DEVELOPMENT AND IMPLEMENTATION OF A WATERSHED-SCALE CUMULATIVE EFFECTS FRAMEWORK FOR THE ATHABASCA RIVER BASIN

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ABSTRACT

Over the recent decades, there has been an increase in human-related negative environmental impacts on aquatic ecosystems worldwide. These impacts are varied and can include climatic variations as well as increased urban and industrial developments. These impacts can be challenging to manage as they can manifest themselves in a cumulative manner over very large spatial (watershed) and temporal (decadal) scales. In response to these challenges, scientists have been developing methods which attempt to assess the increasingly complex interactions between our environment and the current and future demands of society. This thesis proposes a framework for quantifying cumulative changes in water quality and quantity and demonstrates its implementation in an entire watershed, the Athabasca River basin in Alberta, Canada. The Athabasca River basin is an ideal watershed for this study as it has undergone significant increases in urban and industrial developments which have the potential to impact this aquatic ecosystem.

The province of Alberta, Canada is currently experiencing significant economic growth as well as increasing public awareness of its dependence on water. The Athabasca River is a glacial fed river system with a basin covering 157 000 km^2 and is the longest (1538 km) unregulated river in Canada. The basin holds significant cultural and economic importance, supporting more than nine First Nation groups, and providing water to hundreds of industries. There has been an increasing level of industrial, urban and other land-use related development (pulp and paper mills, oil sands developments, agriculture, and urban development) within the Athabasca River basin. Many of the historical water quantity and quality data for this basin have not been integrated or analyzed from headwaters to mouth, which affects development of a holistic, watershed-scale cumulative effects assessment.

The main goal of this thesis was to develop and apply a quantitative approach (framework) to assess and characterize the cumulative effects of man-made stressors (e.g. municipal effluent, pulp and paper effluents, oil sands) on indicators of aquatic health (water quality and biological responses) over space and time for a model Canadian river, the Athabasca River, Alberta. This framework addresses the problems of setting an historical baseline, and comparing it to the current state in a quantitative way. This framework also creates the potential for the prediction of future impacts by creating thresholds specific to the study area. The outcome of this framework is the identification and quantification of specific stressors (dissolved

sodium, chloride and sulphate) showing significant change across the entire Athabasca River basin, as well as the development of thresholds for these parameters.

The first part of this framework was to quantify any spatial and temporal changes in water quality and quantity across the entire basin from headwaters to mouth, across two time periods, historical and current, over a period of 40 years. Data were collected from several federal, provincial and non-government sources. A 14-30% decrease in discharge was observed during the low flow period in the current time period in the lower three river reaches with the greatest decrease occurring at the mouth of the river. Dissolved sodium, sulphate and chloride concentrations in the second time period were greater than, and in some cases double, the 90th percentiles calculated from the first time period in the lower part of the river.

Based on these findings, the second portion of the framework involved developing basin-specific thresholds for these parameters using the partial life-cycle fathead minnow (*Pimephales promelas*) bioassay. Two laboratory experiments (dissolved sodium and dissolved chloride) and three field experiments (sodium chloride, dissolved sulphate and water collected downstream of industrial inputs at the mouth of the Athabasca River) were conducted using a diluter system allowing for the dilution of a 100% test solution (based on the highest recorded concentration of the respective parameter in the river) down to 50%, 25%, 12.5%, 6.25% and a 0% control. Significant changes in egg production were identified and linked to changes in gill diffusion distances illustrating the chronic impacts of these parameters on fathead minnow (FHM). The threshold range determined in the laboratory for dissolved sodium was between 12.5% (36.11 mg/L) and 50% (57.00 mg/L) and dissolved chloride less than 22.22 mg/L. At levels outside or above these ranges a possible decrease in reproductive output may occur. The threshold range determined in the field studies for dissolved sulphate concentration was between 23.33 mg/L and 100 mg/L and for the sodium chloride experiment the greatest increases in reproductive output occurred in the highest treatment (25.43 mg/L Na and 38.90 mg/L Cl) which was similar to the range identified in the laboratory study for dissolved sodium.

These results showed that significant changes have occurred in both water quantity and quality between the historical and current day Athabasca River basin. It is known that in addition to climatic changes, rivers which undergo increased agricultural, urban and industrial development can experience significant changes in water quantity and quality due to increased water use, discharge of effluents and surface run-off. Using the results from this thesis, we can begin to quantify dominant natural and man-made stressors affecting the Athabasca River basin as well as place the magnitude of any local changes into an appropriate context relative to trends in temporal and spatial variability. This thesis is a significant contribution to method development for watershed-scale cumulative effects assessment including development of whole river benchmarks for sublethal exposures of fish to increasing salinity for a river of economic and cultural importance and experiencing significant development pressure.

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DEDICATION

For Nan

Theresa Butler

(August 13, 1934-August 21, 2011)

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LIST OF ABBREVIATIONS

%	Percent
˚C	Degree Celsius
<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to
µS/cm	Microsemien per Centimetre
A	Athabasca Reach
AB	Alberta
AENV	Alberta Environment
amp	Ampere
ANOVA	Analysis of Variance
Aug	August
BASINS	Better Assessment Science Integrating Point and Nonpoint Sources
BET	Basal Epithelium Thickness
CCME	Canadian Council of Ministers of the Environment
CE	Cumulative Effects
CEA	Cumulative Effects Assessment
Cl	Dissolved Chloride
CWQG	Canadian Water Quality Guidelines
Dec	December
DN	Dissolved Nitrogen
DOC	Dissolved Organic Carbon
EEM	Environmental Effects Monitoring
EIA	Environmental Impact Assessment
Feb	February
FHM	Fathead Minnow
GIS	Geographic Information System
GSI	Gonadal Somatic Index
H	Hinton Reach
H_O	Null Hypothesis
HW	Headwaters Reach
HYDAT	Hydroclimatological Data Retrieval Program
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
J	Jasper Reach
Jan	January
kg/day	Kilogram per Day
km	Kilometre
km^2	Square Kilometre
KS	Kolmogorov Smirnov
LSI	Liver Somatic Index
M	Mouth Reach

m^3	Cubic Metre
m^3/sec	Cubic Metres per Second
m^3/year	Cubic Metres per Year
mg/L	Milligrams per Litre
mm	Millimetre
Mo	Molybdenum
N	Nitrogen
NA	Naphthenic Acid
Na	Dissolved Sodium
NRBS	Northern River Basins Study
NREI	Northern River Ecosystem Study
NTU	Nephelometric Turbidity Unit
Oct	October
P	Phosphorous
p	P-value of a test of significance
RAMP	Regional Aquatic Monitoring Program
RO	Reverse Osmosis
SEA	Strategic Environmental Assessment
SK	Saskatchewan
SLL	Secondary Lamella Length
SLW	Secondary Lamella Width
SO_4	Dissolved Sulphate
SSC	Secondary Sexual Characteristics
T1	Time Period One (1966-1976)
T2	Time Period Two (1996-2006)
THREATS	The Healthy River Ecosystem Assessment System
TMDL	Total Maximum Daily Load
TOC	Total Organic Carbon
TP	Total Phosphorous
TSS	Total Suspended Solids
US EPA	United States Environmental Protection Agency
V	Vanadium
YESAB	Yukon Environmental and Socio-economic Assessment Board

CHAPTER 1: GENERAL INTRODUCTION AND RESEARCH OBJECTIVES

1.0 GENERAL INTRODUCTION

1.1 CUMULATIVE EFFECTS ASSESSMENT

The field of ecotoxicology has undergone increased expansion and scrutiny in recent decades. There have been many new challenges to face, such as climate variations and increased urban and industrial developments, which are coupled with increased public awareness of environmental issues. In order to address these challenges, ecotoxicologists have been developing novel methods which attempt to assess the increasingly complex interactions between our environment and the current and future demands of society. As these demands manifest in a cumulative manner over broad temporal and spatial scales, methods which address these types of effects must be developed.

There are many definitions to what cumulative effects are and how they should be assessed. The definition used in the Canadian Cumulative Effects Assessment Practitioners Guide published in 1999 states that cumulative effects "are changes to the environment that are caused by an action in combination with other past, present and future human actions" (Hegmann et al. 1999). In the recent academic literature, cumulative effects have been defined as "the incremental effects of a single action assessed in the context of past, present and future actions, regardless of who undertakes the action" (Ma et al. 2009).

For the purposes of this thesis cumulative effects are several environmental changes associated with multiple activities (man-made and natural) which exist over space and persist over time (Spaling and Smit 1995; MacDonald 2000; Harriman and Noble 2008). An effect is defined as "a change" relative to a chosen benchmark determined by comparing temporally or spatially differing points of reference (Dubé et al. 2006). Cumulative effects assessment (CEA) is the process of systematically assessing effects (changes) resulting from incremental, accumulating and interacting stressors (Reid 1993; Dubé et al. 2004).

1.1.1 History

In Europe, the European Union Environmental Impact Assessment directive was introduced in 1985, as was amended in 1997 to include the requirement for cumulative effects assessment (Connelly 2011). It was this directive that helped to initiate the requirement of cumulative effects assessments (CEA) as part of the environmental impact assessment (EIA) process in many countries (Cooper and Sheate 2002; Canter and Ross 2010). In the United Kingdom this resulted in a description of any possible cumulative effects being required under the Town and Country Planning Regulations in 1988. However, in the UK the assessment of cumulative effects as part of the EIA process has faced several challenges. The most significant of these is the lack of clear methods or frameworks for the assessment of cumulative effects (Cooper and Sheate 2002)

In Sweden, the assessment of cumulative effects is not well legislated and as a result is seldom described in environmental assessments. In fact, the term 'cumulative effects' is not used in any current existing relevant environmental legislation (Warnback and Hilding-Rydevik 2009). Similar to other parts of the world, in Sweden it falls to the proponent (developer) to conduct and report the finding of a cumulative effects assessment as part of a larger environmental impact assessment required for existing and/or proposed developments. The requirement to conduct an environmental assessment was first legislated in 1981 and further formalized in 1987 (Warnback and Hilding-Rydevik 2009). These requirements fall under what is called framework laws wherein the actual implementation is dependent on the interpretations of individual professionals carrying out the assessments. Therefore there is no accepted legislated method of conducting these types of assessments in Sweden.

Recently China has made considerable progress in the implementation of cumulative effects assessment. Environmental impact assessment started in China in the 1970s, progressing to the requirement of strategic environmental assessment in 2003 as part of the Environmental Impact Assessment Law (Bina et al. 2011a). Many provinces as well as the Ministry of Environmental Protection have released several guidelines and plane in the last decade to aid in the implementation of this requirement (Bina et al. 2011b). The most influential of these was the Planning environmental impact assessment ordinance published in 2009 which provided the detailed procedures for many of the aspects of conducting environmental impact assessments (Bina et al. 2011a).

In Canada cumulative effects assessment started to emerge as a growing issue in 1984 with the creation of the Canadian Environmental Assessment Research Council. This council eventually became what is known today as the Canadian Environmental Assessment Agency. In 1995 the assessment of cumulative effects because a legal requirement of every environmental assessment in the Canadian Environmental Assessment Act (Connelly 2011; Duinker and Greig

2006). Under this context, CEAs are required when a new development project (e.g., pipeline, oil sands mining) of significance (i.e., that is triggered to conduct an EIA under the Act) is proposed. Project developers are required to inform regulatory agencies about potential environmental consequences of the development activities and in consideration of projects developed in the past, present and proposed for the future.

1.1.2. CEA within EIA

As a result of CEA being a requirement of EIA the methods used for these assessments are specific to the proposed development project and consequently are typically causal or stressor-based (Spaling and Smit 1995). Stressor-based CEAs typically focus on local project activities (e.g., construction of a dam), the stressors associated with these activities (e.g., siltation), and predict the likely effects on the environment caused by the stressors. These approaches are criticized for assuming that all stressors and stressor-effect linkages are known.

It has been recognized that there is a need to shift from local, project scale CEA to broader, landscape or regional scale assessments to accurately assess cumulative effects (Duinker and Greig 2006). However, there is not currently a mechanism for this to occur under the EIA process. It is unrealistic to expect single proponents to conduct an adequate CEA on a wide enough scale to actually be an effective assessment. This is mainly due to the fact that proponents which are conducting an EIA are doing it for project approval purposes and do not necessarily have the resources or expertise to expand their focus beyond the specifics of their particular projects (Gunn and Noble 2011; Hegmann and Yarranton 2011). Many of the cumulative effects that are measured in a study area are not necessarily due to the presence of an individual proponent (Gunn and Noble 2011). In fact cumulative effects can be attributed towards many different man-made sources as well as natural processes (Dubé 2003). This creates a certain level of uncertainty in these types of assessments and further illustrates the need for a widely-accepted framework for cumulative effects assessment.

Effects-based methods for regional Canadian CEA have developed largely through watershed studies conducted by university and government researchers (Dubé 2003). Although these assessments may be conducted on a larger regional scale, they are still focused on a specific region with specific known stressors and interactions (Harriman and Noble 2008). Effects-based assessments focus first on measuring changes in the aquatic environment (i.e.

determining the existing environmental state) over a broader spatial scale and second, on determining the cause or stressor of the effect if effects are measured. This approach is founded on the premise that if the “performance” or “health” of the environment is affected by the cumulative insults of man-made activities, then mitigation is required, and any new project proposals must ensure development activities do not affect the environment further. Examples of larger-scale effects-based CEAs include the Moose River basin study (Munkittrick et al. 2000), and studies under the Northern Rivers basin Study (Culp et al. 2000a), and the Northern Rivers Ecosystem Initiative (Dubé et al. 2006) which focused on the Athabasca Region in Alberta, Canada. Although these effects-based approaches are conducted at the appropriate scale for CEA and are effective for determining the “health” of a system, development of predictive models to understand how the system may respond to future development pressures is difficult as linkages between stressors and their effects are often unknown.

Despite the fact that it is globally acknowledged that cumulative effects are a growing issue which must be addressed (Schindler 1998; Duinker and Greig 2006), there is currently not a single conceptual approach or even several general principles which are accepted by scientists and managers (Harriman and Noble 2008). Much of the confusion in assessing cumulative effects on aquatic ecosystems is due to poorly defining the resources of concern and the spatial and temporal scales of the analysis (MacDonald 2000). The most powerful and appropriate approach to CEA is to combine general scientific frameworks and methodologies (Risser 1988).

1.1.3. The Importance of Watershed Scale Studies

Historically CEA methodology has been criticized for being conducted at too small a spatial scale over too short a time period (Ziemer 1994). Consequently, there has been a recognized need for methodology to be developed at a regional scale (Duniker and Greig 2006). However, most attempts at developing this methodology fall short due to their failure to define their terms, methods or reference (threshold) points and thus can be severely influenced by the assessors or regulators (Foden at al. 2008). Therefore, prior to conducting an environmental assessment at a regional or watershed scale it is important to define what the assessment will include, the methods with which to conduct it and the standards against which effects will be determined (Foden et al. 2008).

The authors Duniker and Greig (2006) itemize several of the most pressing deficiencies of current CEA methodology. Among them is the focus on project approval and the separation of project-specific impacts from cumulative effects. Industry is required to perform CEA within their project-level EIA. This is seen by many authors as being an unrealistic expectation for many reasons; one of which is the inability to obtain the necessary data to conduct such assessments. Even if projects currently exist in the proposed area, most practitioners are unable to obtain the necessary details about these projects to include them in an assessment and are therefore unable to reliably carry out a larger-scaled assessment. In reality, it can be seen as the regulators responsibility to carry out these assessments since they can obtain access to the necessary information and are more adequately informed of future development proposals in the area. In addition, since one of the main outcomes of any regional or watershed scale assessment should be to provide guidance on the acceptable limits of development in the area, who better to conduct these assessments than the ones in charge of enforcing these limits?

Including an assessment of the entire watershed (headwaters to mouth) is an important aspect of cumulative effects assessment. Limited spatial magnitudes generally narrow impact analysis to considerations of single stressors and simple cause-effect relationships which can be attributed towards a specific environmental attribute and an individual site (Spaling and Smit 1995). To minimize bias, the spatial scale of the assessment should be defined by the spatial scale of the processes (i.e. the industry, land uses) that control the resources of concern (i.e. the catchment or watershed) (MacDonald 2000). Many of the effects that occur (and are detected in project-specific CEA) can also be attributed towards natural changes that occur within the system (ref). Incorporating these natural changes will help to differentiate between those effects that are man-made and those which are not. On this basis then, a CEA method for rivers should include impacts accumulating along the river continuum. Impacts should also be considered over multiple scales such as reach, catchment, and regional landscape (Schindler 1998). Obviously, this level of assessment (e.g., at the watershed scale) is beyond the capacity of single project proponents to conduct under the existing Canadian EIA process.

1.2 THE ATHABASCA RIVER BASIN

The Athabasca River basin covers 157 000 km^2, accounting for approximately 22% of the landmass of Alberta (Gummer et al. 2000). It originates at the Columbia Ice Fields in Jasper

National Park and flows northeast 1300 km across Alberta until it terminates in Lake Athabasca (Figure 1.1). The Athabasca River is a 6th order stream which flows through four major physiographic provinces: Rocky Mountains, Great Plains, Athabasca Plain and Bear-Slave-Churchill Uplands (Culp et al. 2005). The peak flows and flooding events of this river basin are associated with the snowmelt and approximately 70% of the moisture input into the basin originates from precipitation (Culp et al. 2005). The average water flows and temperatures throughout the river (from headwaters to mouth) range from 86.4 m^3/sec to 438.9 m^3/sec and 1.1 ºC to 14.5 ºC.

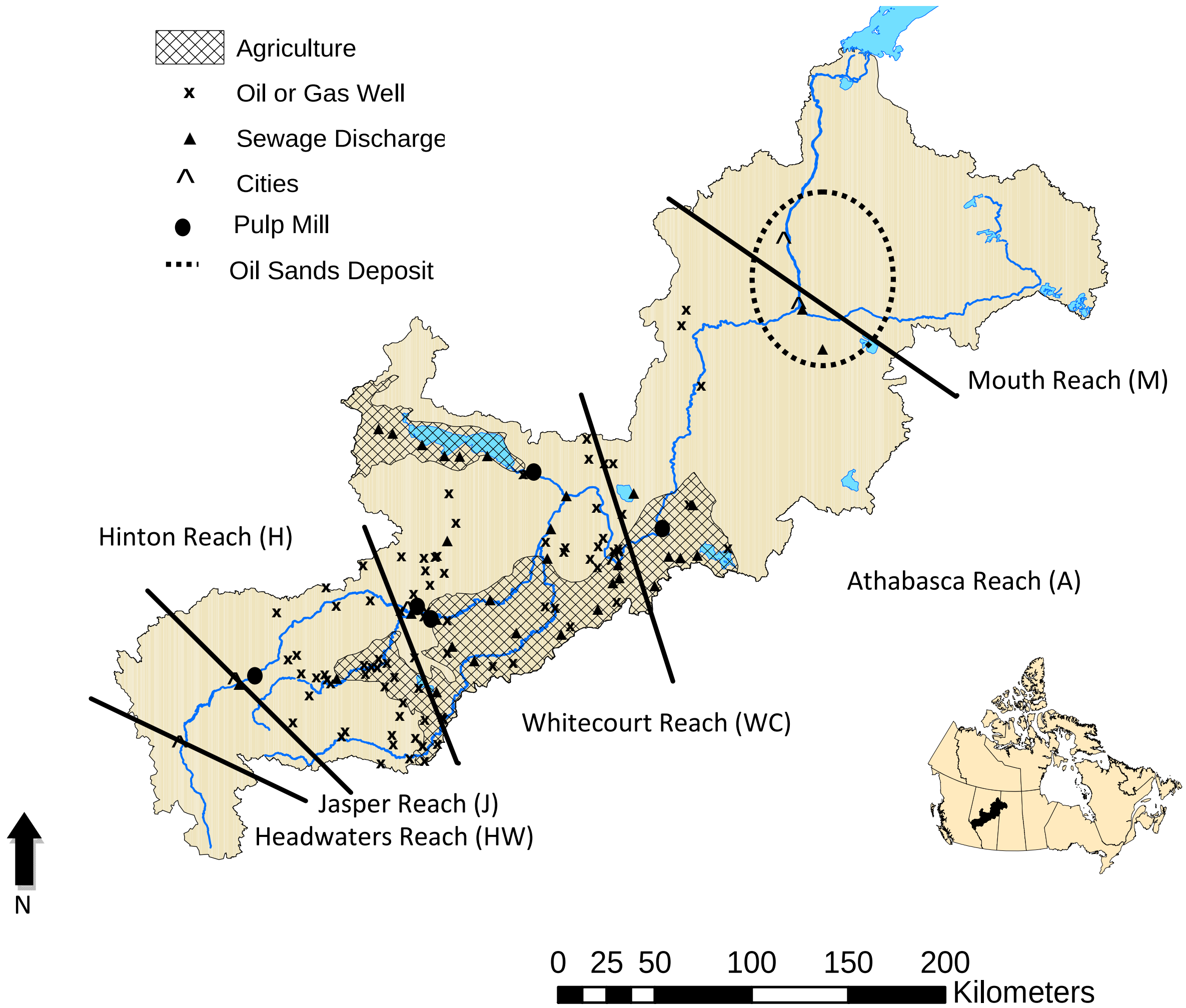

Figure 1.1: Map of the Athabasca River basin showing locations of agricultural, municipal and industrial development as known in 2006. (Adapted from Squires et al. 2010)

1.2.1 Stressors

The Athabasca River serves as a good model to develop a methodology for aquatic CEA as it has experienced an increasing level of land use related development over the past several decades. These developments include including forestry/pulp and paper, coal mining, oil and natural gas, agriculture, tourism, wildlife trapping, hunting and oil sands mining (Wrona et al. 2000; Culp et al. 2005). As a result of these developments, the water quality and quantity of Athabasca River has been monitored by both the federal and provincial governments and private environmental groups. Consequently there is a wide range of data available along the length of the Athabasca and over many decades. It is known that rivers which undergo increases in agricultural and urban development experience increased peak flows, decreased summer flows, increased water temperatures, as well as increases in the concentrations of total dissolved solids (TSS), nutrients and contaminants (van Sickle et al. 2004). There are several major sources of contaminants of concern in the Athabasca River basin which could be considered in CEA (Table 1.1).

Table 1.1: Historical and current major contaminants and their primary sources in the Athabasca River basin.

Contaminant	**Source(s)**
Salinity	Oil sands tailings water, municipal waste
Total Suspended Solids (TSS)	Pulp mill effluent, oil sands waste, municipal waste
Napathenic Acids (NAs)	Oil sands tailings water
Polycyclic Aromatic Hydrocarbons (PAHs)	Oil sands waste (alkylated), municipal waste
Chlorinated Organic Compounds (phenolics)	Pulp mill effluent, municipal waste
Metals (e.g. V, Mo)	Oil sands waste (tailings, coke)
Pesticides	Agriculture, forestry (pest control)
Dissolved Oxygen/ Biochemical Oxygen Demand	Pulp mill effluent, municipal waste
Nutrients (Phosphorous and Nitrogen)	Pulp mill effluent, agriculture, oil sands waste, municipal waste
Dioxins/Furans	Pulp mill effluent

1.2.1.1 Pulp and Paper Mills

The first pulp mill opened on the Athabasca River in 1957 (NRBS 1996a). Currently five pulp and paper mills are discharging directly into the Athabasca River or its major tributaries. Pulp effluents can have confounding positive and negative effects on invertebrates through the opposing interactions of nutrients and contaminants (Culp et al. 1996). Pulp mill effluent has also been shown to affect reproductive processes in fish (Rickwood et al. 2006). Canadian pulp mills are required to test their effluent and measure effects in the river on benthic invertebrates and fish every 3 years under the Environmental Effects Monitoring program (EEM) of the Fisheries Act. Three cycles of testing have been completed (1996, 2000, and 2004 respectively). These programs, along with other studies have generated water quality and biological data in the Athabasca River basin (Gibbons et al. 1998).

1.2.1.2 Municipal Discharges

Urbanization can affect stream hydrology, morphology, water quality, habitat and the ecology of river basins (Baer and Pringle 2000). Examples of impacts include increased frequency and magnitude of floods, erosion and deposition in stream channels, pollution from sewage and industrial effluents and invasion by exotic species. Stormwater runoff from urban areas is often high in automobile wastes, fertilizers and pesticides. In addition, poorly constructed landfill sites have the potential to leak contaminants into groundwater and local surface water bodies (NRBS 1996a). Although the population density of the Athabasca River basin is less than one person per square kilometre of land (a total of approximately 100 000 people), it is limited to a few town sites. Fort McMurray is the largest city in the basin followed by Hinton, Whitecourt, Jasper and Athabasca. The majority of municipalities with populations over 500 employ at least secondary sewage treatment. The amount of nutrients and other contaminants in municipal effluent depends upon the level of sewage treatment. A study conducted by Glozier et al. (2004) assessed the impact of nutrient and contaminant outputs from municipal effluents in the Banff and Jasper National Parks. They found that the Alberta Water Quality Guideline for total phosphorous (0.05 mg/L) was exceeded on a number of occasions in Jasper National Park. Dissolved phosphorus (generally thought to be the more environmentally relevant form) however was less than 0.01 mg/L. A wide variation in dissolved nitrogen was also reported (from 0.09 to 0.12 mg/L) in the headwaters of the Athabasca River basin. In Jasper

National Park, the town of Jasper underwent an overhaul of their water treatment system in 2003 which has aided in the improvement of water quality in this section of the Athabasca River (Squires et al. 2010).

1.2.1.3 Agriculture

The agricultural lands along the southern portion of the Athabasca River basin (Pembina River basin to Lac La Biche River) outline the perimeter of Alberta's main agricultural region to the south. Canola, peas, oats and forage crops dominate this area, with significant stands of barley and some wheat. Beef cattle are the dominant form of livestock, with some hogs, elk and bison (NRBS 1996a). The main water quality concerns related to agriculture in this region are soil erosion and the sediments and chemicals in agricultural runoff (NRBS 1996b). These issues are linked to the natural characteristics of northern soils and the impact of present day farming techniques. Solonetzic and Luvisolic soils, which cover large expanses of the northern agricultural area of the Athabasca River basin, are naturally susceptible to erosion. Extensive land clearing and drainage expose the soil to the erosive forces of wind and water. With increased water erosion, the potential for soils, nutrients, pesticides and herbicides to enter local water bodies increases (NRBS 1996a).

1.2.1.4 Oil Sands Industry

Oil sands, also known as tar sands are deposits impregnated with dense, viscous petroleum called bitumen. There are four major deposits of oil sands in Alberta; Athabasca, Peace River, Cold Lake, and Wabasca. The largest of these, and the only one currently amenable to surface mining, is the Athabasca deposit (31 000 km^2) (Barton and Wallace 1979). Oil sands production in the Athabasca region exceeds one million barrels/day, and is forecasted to reach 3.5 million barrels/day by 2015 and 4.0 million barrels/day by 2020, accounting for more than 80 per cent of Canadian oil production (CAPP 2006). Extraction of bitumen from the oil sands deposits requires large volumes of water (about 0.65 m^3 wastewater for each ton of oil sands processed) and produces a waste product called mature fine tailings (MFT) (Matthews et al. 2000). Tailings contain a variety of trace metals and organic compounds such as polycyclic aromatic hydrocarbons (Madeill et al. 2001) and water from Syncrude tailings waste is acutely toxic to aquatic organisms (*Selenastrum capricornutum*, Microtox®, *Daphnia magna*, *Hyalella azteca,* and *Onchorhynchus mykiss*) (Herman et al. 1994; Leung et al. 2001).

1.2.2 Previous Assessments in the Athabasca River basin

In Alberta, the environmental assessment legislation currently used for the assessment of cumulative effects was enacted in 1993 within the Alberta Environmental Protection and Enhancement Act and is enforced and managed by the Alberta Environment (Johnson et al. 2011). This legislation is very project-focused with its primary goal being to gather information necessary for regulatory decision making and management processes. While these types of assessments are useful when determining the localized impacts a particular project may have in an area, they do not provide a comprehensive assessment of how the proposed project while contribute to the overall health of the ecosystem in combination with any existing or proposed projects in the surrounding area.

A prominent example of a project-focused EIA on the Athabasca River which attempted to assess cumulative effects was the joint federal-provincial review conducted for the Alberta-Pacific Pulp Mill in 1990 (Ross 1998). For this project the major concerns focused on the biological oxygen demand and the chlorinated organic compounds which may be released into the surrounding environment through the effluent (Ross 1998; Griffiths et al. 1998). Of these two issues only the biological oxygen demand (BOD) was identified to be of concern when considering the cumulative impact of the surrounding pulp mill operations already discharging upstream in the Athabasca.

In the lower Athabasca River the development of oil sands deposits has been occurring since the 1960's. In the mid-1990s several environmental impact assessments were conducted on some of the major companies currently mining in this area. Syncrude Canada Inc. submitted an EIA in 1996 which was approved in 1997 without a public hearing. This EIA was a result of intervention by the Pembina Institute, Department of Fisheries and Oceans and Environment Canada as well as other proponents in the area. Some of the main concerns raised were contingency plans in the event of a breach of one of the tailings ponds as well as air and water quality concerns, post reclamation monitoring activities and resource use (Griffiths et al. 1998). The method used to assess the cumulative impacts of these concerns was primarily through modeling (air and water quality) and in all cases no significant impacts to the surrounding environment were expected to occur.

On a regional scale, to date, there have been three major regional assessments for parts of the Athabasca River basin including the Northern River Basins Study (NRBS), the Northern River Ecosystem Initiative (NREI) and the Regional Aquatic Monitoring Program (RAMP). NRBS and NREI were a series of research studies by various scientists over a 5 year program. CEAs were conducted at the end of the program and consisted of a qualitative synthesis of conclusions from the various researchers (Culp et al. 2000a; Dubé et al. 2006). The RAMP program is specific to the oil sands industry and operates over the development region for the longer term (RAMP 2005). These CEAs have been conducted on a portion of the basin, but as of yet, no attempt has been made to assess cumulative effects from headwaters to mouth (Lawe et al. 2005).

RAMP has undergone two separate peer reviews, the most recent being completed in January 2011. While some of the deficiencies outlined in the first review were addressed, there were still several areas of this monitoring program that were found to be deficient (RAMP 2011). Most of these criticisms centered on the lack of consistency in sampling times and locations across years. There was also a strong lack of cohesion between the different components being monitored (water quality, air quality, biological quality).

Most recently the government of Alberta released a draft document outlining their surface water quality management framework for the lower Athabasca region (AENV 2011). This framework aims to provide ambient surface water quality triggers and limits for the region with the goal towards protecting surface water quality and addressing cumulative effects. The main structure of this framework is a management feedback loop based on the formulation of water quality “triggers” and “limits” which once exceeded, will induce a certain management action geared towards mitigating any future impacts.

1.2.3 Known Effects on Fish in the Athabasca River basin

Previous studies on fish health in the Athabasca River basin have been conducted as part of both NRBS and NREI. In both of these assessments, the primary focus was on the effects of pulp and paper mill effluent discharge on the health and reproductive potential of certain sentinel fish species (Cash et al. 2000; NREI 2004). The general conclusion from NRBS was that although certain contaminants (dioxins, furans, PCB and mercury) were present in surface waters and available to fish populations, they were present at low levels within fish tissues, only rarely exceeding Canadian guidelines for human and aquatic health at certain times and in certain locations (Cash et al. 2000). Nevertheless, the study did show that sex hormone levels in burbot and longnose sucker were significantly depressed in areas near pulp mill effluent discharges.

Fish health studies conducted by NREI sought to expand this knowledge and verify the NRBS findings. This study assessed the temporal trend of certain contaminants (PCBs, DDT, Dioxins) over a 4-7 year period (between 1994-1998 or 1992-1999 depending on the contaminant) (NREI 2004). Generally these contaminants were found to either not change or to decrease between the years. The only exception was DDT in longnose sucker muscle which was found to increase downstream of a pulp mill discharge near Hinton.

As part of the NREI, a separate fish study was done which focused on the fish health effects from oil sands wastewater and naturally occurring oil sands compounds (NREI 2004). This study found that slimy sculpin from an oil sands mining site on the Steepbank River (a tributary to the Athabasca) had decreased sex steroid production (in both sexes) compared to those from upstream (Tetreault et al. 2003). This could indicate the potential for population-level effects in the oil sands area (NREI 2004). These authors also conducted acute toxicity studies using FHM and white sucker larvae and found that a small amount (0.2 mg/L) of sediments from wastewater ponds caused an increase in larval deformities. Sediments from naturally occurring oil sands areas also showed these effects, but about 10 times higher amounts of sediment were required (NREI 2004).

Many independent academic studies have also been done using sediments and waste products from the oil sands region of the Athabasca River Basin. Native fathead minnows (FHMs) (*Pimephales promelas*) reared in oil sands wastewater showed reduced growth compared to fish growing in non processed water (Siwik et al. 2000). Exposure of early life stages of FHMs and other species to natural bitumen and wastewater pond sediments affected hatching success, growth, and deformities (Colavecchia et al. 2004; 2006; 2007).

1.3 FATHEAD MINNOWS AS A TEST SPECIES

The FHM (*Pimephales promelas*) is widely distributed across North America. It is a freshwater species that is easy to raise and breed under laboratory conditions due to its relatively rapid life cycle. It is a species that is commonly used in standard toxicity testing and several protocols have been developed for culturing and handling as well as toxicity testing (Ankley et al. 2001; Environment Canada, 1992).

It is a species that has a relatively short life-cycle and is able to breed at 6-9 months of age. For this reason it has also been used in multiple-generation toxicity testing. The conditions ideal for breeding include having a light:dark cycle of approximately 16:8, a temperature of 26 ± 1˚C and the presence of a breeding substrate (usually a short piece of pvc pipe cut lengthwise) on which the female can deposit the eggs.

The FHM has been used as the main test species in artificial stream experiments which are part of the National Environmental Effects Monitoring Program (EEM) as regulated by Environment Canada. Artificial streams provide both control over relevant environmental variables and true replication in treatments to help establish cause where stressor-specific effects are measured on test species under controlled yet environmentally-relevant exposure conditions (Culp et al. 1996). For decades these systems have been used to test the responses of organisms to both single contaminants and complex mixtures (Dubé et al. 2002). Artificial stream studies have been used to measure changes in algal and benthic invertebrate community structure as well as acute and chronic endpoints (e.g., reproductive performance, growth, behaviour) in sentinel fish species after exposure to various contaminants (Culp et al. 2003; Hruska and Dubé 2004; Dubé et al. 2004).

The FHM reproductive bioassay provides an opportunity to assess parental and offspring responses to contaminant exposure as well as to link parent responses to their offspring directly. In addition, rather than using surrogate endpoints that estimate reproductive output (e.g., size of gonads), actual reproductive output such as egg production can be assessed. The sustainability of a population is determined by its ability to reproduce under changing and sometimes challenging environmental conditions. Using a bioassay that can directly measure reproductive output in response to controlled contaminant exposure is a valuable tool to assess the potential for contaminants to affect fish populations.

1.3.1 The Fathead Minnow Reproductive Bioassay

One of the key concepts in toxicology and risk assessment is the dose-threshold, based on the assumption that compounds can only cause effects above a certain dose level (Slob 1999). The probability of observing an effect increases with its magnitude, therefore a problem exists to determine the magnitude of response that represents an ecologically relevant biological effect. In toxicology, it is most common to derive a threshold for a particular stressor from a single species toxicity test (Cairns 1992). Optimally however, ecosystem quality should be assessed by including *in situ* toxicity test methods as these will increase the ecological relevance of the thresholds developed (Tucker and Burton 1999).

The most precise way to determine the threshold concentrations most relevant to the Athabasca River basin is to conduct controlled experiments in the laboratory and field. To assess potential changes in fish reproduction the FHM partial life-cycle bioassay was utilized in this thesis. These experiments were based on partial life cycle tests originally developed by Ankley et al. (2001) and further refined by Rickwood (2006). This assay allows us to assess the reproduction of FHMs, as well as aspects of their early development in a time frame much shorter than a traditional life cycle bioassay.

Experiments are conducted using 6-9 month old FHMs. The first phase of these experiments was a pre-exposure phase lasting 7-10 days. During this phase, approximately twice the number of breeding trios (1 male:2 females) of FHMs required for the exposure phase were placed in a tank with a breeding tile and every 24 hours, each breeding tile was checked for the presence of eggs. After this phase, breeding trios that met test criteria (minimum of 80% fertilization, bred at least once, and adults survived the entire pre-exposure period) were randomly assigned to each treatment. This was done to ensure treatments were not statistically different from each other ($p > 0.05$) and therefore, prior to exposure, each treatment had the potential for similar egg production.

The second phase of these experiments was an exposure phase lasting 21 days. During the exposure period each of the breeding trios that were randomly assigned in the pre-exposure phase were exposed to a particular treatment. Throughout the exposure phase, breeding tiles were checked daily for eggs and kept until 5-days post hatch. The total number of eggs, number of fertilized eggs, time to hatch, and number of larvae (alive, deformed and dead) after 5-days post-hatch were recorded. After 21 days of exposure, the adult fish were euthanized and the secondary sexual characteristics, total body weight, total length, carcass weight, liver weight and gonad weight were recorded.

1.3.2 Diluter Systems

To expose fish to test solutions, a diluter system was used that allowed for a six time dilution of a 100% test solution with three replicates per dilution (Figure 1.2). The basic operation of these diluter systems includes mixing tanks, march pumps, solenoid valves and mixing tubes. These systems were designed and custom built to be used both in the laboratory setting and in The Healthy River Ecosystem Assessment System (THREATS) trailer system (see section 1.4.3). They require two external mixing tanks, one is for the control or dilution water (or 0%) and the second is for the exposure (or 100%) solution. For all the experiments performed with this system as part of this thesis we used tanks big enough to allow for 4 turnovers per day of each replicate tank.

1.3.3 THREATS Trailer System

The THREATS trailer was used to conduct three dose-response experiments at the headwaters of the Athabasca River in Jasper National Park, Alberta, Canada (Figure 1.3). Please see Chapter 5 for further details on these experiments. The trailer is a self-contained 44-foot long 5th wheel trailer that can be transported to allow for experiments to be conducted on-site. This is an ideal requirement especially for industrial sites where regular testing must be conducted to meet regulatory requirements. It requires access to a power input by either a plug-in (50 amp) or a wired directly into the trailer (up to 200 amps).

Figure 1.2: Picture of the diluter systems used to conduct both the laboratory and field experiments in this thesis.

Figure 1.3: Picture of The Healthy River Ecosystem Assessment System (THREATS) trailer as it was setup and used for all experiments conducted at Jasper National Park between June-August 2009.

1.5 RESEARCH GOAL AND OBJECTIVES

Alberta is a province experiencing significant economic growth as well as increasing awareness of water dependencies. Consequently, novel approaches to address cumulative effects in their aquatic ecosystems are required. The Athabasca River basin is an excellent model to develop an approach for CEA because of its landscape characteristics, changes in land use along its continuum, and the opportunity to integrate a large amount of existing information in a cumulative effects context. There has been an increasing level of industrial (forestry/pulp and paper, coal mining, oil, natural gas and oil sands mining), urban and other land-use related development (agriculture, tourism, wildlife trapping, and hunting) within the Athabasca River basin (Wrona et al. 2000; Culp et al. 2005).

CEA is the process of systematically assessing impacts resulting from incremental, accumulating and interacting stressors (Dubé et al. 2006). This is an especially important process for an area such as the Athabasca oil sands which undergoes constant development both industrially (over 17 existing, approved, or planned oil sands projects) and municipally, with the constant expansion of cities such as Fort McMurray. As of yet, integration of stressor concentrations and their resulting effects on biota has not previously been done across the entire Athabasca River basin from its headwaters to its mouth. Therefore, there is a need to produce research which studies the cumulative impacts of stressors across the entire basin.

1.5.1 Research Goal

Develop and apply a quantitative approach (framework) to assess and characterize the cumulative effects of man-made stressors (e.g. municipal effluent, pulp and paper effluents, oil sands) on indicators of aquatic health (water quality and biological responses) over space and time for a model Canadian river, the Athabasca River, Alberta.

1.5.2 Research Objectives

1.5.2.1 Part I: Develop a framework for watershed scale aquatic CEA

Objectives:

I. To identify the desired attributes of a CEA methodology for aquatic systems.

II. To construct a viable framework to assess multiple impacts to water quality and quantity on a watershed scale.

1.5.2.2 Part II: Quantification of spatial and temporal changes in water quality

Objectives:

I. To quantify spatial and temporal changes in water quantity and quality over space (along the river continuum) and time (historical and present day) in a model river.

II. To evaluate the significance of any changes relative to existing benchmarks (e.g. water quality guidelines).

1.5.2.3 Part III: Determination of thresholds for biological indicator species exposed to selected water quality variables

1.5.2.3.1 Laboratory Studies

Objectives:

I. To assess changes in FHM (*Pimephales promelas*) indicators associated with increasing concentrations of dissolved chloride and sodium at concentrations deemed to be of importance to the Athabasca River.
FHM

II. To determine effect thresholds for FHM exposed to dissolved chloride and sodium.

1.5.2.3.2 Field Studies

Objectives:

I. To verify the changes in FHM (*Pimephales promelas*) indicators as seen in previous laboratory studies with concentrations of sodium chloride and dissolved sulphate deemed to be of importance to the Athabasca River.
FHM

II. To determine the sublethal effects on aquatic biota using water from both the headwaters and mouth of the Athabasca River.

1.5.3 Format of thesis

This thesis has been organized in a manuscript format for publication in scientific journals. Therefore there may be some repetition of introduction, materials and methods and figures throughout.

Chapter 2 was submitted to *Integrated Environmental Assessment and Management Special Issue on Watershed Cumulative Effects Assessment.*

Chapter 3 was published in *Integrated Environmental Assessment and Management* (2010) Volume 6, Issue 1, pages 119-134.

Chapter 4 was submitted to *Environmental Toxicology and Chemistry.*

Chapter 5 was submitted to *Environmental Toxicology and Chemistry.*

CHAPTER 2: DEVELOPMENT OF AN EFFECTS-BASED FRAMEWORK FOR WATERSHED SCALE AQUATIC CUMLUATIVE EFFECTS ASSESSMENT

Chapter 2 was submitted to *Integrated Environmental Assessment and Management Special Issue on Watershed Cumulative Effects Assessment.*

2.1 WHAT ARE CUMULATIVE EFFECTS?

The field of environmental science has undergone increased expansion and scrutiny in recent decades. There have been many new challenges to face, such as climate variations and increased urban and industrial developments, which are coupled with increased public awareness of environmental issues. In order to address these challenges, scientists have been developing novel methods which attempt to assess the increasingly complex interactions between our environment and the current and future demands of society. As these demands manifest in a cumulative manner over broad temporal and spatial scales, methods which address these types of effects must be developed.

