SUITABILITY OF SMALL FISH SPECIES FOR MONITORING THE EFFECTS OF PULP MILL EFFLUENT ON FISH POPULATIONS

by

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ABSTRACT

SUITABILITY OF SMALL FISH SPECIES FOR MONITORING THE EFFECTS OF PULP MILL EFFLUENT ON FISH POPULATIONS

Research was conducted to assess the suitability of small fish species as sentinel species for monitoring the effects of pulp mill effluent on fish populations. There has been recent concern that larger fish species may not be suitable monitors of local environments because they are mobile and capable of extensive movement beyond effluent exposure areas. Smaller, resident and non-migratory species (e.g., many minnows, sculpins, darters) were proposed as alternate sentinel species because they exhibit reduced mobility relative to larger species, tend to be more numerous, and are not subject to commercial or sport fishing pressure. Evaluation of small species was based on their response to mill effluent, as well as the abundance and capture efficiency of each species. Responses were described by comparing whole organism (growth, reproduction, energy storage, age) and physiological (MFO induction, sex steroid levels) measurements in fish exposed to pulp mill effluent with comparable reference fish populations not exposed to effluent.

Research focused on spoonhead sculpin (*Cottus ricei*) and lake chub (*Couesius plumbeus*) of the Athabasca River, Alberta, to determine whether changes in whole-organism and physiological measurements could be detected in small fish species exposed to pulp mill effluent. Both species exhibited responses reflecting instream effluent conditions. Spoonhead sculpin exposed to kraft mill effluent showed an overall increase in body and organ size, reproductive commitment, MFO induction and steroid production relative to unexposed fish. As well, graded responses were observed in sculpin exposed to different concentrations of effluent, and persisted downstream for at least 50 km. In contrast, lake chub exposed to effluents from a thermomechanical and a chemithermomechanical mill exhibited few changes relative to reference chub. It was concluded that the response (i.e., little change) of lake chub reflected the low instream concentrations of non-kraft, totally chlorine free effluent in the area.

Research at the Moose River system, Ontario, investigated the consistency and relative sensitivity of responses between trout-perch (*Percopsis omiscomaycus*) and the larger white sucker (*Catostomus commersoni*) under similar conditions of pulp mill effluent exposure and mobility. At this site potential differences in mobility were minimized by the presence of upstream and downstream dams. Both species exposed to effluent from a thermomechanical mill exhibited differences in whole-organism and physiological measurements relative to reference fish. However, the response of trout-perch was not consistent with the response of white sucker. The inconsistency between responses made it difficult to determine how the opposing responses of each species were related, and which species was most sensitive to the instream conditions.

A more detailed examination of fish responses was necessary to facilitate the interpretation of responses, and the comparison between trout-perch and white sucker. An existing monitoring framework was revised and further developed to provide a synthesis of fish responses documented in the literature, and a greater understanding of the progression of fish responses to common mechanisms of stressors. By categorizing fish responses according to age structure, energy expenditure, and energy storage it was possible to simplify the responses, determine which part of the populations were being affected, and identify the probable mechanisms leading to the responses. From this information, it was evident that the responses of trout-perch and white sucker highlighted similar mechanisms of change despite the difference in response patterns, and that either species could be used as a sentinel species at this site. In addition, the framework also indicated that the response of spoonhead sculpin was consistent with information described in previous studies investigating water quality, algal growth and benthos communities.

ACKNOWLEDGEMENTS

I remember when I had finished my M.Sc. degree I had decided that there was no way I was going back to school for a Ph.D. degree . . . no chance . . . never! Perhaps I was too insistent . . . damn, I hate that! Most likely I failed to recognize that over time attitudes change and opportunities have a way of altering one's plans. Whatever the reason, I am now at a stage in my thesis where I am able to extend my appreciation and thanks to all those who have made the past 4 years very enjoyable and meaningful.

Firstly, I would like to thank Dr. Kelly Munkittrick for providing me the opportunity to undertake my Ph.D. degree. Over the past 8 years that we have known each other, Kelly has continuously shown faith in my abilities (even when I am unsure of them) and has been thoroughly supportive in all aspects of my research. His enthusiasm and optimism have been greatly appreciated, as has the friendship that has developed over this time. In all honesty, if it wasn't for him I'd still be stuck in Vancouver in total conflict on whether to go skiing, fishing or hiking day after day after day. Thanks for getting me out of that rut!? I would also like to extend a big thank you to Patty, Sara, and Jessica of the Munkittrick household for welcoming me into their family and providing a room at the ever busy "Munkittrick Hotel" when I first moved back to Ontario.

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Field work was a big part of my thesis research and I was lucky enough to have the company of some great people. Special thanks to Dana Boyter and Len Eklund (along with his sidekick "Porkchop") who accompanied me during most of my field trips. We had some long field trips with many long days and their consistently happy demeanour, optimism and hard work (even while fishing until 1:30 am) really made things much easier on me and attributed greatly to the success of each trip. Of course, periodic soaks in the hot springs certainly wasn't the worst way in the world to try and alleviate any field stress one might be feeling. In addition to these two characters, there were several other characters who at some time accompanied me into the field. For their help I would like to thank Kelly Munkittrick, Janet Jardine, Mark Hewitt, Cam Portt, Peter Flett, Ken Oakes, Mark McMaster, and Bruce Kilgour. Additional thanks goes to Bruce Kilgour who has been a close friend for many years and who is always willing to chat about research, life and the universe; preferably over a cold beer or two (...but who's counting).

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My research on the Athabasca River was funded by the Northern River Basins Study (NRBS), Edmonton, Alberta, awarded to Dr. Kelly Munkittrick. Thanks to Fred Wrona, Ken Crutchfield, Greg Wagner and Richard Chabaylo of the NRBS office for their help during these studies. Research on the Moose River system was funded by the Canadian Electricity Association, Ontario Hydro and the Department of Fisheries and Oceans (Green Plan Toxics Fund, PERD). Additional funding was provided by a personal scholarship from the Natural Sciences and Engineering Research Council (NSERC). Thanks also to personnel from the pulp mills at Hinton, Whitecourt, Kapuskasing and Smooth Rock Falls for their cooperation and assistance.

I would also like to thank everyone in my family for the support they have given me throughout my university studies. I am quite sure they were beginning to think I had become a professional student (kindergarten to grade 23), but fear not, I will get a job some day (but not too soon...I need a break!). I would especially like to thank my parents who have always been there for me and provided encouragement and faith in the ever changing path that I have chosen. It has meant a lot to me. Finally, my deepest thanks goes to my wife, Ruth. Her happy-go-lucky personality is contagious and she has added much laughter and love to my life. I hope the burden of the thesis has not been excessive, and that I am forgiven for not providing the amount of chocolate accolades she deserved. Thanks also for trying to show me the importance of enjoying the journey and not just the destination, something I am just beginning to recognize.

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CHAPTER 1

INTRODUCTION

1.1 Background

Scandinavian studies conducted during the latter part of the 1980's documented impacts of bleached kraft mill effluent on fish community structure (Neuman and Karås 1988), fish growth and reproduction (Sandström et al. 1988), and fish biochemistry and physiology (Andersson et al. 1988) (also reviewed in Södergren 1989; Tana and Lehtinen 1996). However, due to the lack of supporting studies conducted in Canada, the applicability of the Scandinavian results to Canadian receiving environments was uncertain (Sprague and Colodey 1989), and prompted substantial research effort assessing the impacts of pulp mill effluent on fishes in Canada. Soon thereafter, and continuing today, results from this research have documented numerous biochemical and physiological alterations, impacts on growth and reproductive investment, gross pathologies, and increased chemical accumulation/contamination in wild fish exposed to mill effluents (e.g., McMaster et al. 1991; Munkittrick et al. 1991a, 1992b; Gagnon et al. 1992; Hodson et al. 1992; Kahn et al. 1992; Servizi et al. 1993). Consequently, there has been greater acceptance that pulp mill effluents may constitute a threat to fishes in Canadian receiving waters (Sandström 1996).

In response to the growing evidence of mill effluent impacts on fish, all pulp and paper mills and regulated off-site treatment facilities in Canada are required to conduct an Environmental Effects Monitoring (EEM) program (Environment Canada and Department of Fisheries & Oceans 1992). An integral component of the program includes monitoring fish to assess the effects of pulp mill effluents on receiving waters. The approach adopted was to conduct a detailed assessment of fish (i.e., an Adult Fish Survey) using a sentinel fish species to determine the biological significance of effluent effects. The underlying premise of the approach is that the status of the sentinel species is a reflection of the overall condition of the aquatic environment in which the fish reside (Munkittrick 1992b). The general design of the assessment is a comparison of fish exposed to effluent (e.g., downstream of a mill outfall in lotic systems), with comparable reference or control populations not exposed to effluent (e.g., upstream of the outfall).

The success of a sentinel species monitoring program depends heavily on the selection of an appropriate sentinel fish species. In Canada, large fish species have been extensively used as sentinel monitors for freshwater environments, for example: longnose sucker (Gibbons et al. 1991; Gibbons et al. 1992; Swanson et al. 1994); white sucker (Munkittrick et al. 1991a; McMaster et al. 1992b); lake whitefish (Munkittrick et al. 1992b); mountain whitefish (Munkittrick et al. 1990; Kilgour and Gibbons 1991; Swanson et al. 1994); burbot (Brown et al. 1996); and walleye (Swanson et al. 1996). Factors considered when these species were selected included: benthic behaviour; community dominance; contaminant levels; abundance; and commercial importance. However, despite their common usage and careful selection, large species may not be appropriate for all monitoring circumstances.

An important assumption of a sentinel monitoring program is that fish collected at specific sites exhibit responses or characteristics that reflect their local environment. As such, a primary consideration when selecting a sentinel species is the residency time of that species relative to effluent exposure (Environment Canada and Department of Fisheries & Oceans 1992, 1993). This issue is particularly important in open receiving waters such as large river systems, lakes and marine environments (Brown et al. 1993; Gibbons and Munkittrick 1993; Swanson et al. 1994). These types of aquatic systems have always been difficult to monitor because they allow free movement of mobile organisms over considerable distances (Gerking 1950). In general, the greater the likelihood that a fish species is continuously exposed to the effluent, the greater its value as a sentinel species for monitoring the potential effects of the effluent.

Many large fish species may not be suitable for monitoring open systems because they can be very mobile and capable of extensive movement beyond effluent exposure areas. Successful demonstration of responses of wild fish has been seen in Central Canada, but predominantly in environments where movement of fish was restricted by habitat (e.g., Munkittrick et al. 1991a), or man-made barriers (e.g., Hodson et al. 1992). Recent studies in large, open rivers have documented the potential of large species for extensive movement. During a recent pulp mill monitoring study by Swanson et al. (1994) on the Wapiti River, Alberta, radiotelemetry and mark-recapture data showed that the movement of some mountain whitefish was extensive (maximum of 188 km) and rapid. Although much of the large scale movement of this species could be attributed to seasonal spawning activity, it was still obvious that mountain whitefish were capable of moving a considerable distance, and that the difference in exposure between fish collected at the mill and reference sites was uncertain. R.L. & L. Environmental Services Ltd. (1993) conducted a fish radiotelemetry study on the upper reaches of the Athabasca River during the spring and summer season. Among species that were successfully tracked, burbot was found to be relatively sedentary; however, both bull trout and mountain whitefish moved substantial distances (up to 100 km) during the study period. Similarly, the distinction between exposed and reference fish was questioned when longnose sucker in the Athabasca River near Whitecourt were floy tagged within a pulp mill exposure zone, but were recaptured in the upstream reference zone (Sentar Consultants Ltd 1996b).

Capture success of a particular fish species is very site-specific, or more accurately, habitat-specific. In some instances, the density of fish in large rivers prohibits capture of large samples. Several researchers conducting effluent monitoring studies in large river systems have found that many large fish species could not be captured in sufficient numbers to adequately evaluate fish responses. For example, a pilot study on the St. Maurice River, Quebec (Hodson et al. 1992) sampled seven species of fish (white sucker, northern pike, walleye, brown bullhead, fallfish, longnose sucker, smallmouth bass) from 5 sites. Unfortunately, only white sucker were captured in numbers (n=8-15 fish) close to their conservative target of 15 fish per site, sexes combined. Brown et al. (1996) investigated sex steroid levels and gonad morphology of burbot, longnose sucker, northern pike and flathead chub exposed to mill effluent in the Peace, Athabasca and Slave River drainages. Although they had an *a priori* plan to pool several sites for analyses, capture success for any species at the individual sites was, on average, less than 5 fish per sex per site (not including sites where some species were not found). Suitable habitat for some fish species was limited, or missing in some receiving environments. A study conducted by Gibbons et al. (1995) on the Fraser River near Prince George, BC, found that peamouth chub were abundant in the reference zone, but could not be collected in sufficient numbers in the near-field zones to allow for statistical comparisons. It was concluded that capture success was affected by the lack of suitable habitat within the exposure zones. Similarly, Mah et al. (1989) attempted to collect fish upstream and downstream of 10 mills in British Columbia (Fraser River, Thompson River, Columbia River, Williston Lake) to measure tissue concentrations of dioxins and furans. Only comparisons using largescale sucker could be made consistently at all mills; other species were of limited value because the same species could not always be collected upstream and downstream of a mill site.

Finally, many large fish species are selected as sentinel species because of their importance to the commercial or sport fishing industry (e.g., lake whitefish, walleye, northern pike), and not necessarily because they are an appropriate sentinel for the aquatic environment. Wedemeyer et al. (1984) cautioned against trying to assess possible effects of environmental stress on fish that are also subject to exploitation. Heavy exploitation pressure may obscure or confound effects related to effluent exposure. As well, research sampling represents further fishing pressure on an already exploited stock, and many of these river systems have naturally low productivity and low fish density.

Concerns over the use of larger and potentially mobile fish species as sentinels, especially in open receiving environments, has led me to evaluate the suitability of fish species for monitoring this type of environment. Specifically, I propose that smaller, resident and non-migratory fish species could be used as possible alternative sentinel species. Historically, many of the smaller species of the fish community have been regarded simply as forage for the larger, commercially important fish species and have been overlooked when conducting sentinel monitoring. However, many small fish species exhibit characteristics which may circumvent some of the problems experienced when monitoring larger fish species, and these are summarized below:

a) A primary reason for considering a small species as a sentinel is to improve the certainty that the sentinel species is being exposed to effluent. From the limited number of published studies which have documented the movement and home range of small fish species, species such as mottled sculpin (Bailey 1952; McCleave 1964; Hill and

Grossman 1987), longnose dace and rosyside dace (Hill and Grossman 1987), banded sculpin (Greenburg and Holtzman 1987), and numerous darter species (Winn 1958) exhibited limited mobility relative to what has been documented for larger species, and possess a small home range. This is not to say smaller species are not mobile, but that the degree of movement is less when compared to many larger fish species. In addition, many small fish species exhibit territorial behaviour, particularly in lotic systems (Hynes 1970). For example several species of sculpins and darters have been reported to be territorial (Balon 1975; Roberts 1988 a,b; Nelson and Paetz 1992) and less likely to move far from their guarded habitat. Both characteristics increase the probability that a sentinel species will not move beyond a particular exposure zone, and the observed response of that species will more likely reflect the local environment from which it was caught.

b) There is often a tendency for smaller species (typically the forage base) of the fish community to be more numerous than larger, predatory species (e.g., R.L. & L. Environmental Services Ltd 1994). This tendency follows the ecological generality that smaller fish of lower trophic levels should theoretically be more numerous than larger fish (consumers) of higher trophic levels based on food chain energy flow and efficiency (Lindeman 1942; Odum 1971). What this means for sentinel monitoring is that it should be easier to collect greater numbers of small sentinel species, as well as increase the number of possible sampling sites.

c) Most small species have a shorter life-span than larger species. Fish species which have long life spans can be relatively slow to respond to environmental changes, or can exhibit resilience resulting in a considerable lag time before detection of adverse effects or recovery (Munkittrick 1992b). Species of shorter, intermediate life spans live long enough to show responses, and will show possible alterations in reproduction and growth faster than longer lived species.

d) Small fish species are typically not subject to commercial or sport fishing pressure that may confound possible effects related to effluent.

Despite the apparent advantages of using small species as sentinel species, there has been no known published research which has investigated their application to monitoring the effects of pulp mill effluent on receiving environments. Of specific importance is the assurance that small fish species are capable of exhibiting responses reflecting exposure to environmental stressors like pulp mill effluent. In the absence of such information, there is a need to conduct work describing the response of small fish species to effluent exposure before they can be confidently accepted as alternative sentinel species.

1.2 Objective and Outline of Thesis

The overall objective of my thesis was to evaluate the suitability of small fish species as sentinel species for assessing the effects of pulp and paper mill effluents. In particular, I investigated the response of small fish species to pulp mill effluent in lotic receiving waters.

I described the "response" of sentinel species based on observed differences in whole-organism and/or physiological measurements of exposed fish relative to reference fish. Parameters were chosen to reflect growth (size-at-age), reproductive investment and integrity (gonad size, fecundity, *in vitro* sex hormone production), energy stores (condition, liver weight), and age structure (mean adult age), as well as effluent exposure (hepatic mixed function oxygenase activity). It should be emphasized that a response was derived from a sample of fish from a particular area and/or habitat, e.g., fish collected from a riffle within the effluent exposure zone. For lack of a more appropriate term, the sampled group of fish (of the same species) from a particular site was referred to as a local "population". This use of the term closely resembles the definition described by Wetzel (1983) who stated that a population is "a spatially defined assemblage of individuals of one species . . . that occupy or populate a more localized area, such as a particular lake or section of flowing water".

River systems, particularly large river systems, were chosen as the experimental receiving environment for several reasons: a) many rivers are open, unconstrained systems allowing free movement of fish; b) it is generally easier to track the effluent plume and identify exposure levels and sampling sites in lotic systems by virtue of the unidirectional flow of water; c) many inland pulp mill operations utilize river systems as the receiving environment for effluent; d) larger river systems often receive effluent from multiple pulp mill operations due to high dilution capacity of these systems allowing for multiple study sites within one system and a potential opportunity to assess cumulative effects; and e) rivers are subject to seasonal periods of low-flow conditions (e.g., western rivers - winter flow, eastern rivers - summer flow) which decrease the dilution capacity of the system and increase the exposure of resident fish species to higher effluent concentrations.

To address my objective, the first step was to determine whether it was possible to detect changes in whole organism and physiological measurements in small fish species exposed to mill effluent relative to reference or unexposed fish. Initial efforts focused on a small sentinel species exposed to bleached kraft mill effluent (BKME) from a mill in Hinton, Alberta, discharging to the upper Athabasca River. This type of mill process has been monitored extensively in Canada, United States and Scandinavia, and numerous impacts on fishes have been documented, even after the installation of secondary treatment facilities. Further research was conducted downstream of the Hinton mill near Whitecourt to determine whether the responses observed in Hinton persisted downstream in areas of lower effluent concentrations. This region of the upper Athabasca River also receives effluent from a thermomechanical mill and a chemithermomechanical mill, where responses of small fish species to non-kraft (and chlorine-free) mill process types were studied. A final study on the Athabasca River was conducted at the Hinton site to: a) determine whether the response of the small sentinel species was more dramatic in the spring following high effluent exposure during winter low-flow conditions; and, b) determine the geographical extent of responses as far as 50 km downstream of the mill diffuser. The results of the research on the Athabasca River are presented in Chapter 2, "Small fish species responses to pulp mill effluent".

Following the research conducted on the Athabasca River, the next question addressed was whether responses in small species were comparable to responses in large species, assuming limited mobility for both. This work is presented in Chapter 3, "Comparison between responses of small and large fish species exposed to pulp mill effluent". The study area for conducting this phase of the research was the Moose River system in the vicinity of the pulp mills at Smooth Rock Falls (kraft mill) and Kapuskasing (thermomechanical mill), Ontario. The Moose River system is dammed at several points along its water course, including dams upstream and downstream of the pulp mill operations, which effectively restricts large-scale movement of fish beyond the zones of exposure. For this reason, it has been possible for other researchers to use a larger sentinel fish species (white sucker) for monitoring the effects of pulp mill effluent, without the potential risks associated with mobility. For my purposes, this presented an opportunity to directly evaluate and compare the response of a small fish species with the response of the larger white sucker collected from the same sites and exposure conditions.

Chapter 4, "Interpretation of fish responses" provides a framework for the classification and interpretation of fish responses observed in the thesis. It became obvious from the comparisons discussed in Chapter 3 that it was difficult to directly compare the responses of small fish species and large fish species. The comparison of responses was complicated by differences in longevity, maturation rates, growth rates, etc., that ultimately effect relative response times. More detailed examination of the response patterns of the fish species was required to facilitate comparisons. An interpretation framework examining responses of fish was reorganized and revised in light of new data from the thesis, as well as recent information from the literature. The framework also addressed the interpretation of fish responses as a reflection of possible causative agents and the direction of follow-up investigations.

A final discussion presented in Chapter 5 reflects on the suitability of small fish species for sentinel monitoring in open river systems, and summarizes the conclusions and significant findings of the thesis.

CHAPTER 2

SMALL FISH SPECIES RESPONSES TO PULP MILL EFFLUENTS

2.1 Introduction

Several studies have documented the effects of pulp mill effluent on wild fish species resident in freshwater receiving environments (e.g., McMaster et al. 1991; Adams et al. 1992; Munkittrick et al. 1992; Van Der Kraak et al. 1992; Gagnon et al. 1994). However, to date there has been no published field work focussing on small fish species. There is need to demonstrate that smaller fish species are capable of showing responses to industrial effluents under field conditions before their use as sentinel species can be advanced. To this end, the first stage of my evaluation of small fishes as sentinel species was to determine whether it was possible to detect changes in whole-organism and physiological measurements in small, wild fish species exposed to pulp mill effluent. Specifically, given the differences in life history between small and large fish species (e.g., life span, maturation rates, growth rates, etc.,), are smaller species of the fish community sensitive to exposure to effluent.

Many effluent-related impacts on fish have been associated with bleached kraft mill effluent (BKME). For this reason, it seemed reasonable to focus initial evaluations on this mill process type to increase the probability of observing an effluent-related response. The site chosen was a bleached kraft mill located in Hinton, Alberta. This mill discharges into the upper Athabasca River and has been the site of several effluent-related assessments (e.g., Anderson 1989; Brown et al. 1993; Scrimgeour et al. 1995; Golder Associates 1996; Podemski and Culp 1996). The Athabasca River represents a large, open system which, historically, has been difficult to monitor using large fish species. This was exemplified by a study (Brown et al. 1993) evaluating steroid hormone levels and gonad morphology in white sucker, longnose sucker, mountain whitefish and northern pike exposed to effluent from the Hinton mill. The study was compromised by: a) small sample sizes of most species from the exposed sites; b) poor capture success within the upstream reference zone; and c) uncertainty regarding the mobility of sentinel species and the close proximity of the exposure and reference collection sites. Similarly, a recent study conducted by the mill itself monitoring mountain whitefish and longnose sucker found it necessary to collect reference fish from a separate river system to avoid concerns regarding fish mobility (Golder Associates Ltd. 1996).

The Hinton site was also of interest because, despite problems associated with the field studies on fish, there was evidence that effluent from this mill had the potential to impact fish. Kovacs *et al.* (1995) conducted a life cycle test in the laboratory using fathead minnows exposed to effluent from a bleached kraft mill, identified as the Hinton mill (T. Andrews, Weldwood of Canada Ltd., Hinton, Alberta, personal communication). The study was conducted prior to mill process changes initiated in 1993 which included the switch from 45% to 100% chlorine dioxide substitution (Golder Associates Ltd. 1994). Although there was no effect on growth, survival, and hatching success, effluent concentrations of 1.7% caused a 25% reduction in egg production, and a greater proportion of males than females was observed at effluent concentrations exceeding 5 %. As well, work conducted by Brown et al. (1993) suggested possible depressions in female 17 β-estradiol in wild longnose sucker and mountain whitefish collected downstream of the mill diffuser.

Alberta Environment has a long-term water quality monitoring station for the Athabasca River at Windfall Junction, approximately 180 km downstream of the Hinton mill (L. Noton, Alberta Environmental Assessment, Edmonton, Alberta, personal communication). Prior to upgrading of the Hinton mill, concentrations of chlorinated phenolics, resin acids, adsorbable organic halides (AOX) and a variety of other compounds measured at this site indicated that this part of the upper Athabasca River was still influenced by effluent from the Hinton kraft mill (Noton and Saffran 1995). Even after changes in the mill process, conservative tracers such as chloride, sodium and resin acids confirmed that the effluent was detectable at this site (L. Noton, Alberta Environmental Protection, Edmonton, Alberta, unpublished data). Based on effluent discharge and river dilution, it can be estimated that the average effluent concentration at Windfall ranged from 0.25% during freshet (high flow) to 4% during winter low flow conditions. I conducted further research to determine whether the response of small sentinel species observed within the mill near-field zone could be observed downstream at Windfall Junction.

The region of the upper Athabasca River immediately downstream of Windfall Junction also receives effluent from a thermomechanical pulp mill and a chemithermomechanical pulp mill. These mill processes are totally chlorine free (TCF) and typically produce much lower volumes of effluent discharge than kraft operations (McCubbin and Folke 1993). Although laboratory exposures of fish to these types of effluents have shown moderate induction of mixed function oxygenase activity (Gagne and Blaise 1993; Martel et al. 1996), little work has been done to investigate *in situ* whole-organism responses of fish. I conducted an additional study focussing on these mills to further investigate the response of a small fish species to mill effluent, specifically non-kraft mill process types.

The previous Athabasca field collections were done during the fall when discharge levels of the upper Athabasca River were moderate (≈100-200 m^3/s, Water Survey of Canada) and sampling sites were easily accessible. However, low-flow conditions occur in the Athabasca River during the winter months (≈35-50 m^3/s, Water Survey of Canada), and it is at this time that instream effluent concentrations are highest. Consequently, I conducted a final study on the upper Athabasca River in the vicinity of the kraft mill during the spring, immediately after ice break-up. The objective was to determine whether the responses I had observed in sculpin during the fall were altered after exposure to a prolonged period of high effluent concentrations associated with winter low-flow conditions. Furthermore, since others have documented possible sex hormone depressions in large fish species exposed to effluent from this mill (Brown et al. 1993, 1996), the study provided an opportunity to further evaluate the *in vitro* steroid response of the smaller sentinel species: a) immediately following high effluent exposure; and b) during the prespawning stage at which time the steroid profiles were expected to be most developed. In addition, I also investigated the geographical extent of responses downstream of the mill.

In summary, the objectives of my research on the upper Athabasca River were to investigate whether:

1) smaller fish species were capable of showing responses to bleached kraft and non-kraft mill effluent under field conditions
2) it was possible to document the geographical extent of effluent-related responses of small sentinel species
3) responses of the small sentinel species were altered following a prolonged period of high effluent exposure

2.2 Materials and Methods

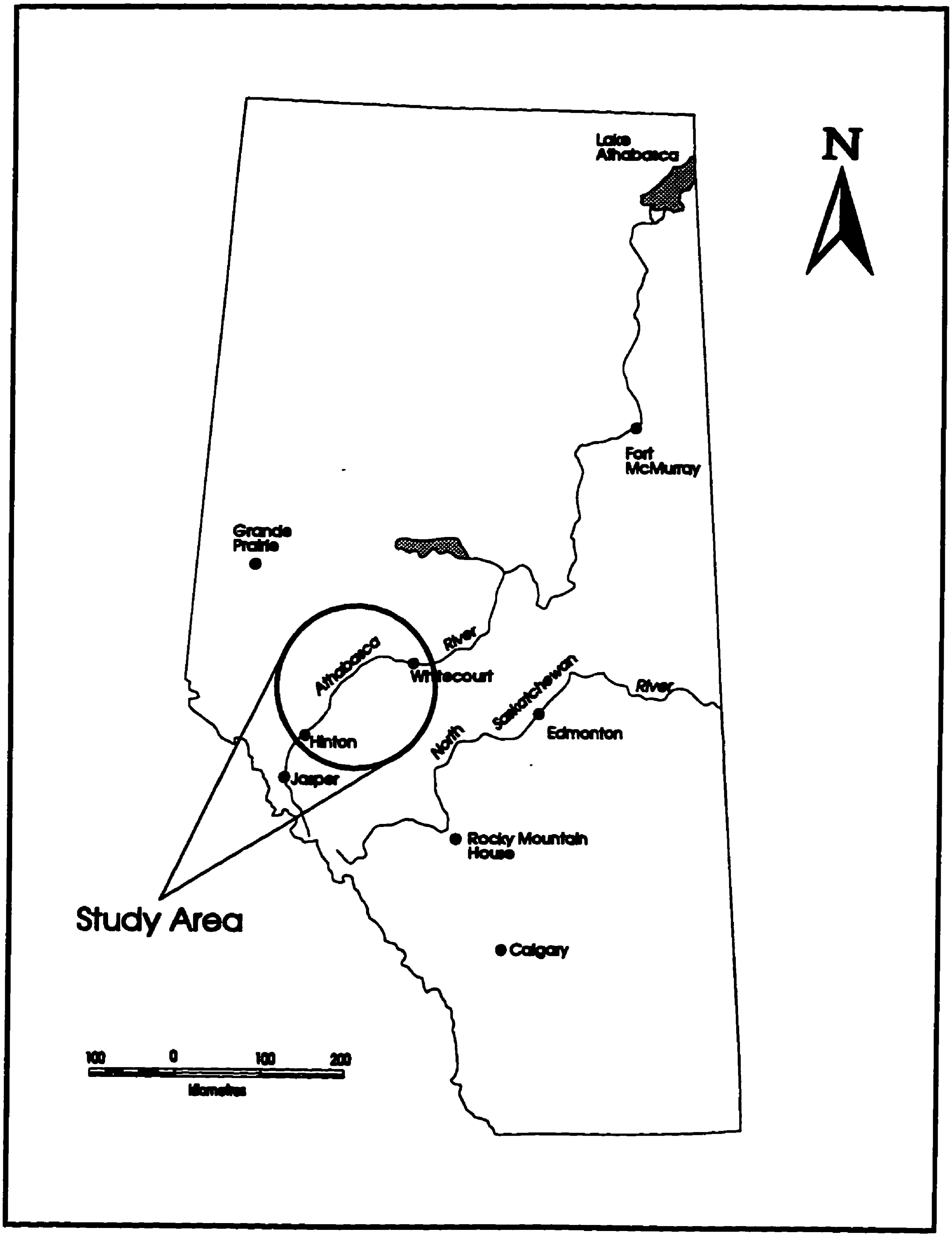
General Study Area and Mill Characteristics

The Athabasca River originates high in the Rocky Mountains in Jasper National Park, flows northeast for approximately 1400 km and terminates at Lake Athabasca, Alberta (Figure 2.1). By virtue of its montane origins, the upper Athabasca River is a cold, oligotrophic system exhibiting high flows during the summer, and low flows during the fall and winter months.

The study areas were the receiving waters of the kraft mill at Hinton and two non-kraft mills near Whitecourt, Alberta. As well, reference zones were located upstream of Hinton and near Windfall Junction upstream of Whitecourt. During the spring study, I sampled additional sites within the far-field zone of the kraft mill, downstream of the Obed Mountain Coal Ltd. conveyor road, and downstream of Emerson Bridge (Athabasca River crossing near Emerson Lakes Forest Recreation Area).

The bleached kraft mill located in Hinton produces approximately 1,100 air dried metric tonnes of bleached softwood kraft pulp per day (Rodden 1995). During the study period, the primary wood sources used by the mill consisted of 55% lodgepole pine, 20% white spruce, 20% black

Figure 2.1. **Map of Alberta and the study area on the Athabasca River.**


Lake Athabasca
N
Fort McMurray
Grande Prairie
Athabasca
River
Whitecourt
North
Saskatchewan
River
Edmonton
Hinton
Jasper
Rocky Mountain House
Study Area
Calgary
100
0
100
200
kilometres

spruce and 5% balsam fir (Kovacs et al. 1996). The bleaching sequence was $DE_{op}(DED)$ (D, reaction with aqueous chlorine dioxide; E, extraction with sodium hydroxide; E_{op}, extraction with sodium hydroxide and the addition of elemental oxygen and peroxide) utilizing oxygen delignification and 100% chlorine dioxide substitution (Golder Associates 1994). Effluent was subjected to treatment in a mechanical primary clarifier followed by secondary treatment in a 5-d aeration stabilization basin prior to discharge to the Athabasca River via an instream diffuser. The effluent consisted of approximately 95% mill effluent and 5% municipal waste water/sewage for a total effluent discharge of 1.2 m^3/s (Noton and Shaw 1989; Kovacs et al. 1996).

The first non-kraft mill is a thermomechanical pulp (TMP) and paper operation located approximately 200 km downstream of the Hinton mill, and 10 km west of Whitecourt. During the study period, the mill used approximately 92% wood fibre and 8% recycled fibre. The primary wood sources consisted of 56% white/black spruce, 36% lodgepole/jack pine and 8% balsam fir (Sentar Consultants Ltd. 1994a). The mill produced 620 tonnes of hydrosulfite-bleached newsprint per day. The effluent was initially treated in a primary clarifier followed by an aerated equalization basin, an activated sludge plant and an aeration polishing basin prior to being discharged into the Athabasca River via a submerged diffuser at a rate of approximately 0.17 m^3/s (Sentar Consultants Ltd. 1994a).

The second non-kraft mill is a chlorine-free chemithermomechanical pulp and paper mill (CTMP) and is located about 10 km downstream of the TMP mill in the town of Whitecourt. During the study period, the mill was capable of utilizing both softwood and hardwood. Softwood consisted of a mixture of 50% lodgepole pine, 30% white spruce, and 20% black spruce. Hardwood consisted totally of trembling aspen (approx. 330,000 m^3/year) (Sentar Consultants Ltd. 1994b). The mill produced approximately 575 ADMT of alkaline peroxide pulp per day (57% softwood pulp, 43% hardwood pulp). Effluent was subjected to treatment in a primary clarifier followed by secondary treatment in an activated sludge system prior to discharge to the Athabasca River via an instream diffuser at a rate of 0.12 m^3/s (Sentar Consultants Ltd. 1994b; Rodden 1995).

Sentinel Species

In the upper Athabasca River system, possible small sentinel species included (Nelson and Paetz 1992):

- longnose dace (*Rhinichthys cataractae*)
- pearl dace (*Margariscus margarita*)
- finescale dace (*Phoxinus neogaeus*)
- lake chub (*Couesius plumbeus*)
- fathead minnow (*Pimaphales promelas*)
- spottail shiner (*Notropis hudsonius*)
- trout-perch (*Percopsis omiscomaycus*)
- spoonhead sculpin (*Cottus ricei*)

These species represent small (maximum adult total length < 15 cm) resident and non-migratory fish species which were assumed to exhibit relatively sedentary behaviour and reduced longitudinal mobility compared to larger fish species. Several habitat types (backwater, riffle, littoral zone, pools, runs, etc.) were sampled in an effort to collect any of the above listed species. Sampling occurred during both day and night (easily accessible sites) using a beach seine (30 m x 2.5 m, mesh size 6 mm) and backpack electrofisher (Smith-Root Type VII). Final selection of a sentinel species was evaluated based on abundance and capture efficiency.

Sampling Procedure

The initial research in the vicinity of the kraft and non-kraft mills was conducted from September 18 - October 6, 1994. In the kraft mill study area, fish were collected from three sites. A reference site (site RL - reference, left upstream bank) was located immediately upstream of the mill pumphouse along the left upstream bank. A site within the effluent plume of the kraft mill (site KL - kraft mill, left upstream bank) was located along the left upstream bank of the river approximately 0.5 km downstream of the Helge-Nelson Bridge. An additional site was sampled across the river from plume site along the right upstream bank (site KR) at the Helge-Nelson Bridge. This site was sampled to investigate whether fish captured across the river from the effluent plume and exposed to lower effluent concentrations exhibited characteristics similar to reference fish or exposed fish. It was hoped that the results from this site might provide some preliminary indication regarding the mobility of the sentinel species relative to the plume, and whether spoonhead sculpin would exhibit a graded response consistent with the gradient in effluent concentration.

In the vicinity of the non-kraft mills, sentinel fish were collected from four sites. A site at Windfall Junction (site WF) was located at the boat launch immediately downstream of the Windfall Junction Bridge along the left upstream bank. This site was also used as a reference site for the study involving the downstream TMP and CTMP mills. A second reference site (site R2) was located approximately 16 km downstream from the Windfall Junction Bridge (2 km upstream of the TMP mill diffuser) along the right upstream bank. A site within the effluent plume of the TMP mill was located approximately 3 km downstream of the TMP mill diffuser (site TMP) along the right upstream bank. A site within the effluent plume of the CTMP mill was located 2.25 km downstream of the CTMP mill diffuser (site CTMP) along the left upstream bank.

Additional research at the kraft mill study area was conducted the following spring from April 05-20, 1995. Sentinel fish were collected from seven sites on the Athabasca River. Reference sites were located immediately upstream of the mill pumphouse along the left (RL) and right (RR) upstream bank. These sites were chosen to further assess the lateral mobility of the sentinel species, and increase the number of sampling sites within the reference zone. As in the fall study, fish were again collected from within the effluent plume (KL) and across the river from the effluent plume (KR). To investigate the downstream extent of responses, fish were also collected from effluent exposure sites located 21 km and 48 km downstream of the kraft mill diffuser. The first site was 0.75 km downstream of the Obed Mountain Coal Ltd. conveyor road. Initially, fishing occurred along the left upstream bank of the river; however, poor capture success necessitated sampling the right upstream bank as well (site C). Data from fish collected from both sides of the river were pooled (site C). The more distant effluent exposure site was located about 0.75 km downstream of Emerson Bridge along the left upstream bank (site E).

The exact location (longitude/latitude coordinates), and distance downstream of the respective mill sites, of each sampling site is presented in Table 2.1. Schematic maps showing the location of each sampling site have also been provided in Appendix A. Specific habitat characteristics of each sampling site within the Hinton and Whitecourt study areas have been described by Gibbons et al. (1996).

Fish Measurements

Each adult sentinel fish was rendered unconscious by concussion and total length (± 0.1 cm), fork length (± 0.1 cm), body weight (± 0.01 g), carcass weight (i.e. eviscerated)(± 0.01 g), gonad weight (± 0.001 g) and liver weight (± 0.001 g) were recorded.

Otoliths from sculpin species, and scales and the left operculum from minnow species, were removed from each fish for ageing (i.e. annulus count). Ageing measurements were obtained following procedures outlined in MacKay et al. (1990). Otoliths were cleaned in hot water, placed in propylene glycol for at least 24 h before reading under a dissecting microscope (reflected light). If annuli were difficult to count, the otoliths were ground thinner using silicon carbide grinding paper (grit # 400) until the annuli were visible. Opercular bones were difficult to age and scales were used instead. Scale reading was facilitated by making acetate impressions of 8-10 scales per fish and magnifying the impressions under a microfiche reader. All ageing structures were aged at least twice. The accuracy of ages from at least 10 % of all fish were verified by an independent researcher.

Following setup for *in vitro* steroid incubations (see below), the remaining ovarian tissue from mature females was frozen for fecundity analyses. In the laboratory, frozen ovarian tissue was thawed, blotted dry and reweighed (±0.001 g). The total number of eggs was counted and these results were used to estimate the total number of eggs per fish (total fecundity) and egg weight. As well, for each fish, the diameter of 10 individual eggs was determined as an alternate measure of egg size.

Table 2.1. **Location and general description of each site sampled during studies conducted during the fall 1994 and spring 1995 on the Athabasca River, Alberta.**

Site	Location (latitude / longitude)	General Description	River Distance from Diffuser(s)
Hinton Study Area			
RL	N 53°24.25'/ W 117°35.49'	Reference site.	-0.50 km
RR	N 53°24.34'/ W 117°35.56'	Reference site - across river	-0.65 km
KR	N 53°24.63'/ W 117°33.46'	Across river site	1.0 km
KL	N 53°25.73'/ W 117°33.17'	Kraft mill effluent plume	1.25 km
C	N 53°31.95'/ W 117°20.76'	Downstream exposure site	21.25 km
E	N 53°42.46'/ W 117°10.13'	Downstream exposure site	47.5 km
Whitecourt Study Area			
WF	N 54°12.16'/ W 116°04.15'	Kraft mill far-field/ non-kraft reference site	180 km / - 18 km (TMP)
R2	N 52°11.28'/ W 115°50.31'	Non-kraft mill reference site	-2.0 km (TMP)
TMP	N 54°09.79'/ W 115°45.54'	TMP mill effluent plume	3.5 km (TMP)
CTMP	N 54°09.95'/ W 115°40.10'	CTMP mill effluent plume	2.25 km (CTMP)

Recent field studies have shown reductions in circulating levels of sex steroids in fish exposed to BKME (McMaster et al. 1991; Munkittrick et al., 1991a; Hodson et al. 1992; Adams et al. 1993). Reductions in steroid levels have also been found to correlate with alterations in gonad size, egg production, age to maturity and expression of secondary sexual characteristics in some species (McMaster et al. 1991; Munkittrick et al. 1991a, 1992a,b). In an effort to evaluate possible reproductive impairment in exposed small species, *in vitro* production of steroid hormones was measured. Due to the small size of fish, insufficient volume of whole blood was available to measure circulating levels of sex steroid. However, *in vitro* production of steroids has been used as a reasonable surrogate measurement of circulating levels (McMaster et al. 1995).

During the fall study, *in vitro* steroid analyses were conducted on female fish only. Steroid analyses were conducted on both males and females during the spring study. Gonadal tissues from adult sentinel fish were excised, weighed (± 0.001 g) and immediately placed in Medium 199 supplemented with 25 mM Hepes, 4.0 mM sodium bicarbonate, 0.01% streptomycin sulphate, and 0.1% serum albumin (pH 7.2) and stored at 4°C for < 2 h prior to incubation. Incubations of gonadal tissues were conducted according to procedures outlined in McMaster *et al.* (1995). During the fall study, triplicate samples of 20 follicles were incubated in Medium 199 for 24 h at 18°C. For the spring study, triplicate samples of only 10 follicles were incubated because the eggs from the prespawning fish were approximately twice as large as eggs from the previous fall. For males, 20 mg of testicular tissue per replicate was incubated. During the spring, additional incubations in Medium 199 supplemented with 10 μM forskolin solubilized in ethanol were conducted in triplicate. Forskolin activates adenylate cyclase, thereby mimicking GtH by bypassing the GtH receptor and increasing cyclic adenosine monophosphate (cAMP) production, leading to stimulation of steroid production. As such, the level of forskolin-stimulated steroid production provides further information regarding the integrity of the steroid production pathway. Testosterone and 17β-estradiol (females only) production was measured during both fall and spring studies. *In vitro* production of testosterone and 17β-estradiol released to the medium were quantified by radioimmunoassay procedures described in Van Der Kraak and Chang (1990) and Van Der Kraak et al. (1989), and further outlined in McMaster et al. (1995). All samples were assayed in duplicate, and interassay variability was <15% for each steroid.

