks rather than annualized, arguing that, for example, the target 10^{-6} daily infections per person per year would be similar to 10^{-4} annual infections per person per year, and would cover the extent of short-term risk fluctuations. Furthermore, the authors suggest using risk management that is directed to pre-identifying

condition-based hazardous event scenarios for emphasizing a daily risk target.

For recommendations, chlorination can be enhanced during periods of cold water, particularly, against *Giardia*. An increase of the parasite concentration can be unexpected during a cold season, when low source water turbidity usually is observed (Figures 5.5; 5.6; 5.7; 5.8; 5.9), which is commonly being perceived as a period of good source water quality. Also, Hipsey and Brookes (2013) suggest that WTP personnel armed with knowledge about peak periods related to hazardous environmental events can respond with decisions on the operational regimen that target attenuation of inflows. In particular, the authors recommend that manipulation of the offtake depth may allow for selectively choosing source water of the best quality in reservoirs due to spatial variability in which stratification may occur during inflow after snowmelt or rain run-off events. Simply knowing at which depth inflow water may be more contaminated with parasites allows for powerful risk management and mitigation strategies to be used (Hipsey and Brookes, 2013).

6.1.6 Modelling of *Giardia* occurrence can add predictability for controlling risk, and can be integrated into QMRA models

It is generally assumed that the presence of *E. coli* in water indicates faecal contamination and poor water quality. However, a growing body of literature suggests that the relationship of indicator bacteria with parasites is obscure. Several studies have failed to demonstrate the relationship of indicator bacteria and parasite occurrence (Lemarchand & Lebaron, 2003; Harwood *et al.*, 2005; Medema *et al.*, 2006; Horman *et al.*, 2004). The group of bacteria

known as total coliforms, once thought to be restricted to fecal origin, are now known to be composed of diverse bacterial genera and species, many of which are naturally found in the environments (Toranzos *et al.*, 2007; Bonadonna *et al.*, 2002; U.S. EPA, 1986; Myers *et al.*, 2003; Gilliom *et al.*, 2002; Wade *et al.*, 2003). Similarly, *E. coli* was once thought to be highly specific to growth and survival in the gastrointestinal system of warm-blooded animals, now appears to be capable of replicating in the natural environments under certain conditions (Osborn *et al.*, 2004). *Escherichia coli* has been shown to replicate in natural water sources and soils in tropical areas (Byappanahalli & Fujioka, 1998; Carrillo *et al.*, 1985). This finding challenges the validity of the universally applied *E.coli*-based water quality standards (Domingo & Ashbolt, 2012; U.S. EPA, 2007). Analyses of waterborne outbreaks caused by parasites have shown that in 50 % of water samples taken during outbreaks, total coliforms were not detected (Craun & Frost, 2002). Atherholt *et al.*, (1998) found that faecal bacteria die off sooner than encysted *Giardia* or *Cryptosporidium* in environmental waters. Therefore, testing for indicator bacteria is useful but cannot fully characterize microbial hazards in water.

A large number of fresh surface water sources have increasingly become polluted (Hranova, 2006), requiring a systematic approach to monitor microbial water safety with an improved resolution. Testing for all pathogens is not a solution at the present time. *Giardia* and *Cryptosporidium* can be used as reference organisms; however, their usefulness is flawed by expensive testing and the inherent uncertainty in the Method1623 test (Higgins *et al.*,

2003; McCuin & Clancy, 2003; Simmons *et al.*, 2001). Current research calls for developing a better understanding of using environmental factors as predictors of parasite contamination of source waters. Knowing how the environment can influence a parasite's survival and better understanding routes of mobilisation would help improve forecasting of parasite occurrence in environmental waters.

The research presented in this thesis, to some extent, succeeded to demonstrate the possibility for accommodating environmental factors for forecasting of *Giardia* occurrence. It is important to note that much of the environmental data collected for this project was not originally tailored for the purpose of predictive modeling and, thus, inherited additional uncertainty compared to if the data was collected for the specific purpose. Nevertheless, it was demonstrated that rain and snowmelt run-off could be used to predict concentration of *Giardia* for the Glenmore WTP. Implementation of a watershed safety program, inclusive of parasite monitoring, would be valuable in the context of detecting changes in patterns of parasite occurrence associated with ecosystem disturbances and estimating risks associated with water treatment purposes.

6.2 Summary and additional considerations

The current research has identified a set of challenges and caveats related to production of safe drinking water in respect to waterborne pathogenic protozoa. As outlined previously for the case of waterborne disease outbreaks, monitoring for bacterial indicators of water quality is a poor predictor of waterborne *Giardia*sis and cryptosporidiosis (Craun, 2012). Although indicator bacteria testing is

still valuable it is also is considered out-dated in view of the awareness that some pathogenic protozoa, as well as some viruses, can survive much better through water treatment processes than indicator bacteria. Sufficient treatment-based reduction of *Giardia* and *Cryptosporidium* was historically believed to be the best approach to managing risks and is also likely to be effective against all other waterborne pathogenic protozoa (Health Canada, 2012). However, it was noted in the research that AESRD treatment standards may be excessively stringent against *Giardia,* and, thus, these standards do not address treatment requirements against *Giardia* proportionally to the risk from this pathogen. U.S. EPA's LT2 Rule specifies using *Cryptosporidium* as the reference protozoa in an exclusive manner, and does not account for varied infectivity and viability of *Cryptosporidium* (U.S. EPA, 2006). The Health Canada QMRA model may provide better options for harmonizing risk to treatment requirements against both pathogens, but this model has caveats also.

A major limitation to all approaches is that they are biased to viewing risk as homogeneous (i.e., arithmetic mean over a long period). Risks in well-engineered drinking water systems nearly always results from periods of high pathogen challenge, along with sub-optimal treatment performance. Therefore while it is important that annual risks are acceptable, models that only report on annual risk really miss the critical point of risk management (Signor & Ashbolt, 2006). Health Canada provides a reasonably good health risk assessment framework, but only after the point at which a concentration of parasite is estimated and is ready for the input. The

potential of environmental factors to predict parasite concentration in surface waters could be accommodated into the Health Canada QMRA model. In Chapter 5, it was demonstrated that by regressing *Giardia* concentration data against rain and snowmelt run-off 60-days running averages, it was possible to develop a model that can provide near real time concentration estimates for *Giardia* and that can be used as inputs for probabilistic models for estimating daily risks.

An advantage of using the Health Canada QMRA model is in the use of a DALY as a quantification metric of health risk. Internationally two alternatives have been established for quantitatively defining microbial safety. The U.S. EPA uses performance targets set in drinking water standards that are referenced to an upper limit of one infection or illness per 10,000 population per year (U.S. EPA, 1989b, 2002, 2006). The WHO has adopted the metric of DALYs and defined tolerable health risk target as being $< 1 \times 10^{-6}$ DALYs per person per year (WHO, 2004; 2006; 2008; 2010; 2011). As noted by the American Academy of Microbiology (2006), while benchmarks based on infection or illness rates currently employed by U.S. EPA are useful as a more descriptive endpoint of disease, taking into account severity of an infection, may be more advantageous. The difference between the two metrics is that application of DALYs includes an additional step. After calculating the likelihood of infection, the additional step is to consider a likelihood and severity of a resulting disease. In this way, DALYs recognise that not all pathogens cause the same level of disease severity. The DALY has the distinct advantage as being a common 'currency' for many public health issues, allowing for comparative assessments of health

outcomes. Consequently, the DALY can also be used to account for investment that has been made into prevention of disease prevalence and mitigating burden of disease across different public health issues (Bichai & Smeets, 2013; Murray & Lopez, 1996). Even in the context of drinking water the DALY approach can be used for comparing between burdens of illness associated with disinfection by-products versus pathogens (Havelaar *et al.*, 2000).

Source water usually contains a "cocktail" of parasites. *Giardia* and *Cryptosporidium* are a very diverse group of parasites that include many species and genotypes (Xiao *et al.*, 2008). Although, they appear to display some host specificity, this is a relative term that implies that a particular type of parasite predominantly infects a major host species, but may infect a minor host species as well (Fadyen, 2008). In addition, infectivity of certain strains vary accordingly. The ID_{50} of *Cryptosporidium parvum* isolates TAMU, UCP and Iowa were estimated at 12.1, 2066 and 132 oocysts, respectively (Messner *et al.*, 2001), hence the uncertainties associated with just the dose-response aspects of a QMRA model are large, but probably less than the uncertainties associated with treatment (Hijnen & Medema, 2007). Therefore, QMRA frameworks should utilize not only hazard variability but also the uncertainties in estimating a hazard. The Health Canada QMRA model (version 2.0) accommodates an option of choosing a fraction of infectious (oo)cysts in source water. However, it is a simplistic way to address varied infectivity and its uncertainties, because it is user-predetermined, and dealing with varied infectivity (and all other key parameters) stochastically might be a better solution.

Another problem inherent to the practice of estimating burden of enteric disease in a population when considering a specific route of exposure, is that the etiological agent can actually transmit through multiple routes. In particular, the potential reductions in health burden available by improving drinking water quality may not be a good representation for making a difference in the overall disease burden (Hunter & Fewtrell, 2001). For a disease, exposure to which is almost universal across different routes, such as cryptosporidiosis and *Giardia*sis, reducing the disease burden targeting one particular route may have a little impact on the overall burden seen in society. *Giardia* and *Cryptosporidium* are not purely waterborne infections; other important routes of exposure to the parasites exist also. People who have not been exposed to infection (and hence degree of immunity) from drinking water may acquire their infection through other routes, such as daycare, swimming in public pools, recreational swimming in fresh water lakes and rivers and from which a greater contribution to disease burden may exist compared to exposure from drinking water (Pintar et. al., 2011). Exposure by means of these routes is associated with mostly human infectious species of *Cryptosporidium* and *Giardia*, because these routes of exposure are linked to sources such as humans (i.e., *C. hominis* in swimming pools) and farm animals (i.e., *C. parvum* in agriculturally-impacted recreational water [beaches]). Additionally, tracking actual disease burden attributable to a particular route of exposure for a particular pathogen is impractical. For example, prevalence of cryptosporidiosis is likely to be underestimated due to underreporting (Perz *et al.,* 1998). Accordingly, detection of outbreaks is limited also. In the US, it

is estimated that only 10-33 % of all outbreaks are detected (Frost *et al.*, 1996). The large Milwaukee drinking water outbreak was initially detected by increased sales of anti-diarrhoeal medication rather than disease reporting due to under-notification and infrequent testing (MacKenzie *et al.,* 1994).

The DALY approach also allows for a balanced approach to managing health risks from different factors in drinking water (i.e., disinfection by products [DBP] compared to pathogens). Although DBPs are formed during water disinfection using chemicals such as chlorine, chloramine, ozone, or chloride dioxide, it is the addition of these chemicals that is critical for pathogen control. These chemicals react with natural organic and inorganic matter in water. The trihalomethanes (THMs) are a prominent group of disinfection by-products, including chloroform, bromoform, bromodichloromethane, and chlorodibromomethane. Health Canada (1996) estimates that THMs comprise up to 50 % by weight of all disinfection by-products in drinking water. Among the trihalomethanes, the most common, based on frequency of detection and concentration in drinking water, is chloroform, followed by bromodichloromethane, then chlorodibromomethane, and bromoform (WHO, 1994; U.S. EPA, 1998b; Health Canada, 2006). Chloroform is a well-studied THM because it occurs in high levels in drinking water and because administration of chloroform results in statistically significant increases in tumors in rodents (Howd & Fan, 2008). In addition to cancer, toxic effects have been observed in the liver and kidney of exposed animals (ATSDR, 1997). EPA proposed a MCLG for chloroform of 0.07 mg/L based on a cancer reference dose (RfD),

assuming that a person drinks 2 liters of water per day (the 90th percentile of intake rate for the U.S. population) and a relative source contribution of 20 percent (Howd & Fan, 2008). Another important class of disinfection by-products are the haloacetic acids, which include dichloroacetic acid and dichloroacetate. Recently, it has been reported that the disinfection using ozone, chloride dioxide, two or more disinfectants can produce mixes of DBPs including iodo- and brome-containing chemicals that induce greater health risk than the currently regulated THMs and haloacetic acids (Richardson *et al.*, 2007). Source water rich with brome-containing substances is a risk factor for high concentrations of brome containing DBPs in produced water (Richardson *et al.*, 2003). Therefore, drinking water disinfection targeting pathogens must be balanced against DBP risks to prevent unnecessary exposure of consumers to DBPs.

6.3 Recommendations

It is recommended that the recent and noticeable increase of *Giardia* contamination in the Bow River be investigated more thoroughly, in addition to developing a better understanding of peak levels of parasite occurrence in the Elbow River. Although an apparently large margin of safety appears to exist in terms of risks associated with *Giardia* at both the Glenmore and Bearspaw WTPs, the non-homogeneous occurrence of the parasites (i.e., the potential for higher parasite numbers to exist during certain periods of time such as rain run-off and snowmelt run-off) warrants that a synoptic approach to monitoring and water sampling be considered. The data in this thesis helps identify conditions under which parasite levels are likely to be high. Increased sampling during these periods may help

identify how high parasite concentrations may reach during environmental conditions that mobilize parasites. The fact that the patterns of occurrence of *Giardia* at the Glenmore WTP during the entire period, and at the Bearspaw WTP before 2007, were similar, suggests some consistency in parasite mobilization processes across the watersheds, and that potentially this may represent a natural state of parasite occurrence in western North American rivers located at a similar latitude. Moreover, long term monitoring can also assist in identifying new threats to water safety. For example, the pattern of *Giardia* occurrence at the Bearspaw WTP manifested after 2007 suggest that contamination patterns can suddenly change, likely due to disturbance in the ecosystem, possibly, of anthropogenic origin. This is important, because the shift results in increasing uncertainty due to unpredictability of occurrence of *Giardia*. The data shows that although the health risk attributed to *Giardia* was low, recent changes in the Bow River ecosystem (and future development in this watershed for that matter) may affect overall levels and spatiotemporal patterns of parasite occurrence, ushering the need for deploying potential watershed control programs as soon as possible.

Microbial communities are the most sensitive part of ecosystems. Microbial composition in the environment changes prior to an environmental impact becoming clearly visible (Steinweg, 2011). The QMRA framework can be used for comparing health risks from presumed hazards before and after foreseen disturbances in ecosystems occur. For example, urban development is an important source of parasite burdens in the environment. However, concerns about source water pollution associated with this types of

development are usually raised only after an estimation of the extent of pollution is observed, but the overall effect is often obscured by the lack of evidence about background pollution in the environment before the disturbance.

A different pattern of parasite occurrence likely implies different sources, and consequently, pathogenicity of parasites from new sources is unknown. Genotyping of both *Cryptosporidium* and *Giardia* may assist in identifying potential host sources of parasites and assess whether human infectious species may be present or not. In addition, particular species of parasites can be more prevalent at different periods of a year, depending on their sources.

The quality of water and associated health risk from source water within the catchment will reflect land-use practices and their spatial locations within the watershed (Hipsey & Brookes, 2013). To effectively cope with health risk from parasites, source water protection should be considered a top priority for risk reduction, since reducing the burden of parasites in the environment can help manage overall levels of occurrence, including during peak contamination events. Understanding sources of parasites in the environment and how they are mobilized into water sources will greatly assist in reducing risks associated with *Cryptosporidium* and *Giardia* to drinking water supplies.

References:

Adam, R.D. (2001). Biology of *Giardia lamblia*. *Clinical Microbiology Review* 14(3), 447–475.

AESRD A guidance framework for the production of drinking water safety plans; Alberta environment and sustainable resources development: Http://www.environment.alberta.ca/apps/regulateddwq/dwsp.aspx, Accessed April 2014; p 15.

Ajjampur, S. S., Sarkar, R., Sankaran, P., Kannan, A., Menon, V. K., Muliyil, J., Ward, H., & Kang, G. (2010). Symptomatic and asymptomatic *Cryptosporidium* infections in children in a semi-urban slum community in southern India. *The American Journal of Tropical Medicine and Hygiene*, *83*(5), 1110-1115.

Alberta Environment (2006). *Standards and guidelines for municipal waterworks, wastewater and storm drainage systems (T/840)*. Edmonton, Alberta, Canada: Alberta Environment, Drinking Water Branch.

Alegre, H. (Ed.). (2006). *Performance indicators for water supply services*. (Second Edition). IWA publishing. (Retrieved Sept. 11, 2014, from: http://books.google.ca/books?hl=en&lr=&id=nfSZiheXeRsC&oi=fnd&pg=PR8&dq=Performance+indicators+for+water+supply+services&ots=3g6-sG5zzD&sig=ZzXEmIvj-DHnwWG9yrzeryjE7T0#v=onepage&q=Performance%20indicators%20for%20water%20supply%20services&f=false).

Allen, M. J., Clancy, J. L., & Rice, E. W. (2000). The plain, hard truth about pathogen monitoring. *American Water Works Association, 92*(9), 64-76.

Amar, C. F., Dear, P. H., Pedraza-Diaz, S., Looker, N., Linnane, E., & McLauchlin, J. (2002). Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia duodenalis* in human feces. *Journal of Clinical Microbiology, 40*(2), 446-452.

American Academy of Microbiology. (2006). *Clean water: What is acceptable microbial risk?* Washington, DC: American Academy of Microbiology. (Retrieved Sept. 11, 2014, from: http://academy.asm.org/images/stories/documents/cleanwatermicrobialrisk.pdf).

Amoah, K., Craik, S., Smith, D., & Belosevic, M. (2005). Inactivation of *Cryptosporidium* oocysts and *Giardia* cysts by ultraviolet light in the presence of natural particulate matter. *Aqua*, *54*, 165-178.

Anonymous. (2001). *Besluit van 9 januari 2001 tot wijziging van het waterleidingbesluit in verband met de richtlijn betreffende de kwaliteit*

van voor menselijke bestemd water. Staatsblad van het koninkrijk der nederlanden 31, 1-53.

Ashbolt, N. J. (2004). Risk analysis of drinking water microbial contamination versus disinfection by-products (DBPs). *Toxicology, 198*(1–3), 255-262.

Atherholt, T. B., LeChevallier, M. W., Norton, W. D., & Rosen, J. S. (1998). Effect of rainfall on *Giardia* and *Cryptosporidium. American Water Works Association, 90*(9), 66-80.

ATSDR. (1997). *Toxicological Profile for chloroform (update).* Atlanta, GA: Public Health Service, U.S. Department of Health and Human Services.

Atwill, E. R., Hou, L., Karle, B. M., Harter, T., Tate, K. W., & Dahlgren, R. A. (2002). Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. *Applied and Environmental Microbiology, 68*(11), 5517-5527.

Bertrand, I., Albertini, L., & Schwartzbrod, J. (2005). Comparison of two target genes for detection and genotyping of *Giardia lamblia* in human feces by PCR and PCR-restriction fragment length polymorphism. *Journal of Clinical Microbiology, 43*(12), 5940-5944.

Betancourt, W. Q., & Rose, J. B. (2004). Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. *Veterinary Parasitology, 126*(1–2), 219-234.

