CHAPTER 1

Introduction

Lakes, reservoirs and freshwater wetlands are a significant part of the Colorado landscape to such an extent that more than 1 500 lakes and reservoirs comprises about 66 368 hectares (~164 000 acres) in the state of Colorado (Colorado Department of Health and Environment, 2000). A recent survey by scientists of the water resources in Colorado, USA revealed the presence of several forms of pollutants present in streams and groundwater sources (Stevens 2003; also see http://co.water.usgs.gov, January 20, 2005) and thus, that the quality of Colorado's waters is deteriorating. The water resources in Colorado is affected by both natural and anthropogenic factors. The natural conditions include geological formations, the topographic and climatological factors controlling the hydrologic regime, and the density of vegetation within watersheds. Anthropogenic factors are mainly the result of discharges of pollutants, polluted runoff from various land uses and development in irrigation systems and floodplains (Stevens 2003).

The Cache la Poudre River in the South Platte River Basin serves as one of two surface-water sources (together with the Horsetooth Reservoir) for the city of Fort (Ft.) Collins in Larimy County, Colorado (Fig. 1.1). The Cache la Poudre River originates in the northern Colorado near the Continental Devide. The river flows out of the Rocky Mountain National Park, through the city of Fort Collins, and eventually into the South Platte River near Greeley, Colorado. Snowmelt provides most of the water to the river, with storm runoff in the summer months and ground-water inflow throughout the year (Collins & Sprague 2005). During the summer months, the Cache la Poudre River is supplying raw water to Sheldon Lake in Ft. Collins (Lake Drainage Improvement Project 2002, 2003a, b). Both these water bodies are extensively utilized for recreation and irrigation purposes, while the Cache la Poudre River also provides for drinking water to the city of Ft. Collins (Collins & Sprague 2005).

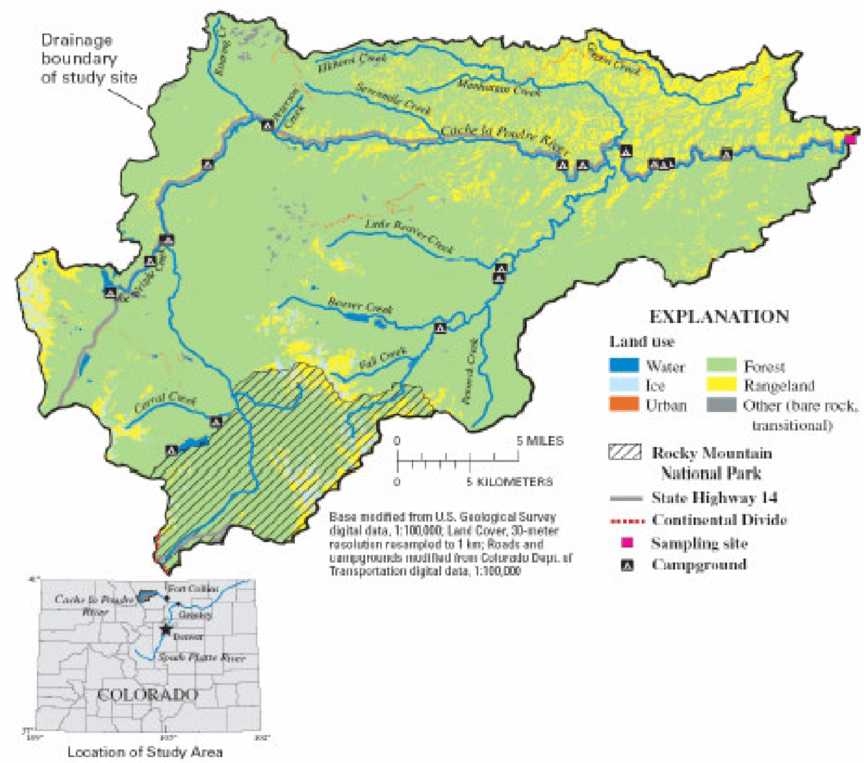

Figure 1.1 Map indicating the catchment area of the Cache la Poudre River (Adapted from Collins & Sprague 2005).

In my Ph.D. thesis, I proposed that anthropogenic activities have a negative impact on selected water resources in Colorado, USA. The main objectives of this study were (i) to investigate the impact of anthropogenic activities on two water bodies in Ft. Collins (e.g., Cache la Poudre River and Sheldon Lake); (ii) to gain scientific knowledge on management and restoration practices, and the effects thereof; and (iii) *to provide a fast, accurate, robust and relatively easy genetic/biological marker system for assessment of the water quality in water resources in general.*

For my experimental model, I have selected two water resources in Ft. Collins, since the Cache la Poudre River is one of the main drinking water sources for near >200 000 residents of Larimy County (including Ft. Collins), Colorado. The city of Ft. Collins water-treatment facility produces more than 37.9 billion liters of treated water annually to serve the needs of the community's residents (Collins & Sprague 2005). The Cache la Poudre River is further the main supplementing water source to the urban Sheldon Lake during the summer months. In contrast to Sheldon Lake that is only receiving water as a drainage system, Cache la Poudre Rive is a free-flowing river system, that supplies water to several urban lakes and the South Platte River. Both aquatic ecosystems are model systems for the study of the impact of anthropogenic activity that necessitated restoration, and then recovery after restoration. Sheldon Lake was restorated prior to the study, while the Cache la Poudre River is presently under restoration with the support of the U.S. Environmental Protection Agency. Restoration of both water bodies was necessary due to poor water quality as a result of anthropogenic pollution.

The chapters of the thesis are independent units by themselves; however, they converge towards addressing more or less the impacts of anthropogenic activity on aquatic ecosystems, using the above two study areas, it further use biomarkers and molecular markers to assess raw water health and the dynamics and diversity in the aquatic ecosystem.