Several studies have documented induction of hepatic mixed function oxygenase activity in wild fish exposed to pulp mill effluent (Andersson et al. 1988; Rogers et al. 1989; Lindström Seppä and Oikari 1990; McMaster et al. 1991; Munkittrick et al. 1991a; Smith et al. 1991; Hodson et al. 1992). Although little success has been achieved in linking this biochemical response to whole-organism changes in exposed fish (Munkittrick et al. 1994), induced MFO activity has been successfully used as a positive indicator of exposure to effluent. It is for this reason MFO activity was measured in these studies. During each field collection, whole livers were removed from each sentinel fish and placed in a cryovial and frozen immediately in liquid nitrogen. In the laboratory, liver samples were thawed on ice and analysed for hepatic cytochrome P450IA-dependent MFO activity using the catabolism of 7-ethoxyresorufin-o-deethylase (EROD) as described in van den Heuvel et al. (1995). For the fall field samples, all livers were homogenized and subsequently centrifuged at 10,000 x g for the purpose of isolating and extracting the microsomal fraction from the liver. However, low levels of EROD activity among reference and exposed fish suggested that the extraction of the microsomal fraction was incomplete, probably due to the small size of the individual livers (0.1-0.3 g). In an effort to ensure all microsomes were present in the sample, liver samples collected during the spring field trip were assayed using the whole homogenate, omitting the centrifugation procedure.

Data Analyses

Means, standard errors and sample sizes were calculated for all fish measurements for each sampling zone. For presentation purposes, common indices describing relationships between body metrics were also calculated. These indices included:

Condition factor (k)=100(carcass weight/fork length^3)
Gonadosomatic Index (GSI)=100(gonad weight/carcass weight)
Liversomatic Index (LSI)=100(liver weight/carcass weight)

With the exception of immature fish (not dissected), carcass weight (i.e. eviscerated) was used in the above calculations because of possible differences in organ weight among sites. Using carcass weight instead of body weight eliminated possible confounding effects of altered organ weight (e.g. gonad weight, liver weight) on interpretation of variables related to body weight. Total body weight was used when calculating condition of immature fish. For species which do not have a forked tail (e.g., cottidae), total length was used instead of fork length when calculating condition factors.

All parameters were regressions of one variable on another. In the case of liver weight, fecundity, egg size and gonad weight; carcass weight was used as a covariate to adjust for any differences in size and placed these variables on a relative scale. The basic design for the analysis of fish data was an Analysis of Covariance (ANCOVA) with site as a factor. An assumption of the ANCOVA model is that the slopes of the regression lines are equal among sites. Therefore, differences in slopes were tested prior to conducting the ANCOVA. Generally, ANCOVA is fairly robust even when slopes are not equal, so slopes were considered different when $p < 0.01$ (Hamilton et al. 1993). Analysis of variance (ANOVA) was used to compare body size (body weight, length and carcass weight) and egg size (egg weight and diameter) estimates among sites. All data were $\log_{10}$ transformed and sexes were analyzed separately. Nonparametric Kruskal-Wallis tests were used to compare MFO activity and *in vitro* steroid levels between reference and exposed fish. All data analyses were done using SYSTAT statistical software (Wilkinson 1990).

Water Chemistry

As further confirmation that fish were being exposed to pulp mill effluent, water samples were collected at each fish collection site for chemical analyses during both fall and spring studies. At each site, water was collected in 3-125 mL Nalgene bottles for analyses of major ions including chloride, sodium, potassium, sulphate, and silica conducted by the National Laboratory for Environmental Testing (NLET), Environment Canada, Burlington, Ontario. Chloride (method detection limit (MDL)=0.05 $mg \cdot L^{-1}$), sulphate (MDL=0.02 $mg \cdot L^{-1}$) and silica (MDL=

0.02 mg·L^{-1}) were measured using a colorimetric method (Cl, ferric thiocyanate method; SO_4, methyl thymol blue; silica, heteropoly blue) and concentrations of sodium (MDL=0.02 mg·L^{-1}) and potassium (MDL=0.02 mg·L^{-1}) were determined by flame emission photometric methods. All analyses and quality assurance/quality control procedures where conducted according methods outlined by NLET (1994).

During the fall study, water was also collected in 3-125 mL Nalgene bottles for adsorbable organic halide (AOX) analysis. Water samples for AOX analyses were acidified (pH 2) with concentrated sulphuric acid. All samples were put on ice immediately after collection. Analyses were conducted by Wastewater Technology Centre (WTC), Burlington, Ontario by adsorbing the samples on granular activated carbon (GAC), removing inorganic halides by washing with a nitrate solution, pyrolizing the GAC-adsorbed halide complex in a combustion furnace and determining the resulting halides by microcoulometric titration (WTC, 1990). The method detection limit for this analysis is 0.5 μg·mL^{-1}. Quality assurance/quality control measures included a method blank (water) and reference blank (trichlorophenol standard solution) as well as other measures outlined in the AOX protocol (WTC, 1990).

2.3 Results

2.3.1 Sentinel Species Collection

Of the possible sentinel species captured in the Hinton study area, spoonhead sculpin (*Cottus ricei*) was the most abundant and widely distributed species (Table 2.2). I sampled for spoonhead sculpin during the day and night (easily accessible sites); however, fishing during the evening (2000-0100 h) proved to be the best time to collect mature sculpin. Although a variety of habitats were sampled, greater success (especially capturing adults) was achieved when sampling faster runs and riffles (≈1.1-1.5 m·s^{-1}) approximately 0.5-0.75 m deep with boulder/cobble substrates. The sampling technique consisted of holding a pole seine (2 m x 1.2

Table 2.2. Number of each potential sentinel fish species caught at the Hinton and Whitecourt study areas during the fall 1994 field study, Athabasca River, Alberta.

Species	Hinton Sites			Whitecourt Sites			
	RL	KR	KL	WF	R2	TMP	CTMP
Trout-perch				7	6	8	5
Lake Chub				57	45	33	28
Longnose Dace		2					4
Spoonhead Sculpin	79	57	57				13

m, 6 mm mesh size) approximately 2 m downstream of a second researcher electrofishing the large cobble/boulder substrate. Fish were shocked by the electrofisher and swept downstream by the current into the pole seine.

Spoonhead sculpin occurs in rivers and lakes from northeastern British Columbia to eastern Quebec in regions of Canada that were glaciated during the Wisconsin Ice Age (Nelson and Paetz 1992). In Alberta, they are primarily a stream species in systems of the foothills and adjacent prairies (Nelson and Paetz 1992). Spoonhead sculpin can reach a maximum size of 13.5 cm total length (Roberts 1988a) and live up to 5-6 years. Based on capture efficiency in the current study, it appears that sculpin actively foraged during the evening and night hours. Roberts (1988b) suggested that spoonhead sculpin spawn during the spring (April-May) at water temperatures around 6°C. Courted females attach adhesive eggs (280-1200 eggs) to the underside of a rock which are guarded and fanned by the male until hatch (2-3 weeks). During the spring study, egg masses and guarding males were observed within the mainstem of the Athabasca River suggesting this species does not migrate to tributary or feeder streams for spawning.

Spoonhead sculpin were not found downstream at Windfall Junction (Table 2.2). Attempts to collect sculpin at sites near the Windfall location were limited because they could not be safely accessed during the preferred evening sampling hours. Plans to evaluate the response of spoonhead sculpin at this site were abandoned. Further effort was made to collect an alternative sentinel species for the purpose of evaluating fish responses to effluent from non-kraft mill operations located downstream near Whitecourt.

Lake chub (*Couesius plumbeus*) was the most abundant species collected in the Whitecourt study area (Table 2.2). Lake chub were collected by electrofishing the quieter margins of the river during the day. Greatest success was achieved in sampling sites consisting of large cobbles and free boulders. The substrate was gently lifted and replaced while electrofishing and the shocked fish were captured with a small, long handled dip-net.

Lake chub has been found in streams, rivers and lakes throughout Canada and in scattered locations in northern United States (Scott and Crossman 1973). The largest lake chub ever recorded in Alberta was 16.6 cm in fork length; however, most mature adults collected from the Athabasca River were 8-10 cm in fork length. Few lake chub live beyond 5 years (Nelson and Paetz 1992) and age at maturity is probably 2 or 3 y. There is little information on the spawning activity of lake chub in river systems, although it has been suggested that they are probably batch or multiple spawners between June-August (M. Spafford, Alberta Pacific Ltd, Athabasca, Alberta, personal communications). Lake chub feed on crustaceans, aquatic insects and algae (Nelson and Paetz 1992).

2.3.2 Kraft mill effluent, Hinton study area

2.3.2.1 Fall Study

Variations in age, body size and organ metrics for spoonhead sculpin at each study site are presented in Table 2.3 and Table 2.4. Estimates of egg size were limited because a large proportion of eggs were used for measuring *in vitro* steroid production. Many of the remaining preserved eggs broke easily when handled during counting and weighing. Univariate comparisons of eggs size were conducted; however, bivariate relationships with carcass weight (i.e. ANCOVA) were not calculated because of insufficient sample sizes. Independent verification of age estimates on a subsample of 20 fish (18% of total number aged) indicated an error rate (i.e. precision) of 5%.

Sculpin were longer, heavier and older at sites KL and KR relative to reference fish (RL) (Table 2.3). There were no differences in these parameters for fish collected across river (KR) from the effluent plume site (KL). Interestingly, size-at-age of sculpin among all three sites were similar (Table 2.5) suggesting that sculpin at the plume site and across river site were longer because they were older, not because of increased growth in length. Condition of both male and female

Table 2.3. Mean and SE (n) of length, weight, condition (K) and age of spoonhead sculpin (*Cottus ricei*) collected at each sampling site during the fall 1994 study on the Athabasca River, Hinton, Alberta. Statistical results for variables analysed using ANOVA are presented. Within a column differences ($p<0.05$) among sites are denoted by different alphabetical superscripts.

Site	Total Length (cm)	Body Weight (g)	Carcass weight (g)	K	Age (y)
Male					
Reference (RL)	8.7 ± 0.2 (26) A	6.61 ± 0.45 (26) A	5.87 ± 0.39 (26) A	0.86 ± 0.02 (26)	3.4 ± 0.2 (25) A
Across River (KR)	9.8 ± 0.3 (14) B	10.62 ± 1.24 (14) B	9.42 ± 1.09 (14) B	0.95 ± 0.02 (14)	4.4 ± 0.2 (14) B
Effluent Plume (KL)	9.5 ± 0.4 (13) B	10.03 ± 1.23 (13) B	8.91 ± 1.12 (13) B	0.98 ± 0.03 (13)	4.2 ± 0.3 (13) B
Female					
Reference (RL)	8.3 ± 0.2 (22) A	5.35 ± 0.45 (22) A	4.77 ± 0.40 (21) A	0.79 ± 0.01 (22)	3.3 ± 0.2 (22) A
Across River (KR)	9.5 ± 0.4 (12) B	9.32 ± 1.39 (12) B	8.0 ± 1.21 (12) B	0.85 ± 0.03 (12)	4.2 ± 0.3 (9) B
Effluent Plume (KL)	9.5 ± 0.4 (13) B	9.35 ± 1.32 (13) B	8.14 ± 1.18 (13) B	0.87 ± 0.02 (13)	4.0 ± 0.3 (13) B

Table 2.4. Mean and SE (n) of liversomatic index, gonadosomatic index, fecundity and egg size of spoonhead sculpin (*Cottus ricei*) collected at each sampling site during the fall 1994, Athabasca River, Hinton, Alberta.

Site	LSI (%)	GSI (%)	Fecundity (# eggs)	Egg Weight (mg)	Egg Diameter (mm)
Male					
Reference (RL)	1.45 ± 0.07 (26)	2.63 ± 0.10 (26)			
Across River (KR)	1.82 ± 0.14 (14)	2.53 ± 0.12 (14)			
Effluent Plume (KL)	1.86 ± 0.07 (13)	2.85 ± 0.08 (13)			
Female					
Reference (RL)	2.28 ± 0.12 (22)	3.08 ± 0.09 (22)	960 ± 229 (4)	0.21 ± 0.02 (4)	0.66 ± 0.02 (5)
Across River (KR)	2.94 ± 0.15 (12)	3.58 ± 0.22 (12)	1081 ± 292 (5)	0.33 ± 0.05 (5)	0.68 ± 0.06 (5)
Effluent Plume (KL)	2.98 ± 0.14 (13)	4.23 ± 0.26 (13)	872 ± 133 (6)	0.43 ± 0.06 (6)	0.73 ± 0.03 (7)

Table 2.5. **Site comparisons (ANCOVA) of size-at-age, condition, gonad weight and liver weight for spoonhead sculpin, fall 1994, Athabasca River, Hinton, Alberta. Abbreviations: I - intercept; S - slope; p - pooled over sites. Subscripts refer to sites. Interaction terms were considered significant at $p<0.01$.**

		Probability value (p)			
Parameter	Sex	Slope	Intercept	Log_{10} Mean Estimate	
Site RL (reference) *vs* Site KL (effluent plume)					
Size-at-age	male	0.10	0.93	S_p=0.35,	I_p=0.76
	female	0.13	0.09	S_p=0.43,	I_p=0.71
Condition	male	0.53	0.001	I_{rl}=0.78,	I_{kl}=0.83
	female	0.25	0.009	I_{rl}=0.71,	I_{kl}=0.75
Gonad vs. carcass wt.	male	0.94	0.002	I_{rl}=-0.806,	I_{kl}=-0.715
	female	0.21	<0.001	I_{rl}=-0.791,	I_{kl}=-0.641
Liver vs. carcass wt.	male	0.03	<0.001	I_{rl}=-1.062,	I_{kl}=-0.914
	female	0.32	0.05	I_{rl}=-0.915,	I_{kl}=-0.829
Site KR (across river) *vs* Site KL					
Size-at-age	male	0.63	0.83	S_p=0.45,	I_p=0.70
	female	0.30	0.25	S_p=0.52,	I_p=0.66
Condition	male	0.10	0.16	S_p=3.35,	I_p=-2.36
	female	0.36	0.22	S_p=3.39,	I_p=-2.45
Gonad vs carcass wt.	male	0.55	0.03	I_{kr}=-0.686,	I_{kl}=-0.629
	female	0.35	0.03	I_{kr}=-0.605,	I_{kl}=-0.523
Liver vs. carcass wt.	male	0.002	-	S_{kr}=0.930,	S_{kl}=1.430
	female	0.15	0.83	S_p=1.182,	I_p=-1.689

Table 2.5. Continued.

Parameter	Sex	Probability value (p) Slope	Intercept	Log_{10} Mean Estimate
Site RL (reference) *vs* Site KR (across river)				
Size-at-age	male	0.59	0.48	S_p=0.33, I_p=0.77
	female	0.08	0.63	S_p=0.42, I_p=0.71
Condition	male	0.035	0.08	S_p=3.38, I_p=-2.42
	female	0.08	0.39	S_p=3.33, I_p=-2.40
Gonad vs carcass wt.	male	0.88	0.50	S_p=0.999, I_p=-1.593
	female	0.90	0.08	S_p=1.095, I_p=-1.561
Liver vs carcass wt	male	0.95	0.64	S_p=1.443, I_p=-2.176
	female	0.85	0.12	S_p=1.343, I_p=-1.861

sculpin from the effluent plume site were greater than values found at the reference site (RL) (Table 2.5). However, condition of sculpin from site KR was intermediate between values estimated for reference and effluent plume fish.

Male and female sculpin from the effluent plume site had larger gonads than sculpin from the reference site or site KR (Table 2.5). Sculpin collected across river from the effluent plume site had gonad weights similar to reference fish. Egg weight was heavier at site KL relative to reference fish ($p<0.01$), however, there were no differences in egg weight between sites KR and RL ($p=0.12$), and sites KL and KR ($p=0.24$). There were no differences in egg diameter among the three sites ($p>0.10$).

Liver weight was greater in sculpin from the effluent plume relative to reference fish (Table 2.5). Liver size of sculpin from site KR was intermediate between values estimated for reference and effluent plume fish. For male liver weight, the slope of the regression line (liver weight. *vs* carcass weight) at site KL was greater than at site KR. Males from the effluent plume had smaller liver weights for most of the range in carcass weight, until the relationship reversed at a carcass weight of about 9.5 g (Figure 2.2).

Mean hepatic EROD activity in sculpin from the effluent plume site was approximately 2.5 fold higher than reference fish (Figure 2.3). EROD activity in males from site KR was similar to reference levels, whereas activity in females was similar to levels found at the effluent plume site. *In vitro* production of testosterone (Figure 2.4a) and 17β-estradiol (Figure 2.4b) by follicles of female sculpin from sites KR and KL was higher than reference levels. Testosterone production at the effluent plume site was greater than production at site KR, whereas levels of 17β-estradiol were similar (Figure 2.4).

Water Chemistry

Adsorbable organic halide (AOX) was not detected (<0.5 μg/mL) in water samples collected at

Figure 2.2. Regression lines of $\log_{10}$ liver weight *vs* $\log_{10}$ carcass weight (condition) for male spoonhead sculpin (*Cottus ricei*) from the effluent plume site (KL) (solid line, r^2=0.93) and across river site (KR) (dashed line, r^2=0.93), fall 1994, Athabasca River, Hinton, Alberta.

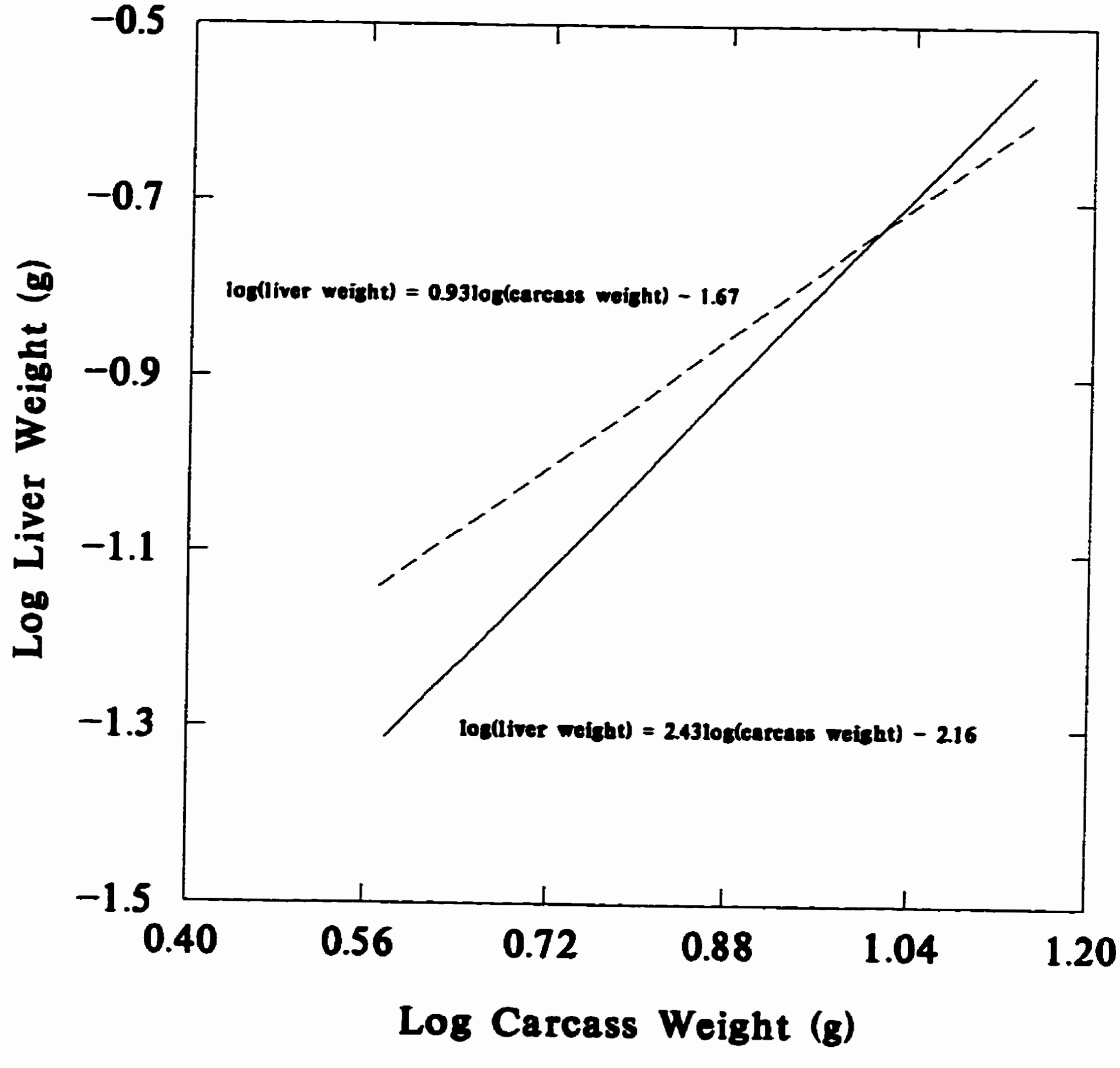
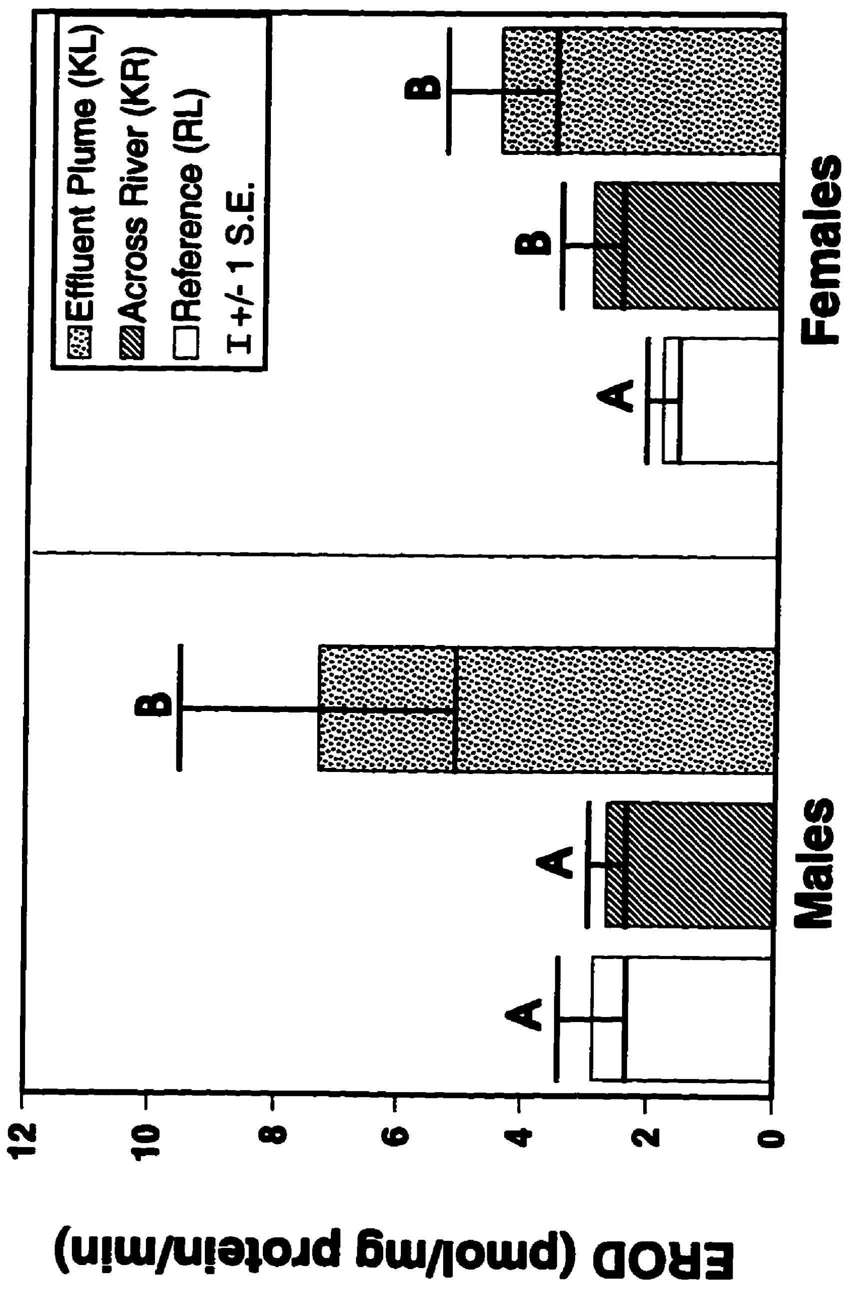
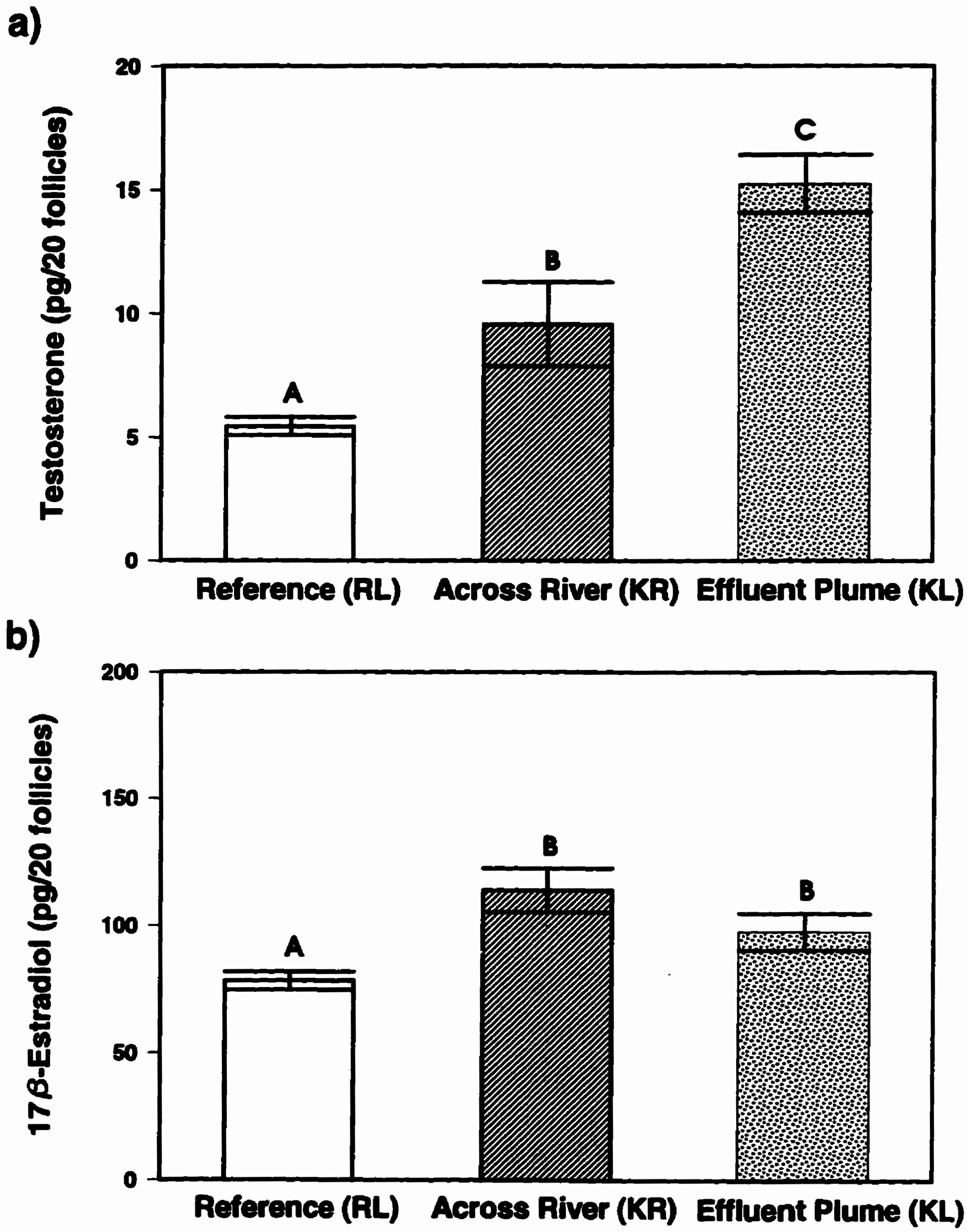

-0.5
-0.7
-0.9
-1.1
-1.3
-1.5
Log Liver Weight (g)
log(liver weight) = 0.93log(carcass weight) - 1.67
log(liver weight) = 2.43log(carcass weight) - 2.16
0.40
0.56
0.72
0.88
1.04
1.20
Log Carcass Weight (g)

Figure 2.3. EROD activity in male and female spoonhead sculpin (*Cottus ricei*) from the reference, across-river (KR) and effluent plume (KL) sites during the fall 1994 study, Athabasca River, Hinton, Alberta. Values represent the mean ± SE. Bars with different uppercase alphabetical superscripts are statistically ($p<0.05$) different from one another.



Effluent Plume (KL)
Across River (KR)
Reference (RL)
I +/- 1 S.E.
A
A
B
A
B
B
Males
Females
12
10
8
6
4
2
0
EROD (pmol/mg protein/min)

Figure 2.4. *In vitro* production of a) testosterone and b) 17β-estradiol by egg follicles of female spoonhead sculpin (*Cottus ricei*) from the reference site, across river site (KR) and effluent plume site (KL) during the fall 1994 study, Athabasca River, Hinton, Alberta. Values represent the mean ● SE. Bars with different uppercase alphabetical superscripts are statistically ($p<0.05$) different from one another.


a)
20
15
10
5
0
Testosterone (pg/20 follicles)
A
B
C
Reference (RL)
Across River (KR)
Effluent Plume (KL)
b)
200
150
100
50
0
17β-Estradiol (pg/20 follicles)
A
B
B
Reference (RL)
Across River (KR)
Effluent Plume (KL)

any study site. The reference blank of trichlorophenol indicated 94.2% recovery. With the exception of silica, major ion concentrations were higher at the effluent plume site compared to the reference site and site KR (Table 2.6). Concentrations of all ions at site KR were similar to concentrations measured at the reference site.

2.3.2.2 Spring Study

Variations in age, body size and organ metrics for spoonhead sculpin collected at each of the study sites are presented in Table 2.7 and Table 2.8. A limited number of female sculpin at site E prevented calculations of bivariate fish parameters; however, univariate comparisons of fish metrics could still be conducted. At some sites, both pre-ovulatory and spent females were collected. Although this had little bearing on sample sizes when calculating parameters such as size-at-age, condition and liver weight, sample sizes were reduced when calculating reproductive parameters (e.g. gonad weight, fecundity, egg size) of pre-ovulatory females. As such, numbers of pre-ovulatory fish at sites KR and E were limited, and site comparisons of reproductive parameters adjusted for carcass weight (i.e. ANCOVA) were not conducted. Independent verification of age estimates on a subsample of 50 fish (19% of total number aged) indicated an error rate (i.e. precision) of 12% (in all cases a difference of one year).

Reference Site Comparisons

Prior to conducting comparisons between reference and downstream fish, preliminary comparisons were conducted between the two reference sites.

From the analyses, it was evident that there were differences between sites within the reference zone. Both male and female sculpin exhibited differences in condition and gonad weight between the two sites (Table 2.9). As well, there were differences in male liver weight and female body and carcass weight (Table 2.7, 2.9). Estimates of egg size were not related to carcass weight (egg weight, $p=0.96$; egg diameter, $p=0.14$), and there was no difference in the

Table 2.6. Mean ± SE (n=3) of major ion concentrations ($mg \cdot L^{-1}$) in water samples collected at each sampling site during the fall 1994 study, Athabasca River, Hinton, Alberta.

Site	Cl	SO_4	Na	SiO_2	K
Reference (RL)	0.68 ± 0.04 A	37.7 ± 0.2 A	1.13 ± 0.01 A	2.5 ± 0.02 A	0.40 ± 0.01 A
Across River (KR)	0.69 ± 0.01 A	37.6 ± 1.3 A	1.20 ± 0.03 A	2.28 A	0.40 ± 0.003 A
Effluent Plume (KL)	1.98 ± 0.02 B	45.7 ± 0.4 B	4.07 ± 0.02 B	2.47 ± 0.01 A	0.47 B

Table 2.7. Mean and SE (n) of length, weight, condition (K) and age of spoonhead sculpin (*Cottus ricei*) collected at each sampling site during the spring 1995 study on the Athabasca River, Hinton, Alberta. Statistical results for variables analysed using ANOVA are presented. Within a column, a difference ($p<0.05$) between reference sites RL and RR is denoted by an asterisk (*). Differences ($p<0.05$) among the pooled reference sites and sites KL and KR are denoted by different alphabetical superscripts. Differences ($p<0.05$) among sites KL, C and E are shown by different numerical superscripts.

Site	Total Length (cm)	Body Weight (g)	Carcass Weight (g)	K	Age (y)
Male					
RL	9.0 ± 0.2 (32) A	7.90 ± 0.47 (32) A	6.81 ± 0.41 (32) A	0.90 ± 0.01 (32)	4.1 ± 0.2 (32) A
RR	8.8 ± 0.2 (22)	6.81 ± 0.43(22)	5.83 ± 0.37 (22)	0.84 ± 0.01 (22)	4.0 ± 0.2 (22)
KR	8.9 ± 0.2 (15) A	8.37 ± 0.70 (15) A	7.08 ± 0.61 (15) A	0.96 ± 0.04 (15)	4.3 ± 0.3 (15) A
KL	9.8 ± 0.2 (19) B,1	10.84 ± 0.75 (19) B,1	9.54 ± 0.66 (19) B,1	1.00 ± 0.02 (19)	3.8 ± 0.2 (19) A,1
C	9.5 ± 0.2 (30) 1	10.44 ± 0.73 (30) 1	9.00 ± 0.66 (30) 1	1.00 ± 0.01 (30)	4.1 ± 0.1 (30) 1
E	10.6 ± 0.3 (15) 2	16.31 ± 1.57 (15) 2	14.01 ± 1.37 (15) 2	1.12 ± 0.02 (15)	4.6 ± 0.3 (15) 2,1†
Female					
RL	8.5 ± 0.2 (12) A	5.62 ± 0.43 (10) A	4.30 ± 0.40 (12) A	0.68 ± 0.02 (12)	3.6 ± 0.2 (12) A
RR	8.0 ± 0.2 (23)	4.49 ± 0.18 (17)*	3.37 ± 0.21 (23)*	0.63 ± 0.01 (23)	3.6 ± 0.10 (23)
KR	9.0 ± 0.3 (16) B	7.28 ± 1.32 (5) B	5.36 ± 0.54 (16) B	0.70 ± 0.01 (16)	4.1 ± 0.3 (16) A
KL	8.6 ± 0.4 (9) A,B,1	8.25 ± 1.46 (9) B,1	5.20 ± 0.89 (9) B,1	0.76 ± 0.02 (9)	3.7 ± 0.3 (9) A,1
C	9.1 ± 0.2 (21) 1	8.13 ± 0.29 (8) 1	5.88 ± 0.36 (21) 1	0.77 ± 0.01 (21)	3.7 ± 0.2 (21) 1
E	9.5 ± 0.7 (6) 1	11.97 ± 2.78 (6) 1	7.89 ± 1.81 (6) 1	0.84 ± 0.03 (6)	4.2 ± 0.5 (6) 1

† age similar to males at site C, but significantly greater than males of site KL.

Table 2.8. Mean and SE (n) of liversomatic index, gonadosomatic index, fecundity and egg size of spoonhead sculpin (*Cottus ricei*) collected at each sampling site during the spring 1995 study on the Athabasca River, Hinton, Alberta.

Site	LSI (%)	GSI (%)	Fecundity (# eggs)	Egg Weight (g)	Egg Diameter (mm)
Male					
RL	1.79 ± 0.08 (32)	2.05 ± 0.08 (32)			
RR	1.54 ± 0.09 (22)	1.40 ± 0.12 (22)			
KR	2.28 ± 0.17 (15)	1.19 ± 0.13 (15)			
KL	1.83 ± 0.13 (19)	1.54 ± 0.11 (19)			
C	2.65 ± 0.15 (30)	1.03 ± 0.07 (30)			
E	2.78 ± 0.22 (15)	2.37 ± 0.27 (15)			
Female					
RL	2.97 ± 0.19 (12)	27.96 ± 1.57 (10)	243 ± 25 (9)	4.69 ± 0.42 (9)	1.73 ± 0.08 (9)
RR	3.02 ± 0.14 (23)	33.84 ± 1.62 (16)	193 ± 11 (17)	5.23 ± 0.25 (17)	1.86 ± 0.03 (17)
KR	3.20 ± .17 (16)	36.52 ± 3.54 (5)	356 ± 52 (5)	4.65 ± 0.55 (5)	1.68 ± 0.14 (5)
KL	3.94 ± 0.29 (9)	41.40 ± 1.26 (9)	353 ± 38 (9)	5.91 ± 0.49 (9)	1.96 ± 0.04 (9)
C	4.06 ± 0.13 (21)	41.18 ± 0.90 (8)	438± 29 (8)	4.88 ± 0.36 (8)	1.65 ± 0.05 (8)
E	4.45 ± 0.23 (6)	32.33 ± 1.88 (6)	568 ± 98 (6)	4.35 ± 0.47 (6)	1.61 ± 0.09 (6)

Table 2.9. **Comparison (ANCOVA) of size-at-age, condition, gonad weight and liver weight for spoonhead sculpin at sites (RL+RR), KL and KR, spring 1995, Athabasca River, Hinton, Alberta. Abbreviations: I - intercept; S - slope; p - pooled over sites. Subscripts refer to site. Interaction terms were considered significant at p<0.01.**

		Probability value (p)			
Parameter	Sex	Slope	Intercept	Log_{10} Mean Estimate	
Site RL *vs* Site RR (reference sites)					
Size-at-age	male	0.28	0.37	S_p=0.31,	I_p=0.76
	female	0.23	0.05	I_{rl}=0.93,	I_{rr}=0.90
Condition	male	0.13	0.003	I_{rl}=0.80,	I_{rr}=0.77
	female	0.81	0.01	I_{rl}=0.57,	I_{rr}=0.54
Gonad *vs* carcass wt.	male	0.12	<0.001	I_{rl}=-0.906,	I_{rr}=-1.119
	female	0.65	0.01	I_{rl}=-0.076,	I_{rr}=0.038
Liver *vs* carcass wt.	male	0.53	0.01	I_{rl}=-0.969,	I_{rr}=-1.061
	female	0.16	0.62	S_p=0.761,	I_p=-1.402
Fecundity *vs* carcass wt.	female	0.50	0.40	S_p=0.94,	I_p=1.80
Site (RL+RR) (pooled reference) *vs* Site KL (effluent plume)					
Size-at-age	male	0.98	<0.001	I_{ref}=0.95,	I_{kl}=0.99
	female	0.14	0.18	S_p=0.35,	I_p=0.72
Condition	male	0.42	<0.001	I_{ref}=0.82,	I_{kl}=0.86
	female	0.76	<0.001	I_{ref}=0.56,	I_{kl}=0.63
Gonad *vs* carcass wt.	male	0.41	0.67	S_p=0.747,	I_p=-1.583
	female	0.62	0.002	I_{ref}=0.039,	I_{kl}=0.163
Liver *vs* carcass wt.	male	0.39	0.02	I_{ref}=-0.976,	I_{kl}=-0.885
	female	0.61	<0.001	I_{ref}=-0.967,	I_{kl}=-0.813
Fecundity *vs* carcass wt.	female	0.11	0.02	I_{ref}=2.33	I_{kl}=2.44

Table 2.9. Continued.

		Probability value (p)			
Parameter	Sex	Slope	Intercept	Log_{10} Mean Estimate	
Site KR (across river) *vs* Site KL					
Size-at-age	male	0.99	<0.001	I_{kr}=0.94,	I_{kl}=0.99
	female	0.55	0.95	S_p=0.45,	I_p=0.68
Condition	male	0.78	0.57	S_p=3.28,	I_p=-2.28
	female	0.65	0.01	I_{kr}=0.68,	I_{kl}=0.71
Gonad vs carcass wt.	male	0.34	0.002	I_{kr}=-1.106,	I_{kl}=-0.902
	female	-	-	unavailable	
Liver vs. carcass wt.	male	0.50	0.32	S_p=0.540,	I_p=-1.280
	female	0.62	0.02	I_{kr}=0.154,	I_{kl}=0.187
Site (RL+RR) *vs* Site KR					
Size-at-age	male	0.95	0.61	S_p=0.31,	I_p=0.76
	female	0.22	0.05	I_{ref}=0.92,	I_{kr}=0.94
Condition	male	0.83	<0.001	I_{ref}=0.79,	I_{kr}=0.83
	female	0.87	0.003	I_{ref}=0.58,	I_{kr}=0.62
Gonad vs carcass wt.	male	0.07	0.001	I_{ref}=-0.986,	I_{kr}=-1.158
	female	-	-	unavailable	
Liver vs carcass wt	male	0.97	0.002	I_{ref}=-1.0,	I_{kr}=-0.856
	female	0.96	0.02	I_{ref}=-0.950,	I_{kr}=-0.885

univariate estimate of egg size (egg weight, $p=0.16$; egg diameter, $p=0.07$). Mixed function oxygenase (EROD) activity in male sculpin was significantly higher at site RR (Figure 2.5); however, there were no differences in activity in preovulatory and spent (low sample size) female sculpin between reference sites (Figure 2.6a,b). *In vitro* production of testosterone in male sculpin was lower at site RR (Figure 2.7a,b), whereas there was no site difference in female production of testosterone (Figure 2.8a,b) or 17β-estradiol (Figure 2.9a,b) in female sculpin. For males, forskolin significantly increased *in vitro* production of testosterone at both sites (RL and RR, $p<0.001$); however, forskolin had no effect on production of testosterone (RL, $p=0.29$; RR, $p=0.11$) or 17β-estradiol (RL, $p=0.50$; RR, $p=0.11$) production in female sculpin.