Cumulative effects occur as several environmental changes associated with multiple activities (man-made and natural) which exist over space and persist over time (Spaling and Smit 1995; MacDonald 2000; Harriman and Noble 2008). An effect is defined as "a change" relative to a chosen benchmark determined by comparing temporally or spatially differing points of reference (Dubé et al. 2006). Cumulative effects assessment (CEA) is the process of systematically assessing effects (changes) resulting from incremental, accumulating and interacting stressors (Reid 1993; Dubé et al. 2004). The objectives of this paper are to 1) review the desired attributes of a cumulative effects assessment framework for aquatic systems 2) propose a quantitative framework for watershed-scale cumulative effects assessment and 3) summarize the implementation of this framework in a model river.

2.2 HISTORY OF CUMULATIVE EFFECTS ASSESSMENT

In Europe, the European Union Environmental Impact Assessment directive was introduced in 1985, as was amended in 1997 to include the requirement for cumulative effects assessment (Connelly 2011). It was this directive that helped to initiate the requirement of cumulative effects assessments (CEA) as part of the environmental impact assessment (EIA) process in many countries (Cooper and Sheate 2002; Canter and Ross 2010). The original context of CEA in Canada originated and existed within the environmental impact assessment (EIA) process defined under the Canadian Environmental Assessment Act (Duinker and Greig 2006). Under this context, CEAs are required when a new development project (e.g., pipeline, oil sands mining) of significance (i.e., that is triggered to conduct an EIA under the Act) is proposed. Project developers are required to inform regulatory agencies about potential environmental consequences of the development activities and in consideration of projects developed in the past and proposed for the future. In this context, CEA methods are causal or stressor-based and are specific to the proposed development project (Spaling and Smit 1995). Stressor-based CEAs typically focus on local project activities (e.g., construction of a dam), the stressors associated with these activities (e.g., siltation), and predict effects in the environment caused by the stressors. These approaches are criticized for assuming that all stressors and stressor-effect linkages are known. Stressor-based approaches are also not conducted at spatial and temporal scales required for CEA.

It has recently been recognized that there is a need to shift from local, project scale CEA to broader, landscape or regional scale assessments to accurately assess cumulative effects (Duinker and Greig 2006). However, there is not currently a mechanism for this to occur under the EIA process. Effects-based methods for aquatic regional Canadian CEA have developed largely through watershed studies conducted by university and government researchers (Dubé 2003). Although these assessments may be conducted on a larger regional scale, they are still focused on a specific region with specific known stressors and interactions (Harriman and Noble 2008).

Effects-based assessments focus first on measuring changes in the aquatic environment (i.e. determining the existing environmental state) over a broader spatial scale and second, on determining the cause or stressor of the effect if effects are measured. This approach is founded on the premise that if the "performance" or "health" of the environment is affected by the cumulative insults of man-made activities, then mitigation is required, and any new project proposals must ensure development activities do not affect the environment further. Examples of effects-based CEAs include the Moose River basin study (Munkittrick et al. 2000), and studies under the Northern Rivers basin Study (Culp et al. 2000a), and the Northern Rivers Ecosystem Initiative (Dubé et al. 2006). Although effects-based approaches are conducted at the appropriate scale for CEA and are effective for determining the "health" of a system, development of predictive models to understand how the system may respond to future development pressures by linking the stressors responsible for these effects is much more difficult to accomplish and thus far has not occurred.

Despite the fact that it is globally acknowledged that cumulative effects are a growing issue which must be addressed (Schindler 1998; Duinker and Greig 2006), there is currently not a single conceptual approach or even several general principles which are accepted by scientists and managers (Harriman and Noble 2008). Much of the confusion in assessing cumulative effects on aquatic ecosystems is due to poorly defining the resources of concern and the spatial and temporal scales of the analysis (MacDonald 2000). The most powerful and appropriate approach to CEA is to combine general scientific frameworks and methodologies and adopt a recognized framework with clear standards and objectives (Risser 1988; Foden et al. 2009).

2.3 DESIRED ATTRIBUTES OF A CEA FRAMEWORK FOR AQUATIC SYSTEMS

2.3.1 The Issue of Scale

According to our definition of cumulative effects, an acceptable methodology for river systems should consider the concept of both time and space. Limited temporal and spatial dimensions generally narrow impact analysis to considerations of single perturbations, simple cause-effect relationships, first-order impacts, immediate effects, a specific environmental attribute and an individual site (Spaling and Smit 1995). To minimize bias, the spatial scale of the assessment should be defined by the spatial scale of the processes (i.e. the industry, land uses) that control the resources of concern (i.e. the catchment or watershed) (MacDonald 2000). On this basis then, a CEA method for rivers should include impacts accumulating along the river continuum as measured over history to the present day. Impacts should also be considered over multiple scales such as reach, catchment, and regional landscape (Schindler 1998). Obviously, this level of assessment (e.g., at the watershed scale) is beyond the capacity of single project proponents to conduct under the existing Canadian EIA process which is apparent in examples below.

Several stressor-based studies have been conducted in the Puget Sound Lowland Ecoregion (Available at: http://www.psat.wa.gov/index.htm). In one of these studies, the effects of urbanization on salmon populations were assessed to establish links between landscape-level condition, and instream habitat characteristics. However, only one measure of watershed urbanization (total impervious surface area) was used, instead of considering the interacting effects of other landscape-scale characteristics (e.g. forest cover, protected parkland, and water surface area). Also a sub-sample of 22 small-stream watersheds was selected over the lower ecoregion to represent the range of urbanization seen over the entire ecoregion. This may not be as accurate as historical data over the entire region to determine the effects of urbanization.

Piper (2000) reviewed the possible cumulative effects of five new proposals that were submitted in one year for developments over 5 km of the north bank of the Humber River (United Kingdom). His approach was to first determine the valued ecosystem components (VEC) (i.e. ecologically important organisms), and then conduct an assessment of cumulative impacts based on the data and schedules for the various developments. The result of this CEA was a series of tables and matrices which included a combined timetable of the required major construction relative to the disturbance potential of the different bird species. This is a widely accepted stressor-based approach which has received numerous validations. However, Piper's use of the approach lacked adequate incorporation of broad time and space components in that it primarily encompassed the immediate area and timeframe of the planned construction projects.

Effects-based assessments are generally conducted over broader spatial and temporal scales than stressor-based assessments (Dubé et al. 2006). Two examples of effects-based CEAs that were previously conducted occurred in the Athabasca River basin, Alberta, Canada are the Northern River Basins Study (NRBS) and its successor, the Northern Rivers Ecosystem Initiative (NREI). These two assessments (approximately 5 years each) were conducted over very large spatial scales and consisted of several independent studies looking at different aspects of the basin (water quality, biological health, hydrology, traditional knowledge and contaminant distribution). At the end of the NRBS, conclusions from each of the independent studies were integrated using a weight-of-evidence approach to provide statements concerning the overall health of the river for use by managers and society (Culp et al. 2000a). Ultimately, the conclusion from each study was qualitatively rated as being an area of concern, caution or minimal concern for five topic areas (dissolved oxygen, nutrient enrichment, hydrologic regime, human health implications and contaminants). The results from the NRBS led into the succeeding study, the NREI. Several independent research studies were again conducted concentrating on priority areas that were identified during the NRBS. A CEA was conducted using decision-support software where both water quality and biological health data from multiple sources were integrated along the river continuum and compared to existing guidelines in a more quantitative manner to assess changes in the river (Dubé et al. 2006). In both of the NRBS and NREI studies, the time period of the CEA was short (5 years each), indicators were not consistently measured across the studies, and there was no mechanism identified during these studies to carry CEAs on at defined intervals into the future.

2.3.2 Establishing a Baseline or Reference Condition

The establishment of a baseline or reference condition is an area of much discussion in CEA science. The main focus of determining a reference area is to provide an indication of what the health of the ecosystem would be in the absence of the stressor in question (Stoddard et al. 2006). Often, the argument is made that there are no such areas that are pristine enough to term "reference" as much of the earth has been touched by human activity (Dubé 2003). This is especially the case when assessing changes on a watershed-scale as it is unlikely to find an entire "reference" watershed that is untouched by human influences.

The most effective method is to conduct sampling in the area of proposed development is previous to any human influence so as to provide for some before-after comparisons in aquatic ecosystem health. However, this type of sampling is typically not possible, especially in larger watershed systems. For example, if human influence has already resulted in a permanent change in the aquatic landscape (e.g. a dam) we must take this into account when establishing a reference condition for a new development in the same area.

There are however, several methods that can be used to establish a reference area to provide baseline conditions even in areas which are under heavy development. The most accepted method is to sample directly upstream from the proposed development or stressor and compare this to downstream of the development or stressor (Dubé 2003). In the case of multiple stressors in this same area, sampling upstream of each stressor and comparing this to downstream of the development under focus will allow for comparisons differentiations between the impacts of each stressor or development.

Historically, a reference condition was referred to as a fixed number to compare the current state of the ecosystem against. However, the term "reference condition" should actually be used to describe the variation or range of ecological indicators in the absence of human influence (Stoddard et al. 2006). If the variation of the impacted area exceeded the variation in the reference area, then it can be considered significantly different from what would be expected and management action can take place.

One such method is the reference-condition approach which required the identification of several "reference" sites. The condition (e.g. biological diversity) at these sites are then averaged and this value used as the reference value in the assessment (Bailey et al. 1998). This method allows for some flexibility in choosing reference areas, however the variability when all the reference areas are averaged is often large and can mask any significant differences between reference and impacted site conditions.

Since reference areas are often impacted in some way by humans, how then can we determine what the pre-development or un-impacted state would be? A common practice in CEA is to use the state of the current environment as the baseline. However, this cannot be considered the most appropriate way to conduct a CEA since doing so makes the effects of past and present actions part of the baseline rather than contributors to the cumulative impacts (McCold and Saulsbury 1996). Instead, it is argued that the most appropriate baseline for considering the significance of cumulative impacts is that time in the past when the valued environmental attribute (or resource) was most abundant or not affected. Assessing changes in a river over a significant period of time provides a better comparison to evaluate if changes have occurred. It also provides the opportunity to relate changes in biophysical indicators to the occurrence of man-made stressors. These relationships are important when developing predictive models (based on past changes), which account for how future man-made developments on the landscape might affect the biophysical attributes of a river (Duinker and Greig 2006).

2.3.3 Appropriate Indicators

One of the major challenges of developing and applying thresholds in a cumulative effects sense is the lack of understanding about the interactions between ecosystem components, which can be very complex (Ziemer 1994). Measuring cumulative effects in a river requires that consistent indicators be evaluated over space and time. It also requires that indicators be selected that are responsive to man-made stressors and representative of the biophysical condition of the river (Munkittrick et al. 2000).

The current standard in EIA practice is to focus on certain indicators that are considered important to communities and stakeholders and are termed valued ecosystem components

(VECs) (Duinker and Greig 2006). In EIA, selection of these VECs are based on both the types of stressors present in the area under study as well as any that are deemed to have societal or ecological importance (Ball 2011). In contrast, VECs which are used in watershed-scale assessments tend to focus more on the effects side of CEA versus the stressors such as in EIA. There is a definite need to standardize a list of VECs for both watershed-scale CEA and project-level EIA in a given watershed so that there is a common link unifying the two scales of assessment (Ball 2011).

2.3.4 Appropriate Thresholds

In addition to the appropriate boundaries and indicators, CEA requires that changes in indicators be measured and the magnitude of the changes evaluated relative to some benchmark of acceptability. However, there are many types of benchmarks such as physical, biological and social (Ziemer 1994). A threshold is defined in Webster's dictionary as "the point at which a physiological or psychological effect begins to be produced". CEA requires development of thresholds to determine the point, at which environmental change has occurred, is close to occurrence, may no longer be acceptable, and may require management action. Thresholds can be degrees of change in an indicator but they also have to be linked to a decision-making process so that if a threshold is approached or exceeded, decision-makers know the action they will take (i.e., adaptive management). Thresholds are most successfully adopted if they are agreed to in advance by parties and if, once triggered, they suggest a management evaluation as opposed to the end of a development.

Many examples of benchmarks exist to measure change in Canadian rivers. However, the scientific community has been hesitant to assign "thresholds" to limit the amount of change that is acceptable for a river exposed to man-made developments. This is largely because the word "threshold" assumes that sufficient scientific knowledge exists to understand when the assimilative capacity of a river has been exceeded and further change will result in unacceptable deterioration. Due to the complexity of biological systems, thresholds for rivers do not exist. However, many benchmarks do exist that can be the first starting point to quantify the level of change in a river over space and time (Dubé 2003; Kennett, 2006; YESAB 2006).

As our understanding of how aquatic and terrestrial ecosystems change and adapt, our thresholds of effect must also change and adapt. A threshold defined for a region must be sensitive to the particular stressors and biota found in that area which can be adaptive to changes in population tolerance over time. This is especially apparent in areas such as the Canadian oil sands in Alberta, Canada where benthic communities in the rivers of this natural oil deposit have been adapting to the presence of this deposit for many years (Whelly 1999) and whose tolerance makes defining a realistic adaptive regional threshold imperative. As our influence on and knowledge of these ecosystems continues, our thresholds and management strategies must be able to adapt in order to provide effective management to protect these areas (Doremus et al. 2011).

The most common stressor-based threshold in ecotoxicology is the LC_{50} (the concentration of a single contaminant at which 50% of the affected organisms die) (Cairns 1992). Other examples of toxicity-based thresholds are the EC_{50} (the concentration at which 50% of the organisms show an effect) the NOEC (no observable effect concentration) and the LOEC (lowest observable effect concentration). Stressor-based thresholds have been extremely valuable to compare the relative toxicity of different chemical contaminants. However, as these thresholds have been developed based on highly controlled laboratory exposures using single contaminants and single species, their relevance for CEA in highly dynamic riverine environments receiving multiple stressors, is unknown. Certainly these measures have some value for understanding chemical stress and when incorporated with field and artificial stream studies have improved environmental relevance (Culp et al. 2000b; Dubé et al. 2002).

Laboratory-based sublethal toxicity thresholds (e.g., EC_{50}) have been applied to Canadian waters in an effort to protect aquatic life. The Canadian Water Quality Guidelines (CWQG) for the protection of freshwater aquatic life are toxicity-based thresholds for different contaminants. Although these guidelines were developed in the lab, they are applied to waters for the most sensitive species of plants and animals and act as science-based benchmarks for the protection of 100% of aquatic species 100% of the time (CCME 2005). However, in the absence of adequate toxicity data, these guidelines may not be the most sensitive if they are not able to account for sublethal effects (e.g. reproduction and growth) which can impact the biological integrity of a basin, even at very low levels. It has also been reported that depending upon the natural habitat of a location, often pristine waters unaffected by human development can exceed CWQG for certain water quality parameters if for example, the waters run through an area of high natural metals. In an effort to improve the development of guidelines to assess changes in waters, other methods have been considered such as summarizing background concentrations of parameters at pristine and undeveloped sites in a region and using the 90th percentile as a site-specific benchmark (Glozier et al. 2004). A major limitation of existing CWQG for the use in CEAs is that guidelines exist for only a small subset of the contaminants affecting waters and sediments.

When considering stressor-based CEA, the integration of landscape drivers as stressors are important. In assessments of whole watersheds, single-contaminant thresholds are often not as applicable due to the large spatial scale of these types of assessments. Instead, assessments on a watershed scale can rely on indicators of landscape development (e.g. road density, population density, forest cover) as stressor-based thresholds. Identifying which key landscape drivers and appropriately assessing their change over space and time are dependent on the study design and the questions being asked (Seitz et al. 2011). Methods which can incorporate these large-scale landscape changes include geographic information systems (GIS) and remote sensing technologies. These methods allow for the establishment of a link between effects on the aquatic environment and stressors on the landscape.

A report released by the Environmental Studies Research Funds showed how using landscape indicators can apply to large-scale assessments of change in the Northwest Territories, Canada (Antoniuk et al. 2009). This report focused on identifying landscape variables that can be linked to the pressures of the oil and gas industry in the north. The authors identified several landscape parameters (total area disturbed, total corridor density, riparian habitat, etc.) that could be linked directly to the land disturbances caused by oil and gas development. A tiered management plan addressing significant change in these indicators was also developed in order to manage any future development in the area.

Effects-based approaches to CEA have developed benchmarks to evaluate change in a river based upon the magnitude, direction, and consistency of changes observed in biological organisms over space and time relative to a lesser impacted site or point in time. Effects-based approaches focus on measuring effects on biological organisms in a river based on the premise that measuring change in the inhabitants of a river is the most direct and relevant (Munkittrick et al. 2000).

In the National Environmental Effects Monitoring (EEM) program, core indicators of biological organisms are measured at reference sites and sites downstream of a contaminant discharge. For benthic invertebrates, a change in the structure of the communities (an indicator of ecosystem structure) is assessed based on four metrics (total invertebrate density, taxon richness, Simpson's diversity index and Bray-Curtis index). For fish populations five indicators are measured at reference and exposed sites (age, condition, liver size, gonad size, and size-at-age) for both sexes of two sentinel species. These indicators represent fundamental biological properties and changes in these indicators are considered to be of importance. These indicators have been successful in identifying significant changes in biological communities exposed to pulp and paper and mine effluents across Canada (Environment Canada 2003).

The National EEM program compares fish and benthic invertebrate endpoints at reference sites and sites exposed to human developments (Environment Canada 2003). If there is a statistically significant difference in endpoints at exposure sites compared to reference sites, then an "effect" as defined under the regulated program has occurred. Furthermore, the EEM program also calculates the percentage difference of an endpoint at an impacted site compared to the reference site to determine the magnitude of the change and if it exceeds a "critical effect size" (Environment Canada 2003). For example, if gonad sizes in the sex of a sentinel fish species are reduced by 25% or more compared to a reference site, then the critical effect size has been exceeded which triggers a specific management action. This is one of the few, if only, examples where benchmarks for biological organisms have been established and used in a regulatory program to measure change in an effects-based context.

In addition to stressor-based and effects-based thresholds, value-based thresholds are the most commonly used in the EIA process. Traditionally, thresholds have not been based on ecological effects, but more commonly on public perceptions of risk (Piper 2002). More recently, some have argued that measurements of environmental change and determining acceptability of those changes are separate processes; one being the role of CEA science and the other a more public, stakeholder-driven process (Dubé and Munkittrick 2001; Dubé 2003).

In summary, many examples of benchmarks exist to evaluate change in Canadian waters and if integrated, could serve as a starting point for application in CEA. Research should be conducted to determine the types of indicators, thresholds and environments most useful for assessing and managing different kinds of cumulative effects (Risser, 1988). The thresholds should consider past, present and future impacts as well as the impacts occurring over different spatial scales (habitat to landscape). These impacts can also be further defined through the use of dose-response studies which can develop site-specific data upon which to base the development of thresholds (Adams 2003).

2.4 A PROPOSED CUMULATIVE EFFECTS ASSESSMENT FRAMEWORK FOR AQUATIC SYSTEMS

Simply expanding the spatial and temporal boundaries of an assessment does not necessarily provide the appropriate approach to regional CEA (Harriman and Noble 2008). As argued, regional CEA cannot be conducted using current EIA processes; instead we must focus on developing strategic methods of conducting these assessments. A proactive approach that attempts to address environmental issues of a cumulative nature is required (Harriman and Noble 2008). To do this it is important to develop and incorporate well-designed specific goals and objectives that will drive the assessment towards a realistic conclusion providing the regulators, industry users and public with functional information.

A CEA framework for riverine systems should include assessments of biophysical changes over space and time as well as an assessment of the drivers or stressors responsible for those changes. An approach that combines effects based and stressor based approaches to CEA that can account for a variety of development pressures is desired (Dubé 2003; Harriman and Noble 2008). While there are currently few frameworks which incorporate strategic regional CEA, we have proposed a framework which can incorporate these assessments in a very goal-orientated way (Figure 2.1). This framework has been applied to a model river basin, the Athabasca River (Squires et al. 2010 (Chapter 3 this thesis); Squires and Dubé 2011 (Chapter 4 this thesis); Squires et al. 2011 (Chapter 5 this thesis)). The following sections will discuss each aspect of the proposed framework and show how it has been applied to the Athabasca River basin.

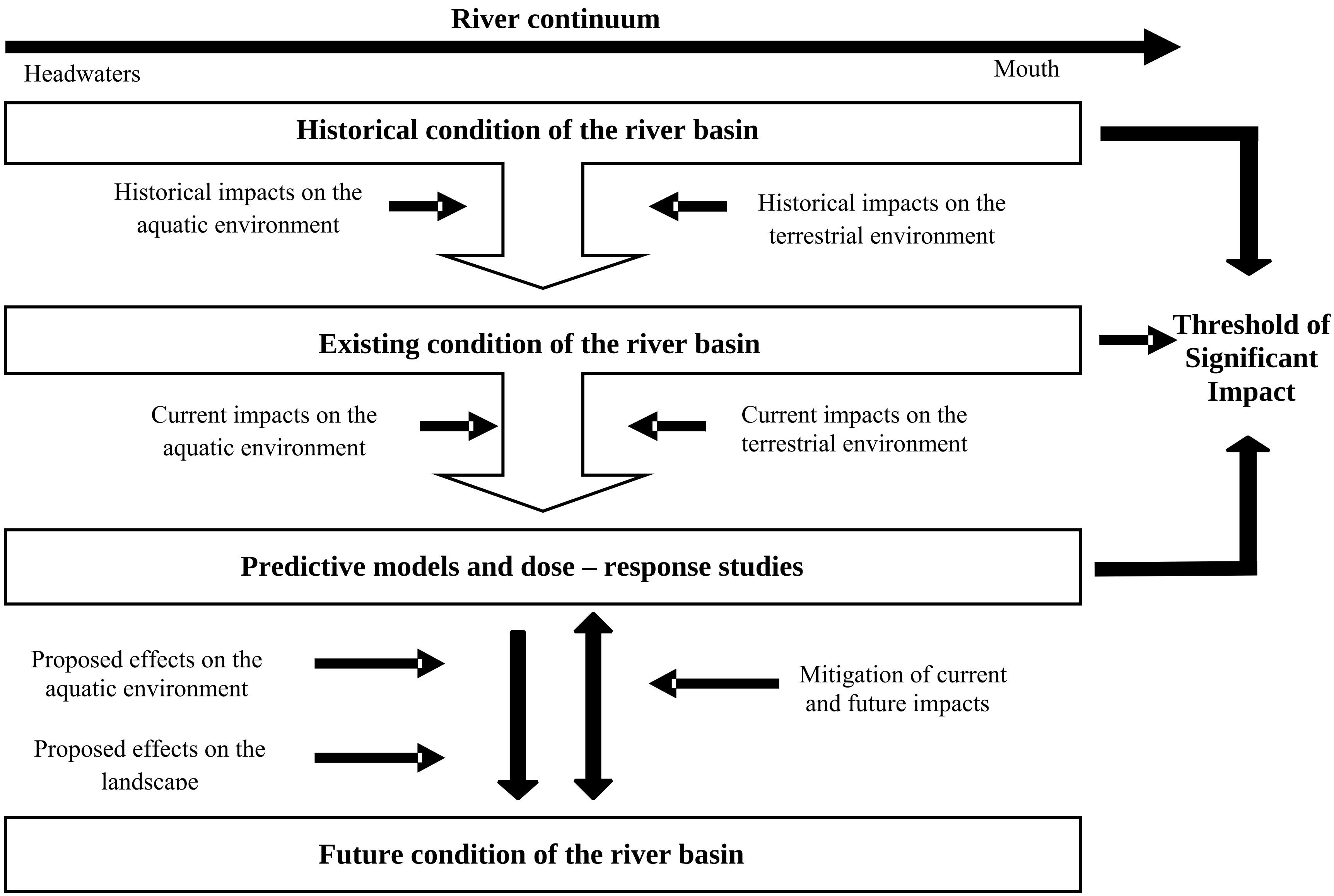

Figure 2.1: A proposed conceptual framework for cumulative effects assessment underscoring the importance of utilizing effects over space and time to predict future impact.

The Athabasca River basin covers 157 000 km^2, accounting for approximately 22% of the landmass of Alberta (Gummer et al. 2000). It originates at the Columbia Ice Fields in Jasper National Park and flows northeast 1300 km across Alberta until it terminates in Lake Athabasca. The Athabasca River is a 6th order stream which flows through four major physiographic provinces: Rocky Mountains, Great Plains, Athabasca Plain and Bear-Slave-Churchill Uplands (Culp et al. 2005). The peak flows and flooding events of this river basin are associated with the snowmelt and approximately 70% of the moisture input into the basin originates from precipitation (Culp et al. 2005). The Athabasca River serves as a good model to develop a methodology for aquatic CEA as it has experienced an increasing level of land use related development including forestry/pulp and paper, coal mining, oil and natural gas, agriculture, tourism, wildlife trapping, hunting and oil sands mining (Wrona et al. 2000; Culp et al. 2005).

2.4.1 Appropriate Temporal and Spatial Boundaries

In the proposed framework, we have identified the entire Athabasca River (headwaters to mouth) as the most appropriate spatial scale to use in these assessments. Using the entire river in the assessment is important in order to better assess the important components (stressors and effects) to focus on in the assessment. Previous assessments of the Athabasca River basin have shown that contaminants discharged in the upper reaches of the river can be found near the mouth of the river, many hundreds of kilometres downstream (NRBS 1996a). Not including the entire river in the assessment prevents the accurate conclusions of stressor effects and limits our ability to effectively manage the watershed.

Including the entire river in an assessment is also vital in quantifying the level of change which can naturally occur in different types of biophysical stressors (water quality and quantity) which are not solely influenced by human activities. The river continuum concept is a fundamental ecological concept that characterizes how a river naturally changes along its length from headwaters to mouth (Vannote et al. 1980). This theory states that in the headwaters, rivers are characterized by high slope, large substrate (mainly from terrestrial leaf litter), clear waters, highly diverse benthic communities (grazers, predators, shredders and collectors). At the mouth, rivers tend to be more turbid with a lower primary productivity, larger width and a less diverse benthic community composition (collectors and predators). Thus there are naturally occurring accumulated changes in biophysical attributes of a river along its length that must be accounted for in a CEA framework. Further, by assessing changes along a river's length, drivers of those changes can be better assessed. While the river continuum concept best applies to unregulated rivers, it also holds value and relevance for regulated systems. While flow regulated systems (i.e., dams) have significantly modified hydrology and ecology (Nilsson et al. 2005) the continuum is still apparent and measurable in terms of biophysical condition (Stanford et al.1996).

Previous assessments of cumulative effects on the Athabasca River have mainly focused on the lower half of the river system (Northern River Basins Study (NRBS) and the Northern River Ecosystem Initiative (NREI)) or in the region of closest proximity to the oil sands (Regional Aquatic Monitoring Program (RAMP)). While focusing on a smaller specific area of a river basin (i.e. a region known to have an increased amount of human development) may make it easier to conduct a more detailed assessment, it does not allow us to accurately incorporate the cumulative effects of human induced or natural changes which occur elsewhere in the watershed and can affect the region under study. These natural changes can include climate change which is becoming an increasingly important variable to consider in these assessments. Rivers that are glacial fed for example are known to be highly sensitive to climate and there is significant regional variability to climate within a watershed system (Johnson and Weaver 2009; Schindler 2001). This variability becomes much more important when trying to quantify the historical or baseline state of the natural system.

For example, in Squires et al. (2010) a significant decrease in water flow was observed in the lower most reach of the Athabasca River relative to historical levels. In addition, a smaller yet also significant decrease in flow also was observed in the upper most reach of this basin, indicating the impacts of climate change are exerting a significant impact on the water flow of the entire basin. If the authors had not also assessed the difference in water flow in these upper reaches (i.e. the headwaters) the assumption would have been that the difference is water flow in the mouth of the basin could be contributed almost exclusively to the human developments in the lower part of the basin. While these developments certainly contribute to the majority of this decrease in flow, they are not the only cause of the problem and the impact of climate requires consideration.

CEA in watersheds must also consider the accumulation of changes over time. Assessing the historical condition of the river basin provides a baseline to determine if the existing condition differs from the past and potential drivers of those changes. The condition of a river changes over time due to historical and current impacts from man-made developments affecting both terrestrial and aquatic systems over multiple scales (reach, stream, catchment and basin).

Determination of what would constitute the "historical" condition should be based on: 1) the availability of data for the area under study and 2) the presence of human-induced stressors in the area and when they commenced. Based on these criteria, the historical condition used in this assessment of the Athabasca River was deemed to be between the years 1966-1976. Prior to this time period, there is only one major industrial development on the river mainstem (Hinton pulp mill which opened in 1955). Also, there is still enough water quality and quantity data available along the entire river to provide for a reliable quantitative assessment of change. The existing or current condition time period (1996-2006) was based on the most recent data available at the time of the assessment.

2.4.2 Quantitative Measures of Change-Historical and Existing Conditions

Evaluation of indicators (water quality parameters, sediment quality parameters, indicators of biological populations of communities) along the river continuum over time can reveal those that have changed and the magnitude of the change. Correlations between these indicators and man-made stressors (e.g., changes in landuse, loadings of point sources, loadings of nutrients) can identify potential drivers (stressors) of the change. It is important to have a quantitative method to measure these changes over larger time and space scales when conducting a watershed scale CEA. To do this, we must not only identify the appropriate time and space scales, but we must also determine which indicators best represent the health of the ecosystem and would provide us with the best indication of change. These indicators can be biophysical (water quality, water quantity, climate) or biological depending on the available data for the region and the particular stressors present in the area over the time of the assessment.

When matching stressors to responses at the appropriate scale, it is critical to consider space and time lags. In situations where effects are not seen until decades after a particular disturbance (e.g. climate change) or are still observed several reaches downstream of a disturbance (e.g. nutrient loading) the concept of space and time lags becomes even more important (Fenton et al. 2011; Ziemer 1994; Reid 1998). For example, recent observations of marine reserves have shown that certain indirect effects on many species (resulting from direct effects on targeted taxa through trophic interactions) can experience time lags that are decades long (Babcock et al. 2010). Therefore it is crucial that the spatial and temporal extent of the assessment match the stressors under consideration.

In Squires et al. (2010) such measures of change were identified using data across a historical time period (1966-1976) and compared to the current time period (1996-2006). For the Athabasca River, data were obtained and compiled from 5 different sources and amounted to over 5 million data points (Squires et al. 2010). Despite this, the indicators selected for assessment of change were constrained on data availability due to the reliability of the data (frequency of collection and the changes in analytical methods of analyses over time). Consequently due to the lack of biological data available during these time periods in the basin, biophysical endpoints were selected and assessed for significant changes across both time periods from headwaters to mouth.

Based on the examination of available data at several water quantity and quality stations along the Athabasca River and the locations of several urban, agricultural and industrial inputs, the basin was divided into six reaches (Figure 2.2). For each reach, mean weighted averages and standard errors were calculated for each parameter (water quality variables and flow) across each time period to assess the differences in trends between periods. Water quality parameters selected for further analyses were total organic carbon (TOC), dissolved nitrogen (DN), turbidity, total phosphorous (TP), conductivity, dissolved chloride (Cl), dissolved sodium (Na) and dissolved sulphate (SO_4). These parameters were selected not only because data coverage met the spatial and temporal boundaries selected for the cumulative effects assessment in the basin, but also because they are either potentially influenced by industrial developments in the basin or by the natural change along the river continuum. One discharge (flow) data station at the most downstream point of each reach was selected to represent the flow for that entire reach. These data were analyzed on a monthly time step for each reach over each time period.

Complete results from this analysis are available in Squires et al. (2010) (Chapter 3 this thesis). The water quality and quantity parameters which showed the greatest change are summarized here (Figure 2.3). There were significant decreases in flow in the headwaters, Athabasca and mouth reaches in the Athabasca River in the current day (1996-2006) time period when compared to levels in the historical time period (1966-1976) ($p<0.05$). A 14-30% decrease in discharge was observed during the low flow period in the second time period in the lower three river reaches with the greatest decrease occurring at the mouth of the river.

The water quality parameters which showed significant increases in the current day time period across various reaches included dissolved sodium, dissolved chloride, dissolved sulphate, total organic carbon as well as dissolved phosphorous and nitrogen ($p<0.05$). While all of these parameters have shown significant treads, dissolved chloride and sodium stood out as parameters warranting further investigation since concentrations were in the top 10% of concentrations observed 30 years ago (Squires and Dubé, 2011 (Chapter 4 this thesis)).

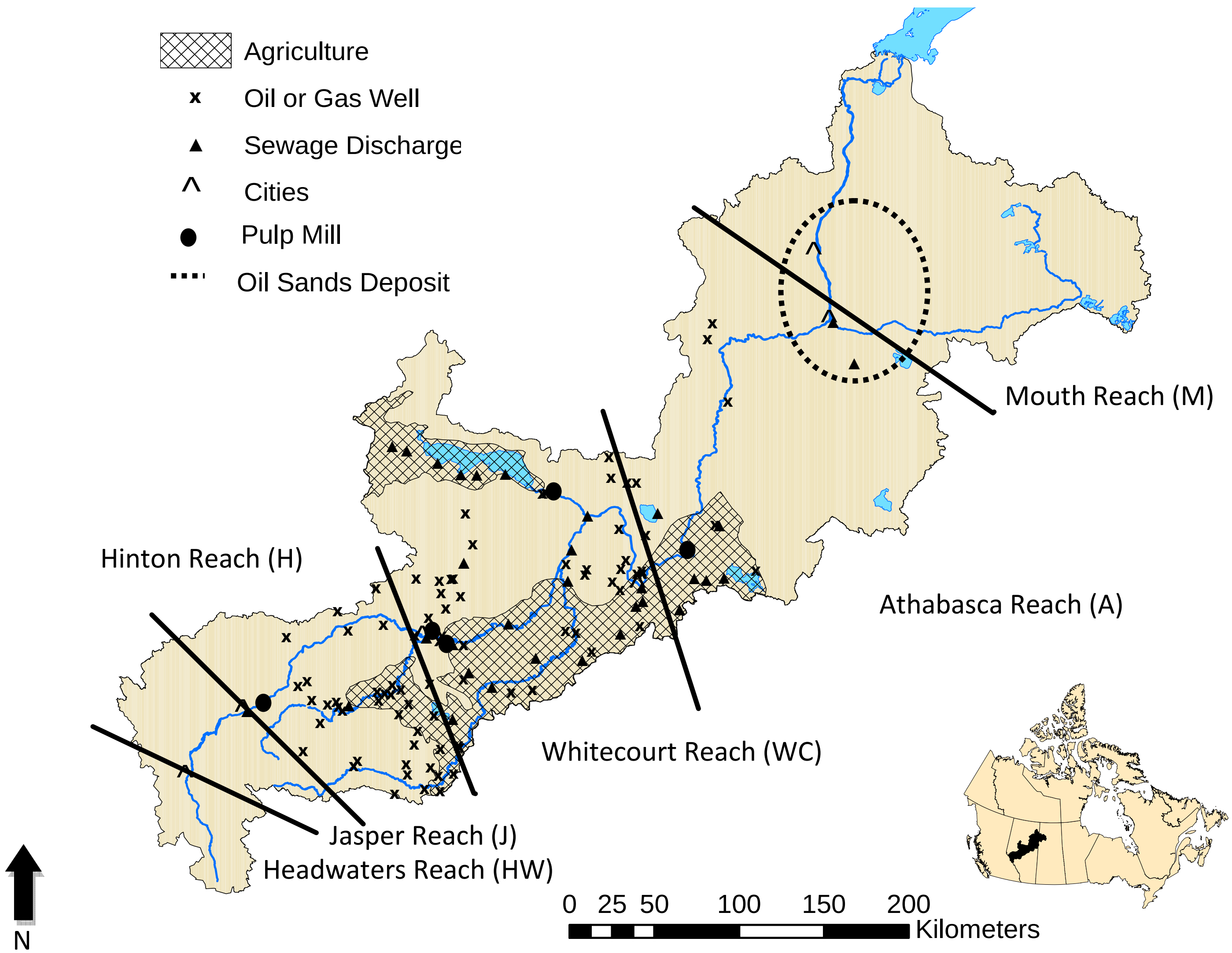

Figure 2.2: Map of the Athabasca River basin showing locations of agricultural, municipal and industrial development. (Adapted from Squires et al. 2010).

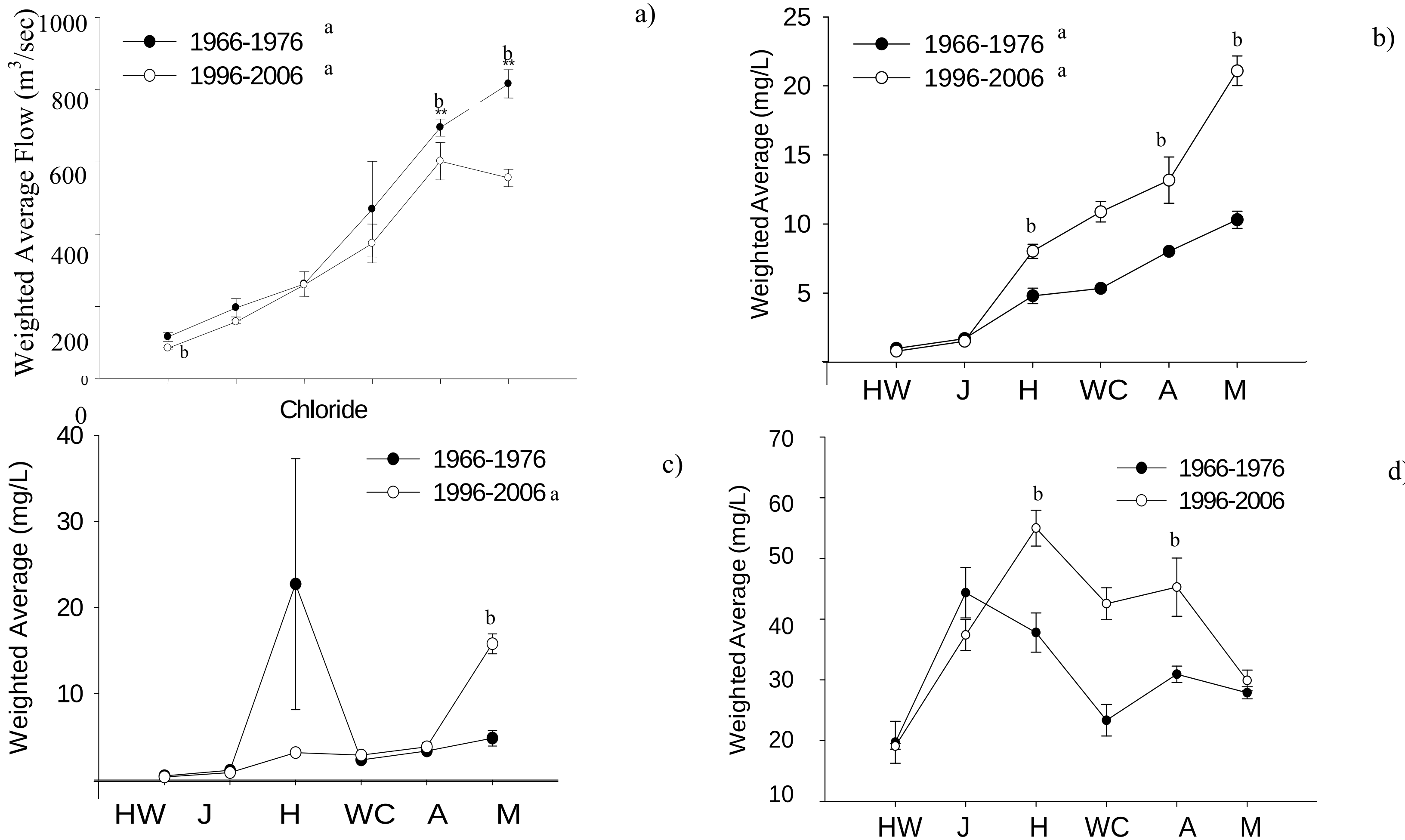

Figure 2.3: Weighted average (± SE) at stations along the Athabasca River continuum across two time periods historical (1966-1976) and current day (1996-2006) for a) Flow b) Sodium c) Chloride and d) sulphate. Reach names are abbreviated as: HW=Headwaters, J=Jasper, H=Hinton, W=Whitecourt, A=Athabasca, M=Mouth. Statistical differences across reaches were assessed using the nonparametric Mann-Kendall trend test and to test between the two time periods the nonparametric Kruskal-Wallis unpaired t-test was performed. For each weighted average parameter, [a] in each legend indicates a significant trend across reaches ($p<0.05$); [b] for a reach denotes a significant difference between time periods in that parameter at that reach ($p<0.05$). Adapted from Squires et al. 2010.

2.4.3 Predictive Models/Dose Response Studies

Another important feature of this conceptual model is that it incorporates the development and implementation of thresholds that quantify the significance of an impact. There is a definite need for research to develop thresholds which encompass meaningful time and space scales (Duniker and Greig 2006). The historical and current conditions of the basin must be incorporated into the threshold development as thresholds may move over time as populations adapt and become more resistant to certain impacts, especially on a chronic level. Dose-response studies will help to better quantify these thresholds by establishing a pattern of effect for single and combinations of stressors (water quality and landscape) over multiple trophic levels.

Squires and Dubé (2011) (Chapter 4 this thesis) and Squires et al. (2011) (Chapter 5 this thesis) conducted several dose-response experiments in both the laboratory and at the headwaters of the Athabasca River using stressors identified to be of concern in the quantitative assessment of change between the historical and current conditions. These were identified to be water quality parameters which showed significant change across space (between the different reaches across the entire river) and across time (between the historical and current day time periods). Parameters selected for these studies included dissolved sodium, dissolved chloride and dissolved sulphate (Squires and Dubé 2011 (Chapter 4 this thesis); Squires et. al., 2011 (Chapter 5 this thesis)).

To develop thresholds for these parameters which are relevant to the Athabasca River, we must establish at what levels we can see effects on the populations of aquatic organisms in the river. To do this, the partial life-cycle FHM assay was performed. These experiments were based on partial life cycle tests originally developed by Ankley et al. (2001) and further refined by Rickwood (2006). This assay allows us to assess the reproduction of FHMs, as well as aspects of their early development in a time frame much shorter than a traditional life cycle bioassay. The maximum concentrations of dissolved chloride and sodium across the years 1996-2006 as was assessed in the previous trend study by Squires et al. (2010) were used as the most concentrated (100%) treatment.

Using this assay the concentrations at which we start to see a detrimental effect on the reproductive output can be established. These levels can be useful when setting thresholds for these parameters within the Athabasca River for use in future cumulative effects assessments assuming we understand how development activities increase concentrations of these parameters. To expose the FHMs to these parameters the use of diluter systems which can dispense an accurate amount of six different concentrations (0%, 6.25%, 12.5%, 25%, 50% and 100%) of a certain parameter(s) were employed. An additional 10-fold concentration (10-fold higher than the 100% concentration used) was added as a potential "worst-case" scenario.

Results from these laboratory studies are discussed in Squires and Dubé (2011) (Chapter 4 this thesis) and only a brief summary are presented here. Significant increases in cumulative egg production occurred at the 25% treatment for dissolved sodium. However all dissolved chloride treatments showed significant decreases in cumulative egg production compared to the control. Using the percent change from control for total eggs/female/day we found that the ideal range of dissolved sodium is between the 12.5% (36.11 mg/L) and 50% (57.00 mg/L) and dissolved chloride between 22.22 mg/L and 49.56 mg/L. At levels outside or above these ranges a possible decrease in reproductive output may occur.