Bichai, F., & Smeets, P. W. M. H. (2013). Using QMRA-based regulation as a water quality management tool in the water security challenge: Experience from the Netherlands and Australia. *Water Research, 47*(20), 7315-7326.

Bingham, A. K., Jarrell, E. L., Meyer, E. A., & Raduleseu, S. (1979). Induction of *Giardia* excystation and the effect of temperature on cyst viability as compared by eosin-exclusion and in vitro excystation. *Waterborne transmission of Giardiasis, report no. EPA-600/9-79-001. US Environmental Protection Agency, Cincinnati, Ohio*, 217-229.

Biondini, R. (1976). Cloud motion and rainfall statistics. *Journal of Applied Meteorology, 15*(205), 224.

Block, S. S. (Ed.). (2001). *Disinfection, sterilization, and preservation*. Philadelphia, PA: Lippincott Williams & Wilkins.

Bonadonna, L., Briancesco, R., Coccia, A. M., Semproni, M., & Stewardson, D. (2002). Occurrence of potential bacterial pathogens in coastal areas of the Adriatic Sea. *Environmental Monitoring and Assessment, 77*(1), 31-49.

BRBC. (2010). Profile of the Bow River basin. (Retrieved Jun. 04, 2013, from http://brbc-wsow.ca/brbc/index.php?option=com_content&view=article&id=81&Itemid=83).

Brookes, J. D., Hipsey, M. R., Burch, M. D., Regel, R. H., Linden, L. G., Ferguson, C. M., & Antenucci, J. P. (2005). Relative value of surrogate

indicators for detecting pathogens in lakes and reservoirs. *Environmental Science & Technology, 39*(22), 8614-8621.

Bukhari, Z., McCuin, R. M., Fricker, C. R., & Clancy, J. L. (1998). Immunomagnetic separation of *Cryptosporidium parvum* from source water samples of various turbidities. *Applied and Environmental Microbiology, 64*(11), 4495-4499.

Byappanahalli, M. N., & Fujioka, R. S. (1998). Evidence that tropical soil environment can support the growth of *Escherichia coli. Water Science and Technology*, *38*(12), 171-174.

Cacciò, S. M., De Giacomo, M., & Pozio, E. (2002). Sequence analysis of the β-giardin gene and development of a polymerase chain reaction–restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. *International Journal for Parasitology, 32*(8), 1023-1030.

Cacciò, S. M., Thompson, R. C. A., McLauchlin, J., & Smith, H. V. (2005). Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends in Parasitology, 21*(9), 430-437.

Carrillo, M., Estrada, E., & Hazen, T. C. (1985). Survival and enumeration of the fecal indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a tropical rain forest watershed. *Applied and Environmental Microbiology*, *50*(2), 468-476.

Casman, E. H., Fischhoff, B., Palmgren, C., Small, M. J., & Wu, F. (2000). An integrated risk model of a drinking water-borne cryptosporidiosis outbreak. *Risk Analysis, 20*(4), 495-511.

CCME (2004). From source to tap: guidance on the multi-barrier approach to safe drinking water. Canadian Council of Ministers of the Environment Winnipeg, Manitoba. Available at: www.ccme.ca/assets/pdf/mba _guidance_doc_e.pdf

Chalmers, R. M. (2010). *Investigation of the taxonomy and biology of the Cryptosporidium rabbit genotype.* (No. WT1226). Swansea: NPHS Microbiology Swansea.

Chalmers, R. M., & Davies, A. P. (2010). Minireview: Clinical cryptosporidiosis. *Experimental Parasitology, 124*(1), 138-146.

Chalmers, R. M., Elwin, K., Hadfield, S. J., & Robinson, G. (2011). Sporadic human cryptosporidiosis caused by *Cryptosporidium cuniculus*, United Kingdom, 2007-2008. *Emerging Infectious Diseases, 17*(3), 536-538.

Chappell, C. L., Okhuysen, P. C., Sterling, C. R., Wang, C., Jakubowski, W., & DuPont, H. L. (1999). Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *American Journal of Tropical Medicine and Hygiene., 60*(1), 157-164.

Chauret, C., Nolan, K., Chen, P., Springthorpe, S., & Sattar, S. (1998). Aging of *Cryptosporidium parvum* oocysts in river water and their susceptibility to disinfection by chlorine and monochloramine. *Canadian Journal of Microbiology*, *44*(12), 1154-1160.

City of Calgary. (2011). State of the environment report. (Retrieved Jun. 04, 2013, from http://www.calgary.ca/UEP/ESM/Documents/ESM-Documents/2010-state-of-the-environment-report.PDF).

Clancy, J. L., & Hunter, P. R. (2004). Monitoring of *Giardia* and *Cryptosporidium* in water in the UK and US. In *The Pathogenic Enteric Protozoa: Giardia, Entamoeba, Cryptosporidium and Cyclospora* (pp. 129-139). Springer US. (Retrieved Sept. 11, 2014, from http://www.springer.com/life+sciences/animal+sciences/book/978-1-4020-7794-4).

Clark, R. M., Read, E. J., & Hoff, J. C. (1989). Analysis of inactivation of *Giardia lamblia* by chlorine. *Journal of Environmental Engineering*, *115*(1), 80-90.

Cole, L., Schupp, D. G., & Erlandsen, S. L. (1989). Viability of *Giardia* cysts suspended in lake, river, and tap water. *Applied and Environmental Microbiology*, *55*(5), 1223-1229.

Collier, S. A., Stockman, L. J., Hicks, L. A., Garrison, L. E., Zhou, F. J., & Beach, M. J. (2012). Direct healthcare costs of selected diseases primarily or partially transmitted by water. *Epidemiology & Infection, 140*(11), 2003-2013.

Commonwealth of Australia. (2002). *Guidelines for assessing human health risk from environmental hazards.* Department of Health and Ageing, Population Health Division. Canberra: Australia.

Connell, K., Rogers, C. C., Shank-Givens, H. L., Scheller, J., M. L. Pope, M. L., & Miller, K. (2000). Building a better protozoan data set. *American Water Works Association, 92*(10), 20-43.

Cornwell, D. A., Macphee, M. J., Brown, R. A., & Via, S. H. (2003). Demonstrating *Cryptosporidium* removal using spore monitoring at lime-softening plants. *American Water Works Association, 95*(5), 124-133. (Retrieved Sept. 11, 2014, from http://www.refdoc.fr/Detailnotice?idarticle=8700270).

Corso, P. S., Kramer, M. H., Blair, K. A., Addiss, D. G., Davis, J. P., & Haddix, A. C. (2003). Costs of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. *Emerging Infectious Diseases*, 9(4), 426.

Cox, P., Fisher, I., Kastl, G., Jegatheesan, V., Warnecke, M., Angles, M., Bustamante, H., Chiffings, T., & Hawkins, P. R. (2003). Sydney 1998: lessons from a drinking water crisis. *American Water Works Association*, *95*(5).

Craik, S. A., Weldon, D., Finch, G. R., Bolton, J. R., & Belosevic, M. (2001). Inactivation of *Cryptosporidium parvum* oocysts using medium and low-pressure ultraviolet radiation. *Water Research*, *35*(6), 1387-1398.

Craun, G., Berger, P., & Calderon, R. (1997). Coliform bacteria and waterborne disease outbreaks. *American Water Works Association, 89*(3), 96-104.

Craun, G. F., & Frost, F. J. (2002). Possible information bias in a waterborne outbreak investigation. *International Journal of Environmental Health Research*, *12*(1), 5-15.

Craun, M. F., Craun, G. F., Calderon, R. L., & Beach, M. J. (2006). Waterborne outbreaks reported in the United States. *Journal of Water and Health*, *4*(Suppl. 2), 19-30.

Craun, G., Brunkard, J., Yoder, J., Roberts, V., Carpenter, J., Wade, T., Calderon, R.L., Roberts, J.M., Beach, M.J., Roy, S. (2010). Causes of outbreaks associated with drinking water in the United States from 1971 to 2006. *Clinical Microbiology Review, 23*(3), 507-528. (Retrieved Sept. 11, 2014 from http://europepmc.org/abstract/MED/20610821).

Craun, G. F. (2012). The importance of waterborne disease outbreak surveillance in the United States. *Annali dell'Istituto Superiore di Sanità*, *48*(4), 447-459.

Cummins, E., Kennedy, R., & Cormican, M. (2010). Quantitative risk assessment of *Cryptosporidium* in tap water in Ireland. *Science of the Total Environment, 408*(4), 740-753.

Dahlgren, R., Singer, M., Allen-Diaz, B., & Atwill, E. (1999). Timing, frequency of sampling affect accuracy of water-quality monitoring. *California Agriculture*, *53*(6), 44-48.

Davies, J. M., & Mazumder, A. (2003). Health and environmental policy issues in Canada: the role of watershed management in sustaining clean drinking water quality at surface sources. *Journal of Environmental Management*, *68*(3), 273-286.

Davies, C. M., Ferguson, C. M., Kaucner, C., Krogh, M., Altavilla, N., Deere, D. A., & Ashbolt, N. J. (2004). Dispersion and transport of *Cryptosporidium* oocysts from fecal pats under simulated rainfall events. *Applied and Environmental Microbiology, 70*(2), 1151-1159.

Dechesne, M., & Soyeux, E. (2007). Assessment of source water pathogen contamination. *Journal of Water and Health, 5*(1), 39.

Deitz, V. J., & Roberts, J. M. (2000). National surveillance for infection with *Cryptosporidium parvum*, 1995--1998: What have we learned? *Public Health Reports, 115*(4), 358 - 363.

DiGiorgio, C. L., Gonzalez, D. A., & Huitt, C. C. (2002). *Cryptosporidium* and *Giardia* recoveries in natural waters by using Environmental Protection Agency method 1623. *Applied and Environmental Microbiology*, *68*(12), 5952-5955.

Domingo, S. J., & Ashbolt, N. (2012). Fecal pollution of water. (Retrieved Aug. 19, 2013, from http://www.eoearth.org/view/article/152739).

Douglas, I., Campbell, A., & Emelko, M. B. (2006). Reducing the risk of pathogen passage through filter optimization. *Proceedings of the 12th National Conference on Drinking Water, Saint John, New Brunswick, April 1–4, 2006. Canadian Water and Wastewater Association, Ottawa,* ON.

Douglas, I. (2008). *Health Canada WQHB (rev. April 2008).* Ottawa, ON. Unpublished manuscript.

Dugan, N. R., Fox, K. R., Owens, J. H., & Miltner, R. J. (2001). Controlling *Cryptosporidium* oocysts using conventional treatment: Filtration. *American Water Works Association, 93*(12), 64-76. (Retrieved Sept 11, 2014, from http://www.refdoc.fr/Detailnotice?idarticle=10524961).

DuPont, H. L., Chappell, C. L., Sterling, C. R., Okhuysen, P. C., Rose, J. B., & Jakubowski, W. (1995). The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine*, *332*(13), 855-859.

Edberg, S., Rice, E. W., Karlin, R. J., & Allen, M. J. (2000). *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*, *88*(S1), 106S-116S.

Edzwald, J. K., Tobiason, J. E., Parento, L. M., Kelley, M. B., Kaminski, G. S., Dunn, H. J., & Galant, P. B. (2000). *Giardia* and *Cryptosporidium* removals by clarification and filtration under challenge conditions. *American Water Works Association,* 92(12), 70-84.

Emelko, M. B., Schmidt, P. J., & Reilly, P. M. (2010). Particle and microorganism enumeration data: Enabling quantitative rigor and judicious interpretation. *Environmental Science and Technology, 44*(5), 1720-1727.

Englehardt, J. D., Ashbolt, N. J., Loewenstine, C., Gadzinski, E. R., & Ayenu-Prah, A. Y. (2012). Methods for assessing long-term mean pathogen count in drinking water and risk management implications. *Journal of Water and Health*, *10*(2), 197-208.

Environment Canada. (2014). Climate. (Retrieved Sept. 11, 2014, from http://climate.weather.gc.ca/climateData/dailydata_e.html?Prov=AB&StationID=2205&Year=2003&Month=5&Day=29&timeframe=2).

Fayer, R., Speer, C. A., & Dubey, J. P. (1990). General biology of *Cryptosporidium*. *Cryptosporidiosis of man and animals*, 1-29. (Retrieved Sept. 11, 2014, from http://www.cabdirect.org/abstracts/19910875514.html;jsessionid=D07E7BD0BFF96D39EB6F69AE39883A59?freeview=true).

Fayer, R. (1994). Effect of high temperature on infectivity of *Cryptosporidium parvum* oocysts in water. *Applied and Environmental Microbiology*, *60*(8), 2732-2735.

Fayer, R., & Nerad, T. (1996). Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, *62*(4), 1431-1433.

Fayer, R., C. Speer, & Dubey, J. (1997). The general biology of *Cryptosporidium*, p.1-41. In Fayer, R. (ed.), *Cryptosporidium* and cryptosporidiosis. Boca Raton, FL: CRC Press, Inc.

Fayer, R. J. M. T., Trout, J. M., & Jenkins, M. C. (1998). Infectivity of *Cryptosporidium parvum* oocysts stored in water at environmental temperatures. *The Journal of Parasitology*, 1165-1169.

Fayer, R., Morgan, U., & Upton, S. J. (2000). Epidemiology of *Cryptosporidium*: transmission, detection and identification. *International Journal for Parasitology, 30*(12), 1305-1322.

Fayer, R. (Ed.). (2008). *General biology, p. 1-42. In R. Fayer and L. Xiao (eds.), Cryptosporidium and cryptosporidiosis*. Boca Raton, FL: CRC Press, Inc.

Feng, Y.Y., Ong, S.L., Hu, J.Y., Song, L.F., Tan, X.L. and Ng, W.J. (2003). Effect of particles on the recovery of *Cryptosporidium* oocysts from source water samples of various turbidities. *Applied and Environmental Microbiology*, 69(4), 1898–1903.

Ferguson, C., Husman, A. M. d. R., Altavilla, N., Deere, D., & Ashbolt, N. (2003). Fate and transport of surface water pathogens in watersheds. *Critical Reviews in Environmental Science and Technology, 33*(3), 299-361.

Ferguson, C. M., Croke, B., Ashbolt, N. J., & Deere, D. A. (2005). A deterministic model to quantify pathogen loads in drinking water catchments: pathogen budget for the Wingecarribee. *Water Science Technology, 52*(8), 191-197.

Ferguson, C., Deere, D., Sinclair, M., Chalmers, R. M., Elwin, K., Hadfield, S., Xiao, L., Ryan, U., Gasser, R., El-Osta, Y.A., Stevens, M. (2006). Meeting report: Application of genotyping methods to assess risks from *Cryptosporidium* in watersheds. *Environmental Health Perspectives, 114*(3), 430-434.

Ferguson, C. M., Davies, C. M., Kaucner, C., Krogh, M., Rodehutskors, J. ö., Deere, D. A., & Ashbolt, N. J. (2007). Field scale quantification of microbial transport from bovine faeces under simulated rainfall events. *Journal of Water & Health, 5*(1), 83-95.

Ferguson, C. M., Croke, B. F. W., Norton, J. P., Haydon, S., Davies, C. M., Krogh, M. H., & Ashbolt, N. J.*Modeling of variations in watershed pathogen concentrations for risk management and load estimations. Project # 3124, http://waterrf.org/Pages/Projects.aspx?PID=3124; the water research foundation: Denver, Colorado, 2010; p 288*.

Ferrie, J. E. (2012). Cholera, John Snow and the 2013 bicentennial meetings at the London school of hygiene and tropical medicine, UK. *International Journal of Epidemiology, 41*, 1501-1502.

Filice, F.P. (1952). Studies on the cytology and life history of a *Giardia* from a laboratory rat (University of California publications in zoology).57*(2),* 53-146. Berkeley: University of California Press.

FitzGibbon, J., & Plummer, R. (2004). Drinking water source water protection: a challenge for integrated watershed management. *Canadian Perspectives on Integrated Water Resources Management*, 84-103. (Retrieved Sept. 11, 2014, from http://afeid.montpellier.cemagref.fr/old/ILWRM/Canadian_Perspectives.pdf#page=93).

Fout, G. S., Schaefer, F. W., Messer, J. W., Dahling, D. R., & Stetler, R. E. (1996). *ICR microbial laboratory manual*. Washington, DC: U.S. Environmental, National Exposure Research Laboratory.

Fox, K. R., & Lytle, D. A. (1996). Milwaukee's Crypto outbreak: Investigation and recommendations. *American Water Works Association, 88*(9), 87-94.

Frost, F. J., Craun, G., & Calderon, R. (1996). Waterborne disease surveillance. *American Water Works Association, 88*(9), 66-75.

FWR (Foundation for Water Research). (1996). *Tap water consumption in England and Wales: Findings from the 1995 national survey.* (No. DWI0771). UK.

Gale, P. (1998). Simulating *Cryptosporidium* exposures in drinking water during an outbreak. *Water Science Technology, 38*(12), 7-13.

Gammie, L., Goatcher, L., & Fok, N. (2000). *A Giardia/ Cryptosporidium* near miss? in: Proceedings of the 8th national conference on drinking water. Quebec City, Quebec. (*Canadian Water and Wastewater Association*, Ottawa, ON).

Gilliom, R. J., Mueller, D. K., Zogorski, J. S., & Ryker, S. J. (2002). A national look at water quality. *Water Resources IMPACT*, *4*(4), 12-16.

Gofti-Laroche, L., Potelon, J. L., DaSilva, E., & Zmirou, D. (2001). Description of drinking water intake in French communities (E.MIRA study). *Rev. Epidemiology Sante Publique, 49*(5), 411-422.

Green, C. (2003). *Handbook of water economics: Principles and practice*. The Atrium, Southern Gate, Chichester: John Wiley and Sons.

Guerrant, R. L. (1997). Cryptosporidiosis: an emerging, highly infectious threat. *Emerging Infectious Diseases*, *3*(1), 51.

Gyürék, L. L., Finch, G. R., & Belosevic, M. (1997). Modeling chlorine inactivation requirements of *Cryptosporidium parvum* oocysts. *Journal of Environmental Engineering*, *123*(9), 865-875.

Gyürék, L. L., & Finch, G. R. (1998). Modeling water treatment chemical disinfection kinetics. *Journal of Environmental Engineering*, *124*(9), 783-793.

Haas, C. N. (1983). Estimation of risk due to low doses of microorganisms: A comparison of alternative methodologies. *American Journal of Epidemiology, 118*(4), 573-582.

Haas, C. N., Rose, J. B., Gerba, C., & Regli, S. (1993). Risk assessment of virus in drinking water. *Risk Analysis, 13*(5), 545-552.

Haas, C. N., Rose, J. B., & Gerba, C. P. (1999). *Quantitative microbial risk assessment.* New York: John Wiley and Sons.

Haas, C. N. (2002). Conditional dose-response relationships for microorganisms: Development and application. *Risk Analysis: An International Journal, 22*(3), 455-464.