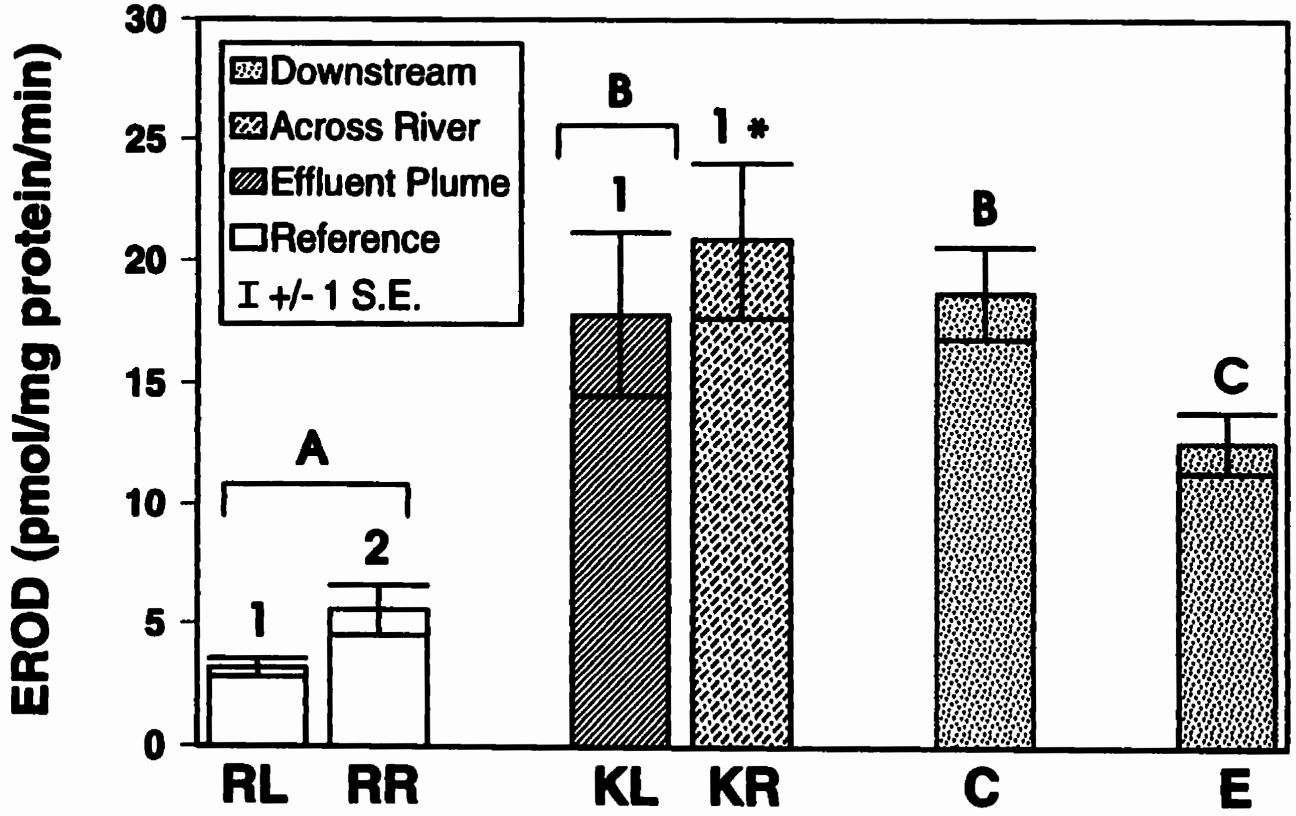
In the absence of anthropogenic stressors, it was likely that the observed differences represented the natural variability in fish characteristics within the reference zone. The results also suggested that the mobility of spoonhead sculpin was not sufficient to minimize these differences. For comparisons between reference and exposed fish, reference sites RL and RR were pooled. Pooling both reference sites was one way to ensure that the full extent of reference variability was included in reference/exposure comparisons.

Near-field Comparisons

Overall there was a general increase in body and organ size at the effluent plume site (KL), whereas sculpin from the across river site (KR) exhibited an intermediate response (i.e similarities to reference and effluent plume fish).

Male sculpin were heavier, longer and had greater size-at-age at the effluent plume site relative to reference and across river males (Table 2.7, 2.9). Mean weight of female sculpin was similar between sites KR and KL, but heavier than reference fish. Female size-at-age was greater at site KR relative to reference fish, whereas size-at-age for females from site KL was similar to site KR and reference fish. There were no differences in the mean age of sculpin among the study sites. Male condition at the effluent plume site was greater than reference and across river males. The condition of female sculpin increased from site RL+RR to KL to KR.

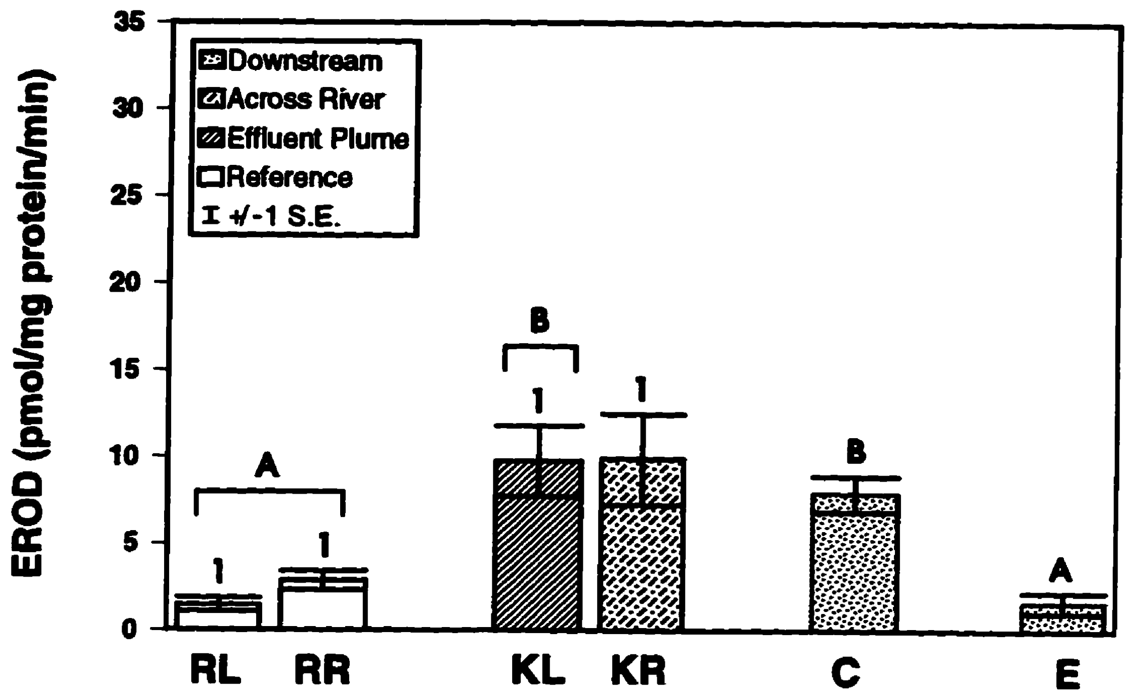
Figure 2.5. EROD activity in male spoonhead sculpin (*Cottus ricei*) from reference and effluent exposure sites during the spring 1995 study, Athabasca River, Hinton, Alberta. Bars with different alphabetical superscripts are statistically different ($p<0.05$). Differences ($p<0.05$) between sites RL *vs* RR and KL *vs* KR are denoted with different numerical superscripts. A difference ($p<0.05$) between site KR and the pooled reference sites is indicated with an asterisk (*).



Downstream
Across River
Effluent Plume
Reference
+/- 1 S.E.
EROD (pmol/mg protein/min)
30
25
20
15
10
5
0
A
1
2
B
1
1 *
B
C
RL
RR
KL
KR
C
E

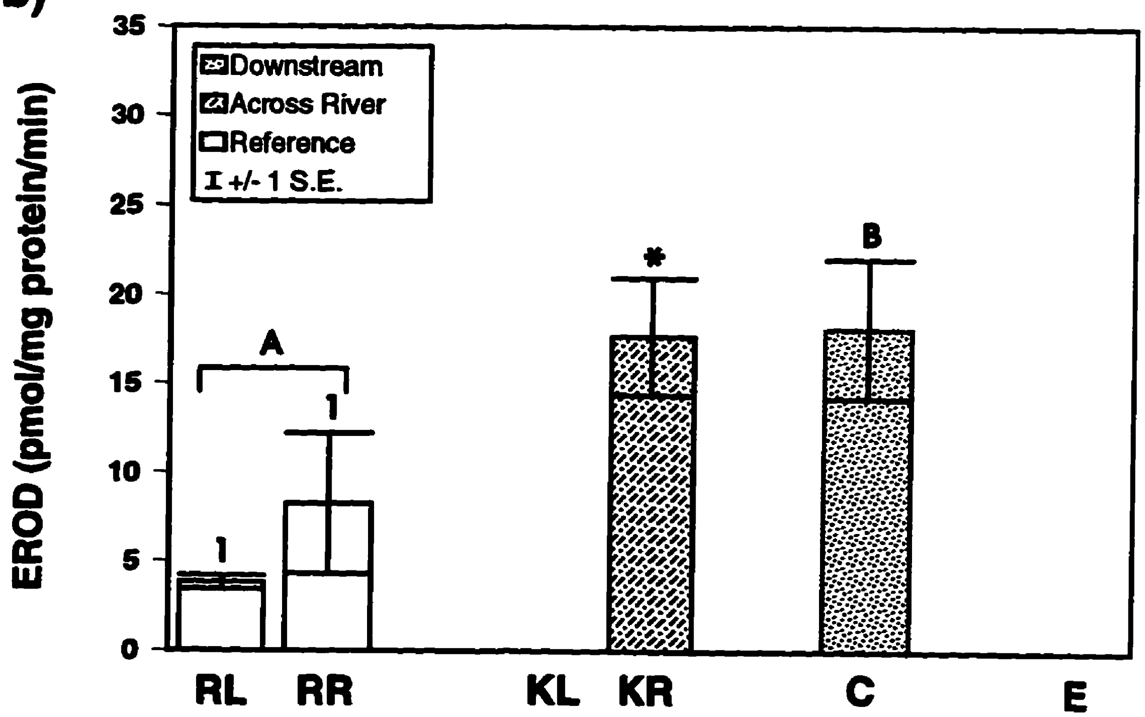
Figure 2.6. EROD activity in a) preovulatory and b) spent female spoonhead sculpin (*Cottus ricei*) from reference and effluent exposure sites during the spring 1995 study, Athabasca River, Hinton, Alberta. Bars with different alphabetical superscripts are statistically different ($p<0.05$). Differences ($p<0.05$) between sites RL *vs* RR and KL *vs* KR are denoted with different numerical superscripts. A difference ($p<0.05$) between site KR and the pooled reference sites is indicated with an asterisk (*).

a)



35
30
25
20
15
10
5
0
EROD (pmol/mg protein/min)
Downstream
Across River
Effluent Plume
Reference
I +/-1 S.E.
A
B
B
A
1
1
1
1
RL
RR
KL
KR
C
E

b)



35
30
25
20
15
10
5
0
EROD (pmol/mg protein/min)
Downstream
Across River
Reference
I +/- 1 S.E.
A
*
B
1
1
RL
RR
KL
KR
C
E

Figure 2.7. a) Basal and b) forskolin-stimulated *in vitro* production of testosterone by testicular tissue of male spoonhead sculpin (*Cottus ricei*) from reference and effluent exposure sites during the spring 1995 study, Athabasca River, Hinton, Alberta. Bars with different alphabetical superscripts are statistically different ($p<0.05$). Differences ($p<0.05$) between sites RL *vs* RR and KL *vs* KR are denoted with different numerical superscripts. A difference ($p<0.05$) between site KR and the pooled reference sites is indicated with an asterisk (*).

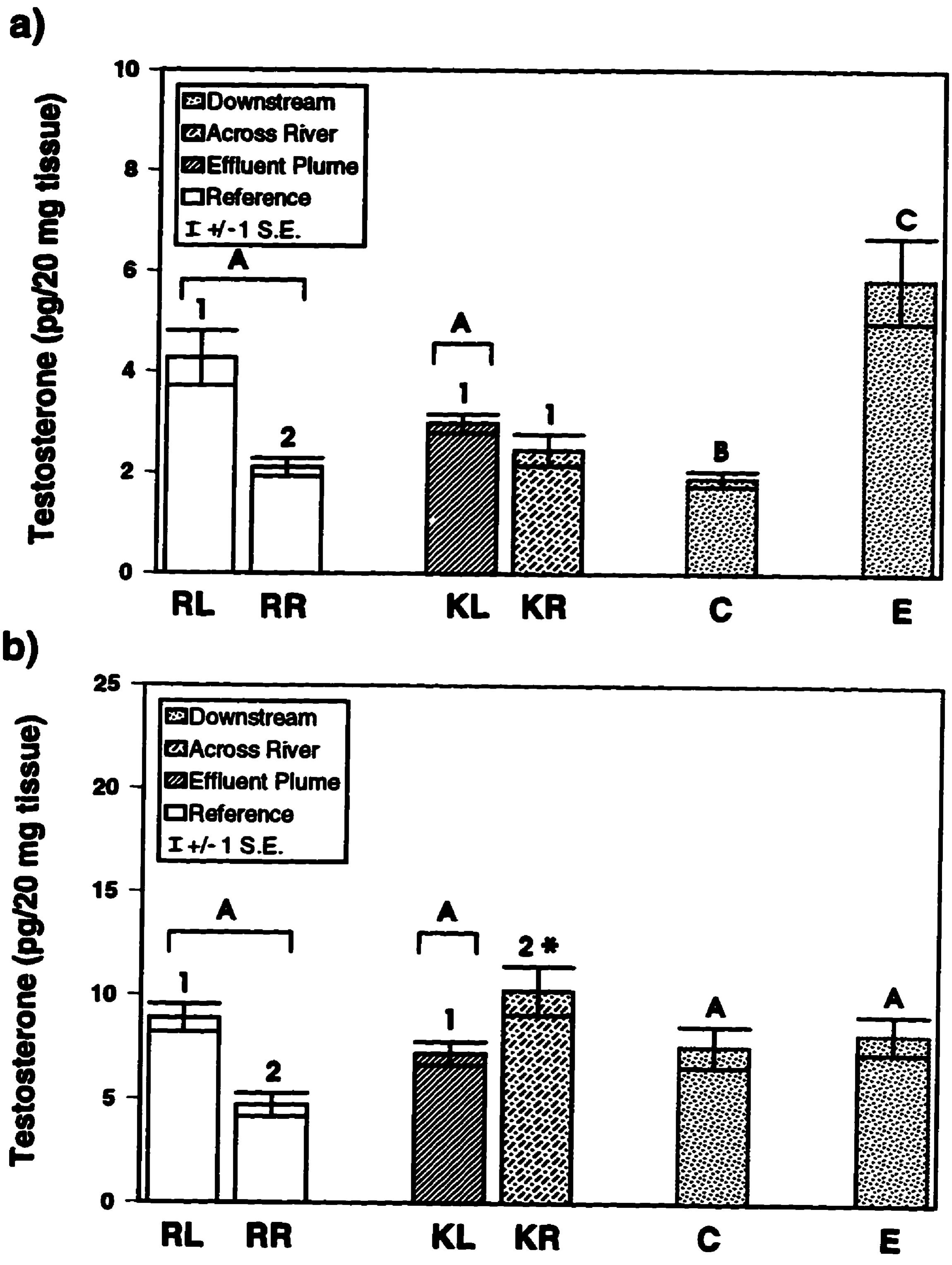

a)
10
8
6
4
2
0
Testosterone (pg/20 mg tissue)
Downstream
Across River
Effluent Plume
Reference
+/- 1 S.E.
A
1
2
A
1
1
B
C
RL
RR
KL
KR
C
E
b)
25
20
15
10
5
0
Testosterone (pg/20 mg tissue)
Downstream
Across River
Effluent Plume
Reference
+/- 1 S.E.
A
1
2
A
1
2 *
A
A
RL
RR
KL
KR
C
E

Figure 2.8. a) Basal and b) forskolin-stimulated *in vitro* production of testosterone by egg follicles of female spoonhead sculpin (*Cottus ricei*) from reference and effluent exposure sites during the spring 1995 study, Athabasca River, Hinton, Alberta. Bars with different alphabetical superscripts are statistically different ($p<0.05$). Differences ($p<0.05$) between sites RL *vs* RR and KL *vs* KR are denoted with different numerical superscripts. A difference ($p<0.05$) between site KR and the pooled reference sites is indicated with an asterisk (*).

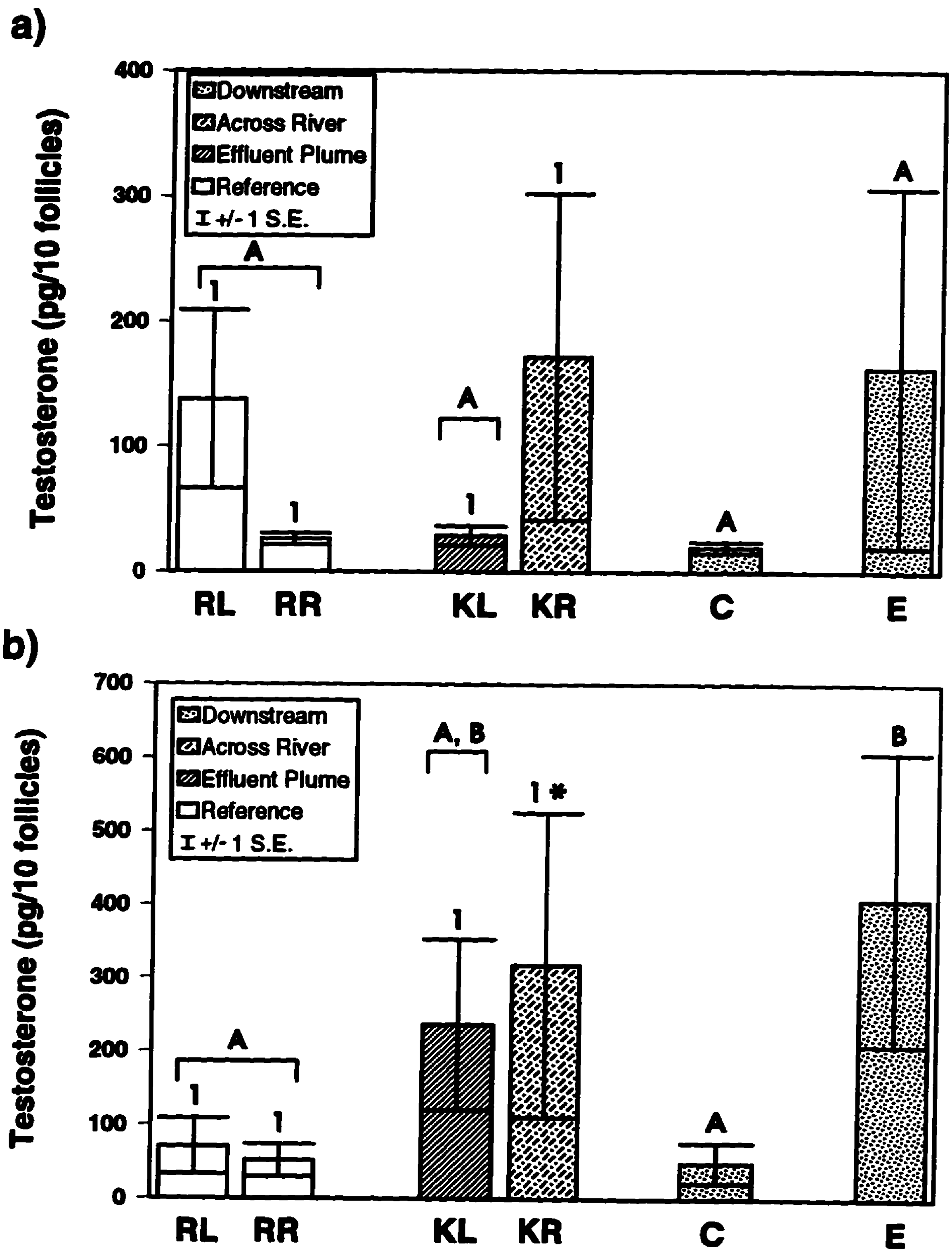
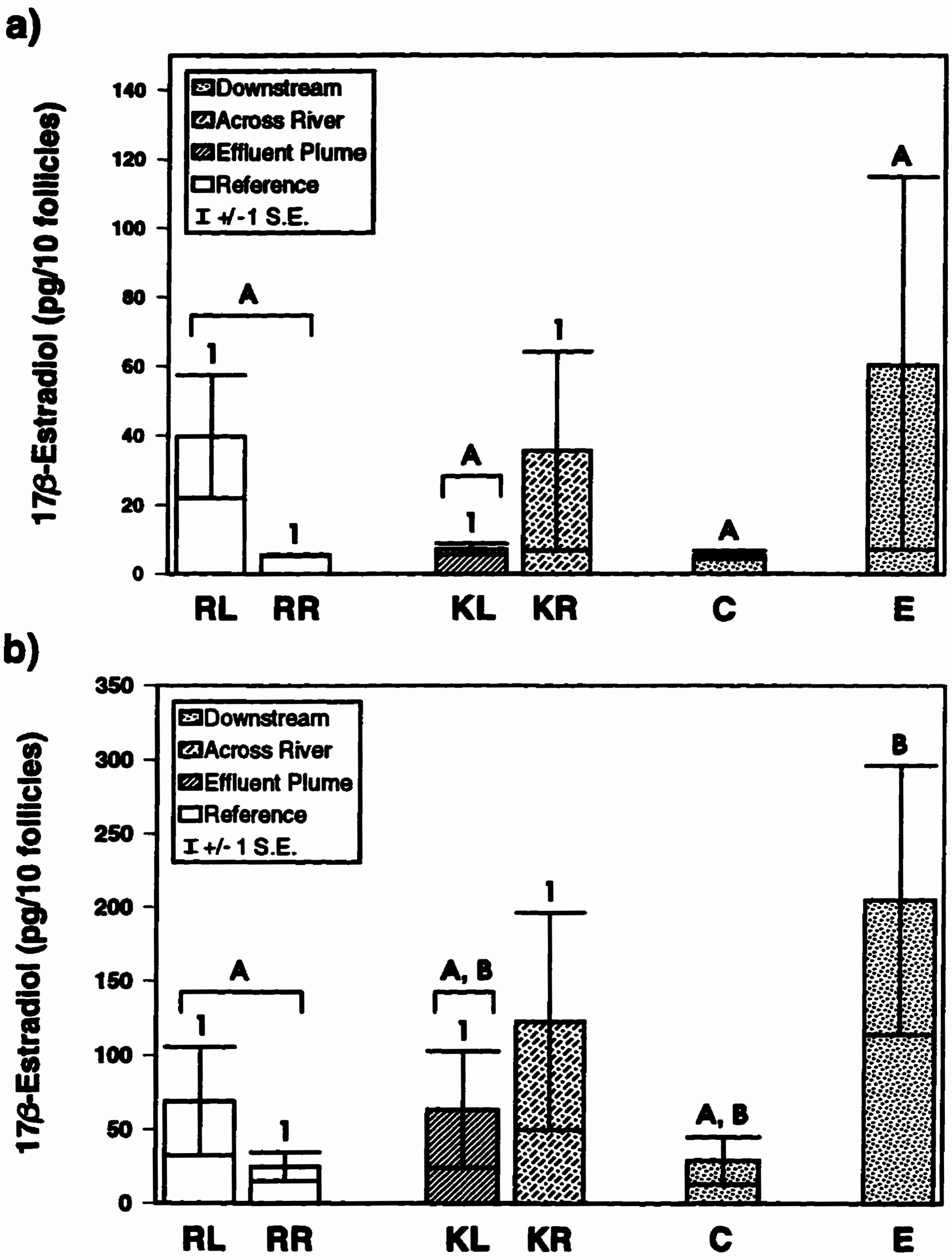

a)
Testosterone (pg/10 follicles)
400
300
200
100
0
Downstream
Across River
Effluent Plume
Reference
+/- 1 S.E.
A
1
A
1
1
1
A
A
RL
RR
KL
KR
C
E
b)
Testosterone (pg/10 follicles)
700
600
500
400
300
200
100
0
Downstream
Across River
Effluent Plume
Reference
+/- 1 S.E.
A, B
B
1 *
1
A
1
1
A
RL
RR
KL
KR
C
E

Figure 2.9. a) Basal and b) forskolin-stimulated *in vitro* production of 17β-estradiol by egg follicles of female spoonhead sculpin (*Cottus ricei*) from reference and effluent exposure sites during the spring 1995 study, Athabasca River, Hinton, Alberta. Bars with different alphabetical superscripts are statistically different ($p<0.05$). Differences ($p<0.05$) between sites RL *vs* RR and KL *vs* KR are denoted with different numerical superscripts. A difference ($p<0.05$) between site KR and the pooled reference sites is indicated with an asterisk (*). Figure Caption 2.5


a)
Downstream
Across River
Effluent Plume
Reference
I +/-1 S.E.
17β-Estradiol (pg/10 follicles)
140
120
100
80
60
40
20
0
A
1
1
A
1
1
A
A
RL
RR
KL
KR
C
E
b)
Downstream
Across River
Effluent Plume
Reference
I +/-1 S.E.
17β-Estradiol (pg/10 follicles)
350
300
250
200
150
100
50
0
A
1
1
A, B
1
1
A, B
B
RL
RR
KL
KR
C
E

Liver weights in sculpin from sites KR and KL were similar, but heavier than in reference fish. There was no change in male gonad weight at the effluent plume site; however, gonad weight was lower at the across river site (Table 2.9). Female gonad weight and fecundity were greater at the plume site relative to the reference site. Estimates of egg size were not different between the reference and effluent plume sites (egg weight, $p=0.62$; egg diameter, $p=0.18$). However, the relationship between egg diameter and carcass weight was borderline ($p=0.06$), and both estimates of egg size were poorly correlated with carcass weight (egg weight, $r=0.40$; egg diameter, $r=0.32$). Remaining site comparisons could not be calculated using ANCOVA due insufficient sample sizes at site KR. Univariate comparisons indicated that there were no differences in egg weight among the study sites ($p>0.13$), but the average egg diameter was greater at site KR relative to eggs from site KL ($p=0.03$).

Hepatic EROD activity in sculpin confirmed that fish were exposed to mill effluent. EROD activity in prespawning sculpin from site KL was approximately 4 fold higher than reference fish (Figure 2.5, 2.6a). Activity in male and spent female (Figure 2.6b) sculpin was also higher at site KR relative to reference levels. There were no differences in EROD activity between sculpin from sites KL and KR.

Comparisons of *in vitro* production of steroid hormones indicated few differences among sites RL+RR, KR and KL. *In vitro* production of testosterone and 17 β-estradiol by gonadal tissues of sculpin collected from the effluent plume site was similar to production by tissues from reference fish (Figure 2.7, 2.8, 2.9). Only forskolin-stimulated production of testosterone was greater at site KR relative to the reference site. As well, steroid production between sites KR and KL was similar with the exception of increased forskolin-stimulated production of testosterone in male sculpin at site KR. Forskolin did not have any effect on testosterone (for each site $p>0.11$) or 17β-estradiol (for each site, $p>0.08$) production in female sculpin.

Far-field Comparisons

Sculpin were collected at sites C and E to investigate whether the responses observed at the kraft mill effluent plume site persisted downstream. Comparisons were made between sites KL *vs* C, KL *vs* E, and C *vs* E. Total numbers of female sculpin collected at site E were limited, and only univariate comparisons were conducted for females.

There was evidence that the effluent response persisted as far downstream as sites C and E; however, some whole-organism parameters had returned to reference levels. Sculpin were similar in length, weight, condition and age between site C and the effluent plume site (Table 2.7, Table 2.10). There were no changes in length, weight and age of female sculpin at site E relative to fish from the effluent plume site or site C; however, male sculpin from site E were longer, heavier and older than fish from either upstream site. Size-at-age of female sculpin was greater at site C relative to females from the effluent plume site, whereas male sculpin from site C showed a decline in size-at-age.

Male testes weight was heavier at site E, but actually declined at site C (Table 2.10). However, there was no difference in ovary weight between sites KL and C. This corresponded with an increased fecundity at site C coupled with a decrease in egg weight. There was no relationship between egg diameter and carcass weight ($p=0.16$); however, an ANOVA indicated that egg diameter at site C was also smaller than at site KL ($p=0.03$). Estimates of egg size (unadjusted for body size) were smaller at site E than site KL (egg weight, $p=0.04$; egg diameter, $p=0.002$), but similar to site C (egg weight, $p=0.38$; egg diameter, $p=0.60$). Liver weights were found to increase downstream in male sculpin from site KL to site E (Table 2.10). Female liver weight at site C was similar to site KL.

Only four parameters at the downstream exposure sites were reduced relative to observations from the effluent plume site: male size-at-age and testes weight at site C; and, female egg weight at sites C and E. As such, these parameters were examined to determine if there was any "recovery" towards reference levels. Comparison of male size-at-age at site C to reference conditions indicated the slope of the regression line for site C was greater ($p<0.001$). Males at site C were shorter than reference males at a given age until age 3-3.5 y (i.e. immature), after which the older mature males from site C were larger at a given age than reference males (Figure

Table 2.10. **Comparison (ANCOVA) of size-at-age, condition, gonad weight, fecundity, egg size and liver weight for spoonhead sculpin at sites KL, C and E, spring 1995, Athabasca River, Hinton, Alberta. Abbreviations: I - intercept; S - slope; p - pooled over sites. Subscripts refer to sites. Interaction terms were considered significant at p<0.01.**

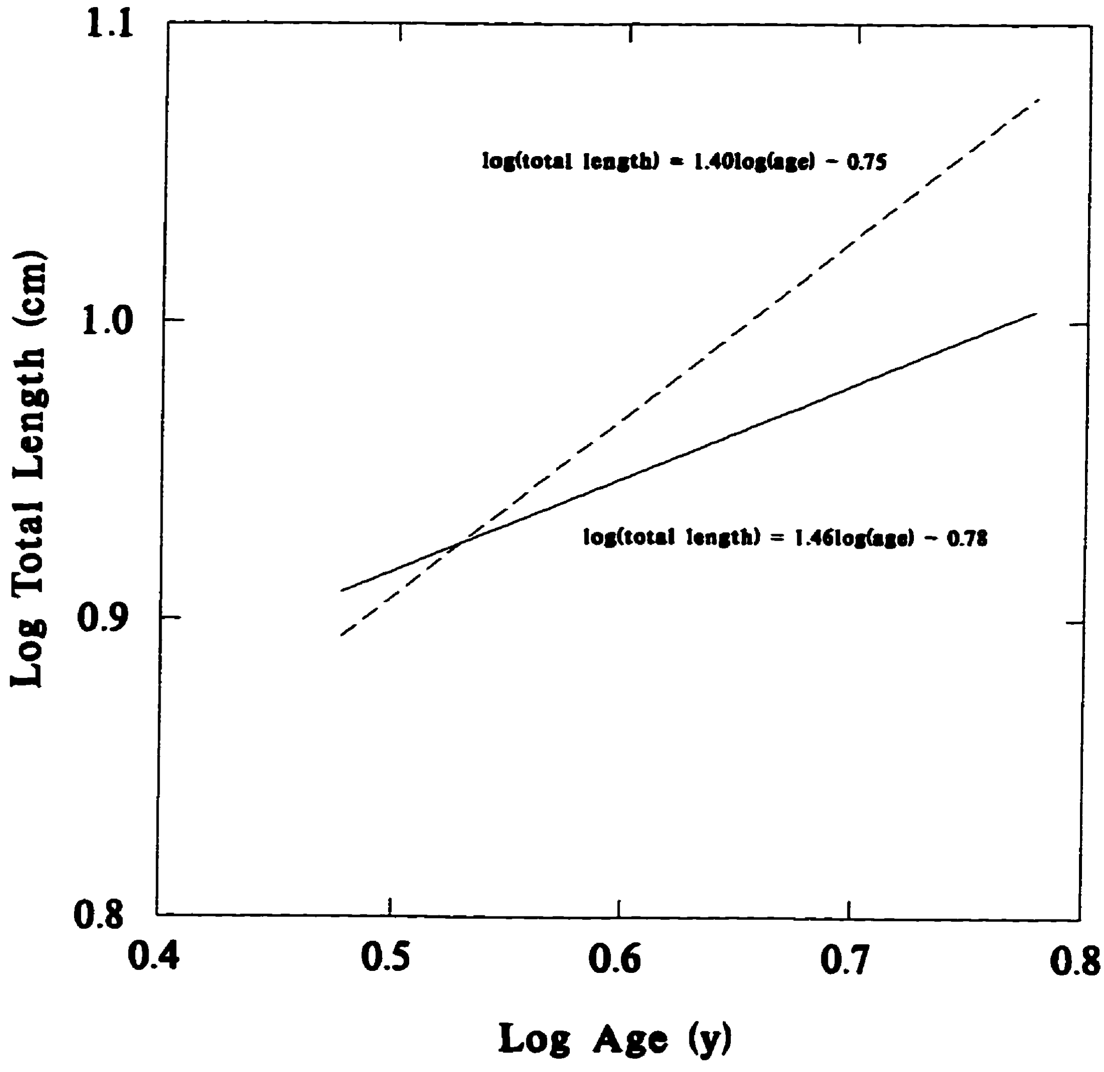
		Probability value (p)			
Parameter	Sex	Slope	Intercept	$\log_{10}$ Mean Estimate	
Site KL (effluent plume) *vs* Site C (downstream site)					
Size-at-age	male	0.02	<0.001	I_{kl}=1.0,	I_c=0.97
	female	0.14	0.03	I_{kl}=0.93,	I_c=0.96
Condition	male	0.65	0.65	S_p=3.24,	I_p=-2.24
	female	0.64	0.73	S_p=2.91,	I_p=-2.04
Gonad *vs* carcass wt.	male	0.64	<0.001	I_{kl}=-0.896,	I_c=-1.085
	female	0.52	0.89	S_p=1.037,	I_p=-0.411
Liver *vs* carcass wt.	male	0.78	<0.001	I_{kl}=-0.812,	I_c=-0.668
	female	0.53	0.12	S_p=0.748,	I_p=-1.216
Fecundity *vs* carcass wt.	female	0.99	0.03	I_{kl}=2.54,	I_c=2.63
Egg wt. *vs* carcass wt.	female	0.77	0.02	I_{kl}=0.76,	I_c=0.67
Site KL *vs* Site E (downstream site) - males only					
Size-at-age	male	0.36	0.82	S_p=0.40,	I_p=0.76
Condition	male	0.89	0.002	I_{kl}=1.01,	I_e=1.05
Gonad *vs* carcass wt.	male	0.54	<0.001	I_{kl}=-0.832,	I_e=-0.602
Liver *vs* carcass wt.	male	0.97	<0.001	I_{kl}=-0.763,	I_e=-0.503
Site C *vs* Site E - males only					
Size-at-age	male	0.19	0.01	I_c=0.98,	I_e=1.01
Condition	male	0.50	<0.001	I_c=0.97,	I_e=1.01
Gonad vs carcass wt.	male	0.76	<0.001	I_c=-1.047,	I_e=-0.637
Liver vs. carcass wt.	male	0.71	0.004	I_c=-0.641,	I_e=-0.531

2.10). Similarly, although male gonad weight at site C was less than at site KL, it was still significantly greater than the reference values ($p<0.001$). Comparisons of egg weight between site C and pooled reference sites indicated that there was no relationship between egg weight and carcass weight ($p=0.13$). Subsequent comparisons of egg weight, unadjusted for body size, showed that egg weight was similar at both sites ($p=0.74$). As well, comparison of egg weight (unadjusted for body size) between site E and the reference sites indicated that there was no difference in egg weight ($p=0.13$).

Hepatic EROD activity suggested that sculpin were still exposed (and responding) to the mill effluent at the downstream exposure sites. EROD activity in sculpin at site C was similar to levels measured in fish from the effluent plume site (Figure 2.5, 2.6). Activity in male sculpin at site E was significantly less than either site KL or site C, but was still induced 3-fold relative to reference males. However, preovulatory females at site E exhibited EROD activity similar to reference conditions.

In vitro production of steroid hormones in gonadal tissues from sculpin sampled at the downstream exposure sites was variable, but it was generally similar to those from the effluent plume site. With the exception of decreased testicular production of testosterone, steroid hormone production by sculpin at site C was similar to those from the effluent plume site and the pooled reference sites (Figure 2.7, 2.8, 2.9). Steroid production by gonadal tissues from fish sampled at site E was less consistent. Basal production of testosterone was higher in male tissues from site E relative to the effluent plume site, site C and the reference sites. However, forskolin-stimulated production of testosterone in testicular tissues of males was similar among the study sites. Similarly, there were no differences in female testosterone and 17β-estradiol production in ovarian tissues from fish sampled at site E relative to sites C and KL and the reference sites, but values were quite variable. Forskolin-stimulated levels of testosterone produced by follicles from site E were similar to levels at site KL, but higher than levels at site C and the reference sites. As well, forskolin-stimulated follicles produced similar levels of 17β-estradiol among fish from sites KL, C and E, but only levels from fish from site E were higher than reference levels. Forskolin stimulated male *in vitro* testosterone production in testisticular

Figure 2.10. Regression lines of $\log_{10}$ total length *vs* $\log_{10}$ age (size-at-age) for male spoonhead sculpin (*Cottus ricei*) from the pooled reference sites (RL+RR) (solid line, r^2=0.46) and site C (dashed line, r^2=0.84), spring 1995, Athabasca River, Hinton, Alberta.


1.1
1.0
0.9
0.8
Log Total Length (cm)
log(total length) = 1.40log(age) - 0.75
log(total length) = 1.46log(age) - 0.78
0.4
0.5
0.6
0.7
0.8
Log Age (y)

tissues from fish from sites C ($p=<0.001$) and E ($p=0.05$), but had no effect on follicle production of testosterone (C, $p=0.92$; E, $p=0.10$), or 17β-estradiol at site E ($p=0.27$). Forskolin also stimulated follicle production of 17β-estradiol in ovarian tissues from fish from site C ($p=0.02$).

Water Chemistry

With the exception of sulphate ($p=0.05$), concentrations of measured ions were similar between reference sites RL and RR (all ions, $p>0.14$) (Table 2.11). When pooled reference samples were compared with water chemistry downstream of the mill diffuser (i.e. site KL), all ions except for silica ($p=0.18$) had increased significantly (each ion, $p<0.001$). Relative to inputs of ions such as chloride or sodium, the concentration of silica contributed by the kraft mill effluent was limited. The increase in ion concentrations at site KL reaffirmed all indications that this site was exposed to mill effluent and was suitable as the effluent plume site. All ions, except silica ($p=0.18$), were higher at site KL (each ion, $p<0.001$) relative to site KR. As well, ion concentrations at the KR site were also greater than levels at the reference sites (SiO_2, $p=0.05$, remaining ions, $p<0.001$), suggesting an intermediate exposure to mill effluent..

To test for a downstream gradient in water chemistry, a linear contrast (-1 0 +1) was used to compare ion concentrations among sites KL, C and E. Concentrations of chloride, sulphate and sodium were found to decrease linearly from site KL downstream to site E (each ion, $p<0.001$). When these ion concentrations at site E were compared with the reference sites, chloride ($p<0.001$) and sodium ($p=0.01$) were still higher than reference concentrations, and sulphate was lower than the reference level ($p<0.001$). There were no differences in silica concentrations among the downstream sites ($p=0.06$). Differences in potassium occurred among the downstream sites ($p<0.001$); however, a quadratic ($p<0.001$) rather than a linear ($p=0.16$) contrast best described the downstream gradient. This indicated that potassium concentrations at site KL were similar to concentrations at site E, but concentrations at site C were lower than either site. It was uncertain why potassium dipped at site C; however, this concentration was still higher than levels measured at the reference sites ($p<0.001$).

Table 2.11. **Mean ± SE (n=3) of major ion concentrations ($mg \cdot L^{-1}$) in whole kraft mill effluent and water samples collected at each sampling site during the spring 1995 study, Athabasca River, Hinton, Alberta.**

Site	Cl	SO_4	Na	SiO_2	K
RL	1.33 ● 0.04	100 ± 1	2.54 ± 0.01	4.19 ± 0.01	0.54 ● 0.01
RR	1.25 ± 0.01	104 ● 1	2.56 ± 0.01	4.19 ± 0.003	0.55 ± 0.003
Effluent	134 ± 0.3	450 ± 9	296 ± 2	6.05 ± 0.21	9.98 ± 0.21
KR*	2.37 ± 0.02	105 ± 1	5.0 ± 0.02	4.12 ± 0.01	0.6 ● 0.003
KL	8.23 ± 0.02	121 ● 1	17.8 ± 0.13	4.29 ± 0.1	0.85 ± 0.01
C	7.06 ± 0.04	109 ± 1	14.2 ± 0.04	4.07 ± 0.04	0.79 ± 0.01
E	5.79 ± 0.08	97.2 ± 0.5	13.8 ± 0.1	4.07 ± 0.04	0.87 ± 0.01

* site KR is located across river from the main plume of the kraft mill effluent.

Instream concentrations of chloride are commonly used as a conservative tracer of pulp mill effluents (mills with Cl bleaching) to estimate the minimum concentration of effluent in receiving waters. Estimated effluent concentrations downstream of the kraft mill diffuser were approximately 5.2 % at site KL and 0.8 % at site KR. Concentrations at the downstream exposure sites were estimated to be 4.3 % at site C and 3.4 % at site E. From these calculations, it was probable that the downstream sites were exposed to mill effluent, and that effluent concentrations remained substantial throughout the study area.

2.3.3 Non-kraft mill effluent, Whitecourt study area

Variations in age, body size and organ metrics for lake chub at each collection site are presented in Table 2.12 and Table 2.13. Independent verification of age estimates on a subsample of 54 fish (59% of total number aged) indicated an error rate (i.e. precision) of 18% (in all cases a difference of one year). This reduced level of precision emphasized the difficulties interpreting some of the scale samples from lake chub collected from any of the survey sites.

Reference Site Comparisons

Lake chub were collected from reference sites WF and R2 to investigate possible differences in fish characteristics among sites within the reference zone. Prior to conducting comparisons between reference and downstream fish, a preliminary comparison was conducted between the two reference sites.

From these analyses, there were only a few differences in lake chub between the two reference sites. Lake chub were similar in length, weight, size-at-age, and gonad weight between sites WF and R2 (Table 2.12, 2.14). Female chub had greater condition and liver size at site R2, but this was not evident in male chub. Male chub collected at site WF were also older than males from site R2. There were also no differences in fecundity and egg size between fish from the reference sites.