Squires et al. (2011) (Chapter 5 this thesis) performed similar experiments at the headwaters of the Athabasca River mainstem in Jasper National Park, Jasper, Alberta, Canada using the The Healthy River Ecosystem Assessment System (THREATS) trailer mobile laboratory system. The parameters used for these experiments included a mixed sodium chloride treatment, a dissolved sulphate treatment and a treatment consisting of water sampled immediately downstream of the major oil sands developments in the mouth of the Athabasca River basin. In addition, the control/dilution water was taken directly from the headwaters of the Athabasca River, providing for a realistic and relevant assessment of the background reproductive potential relative to the increased concentrations of these parameters.

It is important to conduct these experiments using water sampled directly from the Athabasca River in order to verify the potential reproductive effects as were seen in the laboratory studies. The concentrations of the parameters selected for these field experiments were the average concentrations as assessed in Squires et al. (2010) during the current time period (1996-2006). Therefore they were much lower than the concentrations which were selected for use in the laboratory experiments, which were based on the maximum concentrations. This provided the opportunity for assessment of the potential for the existing effects these parameters can have on fish reproduction along the Athabasca river mainstem.

In the dissolved sulphate experiment, the treatments which had the lowest reproductive output were the 6.25% (18.57 mg/L) and the 12.5% (23.33 mg/L) treatments. Current guidelines also state that dissolved sulphate should not exceed 100 mg/L (Elphick et al. 2010). Based on this information, the ideal amount of dissolved sulphate for FHM reproduction was determined to between 23.33 mg/L and 100 mg/L. In the sodium chloride experiment the greatest increases in reproductive output occurred in the highest treatment (25.43 mg/L Na and 38.90 mg/L Cl). This was very similar to the range identified in the laboratory study for dissolved sodium of between 36.11 and 57.00 mg/L and dissolved chloride between 22.22 mg/L and 49.56 mg/L (Squires and Dubé, 2011). Therefore based on these two experiments, the ideal range of sodium should be between 25.43 mg/L and 57.00 mg/L and dissolved chloride should be between 22.22 mg/L and 49.56 mg/L.

Comparing these thresholds developed in both the laboratory and field studies as well as the trend analysis performed on the historical and current day data available we now have a link between these stressors and their potential to impact the aquatic biota in the Athabasca River basin. The next step of this process is to provide a link between the effects of these particular stressors and landscape development in the watershed. This link is essential in order to provide managers with enough information to effectively mitigate any current and future impacts on the Athabasca River basin which may contribute to the concentration load of these specific parameters.

2.4.4 Mitigation of Current and Future Impacts

Aquatic systems are shaped by environmental factors that operate at a variety of spatial and temporal scales (Wang et al. 2003). There is a great deal of interest in developing predictive models which link landscape scale variables (stressors) to the water quality and biota of the ecosystem (effects) in question (Leuven and Poudevigne 2002; Gergel et al. 2002; Allan 2004). Consequently, an understanding of how environmental factors at this scale can affect aquatic ecosystems is essential if management, prevention and restoration activities are to progress effectively. The final component of this framework is to develop links between the stressors identified to be of concern and have shown to have potential negative effects on aquatic biota in the basin (Na, Cl, SO_4) to any current or future development through the use of landscape drivers. This last step effectively integrates the effects-based and stressor-based CEA processes by incorporating assessments of the conditions of the basin (effects) with the future and historical impacts on the basin (stressor).

Alternations to the landscape (agriculture, deforestation, urbanization) can influence aquatic systems by increasing sediment delivery to streams, altering hydrologic and thermal regimes and influencing water chemistry parameters (Argent and Carline 2004). Methods which can link these landscape alterations (stressors) to their eventual biological effects is an area undergoing much development in recent years. Part of the difficulty in establishing these linkages arises from the necessity of using comparable spatial and temporal scales which can sometimes prove challenging when conducting assessments at these larger watershed scales (Pan et al. 2004).

The overall goal in these landscape assessments is to produce a predictive, quantitative model which has value to stream management efforts (Van Sickle et al. 2004). The decision on what landscape variables to focus on and over what time scale is individual to the system under study. However they should be relatively easy to measure and have a clear link to the stressors already identified to be of concern to the impacted biological communities. In addition, the models produced from these variables are most effective when they are relatively simple and easy to use (Jin et al. 2011).

In the Athabasca River basin, previous assessments have shown salinity (Na and Cl) to be a water quality characteristic that has shown significant change over large space and time scales (Squires et al. 2010). Additional research has produced thresholds for these parameters (Squires and Dubé 2011 (Chapter 4 this thesis), Squires et al. 2011 (Chapter 5 this thesis)). In order to effectively mitigate the influence of salinity in the Athabasca River a relationship between landscape change (i.e. industrial development) and increasing salinity must be made.

Research on the use of road salt and their affect on aquatic biota in watersheds have produced several landscape models for watershed managers (Kelly et al. 2008 and Howard and Maier 2007). The most recent of these aims to give accurate estimate of salinity variations based on various input sources (Jin et al. 2011). The model utilized many characteristics including precipitation, groundwater discharge, domestic water softener and road salt usage as well as several land use classifications (% urban, %forest, %short vegetation, %arable). Jin et al. (2011) authors produced a model which accurately predicted both past and current chloride levels in a model watershed. The results of this model demonstrated the possibility of producing an effective predictive tool for watershed managers to mitigate the impacts of salinity on a watershed scale.

2.5 LIMITATIONS OF THE FRAMEWORK

The major limitation of this conceptual model is that it requires significant amounts of data, spanning over a large time period (historical to current) (Squires et al. 2010). For this reason, when applying the framework to the Athabasca River basin we were limited to using water quality and quantity data as the main indicators due to limitations in the availability of complete biological (fish and benthos) and landscape data. This problem will affect most river basins where long-term monitoring stations are not well maintained, sampled frequent enough or do not exist at all. It is important for regulatory bodies to improve our monitoring programs in Canada especially in basins (such as the Athabasca) that are experiencing a significant amount of industrial and urban growth.

Our ability to conduct dose-response threshold studies which are specific to a certain river basin or other aquatic ecosystem is also limited. Each FHM partial life-cycle test is time consuming (28-days) and potentially expensive to perform. A proposed alternative to these types of assays would be to perform a standard invertebrate test such as are done with *Daphnia magna* and *Chironomus dilutus*. These typically require 48 hours to complete and are relatively inexpensive to perform. These types of tests are not chronic in nature and are generally not the best indicator for the overall reproductive potential of the higher trophic levels. While it is universally acknowledged that the longer-term chronic higher-trophic level assays (as were conducted as part of this framework) are more relevant when establishing thresholds, these short-term tests can provide a useful quick preliminary assessment when time and funds are limited.

A significant hindrance to applying this model is the need for leadership in conducting these types of large-scale regional assessments. Currently in Canada there is no regulatory umbrella under which these assessments can take place. The current practice is for a company wanting to develop in a watershed is to produce their own environmental impact assessment (EIA) that focuses on the single development seeking approval. These types of assessments do not provide the necessary expertise, resources and leadership to assess the potential for cumulative impacts to occur in the entire watershed. Therefore, in order for watershed-scale cumulative effects assessment to be effective it must be regulated and authorities (federal and/or provincial governments) who have access to the amount of data and expertise required must take on a leadership role to ensure its successful implementation.

2.6 CONCLUSIONS AND RECOMMENDATIONS

The assessment of the historical and existing conditions of the river basin contributes knowledge about the trends in both space and time for water quality parameters across the entire Athabasca River basin. No study previous to this has attempted to isolate the important water quality variables which have shown significant change across such large time (40 years) and space (entire river mainstem) scales. In addition, this study has demonstrated the link between these water quality parameters and their potential adverse effects of fish reproduction which is important to population dynamics and may drive the biological condition across the basin.

For an assessment to be useful, it must feed back into the regulatory process providing managers with a guide for future assessments and mitigation measures (Foden at al. 2008). To do this it is vital to conduct assessments that can develop thresholds which are specific to the region or watershed under study. Thresholds on a national scale are either too broad or too restrictive and in many cases cannot be realistically enforced in specific regions. The thresholds developed as part of this framework are ideal since they are not specific to an industry like they are in project-based assessments but are specific to the region where they have been shown to change over space and time.

The outcome of this framework ultimately aims to quantify and identify the dominant driving stressors and the corresponding major response patterns on the biota across an entire watershed. The end result will address the need for site-specific *in situ* cumulative effects thresholds in Canadian aquatic environments while developing an approach to assess the cumulative effects of pollutants on rivers. The successful implementation of this framework on the Athabasca River basin shows it's potential to be applied to other river basins worldwide.

CHAPTER 3: AN APPROACH FOR ASSESSING CUMULATIVE EFFECTS IN A MODEL RIVER, THE ATHABASCA RIVER BASIN

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3.0 INTRODUCTION

Cumulative effects assessment (CEA) is the process of systematically assessing impacts resulting from incremental, accumulating and interacting stressors and is regulated under the Canadian Environmental Assessment Act (Reid 1993; Dubé et al. 2004; Duinker and Greig 2006). The original context of CEA methods were causal or stressor-based focused and are specific to a proposed new development project (Spaling and Smit 1995). It has been recognized that there is a need to shift from these local, project scale CEAs to broader, landscape or regional scale assessments to accurately assess cumulative effects across an entire river ecosystem (Duinker and Greig 2006). However, there is not currently a mechanism for this to occur under the current regulatory process.

Effects-based methods for Canadian CEA have developed largely through watershed studies conducted by university and government researchers and focus first on measuring changes in the aquatic environment (i.e. determining the existing environmental state) over a broader spatial scale and second, on determining the cause or stressor of the effect if effects are measured (Dubé 2003). This approach is founded on the premise that if the "performance" or "health" of the environment is affected by the cumulative insults of man-made activities, then mitigation is required, and any new project proposals must ensure development activities do not affect the environment further. Examples of effects-based CEAs include the Moose River basin study (Munkittrick et al. 2000), and studies under the Northern Rivers basin Study (Culp et al. 2000c), and the Northern Rivers Ecosystem Initiative (Dubé et al. 2006). Although effects-based approaches are conducted at the appropriate scale for CEA and are effective for determining the "health" of a system, development of predictive models to understand how the system may respond to future development pressures has not occurred.

Much of the confusion in assessing cumulative effects on aquatic ecosystems is due to poorly defining the resources of concern and the spatial and temporal scales of the analysis (MacDonald, 2000). Limited temporal and spatial dimensions generally narrow impact analysis to inclusion of immediate effects of a specific environmental attribute at an individual site (Spaling and Smit, 1995). To minimize bias, the spatial scale of the assessment should be defined by the spatial scale of the processes (i.e. the industry, land uses) that affect the resources of concern (i.e. the catchment or watershed) (MacDonald, 2000). Impacts attributed to these processes should also be considered over multiple scales such as reach, catchment, and regional landscape (Schindler, 1998). In addition, the river continuum concept is a fundamental ecological concept that characterizes how a river naturally changes along its length from headwaters to mouth (Vannote et al. 1980). These accumulated changes in biophysical attributes of a river along its length must also be accounted for in a cumulative effects assessment (CEA) framework.

A common practice in CEA is to use the state of the current environment as the baseline. This cannot be considered the most appropriate way to assess multiple impacts since this assumes no changes have occurred to date and amalgamates the effects of past and present actions of individual contributors into an accumulated baseline (McCold and Saulsbury, 1996). Instead, it can be argued that a more appropriate baseline for considering the significance of cumulative impacts is that time in the past when the valued environmental attribute (or resource) was most abundant or less affected. Assessing changes in a river over a significant period of time provides an improved baseline from which to evaluate if changes have occurred. It also provides the opportunity to relate changes in biophysical indicators such as water quality parameters, sediment quality parameters and indicators of biological populations or communities to the occurrence of man-made stressors. On this basis then, a CEA method for rivers should include impacts accumulating along the river continuum as measured over history to the present day.

In addition to the appropriate boundaries and indicators, CEA requires that changes in indicators be measured and the magnitude of the change evaluated relative to some threshold value. Thresholds represent a degree of change in an indicator that is linked to a decision-making process. In this context, if a threshold is approached or exceeded, decision-makers know the action they will take (i.e., adaptive management). Due to the complexity of biological systems, thresholds for rivers do not exist. However, many benchmarks do exist that can be the first starting point to quantify the level of change in a river over space and time (Dubé, 2003; Kennett, 2006; YESAB, 2006 Kilgour et al. 2007).

Cumulative effects should not be assumed to be defined exclusively as the combined effects of a number of parameters. A cumulative effect can also occur for a single parameter along a large spatial extent or long temporal scale influenced by multiple stressors affecting a common response. This is especially true when looking at concentrations of specific water quality parameters along a river continuum. The inputs of multiple sources can contribute significant loadings of certain water quality parameters along the river mainstem (nutrients, organic carbon, salinity), resulting in substantially higher concentrations than otherwise would occur in the mouth of these systems.

One way of quantifying these inputs is to calculate the Total Maximum Daily Loads (TMDL) (USEPA, 2009a). TMDLs are specific to one pollutant in a single waterbody and are able to account for seasonality and both point and non-point sources. The main purpose of TMDLs is to be able to calculate the maximum load of a specific parameter in a waterbody so that point source contributions can be better managed. One of the main modelling systems frequently used to calculate TMDLs is the Better Assessment Science Integrating Point and Nonpoint Sources (BASINS) program (USEPA, 2009b). BASINS is an interactive, freely available GIS based software which integrates data, applies existing assessment and planning tools and water quality modelling software which attempts to quantify the inputs of water parameters in a river system. The main purpose of BASINS is to assist in watershed management and TMDL development by 1) characterizing water quality data; 2) identifying pollution sources and 3) allocating loadings (USEPA, 2009b). BASINS is specialized software which focuses on water quantity and quality data needed to calculated TMDLs and does not calculate other types of guidelines or benchmarks which may be required in a watershed-based CEA.

Despite the global acknowledgement that cumulative effects are an expanding issue that must be addressed (Schindler, 1998; Duinker and Greig, 2006), there is currently not a single conceptual approach or even several general principles which are widely accepted by scientists and managers. Therefore methods that can assess these multiple types of effects over a broad spatial and time scale must be developed. The main objectives of this paper are to 1) quantify spatial and temporal changes in water quantity and quality over space (along the river continuum) and time (historical and present day) in our model river, and 2) to evaluate the significance of any changes relative to existing benchmarks (e.g. water quality guidelines).

The Athabasca River in Alberta, Canada serves as a good model to develop a methodology for aquatic CEA as it has experienced an increasing level of land use development over the past decades including forestry/pulp and paper, coal mining, oil and natural gas, agriculture, tourism, wildlife trapping, hunting and oil sands mining (Wrona et al. 2000; Culp et al. 2005). The basin holds significant cultural and economic importance supporting more than nine First Nation groups, and providing water to hundreds of industries, including the multi-billion dollar oil sands mining industry.

3.1 METHODS

3.1.1 Athabasca River basin as a Model River

The Athabasca River basin covers 157 000 km^2, accounting for approximately 22% of the landmass of Alberta (Gummer et al. 2000) (Figure 3.1). It originates at the Columbia Ice Fields, the largest ice field in the North American Rocky Mountains, located in Jasper National Park and flows northeast across Alberta until it reaches Lake Athabasca. Running more than 1538 km, the Athabasca is the longest river wholly contained within Alberta and the longest unregulated river in the prairies.

To date, there have been three CEAs for parts of the Athabasca River basin including the Northern River Basins Study, the Northern River Ecosystem Initiative and the Regional Aquatic Monitoring Program. The Northern River Basins Study and the Northern River Ecosystem Initiative were a series of research studies examining various aspects of water and biota quality over a total of 10 years. CEAs were conducted at the end of the program and consisted of a qualitative synthesis of conclusions from various researchers (Culp et al. 2000c; Dubé et al. 2006). The Regional Aquatic Monitoring Program is specific to the oil sands industry and operates over that development region for the longer term. These CEAs focus on specific portions of the Athabasca River basin, but as of yet, no attempt has been made to assess cumulative effects from headwaters to mouth (Lawe et al. 2005).

The major land-use changes between time period one and time period 2 are outlined in Table 1.1. Point source sewage discharges to the Athabasca River occur on a continuous basis from five larger communities (all with a population >5000), including the Town of Hinton, which has a contract to have its sewage treated and discharged along with the Weldwood of Canada Ltd. pulp mill (Scrimgeour and Chambers, 2000). All of these facilities currently use a minimum of secondary effluent treatment with 1 of 5 practicing tertiary treatment for nutrient removal. In 1993, sewage discharge to the Athabasca River from these facilities (excluding Hinton) totalled 25 046 m^3 (Chambers et al. 2000).

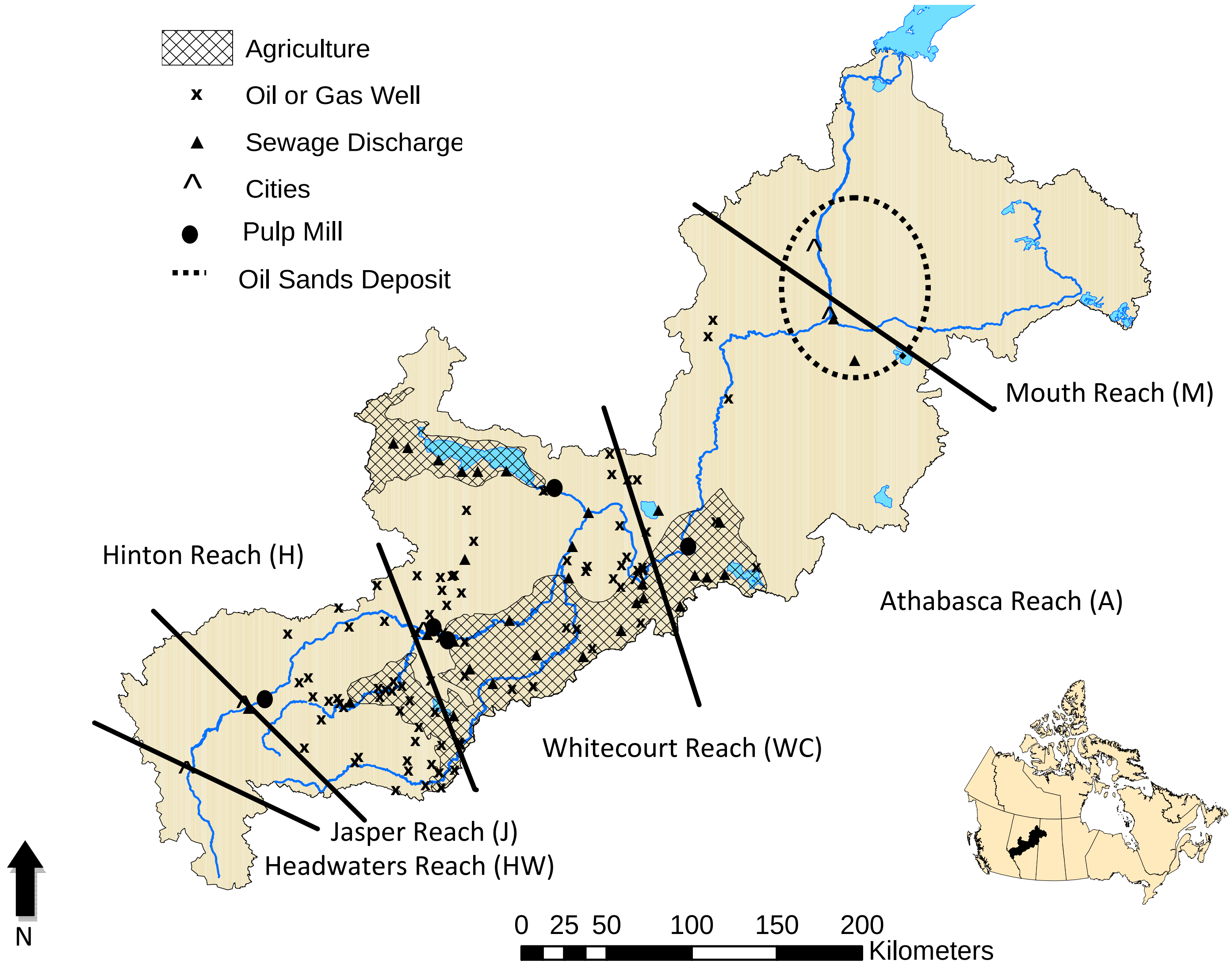


Figure 3.1: Map of the Athabasca River Basin showing locations of the current major industries and urban centres.

There are two bleached kraft pulp mills and three chemi-thermomechanical pulp mills which currently discharge into the Athabasca River and its tributaries (Figure 3.1). The first pulp mill opened in 1957, discharges into the town of Hinton near the headwaters of the river, and is currently operated by Weldwood of Canada Ltd. The next longest operating mill (Miller Western Pulp) opened in 1988, with the remaining three (Alberta Newsprint Co., Slave Lake Pulp and Alberta Pacific Forest Industries) opening in the early 1990s. The combined discharge of these pulp mills in 1993 (including the combined discharge of Weldwood and the town of Hinton) to the Athabasca River basin was 206 658 m^3 (Chambers et al. 2000).

One of the major industries in the lower Athabasca River is the oil sands operations located north of the town of Fort McMurray (Table 3.1, Figure 3.1). The two largest surface mining companies are Syncrude Canada Ltd. and Suncor Energy Inc. and they have been extracting oil sands from this region since 1978 and 1967 respectively. Current mining processes require 2 to 4.5 barrels of water to produce one barrel of oil (Schindler et al. 2007). The oil sands operations must follow a zero discharge policy in regards to their tailings effluent, and consequently these must be stored on site in massive tailings ponds that currently cover over 50 km^2. However there is a recorded discharge of treated utility water from Suncor Energy Inc. of approximately 35 020 m^3/day, amounting to 0.05% of local annual stream flow (Scrimgeour and Chambers, 2000). Existing oil sands mining operations (including the newest Albian Sands operation) are licensed to divert 349 million m^3 of water per year from the Athabasca River with less than 10% of this being returned.

In addition to these major industries, there is an abundance of agricultural and urban development occurring throughout the basin (Wrona et al. 2000). Agriculture consists primarily of forage crops, located in the central portion of the basin. There are more than 200 populated centres with greater than 2 000 people. The total population of the basin is currently 151 750 or 0.96/km^2 when population is divided by the total area of the watershed.

Table 3.1: Land-use changes between the historical (1966-1976) and current (1996-2006) time points for the Athabasca River basin.

Land-Use	1966-1976	1996-2006
Number of pulp mills discharging into basin	1	5
Population[1]	126 345 (1981)	151 750 (2001)
Total Farm Area (Acres)[1]	47 218 170 (1981)	52 058 898 (2001)
Water Withdrawals (m^3/year)[2]	12 069 340	595 580 497
Number of operating oil sands leases[3]	2	3 360
Fertilizer Use (number of acres)[1]	not available	957 651 (2000)

[1] Statistics Canada (2007)
[2] Available through Alberta Environment
[3] Available through Alberta Energy Oil Sands Development Division

3.1.2 Available Data

Cumulative effects assessment requires a significant amount of data with coverage over broad spatial and temporal scales. These are not the type of data available from a single researcher. Consequently, water quality data for this study were collected from several sources including both the provincial (Alberta Environment) and federal (Environment Canada) governments and industrial (Regional Aquatic Monitoring Program for the oil sands) sources. Data from three primary water quality databases covering a fifty year period were examined including over 1,800 variables and 4.5 million sample values. Examination and integration of this multi-source data was facilitated using The Healthy River Ecosystem Assessment System (THREATS) software (Dr. M. Dubé, University of Saskatchewan, Saskatoon, SK, Canada).

All water quality data used in this study were checked for errors and omissions both by the organizations providing the data (Environment Canada and Alberta Environment), and by the authors. Data obtained from Environment Canada are reviewed according to internal protocols and released to the public only after this process is complete. Alberta Environment data are reviewed and validated following Alberta Environment's surface water data validation process involving review by field staff, the project limnologist and finally the data management staff who play an audit role.

Based on the examination of available data at several water quantity and quality stations along the Athabasca River and the locations of several urban, agricultural and industrial inputs, the basin was divided into six reaches (Figure 3.1). For each reach, water quantity and water quality parameters were graphed over two time periods; historical (1966-1976) and current (1996-2006) to assess the differences in trends between periods. The rationale for choosing these two periods is based on both the availability of data and on the start up dates of several major industrial operations including both pulp mill and oil sands developments. Despite the examination of almost 5 million data points, the number of water quality variables available for examination over our selected temporal and spatial boundaries was limited due to the frequency of collection and the changes in analytical methods of analyses over time. There existed many gaps of years where data were collected infrequently or not at all from many of the stations. Despite choosing those stations with the best coverage of data across all years, in some instances there were few data points available for analyses. The data used are described in more detail in Table 3.2. Water quality parameters selected for further analyses were total organic carbon (TOC), dissolved nitrogen (DN), turbidity, total phosphorous (TP), conductivity, dissolved chloride (Cl), dissolved sodium (Na) and dissolved sulphate (SO_4). These parameters were selected because data coverage met the spatial and temporal boundaries selected for the cumulative effects assessment in the basin. In addition, the parameters were potentially influenced by industry (Na, SO_4 and Cl) and were hypothesized to change naturally along the river continuum (conductivity, turbidity, TOC, DN and TP).

Discharge (flow) data were obtained from Canada's national archive for water quantity data (HYDAT), managed by the Water Survey of Canada. Seven HYDAT stations monitor flow in the Athabasca River basin; five of these provide real-time current data. We selected one station at the most downstream point of each reach to represent the flow for that entire reach. These data were analyzed on a monthly time step for each reach over each time period.

Table 3.2: Numbers of samples (high flow/low flow) used to calculate a weighted average for each reach in water quantity (Figure 3.3) and quality (Figure 3.7) analyses for both the historical (1966-1976) and current (1996-2006) time periods for the Athabasca River basin.

Reach	Time Period	Monthly Range	TOC	Dissolved Chloride	Dissolved Nitrogen	Total Phosphorous	Conductivity	Dissolved Sulphate	Dissolved Sodium	Turbidity
Headwaters	1966-1976	Jan-Dec	60/103	59/104	91/117	81/112	----	62/103	59/103	104/121
Jasper	1966-1976	Jan-Dec	44/90	68/163	119/185	95/143	163/195	68/162	69/152	136/189
Hinton	1966-1976	Jan-Dec	4/5	26/54	23/54	7/12	26/31	21/29	26/46	24/54
Whitecourt	1966-1976	Jan-Aug	1/2	1/2	1/2	1/2	3/4	1/2	1/2	1/2
Athabasca	1966-1976	Jan-Dec	9/16	35/76	32/70	8/19	45/81	35/76	36/75	36/76
Mouth	1966-1976	Jan-Dec	14/13	33/50	29/44	12/11	36/56	33/49	33/49	33/50
Headwaters	1996-2006	Jan-Dec	51/93	39/76	51/93	50/95	76/152	39/76	39/76	88/169
Jasper	1996-2006	Jan-Dec	7/6	96/189	98/206	113/242	228/462	96/189	55/114	269/574
Hinton	1996-2006	Feb-Oct	0/2	24/34	4/12	38/71	74/83	26/32	26/32	26/49
Whitecourt	1996-2006	Jan-Oct	0/2	14/14	0/2	20/43	32/59	14/14	14/14	14/21
Athabasca	1996-2006	Jan-Oct	0/2	6/9	5/11	13/20	31/30	6/9	6/9	13/21
Mouth	1996-2006	Jan-Dec	26/35	26/35	75/96	100/154	150/186	102/146	72/108	138/200

Mean weighted averages and standard errors were calculated for each parameter (water quality variables and flow) across each time period. These were weighted based on sample size to remove bias due to unequal contributions of larger sized samples to annual averages (Bland and Kerry, 1998). To assess the presence of a trend in water quality across the six reaches for each time period, the nonparametric Mann-Kendall trend test was performed. This trend test was performed separately for each parameter on the weighted averages across all reaches for each time period (two trend tests per parameter). This test does not require data to be normally distributed, or to have equal variances (Helsel and Hirsch, 2002). To test for a significant difference between the two time periods, the nonparametric Kruskal-Wallis unpaired t-test was performed for each parameter (including flow) at each reach for a total of fifty-four tests.

Average total annual flow could not be calculated due to a lack of data for each time period across all reaches. Cumulative average loadings (kg/day) were calculated for each water quality parameter, with the exception of turbidity and conductivity, by multiplying the weighted average concentration (across the time period) of each water quality parameter by the average discharge (across the time period) at its corresponding HYDAT station for each reach and a conversion factor to account for unit differences. Statistics were not calculated for cumulative loadings due to the spatial dependence of sampling across reaches.

Climate data were obtained from the National Climate Data and Information Archive, operated and maintained by Environment Canada. Average total precipitation (snow plus rain) and average annual temperature were calculated and graphed for each time period as well as for the entire record of data across all reaches. To test for significant differences in temperature and precipitation between the two time periods a parametric paired t-test was performed by pooling data from all reaches to generate a test statistic for the entire time period.

Due to the lack of developed guidelines for the purpose of protecting aquatic life for many of the parameters in this study, the 10^{th} and 90^{th} percentiles were calculated for the first time period. This percentile approach has been used in previous assessments of water quality in portions of the Athabasca River (Glozier et al. 2004). Using the first time period in these calculations is ideal since it is considered to represent a reference-type condition due to the absence of many of the land-use related stressors which are present in the second, more developed time period (Table 3.1). These percentiles can offer some measure of acceptability when compared to the concentrations of these parameters in the second time period. For example, concentrations in the second time period which exceed the 90^{th} percentile can be considered above a threshold of acceptability compared to the first time period.

3.2 RESULTS

3.2.1 Water Quantity

Reach mean discharge for each month during time period 1 (historical) and time period 2 (current day) are shown in Figure 3.2. The Headwaters, Jasper and Hinton peak discharge occurred one month earlier than the Whitecourt, Athabasca and Mouth discharges in time period 1 but not time period 2, suggesting a delayed freshet in the upper reaches during time period 2. The Mouth reach had the highest mean discharge of all the reaches in time period 1, whereas there were only two months (May, Sept) where discharge at the Mouth reach was higher than the Athabasca reach in time period 2. The discharge in the mouth reach in time period 2 was 16% lower on average than in time period 1.

When the high flows (May-August) were averaged at each reach for both time periods, we observe a decrease of 6-356 m^3/s across all reaches from Headwaters to Mouth between time period 1 and time period 2 (Table 3.3). In the Athabasca and Mouth reaches, this translates to a statistically significant decrease of 16% and 26% in high flow, respectively ($p<0.05$). When the low flows (September-April) were averaged at each reach for both time periods, we observe a significant decrease ($p<0.05$) of 14-30% in the lower three reaches (Table 3.4).

Weighted average flow showed a significant increasing trend along the river continuum in both time periods ($p = 0.005$ and 0.015 in time period 1 and time period 2 respectively). The flow was significantly higher in time period 1 compared to time period 2 for the Headwaters ($p = 0.012$), Athabasca ($p = 0.018$) and Mouth ($p < 0.001$) reaches (Figure 3.3).

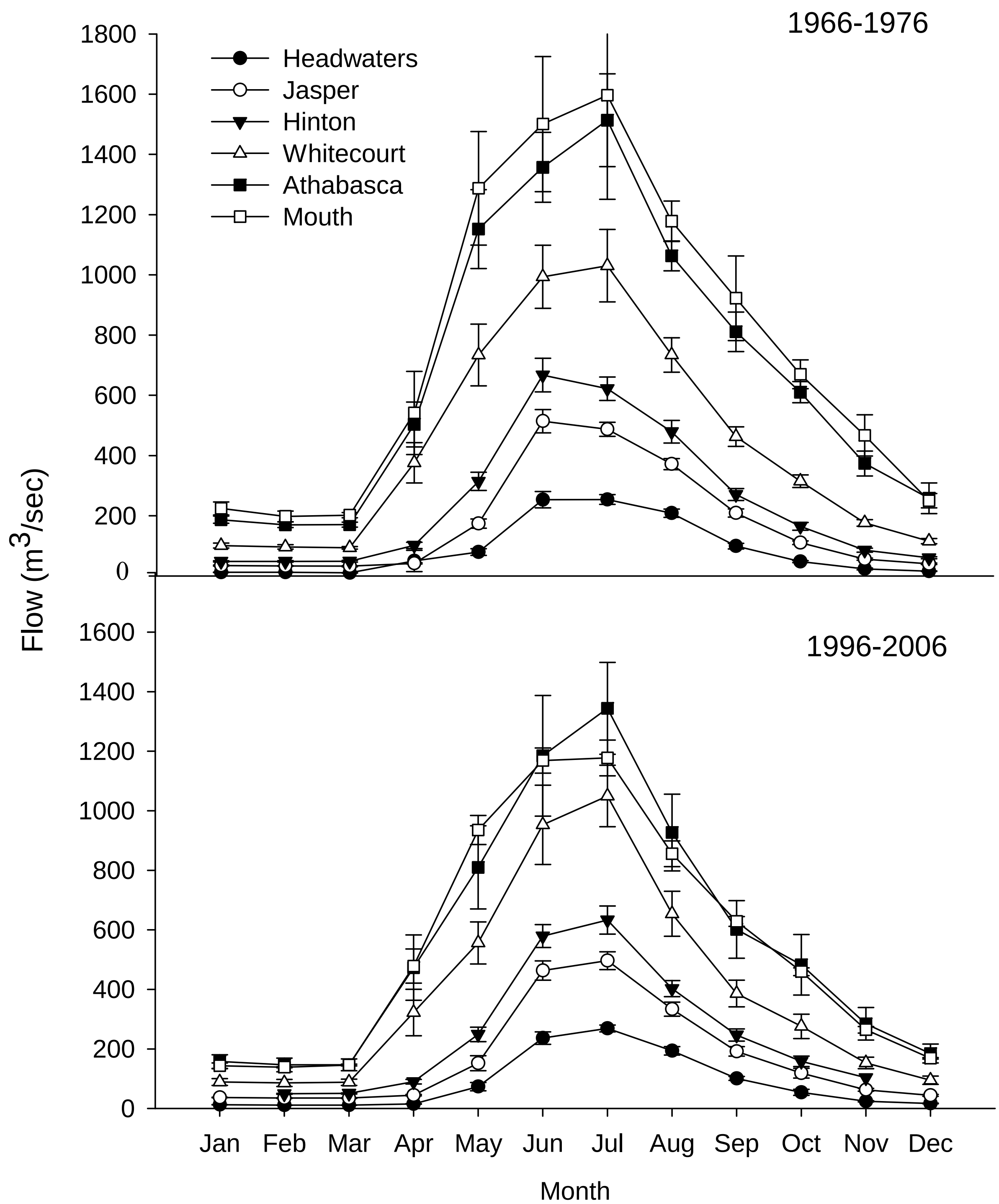


Figure 3.2: Mean monthly (± SE) discharge at HYDAT stations (one per reach) along the Athabasca River continuum in two time periods; historical (1966-1976) and current day (1996-2006).

Table 3.3: Differences in average high flows (May – August) for six reaches in the Athabasca River basin across two time periods (1966-1976 and 1996-2006).

Reach	HYDAT Station	High Flow (m^3/s) 1966-1976	High Flow (m^3/s) 1996-2006	Difference (m^3/s)	Percent Difference (%)
Headwaters	07AA002	198	192	-6	-3.1
Jasper	07AD002	386	361	-25	-6.6
Hinton	07AE001	520	465	-54	-10.5
Whitecourt	07BE001	872	803	-69	-8.0
Athabasca	07DA001	1271	1066	-205	-16.1*
Mouth	See Below[1]	1390	1034	-356	-25.6*

* = significant at $p<0.05$ using a paired samples t-test

[1] Data are provided by HYDAT (07DD001) for time period one and the Regional Aquatic Monitoring Program (S24) for time period two

Table 3.4: Differences in average low flows (September – April) for six reaches in the Athabasca River basin across two time periods (1966-1976 and 1996-2006).

Reach	HYDAT Station	Low Flow (m^3/s) 1966-1976	Low Flow (m^3/s) 1996-2006	Difference (m^3/s)	Percent Difference (%)
Headwaters	07AA002	34	31	-3	-10
Jasper	07AD002	70	71	1	1
Hinton	07AE001	104	116	12	12
Whitecourt	07BE001	217	187	-30	-14*
Athabasca	07DA001	386	310	-76	-20*
Mouth	See Below[1]	434	303	-131	-30*

* = significant at $p<0.05$ using a paired samples t-test

[1]Data are provided by HYDAT (07DD001) for time period one and the Regional Aquatic Monitoring Program (S24) for time period two

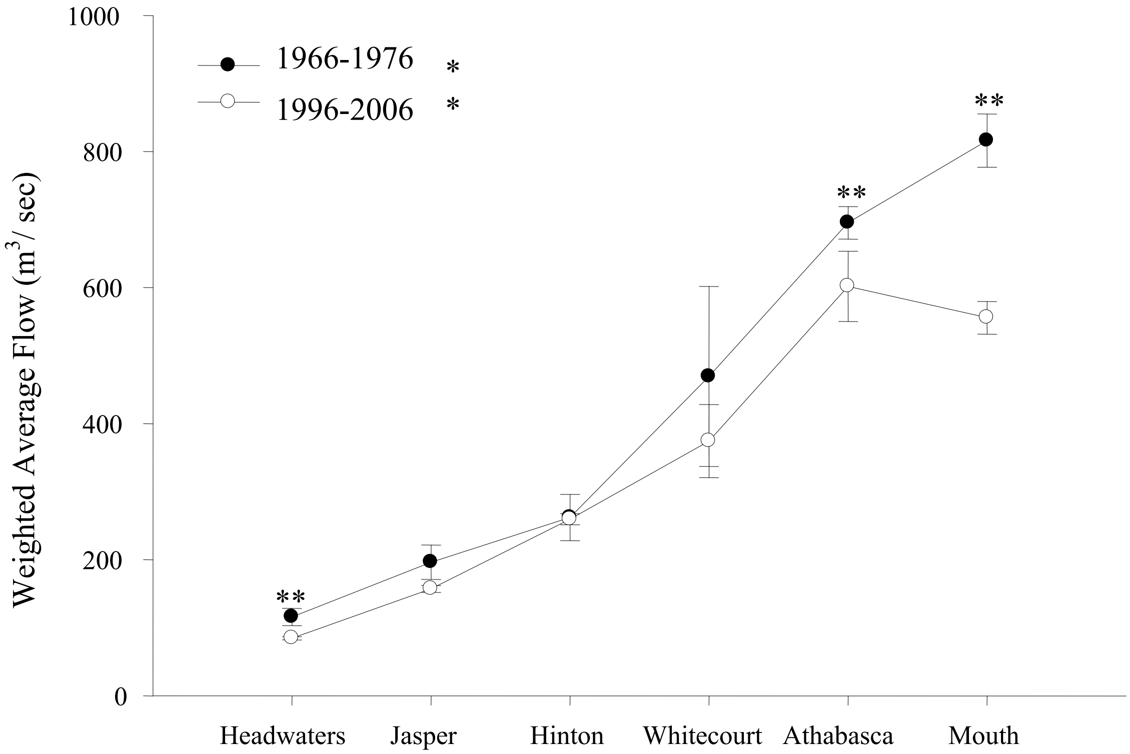


Figure 3.3: Average flow for reaches along the Athabasca River continuum across two time periods: (1966-1976) and current day (1996-2006) calculated from data in Table 2. Statistical differences across reaches were assessed using the nonparametric Mann-Kendall trend test and to test between the two time periods the nonparametric Kruskal-Wallis unpaired t-test was performed. * in the legend indicates a significant trend across reaches ($p < 0.05$) for that time period; ** for a reach denotes a significant difference in flow between time periods at that reach ($p < 0.05$).

3.2.1.1 Climate

Average temperature and total precipitation followed a similar pattern from headwaters to mouth, peaking in the Hinton reach during both time periods as well as over the entire record of data (Figure 3.4). Temperature in time period 2 was consistently warmer than that in time period 1 across all reaches. The average temperature for the entire period of record across all reaches was higher than the average time period 1 temperature, but cooler than the average time period 2 temperature. Total precipitation showed the reverse trend where time period 1 was wetter across all reaches, except the Jasper reach. The average total precipitation for the entire record of data across all reaches was comparable to the trend and amounts seen in time period 1. The peak difference in average temperature was +1.4ºC and the difference in peak total precipitation was approximately -81.8 mm between time periods one and two among all reaches (Figure 3.4).

3.2.1.2 Water Allocations in the Athabasca River basin

The amount of water allocated to be removed and the actual consumptive use of this licensed water in the Athabasca River over both time periods was obtained from Alberta Environment and is shown in Figure 3.5. There was an increase of between 3,482,472 and 455,573,728 m^3/year in the amount of water allocated to be used in the Athabasca River across reaches between the two time periods (Figure 3.5). The greatest increases in licensed allocations have been in the lower three reaches (Whitecourt, Athabasca and Mouth). These trends are mirrored in the actual consumptive use of the allocated water in each reach (Figure 3.5). However, in all reaches the actual consumptive use of water was less than the allowable allocated water amounts. Overall, there was an increase of between 3,178,487 and 413,750,193 m^3/year in the amount of consumptive water use in the Athabasca River across reaches between the two time periods with the greatest increase in the Mouth reach (Figure 3.5). The greatest increases were seen in the industrial, habitat management and municipal sectors where water allocations more than doubled (Figure 3.6).