Harwood, V. J., Levine, A. D., Scott, T. M., Chivukula, V., Lukasik, J., Farrah, S. R., & Rose, J. B. (2005). Validity of the indicator organism

paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology*, *71*(6), 3163-3170.

Hassan, A., Farouk, H., Abdul-Ghani, R., & Hassanein, F. (2012). Contamination of irrigation systems of dental units with *Cryptosporidium* species in Alexandria, Egypt: A neglected disinfection pitfall. *Risk Management Health Policy, 5*, 93-95.

Havelaar, A. H., De Hollander, A. E., Teunis, P. F., Evers, E. G., Van Kranen, H. J., Versteegh, J. F., Van Koten, J.E., & Slob, W. (2000). Balancing the risks and benefits of drinking water disinfection: disability adjusted life-years on the scale. *Environmental Health Perspectives*, *108*(4), 315.

Havelaar, A. H., & Melse, J. M. *Quantifying public health risks in guidelines for drinking-water quality: A burden of disease approach. RIVM: Bilthoven, the Netherlands, 2002; p 46.*

Havelaar, A., & Melse, J. M. (2003). *Quantifying public health risk in the WHO guidelines for drinking-water quality: A burden of disease approach.* (No. 734301022). Rijkinstituut voor Volskgezondheid en Milieu, Bilthoven, Netherlands.

Health Canada (1999). Revised Health Canada risk management framework. Draft for discussion. April 1999. Ottawa, ON, Canada.

Health Canada. (2003). *Guidelines for Canadian drinking water quality: Supporting documentation — turbidity.* Ottawa, ON, Canada: Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada.

Health Canada. (2004). *Guidelines for Canadian drinking water quality: Supporting documentation. Protozoa: Giardia and Cryptosporidium.* Ottawa, ON, Canada: Health Canada.

Health Canada. (2006). *Guidelines for Canadian drinking water quality: Guideline technical document. Escherichia Coli.* Ottawa, ON, Canada: Health Canada.

Health Canada. (2010). *Guidelines for Canadian drinking water quality: Guideline technical document—Enteric Protozoa: Giardia and Cryptosporidium.* Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON, Canada. (Retrieved Sept. 11, 2014, from www.hc-sc.gc.ca/ewh-semt/consult/_2010/giar dia-*Cryptosporidium*/draft-ebauche-eng.php).

Health Canada. (2012). *Guidelines for Canadian drinking water quality: Guideline technical document—Enteric Protozoa: Giardia and Cryptosporidium.* Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON, Canada. (Retrieved Sept. 11, 2014 from http://www.hc-sc.gc.ca/ewh-semt/alt_formats/pdf/pubs/water-eau/protozoa/protozoa-eng.pdf).

Helsel, D. R., & Hirsch, R. M. (1992). *Statistical methods in water resources* (Vol. 49). (Retrieved Sept. 11, 2014, from http://books.google.ca/books?hl=en&lr=&id=jao4o5X1pvgC&oi=fnd&

pg=PP2&dq=Statistical+methods+in+water+resources&ots=QUQzcJjcK_&sig=AXAb9I_FUE6e4Ko-BVio_aodgAc#v=onepage&q=Statistical%20methods%20in%20water%20resources&f=false).

Hey, G., Ledin, A., Jansen, J. l. C., & Andersen, H. R. (2012). Removal of pharmaceuticals in biologically treated wastewater by chlorine dioxide or peracetic acid. *Environmental Technology, 33*(9), 1041-1047.

Higgins, J. A., Trout, J. M., Fayer, R., Shelton, D., & Jenkins, M. C. (2003). Recovery and detection of *Cryptosporidium parvum* oocysts from water samples using continuous flow centrifugation. *Water Research, 37*(15), 3551-3560.

Hibler, C.P., Hancock, C.M., Perger, L.M., Wegrzyn, J.G. and Swabby, K.D. (1987). Inactivation of *Giardia* cysts with chlorine at 0.5 to 5.0 °C. *American Water Works Association*, Denver, CO. 39 pp. (Technical Research Series).

Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research, 40*(1), 3-22.

Hijnen, W. A. M., & Medema, G. J. (2007). *Elimination of micro-organisms by water treatment processes.* (No. 111544.100.002). The Netherlands: Kiwa Water Research.

Hijnen, W. A. M., & Medema, G. J. (Eds.). (2010; p 160). *Elimination of micro-organisms by drinking water treatment processes* (A Review - Third Edition ed.). London: IWA Publishing.

Hipsey, M.R., & Brookes, J.D. (2013). Pathogen Management in Surface Waters: Practical Considerations for Reducing Public Health Risk, Current Topics in Public Health, Dr. Alfonso Rodriguez-Morales (Ed.), ISBN: 978-953-51-1121-4, In Tech. (Retrieved Sept. 11, 2014 from http://www.intechopen.com/books/current-topics-in-public-health/pathogen-management-in-surface-waters-practical-considerations-for-reducing-public-health-risk).

Hirano, S. S., Nordheim, E. V., Arny, D. C., & Upper, C. D. (1982). Lognormal distribution of epiphytic bacterial populations on leaf surfaces. *Applied and Environmental Microbiology*, *44*(3), 695-700.

Hlavsa, M. C., Watson, J. C., & Beach, M. J. (2005). *Cryptosporidiosis surveillance - United States 1999-2002*. Atlanta, Georgia: Atlanta Research and Education Foundation, Division of Parasitic Diseases, National Center for Infectious Diseases, CDC.

Horman, A., Rimhanen-Finne, R., Maunula, L., von Bonsdorff, C. H., & Torvela, N. (2004). *Campylobacter spp.*, *Giardia spp.*, *Cryptosporidium spp.*, noroviruses, and indicator organisms in surface water in southwestern Finland, 2000-2001. *Applied Environmental Microbiology, 70(*1), 87-95.

Houpt, E. R., Bushen, O. Y., Sam, N. E., Kohli, A., Asgharpour, A., Ng, C. T., Calfee, D.P., Guerrant, R.L., Maro, V., Ole-Nguyaine, S., & Shao, J. F. (2005). Asymptomatic *Cryptosporidium hominis* infection among

human immunodeficiency virus–infected patients in Tanzania. *The American Journal of Tropical Medicine and Hygiene*, *73*(3), 520-522.

Howd, R. A., & Fan, A. M. (Eds.). (2008). *Risk assessment for chemicals in drinking water*. Hoboken, New Jersey: John Wiley and Sons.

Hranova, R. (Ed.). (2006). *Diffuse pollution of water resources. Principles and case studies in the southern African region*. London, UK: Taylor & Francis Group plc.

Hrudey, S. E. (2004). Drinking-water risk management principles for a total quality management framework. *Journal of Toxicology and Environmental Health, Part A, 67*(20-22), 1555-1566.

Hrudey, S. E., & Hrudey, E. J. (2007). Published case studies of waterborne disease outbreaks—evidence of a recurrent threat. *Water Environment Research, 79*(3), 233-245.

Huber, M. M., Korhonen, S., Ternes, T. A., & Von Gunten, U. (2005). Oxidation of pharmaceuticals during water treatment with chlorine dioxide. *Water Research, 39*(15), 3607-3617.

Hunter, P. R., & Fewtrell, L. (Eds.). (2001). *Water quality: Guidelines, standards, and health. assessment of risk and risk management to water-related infectious disease* (Fewtrell, L.; Bartram, J. ed.). Padstow, Cornwall, UK: WHO TJ International (Ltd.).

Hunter, P. R., Waite, M., & Ronchi, E. (2010). *Drinking water and infectious disease: Establishing the links.* CRC Press. (Retrieved Sept. 11, 2014, from http://books.google.ca/books?hl=en&lr=&id=79yVekFYTCkC&oi=fnd&pg=PA1&dq=Drinking+water+and+infectious+disease:+Establishing+the+links&ots=gnYOj-rDbk&sig=iifH-9PwMOhvRM2FOFJNO3RurVk#v=onepage&q=Drinking%20water%20and%20infectious%20disease%3A%20Establishing%20the%20links&f=false).

ILSI. (*2000). Revised framework for microbial risk assessment.* An ILSI risk science institute workshop report. International Life Sciences Institute, ILSI press: Washington, D.C., p 22.

Ireland Environmental Protection Agency. (1995). *Water treatment manuals--filtration*. Ardcavan, Wexford, Ireland.

Jaidi, K., Barbeau, B., Carrie`re, A., Desjardins, R., & Pre´vost, M. (2009). Including operational data in QMRA model: Development and impact of model inputs. *Journal of Water and Health, 7*(1), 77-95.

Jakubowski, W. (1990). In *Giardia*sis. In E. A. Meyer (ed.). *The control of Giardia in water supplies* (pp. 336-353). Amsterdam: Elsever.

Jofre, J., Olle, E., Ribas, F., Vidal, A., & Lucena, F. (1995). Potential usefulness of bacteriophages that infect *Bacteroides fragilis* as model organisms for monitoring virus removal in drinking water treatment plants. *Applied and Environmental Microbiology*, *61*(9), 3227-3231.

Johnson, D. C., Enriquez, C. E., Pepper, I. L., Davis, T. L., Gerba, C. P., & Rose, J. B. (1997). Survival of *Giardia*, *Cryptosporidium*, *Poliovirus* and *Salmonella* in marine waters. *Water Science and Technology, 35*(11–12), 261-268.

Kay, D., Watkins, J., Francis, C. A., Wyn-Jones, A., Stapleton, C. M., Fewtrell, L., Wyer, M.D., & Drury, D. (2007). The microbiological quality of seven large commercial private water supplies in the United Kingdom. *Journal of Water & Health, 5*(4), 523-538.

King, B. J., Keegan, A. R., Monis, P. T., & Saint, C. P. (2005). Environmental temperature controls *Cryptosporidium* oocyst metabolic rate and associated retention of infectivity. *Applied and Environmental Microbiology*, *71*(7), 3848-3857.

King, B. J., & Monis, P. T. (2007). Critical processes affecting *Cryptosporidium* oocyst survival in the environment. *Parasitology, 134*(03), 309-323.

Kistemann, T., Claben, T., Koch, C., Dangendorf, F., Fischeder, R., Gebel, J., Vacata, V., & Exner, M. (2002). Microbial load of drinking water reservoir tributaries during extreme rainfall and run-off. *Applied and Environmental Microbiology, 68*(5), 2188-2197.

Kolluru, R. V., & Stricoff, S. (1996). *Risk assessment and management handbook for environmental, health, and safety professionals* (pp. 8-34). New York: McGraw-Hill.

Korich, D. G., Mead, J. R., Madore, M. S., Sinclair, N. A., & Sterling, C. R. (1990). Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied and Environmental Microbiology*, *56*(5), 1423-1428.

Kornacki, J. L., & Johnson, J. L. (2001). *Enterobacteriaceae*, coliforms, and *Escherichia coli* as quality and safety indicators. *Compendium of methods for the microbiological examination of foods*, *4*, 69-82.

Krometis, L. A. H., Characklis, G. W., & Sobsey, M. D. (2009). Identification of particle size classes inhibiting protozoan recovery from surface water samples via US Environmental Protection Agency Method 1623. *Applied and Environmental Microbiology*, 75(20), 6619-6621.

Lalle, M., Pozio, E., Capelli, G., Bruschi, F., Crotti, D., & Cacciò, S. M. (2005). Genetic heterogeneity at the β-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. *International Journal for Parasitology, 35*(2), 207-213.

Lazarova, V., Savoye, P., Janex, M., Blatchley III, E., & Pommepuy, M. (1999). Advanced wastewater disinfection technologies: State of the art and perspectives. *Water Science and Technology, 40*(4), 203-213.

LeChevallier, M. W., Norton, W. D., & Lee, R. G. (1991). Occurrence of *Giardia* and *Cryptosporidium spp*. in surface water supplies. *Applied and Environmental Microbiology, 57*(9), 2610-2616.

LeChevallier, M. W., & Norton, W. D. (1992). Examining relationships between particle counts and *Giardia*, *Cryptosporidium*, and turbidity. *American Water Works Association, 84*(12), 54-60.

LeChevallier, M. W., & Norton, W. D. (1995). *Giardia* and *Cryptosporidium* in raw and finished water. *American Water Works Association, 87*(9), 45-68.

LeChevallier, M. W., Di Giovanni, G. D., Clancy, J. L., Bukhari, Z., Bukhari, S., Rosen, J. S., Sobrinho, J., & Frey, M. M. (2003). Comparison of Method 1623 and cell culture-PCR for detection of *Cryptosporidium spp*. in source waters. *Applied and Environmental Microbiology, 69*(2), 971-979.

Lee, K. J., Kim, B. H., Hong, J. E., Pyo, H. S., Park, S. J., & Lee, D. W. (2001). A study on the distribution of chlorination by-products (CBPs) in treated water in Korea. *Water Research*, *35*(12), 2861-2872.

Lee, M. S., Kang, M. J., & Huh, S. (2013). Causes of death of prisoners of war during the Korean War (1950-1953). *Yonsei Medical Journal, 54(2)* 480-488.

Lemarchand, K., & Lebaron, P. (2003). Occurrence of *Salmonella spp*. and *Cryptosporidium spp*. in a French coastal watershed: Relationship with fecal indicators. *FEMS Microbiology Letters, 218*(1), 203-209.

Levallois, P., Guevin, N., Gingras, S., Levesque, B., Weber, J. P., & Letarte, R. (1998). New patterns of drinking-water consumption: Results of a pilot study. *Science of the Total Environment, 209*(2-3), 233-241.

Lewis, D. J., Atwill, E. R., Lennox, M. S., Pereira, M. D. G., Miller, W. A., Conrad, P. A., & Tate, K. W. (2009). Reducing microbial contamination in storm run-off from high use areas on California coastal dairies. *Water Science and Technology*, *60*(7), 1731.

Limpert, E., Stahel, W. A., & Abbt, M. (2001). Log-normal distributions across the sciences: keys and clues [352 BioScience. *May 2001/Vol. 51 No. 5*] *Bioscience, 51*(5), 341-352.

Lippy, E. C., & Waltrip, S. C. (1984). Waterborne disease outbreaks—1946-1980: A thirty-five-year perspective. *American Water Works Association*, 60-67.

Loper, J. E., Suslow, T. V., & Schroth, M. N. (1984). Log-normal distribution of bacterial populations in the rhizosphere. *Phytopathology*, *74*(12), 1454-1460.

MacKenzie, W., Hoxie, N., Proctor, M., Gradus, M., Blari, K., Peterson, D., Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B., & Davis, J. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *New England Journal Medicine, 331*(3), 161-167.

Macler, B. A., & Regli, S. (1993). Use of microbial risk assessment in setting U.S. drinking water standards. *International Journal of Food Microbiology, 18*(4), 245-256.

Mara, D. (2011). Water- and wastewater-related disease and infection risks: what is an appropriate value for the maximum tolerable additional burden of disease? *Journal of Water and Health*, *9*(2), 217-224.

Masago, Y., Katayama, H., Hashimoto, A., Hirata, T., & Ohgaki, S. (2002). Assessment of risk of infection due to *Cryptosporidium parvum* in drinking water. *Water Science Technology, 66*(11-12), 319-324.

McCuin, R. M., & Clancy, J. L. (2003). Modifications to United States Environmental Protection Agency Methods 1622 and 1623 for detection of *Cryptosporidium* oocysts and *Giardia* cysts in water. *Applied and Environmental Microbiology, 69*(1), 267-274.

McFadyen, S., Douglas, I., & Elliott, J. (2011). *QMRA - probabilistic model to estimate annual risk of illness & DALYs based on source water pathogens and treatment barriers* (version 11 07 ed.). Ottawa, ON, Canada: Health Canada. Unpublished manuscript.

McLauchlin, J., Amar, C., Pedraza-Diaz, S., & Nichols, G. L. (2000). Molecular epidemiological analysis of *Cryptosporidium spp*. in the United Kingdom: Results of genotyping *Cryptosporidium spp*. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. *Journal of Clinical Microbiology, 38*(11), 3984-3990.

McTigue, N. E. (Ed.). (1998). *National assessment of particle removal by filtration*. American Water Works Association. (Retrieved Sept. 11, 2014, from http://books.google.ca/books?hl=en&lr=&id=q8xegppPaa4C&oi=fnd&pg=PR11&dq=National+assessment+of+particle+removal+by+filtration&ots=R-xQEjhe3g&sig=JDJmmkFEpvc95hHTsE_gMNYhj50#v=onepage&q=National%20assessment%20of%20particle%20removal%20by%20filtration&f=false).

Medema, G. J., Bahar, M., & Schets, F. M. (1997). Survival of *Cryptosporidium parvum*, *Escherichia coli*, faecal enterococci and *Clostridium perfringens* in river water: influence of temperature and autochthonous microorganisms. *Water Science and Technology*, *35*(11), 249-252.

Medema, G. J., Hoogenboezem, W., van der Veer, A. J., Ketelaars, H. A. M., Hijnen, W. A. M., & Nobel, P. J. (2003). Quantitative risk assessment of *Cryptosporidium* in surface water treatment. *Water Science Technology, 47*(3), 241-247.

Medema, G. J., Shaw, S., Snozzi, M., Morreau, A., & Grabow, W. (2006). In Dufour A., Robertson W., Waite M., Hunter P., Kirby R. and Anderson Y.(Eds.), *Catchment characterisation and source water quality, p. 111-158. In assessing microbial safety of drinking water*. Cornwall, UK, OECD: TJ International (Ltd), Padstow.

Medema, G. (2013). Microbial risk assessment of pathogens in water. In *Environmental Toxicology* (pp. 361-401). New York, N.Y.: Springer.

Merkhoher, M. W. (1993). *Risk assessment methods: approaches for assessing health and environmental risks*. New York, N.Y.: Springer.

Messner, M. J., Chappell, C. L., & Okhuysen, P. C. (2001). Risk assessment for *Cryptosporidium*: A hierarchical Bayesian analysis of human dose response data. *Water Resources, 35*(16), 3934-3940.

Mons, M. N., Van der Wielen, J. M., Blokker, E. J., Sinclair, M. I., Hulshof, K. F., Dangendorf, F., Hunter, P.R., & Medema, G. J. (2007). Estimation of the consumption of cold tap water for microbiological risk assessment: an overview of studies and statistical analysis of data. *Journal of Water and Health*, *5*, 151-170. (Retrieved Sept. 11, 2014, from http://europepmc.org/abstract/MED/17890843).

Miller, W. A., Lewis, D. J., Pereira, M. D. G., Lennox, M., Conrad, P. A., Tate, K. W., & Atwill, E. R. (2008). Farm factors associated with reducing loading in storm run-off from dairies. *Journal of Environmental Quality*, *37*(5), 1875-1882.

Muirhead, R. W., Collins, R. P., & Bremer, P. J. (2006). Numbers and transported state of *Escherichia Coli* in run-off direct from fresh cowpats under simulated rainfall. *Letters in Applied Microbiology, 42*(2), 83-87.