Table 2.12. Mean and SE (n) of length, weight, condition (K) and age of lake chub (*Couesius plumbeus*) collected at each sampling site during the fall 1994 study on the Athabasca River, Whitecourt, Alberta. Statistical results for variables analysed using ANOVA are presented. Within a column, a difference ($p<0.05$) between reference sites WF and R2 is denoted by an asterisk (*). Differences ($p<0.05$) among the pooled reference sites and sites TMP and CTMP are denoted by different alphabetical superscripts.

Site	Fork Length (cm)	Body Weight (g)	Carcass Weight (g)	K	Age (y)
Male					
WF	9.0 ± 0.2 (10) } A	7.15 ± 0.52 (10) } A	6.45 ± 0.46 (10) } A	0.88 ± 0.02 (10)	1.7 ± 0.2 (10) } A
R2	8.8 ± 0.2 (6)	7.05 ± 0.53 (6)	6.37 ± 0.49 (6)	0.93 ± 0.03 (6)	1.2 ± 0.2 (6) *
TMP	9.2 ± 0.3 (10) A	7.68 ± 0.59 (10) A	6.90 ± 0.52 (10) A	0.89 ± 0.02 (10)	1.8 ± 0.1 (10) A
CTMP	9.0 ± 0.2 (10) A	8.08 ± 0.53 (10) A	7.33 ± 0.49 (10) A	0.99 ± 0.02 (10)	1.4 ± 0.2 (10) A
Female					
WF	9.4 ± 0.2 (16) } A	8.10 ± 0.61 (16) } A	6.71 ± 0.48 (16) } A	0.80 ± 0.01 (16)	1.8 ± 0.1 (16) } A
R2	8.8 ± 0.3 (13)	7.56 ± 0.83 (13)	6.08 ± 0.69 (12)	0.89 ± 0.02 (12)	1.5 ± 0.1 (13)
TMP	9.1 ± 0.3 (15) A	7.73 ± 0.77 (15) A	6.46 ± 0.62 (15) A	0.82 ± 0.01 (15)	1.6 ± 0.1 (15) A
CTMP	8.5 ± 0.4 (7) A	7.18 ± 0.94 (7) A	5.91 ± 0.77 (7) A	0.92 ± 0.02 (7)	1.6 ± 0.2 (7) A

Table 2.13. Mean and SE (n) of liversomatic index, gonadosomatic index, fecundity and egg size of lake chub (*Couesius plumbeus*) collected at each sampling site during the fall 1994 study on the Athabasca River, Whitecourt, Alberta.

Site	LSI (%)	GSI (%)	Fecundity (# eggs)	Egg Weight (g)	Egg Diameter (mm)
Male					
WF	1.60 ± 0.26 (10)	0.96 ± 0.07 (10)			
R2	1.60 ± 0.18 (6)	0.85 ± 0.09 (6)			
TMP	2.02 ± 0.13 (10)	2.15 ± 1.15 (10)			
CTMP	1.62 ± 0.16 (10)	0.91 ± 0.07 (10)			
Female					
WF	1.99 ± 0.11 (16)	8.67 ± 0.52 (16)	2662 ± 391 (13)	0.30 ± 0.02 (13)	0.08 ± 0.02 (13)
R2	2.39 ± 0.18 (12)	7.66 ± 0.70 (12)	2394 ± 267 (9)	0.26 ± 0.02 (9)	0.77 ± 0.03 (9)
TMP	1.98 ± 0.09 (15)	8.07 ± 0.51 (15)	2290 ± 288 (12)	0.27 ± 0.02 (12)	0.74 ± 0.02 (12)
CTMP	2.30 ± 0.34 (7)	8.79 ± 0.64 (7)	2374 ± 290 (6)	0.25 ± 0.01 (6)	0.73 ± 0.02 (6)

Table 2.14. **Site comparisons (ANCOVA) of size-at-age, condition, gonad weight, fecundity, egg size and liver weight for lake chub, fall 1994, Athabasca River, Whitecourt, Alberta. Abbreviations: I - intercept; S - slope; p - pooled over sites. Subscripts refer to sites. Interaction terms were significant at $p<0.01$.**

		Probability value (p)			
Parameter	Sex	Slope	Intercept	$\log_{10}$ Mean Estimate	
Site WF *vs* Site R2 (reference sites)					
Size-at-age	male	0.90	0.32	S_p=0.11,	I_p=0.93
	female	0.76	0.13	S_p=0.22,	I_p=0.91
Condition	male	0.55	0.08	S_p=3.27,	I_p=-2.31
	female	0.59	<0.001	I_{wf}=0.76,	I_{r2}=0.82
Gonad *vs* carcass wt.	male	0.92	0.40	S_p=1.701,	I_p=-2.613
	female	0.94	0.49	S_p=1.714,	I_p=-1.669
Liver *vs* carcass wt.	male	0.74	0.73	S_p=1.737,	I_p=-2.414
	female	0.31	0.05	I_{wf}=-0.929,	I_{r2}=-0.841
Fecundity *vs* carcass wt.	female	0.94	0.77	S_p=0.90,	I_p=2.62
Egg wt. *vs* carcass wt.	female	0.70	0.15	S_p=0.539,	I_p=-1.015
Egg dia. *vs* carcass wt.	female	0.33	0.46	S_p=0.215,	I_p=-0.285
Site TMP *vs* Site CTMP					
Size-at-age	male	0.50	0.38	S_p=0.14,	I_p=0.93
	female	0.78	0.22	S_p=0.16,	I_p=0.92
Condition	male	0.74	0.006	I_{tmp}=0.82,	I_{ctmp}=0.86
	female	0.92	0.005	I_{tmp}=0.75,	I_{ctmp}=0.81
Gonad *vs* carcass wt.	male	0.55	0.13	S_p=1.364,	I_p=-2.332
	female	0.28	0.11	S_p=1.471,	I_p=-1.457
Liver *vs* carcass wt.	male	0.27	0.08	S_p=0.574*,	I_p=-1.398
	female	0.16	0.35	S_p=1.160,	I_p=-1.819
Fecundity *vs* carcass wt.	female	0.47	0.27	S_p=1.20,	I_p=2.37

* pooled liver weight vs carcass weight relationship was poor (r^2=0.181)

Table 2.14. Continued.

Parameter	Sex	Probability value (p)		Log$_{10}$ Mean Estimate	
		Slope	Intercept		
Site (WF+R2) (pooled reference) *vs* Site TMP					
Size-at-age	male	0.25	0.99	S_p=0.13,	I_p=0.93
	female	0.41	0.70	S_p=0.19,	I_p=0.92
Condition	male	0.10	0.67	S_p=2.86,	I_p=-1.91
	female	0.93	0.32	S_p=2.97,	I_p=-2.05
Gonad *vs* carcass wt.	male	0.76	0.35	S_p=1.668,	I_p=-2.572
	female	0.62	0.97	S_p=1.660,	I_p=-1.626
Liver *vs* carcass wt	male	0.02	0.05	I_{ref}=-1.014,	I_{tmp}=-0.896
	female	0.46	0.37	S_p=1.114,	I_p=-1.780
Fecundity *vs* carcass wt.	female	0.28	0.82	S_p=1.0,	I_p=2.53
Egg wt. *vs* carcass wt.	female	0.16	0.97	S_p=0.393,	I_p=-0.893
Egg dia. *vs* carcass wt.	female	0.73	0.09	S_p=0.205,	I_p=-0.285
Site (WF+R2) *vs* Site CTMP					
Size-at-age	male	0.75	0.30	S_p=0.11,	I_p=0.94
	female	0.81	0.11	S_p=0.22,	I_p=0.91
Condition	male	0.23	0.005	I_{ref}=0.81,	I_{ctmp}=0.84
	female	0.99	0.01	I_{ref}=0.77,	I_{ctmp}=0.81
Gonad *vs* carcass wt.	male	0.40	0.51	S_p=1.465,	I_p=-2.436
	female	0.24	0.19	S_p=1.578,	I_p=-1.548
Liver *vs* carcass wt.	male	0.28	0.89	S_p=1.408,	I_p=-2.154
	female	0.40	0.67	S_p=1.256,	I_p=-1.877
Fecundity *vs* carcass wt.	female	0.17	0.39	S_p=0.90,	I_p=2.57
Egg wt. *vs* carcass wt.	female	0.12	0.67	S_p=0.456,	I_p=-0.949
Egg dia. *vs* carcass wt.	female	0.04	0.18	S_p=0.172,	I_p=-0.255

Mean hepatic EROD activity in male lake chub was similar at both reference sites (Figure 2.11); however, activity in female chub was higher at site R2. *In vitro* production of testosterone (Figure 2.12a) and 17β-estradiol (Figure 2.12b) by follicles of lake chub from the two reference sites (sites WF and R2) were not different.

For the most part, lake chub at the two reference sites were similar, although some differences were found. As in the previous study on spoonhead sculpin (see Section 2.3.2.2), both reference sites were pooled to ensure that the full extent of reference variability was included when conducting comparisons between reference and exposed fish.

Near-field Comparisons

There were few differences in whole organism characteristics among the TMP, CTMP and pooled reference sites. Male and female lake chub exhibited increased condition at site CTMP relative to site TMP and the reference sites (Table 2.14). As well, male liver weight was higher at the TMP site relative to reference males. Male liver weight at the CTMP site was intermediate but was not statistically different from either the reference sites or site TMP. All the remaining measurements describing the response of lake chub were similar among the study sites (Table 2.12, 2.14).

EROD activity in exposed lake chub from the TMP and CTMP sites were not induced relative to the reference fish (Figure 2.11). However, activity in male chub from the CTMP site was higher than males from the TMP site. *In vitro* production of testosterone by follicles from TMP females was not significantly different from reference females (Figure 2.12). Follicles from CTMP females; however, produced significantly more testosterone than reference and TMP fish. There were no differences in levels of 17β-estradiol production among sites.

Water Chemistry

Adsorbable organic halide (AOX) was not detected (<0.5 μg/mL) in water samples collected

Figure 2.11. EROD activity in male and female lake chub (*Couesius plumbeus*) from the reference sites, TMP effluent plume site and CTMP effluent plume site during the fall 1994 study, Athabasca River, Whitecourt, Alberta. Values represent the mean ● SE. Bars with different uppercase alphabetical superscripts are statistically ($p<0.05$) different from one another.

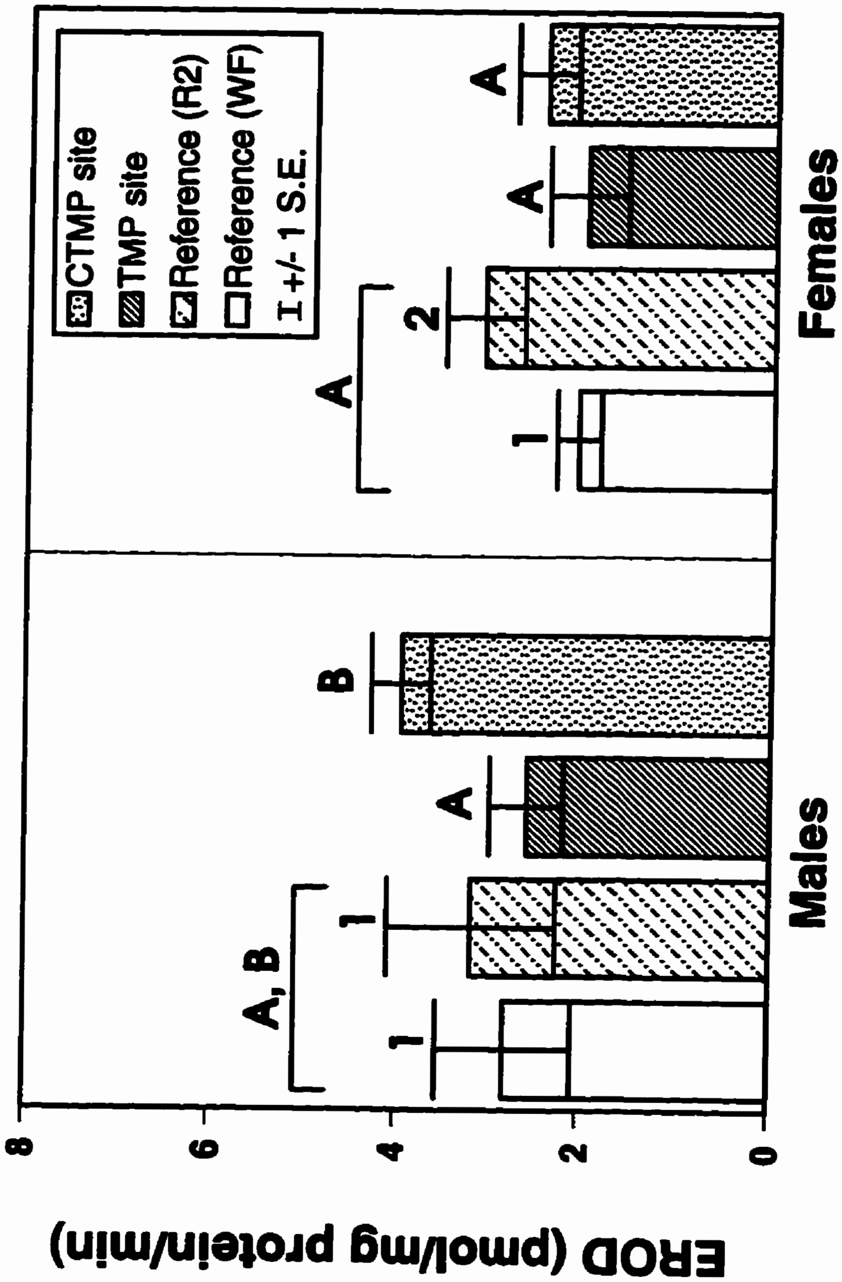
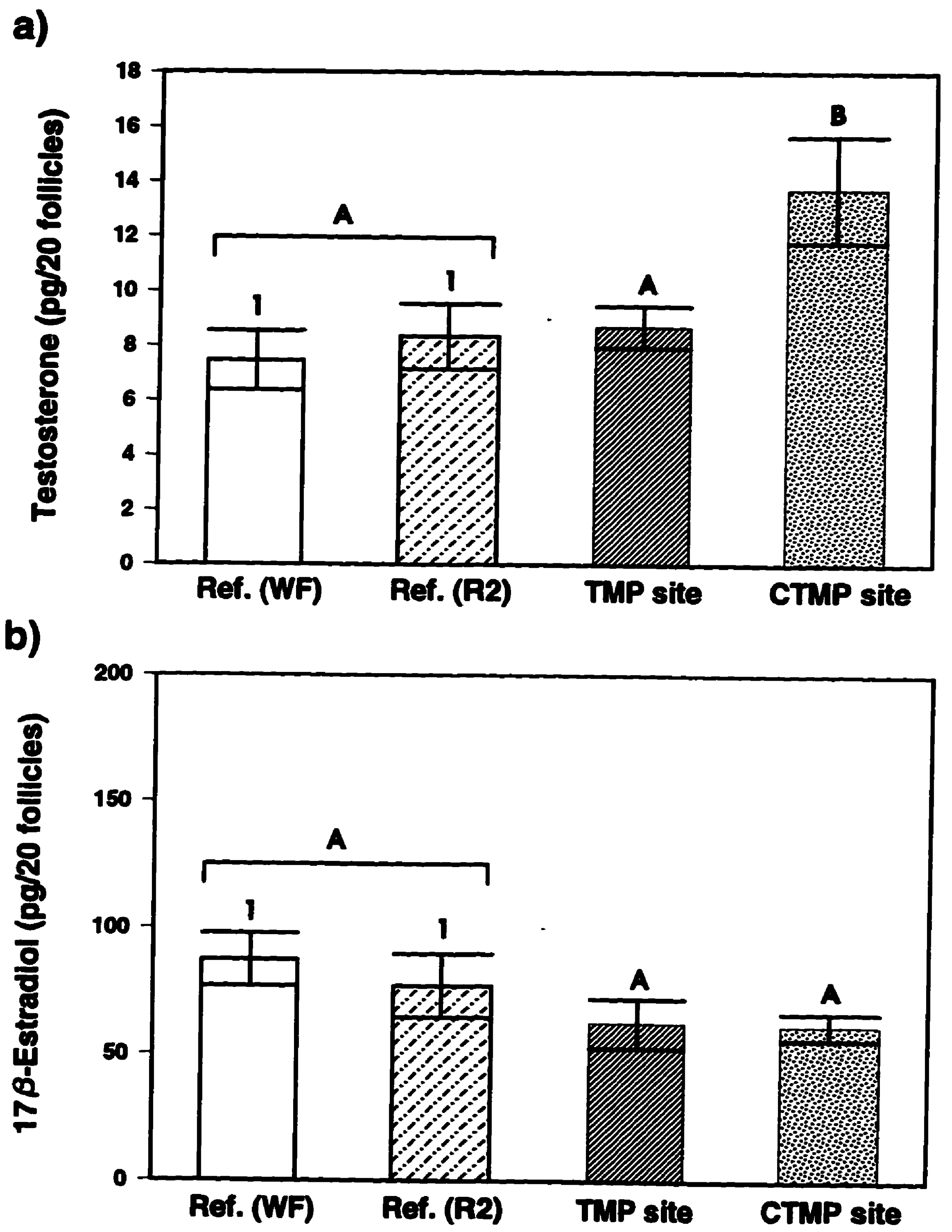

EROD (pmol/mg protein/min)
0
2
4
6
8
CTMP site
TMP site
Reference (R2)
Reference (WF)
I +/- 1 S.E.
A, B
1
1
A
B
Males
A
1
2
A
A
Females

Figure 2.12. *In vitro* production of a) testosterone and b) 17β-estradiol by egg follicles of female lake chub (*Couesius plumbeus*) from the reference sites, TMP effluent plume site and CTMP effluent plume site during the fall 1994 study, Athabasca River, Whitecourt, Alberta. Values represent the mean ± SE. Bars with different uppercase alphabetical superscripts are statistically ($p<0.05$) different from one another.


a)
18
16
14
12
10
8
6
4
2
0
Testosterone (pg/20 follicles)
A
1
1
A
B
Ref. (WF)
Ref. (R2)
TMP site
CTMP site
b)
200
150
100
50
0
17β-Estradiol (pg/20 follicles)
A
1
1
A
A
Ref. (WF)
Ref. (R2)
TMP site
CTMP site

from reference and exposure sites in the Whitecourt study area. With the exception of sodium, concentrations of chloride, sulphate, silica and potassium were similar between the two reference sites (Table 2.15). Sulphate, sodium and potassium all increased downstream from the reference sites to site CTMP. There were no differences in chloride between the reference sites and either mill exposure site; however, chloride was higher at the CTMP site relative to the TMP site. Silica concentrations were also higher at the CTMP site.

2.4 Discussion

Kraft Mill Effluent Response

Several studies on the effects of BKME on fishes have shown increased levels of liver MFO activity (Rogers et al. 1989; McMaster et al. 1991; Munkittrick et al. 1991a; Smith et al. 1991; Hodson et al. 1992). During the fall, the increase in EROD activity in both male and female spoonhead sculpin collected from the site KL indicated: a) that it is possible to measure an increase in EROD activity in spoonhead sculpin, and b) probable exposure to mill effluent. The intermediate levels of activity at site KR suggested that fish were exposed to lower concentrations of effluent. Concentrations of major ions; particularly chloride, sodium, and sulphate; also suggested that the downstream tracking of the effluent was mostly on the left upstream bank of the river. As well, recent plume delineation studies conducted by the mill (Golder Associates 1994) indicated that, during low flow conditions in October, site KR was exposed to approximately 0.05-1% effluent and site KL (effluent plume site) was exposed to about 2-2.5% effluent.

As expected during the spring survey, EROD induction in exposed sculpin was greater (approx. 2 fold greater) than induction observed during the fall season. Estimated effluent concentration at site KL was 5.2 %, twice the concentrations measured during the October, 1993, plume delineation study. Furthermore, the average flow rate of the Athabasca River during the October 1993 study was 100 m^3/s, whereas during the spring 1995 survey it was approximately 31 m^3/s

Table 2.15. Mean ± SE (n=3) of major ion concentrations ($mg \cdot L^{-1}$) in water samples collected at each sampling site during the fall 1994 study, Athabasca River, Whitecourt, Alberta. Within a column, a difference ($p<0.05$) between reference sites WF and R2 is denoted by an asterisk (*). Differences ($p<0.05$) among the pooled reference sites and sites TMP and CTMP are denoted by different alphabetical superscripts.

Site	Cl		SO_4		Na		SiO_2		K	
WF	1.63 ± 0.03	A	38.4 ± 0.03	A	4.03 ± 0.02	A	3.75 ± 0.04	A	0.52 ± 0.003	A
R2	1.58 ± 0.04		39.0 ± 0.2		4.19 ± 0.01*		3.62 ± 0.06		0.52 ± 0.003	
TMP	1.63 ± 0.01	A	39.7 ± 0.2	B	4.32 ± 0.01	B	3.78 ± 0.1	A	0.54 ± 0.01	B
CTMP	1.68 ± 0.01	A,B[†]	25.8 ± 0.1	C	11.9 ± 0.1	C	4.59 ± 0.02	B	0.90 ± 0.003	C

[†]CTMP > TMP, but equal to pooled reference.

(Water Survey of Canada, Calgary, Alberta, unpublished data). High effluent concentration persisted downstream to sites C and E. EROD results indicated that sculpin at site C were exposed to sufficient concentrations of kraft mill effluent to cause induction equal to what was observed at effluent plume site KL. EROD activity declined at site E (48 km downstream); however, for male sculpin this was still higher than reference levels.

Although not formally tested, it was obvious that EROD activity in male sculpin was higher than in preovulatory females, and similar to that of spent females. Decreased levels of EROD activity in females immediately before and during spawning have also been observed in studies monitoring white sucker (McMaster et al. 1991; Munkittrick et al. 1991a; Gagnon et al. 1994). Seasonal differences in EROD activity in female fish and gender differences in prespawning fish have suggested that gonadal steroids, particularly 17β-estradiol, depress total cytochrome P-450 activity. Förlin and Andersson (1984) found that juvenile rainbow trout administered with 17β-estradiol had decreased levels of MFO induction and that testosterone had no effect. Similarly, Stegeman (1982) and Pajor (1990) observed depressed MFO activity in immature brook trout administered with 17β-estradiol. However, contrary to the laboratory findings, Munkittrick et al. (1994) found no relationship between steroid levels and MFO activity in white sucker collected from reference and near-field sites associated with eight Canadian pulp mills. The conclusion from their study was that steroid disruption and MFO induction were independent.

During the fall, spoonhead sculpin from the effluent plume site were older, heavier, fatter and had larger gonad and liver weights relative to reference fish of similar size (i.e. carcass weight). After a prolonged exposure to high effluent concentrations during the winter, the general response of exposed sculpin collected during the spring did not change dramatically from what was observed during the fall. Specifically, sculpin from the effluent plume site were heavier, fatter and had larger liver weights than reference fish. In addition, exposed female sculpin exhibited higher gonad weight and fecundity. Changes in the effluent plume fish from the fall to spring survey included: an increase in male size-at-age, no change in male gonad weight, and similar mean age of adult sculpin. It is uncertain why male sculpin from the effluent plume site exhibited an increase in size-at-age and relative decrease in gonad size i.e., opposite to the fall response. However, the similarity in mean age of prespawning adult sculpin at the reference and near-field sites suggested that the age of maturity in exposed fish had not been affected.

When investigating the response of sculpin collected from the downstream exposure sites, only a few whole-organism parameters were found to decrease downstream (e.g., male size-at-age at site C, egg weight at sites C and E), and many of the changes observed between reference and effluent plume fish had persisted (e.g., male/female condition, female gonad/liver weight), or become more pronounced (e.g. male liver weight, immature condition, female size-at - age/fecundity). Of those parameters found to decrease downstream, only egg weight had returned to reference levels. Induced mixed function oxygenase activity and elevated instream concentrations of chloride suggested that effluent concentrations remained high at the downstream exposure sites. Therefore, if sculpin are showing responses related to effluent exposure, it is not surprising that sculpin from the downstream sites continue to exhibit responses similar to effluent plume fish.

In general, the response of spoonhead sculpin to BKME suggests an overall increase in metabolism (*N.B.* the response will be discussed in more detail in Chapter 4). Although an increase in body size, growth and condition in fish exposed to BKME has been reported, other studies have not found concomitant increases in gonad weight, fecundity or liver weight. Adams et al. (1992) found that redbreast sunfish (*Lepomis auritus*) exposed to BKME in the Pigeon River, Tennessee, showed increased growth and condition, increased mean age, but no change in fecundity. Exposed white sucker (*Catostomus commersoni*) in the St. Maurice River, Quebec also exhibited increased growth and condition, but no change in gonad weight and increased age at maturity (Gagnon et al. 1994). Similarly, Andersson et al. (1988) found that perch (*Perca fluviatilus*) exposed to BKME in the Baltic Sea showed increased growth and condition, but decreased gonad weight, increased age to maturity and decreased egg and sperm viability. In each case, other parameters did not conform to a generalized pattern of increased energetics and indicated possible negative impacts on the exposed fish populations. The pattern of responses to pulp mill effluent commonly seen in other Canadian studies is delayed sexual maturity, smaller gonads, reduced body size (growth and condition) and increased liver size (e.g., McMaster et al. 1991, 1992b; Munkittrick et al. 1991a), and has been associated with metabolic problems and resulted in a negative effect on the exposed fish population. This response is quite different from results reported here where spoonhead sculpin exhibited signs of increased growth (weight and girth not length), increased reproductive commitment and increased energy storage, but did not appear to be negatively affected by the exposure to kraft mill effluent.

Ovarian follicles of fish exposed to BKME have shown reduced production of steroids relative to reference fish at a number of sites (Van Der Kraak et al. 1992; Jardine 1994; McMaster et al. 1994). Depressed levels of steroids can potentially have serious negative effects on the reproductive capacity of some species of fish. Consistent with these observations, Brown et al. (1993) suggested that plasma concentrations of 17 β-estradiol in female longnose sucker (*Catostomus catostomus*) and possibly mountain whitefish (*Prosopium williamsoni*) were depressed in fish collected downstream of the Hinton mill. As well, in a follow-up study evaluating steroid levels in fish basin-wide (Brown et al. 1996), plasma 17 β-estradiol was significantly reduced in burbot (*Lota lota*) and longnose sucker collected near pulp mill sites on the Athabasca and Peace River systems. Neither study found any significant change in gonad size or egg size. From this work, they could not exclude the possibility that pulp mill inputs are producing adverse effects on fish in the Peace/Athabasca drainages.

Results from my work on the Athabasca River with spoonhead sculpin, were not consistent with the above studies using large fish species. During the fall survey, follicles from exposed female sculpin exhibited greater *in vitro* production of testosterone and 17β-estradiol than follicles from reference females. The increased steroid levels closely parallelled the observed trends in whole-organism measurements (e.g., gonad weight and body size, liver size) and seemed consistent with a general increase in metabolism. Reasons for the discrepancies between this work and that of Brown et al. (1993, 1996) are unknown. It is possible that *in vitro* steroid production was not an appropriate surrogate to plasma steroid levels. However, this seems unlikely given a recent study documenting agreement between the two methods of evaluating fish steroid levels (McMaster et al. 1995). Alternatively, Brown et al. (1993) questioned the integrity of their own results due to low sample sizes, poor reference data and mobility of larger fish species. Another possible explanation may be related to differences in species-specific habitat preferences. Concentrations of persistent, lipophilic organochlorine compounds from BKME are known to be highest in sediment accumulation zones of receiving environments. As such, it is possible that the exposure to these types of contaminants by sculpin collected from higher velocity riffle/run areas is quite different from sucker and burbot commonly found in lower-velocity depositional habitats (e.g., pools).

During the spring survey, no differences between reference and effluent plume steroid levels for both male and female spoonhead sculpin were observed. These results were especially interesting because sculpin were collected: 1) at a time when steroid profiles were expected to be most developed, and 2) immediately following a period of high effluent exposure at which time effects on steroids should be most noticeable. One possible explanation may be related to the general increase in metabolism. Sculpin at the effluent plume site may be able to recover from spawning faster (i.e. recover energy losses) and start gonadal development slightly earlier than reference fish. Due to an earlier start, eggs from the effluent plume fish collected in the following fall (fall 1994 survey) may have been at an advanced stage in development, relative to reference eggs, and were able to produce more steroids. However, in the following spring, both reference and effluent plume fish had completed their gonadal development prior to spawning and exhibited similar levels of steroid production. Alternatively, it is possible that site differences in *in vitro* sex hormone production during the spring were difficult to detect given the high degree of variability observed within a site, particularly sites where sample sizes were low (e.g. sites KR, E). Regardless, results from the fall/spring surveys suggest that the kraft mill effluent did not appear to have a negative impact on *in vitro* production of steroids in exposed spoonhead sculpin.

There was no indication that gonadal tissues of spoonhead sculpin incubated with forskolin exhibited marked differences in steroid production between reference and exposed fish. It seemed that the functional integrity of gonads, particularly the stimulation of adenylate cyclase, had not been affected in exposed fish. Interestingly, forskolin was found to stimulate steroid production in male testes, but was less successful stimulating female follicles (both reference and exposed sites). Why forskolin did not stimulate female follicles is uncertain; however, it is possible that female steroid production immediately prior to spawning was maximized such that forskolin had no observable effect (G. Van Der Kraak, University of Guelph, Guelph, Ontario, personal communication.).

A recent Adult Fish Survey (component of an Environmental Effects Monitoring study) was done at Hinton using longnose sucker and mountain whitefish as sentinel species (Golder Associates 1996). Statistical comparisons of responses were not evaluated in the study (not part of the EEM first cycle); however, trends in the data for either large species did not appear consistent with the response described for exposed spoonhead sculpin. Longnose sucker showed evidence of increased condition and liver size (weight and liversomatic index) and no change in mean age, but decreased body size (weight and length), gonad weight, and fecundity. Exposed male whitefish had increased body size, reduced testes weight, no change in mean age and moderately higher liversomatic index (LSI). Conversely, exposed females showed no change in body size or fecundity, but increased ovary weight and moderately higher LSI. Interestingly, the responses of the two large fish species were not the same. Although, both species were collected from similar reference and exposure sites, the difference in responses suggests that factors, other than effluent exposure (e.g., habitat preferences, life-history/spawning strategies, contaminant exposure and uptake, etc.,), may play an influential role. This may also be true when comparing the response of spoonhead sculpin to the larger fish species.

Discrepancies between the Adult Fish Survey data and the response of spoonhead sculpin may be related to the choice of reference site and/or sentinel species. Spoonhead sculpin were collected from a reference site upstream of the mill, whereas reference mountain whitefish and longnose sucker were collected from the North Saskatchewan River (Golder Associates 1996). Reference fish were collected from a separate river system because there was concern that reference and exposure fish populations within the same river system may not be discrete and reflective of localized conditions (*N.B.* although, the solution does not remove the possibility of collecting fish in the exposure area which had just moved downstream from an unexposed area).

An obvious assumption to this solution was that fish collected at the North Saskatchewan River accurately reflected the status of reference fish in the Athabasca River. However, differences in whole organism and physiological measurements of spoonhead sculpin between the North Saskatchewan and Athabasca reference sites suggest that this assumption may not be valid (Table 2.16, modified from Gibbons et al. 1996). Furthermore, if sculpin from the North Saskatchewan River had been used in comparisons with near-field fish during the spring study, one would have concluded that: a) size-at-age for males did not increase but stayed the same, b) male gonad weight was not similar but had declined, c) female size-at-age was not similar but had declined, d) EROD induction in females was much more pronounced, and e) *in vitro* steroid production downstream of the mill was substantially depressed (Gibbons et al. 1996). Overall, the response of sculpin would have been more similar to the responses documented for longnose sucker and mountain whitefish. Why differences existed between fish from the reference populations was not investigated; however, it was probably related to natural differences in habitat characteristics. As well, the collection site on the North Saskatchewan River experiences daily fluctuations in water discharge (water levels at the sampling site changed approx. 0.5-0.75 m) due to regulation of the river by the Big Horn Dam. It is unknown how variable discharge influences fish populations, directly, or indirectly through food resources and habitat quality and availability.

Perhaps for larger fish species, this uncertainty is sufficient reason to consider a different river system from the North Saskatchewan River as a reference site for studies conducted on the Athabasca River. For those monitoring mobile fish species there are few alternatives but to collect reference fish from different river systems. However, this problem does emphasize the benefit of monitoring small fish species, such as spoonhead sculpin, that are unlikely to exhibit large scale mobility between reference and exposure zones.

An additional site (site KR) was sampled across the river from the effluent plume site (site KL) during the fall and spring studies. This site was sampled to investigate whether the across-river sculpin exhibited characteristics similar to reference fish or exposed fish, and to provide some

Table 2.16. Summary of relative change (0, no change; + significant increase; - significant decrease) in whole-organism and physiological measurements of spoonhead sculpin (*Cottus ricei*) from the North Saskatchewan River, relative to sculpin from the reference sites on the Athabasca River (pooled sites RL+RR), spring 1995 (modified from Gibbons *et al.*, 1996). Comparisons significant when $p<0.05$.

	North Saskatchewan River	
Parameter	Male	Female
Weight	+	+
Total Length	+	+
Condition	0	0
Age	0	0
Size-at-Age	+	+
Gonad *vs* carcass wt.	+	0
Fecundity *vs* carcass wt.	na*	0
Liver *vs* carcass wt.	0	0
EROD activity	0	-
Testosterone [forskolin-stimulated]	0 [+]	+ [+]
17 β-estradiol [forskolin-stimulated]	na	+ [+]

* na, not applicable

preliminary information regarding the mobility of the spoonhead sculpin. Results from the fall study indicated that sculpin from site KR appeared to show an intermediate response to exposure relative to fish from the reference and effluent plume sites. In particular, differences between site KR and effluent plume site responses suggested that sculpin did not undergo extensive lateral movements across the river taking them in and out of the effluent plume. Similarly, differences in responses observed between site KR and reference fish suggested that longitudinal movement up and down the river was limited. Although preliminary, these results suggested that spoonhead sculpin did not move large distances and, more importantly, the observed response in sculpin likely reflected the local conditions from which they were caught. Similar results were also observed during the spring study. However, at this time sculpin from site KR exhibited more characteristics which were different from reference fish and they were more similar to the effluent plume fish than observed during the fall. Perhaps this was because site KR experienced greater effluent concentrations during winter low-flow conditions such that differences in effluent exposure at the effluent plume site and site KR were minimized. Conversely, it is possible that ice cover and low-flow conditions during the winter reduced habitat availability (especially at site KR, wide/shallow habitat) displacing fish towards main flow areas of similar effluent exposure. This was especially true during the abnormally low-flow conditions experienced during the winter/spring of 1995 (Water Survey of Canada, Calgary, Alberta, unpublished data). It was also possible that, during times of extreme low-flow, spoonhead sculpin were able to cross the reduced thalweg of the river and access the opposite side.

Non-kraft Mill Effluent Response

Instream concentration of sodium has been used as an inexpensive chemical tracer of effluent from non-kraft mill operations. Results from the fall survey indicated that the concentrations of sodium were higher at the TMP and CTMP sampling sites indicating probable effluent exposure. As well concentrations of sulphate were higher at both sites relative to reference concentrations. Concentrations of chloride, silica and potassium, and especially sodium were higher at the CTMP site than the TMP site. Previous reports have indicated that sodium is present in relatively high concentrations in the CTMP effluent (Sentar Consultants Ltd. 1994b), but in low concentrations in the TMP effluent (Sentar Consultants Ltd. 1994a). As well, the CTMP near-field site was influenced by discharges from CTMP mill as well as the McLeod River. Water samples from the McLeod River were not collected; however, data from historical water quality surveys (Sentar Consultants Ltd. 1994b) indicated that the concentration of sodium in the McLeod River was approximately 2.5-3.3 fold higher than the concentration measured in the Athabasca River upstream of the confluence. The McLeod River was also found to contribute to concentrations of silica and potassium. Results from a plume delineation study by the CTMP mill (Sentar Consultants Ltd. 1994b) confirmed that the CTMP study site was exposed to mill effluent.

Hepatic EROD activity was not induced in lake chub exposed to either TMP or CTMP mill effluent relative to reference activity. However, male lake chub from the CTMP site showed increased EROD activity relative to males from the TMP site. What this latter observation meant was uncertain given that these same fish had similar EROD levels to reference males. Fish exposed to pulp mill effluent (especially effluent from mills with kraft pulping), typically show increased levels of hepatic EROD activity relative to unexposed fish (Munkittrick et al. 1994; Martel et al. 1996). The absence of induction in fish below the TMP and CTMP mills (relative to reference fish) was likely influenced by the high level of effluent dilution even during low river flow conditions. Laboratory tests investigating the effluent thresholds for MFO responses indicate that induced MFO activity is observed at concentrations of 0.5-0.7% for some kraft mill effluents (Robinson et al. 1994). Maximum effluent concentrations immediately below the TMP mill diffuser during low-flow conditions was estimated at approximately 0.15% (Sentar Consultants Ltd 1994a). Maximum effluent concentrations within 155 m of the CTMP mill diffuser was estimated at 0.75%, and quickly dropped to 0.4% approximately 1.5-1.7 km downstream (Sentar Consultants Ltd. 1994b). However, regardless of effluent dilution, the absence of significant EROD induction has also been documented in rainbow trout during laboratory exposures to 100% effluent from the same TMP and CTMP mill facilities (K.R. Munkittrick, Environment Canada, NWRI, Burlington, Ontario, unpublished data).

Overall, there were few differences in whole-organism parameters of male, female or immature lake chub among the reference, TMP and CTMP sites. Adult lake chub downstream of the CTMP mill diffuser did exhibit increased condition relative to TMP and reference fish. A change in condition factor is a common response to pulp mill effluent (e.g. McMaster et al. 1991; Munkittrick et al. 1991a, Swanson et al. 1994). However, unless coupled with other changes in size-at-age, gonad size, fecundity and liver weight, it does not represent a dramatic change. A borderline increase in male liver size of TMP chub (relative to reference males) was also observed, but again, without concomitant changes in other characteristics of male lake chub, it was considered a minor concern. This was especially true when most fish exposed to pulp mill effluent exhibit an increase in liver size (Munkittrick et al. 1994). The meaning of a decrease in liver size is unclear.

Recent Adult Fish Survey's (EEM program) conducted by the TMP mill (Sentar Consultants Ltd. 1996a) and CTMP mill (Sentar Consultants Ltd. 1996b), focussed on lake chub and longnose sucker. Consistent with my findings, few differences were found in whole-organism measurements of either sentinel species between exposed and reference fish. They did find that condition of male and female lake chub caught below the TMP mill was moderately lower; although similar to reference values found in my work. As well, there was a decrease in age of male longnose sucker, and increase in liver size of female sucker downstream of the TMP mill. They also found that condition of exposed female lake chub and male longnose sucker was higher below the CTMP mill. However, there were few concomitant changes that would signify potential problems. The results of this work are particularly interesting because it confirms what I observed during my research, but with the luxury of higher sample sizes (n=25-40/sex/site) and statistical power. As discussed earlier, the high level of effluent dilution minimizes differences in effluent concentrations between reference and exposure sites.

There did not seem to be any negative effects (i.e. reduced production) of effluent exposure on *in vitro* production of testosterone or 17β-estradiol. Instead, there was no difference in testosterone production by lake chub from the TMP site, and increased production of testosterone by chub from the CTMP site. Follicle production of 17 β-estradiol was similar between exposed and reference female chub. The only other work evaluating steroid levels in fish exposed to the mills at Whitecourt, was the study by Brown et al. (1996) who sampled fish approximately 75 km upstream of Windfall Junction (near the Berland River) and about 90 km downstream of Whitecourt near Fort Assiniboine. As mentioned previously this study was affected by low sample sizes; however, preliminary results suggested there were little or no differences in plasma levels of testosterone or 17 β-estradiol in female burbot (*Lota lota*), or testosterone and 11-ketotestosterone in male burbot collected downstream of Whitecourt.

The response of exposed lake chub was described relative to reference chub from Windfall bridge site. Unfortunately, I was not able to test whether reference fish from Windfall Junction were affected by effluent originating from the upstream kraft mill in Hinton. Spoonhead sculpin were not found at the Windfall site, nor were lake chub found in the Hinton study area. The status of Windfall fish is uncertain; however, it is apparent that effluent from the TMP and CTMP mill did not further contribute to any large scale changes in the response of lake chub.

Reference Site Variability

Significant differences in whole-organism and physiological measurements between reference sites of the same river system were observed for spoonhead sculpin and lake chub. Lake chub collected from Windfall Junction and a second site R2, exhibited significant differences in male age and body/organ metrics of female chub. Although the distance between the two reference sites was approximately 18 km, there were no known outfalls, road crossings or other possible pollution sources within this span of river. Similarly, spoonhead sculpin collected from two reference sites located on opposite sides of the river (site RL, RR), showed differences in body size and organ metrics, as well as male EROD activity and *in vitro* testosterone production. The observed differences were especially surprising given the close proximity of the sites. As well, it appeared that the lateral mobility of spoonhead sculpin was not so great as to eliminate these site differences, supporting the assumption of limited mobility.

In each case, fish were collected from reference sites that were as similar as possible in habitat type. However, despite this effort, it is probable that sites were not identical in all characteristics, i.e. possible microhabitat differences. How this affects small fish species like lake chub and spoonhead sculpin is unknown. Assuming there were no other known sources of anthropogenic stressors, it seemed likely the observed differences in lake chub/spoonhead sculpin represented the natural variability in fish characteristics within the respective reference zone. These results emphasized the importance of recognizing the potential variability in small fish species among reference sites. If possible, fish from multiple sites within a reference zone should be sampled to acquire a representative description of reference fish. These reference sites should be as similar as possible to each other (and to the exposure sites, excluding effluent exposure) such that the variability in fish measurements represents natural variability in the reference zone, and not variability inflated by confounding factors.

Synopsis

The main focus of work on the Athabasca River was to determine whether changes in whole-organism and physiological measurements could be detected in small fish species exposed to pulp mill effluent. For the Hinton and Whitecourt study area, the representative small fish species that were studied did exhibit responses reflecting instream effluent conditions. Spoonhead sculpin exposed to kraft mill effluent showed significant changes in whole-organism and physiological parameters relative to unexposed fish. As well, a graded response was observed in sculpin exposed to different concentrations of effluent, e.g., at site KR and the downstream exposure sites. In contrast, lake chub exposed to TMP and CTMP effluent exhibited few changes relative to reference chub. Although, the absence of changes may have indicated that lake chub are not sensitive to effluent exposure, it seems more likely that their response (i.e., little change) reflected the low instream concentrations of non-kraft, totally chlorine free effluent in this area. This interpretation was further supported by Environmental Effects Monitoring data describing a similar response in longnose sucker (Sentar Consultants Ltd. 1996a,b) and laboratory exposures using rainbow trout (K.R. Munkittrick, Environment Canada, NWRI, Burlington, Ontario, unpublished data).