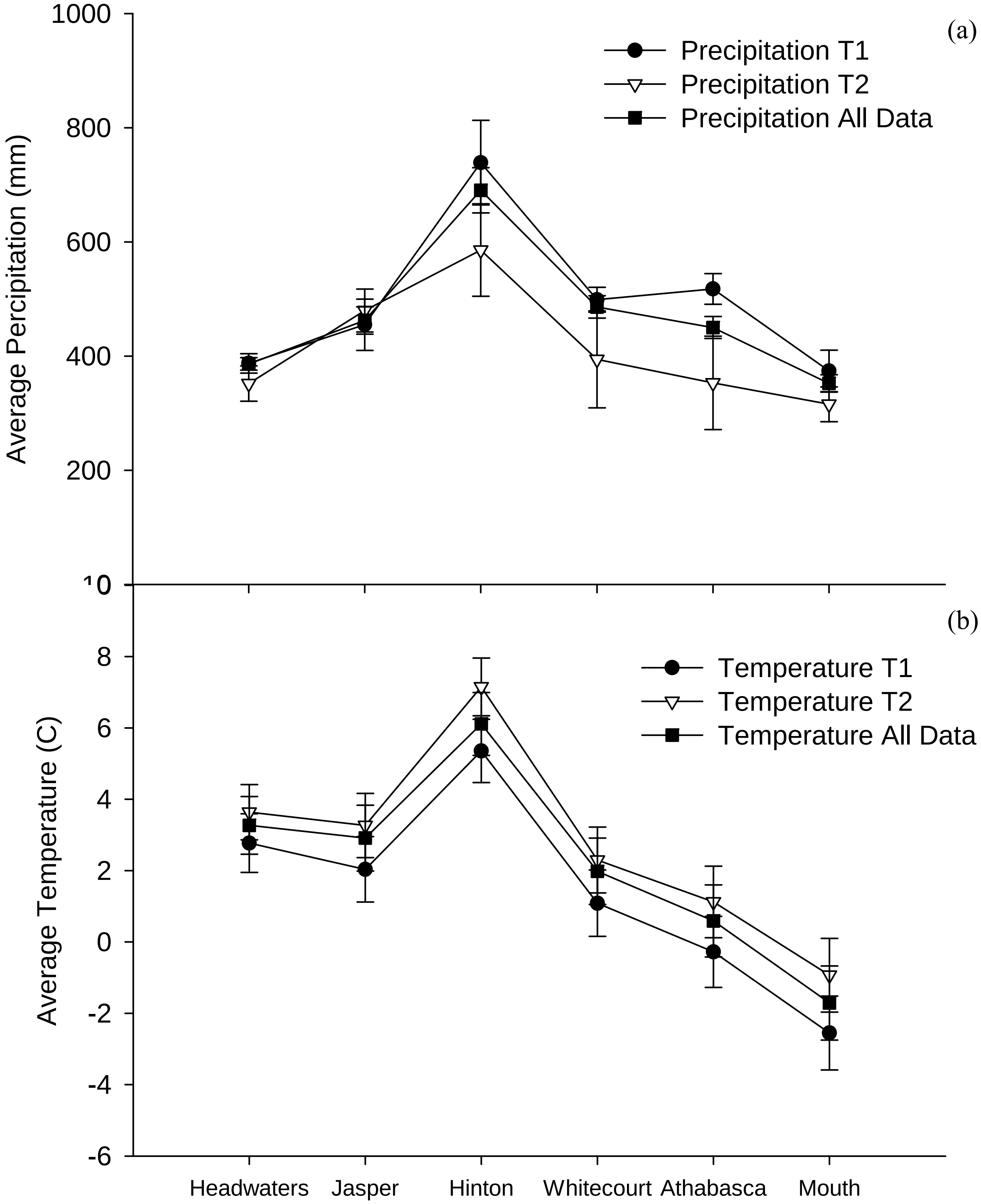


Figure 3.4: Total and average annual precipitation (a) and air temperature (b) at stations along the Athabasca River continuum pre-development or time period 1 (1966-1976), current day or time period 2 (1996-2006) and for all available data (1966-2006). To test for significant differences in temperature and precipitation between the two time periods a parametric paired t-test was performed by pooling data from all reaches to generate a test statistic for the entire time period. There is a significant difference in both average annual temperature (+1.4ºC) and total precipitation (-81.8 mm) ($p<0.05$) between the two time periods.

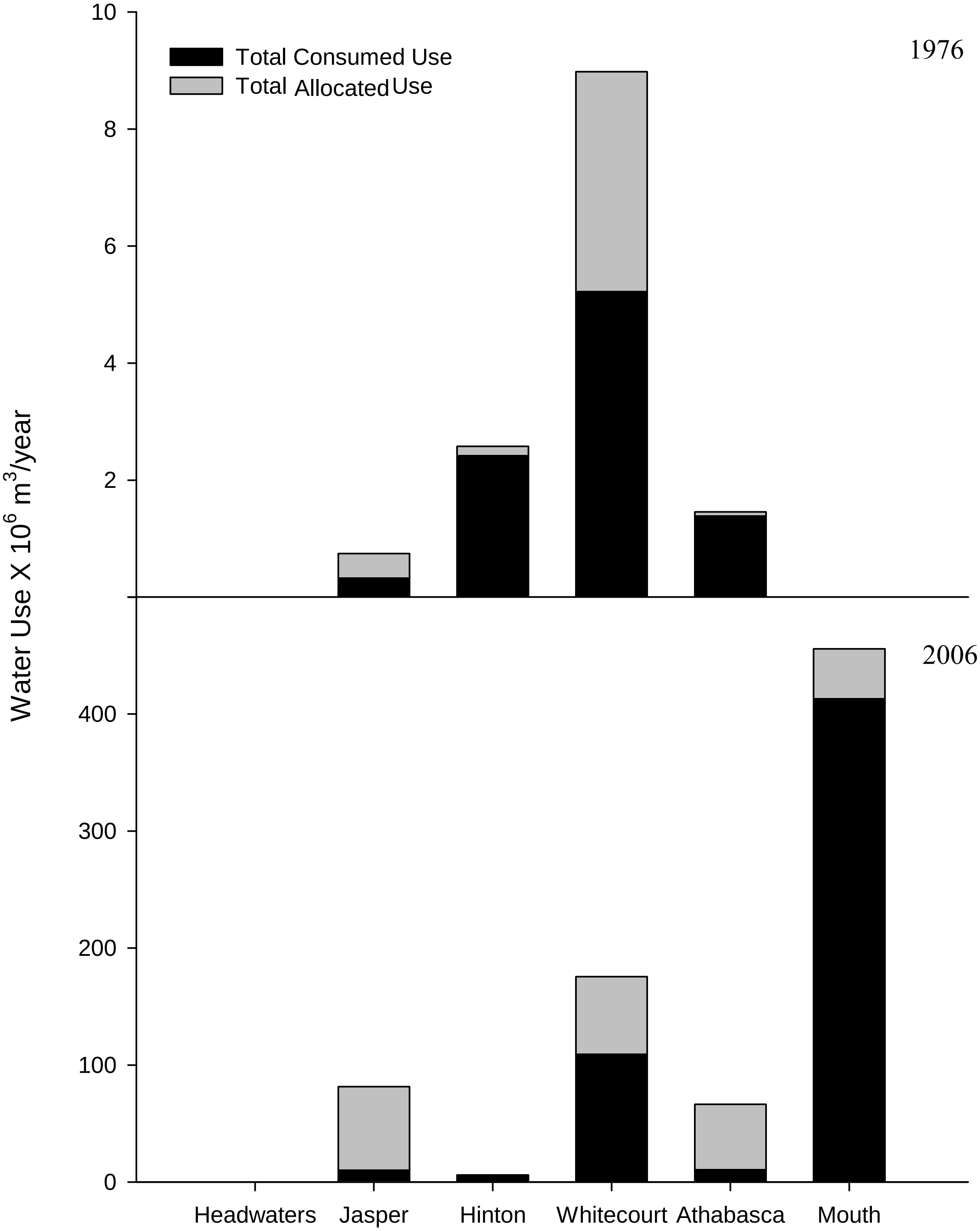


Figure 3.5: The total actual consumption and total allowable allocated consumption of surface water (m^3/year) along the Athabasca River continuum historical (1976) and current day (2006).

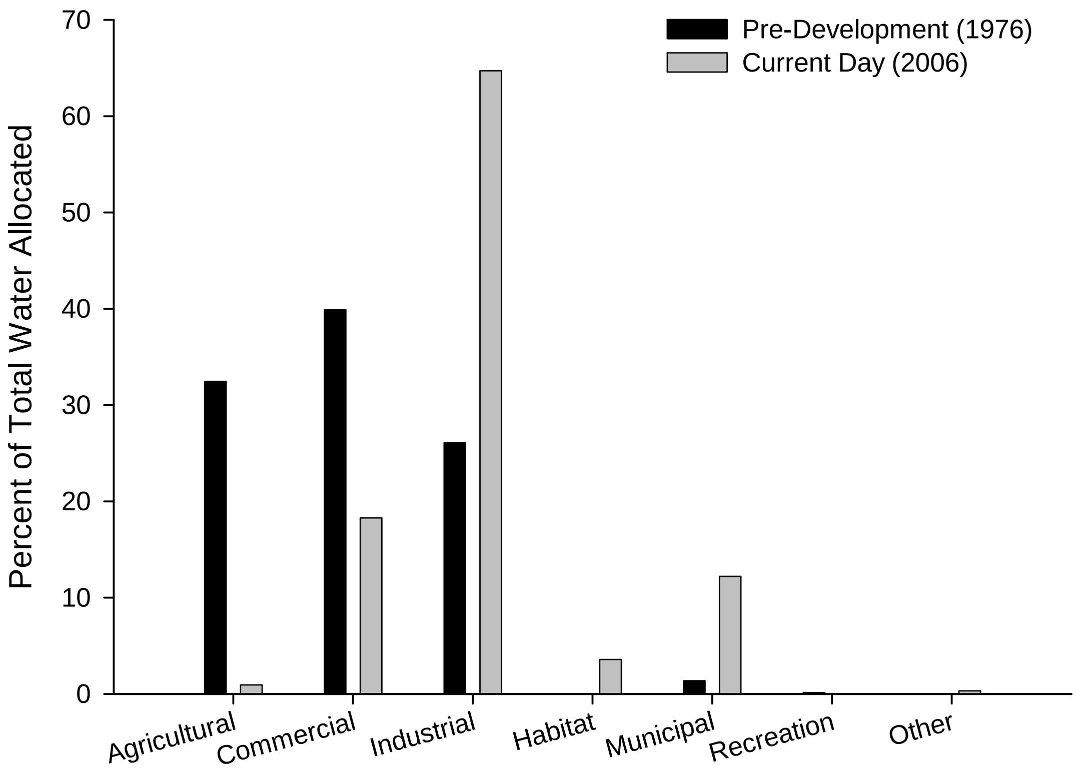


Figure 3.6: Percent of surface water allocated to each sector along the Athabasca River continuum historical (1976) and current day (2006).

3.2.2 Water Quality

3.2.2.1 Mean Concentrations

The weighted average and cumulative loading of the analyzed water quality parameters are shown in Figure 3.7. A significant increasing trend in total phosphorous was observed across all reaches in the second time period ($p = 0.005$). A peak in total phosphorous concentration in time period 1 was noted at the Hinton reach, but was not observed during time period 2. Total phosphorous concentrations were significantly higher in time period 1 in the Jasper ($p = 0.003$) and Mouth reaches ($p = 0.008$), however the reverse was observed for the Athabasca reach ($p = 0.001$). Dissolved nitrogen concentrations peaked in time period 1 at the headwaters followed by a decrease in the Whitecourt reach before rising again to be similar to time period 2 at the mouth reach. Concentrations of dissolved nitrogen were significantly higher ($p = 0.034$) in time period 2 at the Athabasca reach.

In time period 1, average dissolved chloride concentrations peaked at the Hinton reach. In time period 2, this peak was not observed, however concentrations of dissolved chloride were significantly higher ($p < 0.001$) then time period 1 in the Mouth reach. Dissolved chloride did show a significant increasing trend from headwaters to mouth in time period 2 ($p = 0.015$). In time period 1, dissolved sulphate concentrations peaked at the Jasper reach. In time period 2 dissolved sulphate concentrations were significantly higher than in time period 1 for both the Hinton ($p < 0.001$) and Athabasca ($p = 0.017$) reaches. However, even though sulphate concentrations were higher overall in time period 2, they were not significantly different from time period 1 at the Mouth reach.

Average total organic carbon exhibited significant increasing trends across all reaches in time periods one and two ($p = 0.005$ and 0.015), but the concentrations were significantly lower in time period 2 in the Headwaters ($p < 0.001$), Jasper ($p < 0.001$), Athabasca ($p = 0.047$) and Mouth ($p > 0.001$) reaches. Turbidity showed the same pattern across all reaches in both time periods. Conductivity showed a significant increasing trend across the Athabasca River during the second time period. Conductivity values were significantly higher in the Hinton reach in time period 1 ($p = 0.022$) and significantly higher in time period 2 in the Mouth reach ($p = 0.002$). Sodium concentrations showed a significant increasing trend in both time periods one and two ($p = 0.005$ and 0.005) along the river continuum. These concentrations were significantly higher during time period 2 in the Hinton ($p < 0.001$), Athabasca ($p = 0.003$) and Mouth ($p < 0.001$) reaches than time period 1.

3.2.2.2 Cumulative Loadings

The total cumulative loading of sodium was approximately 20 kg/day higher in time period 2. Cumulative total organic carbon and dissolved nitrogen were 13 386 kg/day and 767 kg/day lower across the river basin in time period 2 compared with time period 1, respectively. Cumulative loadings of chloride were 453 038 kg/day higher at the Hinton reach in time period 1 than time period 2 (Figure 3.7). This increase was no longer observed in time period 2; however cumulative dissolved chloride did increase by 757 688 kg/day between the Athabasca and Mouth reaches in time period 2 allowing the levels of chloride to reach a similar cumulative total concentration in the Mouth reach in both time periods.

Cumulative total phosphorous loadings were lower in the Headwater, Jasper and Hinton reaches in time period 2 compared to time period 1. However, this trend reversed in the lower half of the basin where the concentrations in the Whitecourt, Athabasca and Mouth reaches were higher in time period 2 compared to time period 1. Cumulative sulphate loadings showed a similar trend to total phosphorous loadings.

3.2.2.3 Comparison to Benchmarks

A summary of the current water quality guidelines at the Canadian federal level as well as for selected provinces are listed in Table 3.5. Few guidelines for the protection of aquatic life exist for these parameters. However, several guidelines for these variables do exist for agricultural and irrigation waters as well as drinking water for human consumption. Only turbidity and total phosphorous exceeded any of the guidelines listed in Table 3.5 in the time periods considered in this study. Turbidity exceeded both national and provincial guidelines across all reaches in both time periods. Total phosphorous exceeded chronic levels in the Alberta provincial guidelines for the Hinton and Mouth reaches in time period 1 and in the Athabasca and Mouth reaches of time period 2.

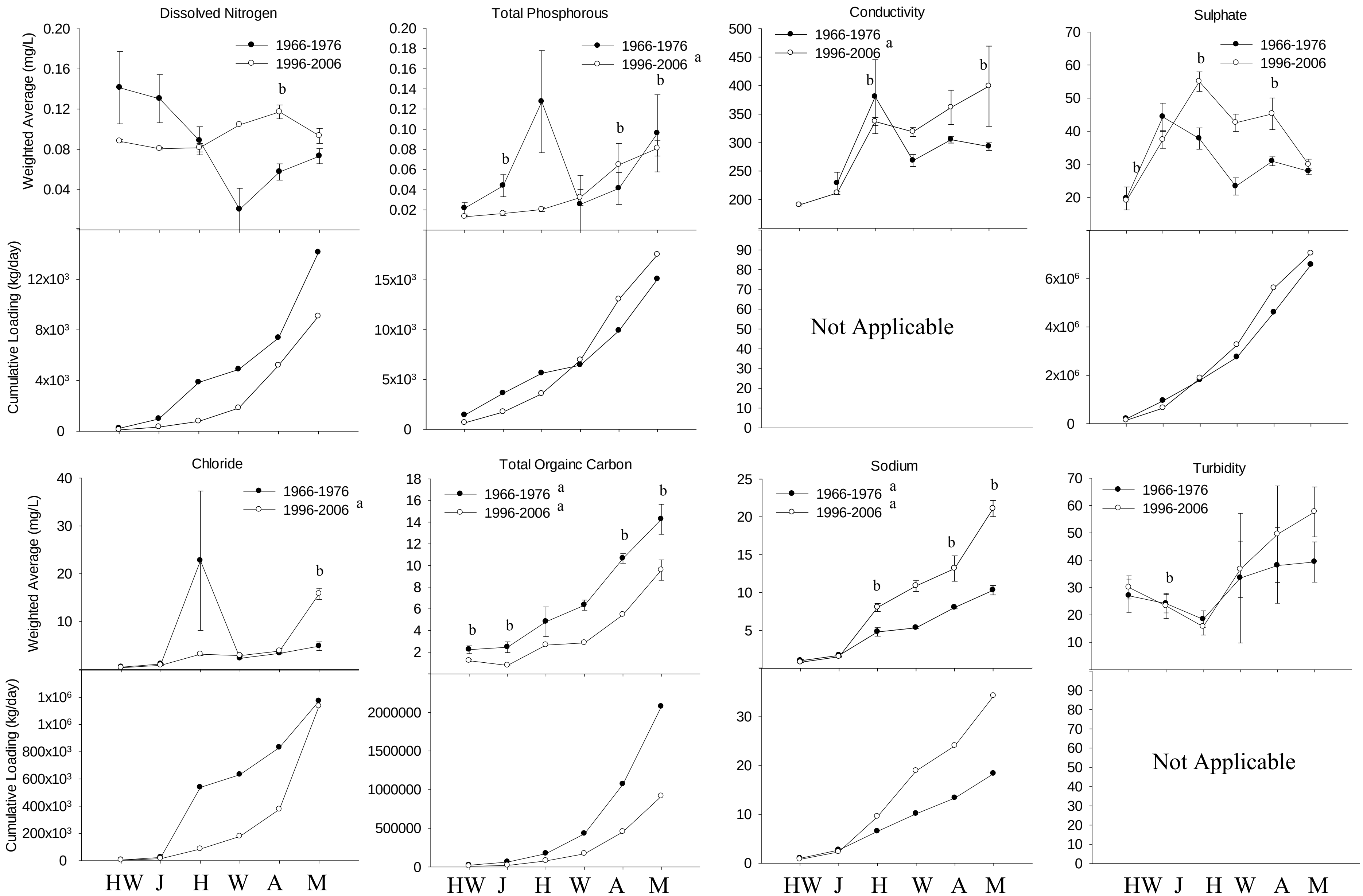


Figure 3.7: Weighted average (± SE) and cumulative loadings for selected water quality parameters at stations along the Athabasca River continuum across two time periods historical (1966-1976) and current day (1996-2006) calculated from data in Table 2. Reach names are abbreviated as: HW=Headwaters, J=Jasper, H=Hinton, W=Whitecourt, A=Athabasca, M=Mouth. Statistical differences across reaches were assessed using the nonparametric Mann-Kendall trend test and to test between the two time periods the nonparametric Kruskal-Wallis unpaired t-test was performed. For each weighted average parameter, [a] in each legend indicates a significant trend across reaches ($p<0.05$); [b] for a reach denotes a significant difference between time periods in that parameter at that reach ($p<0.05$). [1] Units are weighted average (µS/cm) and cumulative (µS/cm). [2] Units are weighted average (NTU) and cumulative (NTU).

Table 3.5: Benchmarks for evaluation of changes in selected water quantity and quality parameters including Canadian federal and provincial guidelines for the protection of aquatic life and objectives calculated for each reach based on the 10th and 90th percentiles of time period one (1966-1976).

Parameter	Canadian (National and Provincial)	Percentile	Headwater Reach	Jasper Reach	Hinton Reach	Whitecourt Reach	Athabasca Reach	Mouth Reach
Average Flow (m^3/sec)		10th 90th	12.34 254.0	33.20 498.0	48.36 667.2	186.5 734.0	170.5 1357.2	202.2 1500.6
Total Organic Carbon (mg/L)	± 20% of median	10th 90th	0.50 5.00	0.50 6.00	1.65 7.80	6.00 6.80	6.00 19.90	9.60 22.40
Dissolved Nitrogen (mg/L)		10th 90th	0.04 0.22	0.04 0.233	0.003 0.13	0.002 0.04	0.003 0.13	0.01 0.18
Dissolved Sodium (mg/L)	0-30[1]	10th 90th	0.40 1.40	0.60 2.50	1.90 9.80	4.14 6.46	4.00 12.49	5.11 17.82
Turbidity (NTU)	2-8[4]	10th 90th	0.60 70.00	1.60 68.40	2.00 37.00	10.52 59.00	1.49 70.60	3.52 89.80
Total Phosphorous (mg/L)	0.05 (chronic) and 0.1-0.3[3]	10th 90th	0.002 0.07	0.003 0.10	0.007 0.38	0.004 0.05	0.004 0.09	0.02 0.08
Conductivity (µS/cm)		10th 90th	No data	131 393	204 439	247 300	216 418	211 415
Dissolved Chloride (mg/L)	100-700[1]	10th 90th	0.20 0.80	0.20 1.96	0.53 9.43	1.76 2.80	1.00 6.52	1.20 7.50
Dissolved Sulphate (mg/L)	100-1000[2]	10th 90th	8.00 42.20	13.33 90.76	19.48 58.82	18.20 27.00	16.48 50.58	17.11 43.04

[1]For agricultural irrigation water (Alberta, British Columbia)
[2]For agricultural livestock water (Federal, Alberta, and British Columbia)
[3]This is an interim guideline (Alberta, British Columbia, and Ontario)
[4]Dependent upon background levels of turbidity (Federal, British Columbia

Percentiles were calculated for each reach using time period 1 as a reference baseline for water quantity (average flow) and water quality (total organic carbon, dissolved nitrogen, dissolved sodium, turbidity, total phosphorous, conductivity, dissolved chloride, dissolved sulphate) parameters in this study (Table 3.5). Average flow in time period 2 fell between the 90^{th} and 10^{th} percentiles from time period 1 in all reaches. Concentrations of total organic carbon were below the 10^{th} percentile in the Whitecourt, Athabasca and Mouth reaches in time period 2. The Whitecourt reach showed concentrations in time period 2 which exceeded the 90^{th} percentile for dissolved sodium, conductivity, dissolved chloride and sulphate. Concentrations of dissolved sodium in the second time period also exceeded the 90^{th} percentiles in the Athabasca and Mouth reaches and are consistent with the significant increases in weighted average concentrations between the two time periods. Concentrations of dissolved chloride in the mouth reach also exceeded the calculated 90^{th} percentile in the second time period.

3.3 DISCUSSION

3.3.1 Water Quantity

In aquatic ecosystems, variability in flow is needed to maintain natural habitat dynamics and support the maintenance and persistence of biota (Baron et al. 2002). Disruption in the natural magnitude, frequency, duration, timing and rate of change of flow in aquatic systems can lead to the elimination of some aquatic environments (Poff et al. 1997). In the Athabasca River, flows in the Mouth reach have decreased by more than 260 m^3/sec in time period 2 compared to time period 1 (Figure 3.3). This is equal to more than the total flows in the headwaters in time period 1 (115 m^3/sec) and time period 2 (85 m^3/sec). The ecological significance and acceptability of this level of water loss is not known in the absence of target in-stream flow needs for the Athabasca River (Schindler et al. 2007). What is known is that a loss of 260 m^3/sec at the mouth of the river over the past 30 years will only increase in the years to come given the increasing developmental pressures, especially in the lower part of the basin (Woynillowciz et al. 2005). Given the development projections, consequences to public health (drinking water), the economy (transportation routes, energy production) and water policy on both provincial and national levels require more serious consideration.

Peak flows and flooding events of the Athabasca River basin are associated with snowmelt and approximately 70% of the moisture input into the basin originates from precipitation (Culp et al. 2005). Decreases in the average and cumulative flows could be attributed to both natural and anthropogenic sources including human water withdrawals, increased warming causing increased evapotranspiration, and decreased winter snowpack (Schindler and Donahue, 2006). Therefore it is important to examine the potential causes for decreasing flows in the Athabasca River, one of the largest unregulated and diversely used freshwater sources in the province of Alberta.

It is widely acknowledged that increasing global temperatures are causing significant decreases in our glacier volumes. Glaciers from the headwaters of the Athabasca River basin and consequently, decreases in their size will impact the quantity of water along the entire river. The average temperature in time period 2 was 1.4°C higher than the average temperature in time period 1 across all reaches (Figure 3.4). This trend is further corroborated with the work of Schindler and Donahue (2006) who showed that since 1970, the Athabasca River has seen an overall increase in air temperature of 2ºC. These authors also show that due to this increase in air temperature, most large glaciers in the headwaters of the nearby Bow and Saskatchewan Rivers, along with the Athabasca River have shrunk by ~25% in the last century. Further glacier decline is expected move the downstream hydrograph toward a snowmelt dominated regime (Comeau, 2008).

In addition to glacial melt, another significant source of water to the Athabasca River is the snow pack. In recent years, climate change has been connected to significant changes in the maximum depths and melting rates of snow packs in the northern region of the province of Alberta (Schindler and Donahue, 2006; Romolo et al. 2006a; Romolo et al. 2006b). In the Peace River basin, north of the Athabasca River, Romolo et al. (2006b) showed that the date of spring snowmelt in this river basin has occurred progressively earlier during 1963-1996. These trends are similar to the Athabasca River basin where we showed that spring melt has been occurring one month earlier in the lower half of the basin in time period 2 (1996-2006) compared with time period 1 (1966-1976). This earlier spring melt can be mostly attributed to the increasing air temperatures between these two time periods which trigger the earlier melt of the snow pack (Schindler and Donahue, 2006; Romolo et al. 2006b).

In the Athabasca River basin, time period 2 saw a lower total precipitation (81 mm) than time period 1 averaged across all reaches (Figure 3.4). These changes, coupled with warming air temperatures and decreasing glacial size at the headwaters will not only cause spring melt to peak sooner in the year, but it will also decrease the amount of spring runoff and thereby affect the overall level and flow rates across the entire basin (Lapp et al. 2005; Schindler and Donahue, 2006). These effects are already taking place as we have observed with the significantly lower average high and low flows during the second time period in the lower half of the Athabasca River basin (Tables 3.3 and 3.4).

Differences in peak and cumulative discharges could also be a result of greater instream flow requirements in this basin during the second time period (more industry, agriculture, urban, population growth, etc.). Allocations for surface water withdrawal along the entire Athabasca River represents 8% of all allocated surface water use in Alberta (Schindler et al. 2007). There is an increasing amount of both industrial and urban land uses in the second time period (Table 1.3). These include four more pulp mills, an increase in the population in the province of Alberta by 40% and an increase in the amount of farm area in Alberta by almost 5,000,000 acres (Statistics Canada, 2007). In response to these increased developments, water allocations have increased dramatically in several reaches (Whitecourt, Athabasca and Mouth) in the second time period (Figure 3.5), coinciding with the observed decrease in flow in the lower half of the river (Figure 3.3). This trend has also been discussed by Schindler et al. (2007) where they argue that in addition to increased water allocation and consumption, the amount of runoff downstream of Hinton has been steadily decreasing over the past 30 years, contributing to our observed trend of decreasing cumulative flow.

Development of the Athabasca oil sands deposit near Fort McMurrary, Alberta (Figure 3.1) is expanding at a tremendous pace. This expanded development requires a greater draw of water from the Athabasca River in the Mouth reach to accommodate oil sands processing. Currently, 76% of allocated water in the Athabasca River is for oil sands extraction (Schindler et al. 2007). To counteract this increased demand, many of the companies currently extracting bitumen from the Athabasca oil sands deposits are making an effort to reduce the amount of water required per barrel of oil. Formally, four barrels of water were required to produce one barrel of oil, currently through the recycling of process water this number has been reduced to two barrels of fresh water for every barrel of oil (Schindler et al. 2007).

3.3.2 Water Quality

Specific conductance (conductivity) is highly correlated with the amount of total dissolved solids in the water and as such, is a representation of the salinity and a measure of the ability of water to conduct an electrical current (Said et al. 2004). An increase in conductivity, as is seen in the second time period, could be an indication that the Athabasca River is becoming more concentrated with salts. This could be a result of the reduced flows also observed in the second time period, or it could be attributed to the increase in industrial and urban development along the river basin.

Turbidity can affect the toxicity of other contaminants by affecting processes such as photoactivation and by binding to, and therefore altering the bioavailability of contaminants (Ireland et al. 1996). High levels of turbidity can also affect the health of aquatic ecosystems by damaging habitats for fish and other aquatic organisms (Said et al. 2004). One aspect of turbidity which has been studied is its affects on the ability of fish to detect prey or mates, thereby affecting the growth and reproduction of fish (Engström-Öst et al. 2006; De Robertis et al. 2003). These can all be future issues of concern if the turbidity in the lower reaches of the river become too great.

The impact of agriculture on water quality can become more evident in areas where agriculture is the most dominant form of land use and the effects of numerous small sources can accumulate to produce a more serious ecological effect (Schroder et al. 2004). While the total area of crop land farmed in the Athabasca River basin specifically was unavailable, in the province of Alberta it has increased by only 1.9% over the past ten years. Applications of herbicides and insecticides have increased by 9.5 and 14.5% respectively (Statistics Canada, 2007). This provides an indication of the increase in intensity of agriculture practices, providing more opportunities for the leaching of nutrients and organic chemicals into the surrounding surface waters. In addition, both nutrients and dissolved organic carbon can be higher during spring runoff due to the flushing of natural organic matter from the soil and shallow groundwater systems (Barber et al. 2006; Schroder et al. 2004). Total phosphorous and total nitrogen have been shown to be strongly linked to particulate matter in the headwaters of the Athabasca River, providing a possible reason for these similar trends we observed between nutrients and dissolved organic matter (Glozier et al. 2004).

Cumulative total organic carbon was lower across the river basin in time period 2 compared with time period 1. Decreased stream flows, due to climate change, allow for increased residence time in the water column and can be a major contributing cause to decreases in dissolved organic carbon. However, drier soils and lower water tables can decrease allochthonous dissolved carbon inputs along the river continuum which can provide the mechanism for settling of organic matter along the river continuum over time (Schindler, 2001).

During periods of low and high flow, concentrations of certain parameters (total phosphorous, TOC and turbidity) can fluctuate. In times of low flow these parameters can become more concentrated thereby exhibiting a greater biological effect. Table 3.2 outlines the number of samples available during high flow (May – August) and low flow (September – April) periods. Although we feel that we have an adequate representation of the yearly variations in concentrations of the most seasonally affected parameters we did not have enough data to calculate the average annual high flow and low flow concentrations in time period 1 across all reaches. A greater dispersion of data is necessary in order to adequately assess the effects of seasonal changes in flow on the concentrations of these parameters.

Glozier et al. (2004) examined nutrient concentrations downstream of the town of Jasper across the years 1973-2002. They found the median total phosphorous levels to be 0.010 mg/L, the median total organic carbon to be 1.17 mg/L and the median dissolved nitrogen to be 0.11 mg/L. Another study conducted as part of the Northern Rivers Ecosystem Initiative, looked at nutrient levels across the lower portion of the Athabasca River from 1985-2000. They found the median concentrations of total phosphorous to be >0.08 mg/L, and dissolved inorganic and total nitrogen to be >0.2 mg/L and >0.7 mg/L respectively across this time period (Chambers et al. 2006). Both of these studies report levels that are similar to, or higher than those in this study. The values stated by Glozier et al. (2004) fall between the first and second time periods of this study (Figure 3.7). The dissolved inorganic and total nitrogen values reported by Chambers et al. (2006) are higher than the dissolved nitrogen values in this study, and their total phosphorous values are higher than the total phosphorous in both time periods with the exception of the Hinton and Mouth reaches of time period 1. The differences in concentrations reported in these two studies and our study could be due to the different time periods and sources of data used for calculations. Both of these variations can skew results, providing us with a different value which may or may not be of concern. These differences highlight the importance of continuous and consistent water quality monitoring programs to monitor long-term changes in this region.

Sources of dissolved nitrogen in surface waters are from both natural and anthropogenic sources. Most of the nitrogen in the cycle is unavailable to the majority of organisms (N_2 gas), therefore the greatest impact humans can have on this cycle is to liberate this fixed nitrogen into more bioavailable forms (Vitousek et al. 1997). Anthropogenic sources can include agricultural fertilizers, municipal effluent discharge, atmospheric transport and bacterial breakdown of in-stream organic matter (Glozier et al. 2004). Previous studies conducted as part of the larger Northern Rivers Ecosystem Initiative study showed nutrient concentrations can increase two fold downstream from secondary treated pulp mill and sewage effluent discharges (Chambers et al. 2006).

The concentrations of dissolved nitrogen in time period 1 were higher than concentrations in time period 2 in the upper half of the basin. This trend in dissolved nitrogen could be due to the better treatment for sewage and pulp and paper mill effluent. The addition of secondary and in some cases tertiary treatment systems can decrease the amount of nutrients and particulate matter which are released directly into the river system (Chambers et al. 2000; Chambers et al. 2006). Downstream of the Whitecourt reach however, cumulative dissolved nitrogen is higher in time period 2. Increased intensity of agriculture in time period 2, along with the addition of a pulp mill between time periods one and two in the Athabasca reach could be contributing factors towards this trend.

Sulphate loadings increased across the river continuum in both time periods; however they decreased substantially after the Whitecourt reach in time period 2, and were markedly lower in time period 2 at the mouth reach. Two major causes of increased sulphate in surface waters are oxidation of sulphide within the sediment, more specifically peat, and the suppression of sulphide reducing activity deeper into the sediment profile through increased oxidation of sediments (Bottrell et al. 2004). In the Athabasca oil sands, the most economical way to remove the bitumen from the ground is through strip mining. This involves the removal of a peat and natural soils overburden layer, usually less than 75 metres in thickness before the bitumen can be removed for processing (Hein and Cotterill, 2006). It is possible therefore, that this initial step of strip mining the peat and natural soil overburden in the oil sands region is decreasing the inputs of sulphur into the overlying waters during the second time period through the removal of the sulphur-containing peat, and the suppression of the sulphur reducing bacteria through the oxidation of the removed layer of natural sediment.

Sodium had higher cumulative loadings in time period 2 downstream from the Jasper reach compared to time period 1. The oil sands area has been shown to have a higher distribution of salinity and there is some evidence to support the idea of the oil sands deposit near Fort McMurray, Alberta to be of marine origin (Adams et al. 2004, Conly et al. 2002, Headley et al. 2005). This provides a natural source of sodium and chloride to this area, and once disturbed through surface mining, these ions may leach into the surrounding surface waters. The MacKay River, which is a major tributary near the mouth of Athabasca River, has shown trends of increasing chloride and sodium concentrations from its headwaters to mouth (Headley et al. 2005).

Chloride can come from anthropogenic sources such as road de-icers and marine sediments (Barber et al. 2006). Chronic concentrations of chloride of 250 mg/L have been recognized as harmful to freshwater life and not potable for human consumption (Kaushal et al. 2005). While concentrations of chloride in this study did not reach these levels, concentrations in the Whitecourt and Mouth reaches during time period 2 did exceed the 90th percentiles from time period 1, and therefore shows the potential to be of greater concern in future years.

Cumulative concentrations of chloride in this study showed a marked increase at the Hinton reach in time period 1 and in the Mouth reach in time period 2. This could be attributed to the use and therefore release of elemental chlorine as the bleaching agent in the Hinton pulp mill during this earlier time period. This spike was no longer observed in time period 2, and this may be primarily due to the elimination of chlorine dioxide as the primary bleaching agent in June 1993 (Chambers et al. 2000). However, chloride concentrations reached a similar cumulative total concentration in the Mouth reach in both time periods because of a marked increase occurring between the Athabasca and Mouth reaches in time period 2.

There are several large tributaries which enter the mainstem of the Athabasca River. These include the Clearwater, Lesser Slave, Pembina and McLeod Rivers. The discharges from these tributaries could either increase or dilute the concentrations of certain water quality parameters in the mainstem. However the extent of these tributary contributions is unknown. It is therefore important to consider the impact of these sources when examining water quality trends along the mainstem.

The greatest changes in water quality and quantity between time period 1 and two have occurred in the mouth reach of the Athabasca River. Both dissolved chloride and dissolved sodium increased significantly between time period 1 and two in the mouth reach (Figure 3.7). The major tributary contributing to this reach is the Clearwater River which enters towards the beginning of this reach. To further explore the contribution of this tributary we plotted the concentrations of dissolved sodium and chloride in each time period and by high and low flows as a function of distance from the Clearwater River confluence (Figure 3.8). In time periods one and two, both sodium and chloride concentrations increase downstream of the confluence, especially during low flow. However these parameters increase even further downstream of the oil sands operations in time period 2. This increase was not observed in time period 1 which occurred prior to the development of any major oil sands operations. Since we lack the spatial resolution of data between the Clearwater tributary and the oil sands operations we cannot determine if concentrations increase at the oil sands beyond the tributary influences. However we can conclude based on the data resolution that we have and through examination of the differences between time periods one and two that the oil sands operations has had an effect over and above the tributary influences. Further consideration should be taken in future work to determine the exact influence of major tributaries on these water quality parameters considering flow and land use.

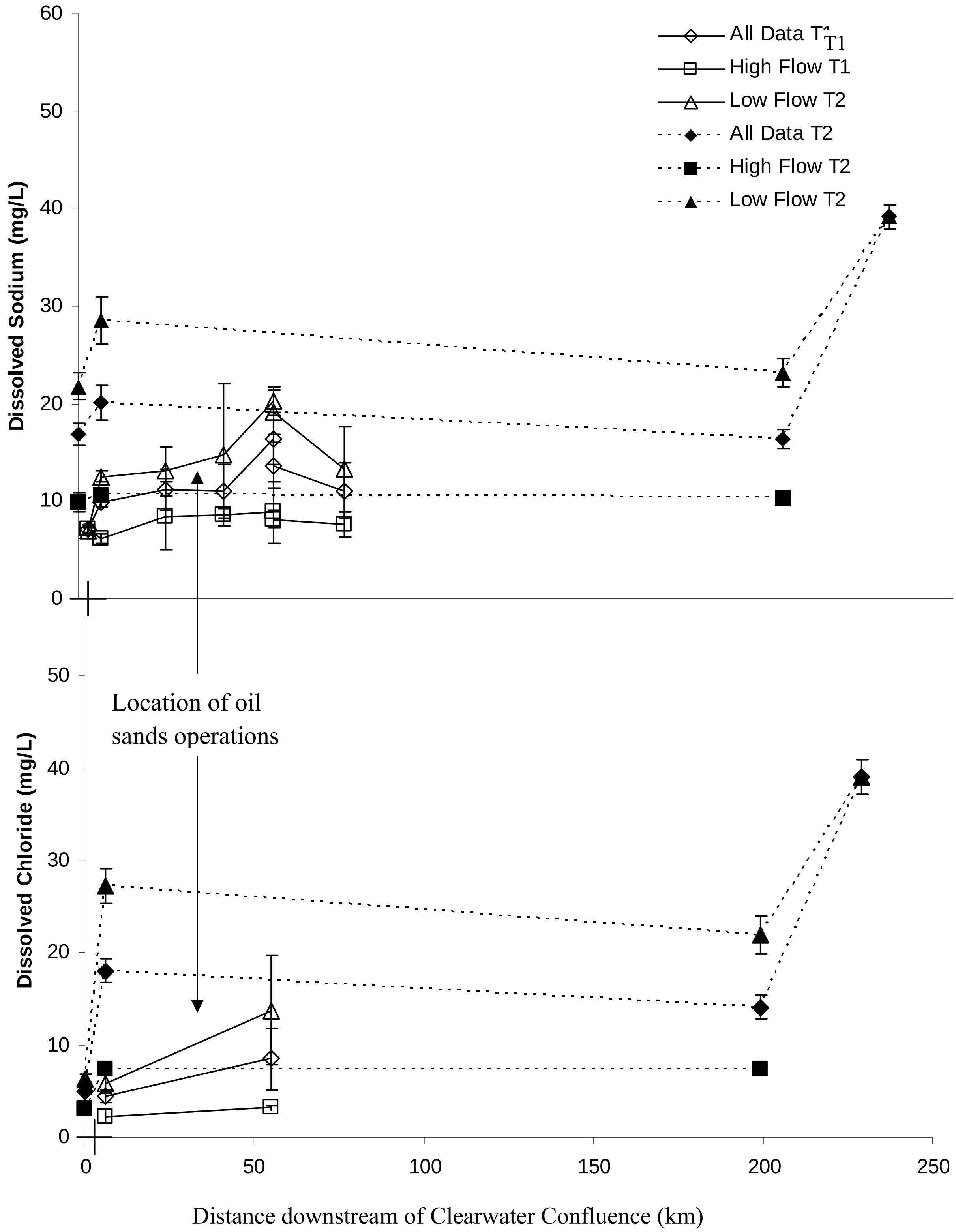


Figure 3.8: Mean concentrations (±SE) of dissolved sodium and chloride (mg/L) in the Athabasca River measured downstream of the Clearwater River confluence in the mouth reach of the Athabasca River Basin

3.3.3 Comparison to Benchmarks

Phosphorous concentrations exceeded the chronic interim provincial guideline (0.05 mg/L) for Alberta in the Athabasca (0.06 mg/L) and Mouth (0.08 mg/L) reaches in time period 2 (Table 3.5). Excessive phosphorus is a major cause of eutrophic conditions in lakes and other water ways (Chambers et al. 2001). Eutrophication can cause a major decrease in the dissolved oxygen levels and increases in the temperature of water, resulting in deaths of fish and other organism populations (Said et al. 2004).

The absence of guidelines for other parameters considered here prevented assessment of the significance of any changes measured between time periods one and two. There is a need to develop site-specific objectives for two reasons: 1) to fill in gaps for parameters where guidelines do not exist; and 2) to account for naturally high levels of some parameters in some [illegible] n proposed to calculate benchmarks for water quality [illegible] dition approaches and percentiles or standard deviates from reference conditions in space or time (Kilgour et al. 2007; Bailey et al. 1998). The reference condition approach for example is largely reserved for biological assessments with greater emphasis placed on benthic macroinvertebrate community structure. It requires the comparison between a system which has undergone stress to a series of reference sites unexposed to this stress (Bailey et al. 1998). To apply this method, the biological condition of several reference sites must be sampled and their variation represents the acceptable range of a reference condition. If the exposed site falls outside of this reference condition, typically in multi-dimensional multivariate space, it is deemed to be impacted. This method is both labour and data intensive and requires many sites with adequate biological data.

It is possible that depending upon the natural habitat of a location, pristine waters unaffected by human development can exceed national or provincial guidelines set for certain water quality parameters (e.g. waters which run through an area of high natural metals, such as the oil sands region). In an effort to improve the development of guidelines to assess changes in waters, other more site-specific methods are being considered, such as summarizing background concentrations of parameters at pristine and undeveloped sites in a region and using the 90th percentile as a site-specific benchmark (Glozier et al. 2004, de Rosemond et al. 2009). This method has the potential to be applied across many indicators (biological and physical) providing the opportunity to make comparisons to a reference condition across different indicators.

Our results showed that Whitecourt reached concentrations in the second time period which exceeded the 90th percentile objective from time period 1 for dissolved sodium, conductivity, dissolved chloride and sulphate (Table 3.5). However, this may be due to the smaller sample sizes available to calculate the 90th percentile in this reach, rather than any stressor-related causes (Table 3.2). Concentrations of dissolved sodium in the second time period exceed the 90th percentile objective from time period 1 in the Athabasca and Mouth reaches. Concentrations of dissolved chloride in the mouth reach also exceeded the calculated 90th percentile in the second time period. These results corroborated the significant differences seen with weighted average concentrations between the two time points. The significance of increased sodium and chloride in the water of the lower Athabasca River is beyond the scope of this paper. What can be said however, is that sodium and chloride concentrations have significantly increased over 30 years with increases in the present day in the top 10% of levels observed 30 years ago.

The data used to calculate these percentiles was the total amount of data over a ten year time period (1966-1976) (Table 3.2). When considering the biological significance of these percentiles we must look at the effects of seasonal changes in flow on the concentrations of these parameters. In times of low flow, concentrations may increase in the river, making their effects more pronounced when compared to high flow periods. A greater distribution of data to provide better seasonal resolution is required to determine if these exceedences are seasonally dependent and if this percentile approach is applicable over a broad time scale.