Murray, C. J. L., & Lopez, A. D. (1996). *The global burden of disease, global health statistics: A compendium of incidence, prevalence and mortality estimates for over 200 conditions, vol. II. Cambridge, MA: Harvard School of Public Health on behalf of the World Health Organization and the World Bank.*

Myers, D. N., Stoeckel, D. M., Bushon, R. N., Francy, D. S., & Brady, A. M. G. (Eds.). (2003). *Biological indicators. In handbooks for water-resources investigations. (eds.) F.D.Wilde.* Reston, VA: *U.S. Geological Survey. TWRI.*

National Academy of Engineering (2000). Greatest engineering achievements of the 20th century. Available from http://www.greatachievements.org/. [Accessed on 03 May 2012].

Neumayerová, H., & Koudela, B. (2008). Effects of low and high temperatures on infectivity of *Cryptosporidium muris* oocysts suspended in water. *Veterinary Parasitology, 153*(3), 197-202.

NHMRCeNRMMC (National Health and Medical Research Council and Natural Resource Management Ministerial Council), 2011. Australian drinking water guidelines. Australian government.

Nieminski, E. C., & Ongerth, J. E. (1995). Removing *Giardia* and *Cryptosporidium* by conventional treatment and direct filtration. *American Water Works Association, 87*(9), 96-106.

NRMMC-EPHC-NHMRC (Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council). (2008). *Australian guidelines for water recycling: Managing health and environmental risks. Phase 2-augmentation of drinking water supplies. Australian government.*

Okhuysen, P. C., Chappell, C. L., Crabb, J. H., Sterling, C. R., & DuPont, H. L. (1999). Virulence of three distinct *Cryptospovidium parvum* isolates for healthy adults. *Journal of Infectious Diseases*, *180*(4), 1275-1281.

Olson, M. E., Goh, J., Phillips, M., Guselle, N., & McAl, T. A. (1999). *Giardia* cysts and *Cryptosporidium* oocyst survival in water, soil and cattle feces. *Journal of Environmental Quality, 28*(6), 1991-1996.

Ong, C., Moorehead, W., Ross, A., & Isaac-Renton, J. (1996). Studies of *Giardia* spp. and *Cryptosporidium spp*. in two adjacent watersheds. *Applied and Environmental Microbiology, 62*(8), 2798-2805.

Ortega, Y. R. (Ed.). (2006). *Foodborne parasites*. Springer. (Retrieved Sept. 12, 2014, from http://download.springer.com/static/pdf/981/bok%253A978-0-387-31197-5.pdf?auth66=1410717084_706e92fe29ba4d7365bdd69691fd97d4&ext=.pdf).

Osborn, M. J., Trussell, R. R., Deleon, R., Fung, D. J. C., Haas, C. N., Levy, D. A., & Yates, M. V. (2004). Introduction and historical background. *Indicators for waterborne pathogens* (pp. 17-52). Washington, D.C.: The National Academies Press. (Accessed Sept. 12, 2014, http://www.nap.edu/catalog.php?record_id=11010&utm_expid=4418042-5.krRTDpXJQISoXLpdo-1Ynw.0&utm_referrer=http%3A%2F%2Fwww.nap.edu%2Fopenbook.php%3Frecord_id%3D11010%26page%3D1).

Ott, W. R., Steinemann, A. C., & Wallace, L. A. (Eds.). (2006). *Exposure analysis*. Boca Raton, FL: CRC Press, Inc.

Parkhurst, D. F. (1998). Peer reviewed: arithmetic versus geometric means for environmental concentration data. *Environmental Science & Technology*, *32*(3), 92A-98A.

Parkhurst, D. F., & Stern, D. A. (1998). Determining average concentrations of *Cryptosporidium* and other pathogens in water. *Environmental Science and Technology, 32*(21), 3424-3429.

Parkin, T., & Robinson, J. (1993). Statistical evaluation of median estimators for lognormally distributed variables. *Soil Science Society of America Journal, 57*(2), 317-323.

Paustenbach, D.J., (1989). A survey of health risk assessment. In: Paustenbach D.J. (ed). *The risk assessment of environmental and human health hazards: a textbook of case studies.* New York, N.Y.: John Wiley and Sons.

Payment, P., Richardson, L., Siemiatycki, J., Dewar, R., Edwardes, M., & Franco, E. (1991). A randomized trial to evaluate the risk of gastrointestinal disease due to consumption of drinking water meeting current microbiological standards. *American Journal of Public Health*, *81*(6), 703-708.

Payment, P., Berte, A., Prévost, M., Ménard, B., & Barbeau, B. (2000). Occurrence of pathogenic microorganisms in the Saint Lawrence River (Canada) and comparison of health risks for populations using it as their source of drinking water. *Canadian Journal of Microbiology*, *46*(6), 565-576.

Payment, P., & Pintar, K. (2006). Waterborne pathogens: A critical assessment of methods, results and data analysis. *Revue des Sciences de l'eau/Journal of Water Science*, *19*(3), 233-245.

Peeters, J. E., Mazas, E. A., Masschelein, W. J., Villacorta Martiez de Maturana, I., & Debacker, E. (1989). Effect of disinfection of drinking water with ozone or chlorine dioxide on survival of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology, 55*(6), 1519-1522.

Peng, M. M., & Xiao, L. (1997). Genetic polymorphism among *Cryptosporidium parvum* isolates: Evidence of two distinct human. *Emerging Infectious Diseases, 3*(4), 567.

Peng, X., Murphy, T., & Holden, N. M. (2008). Evaluation of the effect of temperature on the die-off rate for *Cryptosporidium parvum* oocysts in water, soils, and feces. *Applied and Environmental Microbiology, 74*(23), 7101-7107.

Perz, J. F., Ennever, F. K., & Le Blancq, S. M. (1998). *Cryptosporidium* in tap water: Comparison of predicted risks with observed levels of disease. *American Journal of Epidemiology, 147*(3), 289-301.

Petterson, S., Signor, R., Ashbolt, N., & Roser, D. (2006). *QMRA methodology. Microbiological risk assessment: A scientific basis for managing drinking water safety from source to tap*. Sydney, Australia: University of New South Wales.

Petterson, S. R., Signor, R. S., & Ashbolt, N. J. (2007). Incorporating method recovery uncertainties in stochastic estimates of raw water protozoan concentrations for QMRA. *Journal of Water and Health*, *5*(1), 51.

Pintar, K. D. M., Fazil, A., Pollari, F., Waltner-Toews, D., Charron, D. F., McEwen, S. A., & Walton, T. (2011). Considering of risk of infection by *Cryptosporidium* via consumption of municipally treated drinking water from a surface water source in a Southwestern Ontario community. *Society for Risk Analysis, 32*(7), 1122-1138.

Plummer, J. D., Edzwald, J. K., & Kelley, M. B. (1995). Removing *Cryptosporidium* by dissolved-air flotation. *American Water Works Association*, *87*(9), 85-95.

Potter, J. G. (1965). *Water content of freshly fallen snow. CIR-4232, TEC-569, meteorology branch, dept. of transport, Toronto, ON, Canada, 12 pp. (Available from national snow and ice data center user services, University of Colorado, campus box 449, Boulder, CO 80309-0449).*

Pouillot, R., Beaudeau, P., Denis, J., & Derouin, F. (2004). A quantitative risk assessment of waterborne cryptosporidiosis in France using second-order Monte Carlo simulation. *Risk Analysis, 24*(1), 1-17.

Prado, M. S., Cairncross, S., Strina, A., Barreto, M. L., Oliveira-Assis, A. M., & Rego, S. (2005). Asymptomatic *Giardia*sis and growth in young children; a longitudinal study in Salvador, Brazil. *Parasitology*, *131*(01), 51-56.

Prüss, A., Kay, D., Fewtrell, L., & Bartram, J. (2002). Estimation the burden of disease from water, sanitation, and hygiene at a global level. *Environmental Health Perspectives, 110*(5), 537-542.

Prüss, A., & Corvalan, C. (2006). *Preventing disease through healthy environment. Towards an estimate of the environmental burden of disease.* Geneva, Switzerland: WHO Press.

Read, C. M., Monis, P. T., & Andrew Thompson, R. (2004). Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. *Infection, Genetics and Evolution, 4*(2), 125-130.

Regli, S., Rose, J. B., Haas, C. N., & Gerba, C. P. (1991). Modeling the risk from *Giardia* and viruses in drinking water. *American Water Works Association*, 76-84.

Rendtorff, R.C. (1978). The experimental transmission of *Giardia lamblia* among volunteer subjects. In: Waterborne transmission of *Giardia*sis. W. Jakubowski and J.C. Hoff (eds.). Cincinnati, OH: U.S. Environmental Protection Agency, pp. 64–81 (EPA 600/9-79-001).

Richardson, S. D., Thruston, A. D., Rav-Acha, C., Groisman, L., Popilevsky, I., Juraev, O., Glezer, V., McKague, A.B., Plewa, M.J., Wagner, E. D. (2003). Tribromopyrrole, brominated acids, and other disinfection byproducts produced by disinfection of drinking water rich in bromide. *Environmental Science & Technology, 37*(17), 3782-3793.

Richardson, S. D., Plewa, M. J., Wagner, E. D., Schoeny, R., & DeMarini, D. M. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutation Research/Reviews in Mutation Research, 636*(1–3), 178-242.

Robertson, L. J., Campbell, A. T., & Smith, H. V. (1992). Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Applied and Environmental Microbiology, 58*(11), 3494-3500.

Rose, J. B., & Gerba, C. P. (1991). Use of risk assessment for development of microbial standards. *Water Science & Technology*, *24*(2), 29-34.

Rózsa, L., Reiczigel, J., & Majoros, G. (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology*, *86*(2), 228-232.

Ruecker, N. J., Braithwaite, S. L., Topp, E., Edge, T., Lapen, D. R., Wilkes, G., Robertson, W., Medeiros, D., Sensen, C.W., & Neumann, N. F. (2007). Tracking host sources of *Cryptosporidium spp.* in raw water for improved health risk assessment. *Applied and Environmental Microbiology*, *73*(12), 3945-3957.

Ruecker, N. J., Hoffman, R. M., Chalmers, R. M., & Neumann, N. F. (2011). Detection and resolution of *Cryptosporidium* species and species mixtures by genus-specific nested PCR-restriction fragment

length polymorphism analysis, direct sequencing, and cloning. *Applied and Environmental Microbiology, 77*(12), 3998-4007.

Ruecker, N. J., Matsune, J. C., Wilkes, G., Lapen, D. R., Topp, E., Edge, T. A., Sensen, C.W., Xiao, L., & Neumann, N. F. (2012). Molecular and phylogenetic approaches for assessing sources of *Cryptosporidium* contamination in water. *Water Research*, *46*(16), 5135-5150.

Ruecker, N. J. (2013). *Genotyping Cryptosporidium from water to source track fecal contamination in agricultural watersheds.* (Doctoral dissertation, University of Calgary).

Ryan, U., & Power, M. (2012). *Cryptosporidium* species in Australian wildlife and domestic animals. *Parasitology, 139*(13), 1673-1688.

Ryu, H., Alum, A., & Abbaszadegan, M. (2005). Microbial characterization and population changes in nonpotable reclaimed water distribution systems. *Environmental Science and Technology, 39*(22), 8600-8605.

Samuel, W. M., Pybus, M. J., & Kocan, A. A. (2001). *Diseases of wild mammals* (Second Edition ed.). Ames, Iowa: Iowa State University Press.

Schijven, J. F., Teunis, P. F. M., Rutjes, S. A., Bouwknegt, M., & de Roda Husman, A. M. (2011). QMRAspot: A tool for quantitative microbial risk assessment from surface water to potable water. *Water Research, 45*(17), 5564-5576.

Schmidt, P. J., Emelko, M. B., & Reilly, P. M. (2010). Quantification of analytical recovery in particle and microorganism enumeration methods. *Environmental Science & Technology*, *44*(5), 1705-1712.

Schmidt, P. J., & Emelko, M. B. (2011). QMRA and decision-making: Are we handling measurement errors associated with pathogen concentration data correctly? *Water Research, 45*(2011), 427-438.

Schmidt, P. J., Emelko, M. B., & Thompson, M. E. (2013). Analytical recovery of protozoan enumeration methods: Have drinking water QMRA models corrected or created bias? *Water Research, 47*(7), 2399-2408.

Schoen, M. E., & Ashbolt, N. J. (2011). An in-premise model for *Legionella* exposure during showering events. *Water Research, 45*(18), 5826-5836.

Scholl, M. A., Mills, A. L., Herman, J. S., & Hornberger, G. M. (1990). The influence of mineralogy and solution chemistry on the attachment of bacteria to representative aquifer materials. *Journal of Contaminant Hydrology, 6*(4), 321-336.

Schuler, P. F., & Ghosh, M. M. (1990). Diatomaceous earth filtration of cysts and other particulates using chemical additives. *American Water Works Association*, 82(12), 67-75.

Schuler, P. F., Ghosh, M. M., & Gopalan, P. (1991). Slow sand and diatomaceous earth filtration of cysts and other particulates. *Water Research*, *25*(8), 995-1005.

Selvakumar, A., & Borst, M. (2006). Variation of microorganism concentrations in urban stormwater run-off with land use and seasons. *Journal Water Health, 4*, 109-124.

Seyfried, M. S., & Murdock, M. D. (1997). Use of air permeability to estimate infiltrability of frozen soil. *Journal of Hydrology, 202*, 95-107.

Shin, G.A., Lee, J.K, & Linden, K. G. (2009). Enhanced effectiveness of medium-pressure ultraviolet lamps on human *Adenovirus* 2 and its possible mechanism. *Water Science & Technology, 60*(4), 851-857.

Signor, R. S., & Ashbolt, N. J. (2006). Pathogen monitoring offers questionable protection against drinking-water risks: A QMRA (quantitative microbial risk analysis) approach to assess management strategies. *Water Science Technology, 54*(3), 261-268.

Signor, R., & Ashbolt, N. (2009). Comparing probabilistic microbial risk assessments for drinking water against daily rather than annualised infection probability targets. *Journal of Water and Health, 7*(4), 535-543.

Smith, G. D. (2002). Commentary: Behind the Broad Street pump: aetiology, epidemiology and prevention of cholera in mid-19th century Britain. *International Journal of Epidemiology*, *31*(5), 920-932.

Smith, A., Reacher, M., Smerdon, W., Adak, G. K., Nichols, G., & Chalmers, R. M. (2006). Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992–2003. *Epidemiology and Infection, 134*(06), 1141-1149.

Simmons, O. D., Sobsey, M. D., Heaney, C. D., Schaefer, F. W., & Francy, D. S. (2001). Concentration and detection of *Cryptosporidium* oocysts in surface water samples by Method 1622 using ultrafiltration and capsule filtration. *Applied and Environmental Microbiology, 67*(3), 1123-1127.

Sinclair, J. L. (2000). *Enumeration of Cryptosporidium spp. in water with U.S.EPA Method 1622. Journal of AOAC International, 83*(5), 1108-1114.

Singer, P. C. (1994). Control of disinfection by-products in drinking water. *Journal of Environmental Engineering*, 120(4), 727-744.

Slifko, T. R., Smith, H. V., & Rose, J. B. (2000). Emerging parasite zoonoses associated with water and food. *International Journal for Parasitology, 30*(12–13), 1379-1393.

Smeets, P. W. M. H., van Dijk, J. C., Stanfield, G., Rietveld, L. C., & Medema, G. J. (2007). How can the UK statutory *Cryptosporidium* monitoring be used for quantitative risk assessment of *Cryptosporidium* in drinking water? *Journal of Water and Health, 5*(51), 107-118.

Smeets, P.W.M.H., Rietveld, L.C., Van Dijk, Medema, G.J. (2010). Practical applications of quantitative microbial risk assessment (QMRA) for water safety plans. *Water Science and Technology, 61*(6), 1561-1568.

Smith, H. V., Patterson, W. J., Hardie, R., Greene, L. A., Benton, C., Tulloch, W., Gilmour, R.A., Girdwood, R. W. A., Sharp, J. C. M., & Forbes, G. I. (1989). An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination. *Epidemiology and Infection, 103*(3), 703-715.

States, S., Stadterman, K., Ammon, L., Vogel, P., Baldizar, J., Wright, D., Conley, L., & Sykora, J. (1997). Protozoa in river water: Sources, occurrence, and treatment. *American Water Works Association, 89*(9), 74-83. (Retrieved Sept. 11, 2014, from http://www.jstor.org/stable/41296015).

Steinweg, J. M. (2011). *Sensitivity of microbial community physiology to soil moisture and temperature in an old-field ecosystem* (Doctoral dissertation, Colorado State University).

Stern, D. A. (1996). *Monitoring for Cryptosporidium spp. and Giardia spp. and human enteric viruses in the watersheds of the New York City water supply system. Watershed 96 Conference,* Baltimore, MD. 553-555.

Stewart, I. (1990). *Does god play dice? The mathematics of chaos.* Harmonds-worth: Penguin.

Tang, J., McDonald, S., Peng, X., Samadder, S. R., Murphy, T. M., & Holden, N. M. (2011). Modelling *Cryptosporidium* oocysts transport in small ungauged agricultural catchments. *Water Resources, 45*(12), 3665-3680.

Tate, K. W., Atwill, E. R., George, M. R., McDougald, N. K., & Larsen, R. E. (2000). *Cryptosporidium parvum* transport from cattle fecal deposits on California rangelands. *Journal of Range Management*, 295-299.

Tate, K. W., Pereira, M. D. G. C., & Atwill, E. R. (2004). Efficacy of vegetated buffer strips for retaining. *Journal of Environmental Quality*, *33*(6), 2243-2251.

Teunis, P. F. M., Medema, G. J., Kruidenier, L., & Havelaar, A. H. (1997). Assessment of the risk of infection by *Cryptosporidium* or *Giardia* in drinking water from a surface water source. *Water Research, 31*(6), 1333-1346.

Teunis, P. F. M., & Havelaar, A. H. (1999). *Cryptosporidium in drinking water: Evaluation of the ILSEI/RSI quantitative risk assessment framework. RIVM report no. 284 550 006.* Bilthoven: The Netherlands, National Institute of Public Health and the Environment (RIVM).

Teunis, P. F. M., Xu, M., Fleming, K. K., Yang, J., Moe, C. L., & LeChevallier, M. W. (2010). Enteric virus infection risk from intrusion of sewage into a drinking water distribution network. *Environmental Science & Technology, 44*(22), 8561-8566.

Thomas, S., & Hrudey, S. E. (1997). Risk of death in Canada: What we know and how we know it. University of Alberta: Edmonton, AB.

Thompson, M. C. (2004). The zoonotic significance and molecular epidemiology of *Giardia* and *Giardia*sis. *Veterinary Parasitology, 126*(1-2), 15-35.

Thompson, R.C., & Monis, P.T. (2004). Variation in *Giardia*: implications for taxonomy and epidemiology. *Advanced Parasitology*, 58, 69–137.

Toranzos, G. A., McFeters, G. A., & Borrego, J. J. (2007). In Hurst C. J., Crawford R. L., Garland J. L., Lipson D. A., Mills A. L. and Stetzenbach L. D. (Eds.), *Detection of microorganisms in environmental freshwaters and drinking waters, p. 205-215. In manual of environmental microbiology* (2nd ed.). Washington, DC, USA: ASM Press.