Further work was conducted to investigate whether it was possible to document the geographical extent of responses of small fish species. Unfortunately, it was not possible to determine if the response seen in spoonhead sculpin persisted as far downstream as Windfall Junction and the Whitecourt study area. The absence of sculpin at this site (or lake chub in the Hinton area) emphasized the limits of distribution of the selected small species. Additional work focussing on spoonhead sculpin within the Hinton study area, did indicate that the responses observed at the effluent plume site could be measured downstream for at least 50 km.

During the study on the Athabasca River, several other aspects related to using small fish species as sentinel species were learned:

- The abundance and distribution of the selected sentinel species within each study area facilitated sampling several sites within the reference and exposure zones; however, sampling could be limited by the availability of specific habitat conditions.
- Parameters commonly measured on larger fish species could also be measured on smaller species, including: body and organ metrics, reproductive parameters, age estimates, mixed function oxygenase activity, and *in vitro* steroid production.
- Based on the fall and spring studies, the lateral and longitudinal mobility of spoonhead sculpin seemed limited. These results provided some preliminary support for the assumption of stationary behaviour of some small fish species (at least for spoonhead sculpin), and greatly improved the probability that the observed responses in sculpin reflected the local environment.
- Prespawning sculpin collected following a period of high effluent exposure did not show responses dramatically different than responses observed during the previous fall. The exceptions included: increased hepatic EROD activity suggesting increased effluent exposure, and a disappearance of increased *in vitro* steroid production.
- Spoonhead sculpin and lake chub exhibited significant differences in whole-organism responses among populations within the respective reference zone. In the absence of some other stressor, the differences seemed to represent the natural variability in fish responses, perhaps associated with differences in habitat characteristics. These results emphasized: a)

the potential variation in fish among sites within the reference zone, and b) that care needs be taken to adequately describe the natural variation in the reference zone when making comparisons with fish exposed to effluent.

From initial work conducted on the Athabasca River, the potential to use small fish species appeared high for the purpose of monitoring downstream of pulp mill operations. However, it was also apparent that the response observed in spoonhead sculpin exposed to kraft mill effluent was not consistent with responses documented by other researchers focussing on larger fish species. Possible reasons for the discrepancies may have been related to the mobility of larger species and whether these species accurately reflected local conditions, the use of a separate river system as a reference site for large fish, or possible differences in habitat preferences and associated contaminant exposure among the sentinel species. Regardless, the discrepancies emphasized a need to investigate the responses of large *vs* small fish species in an effort to further evaluate the suitability of small fish species. Specifically, further work needs to be conducted to compare the responses between small and large fish species collected from the same sites, habitat types and exposure conditions. As well, to investigate the relative sensitivity of small and large species to pulp mill effluent, the potential confounding factor of mobility needs to be minimized by selecting a receiving environment with habitat or man-made barriers that restricts the movement of the larger (and smaller) fish species. Under these circumstances, a direct comparison could be made between the responses of small and large fish species to investigate the consistency and relative sensitivity of the responses to pulp mill effluent.

CHAPTER 3

COMPARISON BETWEEN RESPONSES OF SMALL AND LARGE FISH SPECIES EXPOSED TO PULP MILL EFFLUENT

3.1 Introduction

In the previous chapter, spoonhead sculpin exposed to kraft mill effluent in the Athabasca River exhibited alterations in whole-organism characteristics relative to reference sculpin. Studies by other researchers focussing on large species, such as longnose sucker and mountain whitefish, documented responses that were not consistent with the response observed with spoonhead sculpin (Golder Associates Ltd. 1996). There are several possible reasons why the responses were dissimilar, including different reference sites, different exposure conditions for each species, and greater potential mobility of the larger fish species. My study highlighted some advantages of monitoring small species; however, because of the confounding factors, a definitive conclusion regarding the relative response of small and large species was not possible.

Based on my research on the Athabasca River, I recognized that to further evaluate the suitability of small fish species as sentinels, I should focus specifically on comparing the responses of small and large fish species exposed to pulp mill effluent. I therefore conducted a study on the Moose River system in northern Ontario to investigate the consistency and relative sensitivity of fish species responses to effluent. Recent research on several rivers of the Moose River Basin has focussed on monitoring fish populations in the vicinity of pulp and paper mill and hydroelectric facilities (Ruemper and Portt 1994; K.R. Munkittrick, Environment Canada, NWRI, Burlington, Ontario, unpublished data). The river systems are dammed at several points along the water courses, and the presence of dams upstream and downstream of the pulp mill operations restrict large-scale movement of fish beyond the zones of effluent exposure. As well, fish movement is restricted by natural barriers associated with >300 sites in the watershed with potential for hydroelectric development. Due to the barriers to movement, it was possible for the researchers to use white sucker as a sentinel species without the potential risk associated with the mobility of many larger fish species. The ongoing research on the Moose River system with white sucker, along with the restrictions to fish movement imposed by the hydroelectric dams and natural barriers, provided a unique opportunity to directly compare the response of a small fish species to the response of the larger white sucker exposed to pulp mill effluent. As well, it was possible to ensure that the selected small fish species was collected from the same sites at the same time as white sucker to increase the likelihood that each species was exposed to similar concentrations of effluent, water quality, and habitat characteristics.

The research on white sucker also included using one of the river systems without pulp mill development as an alternate reference site for the pulp mill assessments. Two years into the research, a hydroelectric facility was built on the Groundhog River upstream of the fish collection site. Research at this site continued to investigate whether the dam had an effect on the downstream population of white sucker; however, its use as a reference site was questioned. From research I had previously conducted on the North Saskatchewan River with spoonhead sculpin (Chapter 2; Gibbons et al. 1996), there was evidence suggesting that a separate river system, and one with hydroelectric development, was not suitable for reference fish collections. To investigate this issue further on the Moose River system, and given that white sucker data were available, additional data on the selected small fish species were collected from the same "reference" river and site. Comparisons were made to provide information regarding the similarity of fish responses between the recently dammed river and the upstream reference sites on the pulp mill rivers (for each species), as well as the consistency of responses between the small sentinel species and white sucker.

The objectives of the study on the Moose River system were to a) collect field data to describe the response of a small fish species exposed to pulp mill effluent b) collect additional field data of the small sentinel species from the dammed "reference" river, c) describe the response of white sucker from the available raw data, d) determine whether the small fish species exhibited a response to effluent exposure, and whether it was consistent with the response of white sucker, and e) further evaluate the use of the separate river for collecting reference fish for monitoring purposes.

3.2 Materials and Methods

General Study Area and Mill Characteristics

The Moose River basin consists of five major river systems including the Missinaibi River, Kapuskasing River, Groundhog River, Mattagami River and Abitibi River. Each rivers flows northward and eventually converges into the Moose River proper which then flows into James Bay. The Abitibi River, Kapuskasing River and Mattagami River have all undergone development to some extent including both hydroelectric dams and pulp and paper mills. For the purpose of my research, the study areas were confined to the receiving waters of a thermomechanical/de-inked pulp mill on the Kapuskasing River at Kapuskasing, and a kraft mill on the Mattagami River at Smooth Rock Falls. These rivers were previously selected by researchers monitoring white sucker because of pulp mill development and both rivers have dams at the mill site that restrict fish movement. The Groundhog River was the alternate river selected to collect reference data on the selected sentinel fish species. The hydroelectric facility was built at Carmichael Falls approximately 15 km upstream from the fish collection site. The facility began operation in 1993. Comparisons were made between this river and the upstream reference sites on the Kapuskasing River and Mattagami River.

The mill at Kapuskasing has undergone several process changes over the years. Historically, the mill has been a sulphite plant, groundwood pulping facility, magnefite plant and a thermomechanical mill. As well, newsprint and tissue were produced on site. From 1982, operations consisted of the groundwood, magnefite and thermomechanical pulping facilities (B.A.R. Environmental Inc. and Gore and Storrie Ltd. 1993). In 1993, the mill streamlined operations and began producing approximately 820 tonnes of thermomechanical pulp (TMP) per day, 225 tonnes of deinked pulp from old newsprint per day and 1030 tonnes of newsprint per day (Rodden 1996). The primary wood source consists of 98% black spruce and 2% balsam fir (Robinson et al. 1994). The effluent is initially treated in a primary clarifier (since 1971) followed by secondary treatment in an activated sludge basin (since February 1995). The effluent is discharged at a rate of approximately) 0.38 $m^3 \cdot s^{-1}$ into the Kapuskasing River via the tailrace of the electricity turbine downstream of the dam (B.A.R. Environmental Inc. and Gore and Storrie Ltd. 1993).

The kraft mill at Smooth Rock Falls produces approximately 480 air dried metric tonnes of bleached and semi-bleached kraft pulp per day (Acres International Ltd. 1994). At the time of the study the primary wood source consisted of 65% black spruce and 35% jack pine softwood chips (Robinson et al. 1994). The bleaching sequence since 1992 was $ODE_{op}DE_pD$ (O, oxygen delignification; D, reaction with aqueous chlorine dioxide; E_{op}, extraction with sodium hydroxide and the addition of elemental oxygen and peroxide; E_p, extraction with peroxide), utilizing oxygen delignification and 100% chlorine dioxide substitution. Prior to 1992, elemental chlorine was used in the initial bleaching stage of pulp production (Acres International Ltd. 1994). Effluent is subjected to treatment in a mechanical primary clarifier (since 1976) followed by secondary treatment in an aeration stabilization basin (since October 1994) prior to discharge to the Mattagami River via an instream diffuser at a rate of approximately 0.8 $m^3 \cdot s^{-1}$ (Acres International Ltd. 1994).

White Sucker Collections

Data on white sucker from the Kapuskasing River and Mattagami River have been collected for several years from 1991 to 1996 (K.R. Munkittrick, Environment Canada, NWRI, Burlington, Ontario, unpublished data). Since data on the small fish species were collected during the fall 1995, only sucker data from this time period were used for comparisons. Data on white sucker were collected between September 17-30, 1995. Sampling sites were located upstream and downstream of pulp mill and hydroelectric dam operations at Kapuskasing and Smooth Rock Falls. At the Kapuskasing study area, reference fish were collected from a site (site KWF) on the Kapuskasing River upstream of Woman Falls in the vicinity of the public boat launch, approximately 48 km upstream from the town of Kapuskasing. Exposed fish were collected from a site approximately 0.5-1.0 km downstream of the dam facility and pulp mill diffuser (site KDS). At the Smooth Rock Falls study area, reference fish were collected upstream of Smooth Rock Falls on the Mattagami River between Highway #11 and the Canadian National Railway bridge (site MTUS). Sucker exposed to effluent from the mill in Smooth Rock Falls were collected from the Mattagami River in the vicinity of the public boat launch located approximately 0.2 km downstream of the mill diffuser (site MTDS). White sucker were also collected from the Groundhog River at a site immediately downstream of the Highway #11 bridge in the vicinity of the public boat launch (site GH).

Fish were collected using 8.9 cm and 10.2 cm stretch mesh gill nets. Each fish was rendered unconscious by concussion and fork length (± 0.1 cm), body weight (± 0.1 g), gonad weight (± 0.01 g) and liver weight (± 0.01 g) were recorded. Carcass weight was not directly measured, but was estimated by subtracting gonad weight plus liver weight from the total body weight. Fish were aged by counting annuli on cleaned, dried opercular bones. Approximately 1 g liver tissue was placed in a cryovial, frozen immediately in liquid nitrogen, and stored at -80°C pending mixed function oxygenase (MFO) analyses. Measurement of MFO activity was based on the catabolism of 7-ethoxyresorufin-o-deethylase (EROD) as described by van den Heuvel et al. (1995).

To evaluate possible reproductive impairment in exposed sucker, circulating plasma levels of sex hormones were measured. Blood was collected from live fish (prior to dissection) via caudal puncture into a 5.0 mL heparinized Vacuutainers® and placed on ice for 6 to 8 h. Plasma was collected after centrifugation at maximum speed on a IEC centrifuge (International Equipment Company, Needham Heights, MA) for 5 min., frozen in liquid nitrogen and stored at -20°C before analysis of sex steroids. Levels of testosterone and 17 β-estradiol in the blood plasma were measured by radioimmunoassay following ether extraction by procedures described by Van Der Kraak and Chang (1990) and Van Der Kraak et al. (1989), and further outlined in McMaster et al. (1992b). All plasma samples were assayed in duplicate, and interassay variability was <15% for each steroid.

Age data were provided by L. Ruemper (University of Waterloo, Waterloo, Ontario, unpublished data). The remaining data of body and organ measurements, EROD activity and plasma steroid levels were provided by K.R. Munkittrick (Environment Canada, NWRI, Burlington, Ontario, unpublished data).

Small Sentinel Species

Possible small sentinel fish species common to both the Kapuskasing River and Mattagami River systems included (Brousseau and Goodchild 1989; Ruemper and Portt 1994):

- longnose dace (*Rhinichthys cataractae*)
- golden shiner (*Notemigonus crysoleucas*)
- emerald shiner (*Notropis atherinoides*)
- spottail shiner (*Notropis hudsonius*)
- trout-perch (*Percopsis omiscomaycus*)
- johnny darter (*Etheostoma nigrum*)
- logperch (*Percina caprodes*)
- mottled sculpin (*Cottus bairdi*)

As in the Athabasca study, several habitat types (backwater, riffle, littoral zone, pools, runs, etc.) were sampled in an effort to collect any of the above listed species. Sampling occurred during both day and night (easily accessible sites) hours using a bag seine (18 m x 2.5 m, mesh size 5 mm) and backpack electrofisher (Smith-Root Type VII). Final selection of a sentinel species was evaluated based on abundance and capture efficiency.

The field collection of the small sentinel fish species was conducted during the fall low-flow period between October 13 - 20, 1995. Sampling sites were the same as previously described for white sucker. In Chapter 2, significant differences in whole organism and physiological measurements of both spoonhead sculpin and lake chub were found among sites sampled within the reference zones. As a result, an additional reference site on the Kapuskasing River and Mattagami River was included in the sampling design to further evaluate the potential variability in characteristics of small fish species within the reference zone.

Each adult sentinel fish was rendered unconscious by concussion and total length (± 0.1 cm), fork length (± 0.1 cm), body weight (± 0.01 g), carcass weight (i.e. eviscerated)(± 0.01 g), gonad weight (± 0.001 g) and liver weight (± 0.001 g) were recorded. Ageing structures were collected and analysed according to procedures described in Chapter 2. Similarly, fecundity was also estimated according to methods outlined in the previous chapter.

It was not possible to measure circulating levels of sex hormones in the small fish species due to an insufficient volume of blood. *In vitro* steroid production by male and female gonadal tissues was used as a surrogate measurement and was conducted according to methods outlined in Chapter 2. Triplicate samples of 20 egg follicles from females and 20 mg of testicular tissue from males were used for incubations. Additional incubations supplemented with 10 μM forskolin solubilized in ethanol were also conducted in triplicate. Forskolin activates adenylate cyclase, thereby mimicking GtH by bypassing the GtH receptor and increasing cyclic adenosine monophosphate (cAMP) production, leading to stimulation of steroid production. As such, the level of forskolin-stimulated steroid production provides further information regarding the integrity and maximal capacity of the steroid production pathway.

Induced hepatic mixed function oxygenase (MFO) activity was measured as a positive indicator of exposure to effluent following procedures described in Chapter 2. For this analysis, liver samples were assayed using the whole homogenate, omitting the centrifugation procedure, in an effort to ensure that all microsomes were present in the sample.

Data Analyses

Analyses of the small fish species data and white sucker data were identical. As in Chapter 2, the means and standard errors were calculated for all fish measurements for each sampling site. For presentation purposes, common indices describing relationships between body metrics were also calculated. These indices included:

Condition factor (k)=100(carcass weight/fork length^3)
Gonadosomatic Index (GSI)=100(gonad weight/carcass weight)
Liversomatic Index (LSI)=100(liver weight/carcass weight)

Carcass weight (i.e. eviscerated) or corrected weight (white sucker) was used in the above calculations because of possible differences in organ weight among sites. Using carcass/corrected weight instead of body weight eliminated possible confounding effects of altered organ weight (e.g. gonad weight, liver weight) on interpretation of variables related to body weight.

All parameters were regressions of one variable on another. In the case of liver weight, fecundity, egg size and gonad weight; carcass weight (or corrected weight, white sucker) was used as a covariate to adjust for any differences in size and placed these variables on a relative scale. The basic design for the analysis of fish data was an Analysis of Covariance (ANCOVA) with site as a factor. An assumption of the ANCOVA model is that the slopes of the regression lines are equal among sites. Therefore, differences in slopes were tested prior to conducting the ANCOVA. Generally, ANCOVA is fairly robust even when slopes are not equal, so slopes were considered different when $p < 0.01$ (Hamilton et al. 1993). Analysis of variance (ANOVA) was used to compare body size (body weight, length and carcass/corrected weight) and egg size (egg weight and diameter) estimates among sites. All data were $\log_{10}$ transformed and sexes were analyzed separately. Nonparametric Kruskal-Wallis tests were used to compare MFO activity and *in vitro* and plasma steroid levels between reference and exposed fish. All data analyses were done using SYSTAT statistical software (Wilkinson, 1990).

3.3 Results

3.3.1 Small Sentinel Species Collection

Of the possible sentinel species captured in both river systems, trout-perch (*Percopsis omiscomaycus*) was the most abundant and widely distributed species (Table 3.1). Greater numbers of adult trout-perch were captured during the evening hours after sunset (2000-0100 h) using the small bag seine. Although a variety of habitats were sampled, trout-perch were most often found in slow moving water and eddies consisting of fines (silt and clay) and sand substrates.

Trout-perch are widely distributed in lakes and streams from Alaska and northeastern British Columbia to Quebec, and from Kansas to West Virginia (Scott and Crossman 1973; Nelson and Paetz 1992). Greater numbers of trout-perch were captured at night when they appeared to move from deeper waters to the shallow littoral zones to feed on aquatic insects and crustaceans (Nelson and Paetz 1992). Maximum fork length is approximately 15 cm, although most mature adults from the Moose River Basin were 6-9 cm in fork length. Life span of trout-perch is 4 y with sexual maturity as young as 1 y. Spawning in river populations appears to occur in early spring (May), although there is little detailed information describing spawning activity of trout-perch in river systems.

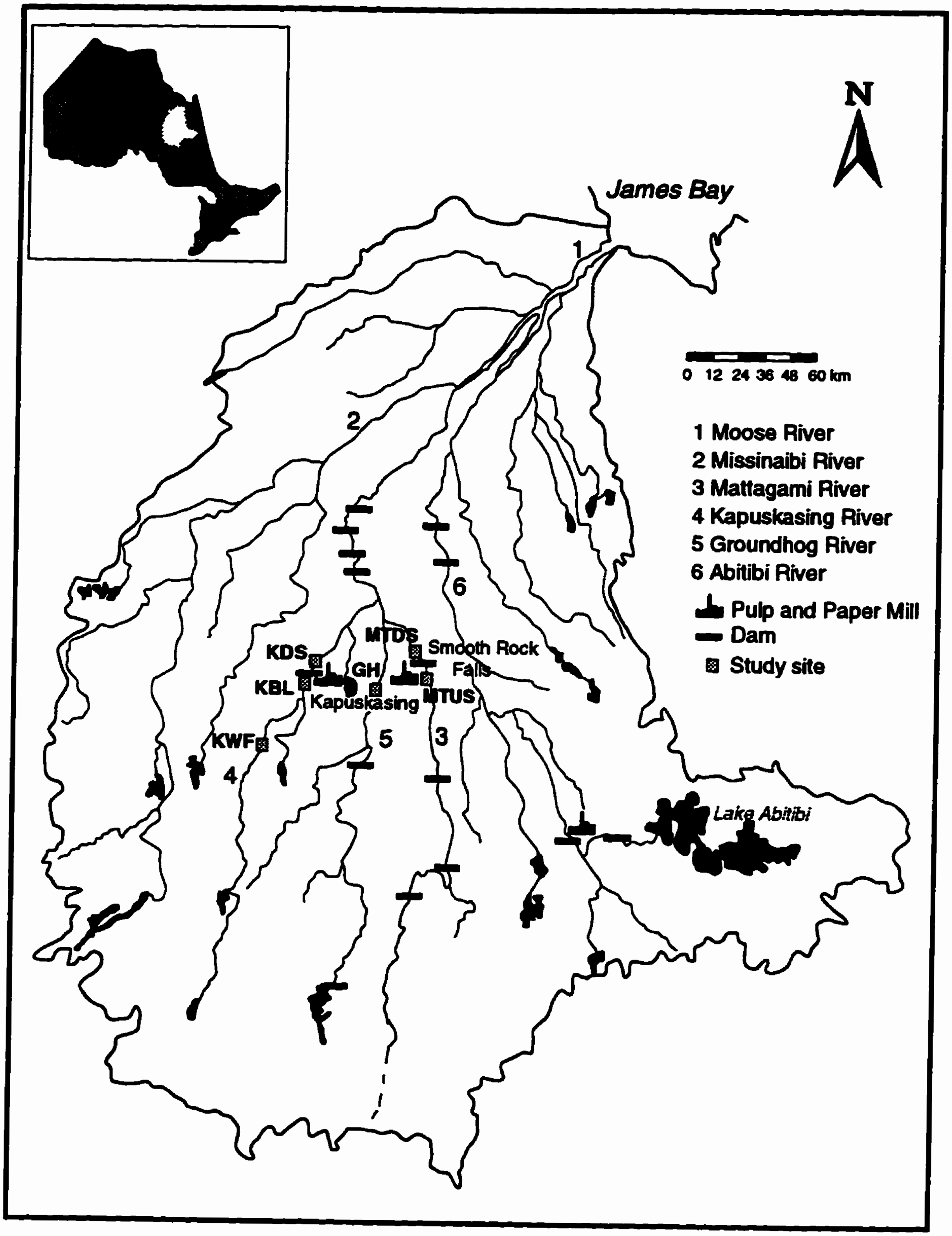
Trout-perch were collected from a total of six sites on the Moose River system (Figure 3.1). At the Kapuskasing study site, trout-perch were collected from the same sites white sucker were collected. As well, reference fish were collected from an additional site (site KBL) located at the public boat launch located immediately upstream of the hydroelectric dam operation in Kapuskasing. Trout-perch were also collected from the Groundhog River at the same site used by Ruemper and Portt (1994) and K.R Munkittrick (Environment Canada, NWRI, Burlington, Ontario, personal communication) to collect white sucker. At the Smooth Rock Falls study area, exposed fish were collected at the public boat launch on the Mattagami River approximately 0.2

Table 3.1. Number of each potential sentinel fish species caught at the Kapuskasing and Smooth Rock Falls study areas during the fall 1995 field study, Moose River system, Ontario.

Species	Kapuskasing River			Mattagami River		Groundhog River
	KWF	KBL	KDS	MTUS	MTDS	GH
longnose dace	1		2			
golden shiner						
emerald shiner	2			2		
spottail shiner	≈190*		50*	≈80*	≈150*	15
trout-perch	39	77	77	14*	86	74
johnny darter	15		1	1	10	11
logperch	10		4			
sculpin sp.	9		1	6		11

* mostly young of the year or juveniles

Figure 3.1. Location of collection sites for trout-perch (*Percopsis omiscomaycus*) and white sucker (*Catostomus commersoni*) on the Kapuskasing River (sites KWF, KBL- trout-perch only, KDS), Groundhog River (site GH) and Mattagami River (sites MTUS, MTDS) of the Moose River system, Ontario, 1995.


N
James Bay
0 12 24 36 48 60 km
1 Moose River
2 Missinaibi River
3 Mattagami River
4 Kapuskasing River
5 Groundhog River
6 Abitibi River
Pulp and Paper Mill
Dam
Study site
1
2
3
4
5
6
MTDS
KDS
KBL
GH
Smooth Rock
Falls
Kapuskasing
MTUS
KWF
Lake Abitibi

km downstream of the mill outfall. Unfortunately, trout-perch (or any other potential small sentinel species) could not be found in sufficient numbers upstream of the mill in Smooth Rock Falls (Table 3.1). Attempts to collect trout-perch at upstream sites on the Mattagami River were limited because of poor access along this stretch of the river, especially during the preferred night sampling hours. As such, plans to evaluate the response of a small fish species to bleached kraft effluent from the Smooth Rock Falls mill were abandoned. The exact location (longitude/latitude coordinates), and distance downstream of the respective mill sites, of each sampling site is presented in Table 3.2. Specific habitat characteristics of the Kapuskasing, Groundhog and Mattagami River systems have been described in Ruemper and Portt (1994).

3.3.2 Site Comparisons

Variations in age, body size and organ metrics for trout-perch and white sucker at each study site on the Kapuskasing River and Groundhog River are presented in Table 3.3 and Table 3.4, respectively. Data for exposed trout-perch collected downstream of the Smooth Rock Falls mill on the Mattagami River have been provided in Appendix B. Independent verification of age estimates on a subsample of 42 fish (12% of total number aged) indicated an error rate (i.e. precision) of 5%. For mature female trout-perch, estimates of fecundity and egg size were not possible. A large proportion of eggs were used for measuring *in vitro* steroid production and many of the remaining preserved egg samples broke easily when handled during counting and weighing. When analysing ovary weight in trout-perch, it became evident that fecund females could be divided into at least two groups: those which were 1 or 2 y old and probably spawning for the first time, and those which were larger, mature females greater than 2 y old. The gonadosomatic indices for these two groups were not similar (first time spawners were less) such that if the first time spawners were included in the analyses, the ovary weight relationships were strongly influenced by the number of these females collected at a particular site. For the purpose of this study, I was interested in comparing the ovary size of adult females with fully mature gonadal development. For this reason, only older (>2 y) and mature (GSI>2%) females were included in the analyses of ovary weight.

Table 3.2. Location and general description of each site sampled during studies conducted during the fall 1995 on the Moose River system, Ontario.

Site	Location (latitude / longitude)	General Description	River Distance from Diffuser(s)
Kapuskasing River			
KWF	N 49°08.00'/ W 82°45.30'	Reference site, Woman Falls	-48.0 km
KBL	N 49°15.40'/ W 82°33.00'	Reference site, boat launch	-0.5 km
KDS	N 49°15.42/ W 82°30.50'	TMP mill exposure site	0.5 km
Mattagami River			
MTUS	na	Reference site	-2.0 km
MTDS	N 49°17.33'/ W 81°38.23'	Kraft mill exposure site	0.20 km
Groundhog River			
GH	N 49°18.82'/ W 82°02.50'	Alternate reference site	na

na, not applicable

Table 3.3. Mean and SE (n) of age, body size and organ metrics of trout-perch (*Percopsis omiscomaycus*) collected from the Kapuskasing River and Groundhog River, fall 1995, Moose River system, Ontario. Statistical results for variables analysed using ANOVA are presented. Within a row, a difference ($p<0.05$) between reference sites KWF and KBL is denoted by different alphabetical superscripts. A difference ($p<0.05$) between sites KWF and GH is shown by different numerical superscripts. A difference ($p<0.05$) between sites KWF and KDS is identified with an asterisk (*).

Sex	Reference		Effluent Plume	Groundhog River
	KWF	KBL	KDS	GH
Male				
Fork Length (cm)	7.2 ± 0.2 (13) A,1	7.0 ± 0.2 (30) A	6.2 ± 0.1 (35) *	5.9 ± 0.2 (27) 2
Body Weight (g)	4.51 ± 0.41 (13) A,1	4.15 ± 0.29 (30) A	2.65 ± 0.07 (35) *	2.58 ± 0.30 (27) 2
Carcass Weight (g)	3.96 ± 0.35(13) A,1	3.81 ± 0.27 (27) A	2.34 ± 0.07 (35) *	2.46 ± 0.28 (25) 2
K	1.03 ± 0.01 (13)	1.0 ± 0.01 (27)	0.98 ± 0.01 (35)	1.06 ± 0.02 (25)
Age (y)	3.6 ± 0.2 (13) A,1	3.4 ± 0.2 (30) A	3.0 ± 0.1 (35) *	2.8 ± 0.2 (27) 2
GSI (%)	3.03 ± 0.15 (13)	3.11 ± 0.09 (26)	2.78 ± 0.10 (34)	2.41 ± 0.17 (25)
LSI (%)	1.69 ± 0.07 (13)	1.60 ± 0.05 (27)	1.71 ± 0.05 (35)	1.83 ± 0.05 (25)
Female				
Fork Length (cm)	6.8 ± 0.3 (12) A,1	7.1 ± 0.2 (24) A	6.3 ± 0.1 (35) *	5.7 ± 0.2 (34) 2
Body Weight (g)	3.69 ± 0.39 (12) A,1	4.35 ± 0.33 (24) A	2.72 ± 0.08 (35) *	2.64 ± 0.32 (26) 2
Carcass Weight (g)	3.25 ± 0.33 (12) A,1	3.80 ± 0.29 (23) A	2.33 ± 0.07 (33) *	2.37 ± 0.28 (26) 2
K	0.98 ± 0.02 (12)	0.97 ± 0.02 (23)	0.95 ± 0.02 (33)	1.0 ± 0.01 (26)
Age (y)	3.4 ± 0.2 (12) A,1	3.5 ± 0.2 (24) A	2.9 ± 0.1 (35) *	2.6 ± 0.2 (34) 2
GSI (%)	5.37 ± 0.24 (10)	5.25 ± 0.14 (19)	4.41 ± 0.11 (27)	4.77 ± 0.34 (12)
LSI (%)	2.29 ± 0.09 (12)	2.23 ± 0.07 (23)	2.30 ± 0.06 (33)	2.52 ± 0.09 (26)

Table 3.4. Mean and SE (n) of age, body size and organ metrics of white sucker (*Catostomus commersoni*) collected from the Kapuskasing River and Groundhog River, fall 1995, Moose River system, Ontario. Statistical results for variables analysed using ANOVA are presented. Within a row, a difference ($p<0.05$) between sites KWF and GH is shown by different numerical superscripts. A difference ($p<0.05$) between sites KWF and KDS is identified with an asterisk (*).

Sex	Reference	Effluent Plume	Groundhog River
	KWF	KDS	GH
Male			
Fork Length (cm)	35.9 ± 0.8 (18) 1	40.2 ± 0.4 (21) *	37.4 ± 0.7 (21) 1
Body Weight (g)	653 ± 46 (18) 1	970 ± 27 (21) *	769 ± 40 (21) 2
Corrected Weight (g)	605 ± 43 (18) 1	884 ± 24 (21) *	711 ± 38 (21) 2
K	1.27 ± 0.02 (18)	1.36 ± 0.02 (21)	1.34 ± 0.02 (21)
Age (y)	8.2 ± 0.5 (17) 1	11.0 ± 0.7 (21) *	8.9 ± 0.8 (21) 1
GSI (%)	6.82 ± 0.36 (18)	8.69 ± 0.40 (20)	7.10 ± 0.36 (21)
LSI (%)	0.95 ± 0.05 (18)	1.45 ± 0.05 (21)	1.11 ± 0.04 (21)
Female			
Fork Length (cm)	39.2 ± 0.6 (25) 1	42.3 ± 0.4 (33) *	39.5 ± 0.5 (39) 1
Body Weight (g)	874 ± 44 (25) 1	1127 ± 34 (33) *	915 ± 39 (39) 1
Corrected Weight (g)	818 ± 41 (25) 1	1069 ± 30 (33) *	864 ± 37 (39) *
K	1.33 ± 0.02 (25) 1	1.40 ± 0.1 (33)	1.38 ± 0.02 (39)
Age (y)	8.6 ± 0.4 (25) 1	10.0 ± 0.6 (25)	8.3 ± 0.4 (31) 1
GSI (%)	5.33 ± 0.16 (25)	5.23 ± 0.15 (25)	5.58 ± 0.15 (32)
LSI (%)	1.41 ± 0.08 (24)	1.66 ± 0.04 (25)	1.66 ± 0.04 (32)

Kapuskasing Reference Sites

Comparisons between trout-perch from the two reference sites (KWF, KBL) on the Kapuskasing River indicated that fish from these sites were very similar, although a few differences were found. With the exception of reduced male condition at site KBL, there were no other differences in whole organism characteristics of trout-perch between the two reference sites (Table 3.3, 3.5). However, hepatic EROD activity was higher in male and female trout-perch collected from site KBL (Figure 3.2). There were no differences in male or female *in vitro* production (basal and forskolin) of testosterone between sites, although basal production of 17 β-estradiol was higher at site KBL (Figure 3.3, 3.4, 3.5).

Despite the similarity in trout-perch from the two reference sites, and contrary to the strategy in Chapter 2, data from these sites were not pooled when conducting comparisons between reference and exposed fish, or fish from the Groundhog River. Because a major objective of the study was to compare the responses between exposed trout-perch and white sucker, I did not want to introduce any potential source of variability that may have confounded the comparison. White sucker were not collected from site KBL; therefore, I decided that only data from sites common to both species should be included in the analyses. For this reason, only reference data from site KWF were used for the reference/exposure comparisons.

Reference vs Exposure Comparison

Both trout-perch and white sucker exposed to TMP effluent from the Kapuskasing mill (site KDS) exhibited significant differences in fish characteristics relative to the corresponding reference populations. There were few similarities between the responses of exposed trout-perch and sucker. A greater change was observed in white sucker, and many characteristics were often in opposition to the response of trout-perch.

Trout-perch at the effluent plume site were shorter, lighter and younger than fish from the reference site (Table 3.3). Conversely, white sucker were longer, heavier and, for male sucker,

Table 3.5. Site comparisons (ANCOVA) of size-at-age, condition, gonad weight and liver weight for trout-perch, fall 1995, Kapuskasing River and Groundhog River, Moose River system, Ontario. Abbreviations: I - intercept; S - slope; p - pooled over sites. Subscripts refer to sites. Interaction terms were considered significant at $p<0.01$.

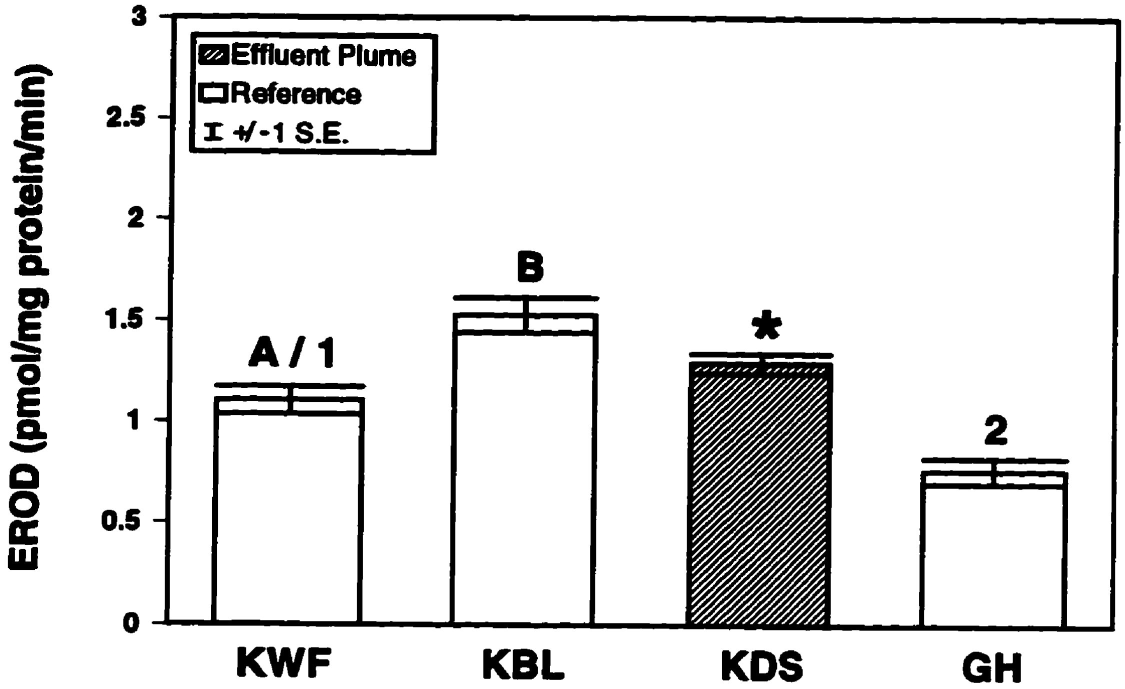
Parameter	Sex	Probability value (p) Slope	Intercept	Log_{10} Mean Estimate	
Site KWF *vs* Site KBL (reference sites)					
Size-at-age	male	0.92	0.93	S_p=0.44,	I_p=0.61
	female	0.38	0.15	S_p=0.47,	I_p=0.59
Condition	male	0.54	0.04	I_{kwf}=0.57,	I_{kbl}=0.55
	female	0.99	0.90	S_p=2.87,	I_p=-1.91
Gonad vs. carcass wt.	male	0.79	0.49	S_p=1.087,	I_p=-1.566
	female	0.79	0.15	S_p=1.274,	I_p=-1.441
Liver vs. carcass wt.	male	0.10	0.34	S_p=1.001,	I_p=-1.790
	female	0.86	0.34	S_p=1.119,	I_p=-1.714
Site KWF *vs* Site KDS (effluent plume)					
Size-at-age	male	0.003	-	S_{kwf}=0.45,	S_{kds}=0.21
	female	<0.001	-	S_{kwf}=0.53,	S_{kds}=0.23
Condition	male	0.81	0.10	S_p=3.21,	I_p=-2.17
	female	0.29	0.29	S_p=3.04,	I_p=-2.05
Gonad vs carcass wt.	male	0.84	0.88	S_p=1.161,	I_p=-1.622
	female	0.58	0.16	S_p=1.406,	I_p=-1.510
Liver vs. carcass wt.	male	0.20	0.52	S_p=1.038,	I_p=-1.791
	female	0.17	0.25	S_p=1.158,	I_p=-1.705

Table 3.5. Continued.