Average flow in time period 2 was compared and was found to be within the calculated 10th and 90th percentiles from time period 1. Although we were able to assess significant changes when comparing the average, peak and cumulative flow between the two time periods, this confirmed when compared to the calculated benchmarks. This could be a result of how these percentiles were calculated. It may be more useful to focus on producing benchmarks for comparing the low and peak flows only, instead of over an entire year or time period. This way, changes in flow during these more ecologically sensitive flow periods can be assessed and managed.

3.4 CONCLUSIONS

It is not yet understood how much water is needed to maintain the ecological integrity of the Athabasca River basin. Therefore, no clear decisions have been made regarding the sustainability of this resource under the high demands of industrial growth. However, we do know that the natural flow dynamic of the Athabasca River must be preserved in order to maintain the ecological integrity of this important river basin. River flow regimes are determined by several factors such as size, climate, geology and vegetative cover. This is a significant unregulated river in Alberta and based on our data, the intensity, timing and volume of flow have changed over the past 30 years. The significance and acceptability of this change requires attention, especially considering development intensity will only increase with time.

Despite there being both federal and provincially managed water quality monitoring programs, there was a limited amount of consistent and reliable data available for use in this study. Our objectives were to measure change in water quality and water quantity at a watershed scale. Considering the increasing emphasis of regional assessments and watershed management, our study shows that data simply do not exist to optimally assess change at this scale. Thus, it is critical to improve our monitoring programs so that data is collected in a continuous and consistent way to facilitate continued broad scale assessments to protect one of our most important natural resources.

Despite it being widely recognized that there is a need to shift from local, project scale CEAs to broader, landscape or regional scale assessments to accurately assess cumulative effects across an entire river ecosystem, no study previous to this has attempted to quantitatively isolate the important water quantity and quality variables which can potentially affect the health and condition of an entire river basin over a period of forty years. This study provides the base for further evaluation of indicators (water quality parameters, sediment quality parameters, indicators of biological populations of communities) along the river continuum. Over time this can reveal indicators that have changed and the magnitude of this change and its potential impact on the Athabasca River basin.

CHAPTER 4: ASSESSMENT OF WATER QUALITY TRENDS CONTRIBUTING TO CUMULATIVE EFFECTS IN THE ATHABASCA RIVER BASIN USING A FATHEAD MINNOW BIOASSAY IN THE LABORATORY

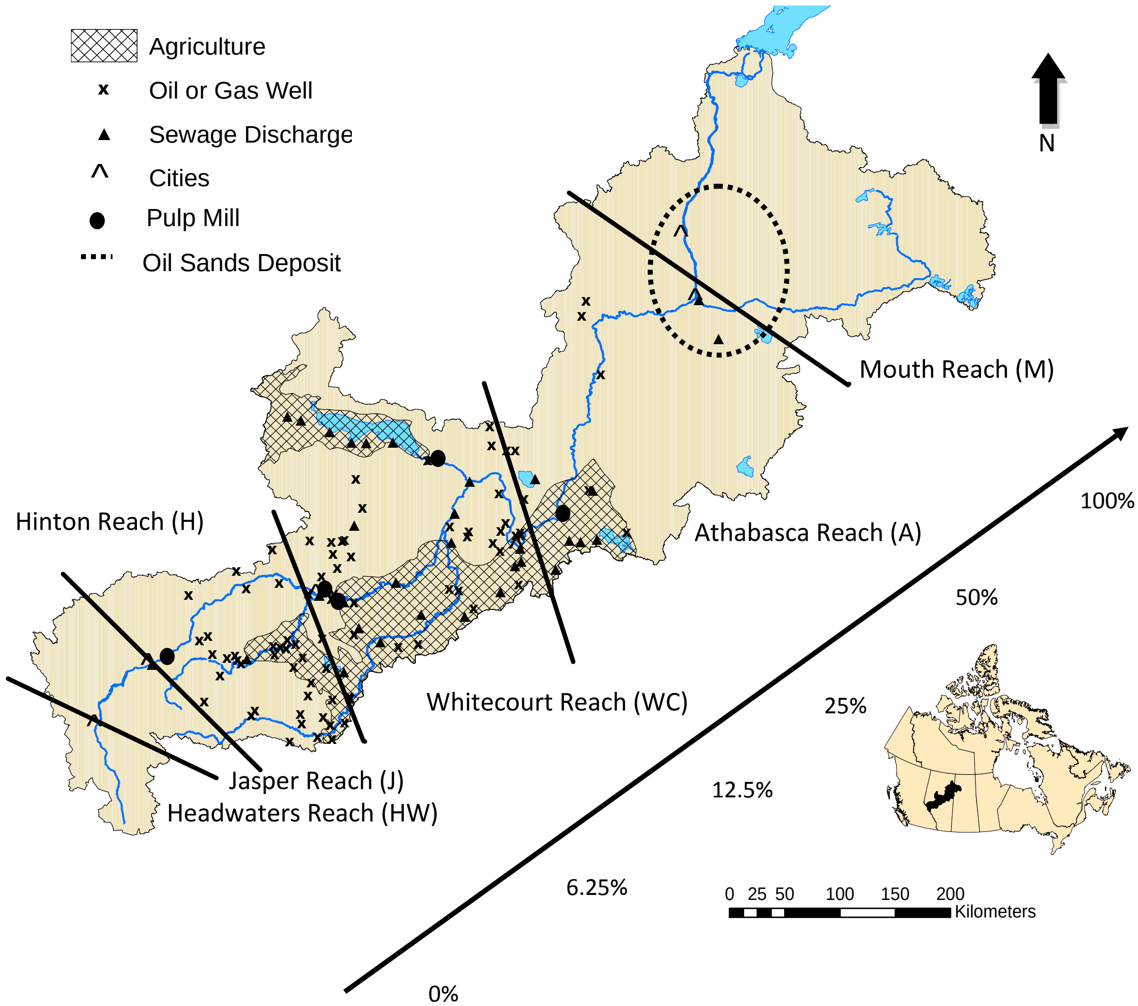
Chapter 4 was submitted to *Environmental Toxicology and Chemistry*.

4.0 INTRODUCTION

The Athabasca River basin covers 157 000 km^2, accounting for approximately 22% of the landmass of Alberta, Canada (Gummer et al. 2000). It originates at the Columbia Ice Fields in Jasper National Park and flows northeast 1300 km across Alberta until it terminates in Lake Athabasca. The Athabasca River is a 6th order stream which flows through four major physiographic provinces: Rocky Mountains, Great Plains, Athabasca Plain and Bear-Slave-Churchill Uplands (Culp et al. 2005).

The Athabasca River has experienced an increasing level of land use related development including forestry/pulp and paper, coal mining, oil and natural gas, agriculture, tourism, wildlife trapping, hunting and oil sands mining (Figure 4.1) (Culp et al. 2005). As a result, several studies have been conducted on portions of the basin and have identified some water quality parameters to be of particular concern (Wrona et al. 2000). Most recently, it has been noted that dissolved sodium and chloride concentrations have significantly increased in the lower reaches of the Athabasca River over 30 years with increases in the present day in the top 10% of levels observed 30 years ago (Squires et al. 2010).

Chloride can come from anthropogenic sources such as road de-icers and marine sediments (Barber et al. 2006). The oil sands area has been shown to have a higher distribution of salinity and there is some evidence to support the idea of the oil sands deposit near Fort McMurray, Alberta to be of marine origin (Adams et al. 2004, Conly et al. 2002, Headley et al. 2005). This provides a natural source of sodium and chloride to this area, and once disturbed through surface mining, these ions may leach into the surrounding surface waters. The MacKay River, which is a major tributary near the mouth of Athabasca River, has shown trends of increasing chloride and sodium concentrations from its headwaters to mouth (Headley et al. 2005). Both sodium and chloride have also been identified as two of the major ions in oil sands tailings release water (Leung et al. 2001).



Dilution Series	Dissolved Sodium (mg/L)	Dissolved Chloride (mg/L)
0 %	22.89 (1.24)	11.11 (0.11)
6.25 %	30.33 (1.84)	22.22 (1.28)
12.5 %	36.11 (0.40)	25.00 (1.02)
25 %	42.89 (0.59)	35.67 (2.50)
50 %	57.00 (0.51)	49.56 (0.11)
100 %	88.00 (0.51)	85.78 (0.11)
10-fold	620.89 (1.74)	829.90 (15.44)

Figure 4.1: Map of the Athabasca River basin showing locations of agricultural, municipal and industrial development. Concentrations as mean +/- (SE) of dissolved sodium and dissolved chloride used per treatment in each respective experiment are listed in the table below. Dilution series is meant to mimic in-river concentrations along the mainstem of the Athabasca River as per Squires et al. (2010).

Concentrations of NaCl in freshwater are typically below 1000μM, however they are variable due to changes in precipitation (Boisen et al. 20003). Human-induced salinization of North American freshwater habitats is recognized as a significant problem (Pistole et al. 2008). Salinity has been shown to be a driving factor in determining the structure of fish assemblages for the past 50 years in prairie streams (Higgins and Wilde 2005). The salinity tolerance of a particular species of fish is related to age and body size (as ratio of gill surface area: body surface area) (Martinez-Alvarez et al. 2002). With chronic exposures to salinity, there is a greater energy cost associated with osmoregulation due to the necessary increase in metabolic rate to repair damage (due to the presence of reactive oxygen species) (Martinez-Alvarez et al. 2002; Pistole et al. 2008).

Salinity is becoming of increasing concern to the industries and its regulators in northern Alberta (Leung et al. 2001). However, the significance of increasing sodium and chloride concentrations on the aquatic biota of the lower Athabasca River is unknown. Freshwater macroinvertebrates maintain their constant internal ionic concentrations in freshwater with passive mechanisms therefore as salinity increases, so does ion intake. Most freshwater macroinvertebrates have an internal ionic concentration of 1,000-15,000 mg/L. At concentrations of 9,000 mg/L, the osmotic gradient across the cell wall causes loss of water from cells via osmosis, eventually causing cell mortality. These effects have also been seen at concentrations as low as 800 mg/L (James et al. 2003).

Fish are dependent on internal (endocrinological and neuroendocrinological) and external (salinity, temperature, photoperiod, etc.) factors to control the many activities and functions contributing to their survival (Boeuf and Payan 2001). Freshwater fish use active processes to regulate their internal ionic concentrations and can tolerate concentrations of 7,000-13,000 mg/L. The presence of low levels of sodium chloride is thought to reduce stress by helping to maintain the osmotic gradient balance by reducing the diffusion of ions in the water (Velasco-Santamaria and Cruz-Casallas 2008). However, when external salinity is greater than internal, these mechanisms begin to fail eventually leading to lower fitness and mortality.

Concentrations of dissolved sodium and chloride have shown significant increases along the Athabasca River over the past decades, and have the potential to become issues of the concern in future assessments, especially if development trends in the basin continue (Squires et al. 2010). It is unknown at this time at what levels we can expect to see adverse sublethal effects on fish and aquatic life. Studies to date have predominately focused on mortality and very few have attempted to assess the sublethal effects on fish with exposure to dissolved sodium or chloride. It is known that the earlier life stages of fish are much less tolerant of exposure to salt concentrations, leading to poor egg and fry survival rates (James et al. 2003). However, it is unknown at what levels these effects may be seen.

The FHM (*Pimephales promelas*) is widely distributed across North America. It is a freshwater species that is easy to raise and breed under laboratory conditions due to its relatively rapid life cycle. It is a species that is commonly used in standard toxicity testing and several protocols have been developed for culturing and handling as well as toxicity testing (Ankley et al. 2001; Environment Canada, 1992). The objectives of this study were to 1) assess changes in FHM indicators associated with increasing concentrations of dissolved chloride and sodium at concentrations deemed to be of importance to the Athabasca River and 2) to determine effect thresholds for FHM exposed to dissolved chloride and sodium.

4.1 METHODS

To assess potential changes in fish reproduction the FHM partial life-cycle bioassay was utilized. These experiments were held under controlled conditions (16:8 light: dark photoperiod and water temperature of 25^{o}C +/- 1^{o}C) and were based on partial life cycle tests originally developed by Ankley et al. (2001) and further refined by Rickwood (2006). This assay allowed us to assess the reproduction of FHMs, as well as aspects of their early development in a time frame much shorter than a traditional life cycle bioassay. All experiments were conducted at the Aquatic Toxicology Laboratory Facility located in the Western College of Veterinary Medicine at the University of Saskatchewan.

4.1.1 Water Chemistry

To expose fish to test solutions, a diluter system was used that allowed for a six time dilution of a 100% test solution with three replicates per dilution. The 100% concentrations chosen for these experiments were based on the highest levels found along the river continuum of the Athabasca River as published in previous research (Squires et al. 2010) and therefore were ecologically relevant to the Athabasca River basin. The frequency distribution of dissolved chloride and sodium concentrations in the Athabasca River mainstem from 1966-1976 and 1996-2006 are shown in Figure 4.2. The concentrations for each dilution series are outlined in Figure 4.1. The dilution series used was 0% (control), 6.25%, 12.5%, 25%, 50% and 100%. In addition to this dilution series, a seventh treatment consisting of a 10-fold concentration of the 100% test solution (also replicated 3 times) was used to simulate potential future worst-case scenario. Based on previous studies, sodium carbonate ($NaCO_3$) was used to provide dissolved sodium to test waters (Mount et al. 1997). To maintain the pH of these test solutions between 7 and 8, sulphuric acid (H_2SO_4) was also added. Due to issues with maintaining hardness and salinity levels, potassium chloride (KCl) was chosen to introduce dissolved chloride into the test solutions. Control water was a 60:40 mixture of treated laboratory tap water and RO water to reduce the background levels of dissolved chloride and sodium in the control treatment. Each test tank was completely turned over 4 times daily.

Samples of water from each treatment were collected daily during the exposure period and analyzed for general chemistry parameters. These included dissolved oxygen, temperature and conductivity (YSI portable meter, Yellow Springs Instrument, Yellow Springs, OH), pH(Oakton phTestr30, San Francisco, CA), hardness (Hatch Test Kit Model 5-EP MG-L, Loveland, CO), and ammonia (Hannah Instruments, Hungary, Europe). In addition, each treatment was sampled on a weekly basis and submitted to ALS Laboratories (Saskatoon, SK) and analyzed for either dissolved sodium (Method APHA 3120 B-ICP-OES) or dissolved chloride (Method APHA 4500 CL-E) to ensure the accuracy of these test solutions throughout the exposure period.

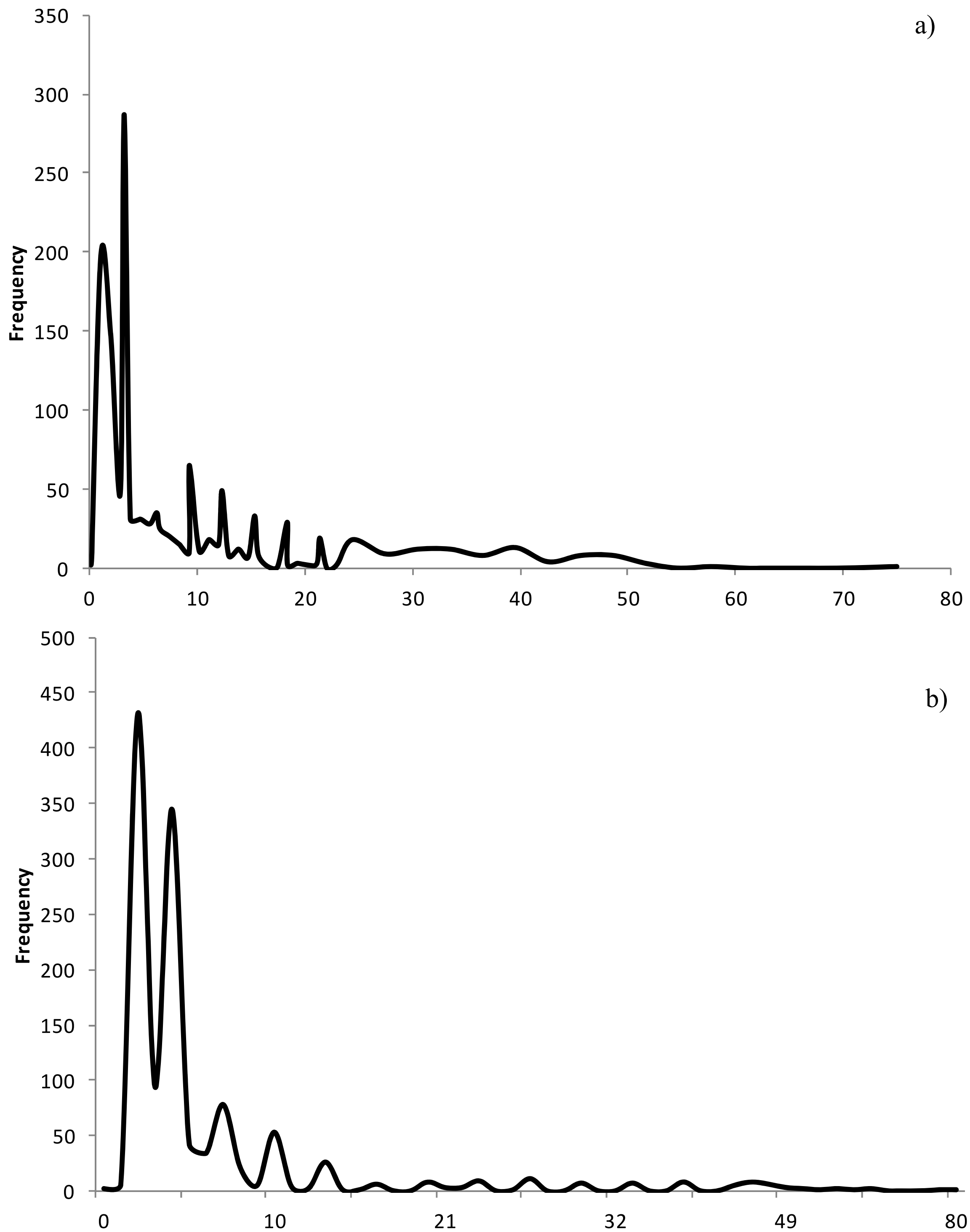


Figure 4.2: Frequency distributions of actual in-river concentrations along the mainstem of the Athabasca River from 1966-19776/1996-2006 for a) dissolved sodium and b) dissolved chloride as used in Squires et al. (2010).

4.1.2 Fish Reproduction

Experiments were conducted using 6-9 month old FHMs obtained from Osage Catfisheries Inc. (Osage Beach, MO, USA). This work was approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use. The first phase of these experiments was a pre-exposure phase lasting 7-10 days. During this phase, approximately twice the number of breeding trios (1 male:2 females) of FHMs required for the exposure phase were each placed into a 9L aquarium with a breeding tile (a section of pvc pipe cut in half length wise) and an air stone. Fish were fed frozen brine shrimp and bloodworms twice daily. The secondary sexual characteristics, total body weight and total length of each fish were recorded prior to addition to the test tanks. Previous research has suggested that length ratio of the male fish to female fish is a good indicator of successful breeding, and therefore each of the females was length matched (ideally 75% of the length of the male) (Pollock et al. 2008). Every 24 hours, each breeding tile was checked for the presence of eggs. If eggs were present they were scraped off the tile and photographed using a Cannon Powershot digital camera (Model A620, Mississauga, ON) and examined using a Vista vision™ (Model 48402-00, VWR International, Mississauga, ON) tri-nocular microscope for counting. After this phase, three breeding trios that meet test criteria (minimum of 80% fertilization, bred at least once, and adults survived the entire pre-exposure period) were randomly assigned to each treatment. Mean egg production and fertilization success of each treatment were tested using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data that did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. This was done to ensure treatments were not statistically different from each other ($p > 0.05$) and therefore, prior to exposure, each treatment had the potential for similar egg production.

The second phase of these experiments was an exposure phase lasting 21 days. During the exposure period each of the breeding trios that were randomly assigned in the pre-exposure phase were exposed to a particular concentration of dissolved sodium or dissolved chloride (see Figure 4.1). Throughout the exposure phase, breeding tiles were checked daily for eggs and removed from the tile and photographed for counting. Eggs were then placed in pvc cups with a mesh bottom and air stone and placed in separate tanks filled with the same concentration of exposure water as the corresponding test tank of their parents. Eggs were checked after 48 hours and then daily after that and the stages of development were recorded (eyed, first hatched, fully hatched). Eggs that were eyed were considered fertilized and the batch of eggs was then photographed for counting (% fertilized) afterwards. The total number of eggs, number of fertilized eggs, time to hatch, and number of larvae (alive, deformed and dead) after 5-days post-hatch were recorded. After five days, post-hatch larvae were anaesthetized using methane tricainesulfonate (MS222, ~1000 mg/L) and preserved in 10% formalin. After 21 days of exposure, the adult fish were anaesthetized using methane tricainesulfonate (MS222, ~1000 mg/L) and then euthanized using spinal severance prior to further processing. Secondary sexual characteristics, total body weight, total length, carcass weight, liver weight and gonad weight were recorded. The second gill arch was removed and preserved in 10% formalin for further histological examination.

4.1.3 Gill Histology

Gill arches were submitted for further processing to Prairie Diagnostic Services (Saskatoon, SK, Canada). Each arch was embedded using routine parafilm processing techniques and then stained using a standard hematoxylin eosin stain. Following staining they were sliced and 2-3 slices were placed on a slide and covered for further examination. Slides were examined using an Axio Observer Z1 microscope (Carl Zeiss MicroImaging GmbH, Gottingen, Germany) and photographed using an AxioCam ICc1. Each photograph was then loaded and processed using Axio Vision Rel. 4.7 software located in the Toxicology Centre, University of Saskatchewan (Saskatoon, SK, Canada). Prior to examination all slides were randomly assigned a number to remove potential for analytical bias. One picture was taken of each gill arch where the third primary lamella from the left side of each arch was selected for further measurement.

On the right side of this primary lamella, three secondary lamellae at the bottom, three in the middle and three at the top were measured for secondary lamellar length (SLL) and secondary lamella width (SLW). In addition, one measurement of the basal epithelium thickness (BET) was taken at each of these three points along the secondary lamella. In an effort to further quantify the diffusion distance across the gills, an additional endpoint was calculated using the average SLW and average SLL for each gill arch to calculate the ratio of width and length of each arch (SLW/SLL). A high ratio of SLW to SLL would mean that the lamella is wide and short, thereby creating a larger distance for ion diffusion across the gill. However, a low ratio of SLW to SLL would mean that the lamella is thin and long, which would reduce the distance required for ion diffusion across the gill.

The averages of all these measurements on each gill arch and the additional endpoint (SLW/SLL) were then analyzed using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. Differences were considered to be significant when $p<0.05$.

4.1.4 Statistics

At the end of the exposure period fish metrics, reproductive endpoints and larval endpoints were analyzed. All statistical analyses were performed using SPSS® 17 (SPSS Inc., Chicago, Il, USA) and graphed using Sigmaplot® Version 11 (San Jose, CA, USA). Results were considered significant when $p<0.05$.

To assess cumulative frequency data the Kolmogorov-Smirnov test was used. These endpoints included: cumulative eggs/female [Cum. # eggs produced per treatment/# of living females/# of days] which factors in the effects of mortality on egg production and represents population effects over time; and cumulative spawning events [Cum. total # spawning events/treatment/day]). One-way ANOVA's or non-parametric equivalent (Kruskal Wallis) tests were conducted on mean egg data. A one-way ANOVA or its non-parametric equivalent was conducted on the following endpoints: hatching success, percent deformities, LSI (liver weight(g)/body weight(g)*100), GSI (gonad weight(g)/body weight(g) * 100), condition factor [(body weight(g)/total length(cm)^3) * 100], mean total egg production [total # of eggs produced per breeding group/ # of females in group/ # of exposure days], mean egg production [mean # eggs produced per stream/ # of females in group/# of exposure days]) and water quality. The one-way ANOVA was used when the data met assumptions (normal distribution and homogeneity of variance) which were analyzed using Levene's and Shapiro Wilk's tests. If data did not meet these assumptions they were transformed (log transformation of continuous or derived data and arcsine transformation of percentage-based or ratio scaled data). If data still did not meet these assumptions, the non-parametric equivalent of the one-way ANOVA (Kruskal-Wallis test) was conducted. Differences among treatment groups were further assessed using a Dunnetts *post hoc* or non-parametric Mann-Whitney-U test.

4.2 RESULTS

4.2.1 Water Chemistry

Water quality parameters for both the dissolved sodium and dissolved chloride experiments are listed in Table 4.1. Dissolved oxygen, temperature, pH, ammonia and conductivity all fell within accepted limits (CCME, 2005). Conductivity was significantly higher in all treatments in both the dissolved sodium and dissolved chloride experiment compared to their respective controls (0%). In the dissolved sodium experiment, pH was significantly higher in the 25%, 50%, 100% and 10-fold treatments compared to the 0% control. In the dissolved chloride experiment, pH was significantly higher in the 12.5%, 25%, 50%, 100% and 10-fold treatments than the 0% control.

Actual measured concentrations of dissolved sodium and chloride in each treatment are listed in Figure 4.1. The concentrations of dissolved sodium ranged from 22.89 mg/L (0% control) to 620.89 mg/L (10-fold). The concentrations of dissolved chloride ranged from 11.11 mg/L (0% control) to 829.90 mg/L (10-fold).

4.2.2 Fish

Survival, condition factor, LSI, GSI, body weight, forklength and secondary sexual characteristics in both male and female fish were not significantly different among treatments in both the dissolved sodium and chloride experiments (data not shown).

Survival was not significantly different among treatments for the dissolved chloride experiment ($p = 0.052$) however there were several deaths recorded throughout the exposure period. These included 2 in the 0% (control), 1 in the 25%, 1 in the 50% and 1 in the 100% treatments. All but 2 males died in the 10-fold treatment. All of the females in the 10-fold treatment died within four days of starting the exposure. There was one death in the 100% treatment of the dissolved sodium experiment.

Table 4.1: General chemistry parameters (pH, temperature, dissolved oxygen, ammonia, and conductivity) averaged for each treatment across the duration of the exposure period (21 days) of each experiment. Mean +/- (SD). Statistical significance denoted by * = $p \leq 0.05$ versus 0% control and ** = $p \leq 0.001$ versus 0% control.

Experiment	Dilution Treatment	Dissolved Oxygen (mg/L)	Temperature (˚C)	pH	Ammonia (mg/L)	Conductivity (µS/cm)
Dissolved Sodium	0 %	7.69 (0.39)	24.0 (0.9)	7.87 (0.20)	0.16 (0.18)	423.8 (3.9)
	6.25 %	7.53 (0.50)	24.3 (1.1)	7.88 (0.17)	0.10 (0.14)	456.2 (6.1) **
	12.5 %	7.54 (0.47)	24.2 (1.1)	7.90 (0.14)	0.09 (0.13)	476.0 (7.9) **
	25 %	7.53 (0.50)	24.3 (1.0)	7.93 (0.11) *	0.09 (0.11)	496.3 (51.2) **
	50 %	7.57 (0.47)	24.2 (1.0)	7.92 (0.22) *	0.14 (0.21)	556.3 (83.7) **
	100 %	7.61 (0.43)	24.1 (0.9)	8.08 (0.16) **	0.08 (0.07)	727.2 (25.9) **
	10-fold	7.73 (0.42)	23.6 (0.5)	8.68 (0.21) **	0.16 (0.21)	2857.8 (119.0) **
Dissolved Chloride	0 %	7.52 (0.25)	24.7 (0.9)	7.93 (0.11)	0.04 (0.06)	379.4 (31.5)
	6.25 %	7.57 (0.24)	24.2 (0.9)	7.93 (0.07)	0.04 (0.07)	415.5 (46.4) **
	12.5 %	7.50 (0.24)	24.3 (0.8)	7.95 (0.05) **	0.06 (0.10)	425.7 (89.2) **
	25 %	7.54 (0.21)	24.3 (0.7)	7.97 (0.04) **	0.07 (0.11)	481.3 (31.8) **
	50 %	7.56 (0.20)	24.3 (0.6)	8.02 (0.04) **	0.02 (0.04)	543.6 (20.5) **
	100 %	7.54 (0.21)	24.4 (0.7)	8.07 (0.05) **	0.03 (0.06)	697.9 (20.0) **
	10-fold	7.56 (0.29)	23.4 (1.0)	8.27 (0.24) **	0.03 (0.06)	3253.1 (349.1) **

4.2.2.1 Reproduction

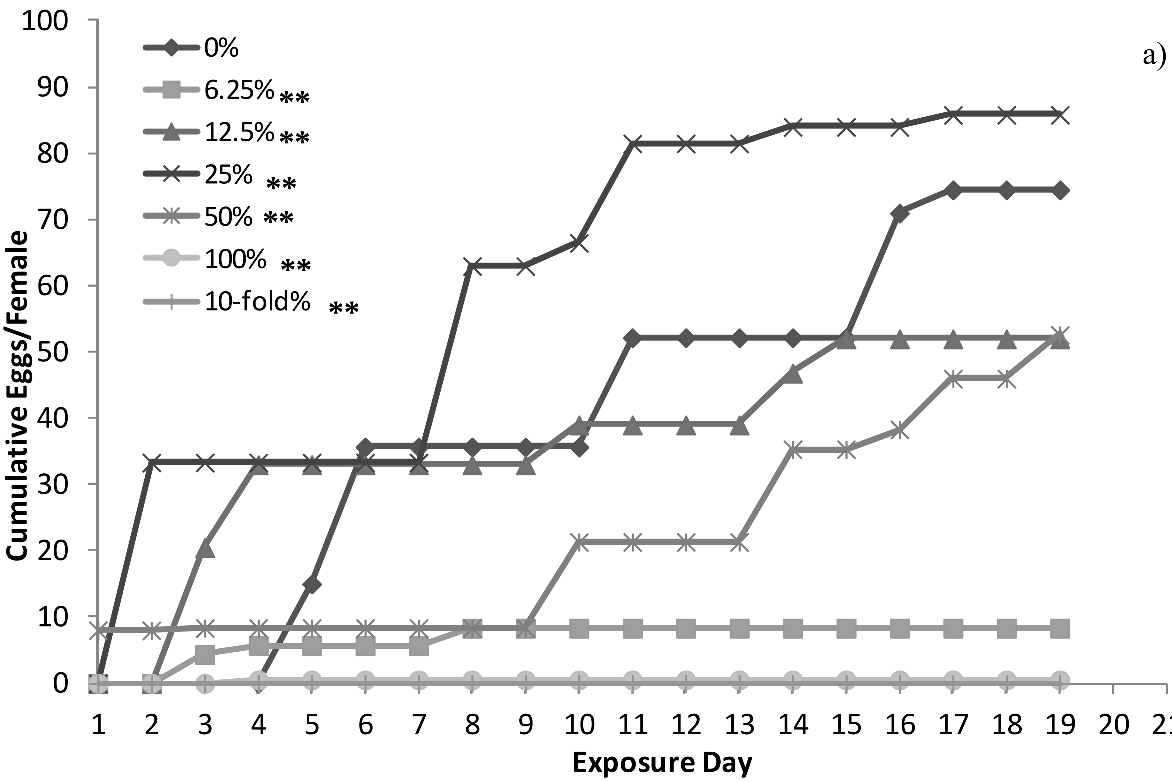
Hatching success, percent deformities, and mean total eggs per female per day showed no significant differences among treatments in either the dissolved sodium or dissolved chloride experiments ($p>0.05$). Due to a power outage, the exposure phase of the dissolved sodium experiment was terminated at 19 days.

For the dissolved sodium experiment, cumulative egg production in all treatments differed significantly from the control (0%) ($p < 0.001$). All treatments had lower cumulative egg production with the exception of the 25% treatment which had significantly higher cumulative egg production than the control (Figure 4.3). There were significantly fewer cumulative spawning events in both the 100% and 10-fold treatments ($p<0.001$) (data not shown).

Mean eggs per female per day (E/F/D) did not show a significant difference among dissolved sodium treatments ($p>0.05$). The highest mean egg production occurred in the 50% treatment, decreasing gradually down to the lower treatments (25%, 12.5%, 6.25%). There was also little to no mean E/F/D in both the 100% and 10-fold treatments. This results in a hormetic trend that is indicative of essential elements such as sodium (Figure 4.3).

Cumulative egg production in all dissolved chloride treatments was significantly lower than the control (0%) ($p <0.05$) (Figure 4.4). Significant decreases in cumulative spawning events were seen in the 6.25%, 12.5% and 10-fold treatments ($p<0.05$) (data not shown). There was no egg production in the 10-fold treatment.

Mean eggs per female per day (E/F/D) did not show a significant difference among dissolved chloride treatments ($p>0.05$). The highest mean egg production occurred in the control followed by the 25% treatment, and then gradually decreasing down in the higher treatments (25%, 50%, 100%). There was no mean E/F/D in the 10-fold treatment (Figure 4.4).



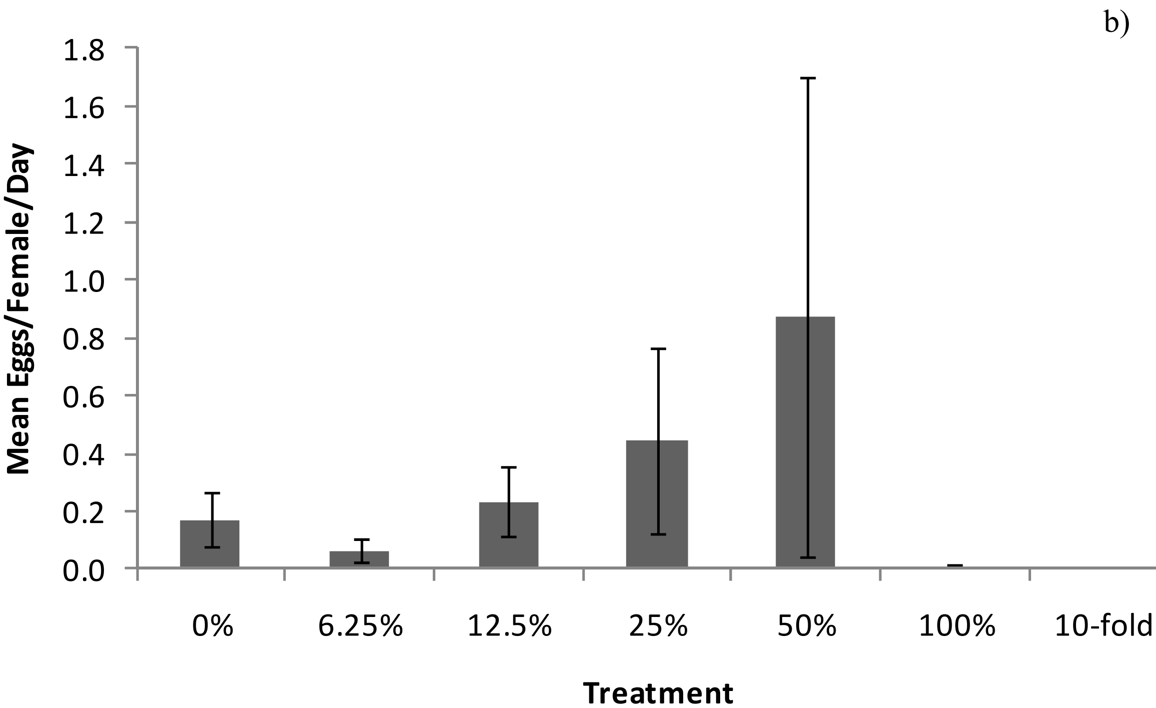


Figure 4.3: Cumulative eggs/female (a) and mean eggs/female/day (b) for the 21 day exposure period in the dissolved sodium experiment. Statistical significance was assessed in the cumulative data using Kolmogorov-Smirnov test and for the mean data using a one-way ANOVA's or non-parametric equivalent (Kruskal Wallis) and differences among treatment groups were further assessed using a Dunnetts post hoc or non-parametric Mann-Whitney-U test. Statistical significance is denoted by ** = p ≤ 0.001 versus 0%.

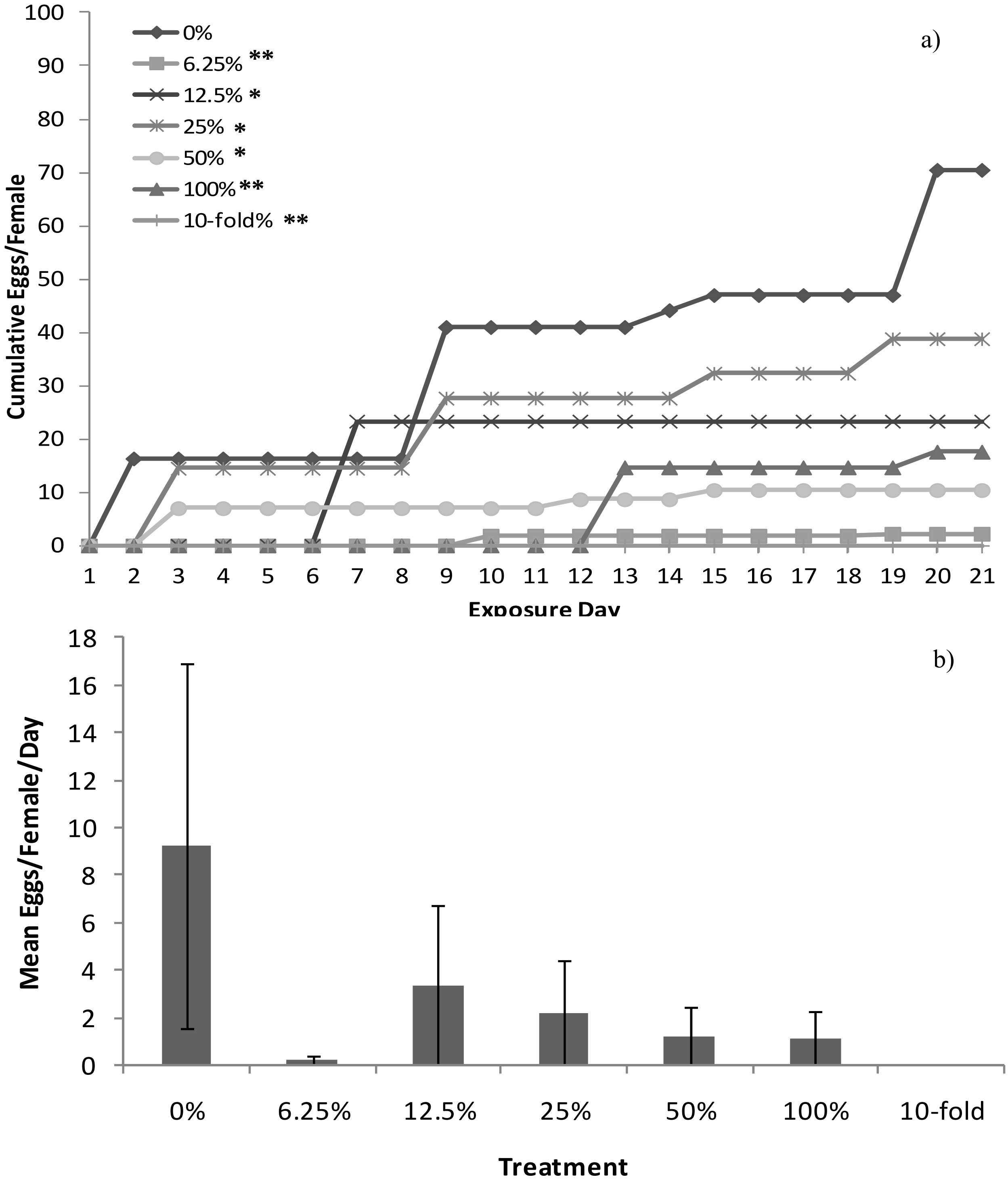


Figure 4.4: Cumulative eggs/female (a) and mean eggs/female/day (b) for the 21 day exposure period in the dissolved chloride experiment. Statistical significance was assessed in the cumulative data using Kolmogorov-Smirnov test and for the mean data using a one-way ANOVA's or non-parametric equivalent (Kruskal Wallis) and differences among treatment groups were further assessed using a Dunnetts post hoc or non-parametric Mann-Whitney-U test. Statistical significance is denoted by * = $p \leq 0.05$ versus 0% and ** = $p \leq 0.001$ versus 0%.

The percent change from control for total eggs/female/day was calculated for both the dissolved sodium and dissolved chloride experiments (Figure 4.5). In the dissolved sodium experiment, the only negative changes were in the 6.25% (30.33 mg/L), 100% (88.00 mg/L) and 10-fold (620.89 mg/L). In the dissolved chloride experiment, all treatments were lower than the control; however the greatest decreases were also observed in the 6.25% (22.22 mg/L), 100% (85.78 mg/L) and 10-fold (829.89 mg/L).

4.2.2.2 Gills

Gill histology endpoints for dissolved sodium and dissolved chloride are shown in Figures 4.6, 4.7 and 4.8. Representative micrograph pictures of a normal and impacted gill arch are shown in Figure 4.6. A negatively impacted gill arch showed signs of fusion and loss of secondary lamella which can impede the transfer of ions across this sensitive area. There was no statistically significant change in the basal epithelium thickness (BET) in either the dissolved sodium or dissolved chloride treatments. For dissolved sodium, the SLW/SLL was significantly different from the control for all treatments excepting the 10-fold treatment (Figure 4.7). The lowest was in the 25% treatment increasing outwards to the 6.25% and 100% treatments. For dissolved chloride, the 10-fold treatment was significantly lower from the control and 12.5%, 25% and 100% were higher than the control for SLW/SLL (Figure 4.8).