Trask, J. R., Kalita, P. K., Kuhlenschmidt, M. S., Smith, R. D., & Funk, T. L. (2004). Overland and near-surface transport of from vegetated and nonvegetated surfaces. *Journal of Environmental Quality, 33*(3), 984-993.

Tyrrel, S. F., & Quinton, J. N. (2003). Overland flow transport of pathogens from agricultural land receiving faecal wastes. *Journal of Applied Microbiology, 94*(s1), 87-93.

UK Drinking Water Inspectorate (2002). Information letter 15/2002. London: DWI.

UK Drinking Water Inspectorate (2005). DWI Information letter 2005. The Water Supply (Water Quality) (Amendment) Regulations 2000, SI No. 3184 England 2001, SI No. 3911 (W.323) Wales: *Cryptosporidium* in Water Supplies, Laboratory and Analytical Procedures. Part 2, June 2005. Protocol containing Standard Operating Protocols (SOPs) for the monitoring of *Cryptosporidium* oocysts in water supplies.

U.S. EPA. (1986). *United States Office of Water. Bacteriological ambient water quality criteria for marine and fresh recreational waters. Agency criteria and standards.* (Environmental Protection Regulations and Standards No. 20460). Washington, DC 20460.

U.S. EPA (1989a). Risk assessment guidance for superfund, Volume 1. Human health evaluation manual, (Part A). Interim Final. EPA/540/1-89/002. Washington, DC: US Environmental Protection Agency, Office of Emergency and Remedial Response.

U.S. EPA (1989b). Drinking water; national primary drinking water regulations; filtration; disinfection; turbidity, *Giardia lamblia*, viruces, *Legionella*, and heterotrophic bacteria; Final Rule, 40 CFRU.S.C. 141 and 142.

U.S. EPA (1992). *Guidelines for exposure assessment; Notice. Part IV.* (Federal Register 29 May 1992).

U.S. EPA (1996). *National primary drinking water regulations: monitoring requirements for public drinking water supplies*; Final Rule. Federal Register, 61:94:24353.

U.S. Army Corps of Engineers. (1998). *Run off from snowmelt. (Retrieved from http://140.194.76.129/publications/eng-manuals/EM_1110-2-1406_sec/Sections/basdoc.pdf*).

U.S. EPA (1998). *National primary drinking water regulations: interim enhanced surface water treatment*; Final Rule. 40 CFR Parts 9, 141, and 142. Federal Register 1998(63)69478--521.

U.S. EPA (2000). *Estimated per capita water ingestion in the United States* (EPA-822-00-008 ed.). Washington D.C.: Office of Science and Technology/Office of Water, U.S. Environmental Protection Agency.

U.S. EPA (2002). *National primary drinking water regulations: Long-term enhanced surface water treatment rule;* Final Rule. Federal register: January 14, 2002.

U.S. EPA (2005). *Method 1623: Cryptosporidium and Giardia in water by filtration/IMS/FA. Office of Water, Office of Science and Technology, Engineering and Analysis Division, U.S. Environmental Protection Agency, Washington, DC (EPA 821-R-01-025).*

U.S. EPA (2006). *National primary drinking water regulations: Long term 2 enhanced surface water treatment;* Final Rule. Federal register: January 5, 2006, 71(3)678–671; 782–783.

U.S. EPA (2007). *Report of the experts scientific workshop on critical research needs for the development of new or revised recreational water quality criteria.* (No. EPA823-R-07-006). Washington, DC: Office of Water Office of Research and Development.

U.S. EPA (2009). *Laboratory quality assurance evaluation program for analysis of Cryptosporidium under the Safe Drinking Water Act (renewal).* Washington, DC: Office of Groundwater and Drinking Water, U.S. Environmental Protection Agency.

U.S. EPA; USDA/FSIS microbial risk assessment guideline: Pathogenic microorganisms with focus on food and water. Prepared by the interagency microbiological risk assessment guideline workgroup. EPA/100/J-12/001; USDA/FSIS/2012-001; U.S. Environmental Protection Agency (EPA) and U.S. department of Agriculture/Food safety and inspection service (USDA/FSIS): Washington D.C., 2012.

Vachon, D. (2005). Doctor John Snow blames water pollution for cholera epidemic. *Old News*, *16*(8), 8-10.

Vakil, N. B., Schwartz, S. M., Buggy, B. P., Brummitt, C. F., Kherellah, M., Letzer, D. M., Gilson, I.H., & Jones, P. G. (1996). Biliary cryptosporidiosis in HIV-infected people after the waterborne outbreak of cryptosporidiosis in Milwaukee. *New England Journal of Medicine*, *334*(1), 19-23.

Van Dyke, M. I., Morton, V., Anderson, W. B., Isaac-Renton, J. L., & Huck, P. (2006). The occurrence of selected bacterial and protozoan pathogens in the Grand River watershed. *Proceedings of the 12th National Conference on Drinking Water,* Saint John, NB, April 1–4, 2006. Canadian Water and Wastewater Association, Ottawa, ON.

Van Keulen, H., Macechko, P. T., Wade, S., Schaaf, S., Wallis, P. M., & Erlandsen, S. L. (2002). Presence of human *Giardia* in domestic, farm and wild animals, and environmental samples suggests a zoonotic potential for *Giardia*sis. *Veterinary Parasitology, 108*(2), 97-107.

Venczel, L. V., Arrowood, M., Hurd, M., & Sobsey, M. D. (1997). Inactivation of *Cryptosporidium parvum* oocysts and *Clostridium perfringens* spores by a mixed-oxidant disinfectant and by free chlorine. *Applied and Environmental Microbiology*, *63*(4), 1598-1601.

VROM-Inspectorate. (2005). *Inspectorate guideline. Assessment of the microbial safety of drinking water. Netherlands.*

Wade, T. J., Pai, N., Eisenberg, J. N., & Colford, J. J. M. (2003). Do U.S. Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. *Environmental Health Perspectives, 11*(8), 1102.

Walker, M., Leddy, K., & Hagar, E. (2001). Effects of combined water potential and temperature stresses on *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, *67*(12), 5526-5529.

Wang, L., Mankin, K. R., & Marchin, G. L. (2004). Survival of fecal bacteria in dairy cow manure. *Transactions of the ASAE, 47*(4), 1239-1246.

Water quality and health council - drinking water chlorination, water disinfection, and safe water. (2014). (Accessed Apr. 16, 2014, http://www.waterandhealth.org/drinkingwater/wp.html).

Weisstein, E. W. (2013). Arithmetic mean -- from wolfram MathWorld. (Retrieved July 07, 2013, from http://mathworld.wolfram.com/ArithmeticMean.html).

Westrell, T., Andersson, Y., & Stenstro, T. A. (2006). Drinking water consumption pattern in Sweden. *Journal of Water and Health, 4,* 511-522.

WHO. (1995). Disinfectants and disinfection by-products. In: WHO seminar pack for drinking water quality. World Health Organization, Geneva, Switzerland (Retrieved May 10, 2014, from www.who.int/water_sanitation_health/dwq/en/S04.pdf).

WHO. (2004). *Guidelines for drinking-water quality* (third edition ed.). Geneva, Switzerland: WHO Press.

WHO. (2006). *Guidelines for drinking-water quality: First addendum to volume 1, Recommendations* (Vol. 1). Geneva, Switzerland: WHO Press.

WHO. (2008). *Guidelines for drinking water quality. Second addendum for third edition.* (Volume 1 recommendations). Geneva, Switzerland: WHO Press.

WHO. (2010). *Health-based targets*. Geneva, Switzerland: WHO Press.

WHO. (2011). *Guidelines for drinking water quality* (*fourth edition ed.*). Geneva, Switzerland: WHO Press.

Wickramanayake, G. B., Rubin, A. J., & Sproul, O. J. (1985). Effects of ozone and storage temperature on *Giardia* cysts. *American Water Works Association*, 74-77.

Wilkes, G., Brassard, J., Edge, T. A., Gannon, V., Jokinen, C.C, Jones, T.H., Neumann, N.F., Pintar, K.D.M., Ruecker, N., Schmidt, P.J., Sunohara, M., Topp, E., & Lapen, D. R. (2013a). Bacteria, viruses, and parasites in an intermittent stream protected from and exposed to

pasturing cattle: Prevalence, densities, and quantitative microbial risk assessment. *Water Research, 1*(14), 1-14.

Wilkes, G., Ruecker, N. J., Neumann, N. F., Gannon, V. P., Jokinen, C., Sunohara, M., Topp, E., Pintar, K.D.M., Edge, T.A., & Lapen, D. R. (2013b). Spatiotemporal analysis of *Cryptosporidium* species/genotypes and relationships with other zoonotic pathogens in surface water from mixed-use watersheds. *Applied and Environmental Microbiology*, 79(2), 434-448.

Wolfe, M. S. (1992). *Giardia*sis. *Clinical Microbiology Reviews*, *5*(1), 93-100.

Xiao, L., Morgan, U. M., Fayer, R., Thompson, R. C. A., & Lal, A. A. (2000). *Cryptosporidium* systematics and implications for public health. *Parasitology Today*, *16*(7), 287-292.

Xiao, L., Fayer, R., Ryan, U., & Upton, S. J. (2004). *Cryptosporidium* taxonomy: Recent advances and implications for public health. *Clinical Microbiology Review, 17*(1), 72-97.

Xiao, L., & Feng, Y. (2008). Zoonotic cryptosporidiosis. FEMS immunol. *Medical Microbiology, 52*(3), 309-323.

Xiao, L., & Fayer, R. (2008). Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *International Journal for Parasitology, 38*(11), 1239-1255.

Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: An update. *Experimental Parasitology, 124*(1), 80-89.

Yakub, G. P., & Stadterman-Knauer, K. L. (2000). Evaluation of immunomagnetic separation for recovery of *Cryptosporidium parvum* and *Giardia duodenalis* from high-iron matrices. *Applied and Environmental Microbiology, 66*(8), 3628-3631.

Yoder, J. S., & Beach, M. J. (2007). *Cryptosporidiosis surveillance - United States, 2003-2005*. Atlanta, GA: Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, CDC.

Yoder, J. S., & Beach, M. J. (2010). *Cryptosporidium* surveillance and risk factors in the United States. *Experimental Parasitology, 124*(1), 31-39.

Yoder, J. S., Wallace, R. M., Collier, S. A., Beach, M. J., & Hlavsa, M. C. (2012). *Cryptosporidiosis surveillance — United States, 2009–2010.* (No. Vol.61, No.5). U.S. Department of Health and Human Services, Atlanta, GA 30333: Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC.

Zeileis, A., & Hothorn, T. (2002). Diagnostic checking in regression relationships. R News 2 (3): 7–10. (Retrieved Sept. 11, 2014, from http://www.icesi.edu.co/CRAN/web/packages/lmtest/vignettes/lmtest-intro.pdf).

Zhou, L., Singh, A., Jiang, J., & Xiao, L. (2003). Molecular surveillance of *Cryptosporidium spp*. in raw wastewater in Milwaukee: implications for understanding outbreak occurrence and transmission dynamics. *Journal of Clinical Microbiology*, *41*(11), 5254-5257.

Appendix

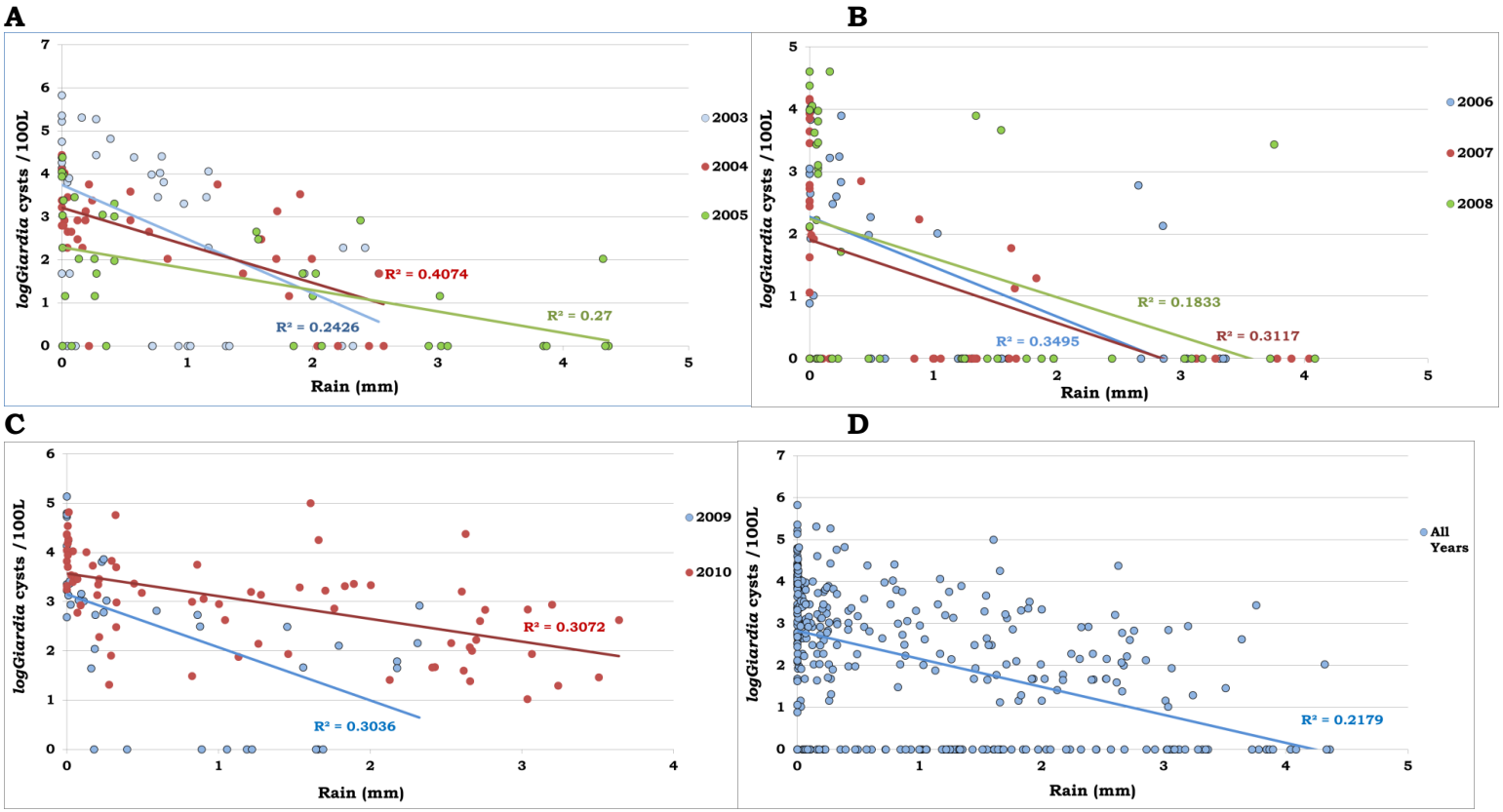

Figure A-1 Relationship between ln (*Giardia* cysts/100 L) concentration in source water and Rain run-off (mm) during 2003, 2004, and 2005 years (**A**), 2006, 2007, and 2008 years (**B**), 2009 and 2010 years (**C**), the entire period of monitoring 2003-2011 years (**D**). Data reflects the sampling campaign for the Glenmore WTP (Elbow River).

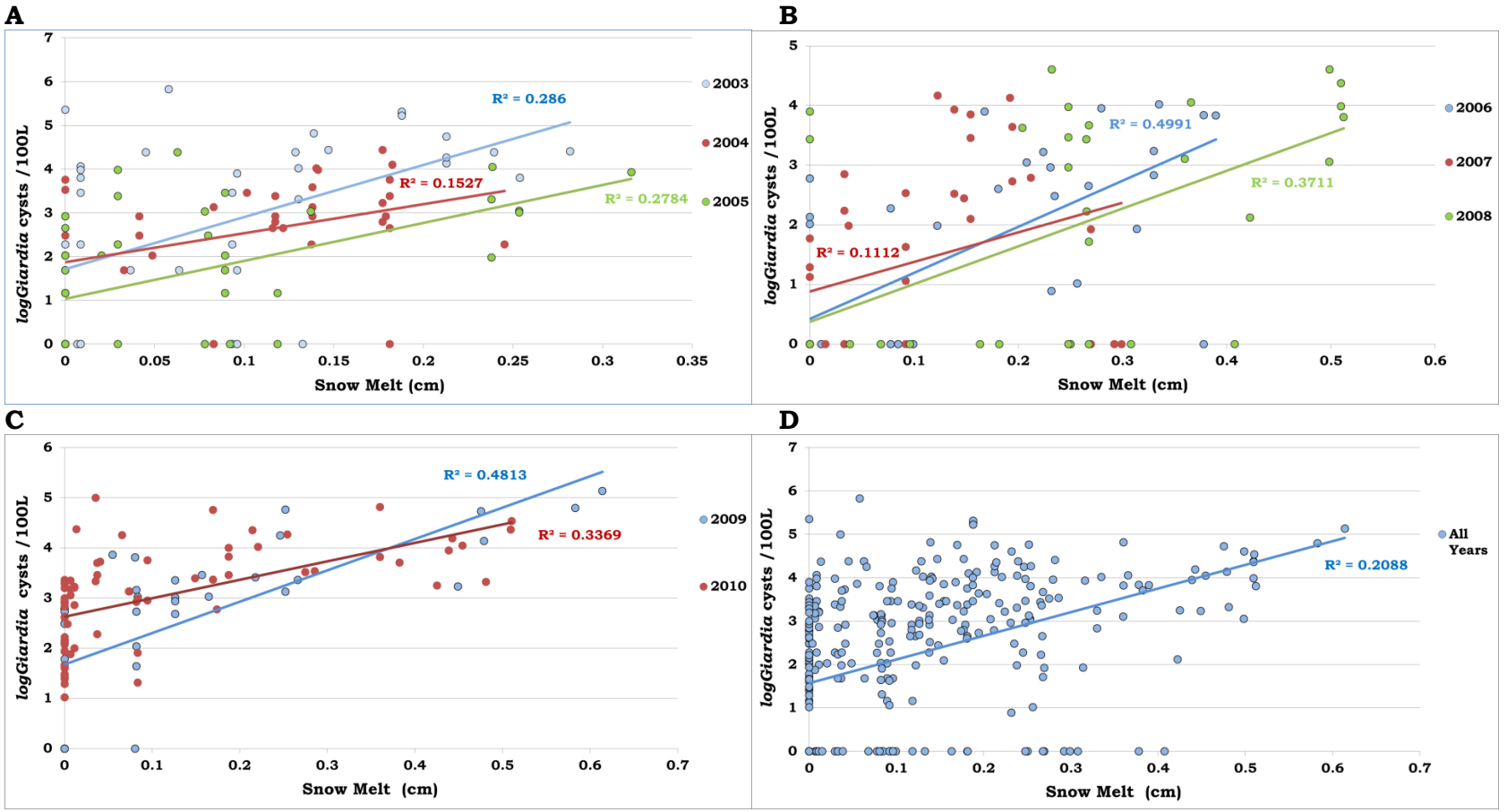

Figure A-2 Relationships between ln (*Giardia* cysts/100 L) concentration in source water and snowmelt run-off during 2003, 2004, and 2005 years (**A**), 2006, 2007, and 2008 years (**B**), 2009 and 2010 years (**C**), the entire period of monitoring 2003-2011 years (**D**). Data reflects the sampling campaign at the Glenmore WTP (Elbow River).