Parameter	Sex	Probability value (p) Slope	Intercept	Log_{10} Mean Estimate	
Site KWF *vs* Site GH (alternate reference)					
Size-at-age	male	0.40	0.03	I_{kwf}=0.81,	I_{gh}=0.78
	female	0.83	0.45	S_p=0.56,	I_p=0.53
Condition	male	0.52	0.97	S_p=2.91,	I_p=-1.91
	female	0.99	0.84	S_p=2.87,	I_p=-1.90
Gonad vs carcass wt.	male	0.23	0.84	S_p=1.389,	I_p=-1.756
	female	0.53	0.12	S_p=1.474,	I_p=-1.557
Liver vs carcass wt	male	0.10	0.11	S_p=0.978,	I_p=-1.744
	female	0.49	0.005	I_{kwf}=-1.303,	I_{gh}=-1.233

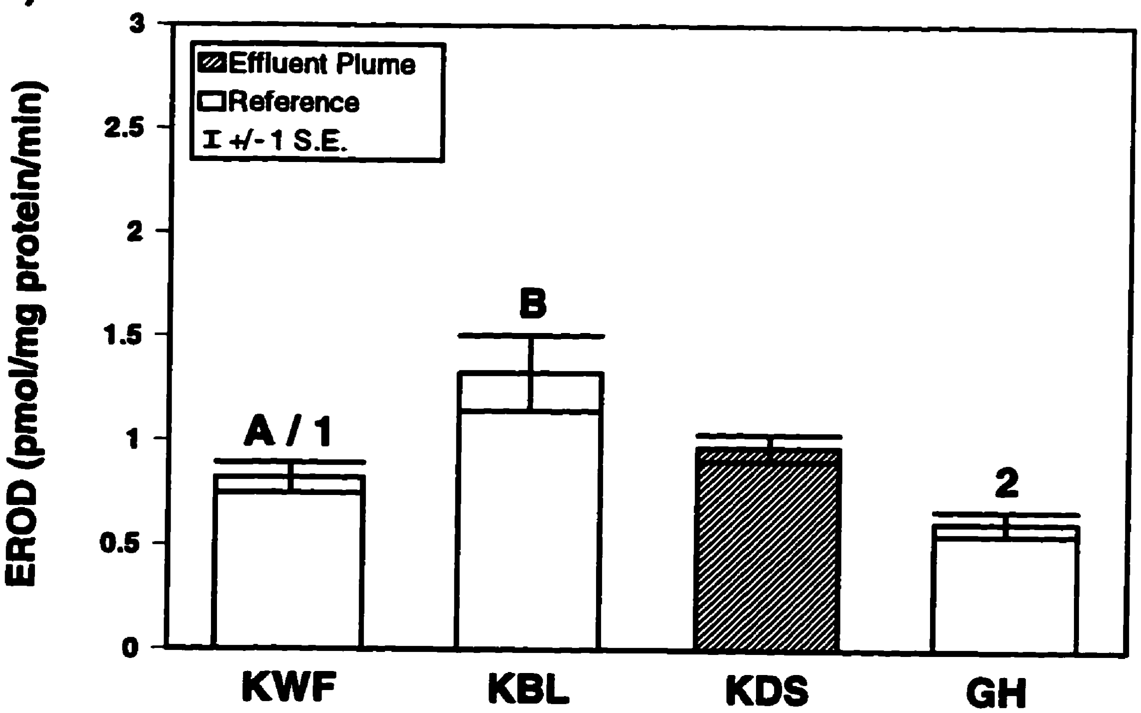
Figure 3.2. EROD activity in a) male and b) female trout-perch (*Percopsis omiscomaycus*) from reference and exposure sites on the Kapuskasing River and site GH on the Groundhog River, fall 1995, Moose River system, Ontario. Values represent the mean ● SE. A difference between reference sites KWF and KBL is denoted by different alphabetical superscripts. A difference between sites KWF and KDS is identified with an asterisk (*) above the KDS bar. A difference between reference sites KWF and GH is shown by different numerical superscripts. Differences were considered significant at $p<0.05$.

a)



Effluent Plume
Reference
+/-1 S.E.
EROD (pmol/mg protein/min)
3
2.5
2
1.5
1
0.5
0
A / 1
B
*
2
KWF
KBL
KDS
GH

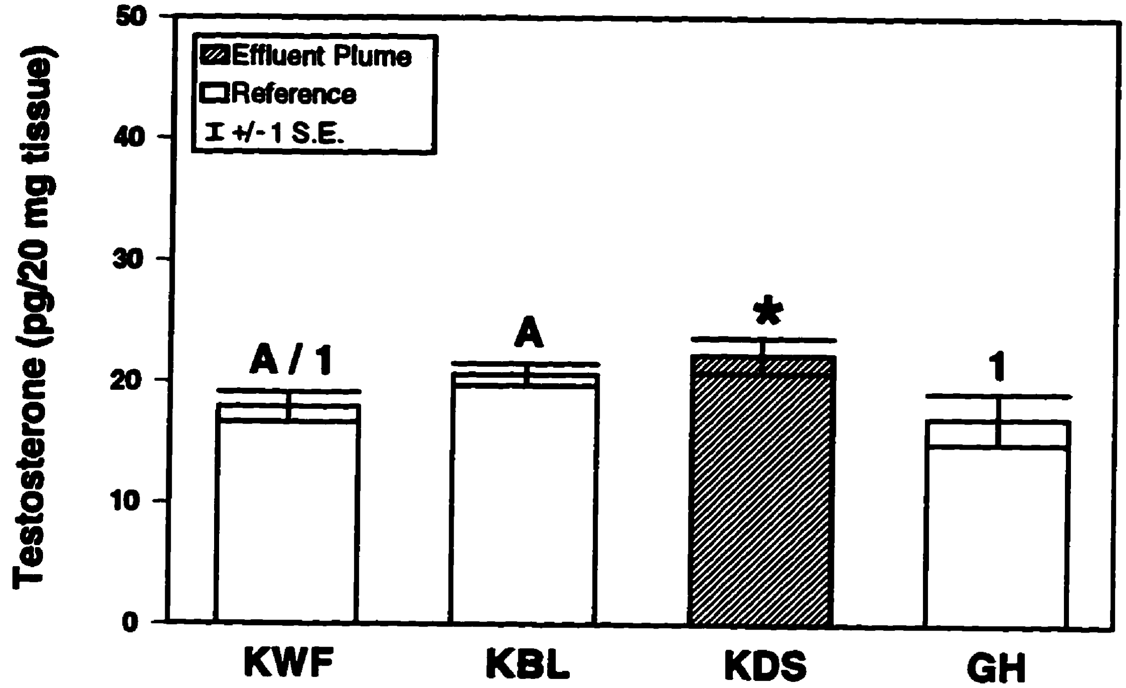
b)



Effluent Plume
Reference
+/-1 S.E.
EROD (pmol/mg protein/min)
3
2.5
2
1.5
1
0.5
0
A / 1
B
2
KWF
KBL
KDS
GH

Figure 3.3. a) Basal and b) forskolin-stimulated *in vitro* production of testosterone by testicular tissue of male trout-perch (*Percopsis omiscomaycus*) from reference and exposure sites on the Kapuskasing River and site GH on the Groundhog River, fall 1995, Moose River system, Ontario. Values represent the mean ± SE. A difference between reference sites KWF and KBL is denoted by different alphabetical superscripts. A difference between sites KWF and KDS is identified with an asterisk (*) above the KDS bar. A difference between reference sites KWF and GH is shown by different numerical superscripts. Differences were considered significant at $p<0.05$.

a)


Effluent Plume
Reference
+/- 1 S.E.
Testosterone (pg/20 mg tissue)
50
40
30
20
10
0
A / 1
A
*
1
KWF
KBL
KDS
GH

b)

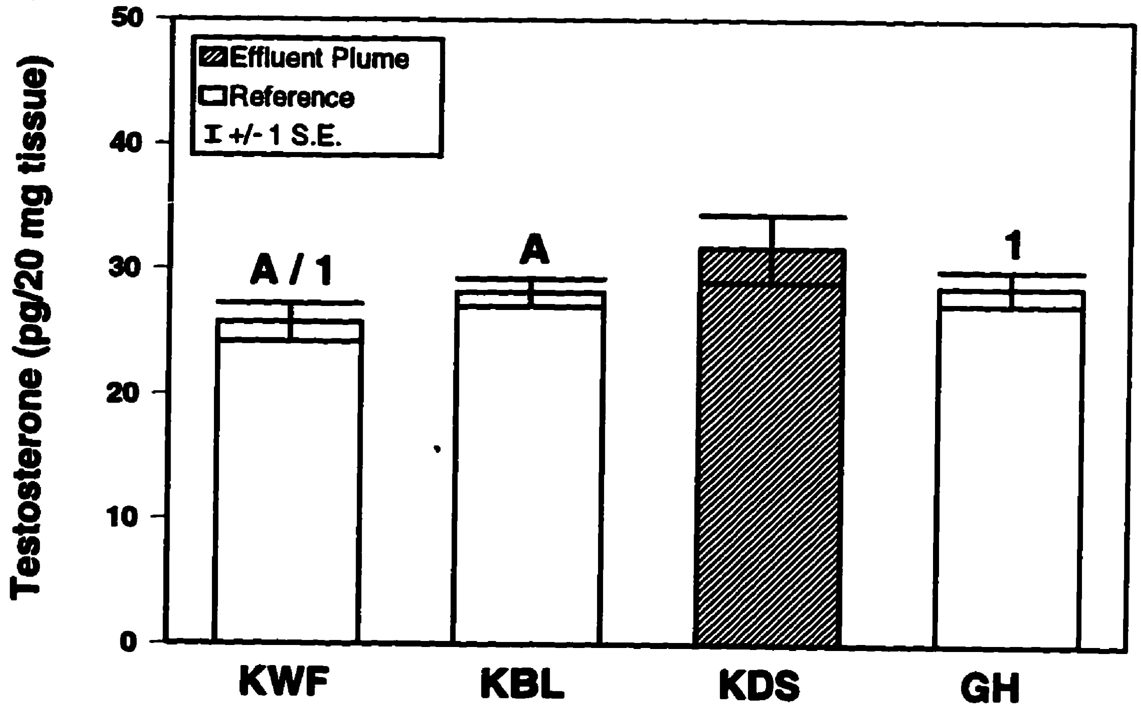

Effluent Plume
Reference
+/- 1 S.E.
Testosterone (pg/20 mg tissue)
50
40
30
20
10
0
A / 1
A
1
KWF
KBL
KDS
GH

Figure 3.4. a) Basal and b) forskolin-stimulated *in vitro* production of testosterone by follicles of female trout-perch (*Percopsis omiscomaycus*) from reference and exposure sites on the Kapuskasing River and site GH on the Groundhog River, fall 1995, Moose River system, Ontario. Values represent the mean ± SE. A difference between reference sites KWF and KBL is denoted by different alphabetical superscripts. A difference between sites KWF and KDS is identified with an asterisk (*) above the KDS bar. A difference between reference sites KWF and GH is shown by different numerical superscripts. Differences were considered significant at $p<0.05$.

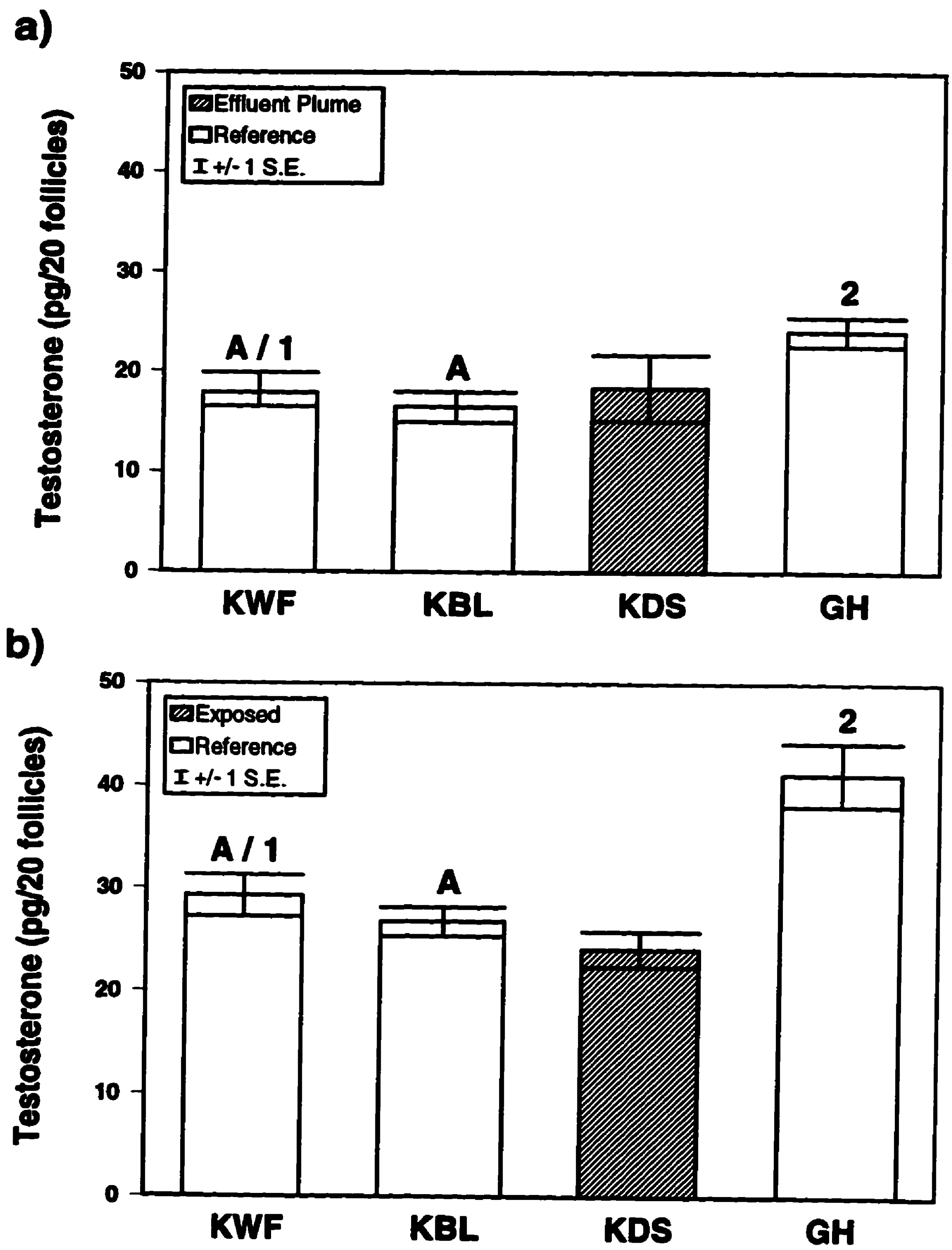
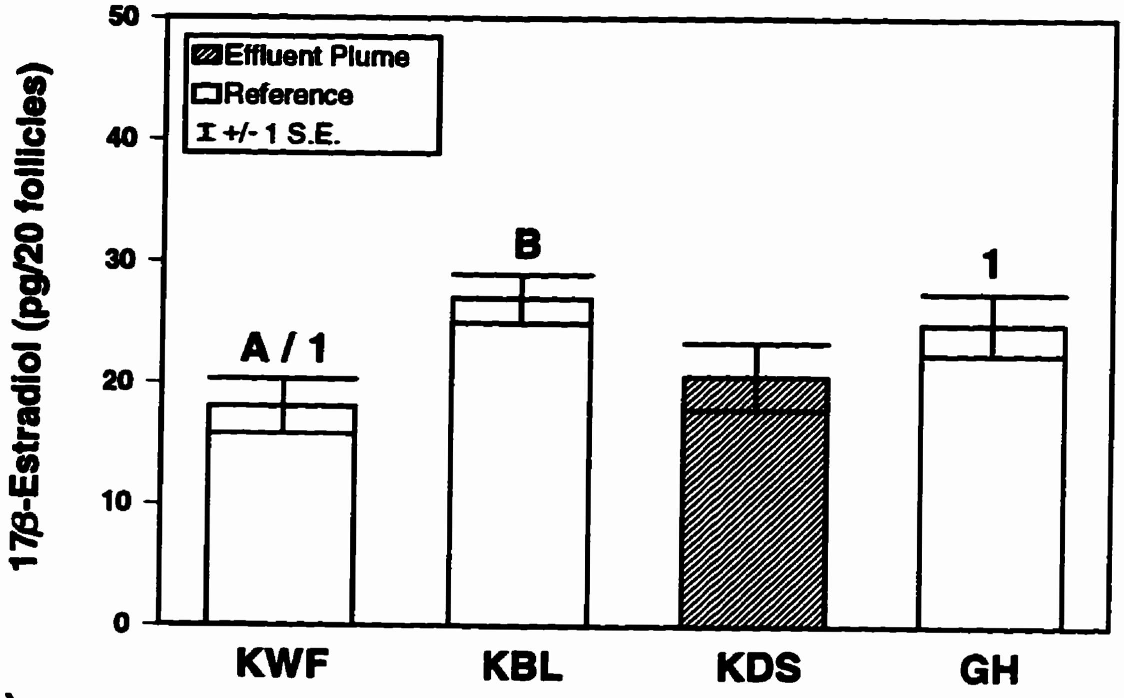

a)
Effluent Plume
Reference
+/- 1 S.E.
Testosterone (pg/20 follicles)
0
10
20
30
40
50
A / 1
A
2
KWF
KBL
KDS
GH
b)
Exposed
Reference
+/- 1 S.E.
Testosterone (pg/20 follicles)
0
10
20
30
40
50
A / 1
A
2
KWF
KBL
KDS
GH

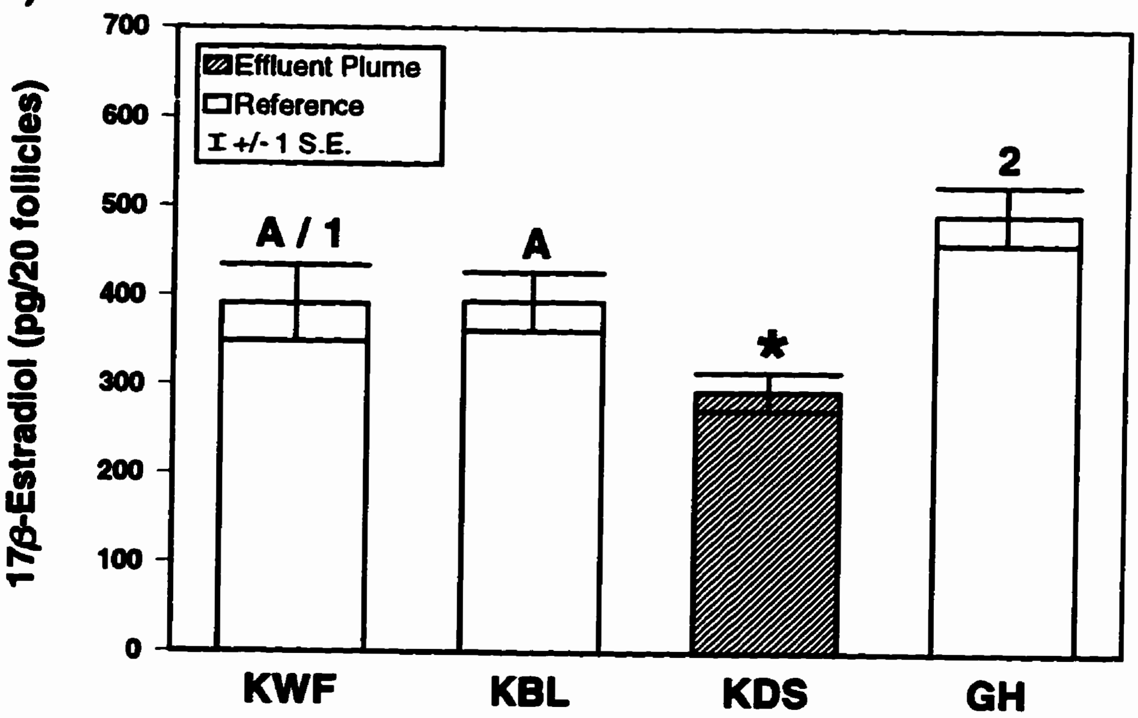
Figure 3.5. a) Basal and b) forskolin-stimulated *in vitro* production of 17 β-estradiol by follicles of female trout-perch (*Percopsis omiscomaycus*) from reference and exposure sites on the Kapuskasing River and site GH on the Groundhog River, fall 1995, Moose River system, Ontario. Values represent the mean ± SE. A difference between reference sites KWF and KBL is denoted by different alphabetical superscripts. A difference between sites KWF and KDS is identified with an asterisk (*) above the KDS bar. A difference between reference sites KWF and GH is shown by different numerical superscripts. Differences were considered significant at $p<0.05$.

a)



Effluent Plume
Reference
+/- 1 S.E.
17β-Estradiol (pg/20 follicles)
50
40
30
20
10
0
A / 1
B
1
KWF
KBL
KDS
GH

b)



Effluent Plume
Reference
+/- 1 S.E.
17β-Estradiol (pg/20 follicles)
700
600
500
400
300
200
100
0
A / 1
A
*
2
KWF
KBL
KDS
GH

older than reference fish (Table 3.4). Condition of sucker was also greater at the effluent plume site; however, there was no change in the condition of exposed trout-perch relative to reference fish. (Table 3.5, 3.6). For male and female trout-perch, the slopes of the regression lines for size-at-age at the reference site KWF were higher than at the effluent plume site (Table 3.4). In both cases, the length of trout-perch was greater at the reference site at any given age after the fish reached an approximate age of 2-3 y old (Figure 3.6, 3.7). Size-at-age of male and female white sucker was greater at site KDS (Table 3.6). There were no differences in gonad size in male and female trout-perch and male sucker between sites KWF and KDS, but sucker ovary size was smaller at site KDS. As well, liver weights of trout-perch were similar between the two sites, whereas liver weights for white sucker were heavier at the effluent plume site.

Only male trout-perch collected downstream of the pulp mill outfall had significantly induced hepatic EROD activity. Activity in female trout-perch and male and female white sucker from site KDS were similar to reference fish (Figure 3.2, 3.8). Similarly, there was an increase in basal *in vitro* production of testosterone by testicular tissue from exposed trout-perch (Figure 3.3). There was no difference in *in vitro* production of testosterone by follicles of female trout perch (Figure 3.4), nor circulating plasma level of testosterone in white sucker (Figure 3.9). Basal *in vitro* production of 17 β-estradiol by follicles from exposed female trout-perch was similar to reference production. Forskolin-stimulated production of 17 β-estradiol was reduced at site KDS, and plasma levels of 17 β-estradiol were depressed in exposed female white sucker (Figure 3.5, 3.10).

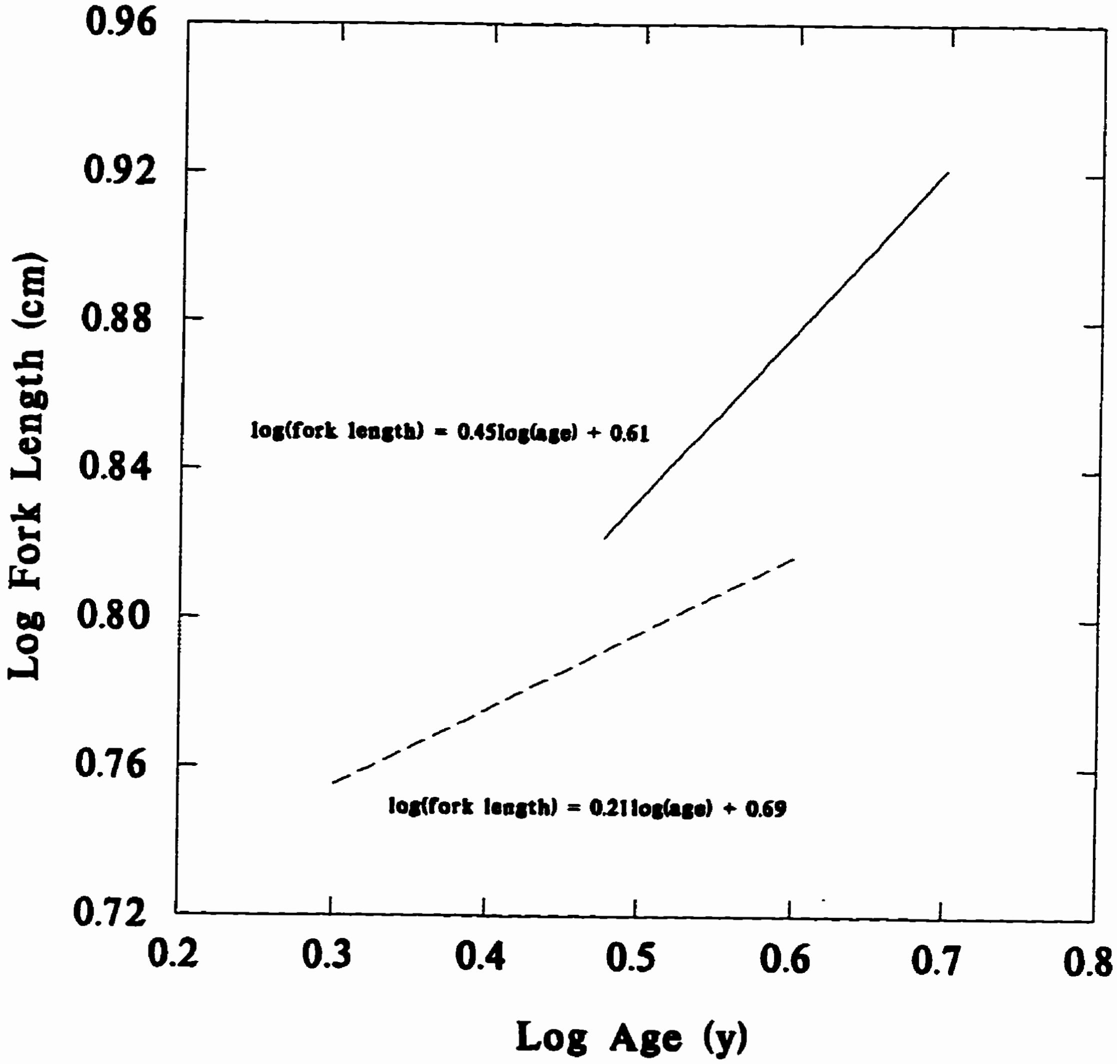
Groundhog River

Originally, the relative response of trout-perch and white sucker collected from the Groundhog River was to be assessed using the upstream reference sites on the Kapuskasing River and Mattagami River. Due to the poor capture success of trout-perch upstream of the Smooth Rock Falls mill, the comparison was only possible with the Kapuskasing site. Overall, there were significant differences observed in the responses of both trout-perch and white sucker between

Table 3.6. Site comparisons (ANCOVA) of size-at-age, condition, gonad weight and liver weight for white sucker, fall 1995, Kapuskasing River and Groundhog River, Moose River system, Ontario. Abbreviations: I - intercept; S - slope; p - pooled over sites. Subscripts refer to sites. Interaction terms were considered significant at $p<0.01$.

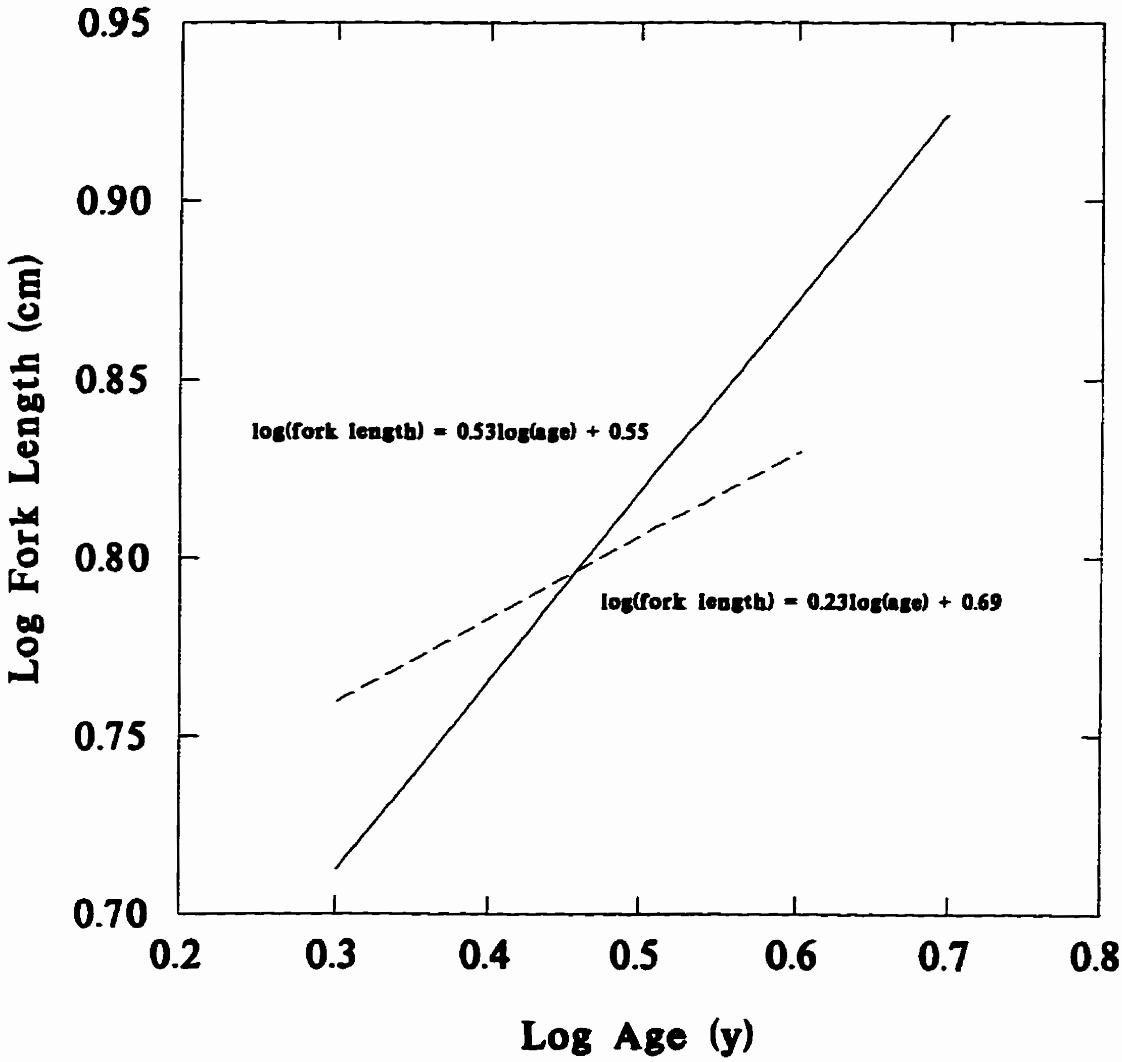
Parameter	Sex	Probability value (p) Slope	Probability value (p) Intercept	Log_{10} Mean Estimate	
Site KWF (reference site) *vs* Site KDS (effluent plume)					
Size-at-age	male	0.04	<0.001	I_{kwf}=1.56,	I_{kds}=1.60
	female	0.02	<0.001	I_{kwf}=1.60,	I_{kds}=1.62
Condition	male	0.02	0.03	I_{kwf}=2.85,	I_{kds}=2.87
	female	0.05	0.001	I_{kwf}=2.96,	I_{kds}=2.98
Gonad vs. carcass wt.	male	0.48	0.07	S_p=1.37,	I_p=-2.19
	female	0.58	0.02	I_{kwf}=1.71,	I_{kds}=1.66
Liver vs. carcass wt.	male	0.94	<0.001	I_{kwf}=0.82,	I_{kds}=1.02
	female	0.23	<0.001	I_{kwf}=1.09,	I_{kds}=1.18
Site KWF *vs* Site GH (alternate reference)					
Size-at-age	male	0.97	0.03	I_{kwf}=1.55,	I_{gh}=1.57
	female	0.21	0.67	S_p=0.21,	I_p=1.41
Condition	male	0.07	0.01	I_{kwf}=2.79,	I_{gh}=2.82
	female	0.67	0.05	I_{kwf}=2.90,	I_{gh}=2.92
Gonad vs carcass wt.	male	0.29	0.33	S_p=1.15,	I_p=-1.58
	female	0.99	0.37	S_p=1.28,	I_p=-2.08
Liver vs. carcass wt.	male	0.61	0.01	I_{kwf}=0.77,	I_{gh}=0.85
	female	0.92	<0.001	I_{kwf}=1.03,	I_{gh}=1.13

Figure 3.6. Regression lines of $\log_{10}$ total length *vs* $\log_{10}$ age (size-at-age) for male trout-perch (*Percopsis omiscomaycus*) from the reference site KWF (solid line) and effluent plume site KDS (dashed line) on the Kapuskasing River, fall 1995, Moose River system, Ontario.



0.96
0.92
0.88
0.84
0.80
0.76
0.72
Log Fork Length (cm)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Log Age (y)
log(fork length) = 0.45log(age) + 0.61
log(fork length) = 0.21log(age) + 0.69

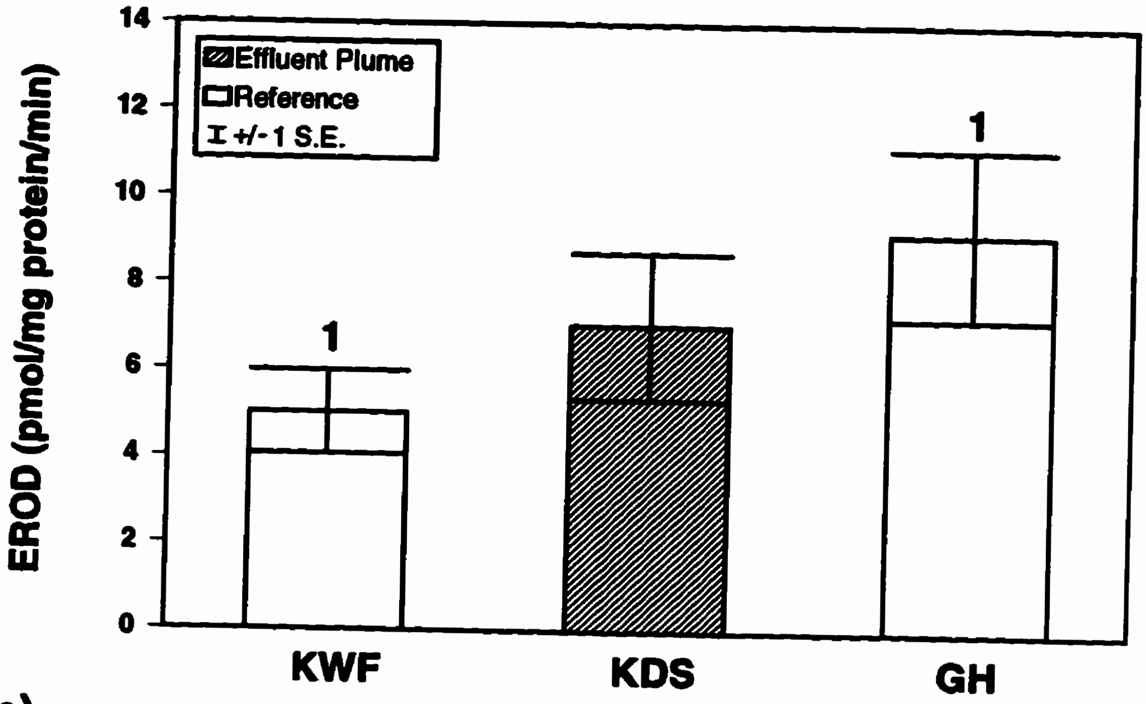
Figure 3.7. Regression lines of $\log_{10}$ total length *vs* $\log_{10}$ age (size-at-age) for female trout-perch (*Percopsis omiscomaycus*) from the reference site KWF (solid line) and effluent plume site KDS (dashed line) on the Kapuskasing River, fall 1995, Moose River system, Ontario.



0.95
0.90
0.85
0.80
0.75
0.70
Log Fork Length (cm)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Log Age (y)
log(fork length) = 0.53log(age) + 0.55
log(fork length) = 0.23log(age) + 0.69

Figure 3.8. EROD activity in a) male and b) female white sucker (*Catostomus commersoni*) from reference and exposure sites on the Kapuskasing River and site GH on the Groundhog River, fall 1995, Moose River system, Ontario. Values represent the mean ● SE. A difference between sites KWF and KDS is identified with an asterisk (*) above the KDS bar. A difference between reference sites KWF and GH is shown by different numerical superscripts. Differences were considered significant at $p<0.05$.

a)


Effluent Plume
Reference
+/- 1 S.E.
EROD (pmol/mg protein/min)
14
12
10
8
6
4
2
0
1
1
KWF
KDS
GH

b)

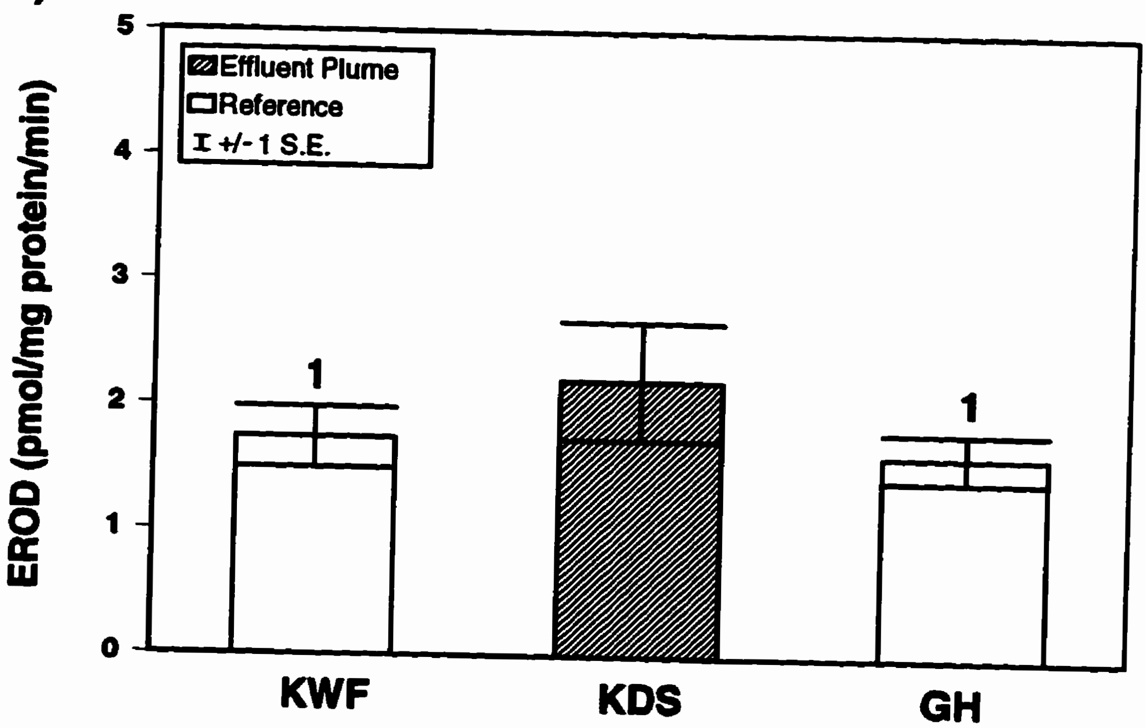

Effluent Plume
Reference
+/- 1 S.E.
EROD (pmol/mg protein/min)
5
4
3
2
1
0
1
1
KWF
KDS
GH

Figure 3.9. Circulating plasma levels of testosterone in a) male and b) female white sucker (*Catostomus commersoni*) from reference and exposure sites on the Kapuskasing River and site GH on the Groundhog River, fall 1995, Moose River system, Ontario. Values represent the mean ± SE. A difference between sites KWF and KDS is identified with an asterisk (*) above the KDS bar. A difference between reference sites KWF and GH is shown by different numerical superscripts. Differences were considered significant at $p<0.05$.

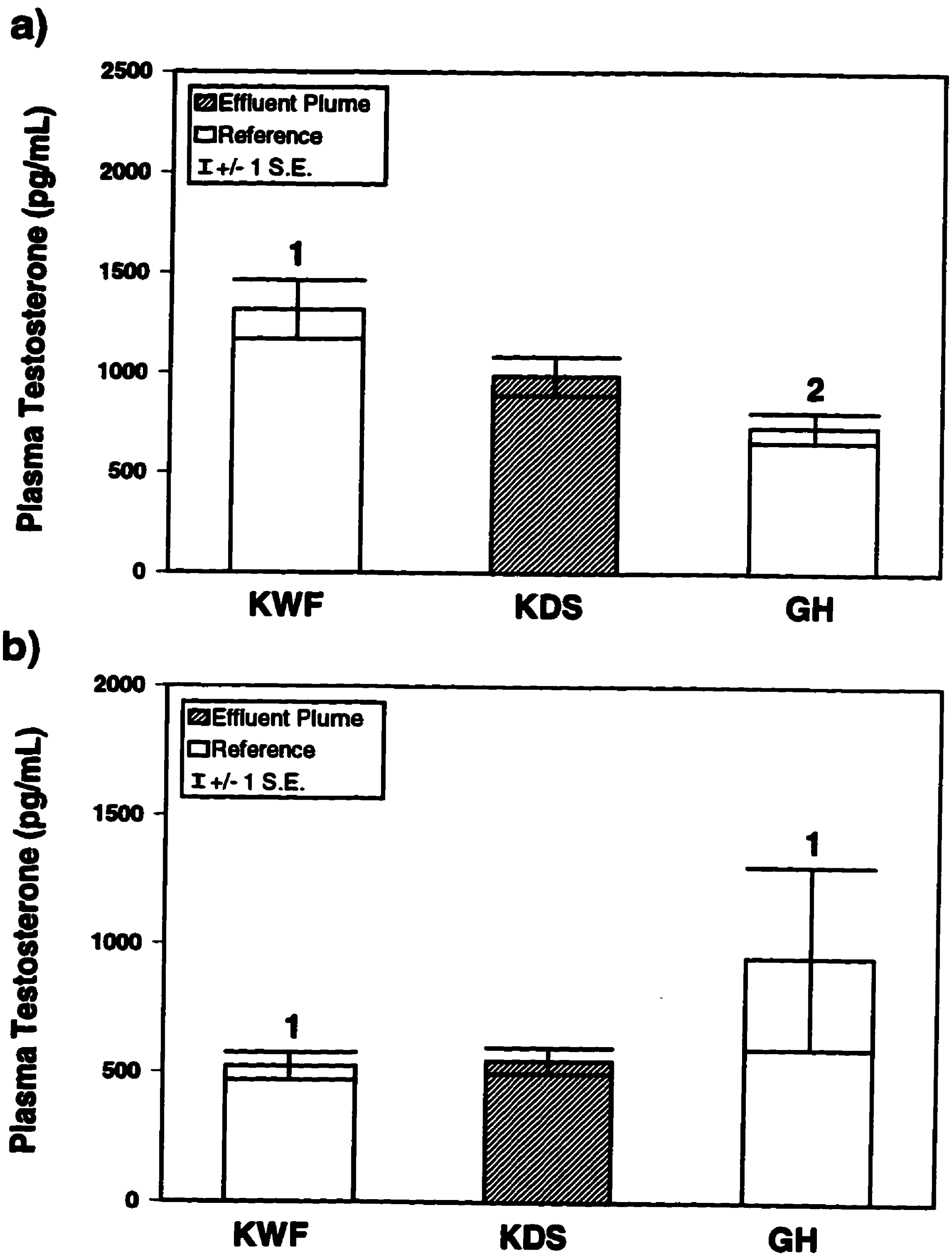
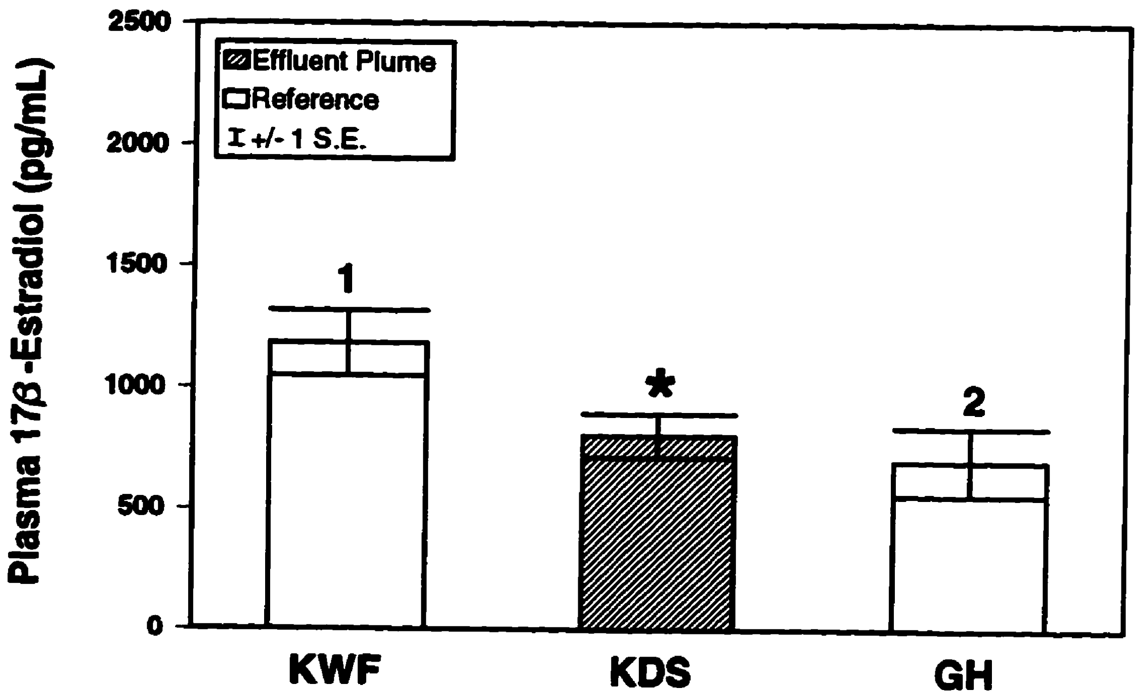

a)
2500
2000
1500
1000
500
0
Plasma Testosterone (pg/mL)
Effluent Plume
Reference
+/- 1 S.E.
1
2
KWF
KDS
GH
b)
2000
1500
1000
500
0
Plasma Testosterone (pg/mL)
Effluent Plume
Reference
+/- 1 S.E.
1
1
KWF
KDS
GH

Figure 3.10. Circulating plasma levels of 17 β-estradiol in female white sucker (*Catostomus commersoni*) from reference and exposure sites on the Kapuskasing River and site GH on the Groundhog River, fall 1995, Moose River system, Ontario. Values represent the mean ± SE. A difference between sites KWF and KDS is identified with an asterisk (*) above the KDS bar. A difference between reference sites KWF and GH is shown by different numerical superscripts. Differences were considered significant at $p<0.05$.