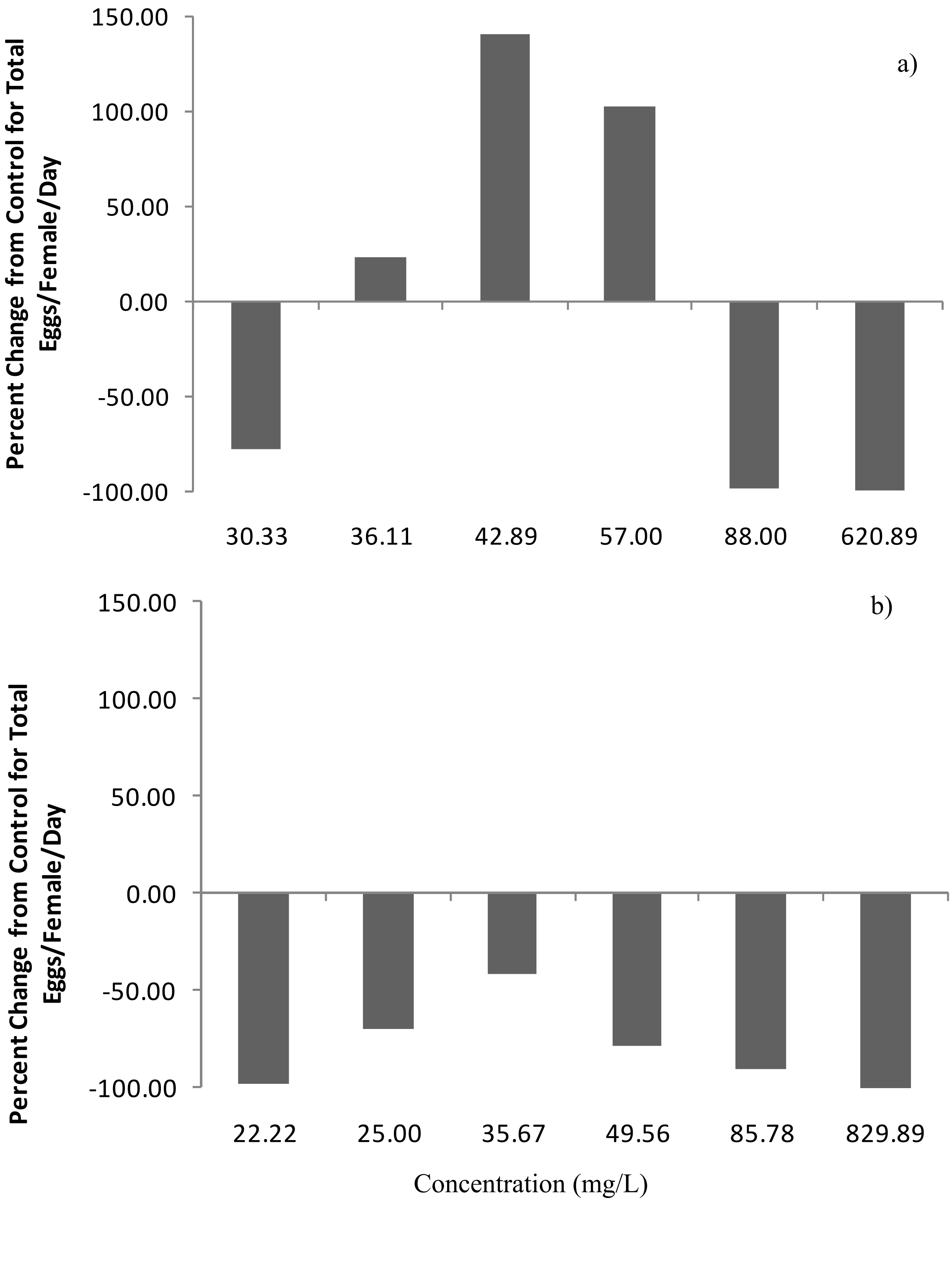
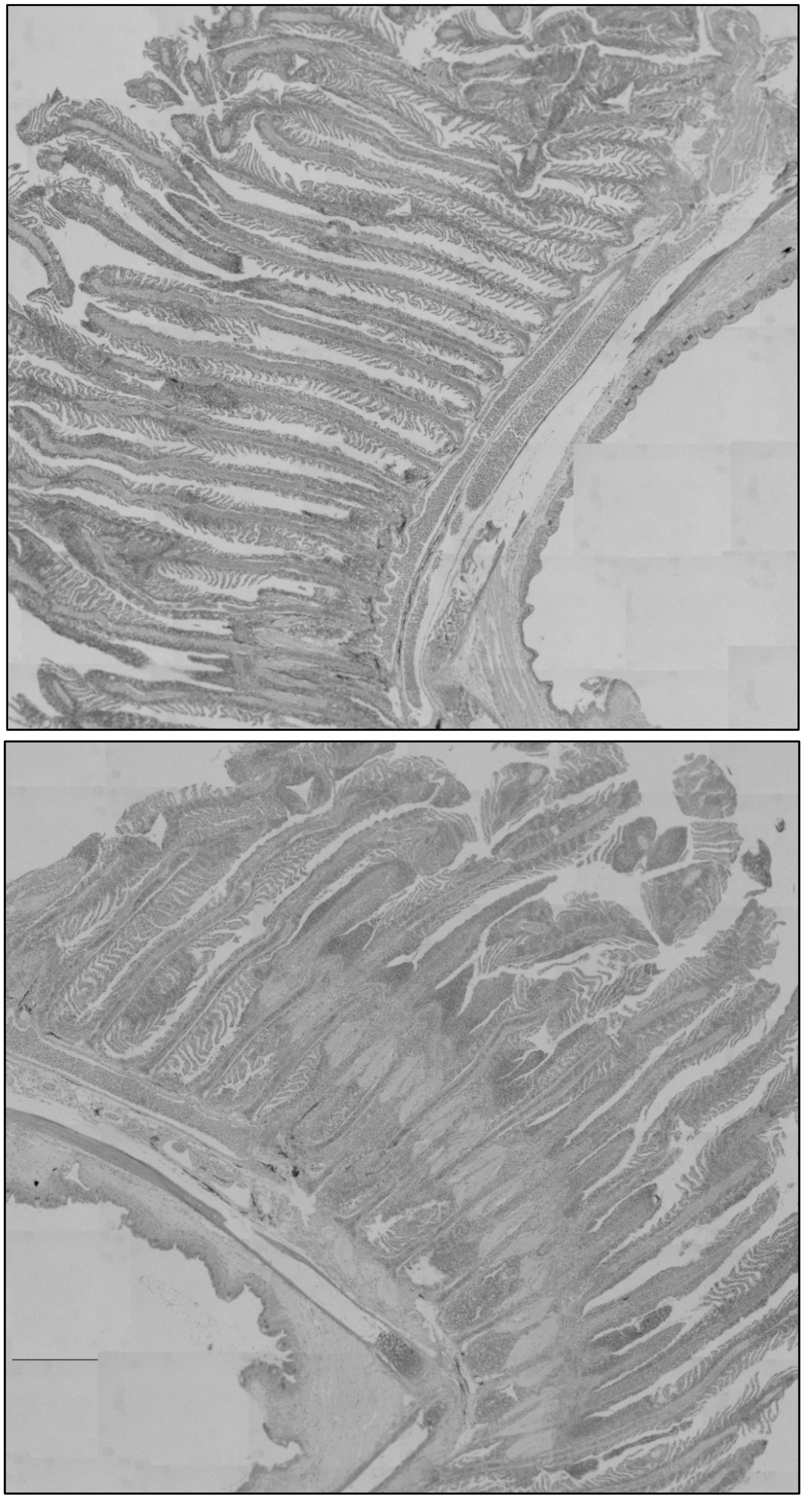


Figure 4.5: Percent change from control for total eggs/female/day versus concentration (mg/L) for (a) dissolved sodium and (b) dissolved chloride.



a)

b)

Figure 4.6: Representative micrograph pictures of a (a) normal and (b) impacted gill arch

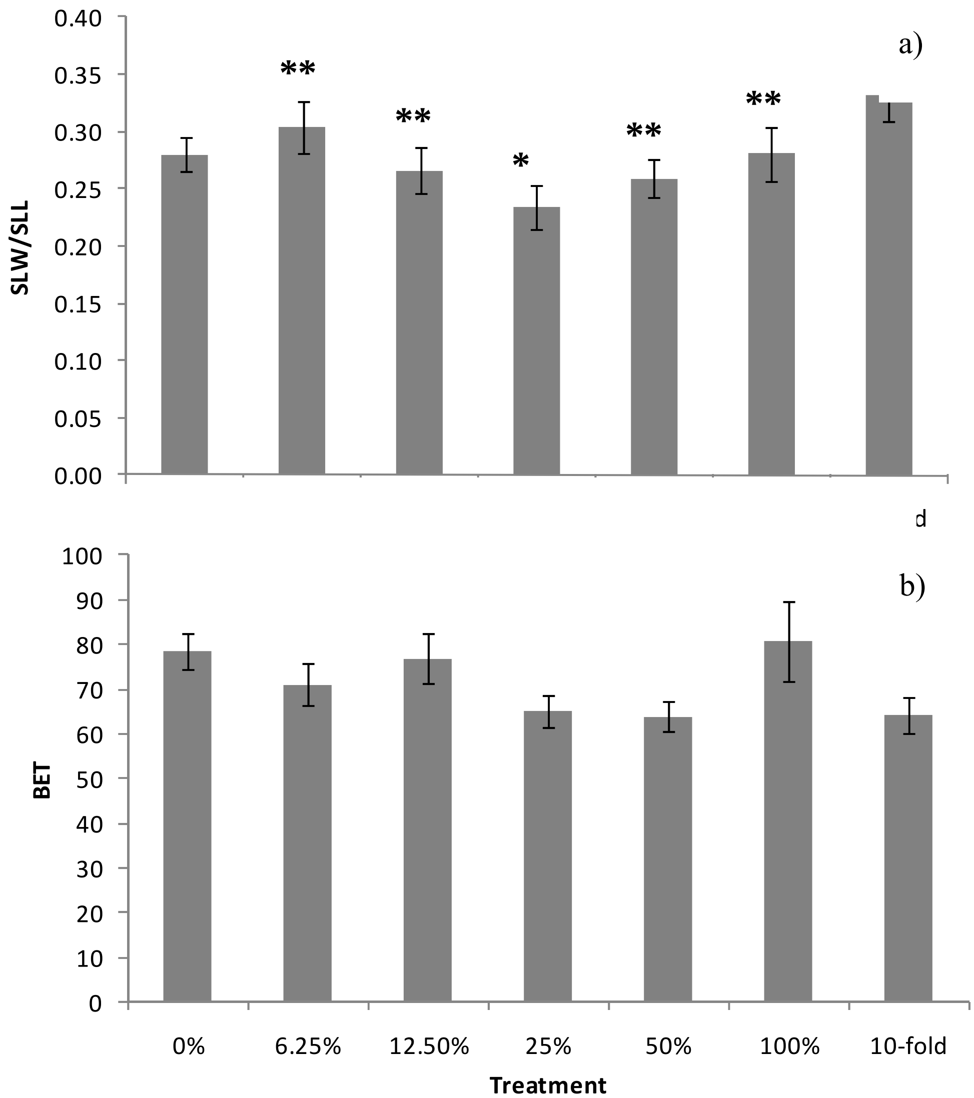


Figure 4.7: The ratio of secondary lamella width (SLW) to secondary lamellar length (SLL) (a) and basal epithelium thickness (BET) (b) for the 21 day exposure period in the dissolved sodium experiment. All data are reported as mean ± SE. Statistical significance was analyzed using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. Differences were considered to be significant when $p<0.05$. Statistical significance is denoted by * = $p \leq 0.05$ versus 0% and ** = $p \leq 0.001$ versus 0%.

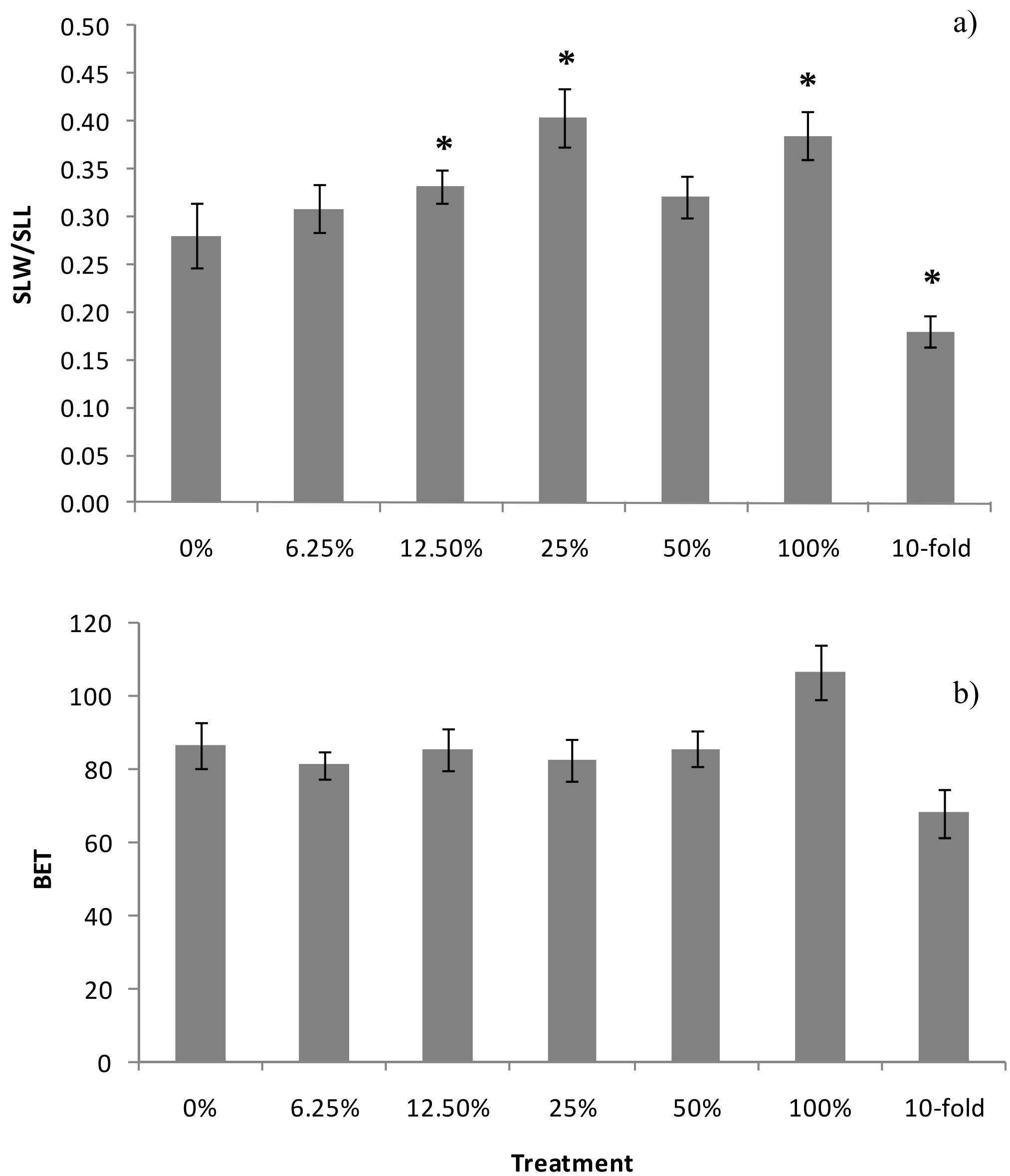


Figure 4.8: The ratio of secondary lamella width (SLW) to secondary lamellar length (SLL) (a) and basal epithelium thickness (BET) (b) for the 21 day exposure period in the dissolved chloride experiment. All data are reported as mean ± SE. Statistical significance was analyzed using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. Differences were considered to be significant when $p<0.05$. Statistical significance is denoted by * = $p \leq 0.05$ versus 0% and ** = $p \leq 0.001$ versus 0%.

4.3 DISCUSSION

4.3.1 Water Chemistry

Conductivity levels increased up to 7 fold and 8 fold in the dissolved sodium and dissolved chloride experiments respectively. This result is not unexpected since conductivity is a measure of the ionic content of water and is therefore dependent on the concentrations of ions such as sodium and chloride. With increasing concentrations of these ions, conductivity is also expected to increase.

Work by Kimmel and Argent (2010) suggests that the threshold for in-stream conductivity impairment to fish communities is 3000 – 3500 uS/cm. The highest levels reached in the dissolved sodium and dissolved chloride experiments were 2857.8 uS/cm and 3253.1 uS/cm respectively (Table 4.1). Therefore it can be reasonably concluded that the increasing conductivity among treatments does not likely have an adverse effect on FHM survival and reproduction. Although significantly higher in higher concentrated treatments, pH levels only reached 8.68 and 8.27 pH units in the 10-fold treatments of the dissolved sodium and dissolved chloride experiments (Table 4.1). These levels are within 1 pH unit of the 0% control treatments in both experiments and are therefore not expected to have any effect on the survival and reproduction of FHMs in this study.

Data collected from various government and industry sources were assimilated and significant trends were found to exist for both dissolved chloride and dissolved sodium both spatially and temporally (1966 to 2006) in the Athabasca River (Squires et al. 2010). From this data, it was determined that the peak concentrations in the Athabasca River during this time period were 72.9 mg/L and 78.1 mg/L for dissolved sodium and chloride respectively (Figure 4.2). The concentrations of dissolved sodium and dissolved chloride in the 100% treatment (88 mg/L and 85.78 mg/L) (Figure 4.1) used in these studies were chosen to mimic these levels.

Worldwide, salinity levels in freshwaters have been increasing over the recent decades. The United Nations GEMS/Water Programme develops and maintains a global freshwater quality information system with a series of national and international partners. This information system includes measured concentrations of dissolved sodium and dissolved chloride concentrations in freshwater river systems worldwide. In our study, we choose concentrations that represent the highest levels of dissolved sodium (72.9 mg/L) and chloride (78.1 mg/L) found along the Athabasca River within the last 40 years.

Of the countries with available dissolved sodium concentrations within the last decade (2000-2008), 41% had measurements which were greater than or equal to 72.9 mg/L (Table 4.2). These countries include Canada (20.0%), India (23.6%), Morocco (95.5%), Pakistan (3.8%), Poland (44.8%), South Africa (13.3%) and United States of America (23.4%). Of the countries with available dissolved chloride concentrations between the years 2000-2009, 50% had measurements which greater than or equal to 78.1 mg/L (Table 4.3). These include Belgium (49.6%), Fiji (2.1%), India (78.5%), Morocco (98.6%), Pakistan (25.0%), Poland (73.3%), Russian Federation (3.2%), Senegal (25.0%), South Africa (15.1%), Sri Lanka (4.7%), United Kingdom (13.1%) and United States of America (20.7%). Levels of dissolved sodium and chloride similar to those found in the Athabasca River basin are also seen in the majority of cases worldwide in past decades and further illustrates the relevance of the concentrations of dissolved sodium and chloride used in this study.

Salinity concentrations within watersheds are expected to increase in the next decades due to increased urban and industrial pressures. Studies conducted on watersheds which have not undergone significant development in recent years have also shown increased (and in some cases doubled) salt concentrations (Findlay and Kelly 2011). Therefore salinity has the ability to be retained within a watershed without undergoing any significant transport or degradation processes (Findlay and Kelly 2011). The influence of increased human-related developments combined with the absence of significant natural movement or breakdown of salts within watersheds demonstrates the real need to develop relevant thresholds for freshwater biota in order to help regulate these levels.

Table 4.2: Summary of the total dissolved sodium measurements 2000-2009 and measurements found to be greater than or equal to the highest levels found in the Athabasca River over 40 years (percent greater than or equal to 72.9 mg/L) in freshwater rivers worldwide. Data provided by the United Nations GEMS/Water Programme.

Country	Total No. Measurements (2000-2008)	% ≥72.9 mg/L
Argentina	90	0.0
Bolivia	29	0.0
Brazil	245	0.0
Canada	15	20.0
Ecuador	29	0.0
French Guiana	46	0.0
India	1131	23.6
Japan	210	0.0
Morocco	89	95.5
Pakistan	53	3.8
Panama	237	0.0
Peru	37	0.0
Poland	569	44.8
Russian Federation	243	0.0
South Africa	2563	13.3
Switzerland	436	0.0
United States of America	3835	23.4

Table 4.3: Summary of the total dissolved chloride measurements 2000-2009 and measurements found to be greater than or equal to the highest levels found in the Athabasca River over 40 years (percent greater than or equal to 78.1 mg/L) in freshwater rivers worldwide. Data provided by the United Nations GEMS/Water Programme.

Country	Total No. Measurements (2000-2009)	% ≥78.1 mg/L
Argentina	138	0.0
Belgium	3719	49.6
Bolivia	28	0.0
Brazil	166	0.0
Canada	858	0.1
Ecuador	27	0.0
Fiji	37	2.7
French Guiana	48	0.0
India	1363	24.6
Japan	227	0.0
Korea	427	0.0
Morocco	212	98.6
Pakistan	76	25.0
Peru	37	0.0
Poland	569	73.3
Russian Federation	655	3.2
Senegal	4	25.0
South Africa	2311	15.1
Sri Lanka	983	4.7
Switzerland	437	0.0
United Kingdom	666	13.1
United States of America	5530	20.7

4.3.2 Fish Reproduction

Cumulative egg production in all treatments differed significantly from the control (0%) in the dissolved sodium experiment. The 25% treatment had higher egg production than all the rest of the treatments, including the control. This would imply that the level of sodium in the 25% treatment (42.89 mg/L) is the ideal amount of this essential element necessary for fish to maintain basic physiological process and reserve enough energy for reproduction. Mean E/F/D did not show a significant difference among treatments, it did however show a trend typical of most essential elements. The greatest production was in the 50% treatment decreasing on either side to the 6.25% and 10-fold treatments.

Cumulative egg production in all dissolved chloride treatments were significantly lower than the control (0%) ($p < 0.05$) (Figure 4.4). Mean egg production did not show a significant difference among treatments (Figure 4.4). Significant decreases in cumulative spawning events were seen in the 6.25%, 12.5% and 10-fold treatments ($p < 0.05$). Next to the 0% control treatment, the highest level of mean E/F/D occurred in the 25% (42.89 mg/L) treatment. Mean E/F/D decreased outwards with increasing dissolved chloride concentration.

Much of the work on salinity effects on fish to-date is focused on marine or brackish water fish species. Marine fish tend to move solutes passively from the water (hypertonic) while freshwater fish will lose ions to the water (hypotonic) (Prodocimo et al. 2007). Despite this, many brackish water fish species are able to adapt to and tolerate a wide variation in salinity by both reducing and reversing the movement of ions through their surface epithelia (Prodocimo et al. 2007). However, the tolerance of many strictly freshwater fish species is unknown.

The reproductive potential of fish can be influenced by the levels of stressors in the surrounding environment (Schreck 2010). This results in an enhancement of biological fitness with the presence of low levels of required nutrients, but also decreased fitness with high (toxic) concentrations of these nutrients. While high levels of sodium can adversely affect freshwater biota in a variety of ways, sodium is also an essential ion that is required at certain concentrations for freshwater biota to survive.

Most of the work on salinity and freshwater biota has focused on determining the acute (lethal) impacts of this parameter in laboratory studies (Findlay and Kelly 2011). For many species, the short-term lethal concentrations of salt are well above levels which are generally seen in most aquatic systems. For example, Ingersoll et al. (1992) showed that salt levels at 8-10 g/L were acutely toxic to FHMs. However, work on a variety of species has determined that the most sensitive taxonomic groups to salinity are amphibians (Findlay and Kelly 2011). Studies conducted on larval wood frogs have shown median lethal concentrations (96hr LC_{50}) to be as low as 2636.5 mg/L (Sanzo and Hecnar 2006). This study also demonstrated that number of larval undergoing metamorphosis decreases with increasing salt concentrations. These results show how dependant the success of these populations is to the increasing levels of salinity in our aquatic ecosystems.

Our study aimed to assess the chronic impacts of low-level salinity on FHM reproduction. Using the percent change from control for total eggs/female/day we found that the optimal range of dissolved sodium for the reproduction of fathead minnows is between the 12.5% (36.11 mg/L) and 50% (57.00 mg/L). In the dissolved chloride experiment, all treatments were lower than the control suggesting ideal chloride concentrations to prevent reduced egg production in FHM to be less than 22.22 mg/L. The greatest decreases in total eggs/female/day were also seen in the 6.25% (22.22 mg/L), 100% (85.78 mg/L) and 10-fold (829.89 mg/L) dissolved chloride treatments suggesting that reducing the most negative effects would be served by maintaining concentrations ≥ 22.22 mg/L and ≤ 49.56 mg/L. These values are much lower than those found in acute studies, where effects are generally seen in the g/L level. However these acute studies generally focus on survival, whereas our sublethal study is focused on the influence of salts on the reproductive potential of freshwater fish populations. Therefore we would expect to see these sublethal effects occurring at much lower concentrations.

A study conducted by Carneiro and Urbinati (2001) illustrated how low levels of salinity can have a positive effect on fish health. This study showed how the presence of low concentrations of salt can influence the stress response in freshwater fish undergoing transport stress. These authors determined that treatments with at least 0.6% NaCl can significantly reduce plasma cortisol levels, blood glucose, and plasma chloride levels. Fish transported with 0.6% NaCl added to their water also recovered significantly faster (within 24 hrs), versus up to 96 hrs for fish that did not have a salt treatment.

A lot of work on the long-term (chronic) effects of salinity on aquatic ecosystems have stemmed from the use of road salts. A recent study looking at the toxicity of these salts to FHM calculated an IC_{25} value for chloride (for the inhibition of weight) to be 1810 mg/L (Corsi et al. 2010). Further studies by these authors with the invertebrate *C. dubia* showed that no young were produced with chloride concentrations exceeding 1770 mg/L. Chronic concentrations of chloride of 250 mg/L have been recognized as harmful to freshwater life and not potable for human consumption (Kaushal et al. 2005). Although a Canadian Federal water quality guideline for the protection of aquatic life has not yet been released, ambient water quality criteria for chloride were developed by the British Columbia Environmental Protection Division which states that the chronic levels should not exceed 150 mg/L (Nagpal et al. 2003).

Our study showed that FHM reproduction can be positively influenced by the presence of much lower concentrations of dissolved sodium (6.25% (30.33 mg/L), 12.5% (36.11 mg/L), 25% (42.89 mg/L) and 50% (57.00 mg/L)) but then decreases at higher concentrations (100% (88.00

mg/L) and 10-fold (620.89 mg/L)). Previous studies on other freshwater fish species (Redbelly Dace and Northern Studfish) have shown that the metabolic rate of these species (measured as oxygen consumption) increased with increasing salinity (Toepfer and Barton, 1992). This increase in metabolic rate is expected and would be necessary to meet the energetic costs of osmoregulation (Pistole et al. 2008). Previous studies by these authors have also demonstrated that the metabolic demands of maintaining this osmolality balance can overwhelm the capabilities of the fish species to adapt, and cause a depression in metabolic rate at higher concentrations which is an indication of the upper limits of the fish's ability to tolerate or accommodate changes induced by exposure to salinity (Pistole et al. 2008). Consequently, while fish could survive these higher salinity levels they were unable to maintain normal regulation and function.

There was no observable reproduction in the 10-fold treatment of the dissolved chloride experiment, and can likely be attributed to the high mortality rate in this treatment. All of the females died within the first four days of exposure, effectively preventing any possibility of breeding with the surviving males. The mortality in this treatment is likely due to the use of KCl as the method of introducing dissolved chloride into the test water. This was considered to be the best option to introduce dissolved chloride into the treatments since it exerted the least amount of influence on other water chemistry parameters such as hardness and salinity which would add other confounding factors (Mount et al. 1997).

The average concentration of potassium in the 10-fold treatment was 913.33 (± 1.35) mg/L and the average concentration of chloride in this same treatment was 829.90 (± 15.44) mg/L. A study conducted by Mount et al. (1997) determined the relative ion toxicity to FHM as being $K^+ > HCO_3^- \sim Mg^{2+} > Cl^- > SO4^{2-}$. Since the toxicity of K^+ is so much greater than Cl^- it is likely that the presence of potassium at such a higher concentration than chloride would make it the dominant ion exerting a toxic effect.

4.3.3 Fish Gills

The fish gill is considered to be one of the most sensitive organs to external pollution exposure due to their direct contact with the water and several methods have been developed to assess morphological and physical changes to this organ (Velasco-Santamaria and Cruz-Casallas 2008). Sodium uptake was previously attributed to a proton exchanger, but most recently has also been attributed to an ATP Na/K channel (Boisen et al. 2003). There is a general agreement that Cl^- is transported across the gills by a Cl/HCO_3 exchanger (Boisen et al. 2003).

For dissolved sodium, the SLW/SLL was significantly lower from the 0% control for the 12.5%, 25% and 50% treatments. The SLW/SLL was significantly higher than the 0% control in both the 6.25% and 100% treatments. A lower ratio of SLW/SLL indicates a shorter diffusion distance across the gill thereby allowing for better osmoregulation. During the dissolved chloride experiment, only the 10-fold treatment was significantly lower than the control for SLW/SLL. This result is not necessarily due to a protective effect; instead it is most likely due to significant gill damage caused by the high concentration of potassium in the 10-fold treatment.

These changes in gill diffusion distances are mimicked in the reproductive output of each treatment. We found that the greatest decreases in the percent change from control for total eggs/female/day for both dissolved sodium and dissolved chloride experiment was in the 6.25%, 100% and 10-fold treatments. These treatments also had the highest diffusion distances in the dissolved sodium experiments. Therefore, in this study there is a demonstrated link between increasing gill diffusion distance and reproductive output in the FHM.

In response to rapid changes in salinity, some freshwater fish species such as Tilapia (*Oreochromis mossambicus*) change the composition of cells in the gills to include more mitochondria-rich cells with greater Na/K ATPase activity (Kammerer et al. 2009). Another factor which contributes to regulation of salinity within fish is the presence of growth hormone which can trigger increases in Na/K ATPase pump activity (Deane and Woo, 2009). An increase in the activity of this pump would allow for faster osmoregulation capabilities, thereby creating a greater tolerance to increasing salinity levels.

Stress can negatively affect the gills ability to maintain the homeostatic balance and as a consequence fish can experience an increased blood flow and permeability of gill membranes (Carneiro and Urbinati 2001). This can result in ion loss and water influx, causing serious and possibility fatal electrolytic disturbances. Severe damage in gill epithelium observed in fish exposed to higher salt concentrations could lead to tissue hypoxia and finally death (Velasco-Santamaria and Cruz-Casallas 2008). The decreasing diffusion distance (SLW/SLL) with increasing reproductive output demonstrates that changes in gill functions can impact the reproductive performance of aquatic biota. This linkage has not previously been demonstrated in FHM.

As discussed, atypically high concentrations of solutes can cause significant stress and damage to fish gill membranes. However, at lower concentrations, this level of stress can cause a protective effect on some aquatic biota. Low levels of stress (such as low concentrations of potentially toxic ions) can induce anti-stress reactions in algae, amphipods and fish including the induction of certain stress proteins which can induce multiple stress resistance (Steinberg et al. 2007).

A study conducted by Contreras-Sanchez et al. (1998) showed that rainbow trout, when exposed to low levels of stress either during the entire maturation process, or during the later stages of maturation, spawned immediately. However, if exposed to that same stress earlier in the maturation process, spawning was delayed. In the present study, stress was introduced much later in the maturation process, and therefore it can be presumed that this may have contributed to the positive effects on reproduction (including egg production).

4.4 CONCLUSIONS

Understanding the consequences of stress on fish populations requires that we understand the effects of stress on individual reproductive output (Schreck et al. 2010). Low levels of dissolved sodium can have a beneficial effect on freshwater fish populations by improving their reproductive performance. Using the percent change from control for total eggs/female/day we found that the ideal range of dissolved sodium is between the 12.5% (36.11 mg/L) and 50% (57.00 mg/L) and dissolved chloride was between 22.22 mg/L and 49.56 mg/L. At levels outside or above these ranges a possible decrease in reproductive output and an increase in gill epithelial damage can occur.

These concentrations are considered to be common in freshwater systems around the world. In the Athabasca River basin concentrations of dissolved sodium along the mainstem of the river were outside of the range 36.11 mg/L and 57.00 mg/L for 94% of the measurements in the time period of 1996-2006 (Squires et al. 2010). The majority (59%) of these measurements outside the range occurred in the low flow months (September-April). Of the concentrations of dissolved chloride measured in the Athabasca River mainstem, 11% exceeded 22.22 mg/L in the time period 1996-2006. Of these, 100% occurred during the low flow months of September-April.

Rivers which experience increased anthropogenic stressors (agricultural, urban and industrial developments) can also experience significant changes in water quality due to increased water use, discharge of effluents and surface run-off. Using laboratory studies, we have determined the importance of the maximum concentrations observed to occur along the Athabasca River mainstem. Further research should be conducted on the Athabasca River to determine the effects of average concentrations of these and other parameters occurring along the mainstem.

CHAPTER 5: ASSESSING THE SUBLETHAL EFFECTS OF IN-RIVER CONCENTRATIONS OF PARAMETERS CONTRIBUTING TO CUMULATIVE EFFECTS IN THE ATHABASCA RIVER BASIN USING A FATHEAD MINNOW BIOASSAY

Chapter 5 was submitted to *Environmental Toxicology and Chemistry*.

5.1 INTRODUCTION

The Athabasca River basin is located in Alberta, Canada and accounts for approximately 22% of the landmass of Alberta (Gummer et al. 2000). It originates at the Columbia Ice Fields in Jasper National Park and flows northeast 1300 km across Alberta until it terminates in Lake Athabasca. The Athabasca River has experienced an increasing level of land use related development over the past five decades including forestry/pulp and paper, coal mining, oil and natural gas, agriculture, tourism, wildlife trapping, hunting and oil sands mining (Wrona et al. 2000; Culp et al. 2005). As a result, several studies have been conducted on portions of the basin which have identified some water quality and quantity issue of particular concern (Wrona et al. 2000; NREI 2004).

Various governmental and non-governmental organizations have conducted independent monitoring programs in this river basin over the past few decades, providing the opportunity to integrate the information in a cumulative effects context at a watershed scale. It is important to assess cumulative effects over large space and time scales since they can occur due to multiple factors including natural phenomena, industrial growth, population growth and economic development.

Near the mouth of the Athabasca River in the Wood Buffalo region of Alberta, Canada there are several oil sands mining operations. As part of the extraction process vast amounts of a slurry called oil sands process water (OSPW) is produced and stored on-site in large lakes and holding ponds (Kavanagh et al. 2011). This OSPW contains several inorganic and organic constituents, some of which have shown to be acutely toxic to aquatic life. Some of these constituents include high levels of salinity, sulphate, ammonia, conductivity and naphthenic acids (Allen 2008). There is the potential for some of these components to make their way into the Athabasca River through seepage and air deposition (Kelly et al. 2010). Previous research has shown that the highest concentrations of sodium and chloride along the Athabasca River mainstem occurred in the mouth reach of the river basin (Squires et al. 2010). Assessing water directly from the mouth of the Athabasca River, downstream of all major inputs would help to better determine the current and potential future impact these developments can have on aquatic biota.

The oil sands deposits are thought to be of marine origin (Conly et al. 2002, Headley et al. 2005). The dominant ions in seawater are chloride and sodium, with other major ions such as sulfate, calcium and potassium (Pillard et al. 2000). As a result, salinity is becoming of increasing concern to the industry and its regulators in northern Alberta (Leung et al. 2001). Recently, it has been noted that dissolved sodium, chloride and sulphate concentrations have significantly increased in the lower reaches of the Athabasca River over 40 years (Squires et al. 2010).

Increases in salinity have been shown to cause both acute and chronic effects at specific life stages. This can result in the exclusion of less-tolerant species which can limit biodiversity and cause a shift in biotic communities (Weber-Scannell and Duffy 2007). To acclimatize successfully to a new salinity involves a large amount of energy to engage several physiological (in the gills, intestine and kidney) and behavioural responses (Serrano et al. 2010). This can often come at the expense of other processes such as growth and reproduction. Laboratory exposures with both sodium and chloride have been shown to alter reproduction in FHM and gill membrane structure (Squires and Dubé 2011 (Chapter 4 this thesis)).

In most freshwater systems, sulphate is found in very low concentrations, the exception being in areas where sulphate-containing ores or anthropogenic activities occur (Davies 2007). Anthropogenic activities which can contribute sulphate include mining, smelting, burning of fossil fuels, agricultural runoff and domestic sewage and may account for up to 90 percent of the sulphur found in surrounding aquatic systems (Davies 2007). Sulfate therefore, is also an ion of interest in the lower Athabasca region.

Based on the present development along the Athabasca River and the results of these previous studies, there is a need to investigate the effects that exposure to sodium, chloride and sulphate at chronic (lower) levels will have on aquatic biota. It is unknown at this time whether or not the levels of these ions that are currently present in the mouth of the Athabasca River are affecting the health and reproduction of aquatic biota living in the area.

The test species chosen for use in this study is the fathead minnow (FHM) (*Pimephales promelas*). It is a freshwater species that is widely distributed across North America. It is easy to raise and breed under laboratory conditions due to its relatively rapid life cycle. Consequently, it is a species that is commonly used in standard toxicity testing and several protocols have been developed for culturing and handling as well as toxicity testing (Ankley et al. 2001; Environment Canada, 1992). The objectives of this study were to: 1) verify the changes in FHM (*Pimephales promelas*) indicators as seen in previous laboratory studies with increasing concentrations of sodium chloride and dissolved sulphate deemed to be of importance to the Athabasca River; and 2) determine the sublethal effects on FHM (*Pimephales promelas*) indicators using water from both the headwaters and mouth of the Athabasca.

5.2 METHODS

To assess potential changes in fish reproduction the FHM (*Pimephalas promelas*) partial life-cycle bioassay was used. This assay allowed for assessment of the reproduction of FHMs, as well as aspects of their early development in a time frame much shorter than a traditional life cycle bioassay and is based on partial life cycle tests originally developed by Ankley et al. (2001) and further refined by Rickwood (2006). All experiments were conducted at the headwaters of the Athabasca River mainstem in Jasper National Park, Jasper, Alberta, Canada using the The Healthy River Ecosystem Assessment System (THREATS) trailer (Figure 5.1) from June-August 2009. The trailer was held under controlled conditions (16:8 light: dark photoperiod and water temperature = $25^{o}C$ +/- $1^{o}C$).

5.2.1 Water Chemistry

To expose fish to test solutions, a diluter system was used that allowed for a six time dilution of a 100% test solution with three replicates per dilution. The 100% concentrations chosen for the sodium chloride and dissolved sulphate experiments were based on the average levels of these parameters found along the river continuum of the Athabasca River as published in previous research (Squires et al. 2010) and therefore were ecologically relevant to the Athabasca River basin. The dilution series used was 0% (control), 6.25%, 12.5%, 25%, 50% and 100%. The salt NaCl was used to generate the 100% test solution for the sodium chloride experiment and the salt $NaSO_4$ was used to generate the 100% test solution for the dissolved sulphate experiment. To obtain enough water to conduct the mouth water experiment, 1800L of water was collected from the Athabasca River mainstem downstream of major oil sand develops and trucked to the experimental site in Jasper National Park. Control water was taken directly from the headwaters of the Athabasca River at the experimental site. Each test tank (replicate) had a complete turnover of water 4 times daily.

Figure 5.1: Picture of the The Healthy River Ecosystem Assessment System (THREATS) trailer that was used for all experiments conducted at Jasper National Park between June-August 2009.

Samples of water from each treatment were collected daily during the exposure period and analyzed for general chemistry parameters. These included dissolved oxygen, temperature and conductivity (YSI portable meter, Yellow Springs Instrument, Yellow Springs, OH), pH(Oakton phTestr30, San Francisco, CA), hardness Hatch Test Kit Model 5-EP MG-L, Loveland, CO), and ammonia (Hagen Ammonia Test Kit A7820). In addition, each treatment was sampled on a weekly basis and submitted to ALS Laboratories (Saskatoon, SK) and analyzed for dissolved sodium (Method APHA 3120 B-ICP-OES) , dissolved chloride (Method APHA 4500 CL-E), total kjeldahl nitrogen (Method APHA 4500N-C-Dig.-Auto-Colorimetry), total organic carbon (Method APHA 5310 B-Instrumental), ammonia (Method APHA 4500-NH3-G), total phosphorous (Method APHA 4500-P-B, C Auto-Colorimetry), total suspended solids (Method APHA 2540 D-Gravimetric), sulphate (Method APHA 3120 B-ICP-OES) and turbidity (Method APHA 2130 B).

5.2.2 Fish Reproduction

These experiments were conducted using 6-9 month old FHMs obtained from Osage Catfisheries Inc. (Osage Beach, MO, USA). This work was approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use. The first phase of these experiments is a pre-exposure phase lasting 7-10 days. During this phase, approximately twice the number of breeding trios (1 male:2 females) of FHMs required for the exposure phase were each placed into a 9L aquarium with a breeding tile (a section of pvc pipe cut in half length wise) and an air stone. Fish were fed frozen brine shrimp and bloodworms twice daily. The secondary sexual characteristics, total body weight and total length of each fish were recorded prior to addition to the test tanks. Previous research has suggested that length ratio of the male fish to females is a good indicator of successful breeding and therefore, each of the females was length matched (ideally 75% of the length of the male) (Pollock et al. 2008). Every 24 hours, each breeding tile was checked for the presence of eggs. If eggs were present they were scraped off the tile and photographed using a Cannon Powershot digital camera (Model A620, Mississauga, ON) and examined using a Vista vision™ (Model 48402-00, VWR International, Missisauga, ON) tri-nocular microscope for counting. After this phase, three breeding trios that met test criteria (minimum of 80% fertilization, bred at least once, and adults survived the entire pre-exposure period) were randomly assigned to each treatment. Mean egg production and fertilization success of each "treatment" before exposure were tested using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. This was done to ensure treatments were not statistically different from each other ($p > 0.05$) and therefore, prior to exposure, each treatment had the potential for similar egg production.

The second phase of these experiments was an exposure phase lasting 21 days. During the exposure period each of the breeding trios that were randomly assigned in the pre-exposure phase were exposed to a particular concentration of dissolved sodium or dissolved chloride (see Figure 2). Throughout the exposure phase, breeding tiles were checked for eggs daily and removed from the tile and photographed for counting. They were then placed in pvc cups with a mesh bottom and air stone and placed in separate tanks filled with the same concentration of exposure water as their parents. Eggs were checked after 48 hours and then daily after that and the stages of development were recorded (eyed, first hatched, fully hatched). Eggs that were eyed were considered fertilized and the batch of eggs was then photographed for counting (% fertilized) afterwards. The total number of eggs, number of fertilized eggs, time to hatch, and number of larvae (alive, deformed and dead) after 5-days post-hatch were recorded. After five days post-hatch, larvae were anaesthetized using methane tricainesulfonate (MS222, ~1000 mg/L) and preserved in 10% formalin. After 21 days of exposure, the adult fish were anaesthetized using methane tricainesulfonate (MS222, ~1000 mg/L) and then euthanized using spinal severance prior to further processing. Secondary sexual characteristics, total body weight, total length, carcass weight, liver weight and gonad weight were recorded. The second gill arch was removed and preserved in 10% formalin for further histological examination.

5.2.3 Gill Histology

Gill arches were submitted for further processing to Prairie Diagnostic Services (Saskatoon, SK, Canada). Each arch was embedded using routine parafilm processing techniques and then stained using a standard hematoxylin eosin stain. Following staining they were sliced and 2-3 slices were placed on a slide and covered for further examination. Slides were examined using an Axio Observer Z1 microscope (Carl Zeiss MicroImaging GmbH, Gottingen, Germany) and photographed using an AxioCam ICc1. Each photograph was then loaded and processed using Axio Vision Rel. 4.7 software located in the Toxicology Centre, University of Saskatchewan (Saskatoon, SK, Canada). Prior to examination all slides were randomly assigned a number to remove treatment bias. One picture was taken of each gill arch where the third primary lamella from the left side of each arch was selected for further measurement.

On the right side of this primary lamella, three secondary lamellae at the bottom, three in the middle and three at the top were measured for secondary lamellar length (SLL) and secondary lamellar width (SLW). In addition, one measurement of the basal epithelium thickness (BET) was taken at each of these three points along the secondary lamella. In an effort to further quantify the diffusion distance across the gills, an additional endpoint was calculated using the average SLW and average SLL for each gill arch to calculate the ratio of width and length of each arch (SLW/SLL). A high ratio of SLW to SLL would mean that the lamella is wide and short, thereby creating a larger distance for ion diffusion across the gill. A low ratio of SLW to SLL would mean that the lamella is thin and long, which would reduce the distance required for ion diffusion across the gill.