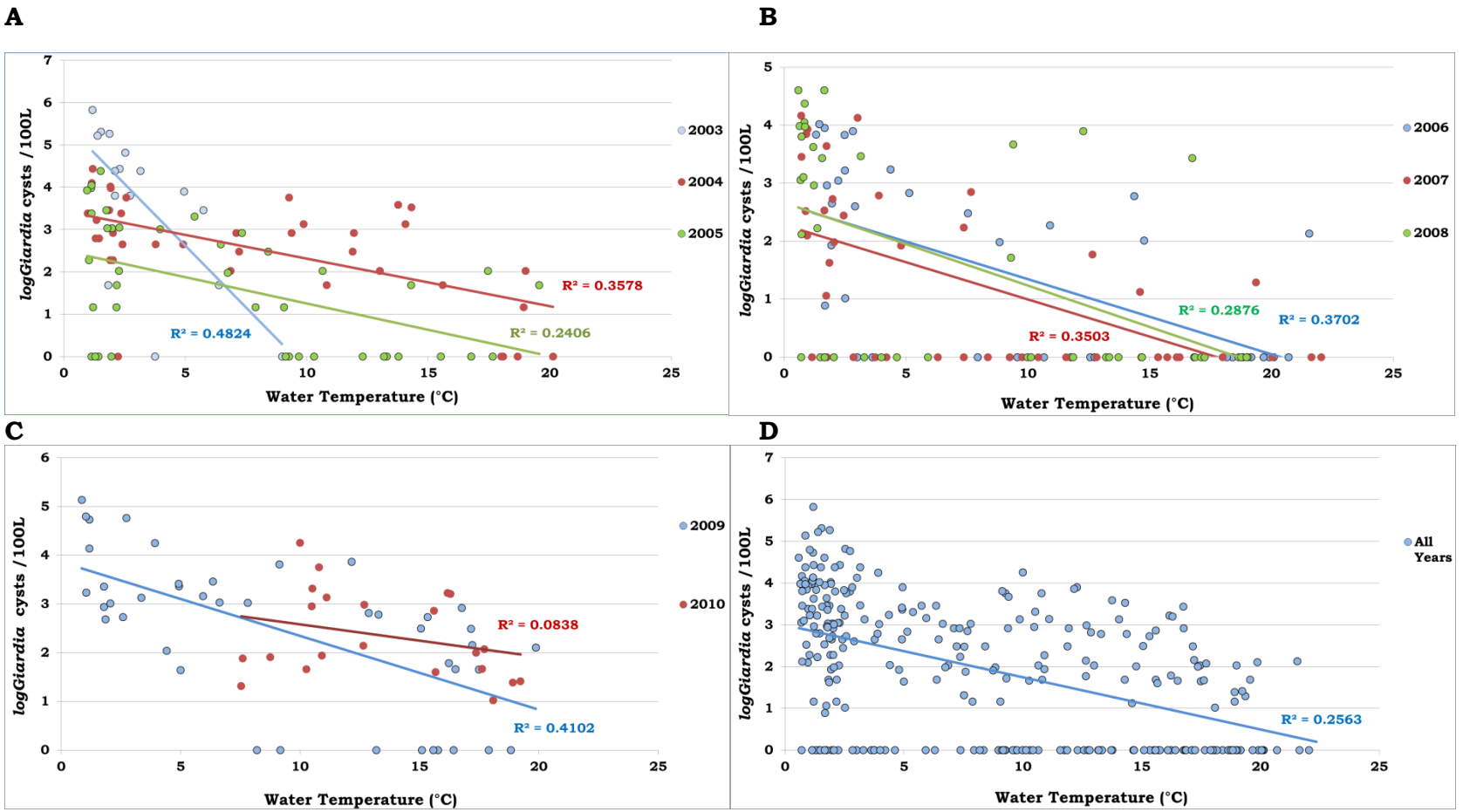

Figure A-3 Relationships between ln (*Giardia* cysts/100 L) concentration in source water and water temperature during 2003, 2004, and 2005 years (**A**), 2006, 2007, and 2008 years (**B**), 2009 and 2010 years (**C**), the entire period of monitoring 2003-2011 years (**D**). Data reflects the sampling campaign for the Glenmore WTP (Elbow River). Water temperature monitoring started on 20 Oct 2003 and ended on 30 Oct 2010.

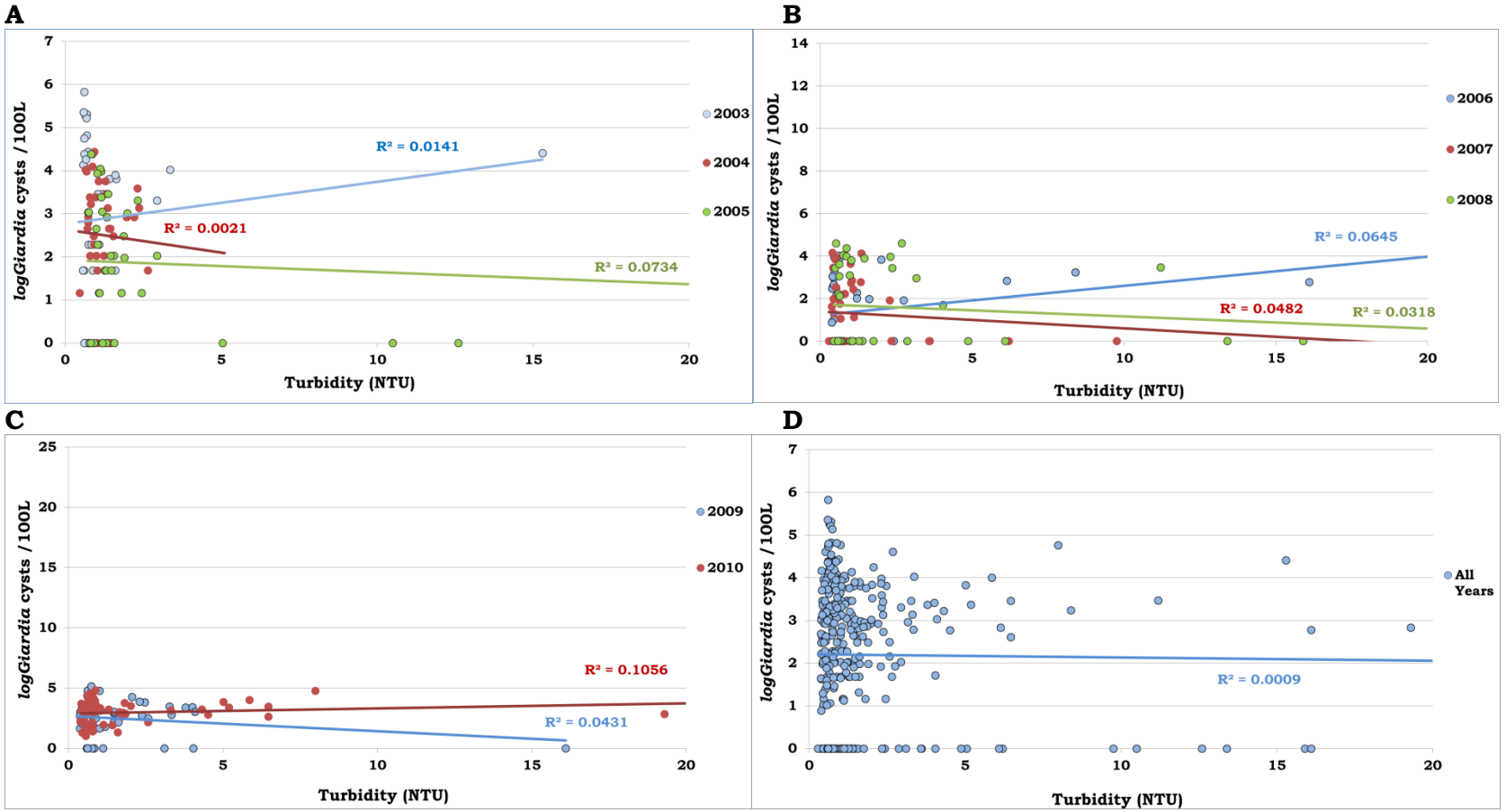

Figure A-4 Relationships between ln (*Giardia* cysts/100 L) concentration in source water and source water turbidity (NTU) during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign at the Glenmore WTP (Elbow River).

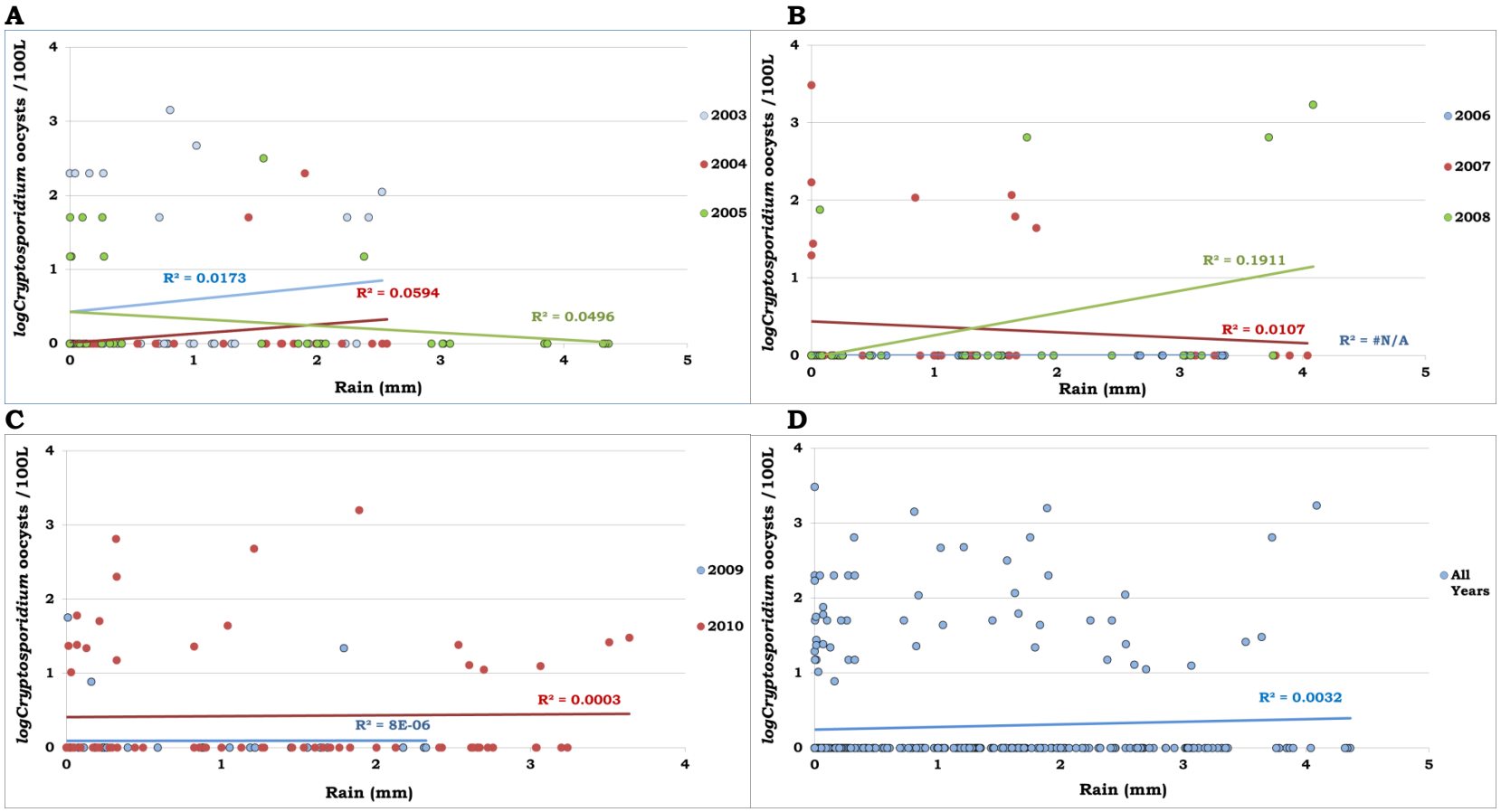

Figure A-5 Relationships between ln (*Cryptosporidium* oocyst/100 L) concentration in source water and rain run-off during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 years (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign at the Glenmore WTP (Elbow River).

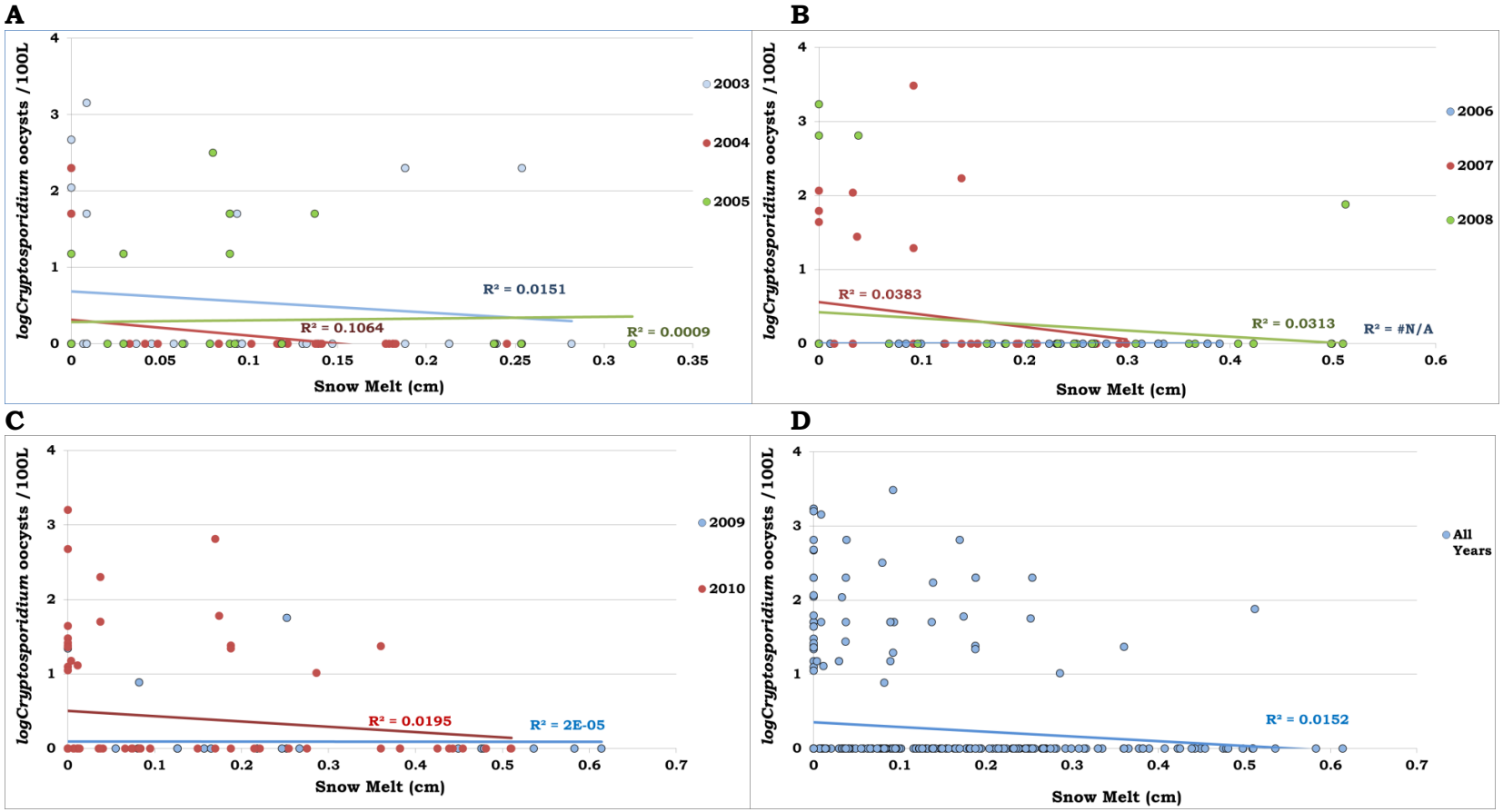

Figure A-6 Relationships between ln (*Cryptosporidium* oocyst/100 L) concentration in source water and snowmelt run-off during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign for the Glenmore WTP (Elbow River).

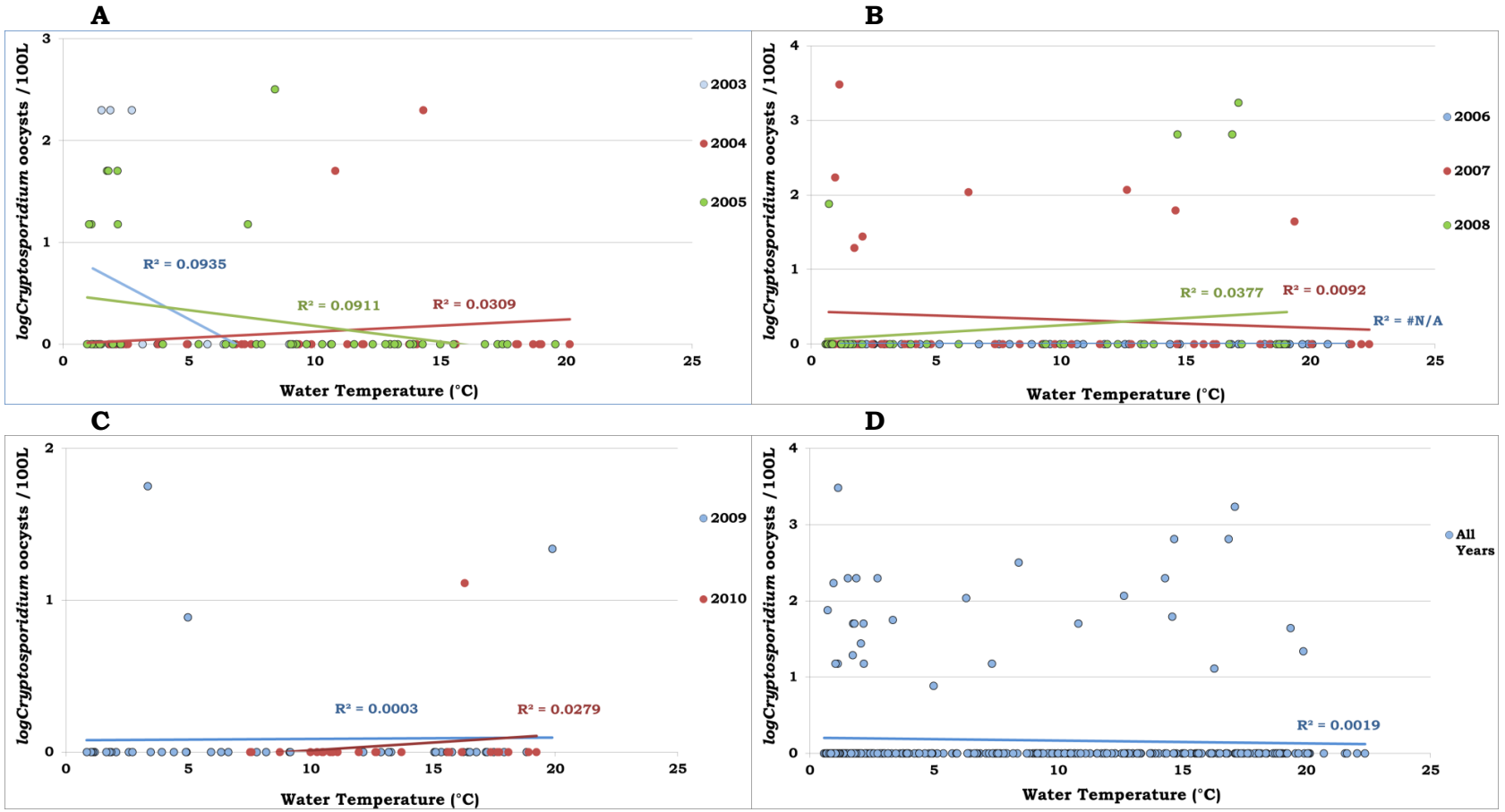

Figure A-7 Relationships between ln (*Cryptosporidium* oocyst/100 L) concentration in source water and source water temperature during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign for the Glenmore WTP (Elbow River).