Plasma 17β-Estradiol (pg/mL)
2500
2000
1500
1000
500
0
Effluent Plume
Reference
+/- 1 S.E.
1
*
2
KWF
KDS
GH

the site on the Groundhog River and the upstream reference site on the Kapuskasing River (KWF). Also, the differences were not consistent between the two species.

Trout-perch from the Groundhog River (GH) were shorter, lighter and younger than fish from site KWF (Table 3.3). There were no differences in length and age of white sucker, or weight of female sucker between sites, but males were heavier at site GH (Table 3.4). There were no differences in size-at-age of female trout-perch and white sucker between sites (Table 3.5, 3.6). However, male trout-perch were smaller at a given age at site GH, and male sucker were larger at a given age at site GH. Condition of sucker was also greater at the Groundhog River; however, there was no difference in the condition of trout-perch between sites. (Table 3.5, 3.6). There were no differences in gonad size in either species between sites KWF and GH. Liver sizes in sucker and female trout-perch were higher at site GH.

EROD activity was lower in trout-perch from the Groundhog River, but no differences in activity were found in white sucker between sites (Figure 3.2, 3.8). *In vitro* production of testosterone in male trout-perch was similar between sites (Figure 3.3). Female *in vitro* production of testosterone and forskolin-stimulated production of 17 β-estradiol was higher at the Groundhog site (Figure 3.4, 3.5). Plasma levels of testosterone were similar in female white sucker between sites, but depressed in male sucker from site GH. Plasma 17 β-estradiol was also lower in female sucker from the Groundhog River.

3.4 Discussion

Results from this study indicated a number of interesting points, including: substantial EROD induction was absent in fish collected from downstream of the pulp mill; both trout-perch and white sucker exposed to mill effluent exhibited differences in whole-organism and physiological measurements relative to reference fish; the relative responses of exposed trout-perch and white sucker were not consistent; use of the Groundhog River as a reference river was questionable due to inconsistencies with reference data from the Kapuskasing River; and, data from the

Groundhog River highlighted the possible influence of the hydroelectric dam on the Kapuskasing River.

Overall, hepatic EROD activities were not substantially induced in trout-perch and white sucker exposed to effluent from the thermomechanical pulp (TMP) mill in Kapuskasing. Activity in male trout-perch was 1.25 fold higher than reference males; however, it has been suggested that EROD induction <2 fold falls within the range of natural biological variation (Martel et al. 1996), and the effluent likely represents only a marginal inducer. Since there has been no published work describing EROD activity in trout-perch exposed to potential inducers, it is possible that trout-perch are not capable of exhibiting a significant EROD response to pulp mill effluent (e.g., as is the case for gold fish (*Carassius auratus*), G.J. Van Der Kraak, University of Guelph, Guelph, Ontario, personal communication). However, this seems unlikely for two reasons: a) induction was also absent in exposed white sucker, a species for which EROD induction has been readily documented (e.g., Hodson et al. 1992; McMaster et al. 1991; Munkittrick et al. 1991a; van den Heuvel et al. 1995); and, b) trout-perch collected downstream of the kraft mill in Smooth Rock Falls (Appendix B) showed EROD activity >2 fold higher than was measured at sites KWF, GH and KDS.

Hepatic EROD activity has been used commonly as a positive indicator of exposure to pulp mill effluent, particularly bleached kraft mill effluent. However, it does not follow that the absence of induction, as observed in the current study, indicates that fish were not exposed to effluent. Several studies have documented a variety of pulp mill effluents not capable of inducing EROD activity, with no clear trends regarding mill process type, treatment facilities or wood type (Munkittrick et al. 1994; Hodson 1996; Martel et al. 1996). As well, previous work conducted in 1991 on the Kapuskasing River did not find significant EROD induction in white sucker downstream of the TMP mill (Munkittrick et al. 1994). This was particularly interesting given their study was conducted prior to the installation of the secondary treatment facility. Furthermore, the effluent concentration at a site approximately 11 km downstream from site KDS was estimated to be >1% (B.A.R. Environmental Inc. and Gore and Storrie Ltd. 1996).

The current study was successful in documenting changes in whole-organism and physiological measurements in another small fish species exposed to pulp mill effluent. Exposed trout-perch were younger and lighter, but also shorter at any given age, than trout-perch from the reference site. Reduced growth (as estimated by size-at-age) has not been a common response observed in wild fish exposed to pulp mill effluents. This may be because effluent discharged by pulp mills commonly contributes to increasing local water temperature and nutrient loadings which often promote rather than limit growth. In a review of fish responses to pulp mill effluent at 18 mill sites in North America and Scandinavia (Sandström 1996), only one site (bleached kraft mill at Jackfish Bay, Canada) exhibited a reduction in growth in exposed white sucker (McMaster et al. 1991; Munkittrick et al. 1991a). Researchers at this site also found that exposed sucker had reduced gonad size and increased condition, leading them to speculate that the resultant response was consistent with problems associated with metabolism and energy allocation (Munkittrick et al. 1991a). However, other than reduced growth, the response of trout-perch was not congruent with the response at Jackfish Bay because trout-perch did not exhibit any concomitant changes in condition or gonad size. It appears that the response of trout-perch exposed to TMP effluent in the Kapuskasing River has not been documented in other fish species at other pulp mill sites.

Several studies have documented reduced *in vitro* production of sex steroids in fish exposed to pulp mill effluent (Van Der Kraak et al. 1992; Jardine, 1994; McMaster et al. 1994). There was no evidence that exposure to mill effluent had a negative effect on testosterone production in trout-perch; however, there was a marginal increase in male testosterone production at the effluent plume site. Increased production has been observed before in spoonhead sculpin exposed to kraft mill effluent on the Athabasca River (Chapter 2), and probably does not represent a concern. In contrast, there was a decrease in forskolin-stimulated 17 β-estradiol production at the effluent plume site. The decrease in forskolin-stimulated production, coupled with similar testosterone levels at both sites (basal and forskolin levels), suggests possible problems associated with the conversion of testosterone to 17 β-estradiol (e.g., availability of aromatase enzyme) under stimulated conditions. Although this may have little bearing on routine production of 17 β-estradiol, it may pose a problem during periods of peak 17 β-estradiol production, i.e. during vitellogenesis. Clearly, more work needs to be conducted to investigate this issue further.

Surprisingly, the response of exposed trout-perch was not similar to the response of white sucker. In fact, in many ways the responses were opposite. White sucker exposed to effluent from the TMP mill were larger in body size and grew faster than reference fish. This response was very similar to what I had previously found in spoonhead sculpin from the Athabasca River (Chapter 2), suggesting an overall increase in metabolism. Increased growth and condition has been a common response observed in fish exposed to pulp mill effluent (Andersson et al. 1988; Sandström et al. 1988; Hodson et al. 1992; Gagnon et al. 1994, 1995). However, the mean age of white sucker downstream of the Kapuskasing mill was also greater than at the reference site, suggesting an overall shift to older and larger fish. This type of response has also been observed in redbreast sunfish (*Lepomis auritus*) exposed to bleached kraft mill effluent in the Pigeon River, Tennessee (Adams et al. 1992). It was speculated that the sunfish were experiencing reproductive and recruitment problems leading to an ageing population and larger individuals due to relaxed resource competition.

17 β-estradiol is responsible for the production of vitellogenin (egg yolk protein) during gonadal recrudescence in fish (Wallace and Selman 1981). Circulating levels of 17 β-estradiol were reduced in female white sucker collected downstream of the mill diffuser. This change was consistent with a decrease in ovary size, and may help explain why the change in ovary weight conflicted with the general response of increased metabolism. Several studies have documented reductions in circulating levels of sex steroids in fish exposed to BKME (McMaster et al. 1991; Munkittrick et al. 1991a; Adams et al. 1992, 1993; Gagnon et al. 1994); however, there has been no known published work documenting a change in steroid levels in fish exposed to TMP mill effluent. Because testosterone serves as a precursor for the production of estradiol, a reduction in estradiol production may result from a reduction in available testosterone, or some impairment associated with the normal conversion of testosterone to estradiol. White sucker downstream of the mill in Kapuskasing had circulating levels of testosterone similar to reference fish. As

such, the reduction in estradiol is most likely associated with the conversion from testosterone. Interestingly, this is the same potential problem identified in exposed trout-perch. Also, reduced estradiol in white sucker was consistent with the steroid response of exposed redbreast sunfish in the Pigeon River (Adams et al. 1992), adding to the similarity in overall responses observed at these two sites.

There is an obvious contrast between the responses of trout-perch and white sucker collected downstream of the mill in Kapuskasing (Table 3.7). This was surprising given both species were collected from the same sites and experienced similar concentrations of mill effluent. The response of white sucker was further substantiated by its consistency with the response documented during the previous fall 1994 survey (K.R. Munkittrick, Environment Canada, NWRI, Burlington, Ontario, unpublished data). However, there is no reason to suspect that the response of trout-perch is inaccurate. The reason for the discrepancies between responses is uncertain. It is possible that the differences in responses are a reflection of differences in species sensitivity and/or life history. For example, the average age of the white sucker collected during the survey was about 5-7 y older than the average age of trout-perch. As such, it is possible that the response observed in sucker more closely reflects historical conditions not experienced by any of the sampled trout-perch (maximum age of 4-5 y). This is especially true for parameters such as growth which integrate changes in biotic and abiotic factors over time (Goede and Barton 1990). As well, the mill has recently streamlined operations from a combination magnefite/groundwood/TMP mill to a TMP mill, and installed a secondary treatment facility. Both of these changes have influenced the effluent profile and quality of effluent discharged to the river.

Both trout-perch and white sucker downstream of the dam on the Groundhog River exhibited differences in whole-organism and physiological measurements compared to fish from the reference site on the Kapuskasing River. Assuming that data from the Kapuskasing River represent the true reference conditions, the current results suggest that the Groundhog River is

Table 3.7. A comparative summary of relative changes (0, no change; + significant increase; - significant decrease) in whole-organism and physiological measurements of trout-perch (*Percopsis omiscomaycus*) and white sucker (*Catostomus commersoni*) collected downstream of the pulp mill and hydro dam facilities, relative to fish from the upstream reference site (KWF), Kapuskasing river, fall 1995, Moose River system, Ontario.

	Trout-Perch		White Sucker	
Parameter	Male	Female	Male	Female
Weight	-	-	+	+
Total Length	-	-	+	+
Condition	0	0	+	+
Age	-	-	+	0
Size-at-Age	-	-	+	+
Gonad *vs* carcass wt.	0	0	0	-
Liver *vs* carcass wt.	0	0	+	+
EROD activity	+/0 *	0	0	0
Testosterone †	+	0	0	0
[forskolin-stimulated]	0	0	na	na
17 β-estradiol †	na	0	na	-
[forskolin-stimulated]	na	-	na	na

na, not applicable
* no change if consider <2 fold induction to be marginal
† *in vitro* steroid product for trout perch, circulating plasma levels for white sucker

not a suitable reference site for either white sucker or trout-perch. This conclusion was also inferred by Ruemper and Portt (1994) who found that data from the Groundhog River substantially inflated the variability in whole-organism measurements of white sucker collected among five reference sites on the Moose River system. Data from the Kapuskasing River were considered more representative of reference conditions than data from the Groundhog River because: a) site KWF is part of the same river system as the exposure site KDS, eliminating potential confounding factors between river systems, b) characteristics of trout-perch from two reference sites on the Kapuskasing River (sites KWF and KBL) were similar suggesting that data from site KWF were consistent with reference conditions of the Kapuskasing River; and, c) the recently built dam on the Groundhog River may directly or indirectly influence the downstream fish populations.

Although both species exhibited changes in characteristics at the Groundhog River, the changes in trout-perch were not consistent with the changes in white sucker. There was a tendency for trout-perch to be shorter, lighter and younger at site GH; whereas white sucker exhibited greater growth, condition and liver size. The reason for the discrepancy is unclear. It is recognized that the effect of a hydroelectric facility on the downstream fish populations can be variable, depending on factors such as the type and size of dam, location of water release (surface *vs* hypolimnion) and site-specific characteristics of the river (Baxter and Glaude 1980; Petts 1984; Rochester et al. 1984). However, differences in responses between species downstream of the same dam facility suggests probable differences in species sensitivity to dam-related changes, and warrants further investigation.

It is noteworthy that the direction of each species-specific response (trout-perch, negative; white sucker, positive) is similar to what was observed during the comparison between exposed and reference fish of the Kapuskasing River. A similarity between these two river systems is the presence of a hydroelectric facility located upstream of the fish sampling sites. Probably at least part of the response observed in fish downstream of the Kapuskasing mill is related to the upstream dam, and not just exposure to pulp mill effluent. It is possible to speculate on the relative influence of each stressor (dam *vs* effluent) by comparing the responses observed at the

Groundhog River (influenced by a dam) to the responses observed downstream of the pulp mill and hydro dam facilities on the Kapuskasing River (Table 3.8). Using this approach, it appears that a great deal of the whole-organism response of trout-perch at site KDS could be attributed to the upstream hydro dam, whereas depressed *in vitro* steroid production was only observed downstream of the mill. Similarly, most of the changes in whole-organism measurements of male white sucker could be attributed to the dam; however, female white sucker seemed to be influenced less by the dam and more by exposure to effluent. In support of the latter observation, subsequent comparisons indicate that the current response of female sucker from the Groundhog River is not substantially different from the response measured in 1991 prior to the installation of the dam (K.R. Munkittrick, Environment Canada, NWRI, Burlington, Ontario, unpublished data). Unfortunately, the sample size of male sucker was insufficient to conduct this comparison, and comparable data for trout-perch were not available.

The above *a posteriori* comparison suggests that the hydroelectric facility on the Kapuskasing River may strongly influence the responses of both sentinel species. However, to directly compare the responses between site KDS (i.e. KWF *vs* KDS) and site GH (i.e. KWF *vs* GH), at least two assumptions were made: a) the site on the Groundhog River is comparable to the upstream site on the Kapuskasing River in all respects, except for the influence of the dam; and, b) the downstream influence of the hydroelectric facility on the Groundhog River is similar to the influence of the dam on the Kapuskasing River. The former assumption could be further clarified by sampling fish upstream of the dam on the Groundhog River to ensure that: i) Groundhog River fish are similar to the upstream reference site on the Kapuskasing River; and, ii) the response measured downstream of the dam on the Groundhog River is reflective of the dam, and not some other confounding factor between the Groundhog and Kapuskasing River.

Synopsis

Overall, the success of using a small fish species as a sentinel species on the Moose River system was mixed. Of significant importance was that it was possible to document changes in the response of trout-perch to potential stressor(s). This was seen downstream of the TMP mill

Table 3.8. The relative change (0, no change; + an increase; - a decrease) in responses of trout perch (*Percopsis omiscomaycus*) and white sucker (*Catostomus commersoni*) between site GH (Groundhog River, hydro dam influence) and site KDS (Kapuskasing River, hydro dam + effluent influence). The resultant response approximates changes at site KDS potentially related only to pulp mill effluent (i.e., KDS-GH). Example: female trout-perch size-at-age at GH (KWF *vs.* GH) = no change (0); at KDS (KWF *vs.* KDS) = decreased (-); resultant response due to effluent alone (KDS-GH) = decreased (-).

	Trout-Perch		White Sucker	
Parameter	Male	Female	Male	Female
Weight	0	0	0	+
Total Length	0	0	+	+
Condition	0	0	0	0
Age	0	0	+	0
Size-at-Age	0	-	0	+
Gonad *vs* carcass wt.	0	0	0	-
Liver *vs* carcass wt.	0	-	0	0
EROD activity	0	0	0	0
Testosterone †	+	-	+	0
[forskolin-stimulated]	0	-	na	na
17 β-estradiol †	na	0	na	0
[forskolin-stimulated]	na	-	na	na

na, not applicable

† *in vitro* steroid product for trout perch, circulating plasma levels for white sucker

and hydroelectric dam on the Kapuskasing River, and the hydro dam on the Groundhog River. It was also of interest to note that trout-perch sampled from different sites within the reference zone were similar in whole-organism characteristics. This is in contrast to what I had found for spoonhead sculpin and lake chub in the Athabasca River (Chapter 2), but increased the likelihood that the full extent of reference variability in trout-perch was adequately represented.

Capture success of trout-perch at sites on the Kapuskasing River and Groundhog River was high (e.g., sufficient numbers of fish captured in one seine haul); however, this was not the case on the Mattagami River. Despite successfully collecting trout-perch downstream of the kraft mill on the Mattagami River, trout-perch (or any other small species) could not be captured in sufficient numbers within the reference zone upstream of the mill. Without reference data, it was not possible to use trout-perch as a sentinel species on this river. As well, based on conclusions from Chapter 2, it was not advisable to use reference data from a separate river system such as the Kapuskasing River. For the Mattagami River, poor capture success precluded the use of a small fish species as the sentinel species, particularly when the larger white sucker could be collected in adequate numbers.

An interesting ancillary issue introduced during the study was that the responses of both trout-perch and male white sucker at site KDS could be largely attributed to the hydroelectric dam, and secondarily to the exposure to pulp mill effluent. As was suggested, further data need to be collected to verify this hypothesis; however, it does illustrate the error in considering the dam facility on the Kapuskasing River as simply a barrier to fish movement without considering the potential influence it may have on downstream fish populations. By sampling the Groundhog River and Kapuskasing River, the study provided a simple example of separating out the possible influence of component sources of a cumulative effect or response.

A primary objective of the study was to compare the consistency and relative sensitivity of responses of trout-perch and white sucker under conditions of similar effluent exposure and mobility. The comparison was, in part, conducted for the purpose of further validating small fish species as sentinel species. It was evident from the results that the responses of the two species were not consistent; although it was not possible to identify reasons for the discrepancy. The inconsistency between responses also made it difficult to determine which species was more sensitive to the instream conditions. It is apparent from these difficulties that a more detailed examination of fish responses is needed that would facilitate the interpretation of responses and the identification of possible causative agents. An interpretation framework has been previously described by Munkittrick and Dixon (1989a,b); however, recent studies (e.g., Hickie et al. 1989; Adams et al. 1992; Munkittrick et al. 1994; Servos et al. 1994) have revealed several limitations and deficiencies in the framework. As well, the framework did not include all of the responses observed in the current study. Without an alternative approach, or a revision and reorganization of the existing framework, the conflicting responses observed in the current study remain difficult to interpret, and the relative suitability of each sentinel species cannot be fully evaluated.

CHAPTER 4

INTERPRETATION OF FISH RESPONSES

4.1 Introduction

While conducting research on the Moose River system (Chapter 3), the observed differences in responses between trout-perch and white sucker exposed to the same potential stressors (mill effluent, hydro dam) presented an unexpected problem in evaluating small fish species as potential sentinel species. Specifically, it was uncertain how the different responses of each species were related, and which species was most sensitive to the stream conditions. The comparison of responses was complicated by species differences in life history, habitat utilization, community dynamics, longevity, etc., that may ultimately influence the relative response times of each species. A more detailed examination of the fish responses was necessary to facilitate the interpretation of responses, and the comparison between the small and large fish species.

Munkittrick and Dixon (1989a, 1989b) described an interpretation framework which focussed on the classification and interpretation of fish responses, as well as the identification of possible causative agents. The approach was amenable to my work; however, the original framework was of limited use because it did not discuss or include the responses observed at the Moose River system, or at the Athabasca River. As well, recent studies in the published literature (Hickie et al. 1989; Adams et al. 1992; Munkittrick et al. 1994; Servos et al. 1994) have also documented responses of fish to environmental stressors which were not considered in the original framework. For my purposes, I decided to use the framework as a starting point from which to develop a revised and reorganized framework that would allow the incorporation of recently described response patterns, provide a better understanding of the fish responses, and simplify the interpretation of fish responses not only for the thesis, but also for other researchers doing similar preliminary fisheries assessments at the individual or population level.

The current chapter has been organized to include: a) background to the original framework; b) brief summary of recently described responses to be included in the revised framework (from thesis work and recently published literature); c) revised framework and a description of the progression of fish responses; and d) interpretation of fish responses observed in the thesis, particularly responses of trout-perch and white sucker from the Moose River system.

4.2 Background to Original Framework

The use of fish populations and communities to assess the state of the environment has received considerable attention in recent years (e.g., Plafkin et al. 1989; Bartell 1990; DeAngelis et al. 1990; Edwards et al. 1990; Fausch et al. 1990; Shuter 1990). Over the last few years, Munkittrick & Dixon (1989a, 1989b) and Munkittrick (1992) have described an environmental monitoring framework which can assist in designing studies aimed at identifying stressors associated with changes in the performance of fish populations. The framework was developed from the original concept of Colby & Nepszy (1981) and Colby (1984), who proposed that the response of a fish species to particular stressors was distinct and predictable, and that this response could be classified and used to define the status of a fish population. Responses of fish to a stressor or stressors were characterized by comparing traditional fisheries data (e.g., growth rate, condition, age at maturity, mean age, size at age, gonad size, fecundity, etc.) to historical data (e.g, data from previous sampling times) or data from reference fish. The framework assumes that density-independent regulatory factors are consistent between the study sites and the reference sites, and observed differences are considered to be responses of the fish to alterations in the environment which are directly or indirectly related to a stressor.

In his original study, Colby (1984) classified the responses of fish to a variety of environmental stressors. All responses could be grouped into five generalized response patterns characteristic of exploitation, recruitment failure, multiple stressors, food limitation and niche shift (Table 4.1).

Table 4.1. Original response patterns of fish to contaminant impacts described by Colby (1984) and modified by Munkittrick and Dixon (1989a, 1989b).

Response Pattern	Growth Rate	Condition Factor	Age at Maturation	Fecundity	Egg Size	Age Structure	Mean Age	Population Size	Stressor/ Mechanism
I	+[1]	+	-	+	-/+[2]	shift towards younger	-	-	Exploitation
II	0/+	0	0	0	0	shift to older	+	-	Recruitment Failure
III	-	-	+	-	-/0	shift to older	+	-	Multiple Stressors
IV	-	-	+	-	-	no change	0	+	Food limitation
V	-	0	+	-	-	no change	0	-/0	Niche Shift

[1] + signifies increase relative to reference or historical data, - signifies decrease, 0 signifies no increase or decrease
[2] response may vary dependent upon the magnitude of alteration within the population

Using published data, Munkittrick & Dixon (1989a, 1989b) reviewed the responses of a wide variety of fish species to changes in eutrophication, acidification, reservoir impoundment, predation pressure and industrial wastes. Results of the literature review categorized all examples into one of the five generalized response patterns and confirmed Colby's original work.

Munkittrick & Dixon (1989a, 1989b) advocated using the generalized response model outlined by Colby (1984) as the basis of a fish monitoring framework for environmental assessment purposes. The monitoring framework recommended focusing on one or two sensitive "sentinel" fish species as an indication of the status of resident fishes. By characterizing the response of adult fish collected from an area of concern, the response could be matched with one of the five generalized response patterns. Recognizing which pattern the observed fish response matched would provide information on the possible impacts on the fish, as well as providing direction and focus for research efforts to identify causal factors which might be related to those responses. The framework was intended as an aid for focusing confirmatory studies, and also to provide a cost-effective method for initial assessment of fish populations living in stressed environments. Additional details on study design and the selection of appropriate sentinel species has been provided in Munkittrick (1992).

4.3 Revised Interpretation Framework

Recent studies have revealed several limitations and deficiencies of the original monitoring framework, including the subsequent description of additional responses of fish in stressed environments. In light of the new information described in the published literature and this thesis, I have revised the monitoring framework to account for these response patterns. In addition, the framework was reorganized to emphasize the relationships among responses in fish, and the theoretical progression from one response to another. The new sentinel monitoring framework was developed to provide a more comprehensive understanding of the responses, possible reasons leading to a response, and appropriate follow-up studies which could be conducted to further identify or confirm causative agents. Although, the focus of this chapter was to facilitate the interpretation of fish responses observed in the Moose River system, an effort was made to elaborate on the applicability of the framework an approach that could be applied to a variety of situations and potential environmental stressors.

4.3.1 Recently Described Response Patterns

Although an extensive literature base was surveyed when testing the original framework, recent studies focusing specifically on the impacts of contaminants on fish have documented other responses that do not conform to the original five generalized response patterns. The identification of these patterns has provided greater insight to the response patterns and a greater understanding of how fish typically respond to contaminants. A summary of the recently described response patterns are provided in Table 4.2.

Chronic Recruitment Failure

Adams et al. (1992) described a population of fish chronically exposed to pulp mill effluent. They concluded that decreased recruitment resulted in an older age structure, and may have caused a reduction in population size and a decrease in competition. The increased availability of food resources meant that more food was available for growth and lipid storage in the surviving fish, resulting in a response pattern showing increased age, increased energy expenditure and increased energy storage. A similar overall increase in fish characteristics was also observed in male white sucker downstream of the pulp mill and hydroelectric facility on the Kapuskasing River, Ontario (Chapter 3).

Metabolic Redistribution

Recent work has suggested that a stressor may directly affect the ability of fish to efficiently convert energy into somatic or reproductive tissue (Hickie et al. 1989). Field studies of fish collected downstream of pulp mill effluent discharges described increases in condition factor

Table 4.2. Summary of new fish responses recently described in the literature and the current thesis research.

Growth Rate	Condition Factor	Age at Maturation	Fecundity	Egg Size	Age Structure	Mean Age	Population Size	Stressor/ Mechanism
-[1]	+	+	-	-	shift towards younger	0/-[2]	0/-[2]	Metabolic Redistribution
+	+	-	0/-[3]	0/-[3]	shift towards older	+	-	Chronic Recruitment Failure[4]
0	0	0	0	0	no change	0	0/-	No response

1 + signifies increase relative to reference or historical data, - signifies decrease, 0 signifies no increase or decrease
2 response may vary dependent upon the magnitude of alteration within the population
3 fecundity and egg size were lower in exposed white sucker collected from the Kapuskasing River (Chapter 3); whereas, Adams (1992) found no differences in theses parameters
4 the response pattern for chronic recruitment failure probably represents the next stage in the recruitment failure response (+00) described by Colby (1984). As recruitment continues to fail, more resources will be available to the remaining fish resulting in an overall increase in energy expenditure and storage (+++)

(Munkittrick et al. 1991a) or lipid levels (Servos et al. 1994) despite a decrease in gonadal sizes (Munkittrick et al. 1994). This resulted in detection of a response pattern showing increased age structure, decreased energy expenditure and increased energy storage. These data suggest some level of metabolic disruption capable of altering growth and reproductive output, which would be detected by inconsistencies in energy allocation (e.g., a decrease in reproductive or growth rates along with an increase in storage of energy, or alternatively, opposite changes in growth and reproduction).

No response

The original framework did not include the provision for detecting a null response (ie. no difference from reference sites in terms of age structure, energy expenditure or energy storage). If significant differences are not observed, and the statistical design of the study is appropriate, there are several possible reasons for the null response: there has been no response of the fish population to the stressor, several stressors are impacting the same sites in opposite directions, or the carrying capacity of the system was reduced and the population has had sufficient time to equilibrate with the new system. Differentiation of no impact from a reduced carrying capacity should be separable by examination of the relative population sizes between the exposed and reference sites (i.e. catch per unit effort data). Identification of conflicting stressors would be much more difficult, experimentally and philosophically: if there has been no detectable response in terms of growth, survival or reproduction, has there been any impact?

4.3.2 Progression of Fish Responses

There are many possible mechanisms through which stressors may affect fish, most of which can be categorized according to their effects on:

- ability to use or process food (energy conversion)
- food and habitat availability and use
- behaviour

- mortality (of adults, juveniles, larvae or eggs)

Each of these effects should result in identifiable changes in the surviving or remaining fish and, to date, there are at least eight response patterns documented in the literature (Table 4.1, 4.2). However, the particular response pattern observed at a specific sampling time reflects only that specific sampling time, and may represent one of several possible stages in the progression of a population response. Identification of a pattern does not provide any information on the stability of the pattern, or direction and speed at which response patterns are changing. In addition, the time that a population remains in any pattern will depend not only upon the degree of habitat change, but also the responsiveness of the species being monitored.

Although there may be additional response patterns which have not been identified in the current framework, the documented patterns can be linked together in a progression of responses to a stressor. The following discussion outlines the progression and direction of fish responses to alterations in food/habitat availability, rates of mortality and physiological impairment. Behaviour has not been included in the framework because it is often difficult to observe and quantify. As well, it is not possible to sample fish which are behaviourally avoiding a location; it is the response of the fish that remain which is important to understand. The response pathways form the basis for interpreting the results of initial sampling and facilitates the identification of the response pattern and pathway, the probable mode of action in the fish population, and the strategy for future follow-up studies. Although the relationships between response patterns may be conceptual, the framework developed from these patterns represents a useful model for interpreting results of fisheries data. The pathways of the framework were developed to help the researcher understand the likely progression leading to a particular response. However, it should be emphasized that they are not intended to be used as predictive tools to identify the future progression of fish responses.

In the accompanying diagrams, fish from a study area are described in relative terms to reference fish or historical data, and may exhibit an increase [+], decrease [-], or no change [0] in response.

There may be many responses of a population to any change, but identification of the response pattern requires an understanding of the key characteristics, since population characteristics are not independent. For instance, an increase in food availability associated with an increased growth rate would also usually be associated with earlier maturity and increased reproductive commitments. It is recommended that any field survey examine all population characteristics to determine their consistency with the response pattern; it is the failure to respond consistently that highlights the areas requiring follow-up study. For ease of presentation, it is possible to reduce the description of characteristics to the basic groups which describe age structure, energy expenditure and energy storage (Table 4.3). For each of the following fish response descriptions, the response pathway begins with fish unaffected by a stressor [fish show no change in age structure, energetic expenditure or energy storage: **000**]. Some patterns described are transitional, and the possibility of their detection in wild fish populations would depend on the time scale, responsiveness of the species being monitored and the severity of impact. The established patterns which have been identified in the literature and thesis are highlighted in the accompanying figures by a shaded boxes.

4.3.2.1 Food/Habitat Availability

The amount and quality of food energy available to an organism will have an effect on its growth rate, reproductive investment, and energy storage (Adams and Breck 1990). One of the dominant habitat characteristics affecting the performance of fish is the quality, quantity and accessibility of food. Obviously, the first priority is to allocate energy for maintenance and survival. Any remaining energy will be used for somatic growth, reproduction, or lipid and glycogen storage. If a change in habitat characteristics increases the availability of food, fish will respond by increasing their expenditure and storage of energy, including an increase in their growth rate. Reductions in food availability would have an opposite effect on fish performance, assuming all other factors remain constant.

Table 4.3. Grouping of characteristics according to the summary categories of age structure, energy expenditure and energy storage.

Common Group	Population Parameters
Age Structure	mean age or age distribution
Energy Expenditure	growth rate
	gonad weight
	fecundity
	age at maturity
Energy Storage	condition
	tissue lipid levels
	liver weight (sometimes variable)

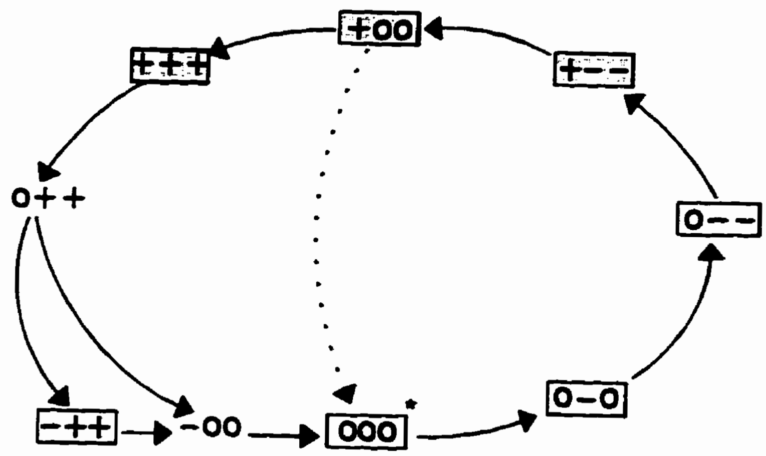
Decreased Availability of Resources

Resource availability may decrease for several reasons, such as an increase in the population size, or a decrease in the availability of habitat and/or a change in the food base. Increased population size can result from a number of factors including increased immigration (Barnes et al. 1984), stocking (Riemers 1958, 1979), improved survival (Hassler 1969; Nelson 1974), or decreased predation rates. Decreased habitat availability can result from a decrease in the quality or amount of food available (Muth and Ickes 1993), increased competition associated with improved survival of competing species (Swanson 1982; also reviewed in Colby 1984; Hanson and Leggett 1986; Persson 1987), reduced water flows, or toxicity effects associated with chemicals (Munkittrick and Dixon 1988; Munkittrick et al. 1991b), solids (Handford et al. 1977), heat, or BOD.

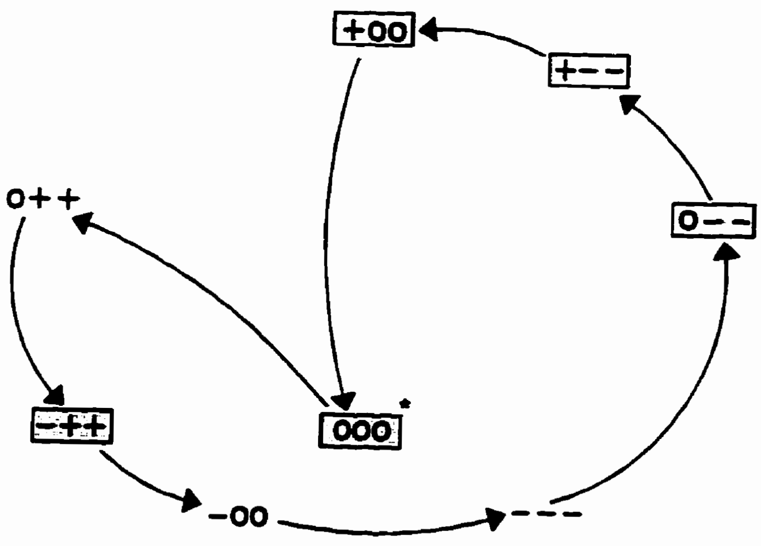
The initial response of a population faced with diminishing resources is to reduce energy expenditures, resulting in a decrease in growth rate and reproductive output. There would be no initial impact on age structure, since the same adult fish are present before and after the change in resources. Sampling the population at this point in time would result in detection of a pattern (based on age structure, energy expenditure and energy storage) which showed a difference only in terms of energy expenditure [0-0] (Figure 4.1a). Further reduction in the food base would decrease the amount of energy available for storage, resulting in a change in response pattern to reflect decreasing energetic storage as well as decreased expenditures [0--]. Since the population has been producing fewer young, the average age of the population would begin to climb, resulting in an eventual increase in mean age [+--]. The change in resource availability would reduce the carrying capacity (the size of the population which can be supported by the environment). When the size of the resident population is reduced to a level consistent with the new carrying capacity, there may be sufficient resources for the remaining fish to reproduce, grow and store energy at a level consistent with their original rates of energy utilization and storage. However, the population would still be older than the original distribution, resulting in a pattern of [+00]. There are two possible outcomes from this pattern:

Figure 4.1. **Progression of fish population response patterns after a a) decrease or b) increase in the availability of food or habitat. The responses are abbreviated in terms of the relative change (- less than, 0 equivalent to, + more than) in age structure, energy expenditure and energy storage when compared with the reference sites or historical data. The asterisk signifies the starting point of the progression [000]; response patterns in shaded boxes represent patterns documented in the literature.**

a)



b)



a) If the age shift is moderate and the ability of the population to respond is high, the population may be able to rebound quickly, resulting in the detection of a population indistinguishable from the reference population [000], except for a change in population size (which may be apparent from the capture data).

b) If the population has a substantially older age structure (or additional external factors are associated with lethal conditions), the mortality rate will be higher. Increased mortality will result in a further decline in population size, which would result in an excess of food resources for the small population of individuals that remain. These fish will respond to the increased resources by increasing energy expenditures and storage [+++]. The increased reproductive output will result in an increase in younger fish, eventually leading to a decrease in the mean age of the sample population (when the fish reach sampling size) [0++]. The increased reproductive rate will result in an eventual further decrease in mean age, which may have two consequences:

i. The ability of the population to respond is high, and the differences slight, allowing the population to return quickly through an intermediate stage [-00] to reference levels [000].

ii. The population responds slowly, demonstrating an intermediate [-++] pattern.

Once the observed response has been characterized, a "predicted" response pattern can be identified based only on the characteristics of age structure, energy expenditure and energy storage. If, as in the present example, a population has been "diagnosed" as showing food limitation [0--], there are a number of additional characteristics which should be responding (Table 4.1, 4.2). Each of the fish characteristics described for the sampled fish population should be compared with the individual characteristics of the predicted response of the population (Munkittrick and Dixon 1989a). If all characteristics respond as would be predicted under food limitation (in this case), high confidence can be placed in the conclusion that food resources are limiting; follow-up studies should attempt to confirm this response and identify the factors involved with the limitation of food resources. If there are discrepancies between predicted and observed responses, these differences highlight the areas requiring follow-up studies.

The absence of detectable effects on age structure, energy expenditure and energy storage would suggest that the population is acting normally in terms of how energy is used or stored. Comparison with the expanded patterns would show that a difference between the original [000] and the final [000], based on population size and capture success (CPUE). This would focus follow-up studies on determining why there has been a change in population size. In the absence of historical data, the definition of a response pattern would be made with appropriate reference sites.

Increased Availability of Resources

Increased availability of resources can occur for one of two reasons, either a decrease in population size or an increase in the amount of available habitat and food resources. Decreased population size may be associated with increased mortality due to increased abundance of predators (Power and Gregoire 1978; Burrough and Kennedy 1979; Henderson 1986), the presence of lethal conditions (Healey 1975; Jensen 1981; Schneider and Leach 1977; McFarlane and Franzin 1978), or an increase in natural mortality due to an ageing population. Similar responses in adult fish could also occur due to increased food availability associated with enrichment or stocking of forage fish, increased habitat availability associated with improving habitat conditions (Martin 1951, 1970; Mills 1985; Erikson and Tengelin 1987; Rosseland 1986; Beggs and Gunn 1986), and decreased competition or increased habitat associated with changes in water flow and flooding.

The initial response of adult fish to increased resources is to increase both energy expenditure and storage (ie. Adams et al. 1992). Since the same fish are present, there is no initial change in population size or age structure [0++] (Figure 4.1b). The length of time spent in transitional stages will vary with a number of factors. The increased reproductive rate results in an increase in younger fish and a decline in mean age [-++]. The increasing population size will result in eventual limitation of the food resources due to increased competition, resulting in a return of energetic expenditure and storage in adults to earlier levels, but with the presence of a much younger population [-00]. As the younger fish enter into competition with the adults for resources, there will be a further strain on resource availability and energetic expenditure and storage will decline [---]. The decreased reproductive output will result in an increase in age, progressing through [0--] to [+--]. The decreasing reproductive rate will result in a decreased population size, reducing the number of fish to a level consistent with the original carrying capacity (if mortality was the original stimulus) or consistent with the new, higher carrying capacity (if improved conditions was the original stimulus) [+00]. In either case, the end result is the eventual return of the population to relatively normal characteristics [000].

It is unclear how long response patterns may persist. The patterns which have been documented (shaded boxes) may represent responses which persist for longer periods of time. Otherwise, the chance of documenting these patterns in wild fish would be based on chance or periodic observations during changes in environmental conditions. Most of these patterns described for increased food availability are indistinguishable in preliminary sampling from the earlier descriptions based on food limitation. It is by comparison of the expanded patterns that population size can be used to discriminate whether food limitation at this stage is associated with too many fish or not enough food. There are several possible explanations for most patterns. For example, the response pattern [+--] can also be associated with persistent food limitation or with the presence of stressors affecting adult survival. The increased mean age could be associated with one stressor affecting early life survival, while a second stressor limiting food availability may be associated with the decreasing energy expenditure and storage (e.g., Swanson 1982). The utility of the framework is that it focuses questions on the key characteristics to be examined and provides hypotheses to direct follow-up testing.

4.3.2.2 Change in Mortality

Adult Mortality

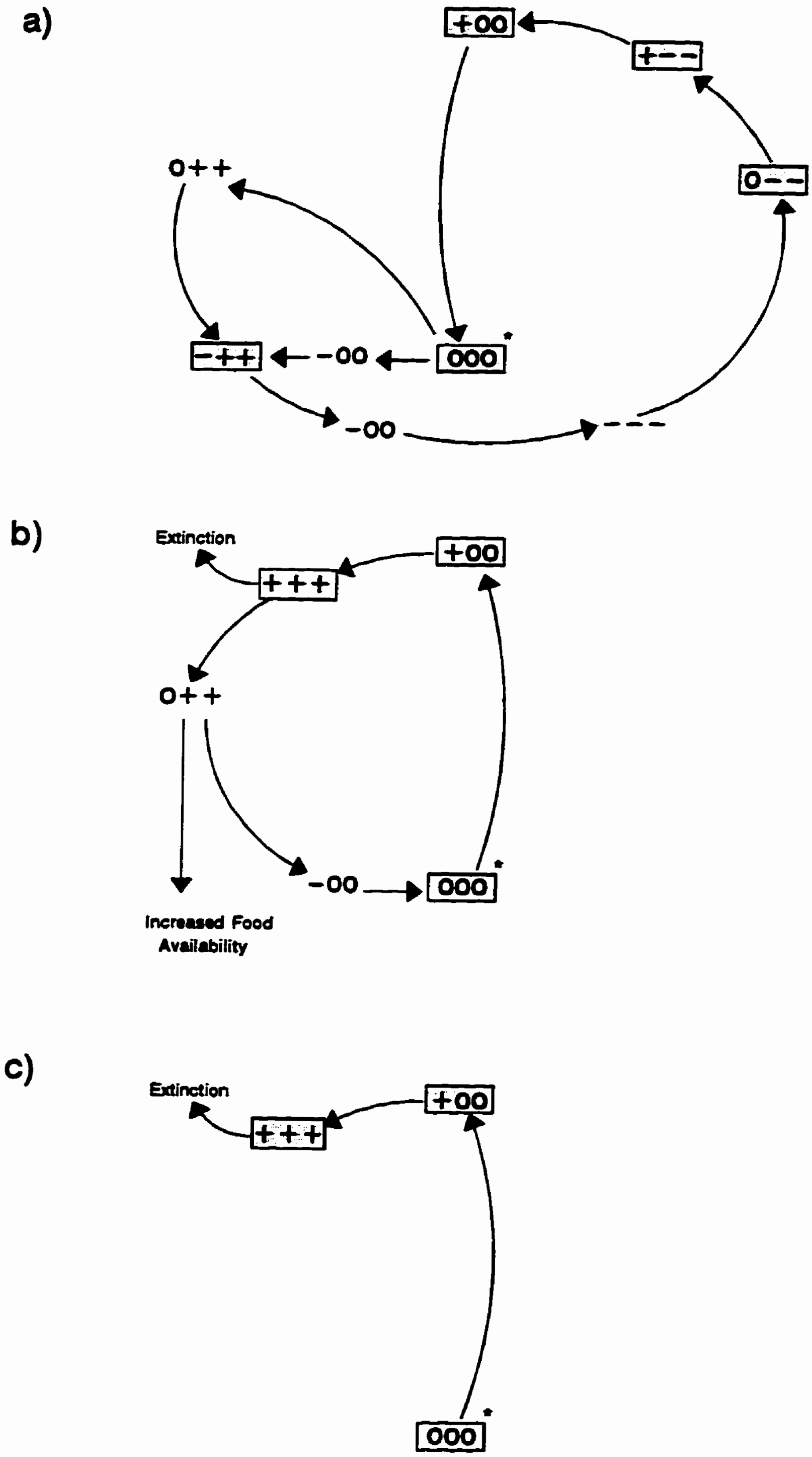
Loss of adults from a population could occur due to mortality associated with contaminants, fishing, or predation. Physical and/or chemical barriers may also prevent immigration. If the loss of adults is age dependent (older fish are more at risk), there is an initial decrease in the mean age of the population [-00] (Figure 4.2a). The decreased competition for food resources results in a relative increase in resource availability, and increased energy expenditure and storage [-++]. If the mortality of adults is independent of age (eg., chemical spill) or the entry of younger fish into the sampling population is sufficiently slow, the population may initially show no change in age structure, but an increased availability of resources [0++].