Outline of Chapters

Chapter 2

In Chapter 2, I give a review on the existing information relating to the impact of anthropogenic activity (e.g., urban run-off, eutrophication) on the quality of water resources in general and specific to the US. Toxic chemical pollutants and cyanobacterial toxins and the risk posed by these compounds to human health are discussed. Economic losses related to the presence of freshwater pollutants are the result of contact with or consumption of water containing toxic substances and/or toxic biological organisms/cells, and these include the costs incurred from death of domestic animals, allergic and gastrointestinal problems in humans and water treatment. I further discuss applied technology for assessment of aquatic health, which includes: the use of biological organisms as bioindicators and other detection methods, specifically the use of PCR-based technology that has a great potential as a fast screening method to detect toxic cyanobacteria strains in water bodies.

Chapter 3

In Chapter 3, I have examined the impact of long-term contamination of coal tar residue on benthic organisms in the Cache la Poudre River, Colorado. Hazardous chemicals such as oils and hydrocarbons are common water pollutants generated from a variety of industrial processes, such as oil refineries, coke ovens and coal gasification. Toxicity of oils to organisms is usually related to their content of non-volatile aromatic hydrocarbons (Zikto & Carson 1970; Neff & Anderson 1975).

Hufford (1971) revealed that the most immediate toxic and subtoxic fractions of oils are those soluble in water. Knowledge of the effects of oil spills on freshwater ecosystems is limited compared to the wealth of information available on oil entering the marine environment. In the United States, 179 freshwater oil spills were reported between 1979 and 1986, and spills exceeding 200 000 L occurred 25 times between 1974 and 1980 (Cronk *et al.* 1990). Petroleum releases into the aquatic environment are a leading cause of fish kills in the United States (Green & Trett 1989). Crunkilton (1984) indicated that one-third of all reported water pollution incidents in Missouri were petroleum-related.

The first objective of Chapter 3 was to apply the Australian river bioassessment system (AUSRIVAS) on a long-term coal tar contaminated area of the Cache la Poudre River, Colorado. This method is based on the presence and absence of aquatic macroinvertebrates, and compares the numbers and types of organisms found at a site with those predicted to be present on the basis of a model derived using data from minimally degraded reference sites. The second objective was to use several test species, belonging to different trophic levels as biomarkers to provide rapid toxicity assessment of the coal tar contaminated area of the Cache la Poudre River. Since the Cache la Poudre River is supplying water to Sheldon Lake, its water quality will be a determining factor in the general "health" of the aqua ecosystem in the urban Sheldon Lake (see Chapters 4 and 5).

Chapter 4

The goal of chapter 4 was to assess the ecological status, as well as the limnological condition of Sheldon Lake a year after restoration. Succession is one of the more obvious and regular features of a lake. Hence, some organisms always occur during spring, whereas others exclusively dominate during summer or in late autumn, forming a succession pattern that is generally repeated every year. Factors that may be affecting seasonal succession are the trophic state of a lake, the different physical or chemical requirements of organisms, including temperature and nutrient or food preferences. Interactions among the organisms themselves are also important, including predation, grazing and competition pressure. Hence, following a seasonal cycle in Sheldon Lake will reveal the abiotic as well as biotic processes acting to shape the observed succession pattern among organisms after restoration.

Chapter 5

The purpose of the study in Chapter 5 was to evaluate the efficacy of artificial mixing in controlling cyanobacterial bloom formation in summer during low wind velocity. In chapter 5, the use of PCR amplification of the *mcy* gene cluster in environmental *Microcystis aeruginosa* samples of Sheldon Lake for early detection of potentially toxic *Microcystis* mass occurrences a year after restoration of this shallow urban lake was assessed. Since microcystins are synthesized nonribosomally by a peptide synthetase polyketide synthase enzyme complex encoded by the microcystin synthetase (*mcy*) gene cluster (Christiansen *et al*. 2003; Dittmann *et al*. 1997; Nishizawa *et al*. 1999, 2000; Tillett *et al*. 2000). Further objectives were to use PCR amplification of the *mcy* gene cluster to detect the toxicity through gene expression of

Microcystis populations. By doing so it will provide us with more information on the microcystin producing genotypes that form the epilimnetic population.

Chapter 6.

In Chapter 6, I investigated the usefulness of a novel DNA fingerprinting technique, AFLP, which is based on the selective amplification of genomic restriction fragments by PCR, to differentiate between geographical unrelated strains of *Microcystis spp.* because some species may occur more widely, but with morphologies that are phenotypically uniform in one area and variable in another. Examples of variable cyanobacteria populations in comparison with other regions are *Woronichinia naegeliana* in Canadian lakes (Komárek & Komarkova-Legnerova 1992) and *Microcystis wesenbergii* in Northern Europe (Cronberg & Komárek 1994). Komárek and Anagnostidis (1989) stated that the feature of more than 50% of the strains in collections do not correspond to the diagnoses of the taxa to which they are assigned.

Chapter 6 further aims to supply insight into the genetic diversity of the newly collected toxic strains in the study (i.e., UPUS1 *M. aeruginosa* and UPUS2 *Woronichinia naegeliana*), since little information is available on Colorado cyanobacterial strains in general. The study, in part, aims to put the Colorado strains in genetic "context" to other reference strains used during the study (i.e., *M. aeruginosa* PCC7806 and *M. aeruginosa* UP37). Both strains tested toxic using ELISA and PP2A methodology, with UP37 the most toxic strain collected in South Africa during the study (see Chapter 5).

Finally, I am presenting a summary of all the observations made through the study. I am also concluding with recommendations with regard to restoration and management practices (see Summary).

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