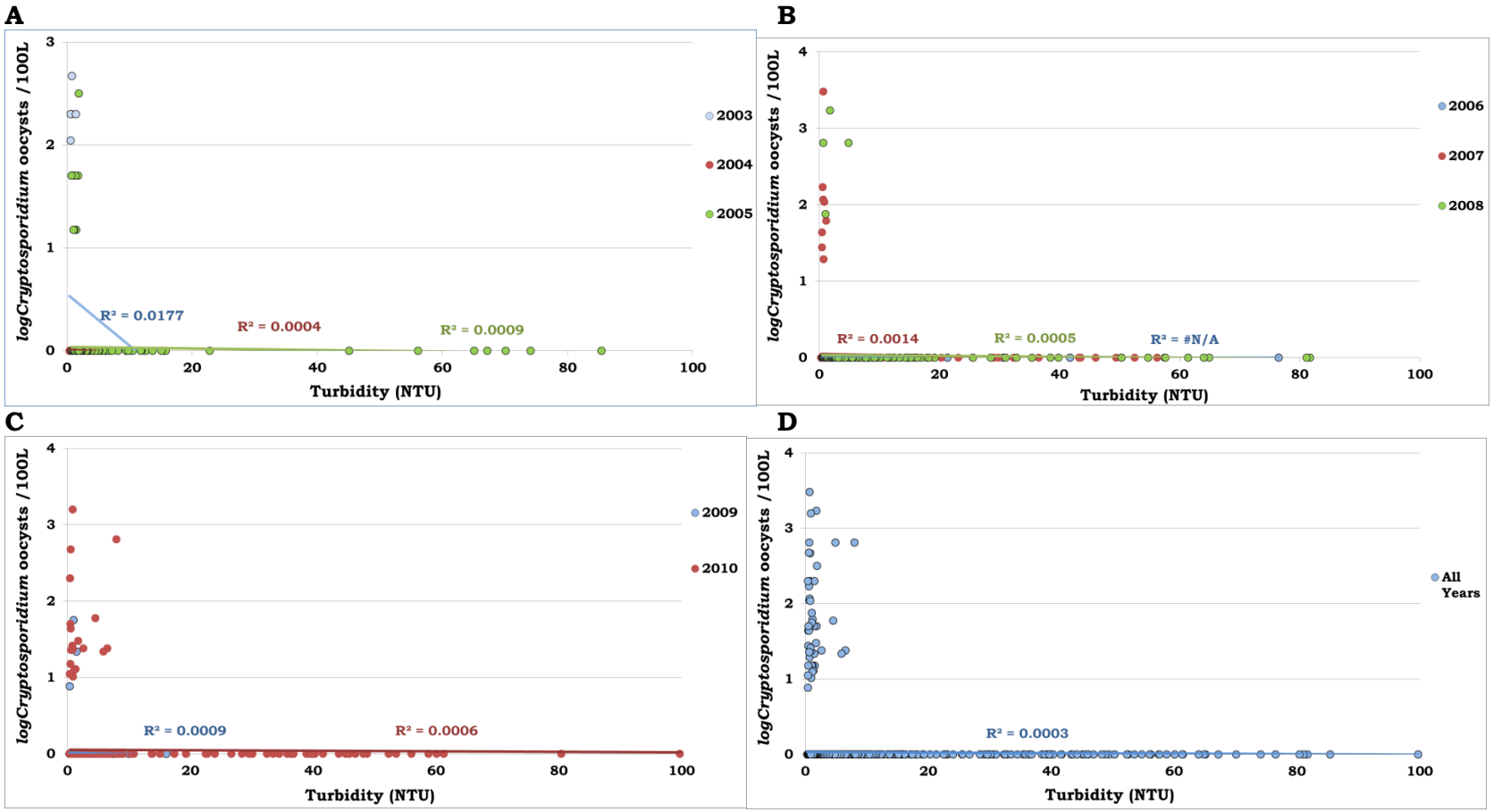

Figure A-8 Relationships between ln (*Cryptosporidium* oocyst/100 L) concentration in source water and source water turbidity during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign for the Glenmore WTP (Elbow River).

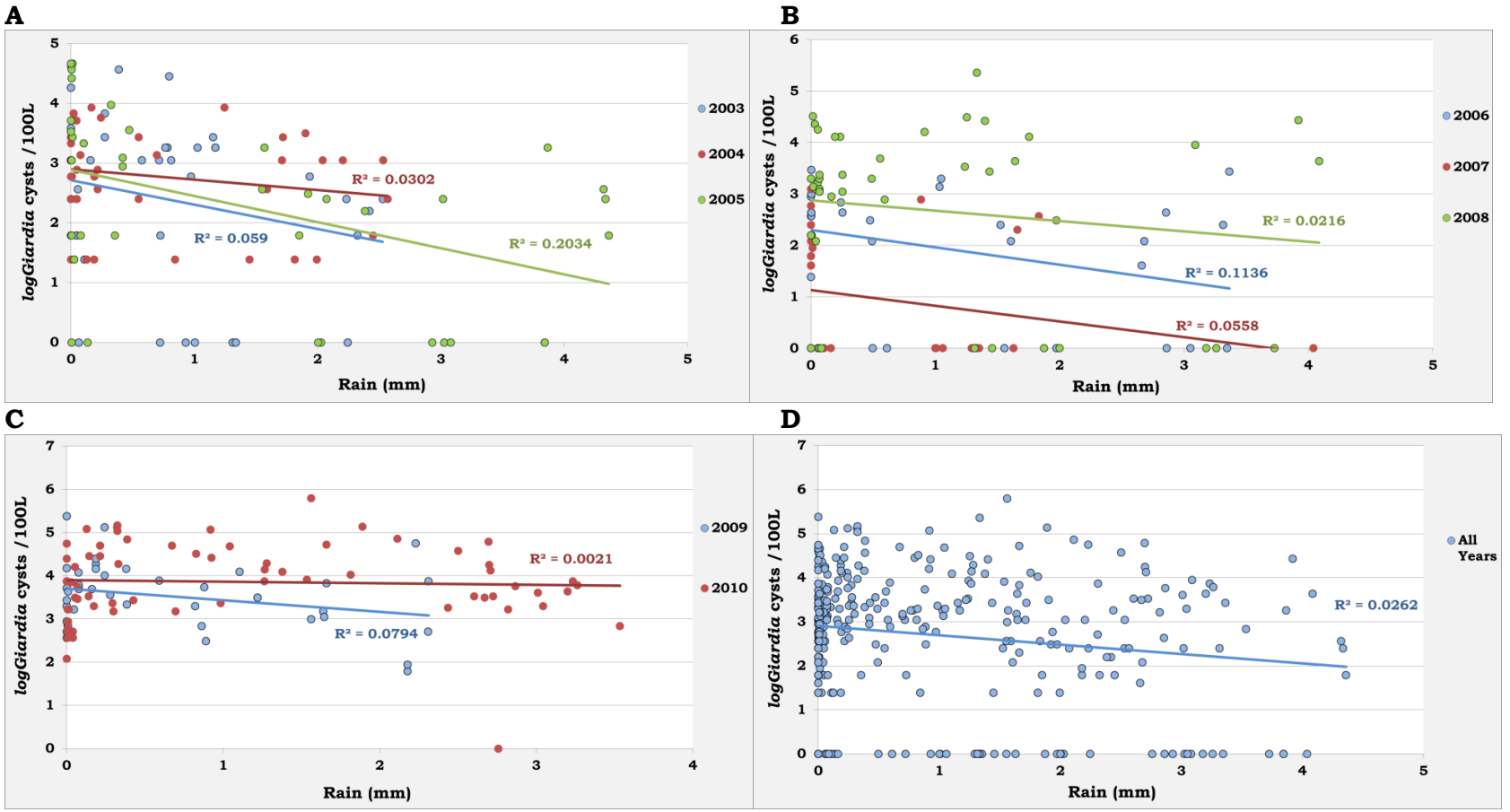

Figure A-9 Relationships between ln (*Giardia* cysts/100 L) concentration in source water and rain run-off during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign for the Bearspaw WTP (Bow River).

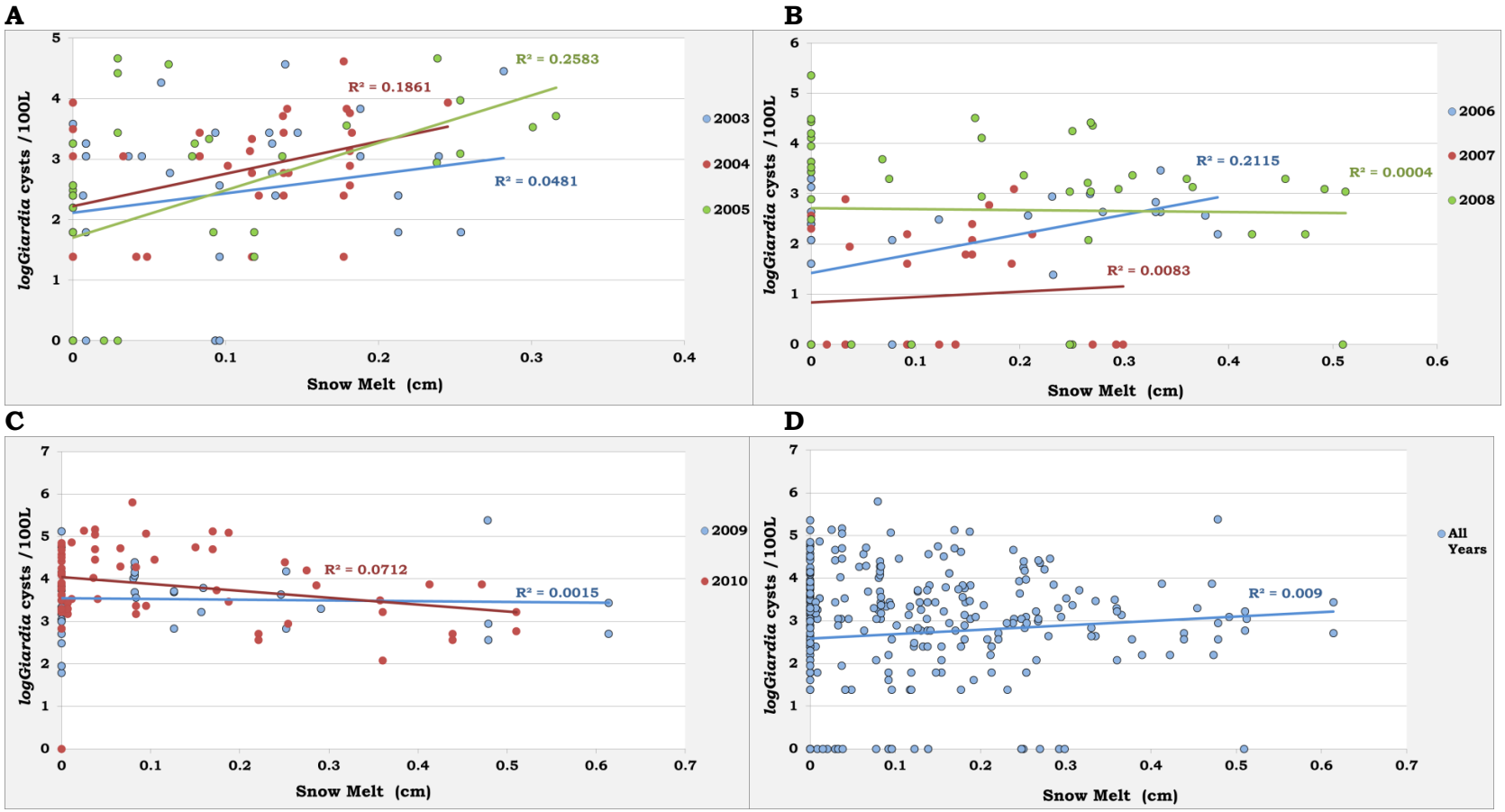

Figure A-10 Relationships between ln (*Giardia* cysts / 100 L) concentration in source water and snowmelt run-off during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 years (**D**). Data reflects the sampling campaign at the Bearspaw WTP (Bow River).

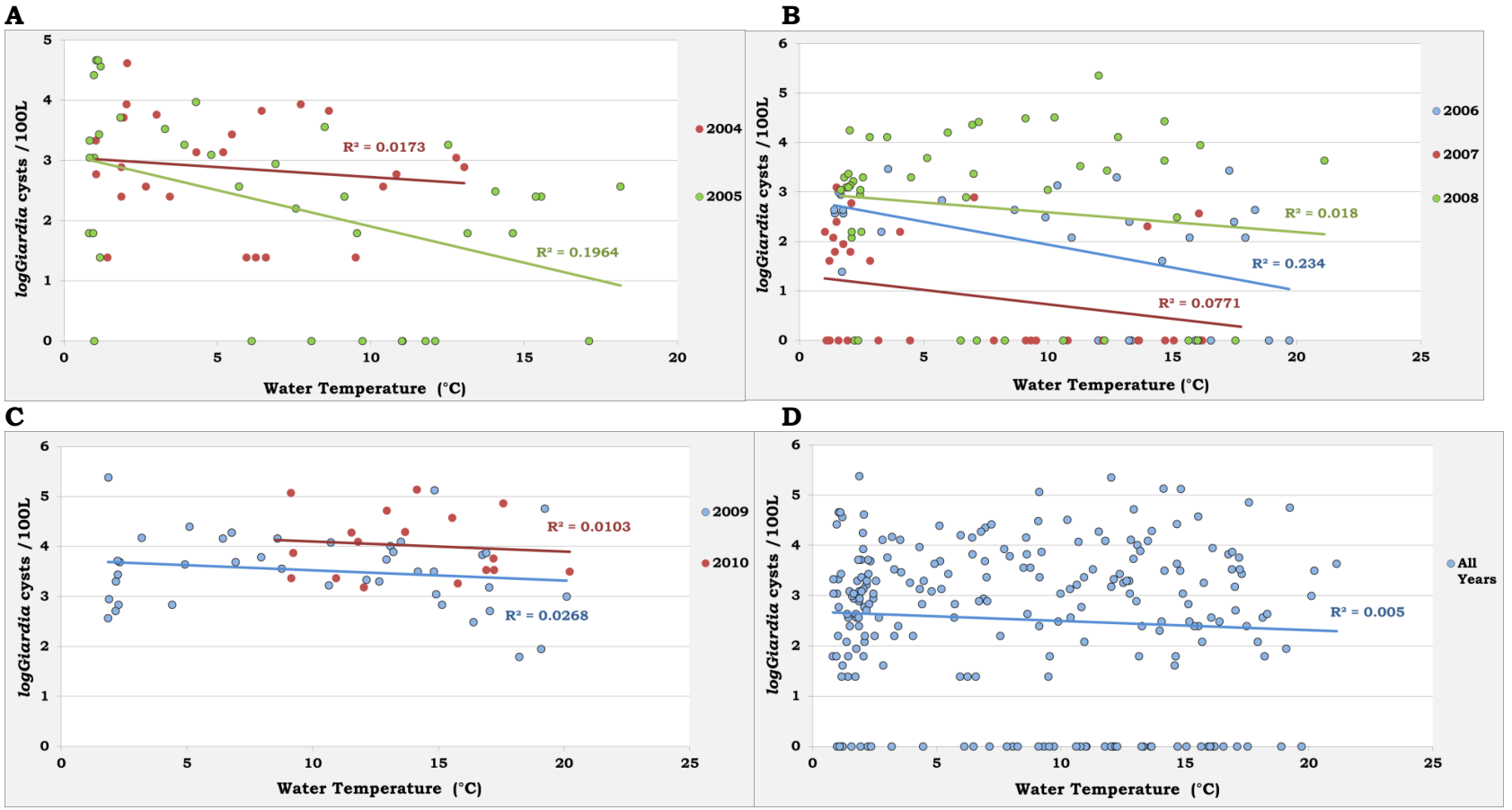

Figure A-11 Relationships between ln (*Giardia* cysts/100 L) concentration in source water and source water temperature in 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), and the entire period of monitoring 2003-2011 (**D**). Data reflects the sampling campaign at the Bearspaw WTP (Bow River).