The averages of basal epithelium thickness (BET) on each gill arch and the endpoint SLW/SLL were analyzed using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. Differences were considered to be significant when $p<0.05$.

5.2.4 Statistics

At the end of the exposure period, fish metrics, reproductive endpoints and larval endpoints were analyzed. All statistical analyses were performed using SPSS® 17 (SPSS Inc., Chicago, Il, USA) and graphed using Sigmaplot® Version 11 (San Jose, CA, USA). Results were considered significant when $p<0.05$.

To assess cumulative frequency data the Kolmogorov-Smirnov test was used. These endpoints included: cumulative eggs/female [Cum. # eggs produced per treatment/# of living females/# of days] which factors in the effects of mortality on egg production and represents population effects over time; and cumulative spawning events [Cum. total # spawning events/treatment/day]). One-way ANOVA's or non-parametric equivalent (Kruskal Wallis) tests were conducted on mean egg data. A one-way ANOVA or its non-parametric equivalent was conducted on the following endpoints: hatching success, percent deformities, LSI (liver weight(g)/body weight(g)*100), GSI (gonad weight(g)/body weight(g) * 100), condition factor [(body weight(g)/total length(cm)^3) * 100], mean total egg production [total # of eggs produced per breeding group/ # of females in group/ # of exposure days], mean egg production [mean # eggs produced per stream/ # of females in group/# of exposure days]) and water quality. The one-way ANOVA was used when the data met assumptions (normal distribution and homogeneity of variance) which were analyzed using Levene's and Shapiro Wilk's tests. If data did not meet these assumptions they were transformed (log transformation of continuous or derived data and arcsine transformation of percentage-based or ratio scaled data). If data still did not meet these assumptions, the non-parametric equivalent of the one-way ANOVA (Kruskal-Wallis test) was conducted. Differences among treatment groups were further assessed using a Dunnetts *post hoc* or non-parametric Mann-Whitney-U test.

5.3 RESULTS

5.3.1 Water Chemistry

Dissolved oxygen, temperature, pH, ammonia and conductivity measurements for all three experiments are listed in Table 5.1. All parameters were within acceptable limits for aquatic life throughout all three experiments (CCME, 2005). Significant increases in conductivity occurred in all three experiments. In the sodium chloride and dissolved sulphate experiments, 6.25%, 12.5%, 25%, 50% and 100% treatments all had significantly higher conductivity than the 0% control. In the mouth water experiment, significant increases in conductivity occurred in the 25%, 50% and 100% treatments. Table 5.2 lists the levels of dissolved sodium, dissolved chloride, dissolved sulphate, total organic carbon and turbidity in all three experiments. Levels of all three of these parameters were similar across all control treatments.

The levels of dissolved sodium and chloride in the 100% treatment in the sodium chloride experiment were 25.43 and 38.90 mg/L respectively. There was an unrepresentative spike in total organic carbon to 14.80 mg/L in the 12.5% treatment in this experiment. There were no detectable levels of chloride in the dissolved sulphate experiment; however there was 43.23 mg/L dissolved sodium and 101.37 mg/L of dissolved sulphate measured in the 100% treatment of this experiment. There was an increase in TOC in the 100% treatment of the dissolved sulphate experiment to 6.90 mg/L.

In the 100% treatment of the mouth water experiment, the levels of dissolved sodium, dissolved chloride and dissolved sulphate reached 8.23 mg/L, 5.73 mg/L and 14.40 mg/L respectively. There was a significant increase in total organic carbon in the 12.5%, 25%, 50% and 100% treatments compared to the 0% control treatment. Although not significant, there was also an increase in turbidity in the 50% treatment to 5.58 NTU.

5.3.2 Fish

Survival, condition factor, LSI, GSI, body weight, forklength and secondary sexual characteristics in both male and female fish were not significantly different among treatments in the sodium chloride, dissolved sulphate or mouth water experiments. One female died in the 6.25% treatment on day 13 in the sodium chloride experiment and two females died in the 0% control in the dissolved sulphate experiment on days 18 and 20.

5.3.3 Reproduction

Hatching success, percent deformities, and cumulative spawning events showed no significant differences among treatments in the sodium chloride, dissolved sulphate or mouth water experiments ($p>0.05$). There were no significant differences among treatments for cumulative eggs/female, total eggs/female/day and mean eggs/female/day for the sodium chloride experiment compared to the 0% control (Figure 5.2a). However, all treatments except the 12.5% treatment had higher cumulative egg production than the 0% control. Although not significant, only the 25% treatment had lower mean egg production than the 0% control in the sodium chloride experiment (Figure 5.2b).

There was no difference among treatments for cumulative eggs/female, total eggs/female/day and mean eggs/female/day in the dissolved sulphate experiment compared to the 0% control. However at the end of the experiment (day 21 of exposure) all treatments had lower cumulative egg production than the 0% control (Figure 5.3a). Although not significant, there was also an increase in mean egg production in the 25% treatment in the dissolved sulphate experiment (Figure 5.3b).

Table 5.1: General chemistry parameters (pH, temperature, dissolved oxygen, ammonia, and conductivity) averaged for each treatment across the duration of the exposure period (21 days) of each experiment. Mean +/- (SD). Statistical significance was assessed using a one-way ANOVA's or non-parametric equivalent (Kruskal Wallis) and differences among treatment groups were further assessed using a Dunnetts post hoc or non-parametric Mann-Whitney-U test. Significant is denoted by a = $p \leq 0.001$ versus 0% control.

Experiment	Dilution Treatment	Dissolved Oxygen (mg/L)	Temperature (˚C)	pH	Ammonia (mg/L)	Conductivity (µS/cm)
Sodium Chloride	0 %	7.43 (0.88)	20.16 (2.02)	8.11 (0.13)	0.15 (0.26)	156.95 (5.72)
	6.25 %	7.36 (0.69)	20.06 (1.73)	8.10 (0.12)	0.15 (0.26)	168.00 (11.13) [a]
	12.5 %	7.15 (1.38)	20.20 (1.76)	8.09 (0.12)	0.17 (0.44)	171.61 (20.12) [a]
	25 %	7.74 (0.56)	20.16 (1.52)	8.10 (0.09)	0.16 (0.30)	196.32 (23.05) [a]
	50 %	7.52 (1.10)	20.08 (1.84)	8.14 (0.10)	0.07 (0.12)	212.29 (9.78) [a]
	100 %	7.42 (1.16)	19.43 (1.91)	8.12 (0.09)	0.27 (0.51)	292.04 (14.77) [a]
Dissolved Sulphate	0 %	7.04 (1.07)	22.15 (1.58)	8.23 (0.20)	0.13 (0.25)	166.23 (22.39)
	6.25 %	7.15 (0.53)	22.69 (1.24)	8.21 (0.15)	0.15 (0.26)	178.95 (19.15) [a]
	12.5 %	6.73 (1.26)	22.88 (1.32)	8.16 (0.16)	0.19 (0.28)	185.78 (9.05) [a]
	25 %	6.96 (1.06)	22.87 (1.33)	8.15 (0.13)	0.10 (0.17)	206.95 (10.74) [a]
	50 %	6.83 (1.29)	22.58 (1.55)	8.13 (0.14)	0.10 (0.16)	261.44 (10.46) [a]
	100 %	6.51 (1.32)	22.48 (1.65)	8.05 (0.16)	0.07 (0.12)	362.99 (23.46) [a]
Mouth Water	0 %	6.99 (1.32)	19.92 (1.73)	8.03 (0.17)	0.27 (0.51)	165.09 (17.24)
	6.25 %	7.55 (0.87)	20.12 (1.73)	8.09 (0.13)	0.12 (0.17)	161.22 (5.52)
	12.5 %	7.19 (1.09)	20.18 (1.69)	8.05 (0.13)	0.11 (0.15)	166.27 (4.55)
	25 %	7.06 (1.32)	20.46 (1.72)	8.03 (0.11)	0.12 (0.17)	173.86 (4.15) [a]
	50 %	6.78 (1.26)	20.63 (1.56)	8.03 (0.13)	0.10 (0.12)	184.97 (4.38) [a]
	100 %	7.03 (0.78)	21.20 (1.51)	8.05 (0.15)	0.16 (0.24)	213.34 (10.28) [a]

Table 5.2: Dissolved sodium, dissolved chloride, dissolved sulphate, total organic carbon (TOC) and turbidity averaged for each treatment across the duration of the exposure period (21 days) of each experiment. Mean +/- (SD) Statistical significance was assessed using a one-way ANOVA's or non-parametric equivalent (Kruskal Wallis) and differences among treatment groups were further assessed using a Dunnetts post hoc or non-parametric Mann-Whitney-U test. Significant is denoted by a = p ≤ 0.001 versus 0% control. Measurements for TOC and turbidity in the 6.25%, 12.5%, 25%, 50% and 100% treatments are single measurements made during the first week of the experiment.

Experiment	Dilution Treatment	Dissolved Sodium (mg/L)	Dissolved Chloride (mg/L)	Dissolved Sulphate (mg/L)	Total Organic Carbon (mg/L)	Turbidity (NTU)
Sodium Chloride	0 %	1.15 (0.15)	ND	12.73 (1.29)	1.30 (0.30)	4.80 (1.40)
	6.25 %	3.67 (0.58)	4.30 (0.59)	12.53 (1.34)	1.30	5.10
	12.5 %	3.23 (0.30)	4.07 (0.20)	12.53 (1.41)	14.80	5.15
	25 %	8.07 (0.26)	10.83 (0.13)	12.50 (1.21)	ND	5.94
	50 %	11.37 (1.09)	16.63 (1.27)	12.80 (1.15)	1.20	6.04
	100 %	25.43 (0.68)	38.90 (0.15)	13.10 (1.08)	2.10	4.42
Dissolved Sulphate	0 %	1.70 (0.50)	ND	15.03 (0.93)	2.80 (1.60)	3.29 (0.86)
	6.25 %	3.67 (0.20)	ND	18.57 (1.37)	1.30	5.13
	12.5 %	6.13 (0.15)	ND	23.33 (1.79)	1.10	5.07
	25 %	11.00 (0.31)	ND	34.43 (1.91)	1.80	4.07
	50 %	22.00 (0.72)	ND	57.13 (3.10)	1.20	3.87
	100 %	43.23 (1.22)	ND	101.37 (4.88)	6.90	3.28
Mouth Water	0 %	2.10 (0.40)	1.40 (0.40)	13.13 (1.89)	2.00 (0.40)	3.19 (0.38)
	6.25 %	1.87 (0.28)	ND	12.97 (1.63)	3.07 (0.61)	3.57 (0.93)
	12.5 %	2.50 (0.26)	1.53 (0.18)	15.30 (2.81)	4.27 (1.17) [a]	3.27 (0.13)
	25 %	3.80 (0.17)	2.30 (0.17)	13.67 (1.12)	4.80 (0.15) [a]	3.50 (0.39)
	50 %	6.20 (0.31)	4.07 (0.09)	13.83 (0.71)	8.20 (0.38) [a]	5.58 (1.22)
	100 %	8.23 (2.52)	5.73 (2.02)	14.40 (0.61)	10.73 (3.62) [a]	4.11 (0.68)

There was no significant difference among treatments in the mouth experiment for cumulative eggs/female/day, however there was a significant increase in both total eggs/female/day and mean eggs/female/day (Figure 5.4b) in the 50% treatment compared to the 0% control. Although not significant, all treatments (except the 25% treatment) had higher cumulative egg production than the 0% control (Figure 5.4a).

The percent change from control for total eggs/female/day was calculated for all three experiments the (Figure 5.5). In the sodium chloride experiment, the only negative changes were in the 6.25% (3.67 mg/L), and 12.5% (3.23 mg/L). In the dissolved sulphate experiment, three treatments were lower than the control, 6.25% (18.57 mg/L), 12.5% (23.33 mg/L) and 100% (101.37 mg/L). In the mouth water experiment, the only negative change from control was in the 25% treatment (4.8 mg/L TOC). There was an increase of 175% in the 50% treatment (8.20 mg/L TOC).

5.3.4 Gills

There was no change in the basal epithelium thickness (BET) in the sodium chloride experiment (Figure 5.6b). However there was a significant increase in SLW/SLL in the 12.5% ($p<0.001$) and 100% ($p<0.05$) treatments compared with the 0% control (Figure 5.6a). In the dissolved sulphate experiment, there was a significant increase in BET in the 50% treatment ($p = 0.039$) (Figure 5.7b) and significant decrease in SLW/SLL in the 6.25%, 12.5% and 100% treatments ($p<0.05$) compared with the 0% control (Figure 5.7a). For the mouth water experiment, there was a significant decrease in BET in the 100% treatment compared to the 0% control ($p = 0.03$) (Figure 5.8b). There were also significant decreases for SLW/SLL in all treatments compared to the 0% control ($p<0.05$ and $p<0.001$) (Figure 5.8a).

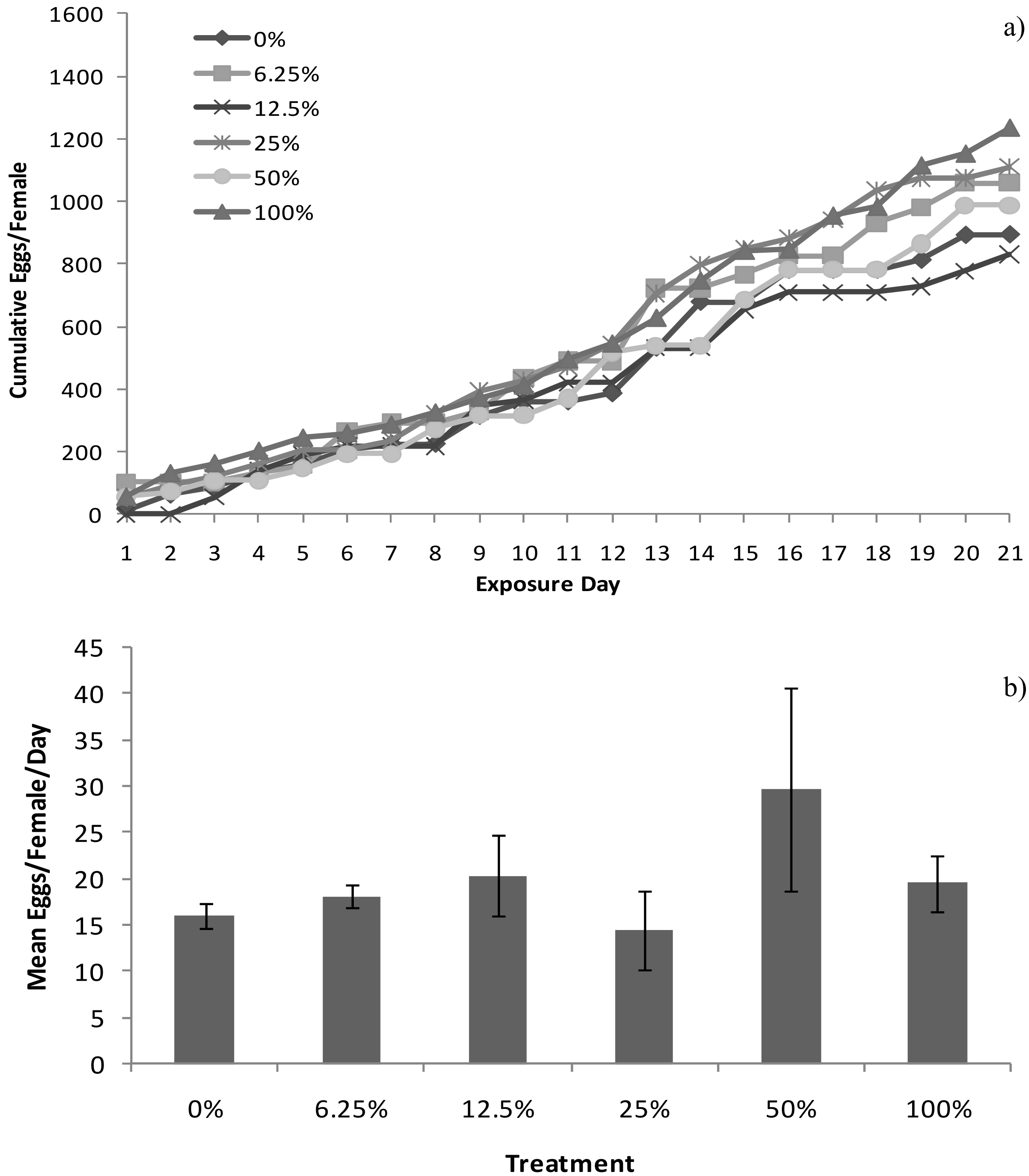
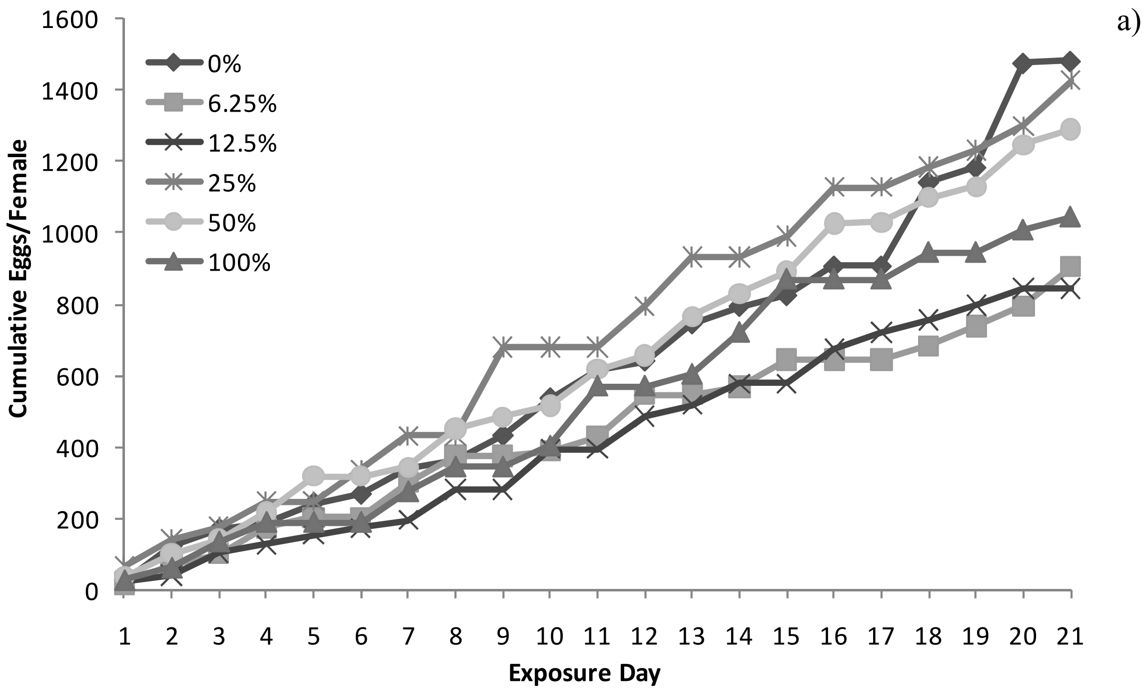


Figure 5.2: Cumulative eggs/female (a) and mean eggs/female/day (±SE) (b) for the 21 day exposure period in the sodium chloride experiment. Concentrations of dissolved sodium and chloride used in each treatment are listed in Table 5.2. Statistical significance was assessed in the cumulative data using Kolmogorov-Smirnov test and for the mean data using a one-way ANOVA's or non-parametric equivalent (Kruskal Wallis).



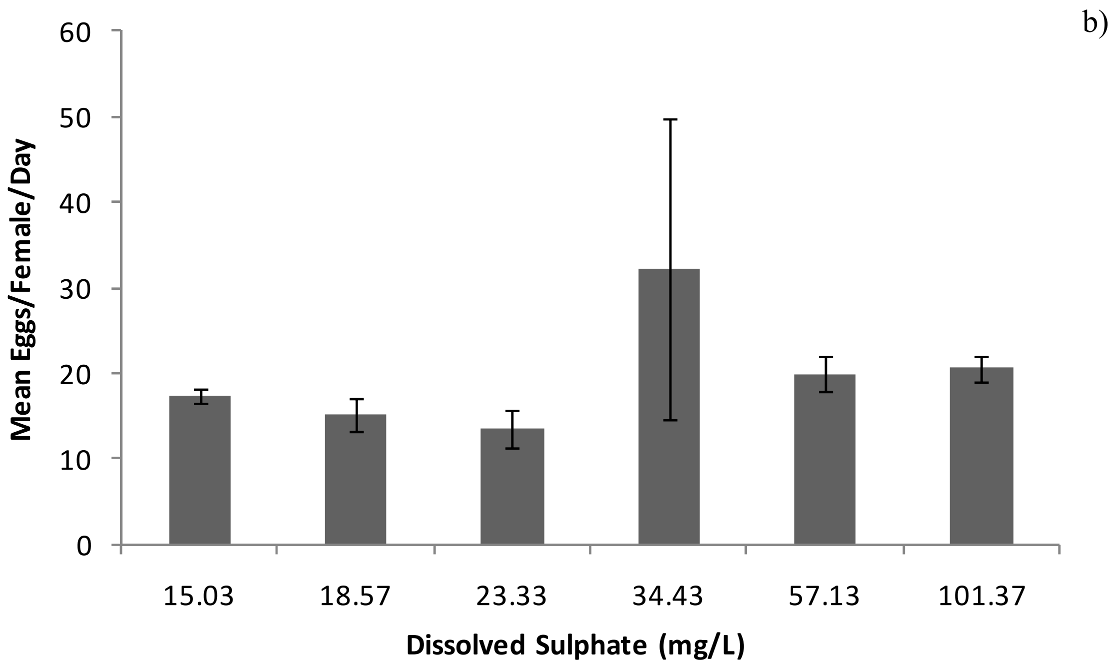


Figure 5.3: Cumulative eggs/female (a) and mean eggs/female/day (±SE) (b) for the 21 day exposure period in the sulphate experiment. Statistical significance was assessed in the cumulative data using Kolmogorov-Smirnov test and for the mean data using a one-way ANOVA's or non-parametric equivalent (Kruskal Wallis).

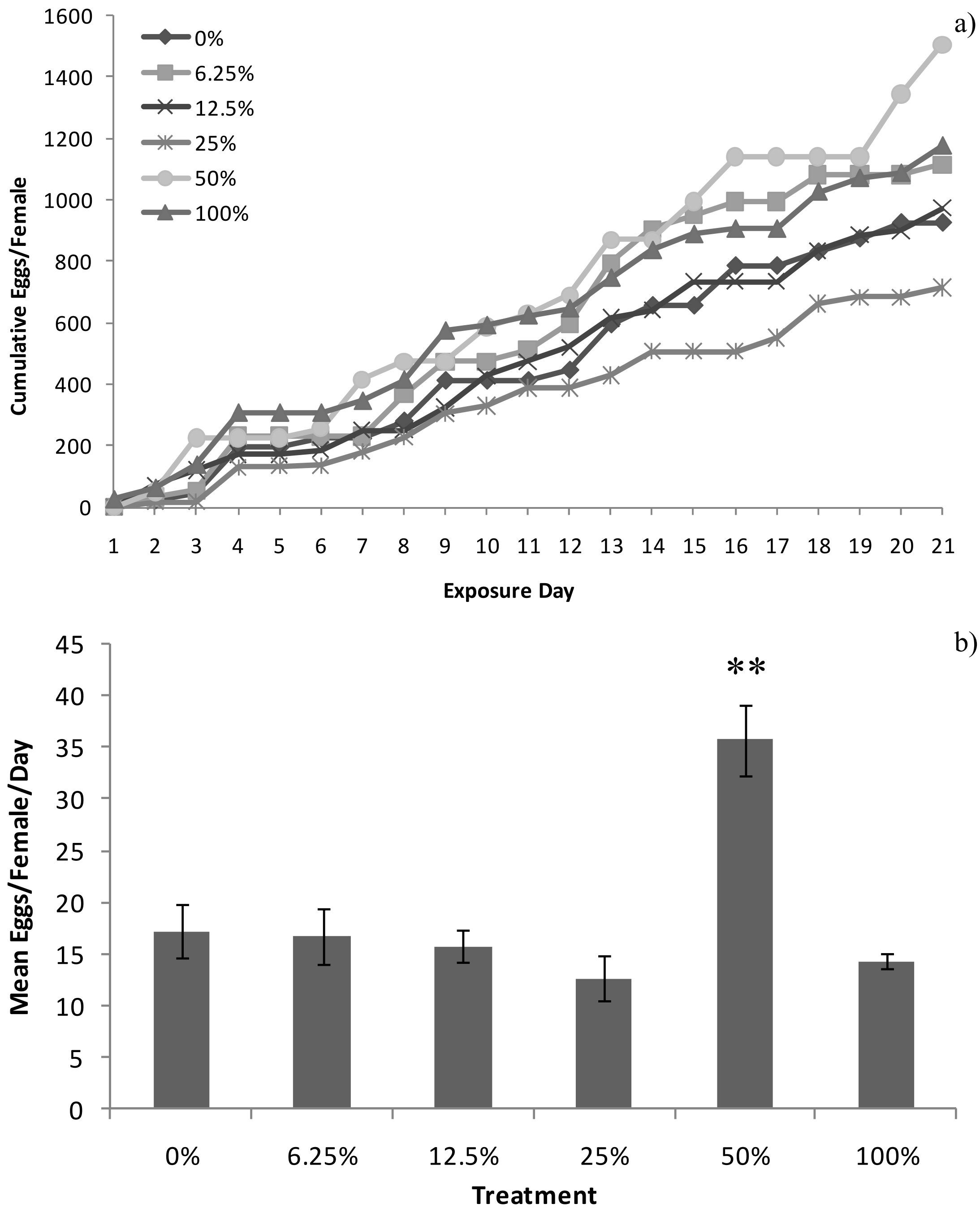


Figure 5.4: Cumulative eggs/female (a) and mean eggs/female/day (±SE) (b) for the 21 day exposure period in the mouth water experiment. Statistical significance was assessed in the cumulative data using Kolmogorov-Smirnov test and for the mean data using a one-way ANOVA's or non-parametric equivalent (Kruskal Wallis) and differences among treatment groups were further assessed using a Dunnetts post hoc or non-parametric Mann-Whitney-U test. Statistical significance is denoted by** = $p \leq 0.001$ versus 0%

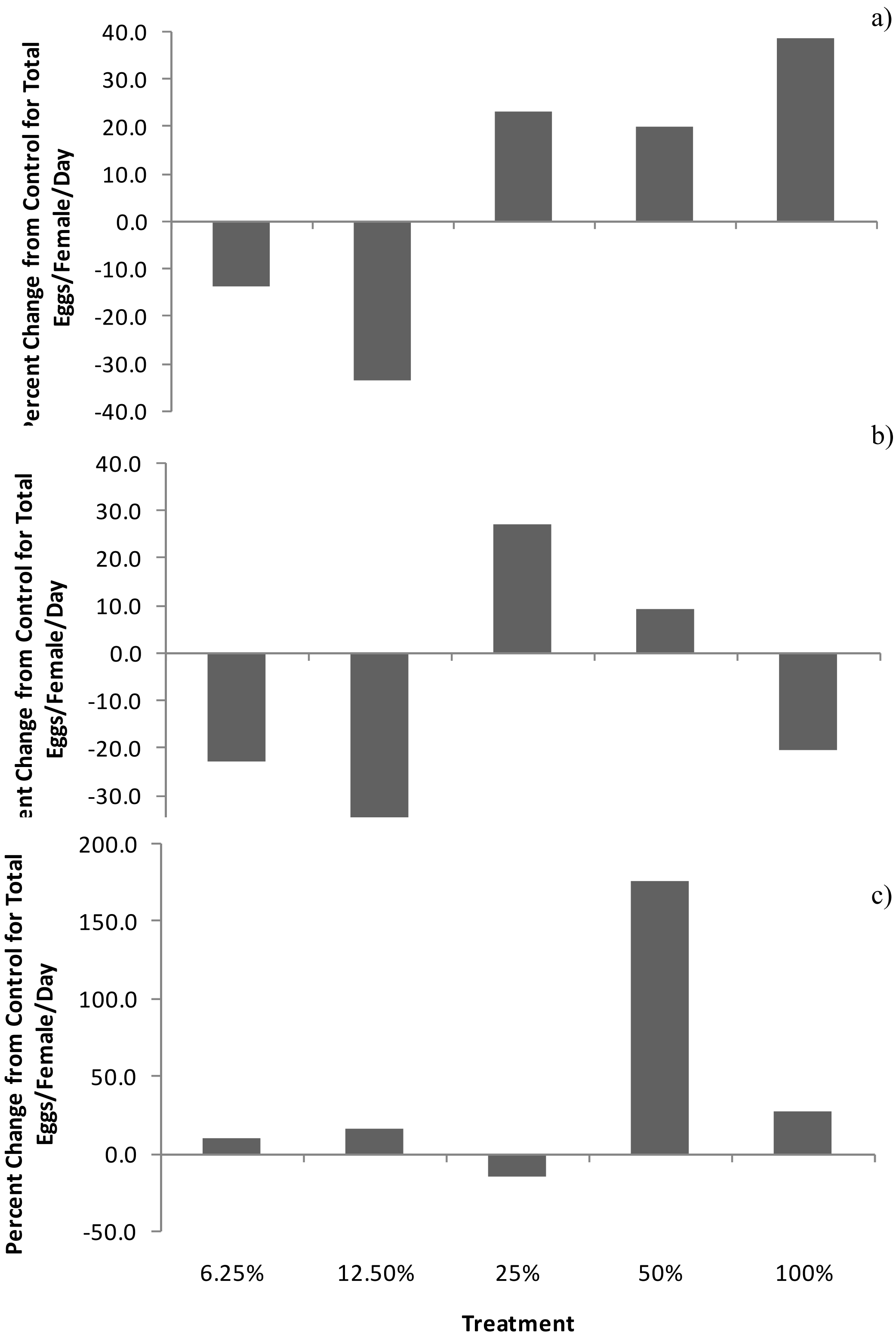


Figure 5.5: Percent change from control for total eggs/female/day for (a) sodium chloride (b) dissolved sulphate and (c) mouth water experiments.

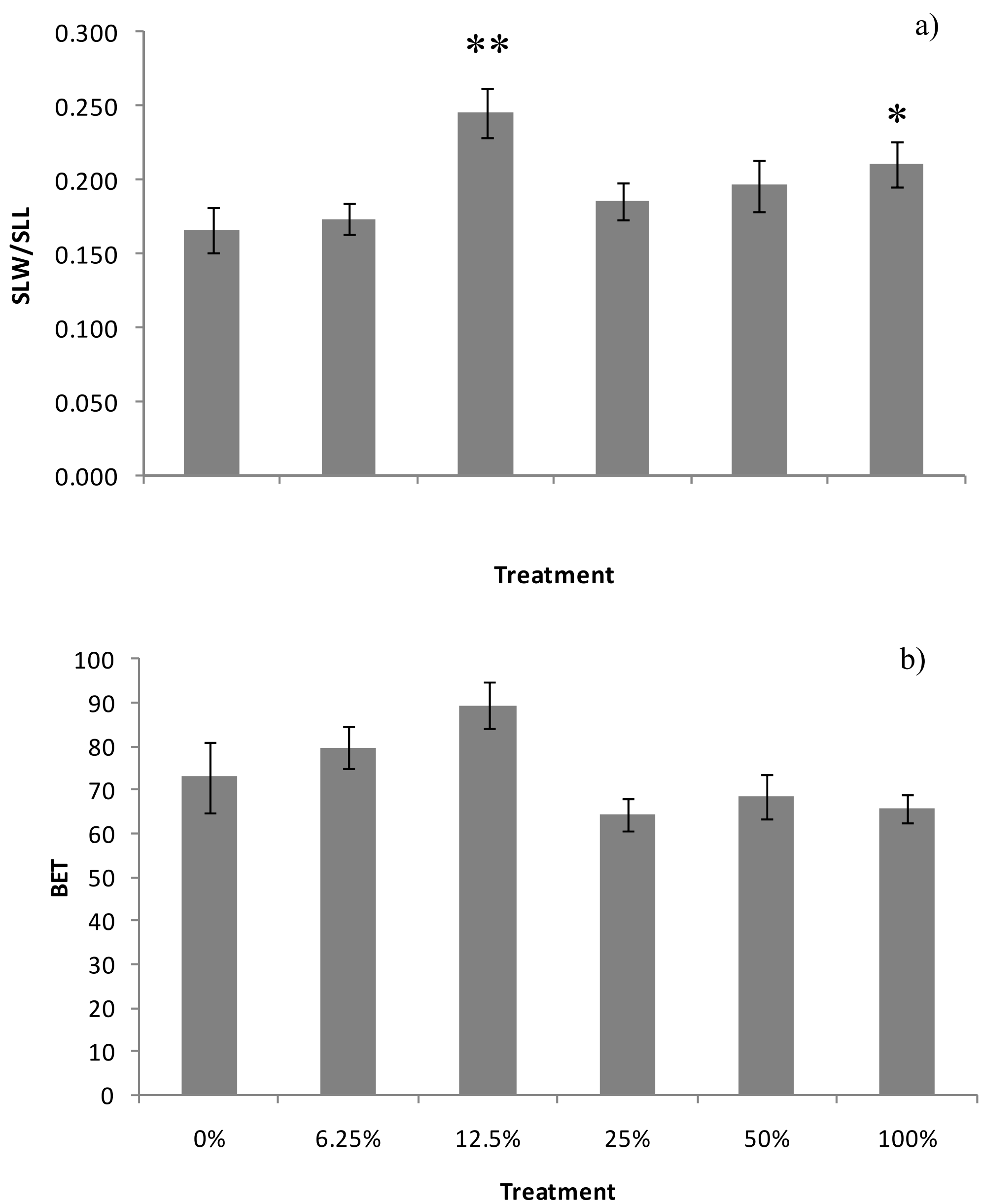


Figure 5.6: The ratio of secondary lamella width (SLW) to secondary lamellar length (SLL) (a) and basal epithelium thickness (BET) (b) for the 21 day exposure period in the sodium chloride experiment. All data are reported as mean ± SE. Statistical significance was analyzed using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. Differences were considered to be significant when $p<0.05$. Statistical significance is denoted by * = $p \leq 0.05$ versus 0% and ** = $p \leq 0.001$ versus 0%.

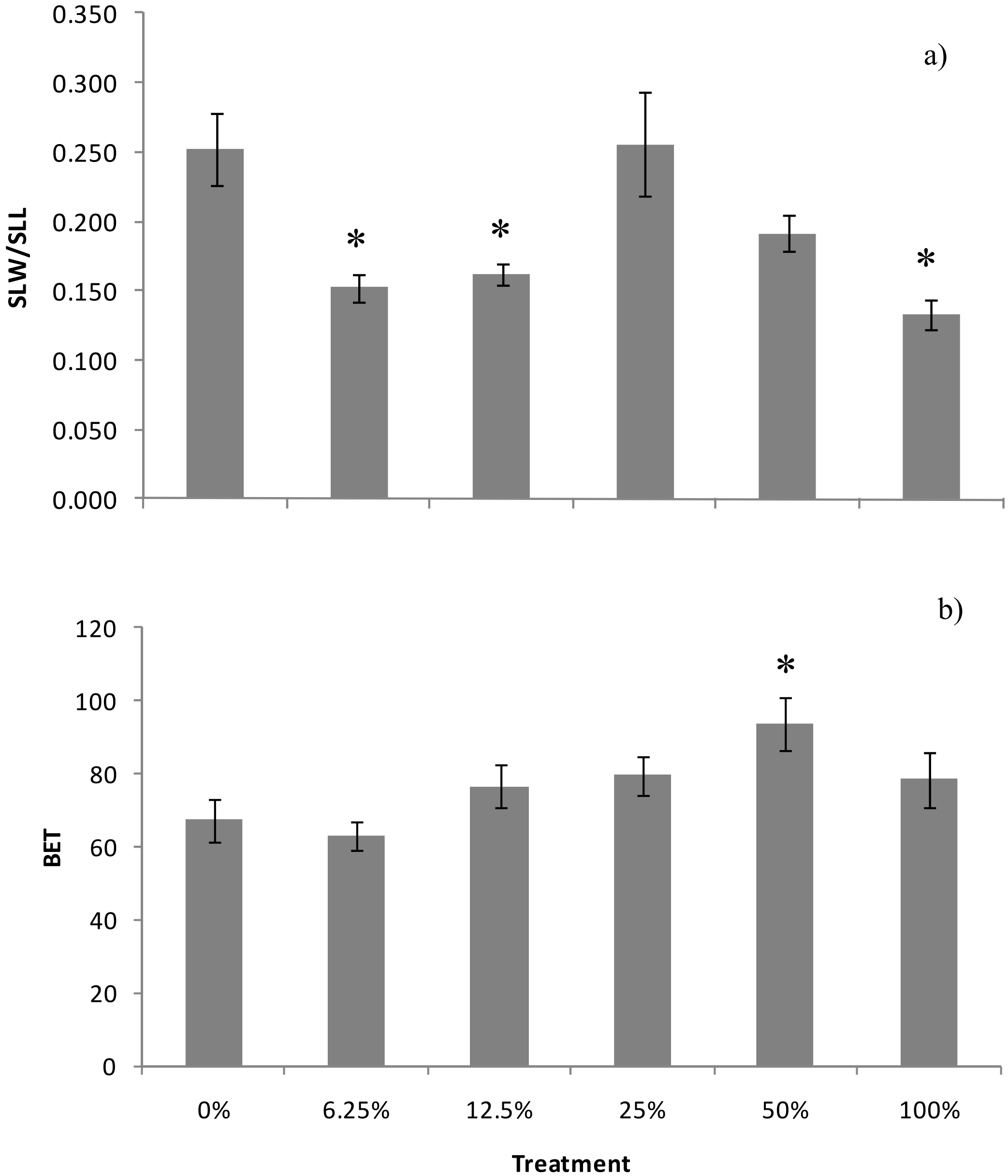


Figure 5.7: The ratio of secondary lamella width (SLW) to secondary lamellar length (SLL) (a) and basal epithelium thickness (BET) (b) for the 21 day exposure period in the sulphate experiment. All data are reported as mean ± SE. Statistical significance was analyzed using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. Differences were considered to be significant when $p<0.05$. Statistical significance is denoted by * = $p \leq 0.05$ versus 0% and ** = $p \leq 0.001$ versus 0%.

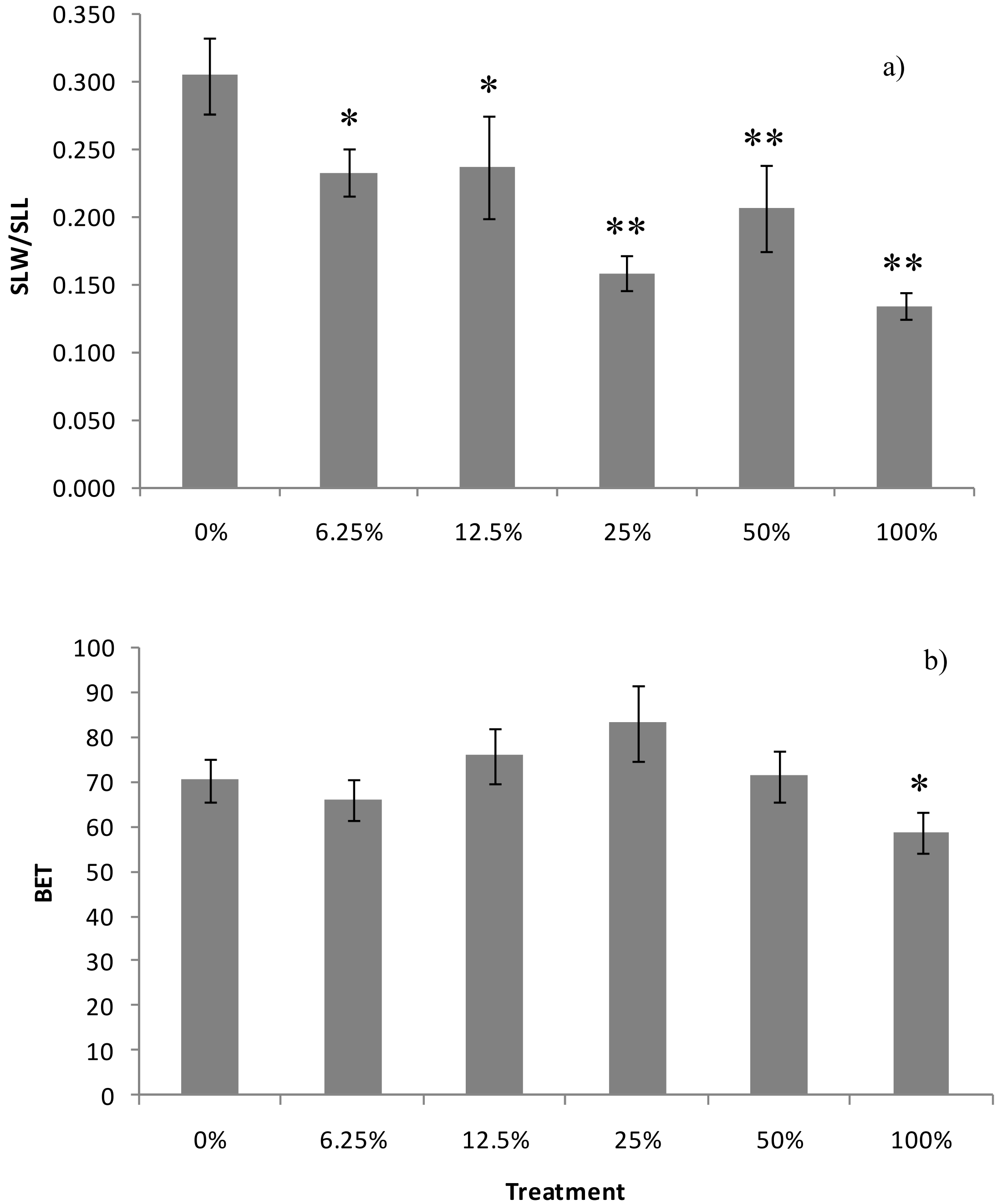


Figure 5.8: The ratio of secondary lamella width (SLW) to secondary lamellar length (SLL) (a) and basal epithelium thickness (BET) (b) for the 21 day exposure period in the mouth water experiment. All data are reported as mean ± SE. Statistical significance was analyzed using a one-way ANOVA providing assumptions of normality (Shapiro-Wilks) and homogeneity of variance (Levene's) were met. For data which did not meet these assumptions the non-parametric equivalent (Kruskal-Wallis test) was used. Differences were considered to be significant when $p<0.05$. Statistical significance is denoted by * = $p \leq 0.05$ versus 0% and ** = $p \leq 0.001$ versus 0%.