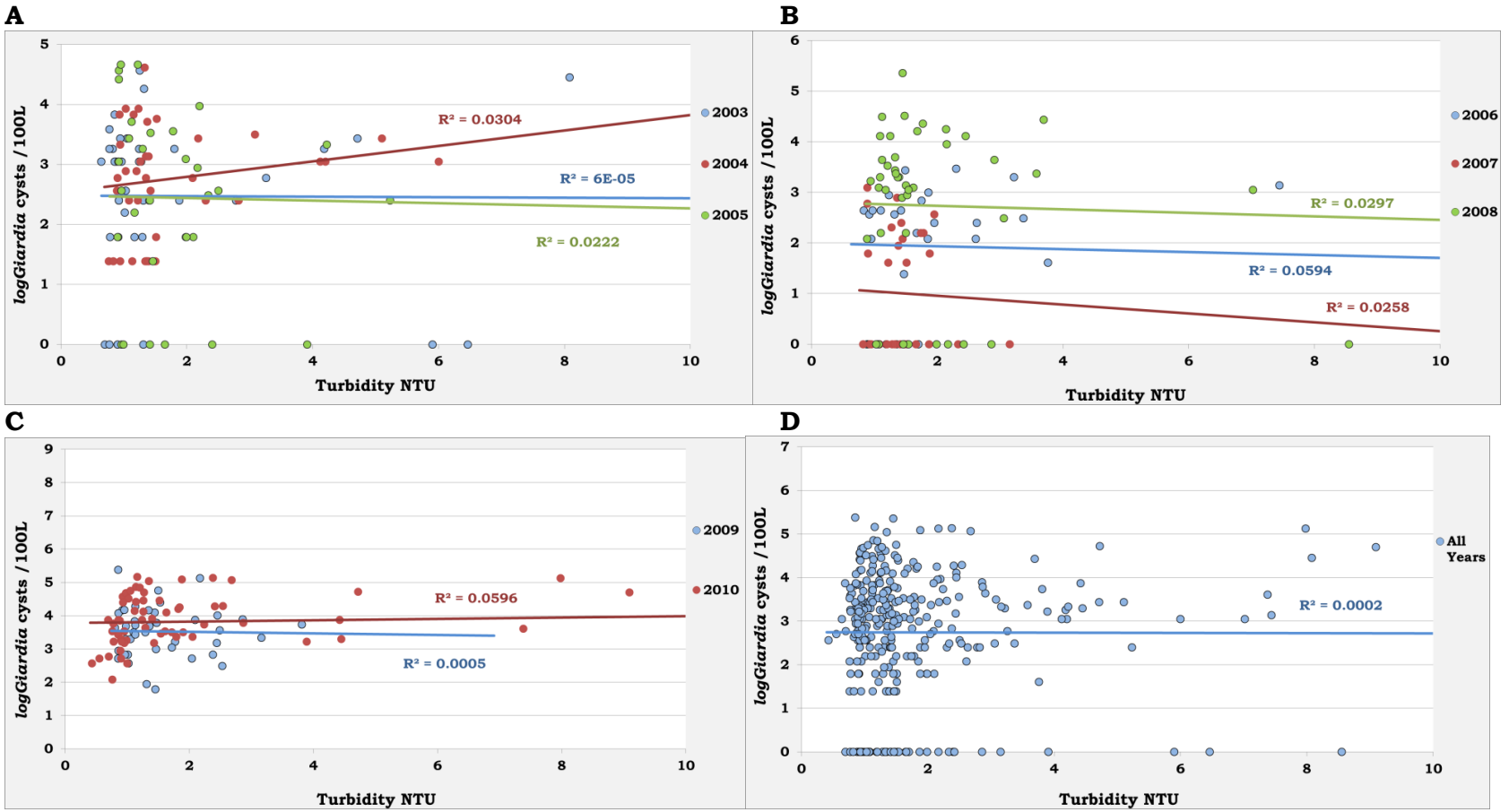

Figure A-12 Relationships between ln *Giardia* cysts/100 L concentration in source water and source water turbidity during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign at the Bearspaw WTP (Bow River).

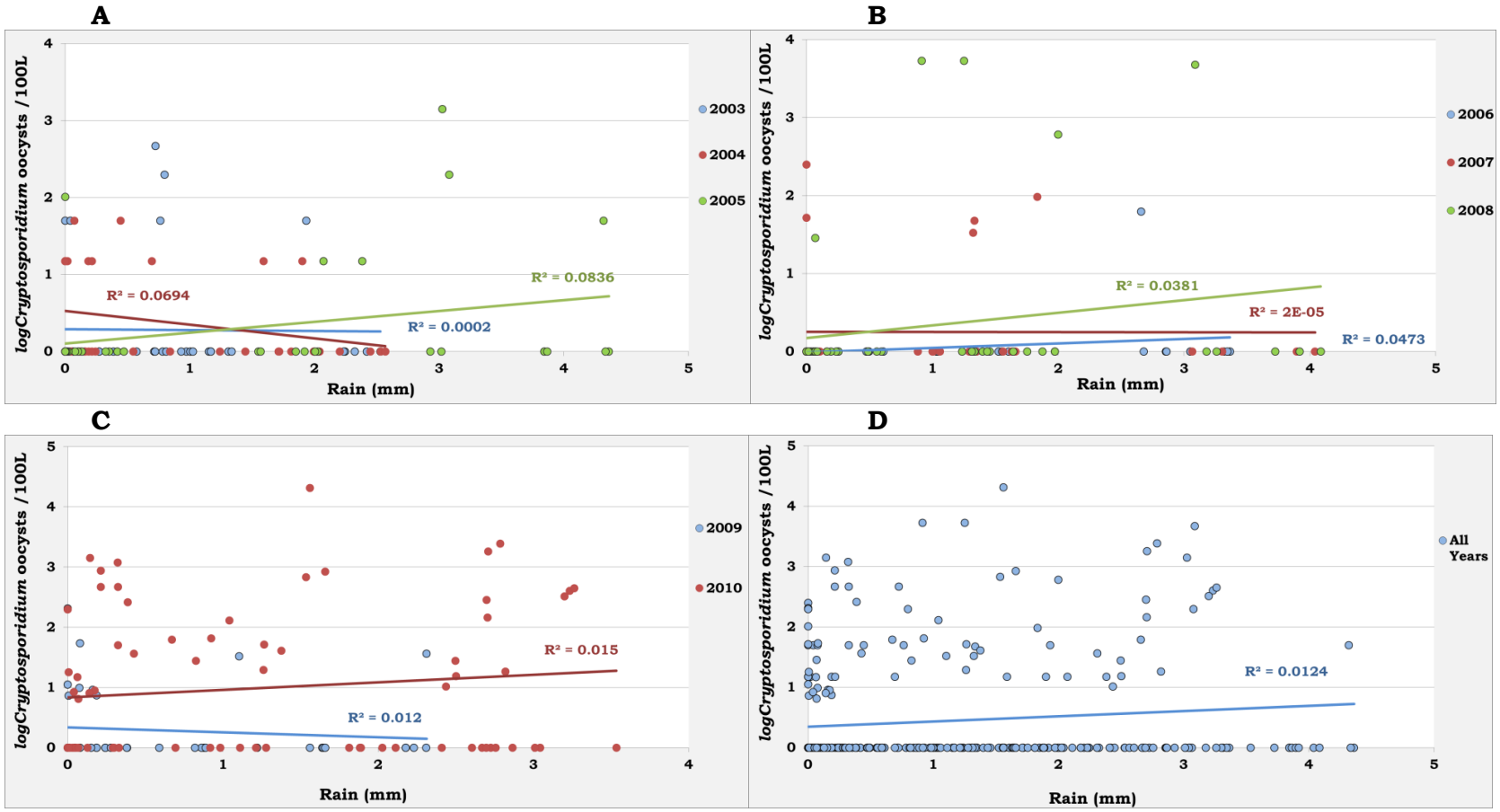

Figure A-13 Relationships between ln *Cryptosporidium* oocyst/100 L concentration in source water and rain run-off in 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign at the Bearspaw WTP (Bow River).

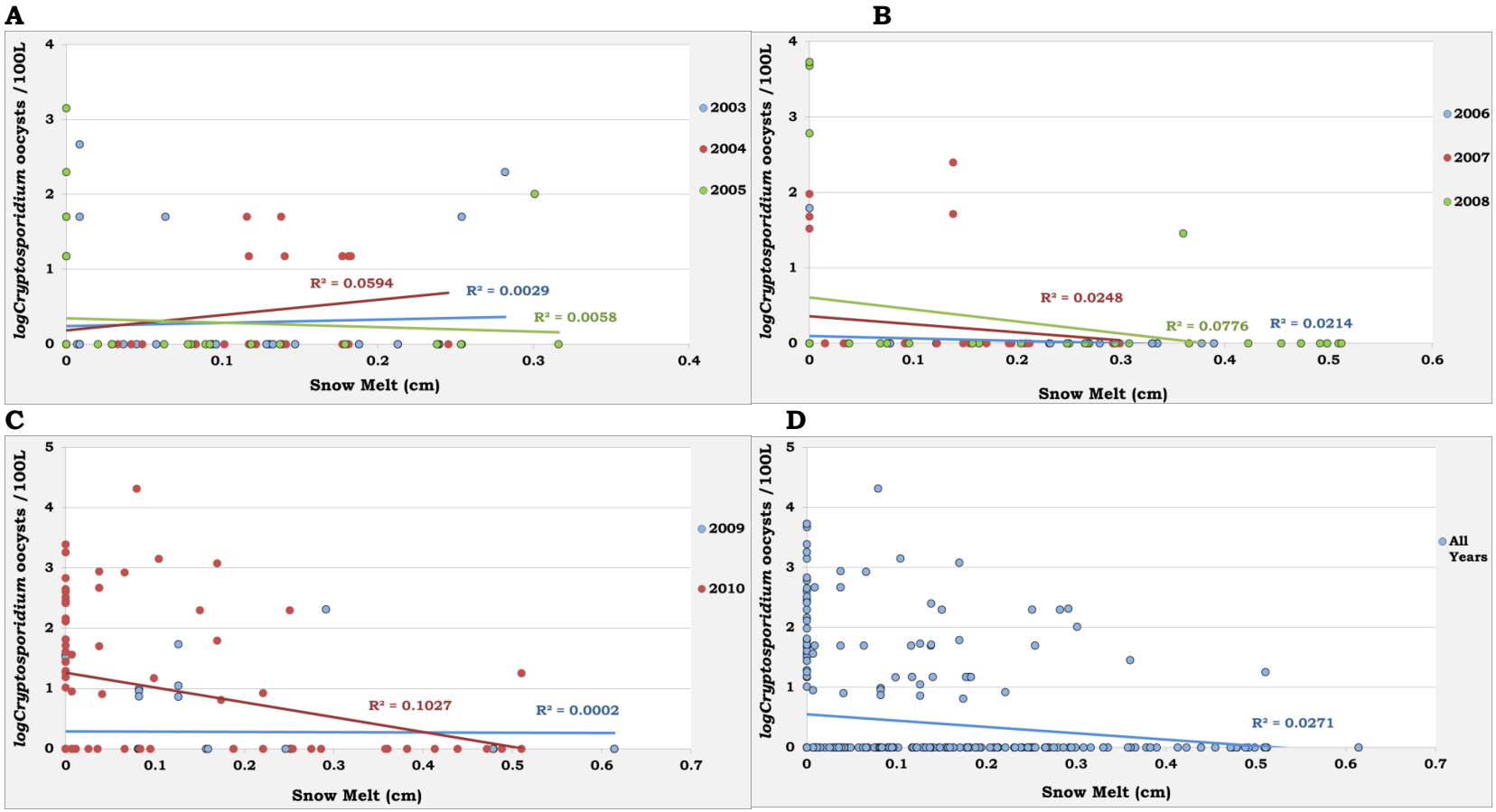

Figure A-14 Relationships between ln (*Cryptosporidium* oocyst/100 L) concentration in source water and snowmelt run-off during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period of monitoring 2003-2011 (**D**). Data reflects the sampling campaign at the Bearspaw WTP (Bow River).

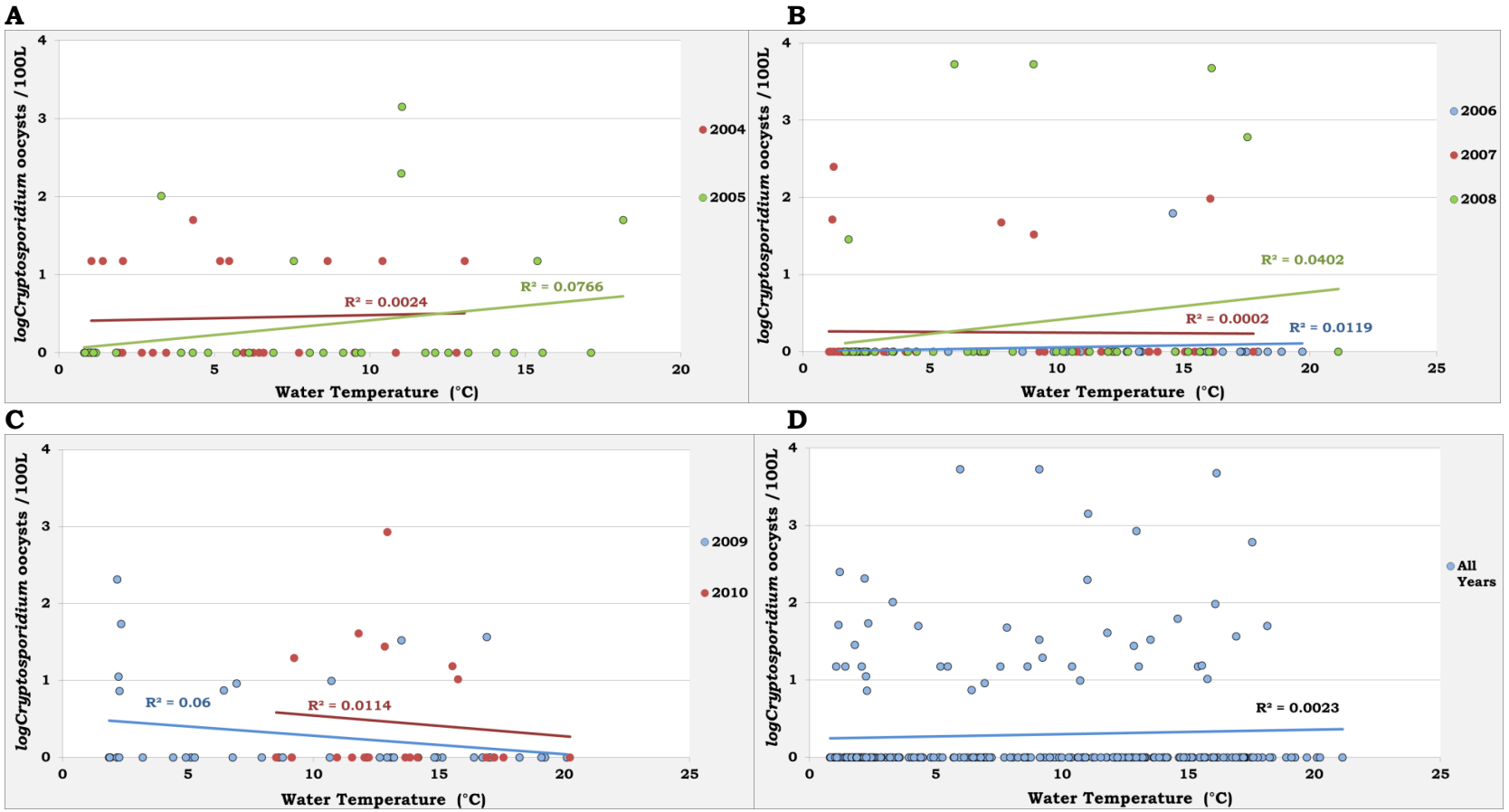

Figure A-15 Relationships between ln (*Cryptosporidium* oocyst/100 L) concentration in source water and source water temperature during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period of monitoring 2003-2011 (**D**). Data reflects the sampling campaign at the Bearspaw WTP (Bow River). Water temperature monitoring ended on 25 October 2010.

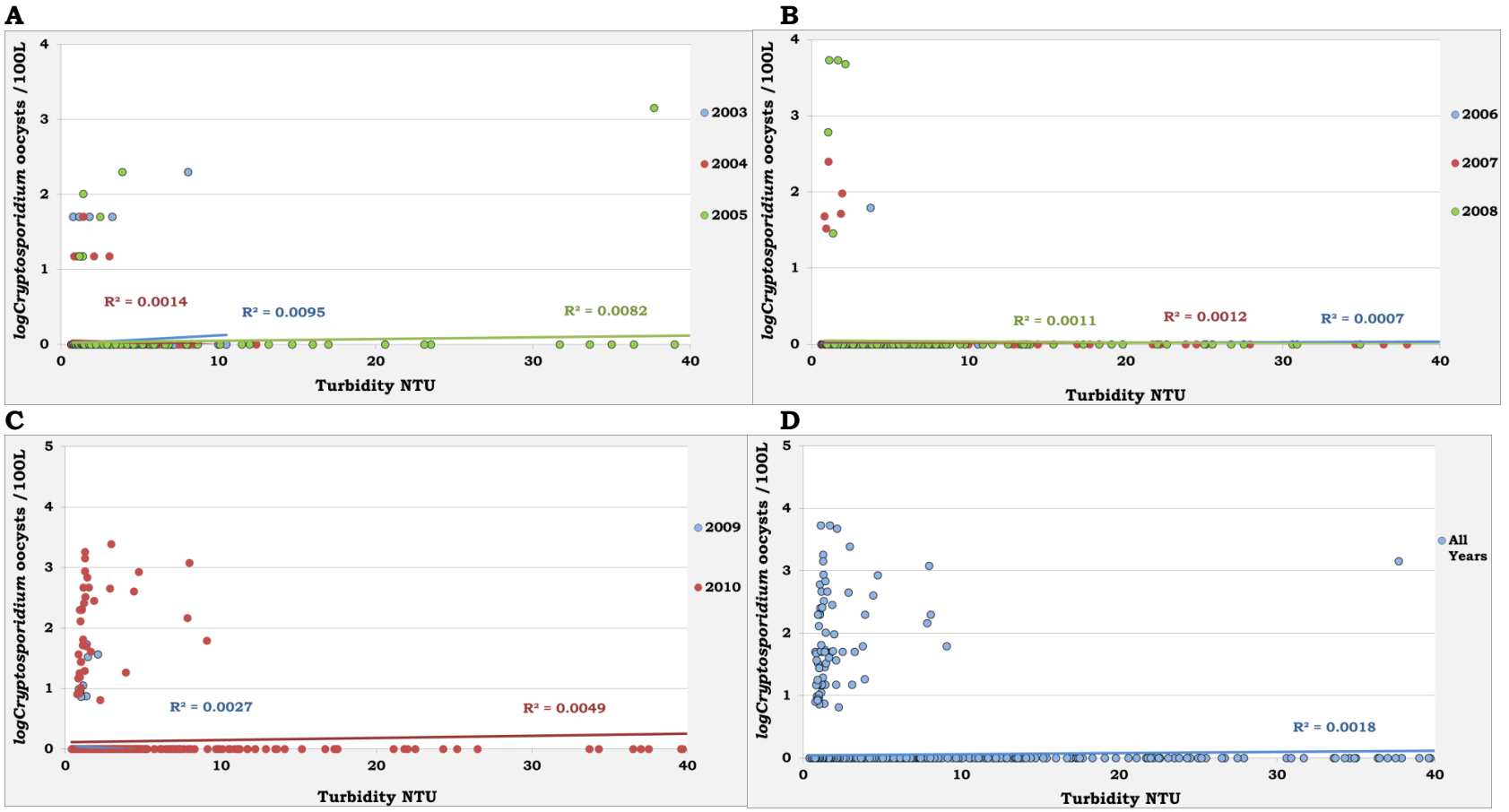

Figure A-16 Relationships between ln (*Cryptosporidium* oocyst/100 L) concentration in source water and source water turbidity during 2003, 2004, and 2005 (**A**), 2006, 2007, and 2008 (**B**), 2009 and 2010 (**C**), the entire period 2003-2011 (**D**). Data reflects the sampling campaign at the Bearspaw WTP (Bow River).