5.4 DISCUSSION

5.4.1 Water Chemistry

Data obtained from various government and industry sources were analyzed and significant trends in average concentrations of dissolved chloride, dissolved sodium and sulphate along the Athabasca River continuum and over time (1966 to 2006) were identified (Squires et al. 2010). Based on these data, the highest average concentrations (across the years 1996-2006) of these parameters in the Athabasca River were 21.09 mg/L dissolved sodium, 22.71 mg/L dissolved chloride and 45.27 mg/L sulphate. The concentrations of dissolved sodium and dissolved chloride in the 100% sodium chloride treatment (25.43 mg/L and 38.90 mg/L respectively) and the concentration of sulphate in the 100% treatment of the sulphate experiment (101.37 mg/L) were chosen to imitate these levels (Table 5.2). Although the concentrations of dissolved sodium and chloride used in this study were slightly higher than was seen in the Athabasca River by Squires et al. (2010), they are still representative of the average concentrations of these parameters in the Athabasca River. The concentration of sulphate in the 100% however was approximately twice the amount of average sulphate measured in the Athabasca River. The concentration of sulphate in the 50% treatment (57.13 mg/L) was much closer to the average concentrations and therefore these levels are still representative of the actually in-river concentrations of these parameters.

There was an unrepresentative spike in total organic carbon to 14.80 mg/L in the 12.5% treatment in the dissolved sodium experiment. However, since this is based on a single measurement, it is unlikely to be representative of the average conditions in this treatment during the entire experiment and therefore is not considered to be of particular concern. This is further realized when this level is outside of the averaged levels seen throughout all treatments in the mouth water experiment. There was an increase in TOC in the 100% treatment of the dissolved sulphate experiment to 6.90 mg/L. While this was also based on a single measurement, unlike the spike seen in the 12.5% treatment in the dissolved sodium this is still within the averaged range seen in the mouth water treatments and could therefore reasonably be considered representative of average conditions for this experiment.

5.4.2 Fish Reproduction and Gill Histology

5.4.2.1 NaCl Experiment

All treatments except the 12.5% treatment had higher cumulative egg production than the 0% control, although statistically not significant. This result is similar to what was seen in the mean/eggs/female/day endpoint where all treatments had higher egg production than the control, except for the 25% treatment. Low levels of salinity are often added in aquaculture practices to help reduce fish stress (due to handling, crowding, etc.) (Velasco-Santamaria and Cruz-Casallas, 2008). It is known that the toxicity of other freshwater constituents (such as ammonia and nitrate) is dependent on salinity levels, decreasing with higher salinity (Sampaio et al. 2002). The induction of anti-stress reactions in algae, amphipods and fish includes the induction of certain stress proteins which can induce multiple stress resistance (Steinberg et al. 2007). At the lowest concentrations used in this study (6.25%) it is possible that we are seeing this protective effect.

In the sodium chloride experiment, the only negative percent change from control for total eggs/female/day was in the 6.25% (3.67 mg/L) and 12.5% (3.23 mg/L) treatments. This result differs from what the cumulative eggs/female and the mean eggs/female/day endpoints where increases in egg production occurred at these levels. Total eggs/female/day is calculated by totalling the number of eggs each replicate breeding trio produced across the entire exposure period, then dividing this by the number of day of the exposure (21) and the number of females per treatment (2 per replicate, 3 replicates per treatment means 6 females per treatment). Since this is a total number and is based on the 21 days of exposure we cannot necessarily extrapolate this value past these 21 days of this specific experiment. The cumulative and mean endpoints are not static totals and instead illustrate the general trend of reproductive output during the experiment. This is considered more realistic of what can be expected in a natural population and can better predict the potential population-level effects of these constituents.

Gills are the major site of respiration and as they are in close contact with the water on a constant basis, they are particularly vulnerable to contaminants such as salinity (Gernhofer et al. 2001). In similarly conducted experiments in the laboratory using dissolved sodium, the SLW/SLL was found to significantly higher than the 0% control treatment in the 6.25% (30.33 mg/L) and the 100% (88.00 mg/L) treatments (Squires and Dubé 2011 (Chapter 4 this thesis)).

In these same experiments, the greatest decreases in the percent change from control treatment for total eggs/female/day the laboratory dissolved sodium experiment was also in the 6.25%, 100% treatments. These results demonstrate a link between increasing gill diffusion distance and decreasing reproductive output in the FHM.

In the present study, there was a significant increase in SLW/SLL in the 12.5% (3.23 mg/L Na) treatment ($p<0.001$) which may have contributed to a non-significant decrease in reproductive ability as measured by cumulative egg production. If the duration of the exposure period in this experiment had been increased past 21 days, it is possible that the decrease in cumulative egg production in the 12.5% treatment would become significant, and reflect the changes seen in the gill epithelium as has been demonstrated by laboratory studies (Squires and Dubé 2011 (Chapter 4 this thesis)).

The greatest increases in reproductive output (cumulative eggs/female, mean eggs/female/day and percent change from the control treatment for total eggs/female/day) in the sodium chloride experiment occurred in the highest treatments (50% and 100%). At these higher concentrations (50% and 100%), it is possible that we are seeing a different type of salinity effect. A study conducted by Cataldi et al. (2005) tested whether or not the presence of certain levels of salinity (freshwater, iso-osmotic water and seawater) would affect the osmolarity capabilities of fish after being exposed to stress. They found that fish held in freshwater had a significant decrease in serum osmolality whereas fish held in seawater had a significant increase in serum osmolality.

The 100% treatment had a significantly larger diffusion distance (SLW/SLL). However, although not significant, the 100% treatment also had a greater cumulative egg production than the control and a positive change from control for total eggs/female/day. In this treatment it is possible that despite the larger diffusion distance, the increase in osmolality which occurs with exposure to higher levels of salinity over a longer period of time (such as the 21 day exposure period used in this study) could have caused a reduction in stress allowing for fish to allot more energy towards reproduction. Additionally, the dissolved chloride concentration in the 100% treatment was 38.90 mg/L. In previous laboratory studies (Squires and Dubé 2011 (Chapter 4 this thesis)) the ideal amount of dissolved chloride for fish reproduction was found to be between 22.22 mg/L and 49.56 mg/L. The only treatment to fall within this range was is the 100% treatment. It is possible that the effects of the presence of this higher level of dissolved chloride (through competitive binding) would offset the effects of dissolved sodium, allowing for an increase in reproduction ion this treatment.

5.4.2.2 Mouth Experiment

The presence of turbidity and/or organic carbon in water collected from the mainstem of the Athabasca River downstream of the oil sands operation and its potential effect on FHM reproduction have not previously been studied. There was a 175% increase in the 50% treatment (8.20 mg/L TOC). The highest level of mean egg production was significantly greater ($p<0.001$) than the 0% control treatment and occurred in the 50% mouth water treatment. The 50% mouth water treatment also had the highest level of turbidity at 5.58 NTU. Turbidity consists of many aspects including sediment, algal cells, dissolved humic substances, dissolved minerals and detrital organic matter (Bilotta and Brazier 2008). Humic substances are very complex organic molecules that can make up to most (80%) of the dissolved organic matter present in freshwater ecosystems (Steinberg et al. 2006). The presence of humic substances can evoke anti-stress reactions in exposed organisms in an effort to try and remove these easily accumulated substances (Steinberg et al. 2007). Low levels of humic substances have been shown to induce phase I and II enzymes, providing a protective effect and in the case of some amphipods, also increase the number of offspring produced (Steinberg et al. 2007). Therefore, exposure to low concentrations of humic substances can help to "train" the defense system and lead to stress resistance, thereby allowing for more energy to be diverted to other activities such as reproduction.

Immediately upstream of the collection site for the mouth water used in this study is located several large oil sands mining and extraction sites. A study conducted by Tetreault et al. (2003) sampled two small forage fish species (slimy sculpin and pearl dace) from several sites along the Athabasca River near the oil sands operations and compared with fish sampled upstream of the oil sands operations. No significant differences in length, weight, condition factor, liver somatic index or gonadosomatic index were found in either species. However some differences in levels of steroid production at sites downstream of the oil sands operations were demonstrated. A more recent study by Kavanagh et al. (2011) assessed the potential of aged oil sands process water (OSPW) on FHM reproduction. These authors demonstrated a significant decrease in cumulative number of eggs for FHMs exposed to aged OSPW compared to reference water. This study also demonstrated a decrease in plasma steroid levels (testosterone, 11-ketotestosterone and 17β-estradiol) in some cases. These studies focused on the impact of aged OSPW, which is currently held on site and not released into the Athabasca River adjacent to the oil sands operations.

Presently, no study has assessed the impact of Athabasca River water downstream of the oil sands operations on the reproductive potential of the FHM. In the present study a negative percent change from the control treatment for total eggs/female/day was seen in the 25% treatment and a positive percent change from the control treatment was seen in the 50% treatment. It is unclear from this study what factors may be attributed to these different changes. However due to the complex nature of the oil sands process water as well as the natural abundance of oil sands in the surrounding area it is important to further clarify the potential impacts of actual in-river concentrations of oil sands related water quality parameters on the reproduction of local fish species.

All treatments had significantly lower diffusion distances (SLW/SLL) than the 0% control. This result would imply that increasing amounts of mouth water have a positive effect on the gill epithelium. One reason for this could be because the ionic potential of the mouth water is closer to equilibrium with the fish versus the ionic potential of the headwater (0% control). A greater difference in electric potential causes increase stress and damage to the animal due to the necessity of a greater energy allocation to osmoregulation (Galvez et al. 2008; Bilotta and Brazier 2008). This is further reflected in the cumulative egg production where all treatments (except the 25%) had higher cumulative egg production than the 0% control treatment. This trend is also similar to what was observed in the laboratory experiments conducted with dissolved sodium where a link between decreased diffusion distance and increased reproductive output was demonstrated (Squires and Dubé 2011 (Chapter 4 this thesis)). Since the trends in cumulative egg production in this study mirrored those seen in this previous laboratory work, it can be reasonably assumed that if the duration of exposure was increased, these trends may become significant.

The primary role of the gill epithelium in aquatic organisms is to support life through gas exchange, acid-base regulation, nitrogenous waste excretion, immunity and ion transport (Galvez et al. 2008). The fish gill is considered to be one of the most sensitive organs to external pollution exposure due to their direct contact with the water and several methods have been developed to assess morphological and physical changes to this area (Velasco-Santamaria and Cruz-Casallas 2008). Previous research has shown a link between decreasing diffusion distance (SLW/SLL) and increasing reproductive output and demonstrated links between gill diffusion distance and reproductive output (Squires and Dubé 2011 (Chapter 4 this thesis)).

There were higher levels of TOC in the 12.5% (4.27 mg/L), 25% (4.80 mg/L), 50% (8.20 mg/L) and 100% (10.73 mg/L) treatments which can also contribute to the trends in gill damage and consequently, reproductive output. Organic matter has been shown to influence the flux of Na+ across gills of freshwater organisms such as Daphnia and fish (Galvez et al. 2008). In past decades, growing recognition that organic matter can affect the physiology of organisms through several mechanisms including activation of glutathione S-transferase, induction of heat shock proteins and CYP1A enzymes as well as changes in behaviour (Galvez et al. 2008). The presence of organic matter at concentrations of 10 mg/L can also alter the fundamental physiological properties of fish gills, by hyperpolarizing gill membranes (Galvez et al. 2008). This happens when humic substances complex with biologically active ions such as free Ca^{2+} from the water. Ca^{2+} is an important constituent of epithelial tight junctions, and reductions in this ion to very low levels are known to cause a general increase in diffusive ion losses, as well as to selectively enhance the permeability of the gills to Na^{+} relative to Cl^{-}, resulting in hyperpolarization (Galvez et al. 2008; Glover et al. 2005).

5.4.2.3 Sulphate Experiment

There was no difference among treatments for cumulative eggs/female, total eggs/female/day and mean eggs/female/day in the dissolved sulphate experiment compared to the 0% control treatment. However at the end of the experiment (day 21 of exposure) all treatments had lower cumulative egg production than the 0% control treatment. Although not significant, there was also an increase in mean egg production in the 25% treatment in the dissolved sulphate experiment.

There have been a few studies on the toxicity of sulphate in the aquatic environment. The water flea, *Hyalella azteca* was found to have an LC_{50} of 512 mg/L SO_4 (Soucek and Kennedy 2005). Toxicity associated with excess SO_4 can be related to indirect effects on calcium availability rather than direct impacts from SO_4 (Pillard et al. 2000). The toxicity of SO_4 to aquatic biota was found to decrease with increasing hardness and chloride concentrations (Soucek and Kennedy 2005). The presence of calcium and chloride can aid in the biota's ability to osmoregulate and therefore tolerate higher ionic solutions (Soucek and Kennedy 2005 and Pillard et al. 2000).

The province of British Columbia, Canada has established a water quality guideline for the protection of aquatic life for sulphate of 100 mg/L (Elphick et al. 2010). In the dissolved sulphate experiment, three treatments had lower percent change from control for total eggs/female/day than the control, 6.25% (18.57 mg/L), 12.5% (23.33 mg/L) and 100% (101.37 mg/L). The highest of these treatments had a concentration which is comparable to this guideline set by British Columbia. This guideline was based on the acute toxicity data generated from several species of invertebrates, fish, algae, moss and amphibians with a 2-fold safety margin. Our results are based on chronic exposures (21-days) to sublethal levels of sulphate to assess the impacts on the reproductive output on FHMs. Since this water quality guideline is based on short-term exposures (< 24 hours) on lower trophic-level species (*C. dubia*) it is unlikely that our lowest concentration where an effect is observed would correlate with this guideline value. It would therefore be worthwhile to conduct further studies in which the sublethal effects of sulphate on the population of higher order species.

In the dissolved sulphate experiment, there was a significant increase in BET in the 50% treatment ($p = 0.039$) and significant decrease in SLW/SLL in the 6.25%, 12.5% and 100% treatments ($p<0.05$) compared with the 0% control treatment. Both BET and SLW/SLL are measures of gill epithelium thickness. Decreases in either of these measurements correspond to decreases in the diffusion distance across the gill. These treatments also all had a negative change from the control treatment for total eggs/female/day. Therefore, we saw decreased gill diffusion distances in the treatments which had the lowest cumulative egg production, which is opposite from what was observed in the sodium chloride experiment in this study as well as was observed in previous laboratory studies (Squires and Dubé 2011 (Chapter 4 this thesis)).

One explanation for these conflicting results is presence of higher turbidity in the 6.25% (5.13 NTU) and 12.5% (5.07 NTU) treatments and higher TOC (6.90 mg/L) in the 100% treatment relative to the rest of the treatments in this experiment (Table 5.2). While we saw increased reproduction of FHM likely in response to increased levels of TOC and turbidity in the mouth water experiment, there are thresholds beyond which adverse effects on fish populations are observed. It is possible that the presence of higher levels of organic matter in these treatments can have a negative effect on fish reproduction.

The reproductive success of salmonid fish are known to be especially sensitive to suspended organic matter since matter depositing on the stream beds will block pores in the gravel which are ideal for the deposition of eggs (Bilotta and Brazier 2008). In general though, high enough levels of suspended organic matter are known to clog fish gills decreasing the ability of the fish for oxygen exchange and osmoregulation (Dunlop et al. 2005). Also, suspended sediments can cause stress by suppressing their immune system which can then lead to susceptibility to disease (Bilotta and Brazier 2008). Therefore sublethal thresholds which account for the effects of turbidity on reproductive potential are necessary and important when considering the impacts of contaminants on the aquatic ecosystem.

5.5 CONCLUSIONS

Previous research has noted that dissolved sodium, chloride and sulphate concentrations have significantly increased in the lower reaches of the Athabasca River over 30 years (Squires et al. 2010). While further laboratory research has shown that both sodium and chloride have shown to alter reproduction in FHM and gill membrane diffusion distances, it was unknown whether these effects would be seen using actual in-river water concentrations (Squires and Dubé 2011 (Chapter 4 this thesis)).

This study aimed to determine whether the thresholds developed in the laboratory could be applied using water taken from both the headwaters and mouth of the Athabasca River. This was done by exposing fish to concentrations of sodium chloride and sulphate relevant to average levels in the mouth of the river. In addition, water taken from downstream of industrial inputs in the mouth of the river was used for threshold development. In the dissolved sulphate experiment, the treatments which had the lowest reproductive output were the 6.25% (18.57 mg/L) and the 12.5% (23.33 mg/L) treatments. Current guidelines also state that dissolved sulphate should not exceed 100 mg/L (Elphick et al. 2010). Based on this information, the ideal amount of dissolved sulphate for FHM reproduction was determined to between 23.33 mg/L and 100 mg/L. The greatest increases in reproductive output (cumulative eggs/female, mean eggs/female/day and percent change from control for total eggs/female/day) in the sodium chloride experiment occurred in the highest treatment (25.43 mg/L Na and 38.90 mg/L Cl). This is very similar to the range identified in the laboratory study for dissolved sodium of between 36.11 and 57.00 mg/L and dissolved chloride between 22.22 mg/L and 49.56 mg/L (Squires and Dubé 2011 (Chapter 4 this thesis)).

Although differences in cumulative egg production in the present study were not found to be significant in the sodium chloride experiment, the trends between gill diffusion distances and cumulative reproduction are similar to those demonstrated in previously conducted laboratory studies using higher concentrations of dissolved sodium and chloride (Squires and Dubé 2011 (Chapter 4 this thesis)). The present study used concentrations where the 100% treatment was 30% of the concentrations in the100% treatment used in the laboratory studies. It is unlikely that at these lower levels we would see the same significant effects on reproduction as were seen in the higher concentrated laboratory studies over the similar 21-day exposure period. However, since we did see a similar connection between gill diffusion distance and reproduction output as was demonstrated in the laboratory study this may over a longer period of time have a more significant impact on reproductive output.

It is important to determine thresholds that are specific to an area or region. Further research would include longer exposure periods to try and determine if the trends seen in this study continue and influence future generations of FHM. It would also be important to use multiple species since there are differing sensitivities among species to these types of exposures. This information would allow us to form a better understanding of how these parameters will affect this river basin in the long-term.

CHAPTER 6: GENERAL DISCUSSION

6.0 GENERAL DISCUSSION

There has been an extraordinary amount of growth and development in the Athabasca River basin over the recent decades. No more so than what has occurred in the mouth of the river, in the oil sands deposit. The overall goal of this thesis work was to develop and apply a quantitative approach (framework) to assess and characterize the cumulative effects of man-made stressors (e.g. municipal effluent, pulp and paper effluents, oil sands) on indicators of aquatic health (water quality and biological responses) over space and time for a model Canadian river, the Athabasca River, Alberta. The framework is explained in greater detail in Chapter 2. The following is a brief summary of the framework and the results of its implementation on the Athabasca River basin.

6.1 EVALUATION OF THE FRAMEWORK

6.1.1 Trends

The first part of this framework allowed for the quantification of trends in water quality and quantity along the entire mainstem of the Athabasca River. This trend analysis is detailed in Chapter 4. It involved the collection of data from 5 different sources which amounted to over 5 million data points (Squires et al. 2010). Despite this abundance of data many of the indicators selected for assessment of change were constrained on data availability. This is because much of the data was unreliable due to the frequency of collection and the changes in analytical methods of analyses over time. There was also a lack of biological data available in the basin (RAMP 2011) and consequently only biophysical endpoints (water quality and quantity) were selected and assessed for significant changes across both time periods from headwaters to mouth.

The Athabasca River basin is one of the most studied river basins in Canada. There are monitoring stations for surface water quality managed by both the federal and provincial governments. Also, there are several industry monitoring programs and non-profit monitoring groups in the lower reaches of the basin. Even though there are many groups monitoring water quality and quantity in the basin (especially near the oil sands region), none of these are done in a consistent, integrated manner. The Athabasca River basin is a large and complex system and integration of all monitoring programs is required (RAMP 2011; Environment Canada 2011).

Collecting and compiling the data used in this trend analysis was no easy undertaking. We found that many of the sources of available data were difficult to obtain (RAMP and AENV) and in some cases we were not able to collect any data at all (namely several industrial users in the basin). Gaining access to these industrial sources of data would have contributed greatly to the overall success of this part of the framework by providing us with additional water quality parameters that are specifically related to the human induced stressors produced by the industrial inputs and are not currently available in the publicly available data we used (i.e. PAHs, metals, naphthenic acids etc.).

It is vital to have easy public access to monitoring data in the Athabasca River basin. Historically, access to data involved sending special requests to the managers of the data and signing confidentially documents before gaining access to a small subset of the data. However now, many monitoring groups are providing access to data on their websites in a format that is free for anybody to download. In addition the newly released federal water quality monitoring plan (Phase 1) for the Lower Athabasca also states that all data collected will be made publicly available (Environment Canada 2011). These are encouraging improvements and will hopefully prevent any future issues with accessing data on the Athabasca River.

Despite these issues with accessing data, the implementation of this portion of the framework on the Athabasca River basin was successful. Several statistically significant changes in many water quality and quantity parameters across several decades of data were found. These parameters then provided the foundation for further dose-response studies that constituted the majority of the rest of the framework. Ultimately these trend analysis can be referred to and updated as new data become available thereby making it a very valuable and useful contribution to the assessment of the aquatic ecosystem health of the Athabasca River and hopefully provide a baseline for future assessments.

6.1.2 Thresholds

As part of the first portion of the framework (trend analysis) concentrations of the parameters identified to have significant trends across space and time were compared to any existing provincial or federal water quality guidelines. However, due to the lack of developed guidelines for the purpose of protecting aquatic life for many of the parameters in this study, the 10th and 90th percentiles were calculated for the first time period. This was done as this time period is considered to represent a reference-type condition due to the absence of many of the land-use related stressors which are present in the second, more developed time period. These percentiles can offer some measure of acceptability when compared to the concentrations of these parameters in the second time period. For example, concentrations in the second time period which exceed the 90^{th} percentile can be considered above a threshold of acceptability compared to the first time period.

It is possible that depending upon the natural habitat of a location, pristine waters unaffected by human development can exceed national or provincial guidelines set for certain water quality parameters (e.g. waters which run through an area of high natural metals, such as the oil sands region). Using the 90^{th} percentile as a site-specific benchmark allows for the accommodation of these areas containing naturally higher levels of the compounds of interest (Glozier et al. 2004, de Rosemond et al. 2009). This method has the potential to be applied across many indicators (biological and physical) providing the opportunity to make comparisons to a reference condition across different indicators.

The second part of this framework was the development of thresholds specific to the Athabasca River basin by conducting dose-response experiments in the laboratory and field. The parameters used in these experiments were based on those found to exhibit significant trends across space and time in the first part of the framework (Chapter 4). These parameters were dissolved chloride, dissolved sodium and dissolved sulphate. An additional treatment of water sampled downstream of most of the industrial development in the mouth of the river was also used in the field study. Both the laboratory and field studies are explained in greater detail in Chapters 4 and 5 of this thesis.

The partial life-cycle fathead reproduction minnow assay was used as an indicator of population-level effects on aquatic biota in the Athabasca River basin. This assay allows for the assessment of the reproduction potential of FHMs, as well as aspects of their early development when exposed to a dilution series. The maximum concentrations of dissolved chloride and sodium as assessed in the previous trend study by Squires et al. (2010) were used as the most concentrated (100%) treatment in the laboratory studies.

Once the laboratory studies were completed, a field study was conducted to provide an additional aspect of environmental realism and to allow for validation of these thresholds in the Athabasca River basin. These field experiments were conducted at the headwaters of the

Athabasca River using concentrations of several parameters deemed to be of significance to the basin as well as water sampled directly from the mouth of the river basin. This allowed us to assess and compare the effects of the actual fluctuating concentrations of stressor combinations at these sites as ecological systems have been shown to exhibit large temporal and spatial variability (Zeimer 1998). These field experiments were done in much the same manner as those conducted in the laboratory.

Results between the laboratory and field experiments had several differences. Number one was that the concentrations of parameters (sodium and chloride) used in the laboratory studies were based on the maximum concentrations found in the mouth of the Athabasca River in the second time period (1996-2006). Whereas the concentrations of these parameters used in the field were based on the average concentrations found in the mouth of the Athabasca River in the second time period (1996-2006) and were consequently much lower.

The threshold range determined in the laboratory for dissolved sodium was between 12.5% (36.11 mg/L) and 50% (57.00 mg/L) and dissolved chloride less than 22.22 mg/L. At levels outside or above these ranges a possible decrease in reproductive output may occur. The threshold range determined in the field studies for dissolved sulphate concentration was between 23.33 mg/L and 100 mg/L. In the sodium chloride experiment the greatest increases in reproductive output occurred in the highest treatment (25.43 mg/L Na and 38.90 mg/L Cl). This was very similar to the range identified in the laboratory study for dissolved sodium of between 36.11 and 57.00 mg/L and dissolved chloride less than 22.22 mg/L.

This aspect of the framework can be considered a success since we were able to meet the objective of developing site-specific thresholds for parameters of concern in the Athabasca River basin. Using these thresholds developed in both the laboratory and field studies we now have the potential to successfully mitigate the impacts of any current or future developments on the basin related to these particular parameters. Ultimately as more data becomes available and new developments occur on the basin, different parameters of concern may arise in the future. The methods as outlined in this thesis can be applied successfully to new parameters allowing us to create a dose-response pattern that can be used as a threshold when considering the approval of any future development scenarios on the basin.

6.1.3 Usability

Overall the framework has proven to be useable when applied to a major river basin in Canada. When we applied it to the Athabasca River basin we were able to compile enough data to determine significant trends across space and time for many water quality parameters and then use this information to produce thresholds specific to the reproductive health of fish populations in this area. There are however a few limitations and challenges when applying this framework which may inhibit its success in certain areas.

The major limitation of this framework is that it requires extraordinary amounts of data, spanning over a large time period (historical to current) (Squires et al. 2010). Since no one researcher, regulatory body or industry can possess the amount of data required, it is imperative that cooperation and data sharing between all of these stakeholders occurs. Nevertheless, data sharing between industries, government and not-for-profit organizations is often difficult in these types of situations. This can often be attributed to the absence of leadership of the assessment since it is not currently the responsibility of any one stakeholder to conduct such a large-scale assessment (Duinker and Greig 2006).

6.2 RECOMMENDATIONS

6.2.1 Monitoring needs

Currently there are only two monitoring programs that have stations along the entire length of the Athabasca River and these are run by Alberta Environment (AENV) and Environment Canada. There are other organizations which sample on the Athabasca and its tributaries but these programs focus mainly on the mouth of the basin near the oil sands deposits. These include the Wood Buffalo Environmental Association, Regional Aquatics Monitoring Program (RAMP) and the Athabasca Tribal Council. In addition several of the industries along the river such as the pulp mill Alberta-Pacific Forest Industries and many urban centers such as the town of Hinton also sample only in the immediate area surrounding their discharge point.

Many of these sampling programs are constrained by the operations they are centered around (oil sands, pulp and paper, municipal) and are not set up to be long-term sampling programs. There are currently three long-term stations currently being maintained on the basin, all three of them by Alberta Environment. Many of the stations have limited years of data available, not all of them sampled throughout each year, and the variables selected for analysis can differ with each sampling event. This lack of consistency in data collection makes it difficult to form any empirical conclusions of what a baseline condition in the Athabasca River basin might be like. Of the three long–term stations currently maintained by AENV only 2 of them existed before the first major oil sands development occurred (i.e. prior to 1976) and both of them are downstream of these major developments.

While there are a number of stations that have a few years of data available prior to when the major amount of industrial development occurred in the basin, the data is in most cases not considered to be useful. This is largely due to the different analytical techniques used across decades of sample analysis. Many of the techniques used in previous decades produced data with higher detection limits, and were not able to be analyzed for certain important industrial related parameters (PAHS, metals, naphthenic acids) and therefore can provide no real comparison to the current day data. In future, it is important to standardize these analytical methods so that data can be compared across decades in a reliable way.

It is strongly recommended that these long-term stations are maintained, and that stations which were known to exist in previous decades be revisited and sampled on a regular basis to aid in the comparison between historical and current conditions. It is also recommended that the number of stations be increased in the areas of greatest development, such as the oil sands deposits. This will allow for the possibility of enough data to give high enough resolution of the area's condition as to differentiate between the impacts each development may be having on the aquatic ecosystem. In addition to increasing the number of water quality monitoring stations, it is also recommended that each water quality station be paired with a water quantity monitoring station. This would not only allow for better assessments of changes in water quantity along the river basin, but would also allow for better loading calculations along the river basin.

It is also important to have a formal data sharing agreement for all industrial partners in the basin. For example, if you draw water from the river, you should have to submit your individual monitoring/assessment results to the provincial government. This would mean that everybody would have access to the data they need to perform the CEA which is currently mandated to be part of the project-specific EIA required for project approval, but most importantly it would contribute greatly to the lack of regular and consistent monitoring data in the region.

6.2.2 Assessment Needs

In the past, the majority of the CEAs conducted in the Athabasca region of northern Alberta have been project-based. A problem with project-based CEA is that a single project approval process has little scope for managing cumulative effects attributable to other projects (Spaling et al. 2000). This is especially true for an area such as the Athabasca oil sands which undergoes constant development both industrially (over 17 existing, approved, or planned oil sands projects) and municipally with the constant expansion of cities such as Fort McMurray.

In response to the impacts associated with a growing industry of surface mining of oil sands and processing of bitumen which include land clearing, disturbance of soil and subsurface strata, changes to surface and groundwater hydrology, effects on fish and wildlife populations, and atmospheric emissions of sulphur and nitrous oxides, regional cumulative effects emerged as an issue in the late 1990s (Spaling et al. 2000). Environment Canada stated: "Although each of these additional projects would also require a CEA, a project-by-project review may not facilitate the most efficient or effective assessment of the effects from the collective development. It is considered critical that consideration of future oil sands projects be undertaken in a regional context". This, combined with the release of the province of Alberta's Regional Sustainable Development (RSDS) for the Athabasca Oil Sands Area in 1999 precipitated the formation of the Cumulative Environmental Management Association (CEMA). This organization was originally comprised of industry, government, aboriginal and academic partnerships with the objective of setting regional environmental thresholds for a variety of areas from air acidification, biodiversity, culture and historical resources, fish habitat, ground level ozone, landscape diversity, reclamation, surface water quality, trace metals and wildlife habitat. Despite its potential, this organization has not yet produced a framework that can set the standard for CEA in the Athabasca River basin.

To date, there have been three major CEAs for parts of the Athabasca River basin including the Northern River Basins Study (NRBS), the Northern River Ecosystem Initiative (NREI) and the Regional Aquatic Monitoring Program (RAMP). NRBS and NREI were a series of research studies by various scientists over a 5 year program. CEAs were conducted at the end of the program and consisted of a qualitative synthesis of conclusions from the various researchers (Culp et al. 2000a; Dubé et al. 2006). These CEAs have been conducted on a portion of the River basin, but as of yet, no attempt has been made to assess cumulative effects from headwaters to mouth (Lawe et al. 2005).

RAMP is an organization that has provided monitoring of water quality, quantity and biological sampling since 1997. It has the best potential to provide the data necessary to conduct regional scale assessments in this including determining baseline conditions for the highly specific area around and within the oil sands deposit. However RAMP has undergone increased criticism in the past few years (Kelly et al. 2010). The most recent third party review was completed in January 2011, and like previous reviews identified many areas which require improvement. These included a lack of consistency and transparency in sampling site selection and data processing, no cohesion between biological and water quality sampling as well as a lack of information regarding traditional ecological knowledge (RAMP 2011).

It is ultimately up to the federal or provincial governments to step up and lead the assessment of the river basin; it should not and cannot be left up to individual project proponents or as in the case of both CEMA and RAMP multi-stakeholder groups. It is imperative that the governing body takes leadership of these assessments to provide these multi-stakeholder groups with stability, leadership and integrity. These assessments must also be regulated, otherwise data sharing between the different stakeholders will not occur and much of the potential of the data collected will be lost.

6.2.2.1 The Provincial Government (Alberta)

In response to this need, the Government of Alberta has recently (March 2011) released the draft document outlining their surface water quality management framework for the lower Athabasca Region (AENV 2011). This framework aims to provide ambient surface water quality triggers and limits for the region with the goal towards protecting surface water quality and addressing cumulative effects. The main structure of this framework is a management feedback loop based on the formulation of water quality “triggers” and “limits” which once exceeded, will induce a certain management action geared towards mitigating any future impacts.

This framework has the potential to provide not only valuable information about the health of the aquatic ecosystem in this region, but it also provides a direct think to management actions to mitigate any current impacts while providing the information necessary to make management decisions concerning future developments in this area. There are however several areas of improvement that must be addressed in this framework which I will outline below:

1. Currently the entire framework is based on forming a baseline "historical" condition using information from only one long-term station well downstream of the oil sands operations (Old Fort monitoring station). This station is potentially confounded by the developments occurring upstream and should not be considered when determining a baseline condition for the area. Instead, I would recommend that additional stations upstream of the development as well as in surrounding undeveloped tributaries be used.
2. The framework states that water quality limits are calculated annually from 12 monthly samples at the Old Fort station. This resolution of data analysis is not satisfactory as it does not account for seasonal variations within the year where exceedences above the baseline condition may occur. As was shown in Squires et al. (2010) seasonality is a factor in this area where low flow months can show the greatest increases in water quality parameters of concern. Yearly averages will mask this seasonality effect creating variability within the endpoint and may in fact prevent the "triggers" set in place for management action to occur.
3. The historical baseline is based on data collected from 1988 up until and including 2008. Therefore in some cases, we would be comparing current data to data generated only 3 years ago. As was shown in Squires et al. (2010) there are data available in this region older than 1988 and these data should be included whenever possible when determining the historical condition of the area. It would also be more realistic to limit how recently the data have been collected when used as part of the historical condition. Ideally this would be determined by when development started to occur in the area, and data before this development is to be the only data that should be used to determine the baseline.
4. It is also recommended that the management actions taken when a water quality trigger is exceeded be clarified. As it states in the framework, Alberta Environment may choose not to initiate an investigation into an exceedence if they feel that not enough evidence is present to indicate that an undesirable trend is developing. The whole purpose behind having these triggers set is to initiate a definite and decisive management action to mitigate any impacts that are occurring and protect the health of the aquatic ecosystem of the Lower Athabasca Region. In this framework,

management actions are not clearly defined and in many cases are left up to the individual to determine what action (if any) would occur in the presence of an exceedence.

5. In the case where an investigation into an exceedence is warranted, the framework allows for the direct participation of the regulated parties that are potentially contributing to the problem. In fact it states that "regulated parties may be asked to carry out portions of the investigation". This type of self-regulation is what has proven ineffective in the past (e.g. RAMP, CEMA) and the Government of Alberta should be solely responsible for any investigations and management actions as they are triggered by exceedences in water quality indicators.

6.2.2.2 The Federal Government

The federal government has also recently (March 2011) released a water quality monitoring plan (Phase 1) for the Lower Athabasca (Environment Canada 2011). The development of this framework primarily involved collaboration between the federal and Alberta provincial governments but also involved the expertise of many scientists both within and outside of the government across Canada in many areas of aquatic science. The main goal of this monitoring program was to provide a comprehensive, integrated monitoring program for the oil sands region (Environment Canada 2011).

There are many aspects of this framework which improves upon the existing monitoring in the Lower Athabasca Region:

1. This framework includes adding additional long-term stations in the area. In addition, many more stations will be added both in the mainstem of the river and its major tributaries. These proposed stations are designed to improve both spatial and temporal resolution of data in order to improve the ability to detect significant changes in the area.
2. This preliminary document focused primarily on the physical and chemical aspects of water quality but additional phases will include biological endpoints to incorporate a more effects-based monitoring assessment. This is important as biological endpoints have proven to be more sensitive to change than physical chemical parameters and

therefore can often provide the first indication of the presence of adverse levels of a particular contaminant.

3. This framework proposes to link water quality and quantity sampling stations. This is an important aspect of water quality assessment which is often neglected. Linking water quality and quantity sampling ensures accurate assessments of chemical loading. This information also provides the opportunity to examine in more detail the movement of chemical parameters during low and high flow time periods.
4. One of the most progressive aspects of this monitoring program is its intention to integrate air, sediment, water (surface and groundwater) and climate monitoring. Establishing relationships between these different matrices and the movement of contaminants between them will allow for a more holistic monitoring approach. It will provide the potential to identify the sources of these contaminants within the basin allowing for a more effective management strategy.

Many aspects of this monitoring framework are positive and create a significant improvement upon the existing monitoring programs in this area of the Athabasca River basin. However the absence of sampling in other areas of the basin prevents this framework from considering the cumulative impacts of industrial developments (such as pulp mills, municipal sewage etc.) which are discharging upstream and potentially contributing to the chemical and biological impacts assessed in the Lower Athabasca region. It is also vital in quantifying the level of change which can naturally occur in different types of biophysical stressors (water quality and quantity) which are not solely influenced by human activities. The river continuum concept is a fundamental ecological concept that characterizes how a river naturally changes along its length from headwaters to mouth (Vannote et al. 1980). At the mouth, rivers tend to be more turbid with a lower primary productivity, larger width and a less diverse benthic community composition (collectors and predators). Thus there are naturally occurring accumulated changes in biophysical attributes of a river along its length that must be accounted for in a CEA framework. Adding additional sampling stations in the upper reaches of the Athabasca River mainstem would help to differentiate between these changes.

A challenge which has faced any monitoring program in the area of the Lower Athabasca has been defining the reference condition or baseline state. Since this area has been under development since the 1960s, it is difficult if not impossible to obtain sufficient monitoring data prior to any human disturbance in the area. In addition due to the nature of the oil sands deposit, there are few if any comparable watersheds in the area which can be used as a "reference". This proposed federal framework acknowledges these difficulties but fails to clearly define how it will be addressed. The addition of more long-term monitoring stations in the area will help to address this problem for future generations, but does not contribute much to this monitoring program. Currently this framework proposes to examine any existing sources of pre-development data in the hopes of obtaining enough to establish a reference condition for the area. As discovered in Squires et al. (2010) while there is publicly available water quality monitoring data prior to major developments in the basin, it is fragmented and typically does not provide information about the oil sands specific water quality constituents which have since been identified. It is hoped that in future phases of this framework the definition of what will constitute the reference condition for this area will be more clearly defined.

6.3 FUTURE WORK

Comparing the thresholds developed in both the laboratory and field studies as well as the trend analysis performed on the historical and current day data available we now have the potential to mitigate any current and future impacts on the Athabasca River basin which may contribute to the concentration load of these specific parameters. These can include both proposed effects on the aquatic environment (new industrial effluent discharges, water allocation pressures) and on the landscape (growth in urban and rural infrastructure).

We have shown salinity (Na and Cl) to be a water quality characteristic that has shown significant change over large space and time scales and we have produced thresholds using the FHM reproductive bioassay for these parameters. In order to effectively mitigate the influence of salinity in the Athabasca River a relationship between landscape change (i.e. industrial development) and increasing salinity must be made.

An understanding of how environmental factors at the landscape can affect aquatic ecosystems is essential if management, prevention and restoration activities are to progress effectively. Although implementing this entire framework is beyond the scope of a single thesis project, the final step in the framework has yet to be completed. The final component of this framework is to develop links between the stressors identified to be of concern and have shown to have potential negative effects on aquatic biota in the basin (Na, Cl, SO_4) to any current or future development through the use of landscape drivers. This last step effectively integrates the effects-based and stressor-based CEA processes by incorporating assessments of the conditions of the basin (effects) with the future and historical impacts on the basin (stressor).

It is also important to continuously update the data used in this assessment as new sources become available. It is also necessary to initiate a rigorous quality control/quality assurance protocol to ensure that the data is free of errors and that issues concerning data quality differences between sources are adequately addressed. It is hoped that in the future industry, government and multi-stakeholder groups which are currently conducting separate monitoring programs will come together and share the data making this type of assessment even more effective. As this occurs, new parameters of concern may emerge and thresholds for these parameters can be generated and used in future planning scenarios.

6.4 CONCLUSIONS

The outcome of this framework ultimately aims to quantify and identify the dominant driving stressors and the corresponding major response patterns on the biota across an entire watershed. The end result of this project addressed the need for site-specific *in situ* thresholds in Canadian aquatic environments while developing an approach to assess the cumulative effects of pollutants on rivers. The successful implementation of this framework on the Athabasca River basin shows it's potential to be applied to other river basins worldwide.

Chapter 3 outlines a significant contribution to knowledge about the trends in both space and time for water quality parameters across the entire Athabasca River basin. While many researchers have identified trends in water quality, no study previous to this has attempted to isolate the important water quality variables which drive the condition (both biological and habitat) across the basin over a period of thirty years. This is especially important when combined with the development of predictive equations for the condition of the basin as it contributes greatly to the reliability and applicability of these equations over both time and space.

The thresholds determined in Chapters 4 and 5 used methods which assess the reproductive potential of a sentinel fish species. This can help to mediate population-level effects on this trophic level. These thresholds can be used as regional-specific indicators of biological effects of these parameters. This information can now be used by regulatory and industrial agencies to help mitigate current and future impacts on the Athabasca River basin.

Some of the deficiencies in how cumulative effects assessment is conducted include issues surrounding the scale (both spatially and temporally) to be used as well as the appropriate indicators and thresholds to consider. As a result, there is not yet a framework for these types of assessments which is accepted and used worldwide. This is not only due to the fact that cumulative effects assessment is still such a debated concept, but also due to the reality that each ecosystem which undergoes assessment is unique. As a result, considerations which are taken and addressed in the assessment of one watershed may or may not be necessary when conducting an assessment in a different watershed.

Since the model system chosen for this thesis work was the Athabasca River Basin, many of the indicators used and thresholds developed (for these indicators) are specific to this particular area. This is a direct result of its unique stressor combination (oil sands, pulp and paper mills, etc.) which may not be found elsewhere in the world. However many of the principles which guided this assessment can be applied to other watersheds worldwide. One such contribution is the utilization of the entire river from headwaters to mouth in this assessment process. Too often many assessments are focused on a particular region due to the presence of a concentration of developments. This can be useful as it allows for an exhaustive detailed assessment of the one region. However as this thesis work has shown, limiting the area of study like this can eliminate our ability to incorporate the natural changes which occur across the length of a watershed. These natural changes can contribute significantly to impacts which are measured further upstream and are missed entirely in these smaller-scaled assessments.

One of the major challenges in conducting any type of environmental assessment at any scale is the determination of a reference area. This is especially difficult which conducting assessments at a watershed scale since no two watersheds are exactly alike. In this thesis work the historical condition of the river basin provided the reference baseline need to then determine if the current existing condition differs from the past and what the potential drivers of those changes were. Determination of what would constitute the "historical" condition should be based on: 1) the availability of data for the area under study and 2) the presence of human-induced stressors in the area and when they commenced.

The overriding goal of this thesis work was to provide a quantitative approach that can assess and characterize cumulative effects of a variety of man-made and naturally occurring stressors over a large space (watershed) and temporal (40 years) scale using an example watershed, the Athabasca River basin. The proposed framework has proved successful and can provide the foundation for other work in basins around the world. Most importantly this framework has proven that quantitative watershed-scale assessments are not only possible but necessary and should be conducted on an ongoing basis if we are to preserve our resources for future generations.

CHAPTER 7: REFERENCES

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