The increased reproductive rate results in a decreasing mean age [-++]. The remainder of this response scenario follows the same response patterns as increased food availability, for the reason that the surviving fish are responding to the increase in food availability, not to the mortality of adults. It is clear and logical that patterns can arise for a number of reasons, but the important output of the framework is how to allocate sampling effort on the areas requiring clarification. For appropriate interpretation, it is not important whether the [-++] pattern has occurred because of increased mortality, increased habitat or any other reason. The important conclusion is that there has been an increase in the amount of resources available, and follow-up studies should be focused on determining why resources have changed and whether the observed changes in population characteristics can be associated with specific stressors impacting the population.

Juvenile Mortality

Mortality of juveniles will eventually result in an increase in the mean age of the sample [+00] (Figure 4.2b). Due to a decreased population size, there is an increase in food resources, and

Figure 4.2. Progression of fish population response patterns after an increase in a) adult mortality, b) juvenile mortality; and, c) mortality of eggs or early life stages. The responses are abbreviated in terms of the relative change (- less than, 0 equivalent to, + more than) in age structure, energy expenditure and energy storage when compared with the reference sites or historical data. The asterisk signifies the starting point of the progression [000]; response patterns in shaded boxes represent patterns documented in the literature.


a)
+00
+--
0--
0++
-++
-00
000*
-00
---
b)
Extinction
+++
+00
0++
-00
000*
Increased Food Availability
c)
Extinction
+++
+00
000*

individual fish respond by increasing energetic expenditure and storage [+++]. If the cause of juvenile mortality is chronic, the population will eventually become extinct. If the mortality has stopped, the increasing reproductive rate results in a gradual lowering of mean age [0++]. Depending upon the degree of alteration, the population should recover (assuming the stressor has been alleviated) after a minor change in mean age as the younger fish mature [-00]. If the amount of resources for early life stages remain limiting, the time for recovery may be extended, limiting our ability to detect intermediate stages. If the change in population size has been severely altered, the patterns will cycle from [0++] through the loop describing an increased food base (Figure 4.1b), again providing that the cause of mortality has abated.

Early Life-Stage Mortality

The absence of younger year classes in a variety of fish populations has been associated with low pH (Beggs and Gunn 1986), prolonged industrial discharges (Munkittrick and Leatherland 1984), mining wastes (Black et al. 1985) and declining water quality (Evans 1978; Colby 1984).

The mortality of early life-stages (embryos, larvae, juvenile stages which do not share food/habitat resources with the adults) will eventually result in an increased mean age [+00] (Figure 4.2c). There is no initial change in energetics since there is no change in the number of adults, the number of fish present, nor their habitat. As the older population begins to die off, there is a relative increase in the food/habitat available, and growth, reproduction and storage of energy increases [+++]. If the cause of early life-stage mortality has not abated, there will be eventual extinction of the population. If the mortality was transient, the population will cycle through the loop for increased food availability, since the remaining fish are responding to decreased competition and not to the earlier egg or larval mortality (Figure 4.1b). If conditions do not change, this response pattern could persist and be detectable for extended time periods.

4.3.2.3 Metabolic Redistribution

This pattern is reflective of a change in the ability of the fish to process energy, resulting in conflicting interpretation between the results of energy storage and expenditures. This pattern results when fish show decreased energetic expenditure but increased storage (or *vice versa*) [0-+]. The decreased reproductive rate will eventually lead to an older population [+-+] (Figure 4.3). This pattern appears to be very stable, consistent with metabolic disruption by foreign chemicals. If the cause of the disruption were abated, the population should recover its reproductive/growth potential [probably through +00]. Appropriate follow-up studies involve detailed physiological investigation of energetics and hormonal regulation of reproduction and growth.

It is also possible that a disruption in energy allocation can be identified by discrepancies between characteristics within the general groups of energy expenditure or storage. For example, female white sucker from the Kapuskasing River downstream of the pulp mill and hydro dam (Chapter 3) exhibited increased growth, but decreased gonad size. Typically, these parameters covary, or there is a decrease in growth in an effort to maintain reproductive investment; however, declining reproductive investment with increased growth suggest a possible problem associated with utilization and/or allocation of available energy.

4.3.3 Summary of Patterns

It is possible to integrate the response patterns and pathways by linking the common relationships among each of the general responses to food and habitat availability, mortality and metabolic redistribution (Figure 4.4). The interconnection among the response patterns provides a more comprehensive theoretical linkage describing how fish respond to direct and indirect stresses. Based on the observed responses of fish, this framework should indicate whether fish exposed to environmental stressors are exhibiting altered characteristics relative to the reference

Figure 4.3. Progression of fish population patterns in response to metabolic disruption. The responses are abbreviated in terms of the relative change (- less than, 0 equivalent to, + more than) in age structure, energy expenditure and energy storage when compared with the reference sites or historical data. The asterisk signifies the starting point of the progression [000]; response patterns in shaded boxes represent patterns documented in the literature.

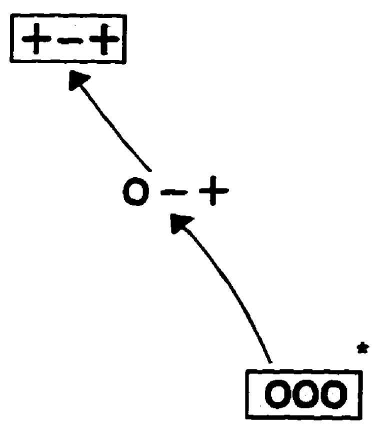

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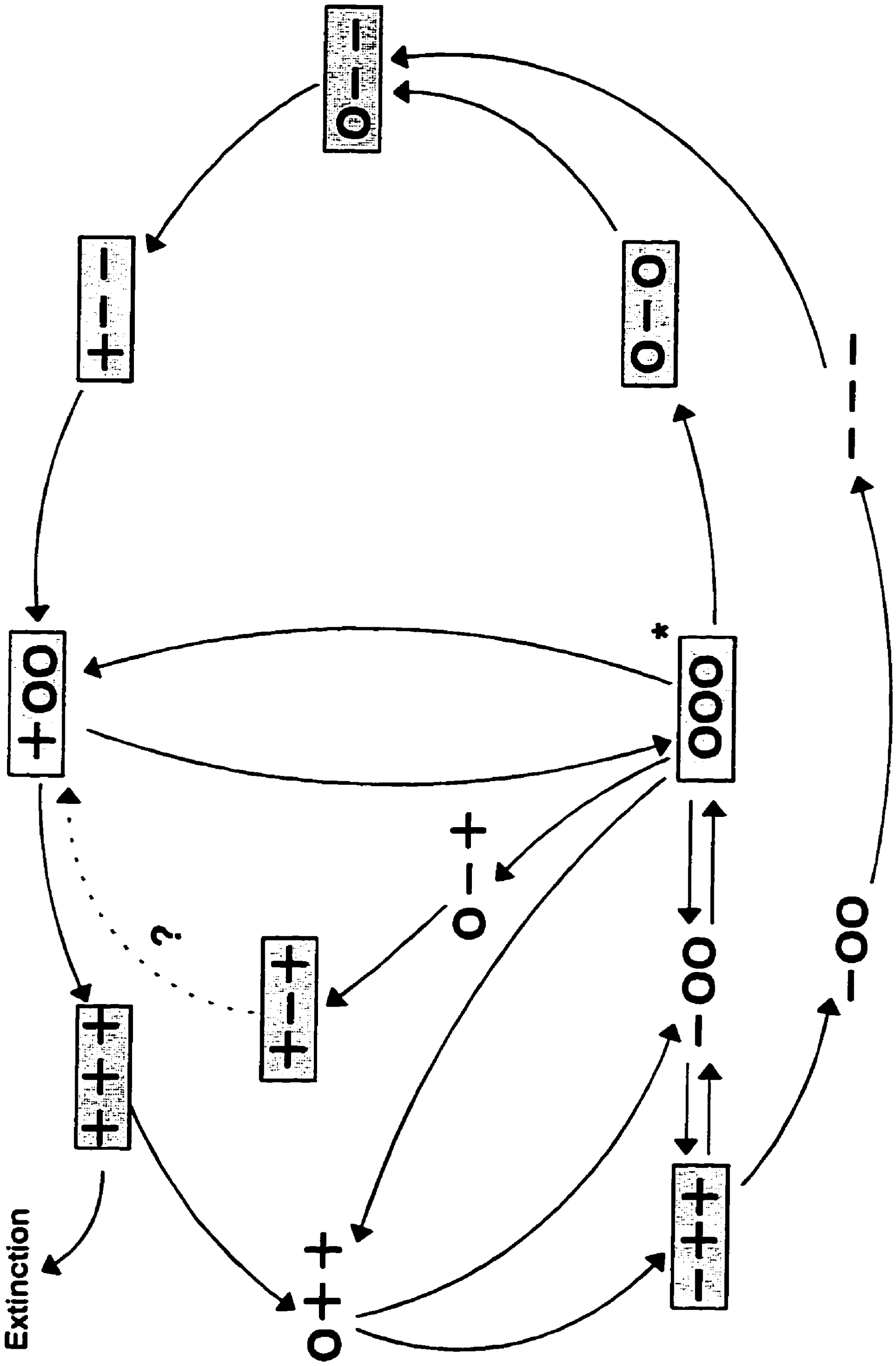
Figure 4.4. Synthesis of all response pathways integrated by linking the common relationships among each of the general responses to food and habitat availability, mortality and metabolic redistribution. The responses are abbreviated in terms of the relative change (- less than, 0 equivalent to, + more than) in age structure, energy expenditure and energy storage when compared with the reference sites or historical data. The asterisk signifies the starting point of the progression [000]; response patterns in shaded boxes represent patterns documented in the literature.


Extinction

populations (or historical data), identify the probable mechanism(s) of change, and indicate the specific portion of the population (if any) that has been affected. This information can then be used to focus the direction of follow-up studies designed to identify causative agents leading to the observed fish responses. For example, a response consisting of a decrease in energy expenditure (growth and reproductive investment) without concomitant changes in age structure or energy storage (0-0) is consistent with a response to decreased availability of food or habitat (Figure 4.1a). As such, appropriate follow-up studies should examine the quality/quantity of food and habitat resources, as well as investigating possible changes in competition for the available resources. Using the same approach, examples of follow-up study design considerations have been described for each of the dominant responses documented in the literature (Table 4.4).

It should be emphasized that this framework will only correctly discriminate patterns if the population being monitored is showing intimate interaction with the habitat in the study area. The framework will not be able to discriminate patterns in populations which are highly mobile or migratory at the time of sampling, since the factors controlling the physical characteristics of these fish are not associated with the habitat being sampled. As well, the success and reliability of the sampling effort depends on the adequacy of reference sites selected. If there are different stressors or density-independent regulatory factors between the exposed and reference sites, the response pattern identified will focus follow-up efforts on the main characteristics which are different between sites. If the conclusions of follow-up studies are that reference sites were not satisfactory, new reference sites will have to be selected, emphasizing greater similarity between reference and exposed sites.

4.4 Interpretation of Thesis Responses

As mentioned previously, the motivation for revising and further developing the response pattern framework was to facilitate the interpretation of some of the responses observed during my thesis research. In particular, the inconsistent response of trout-perch and white sucker from the

Table 4.4. Description of response patterns under the sentinel monitoring framework and examples of follow-up study design considerations.

Response Pattern[1]	Stressor/ Mechanism	Cause	Follow-up Study
-++	Increased food supply	decreased competition associated with increased adult mortality or eutrophication	examine food resource availability and population density
+00	Recruitment Failure	shift to older age classes associated with decreased reproductive success	detailed examination of spawning habitat and utilization, reproductive development
+--	Multiple stressors	independent impacts on reproductive success and food utilization	detailed studies of reproductive development and food resources
0--	Food Limitation	increased competition associated with increased reproductive success or decreased food availability	examine food resource availability and population density
0-0	Niche shift	modest increase in competition for forage base	examine food base and competition aspects
+-+	Metabolic Redistribution	metabolic disruption associated with inability to maximize utilization of available food resources	detailed physiological study of energetics
+++	Chronic Recruitment Failure	shift to older age classes, but food availability remains high	detailed study of reproductive performance
000	No Response		check population size data to see if carrying capacity of the system has changed

[1] based on changes in age structure, energy expenditure and energy storage relative to the reference sites

Kapuskasing River posed a problem, as well as the interpretation of the response of spoonhead sculpin exposed to kraft mill effluent in the Athabasca River. The following section attempts to address these specific issues using the framework as a tool for understanding how fish respond.

Moose River System - Kapuskasing River

The response of trout-perch downstream of the pulp mill and hydroelectric facility on the Kapuskasing River consisted of a decrease in mean age and growth (size-at-age) without concomitant changes in gonad size or condition. Using the response notation used in the framework, the response corresponds to a decrease in age and energy expenditure, but with no change in energy storage (i.e. --0). Initially, it appears that the response is not included in the framework. One might consider it to be a transitional pattern between -00 and --- in the progression of responses to adult mortality or increased food/habitat resource. The change in response from -00 to --- in both these pathways is initiated by a decrease in the amount of available resources resulting in an overall decrease in energy expenditure and storage (see. Section 4.3.2.1, *Increased Availability of Resources*). Although there was a decline in growth of trout-perch, there was no concomitant decline in gonad size, condition, or liver size indicative of an overall decrease in energetics.

Because of the conflicting results between growth and other measurements of energy expenditure and storage, it is helpful to consider each change (age, energy expenditure) of the response separately. Firstly, there is a decline in mean age. From the description of responses outlined in the framework, a decrease in mean age is commonly the result of an increase in adult mortality, or an increase in recruitment. Increased recruitment seems an unlikely mechanism because there is little evidence of increased reproductive effort (e.g., gonad size, steroid production) in the exposed population of trout-perch. In fact, forskolin-stimulated production of estradiol was depressed in females. However, there are other factors which could influence recruitment and need to be considered such as the number and quality of eggs produced, number of successful spawning individuals, availability and quality of spawning habitat, and survival/

growth of juveniles. It is also possible that the response is a function of chronic size-selective mortality of adults and/or faster growing fish; which is consistent with the absence of the older age class at the exposed site. From the data collected in Chapter 3, it is not possible to discern which mechanism is driving the change in age structure without further investigation. However, future follow-up studies should investigate these mechanisms, and increased adult mortality seems most likely.

The second alteration in the trout-perch was a decrease in size-at-age. Because there was no change in gonad size or condition, it is unlikely the decrease in growth was a function of reduced availability of food/habitat resources (see Figure 4.1a). It is possible that the growth response was related to the same mechanism associated with the decline in mean age (e.g., size selective mortality of faster growing and/or older fish), or a separate mechanism specifically affecting growth (e.g., chronic toxicity).

With the help of the framework a better understanding of the response has been achieved, as well as the identification of probable mechanisms leading to the response. From this information, follow-up studies can investigate these mechanisms for the purpose of determining the cause of the response. For example, work should be conducted to investigate adult mortality of trout-perch, specifically investigating related issues such as predation, exploitation or chemical toxicity. As well, aspects related to recruitment (e.g., spawning habitat and utilization, reproductive development) need to be investigated as possible factors influencing population age structure. Additional research should also focus on growth of trout-perch, particularly a physiological study of energetics and hormonal regulation of growth.

The response of exposed white sucker from the Kapuskasing River is not consistent with the response of trout-perch; however, several possible mechanisms leading to the responses are similar. Male sucker exhibited an increase in mean age, growth, condition and liver size (i.e. +++). The response of exposed female sucker was similar except that the increase in mean age was not significant, and there was a decrease in gonad weight (i.e. **0** +/- +). From information described in the framework, an increase in mean age can result from an increase in juvenile and/or early life stage mortality or decreased recruitment. There is evidence of reduced gonad size and depressed estradiol production in sucker suggesting possible reproductive problems which could influence recruitment. However, from the data collected in Chapter 3, size-selective mortality of younger, smaller individuals cannot be ruled out as a possible mechanism. There may even be a connection between juvenile mortality in sucker and adult mortality in trout-perch, given the similarity in size of affected individuals (e.g., predation). In either case, a reduction in population size would reduce competition for available food and habitat. In response, surviving fish should exhibit an increase in energy expenditure and storage, which is evident in white sucker as an increase in growth, condition and liver size. At the Kapuskasing site, it is also possible that the mill effluent and dam facility contribute to the increased availability of food and nutrients (i.e. enrichment effect). This is in part supported by recent studies conducted by B.A.R. Environmental Inc. (1996) which found that benthic fauna were more abundant downstream of the mill/dam and consisted of taxa characteristic of higher nutrient conditions than the reference community. In general, detailed follow-up studies should focus on determining whether white sucker are responding to altered reproductive performance and recruitment, or juvenile mortality. Additional work could also be conducted to investigate a possible enrichment effect related to the mill and/or dam.

Exposed female sucker exhibited increased size-at-age along with decreased ovary size. Both of these parameters are estimates of energy expenditure and tend to increase and decrease together in response to available energy. The conflicting results suggest problems associated with the utilization and allocation of available energy to somatic and gonadal growth (i.e. metabolic disruption). Interestingly, circulating levels of 17 β-estradiol were also reduced in exposed females. Since estradiol is involved in egg development and gonadal recrudescence (Wallace and Selman 1981), depressed levels may be related to the decrease in ovary size, regardless of the amount of energy available. Follow-up studies should focus on investigating aspects related to energetics and energy flow, as well as hormonal control of growth and reproduction.

Despite the differences in responses between trout-perch and white sucker, underlying mechanisms thought to be related to each response were surprisingly similar. The responses of both species highlighted potential problems associated with size-specific mortality and/or recruitment. Female white sucker provided additional evidence of metabolic redistribution that was not evident in trout-perch, although it could be argued that reduced growth in trout-perch coupled with no change in gonad size and condition represents some level of metabolic disruption. It was recognized that there were some differences regarding the direction of response (increased *vs* decreased recruitment), or what part of the population was being affected (adult *vs* juvenile mortality); however, the focus of follow-up studies designed to identify causative agents were similar regardless of what sentinel species was used. This is an important conclusion regarding the successful use of small fish species as a sentinel species. The results of the Moose River study suggest that, in a situation where mobility prevents a reliable interpretation of the response of white sucker, use of trout-perch as the sentinel species would highlight the same dominant areas of concern, and lead to similar follow-up studies.

Athabasca River - Hinton study area

In Chapter 2, research was conducted on the Athabasca River, Alberta, to investigate the response of spoonhead sculpin exposed to kraft mill effluent. After a prolonged period of high effluent exposure during the winter low-flow conditions, prespawning sculpin collected in the spring exhibited an increase in energy expenditure and energy storage relative to reference fish (i.e. 0++). From the description of responses outlined in the framework, the response of spoonhead sculpin seems indicative of an increase in the availability of food/habitat resources. An increase in available energy can occur due to a reduction in population size (i.e. reduced competition), or an increase in the source of food and/or availability of habitat. At the Hinton study site on the Athabasca River, both mechanisms are possible.

Food availability downstream of the mill diffuser may be higher than reference levels due to an enrichment effect related to the mill effluent. Effluent from the kraft mill represents a combination of treated wastewater from the mill (95%) and municipal sewage (5%) from the town of Hinton. Recent research using stream mesocosms has shown that a 1% dilution of the combined effluent from this mill significantly increased algal biomass relative to control streams, and mirrored what was observed in streams treated only with nitrogen and phosphorous (Podemski and Culp 1996). The results indicate that 1% effluent stimulates periphytic growth because of P and N loading from the effluent. An enrichment effect has also been inferred during several instream studies which have documented an increase in nutrient concentrations, algal biomass, benthos abundance, benthos richness, and size of benthic organisms downstream of the kraft mill diffuser (Anderson 1989, 1991; TAEM 1992; Podemski and Culp 1996). As such, the observed responses of spoonhead sculpin downstream of the mill may be related to eutrophication associated with the mill effluent.

Despite evidence of an increased food source, it is also possible that there are fewer fish at the exposure site competing for available food resources (i.e. relaxed competition), relative to the reference site. This hypothesis is partially supported by the catch data of spoonhead sculpin during the spring survey. At that time, approximately 1.29 sculpin were caught per minute of electrofishing at the reference sites compared to 0.62 sculpin per minute at the exposure site (KL) (Gibbons et al. 1996). A similar result was found for the incidental catch of other fish species. Although catch per unit effort (CPUE) is only a rough indicator of relative population size, the lower CPUE at the exposure site suggests that there are fewer spoonhead sculpin at this site. Interestingly, the mean age of exposed sculpin collected during the spring had decreased from the mean age documented during the previous fall collection. Furthermore, the mean age of reference fish was found to increase during the 6 month time period between sampling trips (see Chapter 2). A decrease in age may result from an increase in recruitment, an increase in adult mortality, or migration. Since there was no opportunity to increase recruitment over the winter through reproduction, and evidence collected during the fall and spring study suggest that spoonhead sculpin do not move large distances, an increase in adult mortality is the most likely mechanism. From the preliminary data presented in Chapter 2, the proportion of 5 and 6 y old sculpin declined from 35.5% of the total catch in the fall to only 8.3% in the spring, whereas the proportion of 3 and 4 y adult sculpin increased. It is possible that the observed loss of older sculpin may be related to exposure to high effluent concentrations during the winter low-flow conditions. Natural mortality of older individuals associated with the harsher conditions experienced during the over-wintering period does not seem likely given the absence of similar losses at the upstream reference sites.

Although it is possible that over-wintering mortality influenced the metabolism of spoonhead sculpin collected in the spring, it had no effect on the response of sculpin collected during the previous fall. Yet sculpin in the fall also exhibited a response of increased energy expenditure and storage. The similarity of the fall results suggest that some other mechanism is affecting the population size of sculpin at the exposed site, or that an increase in food source is a dominant influence on the response of sculpin. To determine which of the above mechanisms are influencing spoonhead sculpin exposed to the kraft mill effluent, future research should focus on investigating aspects related to the food base (enrichment), competition and population size, as well as mortality.

4.5 Conclusions

Sentinel Monitoring Framework

The framework is designed as a means to focus preliminary fisheries assessments and provides a mechanism to organize data and analyses. However, it does not represent a mechanism to formulate final conclusions. Field studies should be designed as an iterative process and this framework provides a mechanism to focus and design appropriate follow-up studies. Follow-up studies should focus on a) characterizing the observed response pattern, b) selecting the standardized pattern which is closest to the observed pattern, and c) examining the expanded patterns (individual fish measurements) for discrepancies between observed and predicted responses in the characteristics (Munkittrick and Dixon, 1989a).

The sentinel monitoring framework:

a) provides a mechanism for the use of traditional fisheries measurements to demonstrate significant changes prior to detailed sampling. The approach does not attempt to separate effects from various developments, but emphasizes interpretation of the integrated effects of all stressors on the populations at risk, and therefore ensures that studies are directed in a top-down, effects-oriented manner.

b) allows initial sampling to focus and direct follow-up studies to areas which are clearly affected. Since initial sampling is relatively inexpensive, this approach allows cost and effort to be conserved and subsequently focused on areas requiring detailed studies,

c) places the focus on interpretation of habitat changes associated with damage at the population level, and not on detailed initial characterization of stressors, allowing follow-up studies to focus on the habitat changes which are affecting the populations.

d) prevents expenditure of funds on follow-up studies at sites not showing changes, and therefore prevents collection of data sets on physiological changes and habitat alterations which are not translated to damage at the population level.

Response of Small Fish Species

Use of the sentinel monitoring framework permitted a more detailed interpretation of fish responses documented during my research on the Moose River system, Ontario, and Athabasca River, Alberta. From information described in the framework, I was able to simplify the responses, determine which part of the populations were being affected, and identify the probable mechanisms leading to the responses. Most importantly for my work, the interpretation of responses facilitated the evaluation of small fish species as sentinel species. Regarding the Moose River system, interpretation of the response of trout-perch indicated that the small species highlighted similar mechanisms of change identified by the response of white sucker, despite the obvious differences between the response patterns. The results of this comparison indicated that either species could be used as a sentinel species at this site; an important result for other situations where the mobility of the larger species is uncertain and use of small species is being considered. With respects to the Athabasca River, the interpretation of the response of spoonhead sculpin provided further evidence that a smaller fish species can show responses reflecting local conditions. Although cause-and-effect relationships could not be determined from the preliminary assessment, the interpretation of the response of sculpin was consistent with information described in previous studies investigating water quality, algal growth and benthos communities. In both cases, use of the framework provided an interpretation of responses needed to assess the suitability of the smaller fish as sentinel species.

CHAPTER 5

GENERAL DISCUSSION

The previous four chapters have presented the results of my research designed to conduct a field assessment of small fish species for sentinel monitoring. As outlined in the introduction, the overall objective of the thesis was to evaluate the suitability of small fish species as sentinel species for assessing the effects of pulp mill effluent specifically, and environmental stressors in general. However, it became obvious that additional work regarding the interpretation of fish responses was needed to complete the evaluation. A response framework was developed to facilitate the interpretation of fish responses documented during my thesis research, with the hope that it could be applied by other researchers collecting similar fisheries data. The following discussion reflects on the suitability of small fish species for sentinel monitoring, provides a summary of significant findings of the thesis, comments on the issues related to the framework, and provides some suggestions regarding future needs and research to be considered.

Suitability of Small Fish Species

The suitability of small fish species for sentinel monitoring can be considered from at least two perspectives: 1) do small species exhibit responses which accurately reflect instream conditions related to effluent exposure or other environmental stressors; and, 2) does the use of small fish species reduce problems commonly encountered when monitoring larger fish species. From information gained through my research, I conclude that small fish species may be successfully used as sentinel species, but may not represent the definitive solution for all monitoring situations.

The first and dominant question posed by the thesis was whether small species exposed to pulp mill effluent would exhibit differences in whole-organism and physiological measurements relative to unexposed reference fish. In other words, if small fish species are to be of any use as sentinel species, it is necessary to confirm that they are sensitive and responsive to conditions related to effluent exposure or other environmental stressors. From my research on the Athabasca River (Chapter 2) and Moose River system (Chapter 3) it was evident that the selected small species could show responses to ambient conditions. Spoonhead sculpin from the Athabasca River provided the first evidence of a small fish species exposed to bleached kraft mill effluent (BKME) exhibiting significant differences in age structure, body size, organ metrics, mixed function oxygenase activity and steroid production. Furthermore, graded responses were observed for sculpin exposed to different concentrations of effluent. Trout-perch collected downstream of thermomechanical pulp mill and/or hydrodam facilities on the Moose River system also exhibited significantly different responses from reference fish. Lake chub from the Athabasca River exposed to totally chlorine free pulp mill effluent was the only species examined that did not exhibit substantial differences in response relative to reference fish. Although it appeared that lake chub were not responsive to effluent exposure, water chemistry/plume delineation data and other fisheries data indicated that the response of lake chub was probably an accurate reflection of low instream concentrations of effluent.

Although small fish species were found to be responsive to effluent exposure, I wished to know whether the response of a small species was consistent with the response of larger species commonly used for sentinel monitoring. Although it could be argued that "a fish is a fish" regardless of size, it would be expected that the response of each species to a common stressor would be similar. However, given the differences in life history, longevity, habitat preferences, and resource utilization between many small and large fish species, it also seems likely that these differences may influence the sensitivity and/or tolerance of species to effluent exposure. Research conducted on the responses of exposed trout-perch and white sucker from the Moose River system indicated that the responses were not similar in terms of whole-organism and physiological measurements. Initially, the inconsistency posed a problem: which species response accurately reflected the conditions of the receiving environment? However, with the help of the interpretation framework developed in the thesis, it became more obvious that the responses of both species highlighted similar mechanisms of change and areas of concern. This conclusion provided additional support for the successful use of small species for sentinel monitoring.

The smaller fish species collected during the thesis research exhibited a strong contagious distribution associated with specific habitat (or microhabitat) preferences. For example, spoonhead sculpin were found only in swiftly flowing riffles and runs consisting of larger cobbles and boulders, whereas lake chub were found along the margins of the river where there was minimal water flow and where the substrate consisted of cobbles piled one on top of each other. In both examples, substrate composition and water flow appeared to be dominant characteristics and were useful for identifying potential sampling sites. The contagious or clumped distribution also provided some assurance that the local populations were distinct and that large scale movement between populations was unlikely. This was further supported by comparisons made between reference populations of spoonhead sculpin and lake chub from the Athabasca River which showed significant differences in whole-organism and physiological measurements. Habitat preferences for trout-perch from the Moose River system were less specific. They preferred sandy depositional zones of variable depth and water flow. Interestingly, reference populations of trout-perch were also similar in characteristics. In considering the suitability of small fish species as sentinel species, the ability to sample discrete populations within a small area may represent a distinct advantage when monitoring cumulative effects of multiple discharges within a limited section of a receiving environment.

A contagious distribution may also be a disadvantage in that sampling could be limited by the availability of the preferred habitat. This was true for trout-perch from the Mattagami River within the Moose River system. Although fish were collected downstream of the pulp mill and dam facilities, adult trout-perch could not be collected in sufficient numbers from the upstream reference zone. The poor capture success was attributed to sub-optimum habitat conditions, as well as limitations posed by night sampling and safe access to potential sampling sites. In contrast, white sucker were readily captured from this same area. A similar problem occurred during research I had previously conducted on the Fraser River, BC focusing on peamouth chub (Gibbons et al. 1995). Mature peamouth chub could be readily collected from multiple sites within the reference zone, but capture success was poor downstream of the pulp mills. Despite substantial sampling effort and use of a variety of sampling techniques, the lack of suitable habitat appeared to contribute to the poor sampling success of peamouth chub from this area.

Both examples suggest that use of smaller fish species may not necessarily ensure that capture success will be high. Despite the ecological generality that smaller fish species at lower trophic levels should theoretically be more numerous than larger fish species at higher trophic levels, site-specific conditions may influence the community composition at a particular location. As well, it is possible for a small fish species to be at a similar or higher trophic level relative to many larger species. Although capture of smaller species was successful on the Athabasca River, and for most sites on the Moose River system, use of small fish species may not completely solve the problem of obtaining sufficient sample sizes.

An important factor in assessing the suitability of small fish species is the assumption that smaller fish are generally less mobile than large fish species. I refer to it as an assumption because there are only a limited number of studies which have tried to document movement and home range of small fish species (e.g., Gerking 1959; Greenburg and Holtzman 1987; Hill and Grossman 1987; M. Spafford, Alberta-Pacific Ltd., Athabasca, Alberta, unpublished data). Regardless, by virtue of size and life history, it seems reasonable to expect that many small species do not exhibit the same degree of movement as larger species (Minns 1995). None of my research was designed to directly measure the movement of small species; however, ancillary evidence was collected which suggests that the mobility of the selected sentinel species was limited. For example, I observed significant differences in whole-organism and physiological measurements between reference populations of spoonhead sculpin and lake chub. This was particularly interesting regarding sculpin where the reference populations were on opposite sides of the river and only separated by the thalweg. In addition, the response of spoonhead sculpin collected from a site of intermediate effluent exposure (across river from effluent plume) was significantly different from the response of plume fish, suggesting limited lateral movement, and also different from the response of upstream reference fish, suggesting limited longitudinal movement. Presumably, if the sentinel species underwent extensive movement from one site to another, one would not expect to see distinct differences in fish characteristics reflective of site-specific environmental conditions, i.e. fish collected from any location would be part of the same mobile population.

Longevity of sentinel fish species is an important consideration when monitoring the effects of industrial discharges. It has been suggested that species of intermediate life span are preferred as sentinel species because they live long enough to show responses, but not too long to obscure impacts (Munkittrick 1992). This may have been the reason why I saw evidence of metabolic disruption in female white sucker, but no parallel response in trout-perch sampled in the Moose River system. White sucker live longer than trout-perch and potentially integrate more information over time. However, it is also possible that the response of trout-perch was more representative of current effluent conditions, whereas the response of white sucker may have been influenced by historical contamination or previous conditions of poorer effluent quality. As such, the shorter life span of most small species may be considered an advantage when trying to evaluate recent changes in environmental conditions. This has become more important because most of the pulp and paper industry has implemented various changes in process methodologies, bleaching agents and treatment facilities in an effort to improve the quality of effluent discharged to receiving environments. Since most of these changes have occurred within the last 5 years, the response of smaller and shorter-lived species may more accurately reflect the new effluent conditions, and enable one to evaluate possible improvements in effluent quality.

A secondary conclusion derived from this research was that it was possible to measure the same type of parameters on small fish species that are commonly measured on larger fish species. Obviously, there was no question that simple measures of body size and organ metrics could be collected, but estimates of age, MFO activity and steroid levels were less certain. Ageing of small fish species can be difficult because of the small size of ageing structures and limited life span. Otoliths proved to be the most useful ageing structure (spoonhead sculpin, trout-perch), followed by scales (lake chub). It was recognized that the age estimates may not have accurately reflected the absolute age of each fish (i.e. further validation would be required); however, I am confident that the ageing methodology was consistent, and that the relative ages of fish among sites were accurate. Induction of MFO activity was used in the thesis as a positive indicator of effluent exposure. Unfortunately, there was no published literature which documented measuring MFO activity in wild populations of freshwater small fish species such as cyprinids or cottids. The development of a laboratory protocol for measuring the inducing potential of specific fractions of pulp mill effluent using juvenile rainbow trout (Hewitt 1993) provided the necessary methods for measuring MFO activity of small livers <1.0 g. My research showed that an induced MFO response could be measured in small species exposed to pulp mill effluent, and that the measurement of activity in small livers could be improved if the assay was conducted using whole liver homogenate rather than trying to extract the microsomal fraction by centrifugation. Circulating plasma levels of sex steroids has become a common and informative measurement of fish exposed to industrial discharges (McMaster et al. 1992a). However, it is not possible to collect sufficient volume of blood from small fish to conduct the analysis. *In vitro* steroid production was used as a surrogate measurement and proved to be a successful alternative for my research on small fish species. This was further emphasized during research at the Moose River system where the steroid response of trout-perch estimated by *in vitro* procedures was very similar to the response of white sucker measured directly from blood plasma concentrations. The comparison was facilitated by the fact that both species spawn early in the spring and, therefore, were probably at similar stages of their maturation cycles during the fall survey. In general, the use of small fish species as sentinel species did not limit the type of data collected to describe whole-organism and physiological responses to exposure to pulp mill effluent.

In summary, the selected small fish species exhibited responses reflecting local environmental conditions, provided information similar to larger species, and represented a viable alternative to monitoring highly mobile species. The contagious distribution of the selected species also represents an advantage for the application to cumulative effects assessments. As well, the shorter life span of many small species may prove to be a useful trait for evaluating instream conditions following recent changes to effluent quality. However, I recognize that small fish species may not represent the definitive solution for all monitoring situations. The unsuccessful attempts to collect trout-perch from the upper Mattagami River provides an obvious example. The use of small fish species for sentinel monitoring does, however, represent an additional tool at our disposal to facilitate environmental impact assessments.

Sentinel Monitoring Framework

Although sentinel species monitoring represents a quick and cost-effective approach, the success of this type of monitoring is contingent on understanding what the response of the specific sentinel organism means. For sentinel fish species, this has been facilitated by the wealth of information and literature available describing life history and general biology of numerous species. As well, these traditional fisheries data have been supplemented with a great deal of research investigating the effects of a variety of contaminants and other stressors on fish. However, despite this knowledge base, the response of fish to environmental stressors is not always easily understood or interpreted. The framework described in Chapter 4 was developed to facilitate the interpretation of responses of sentinel fish species using traditional fisheries data. Effort was made to revise and reorganize an existing framework so that it was easier for me, and hopefully others, to understand the responses and the possible mechanisms behind the observed changes in fish characteristics.

An important revision to the previous framework outlined by Munkittrick and Dixon (1989a, 1989b) is the description of the progression of fish responses to changes in food and habitat availability, size selective mortality, and physiological impairment. The progression of responses, or response pathways, effectively links fish responses which have been documented in the literature. However, it should be emphasized that these linkages and response pathways are theoretical. The pathways were developed using traditional fisheries data and knowledge of population dynamics, but no work has specifically tested whether the response of fish would progress in the way described in the framework. As well, many of the intermediate response patterns have not been documented in the literature as yet, and may not represent a stable stage in the progression of responses. Although the relationships between response patterns may be hypothetical, the framework developed from these patterns represents a useful model for interpreting results of fisheries data, and was instrumental in understanding fish responses documented in the thesis.

As with any conceptual model, it was necessary to make a few assumptions in the development of the interpretation framework. One such assumption is that the progression of responses described in the framework result from density-dependent regulation. This is not to say that density-independent factors are not important in regulating the fish populations, but that these factors will generally act equally on the reference and stressed populations under investigation. The strength of this assumption increases when reference fish are collected from the same water body as the receiving waters (e.g., reference zone located upstream of the exposure zone), the sites are as similar as possible in all characteristics except the presence of the stressor, and the distance between reference and exposure sites is minimized. Under these conditions, it is assumed that the response of the monitored fish population will reflect changes associated mostly with density-dependent factors, and the framework was developed under this premise. A more fundamental assumption identified by Munkittrick (1988) was that the stressor or stressors will, in some way, affect growth, survivorship or reproduction of the exposed population. This is a reasonable assumption in that these aspects of life history have been identified as common impact points (e.g., Ricker 1975; Larkin 1978; Colby 1984; Seim et al. 1984; Birge et al. 1985). Alterations to fish behaviour which leads to avoidance of a location may be the most notable exception to this assumption. However, it is the response of those fish which remain and continue to be exposed to the stressor which are being monitored, and which are important to understand.

The framework proved to be instrumental in my understanding of the results of my studies at the Moose River system, as well as the response of spoonhead sculpin from the Athabasca River. Despite the framework's theoretical nature and assumptions, it incorporates a great deal of basic biology and traditional fisheries information into the interpretation, and provides the researcher a better understanding of the possible mechanisms involved.

Future Considerations

As is often the case when conducting research, one is confronted with a host of new questions and considerations arising from the results of the initial investigation. Fortunately, it is this process that allows research to progress. From the point of view of furthering the research on the suitability of small fish species, the sentinel species approach, and the interpretation framework; there are several issues which merit further consideration. The following list of considerations represents some of the issues which became obvious during the development and progression of the thesis research:

- Very little information exists on the growth rates, reproductive strategies, and habitat requirements of small fish species. Baseline data on the biology of these species needs to be collected to facilitate the use of small fish species for monitoring the impacts of industrial discharges.

- Results presented in the thesis demonstrate that there can be substantial variability in whole-organism responses among reference populations of small fish species (e.g., spoonhead sculpin, lake chub). More effort needs to be directed towards establishing the full range of variability associated with reference fish within a monitoring system.

- More information is needed to further evaluate the mobility of smaller fish species. Although small species are less likely to be as mobile as many of the large fish species, it is still necessary to increase our understanding of the degree and pattern of mobility, size of home range and habitat requirements for different seasons and phases of the life cycle.

- Age structure or mean age is an important and influential parameter in the interpretation of fish responses. Because age data can be variable and sensitive to sampling biases, increased sampling effort is needed to obtain an accurate estimate. The amount of effort will depend on the species and receiving environment, but greater consideration needs to be made to ensure the reliability of this estimate. As well, more work is needed to identify appropriate methods for ageing small fish species.

- Although the impact status of all species of fish is important to aquatic ecosystems, perhaps greater consideration should be given to understanding how well the selected sentinel species represents the impact status of other fish species, or the aquatic system. Issues related to sensitivity and tolerance to specific stressors need to be considered so that the most sensitive link in the system is protected.

- Additional research could be conducted to investigate the validity of the response pathways described in the interpretation framework. As mentioned, the progression of fish responses are theoretical constructs developed from existing fisheries data; however, research testing the progression of fish responses would be beneficial.

- A natural progression for the research presented in the thesis is the application to cumulative effects monitoring. To date, there has been difficulty in making definitive statements regarding the source of problem(s) when there are multiple users of a receiving environment. The research on small fish species will be useful for developing methodologies amenable to multiple discharge conditions.

My final comment concerns the future direction of environmental monitoring and assessment. Increasing emphasis has been placed on conducting environmental effects monitoring programs to assess the impacts of industrial discharges on receiving environments. These studies have highlighted a number of problems in water and habitat quality which have resulted in effects at all levels of biological organization. Although my thesis has focused on improving and refining techniques for monitoring, I also feel more emphasis needs to be placed on developing solutions and mitigative measures to the problems which have been documented. Whether solutions take the form of technological advancements, reduction/removal of the contamination source, policy changes or some other approach; the next logical step from monitoring is to address the observed problems (if any) and minimize the environmental risk. In my view, there is little value in monitoring and identifying impacts over time unless we are willing to be involved in the next logical step of addressing the problems.

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APPENDIX A

Maps showing the location of each fish collection site on the Athabasca River, Alberta, 1994-1995.

Figure A.1. Location of spoonhead sculpin (*Cottus ricei*) collection sites RL, RR, KR and KL in the Hinton study area, Athabasca River, Alberta, 1994-1995.

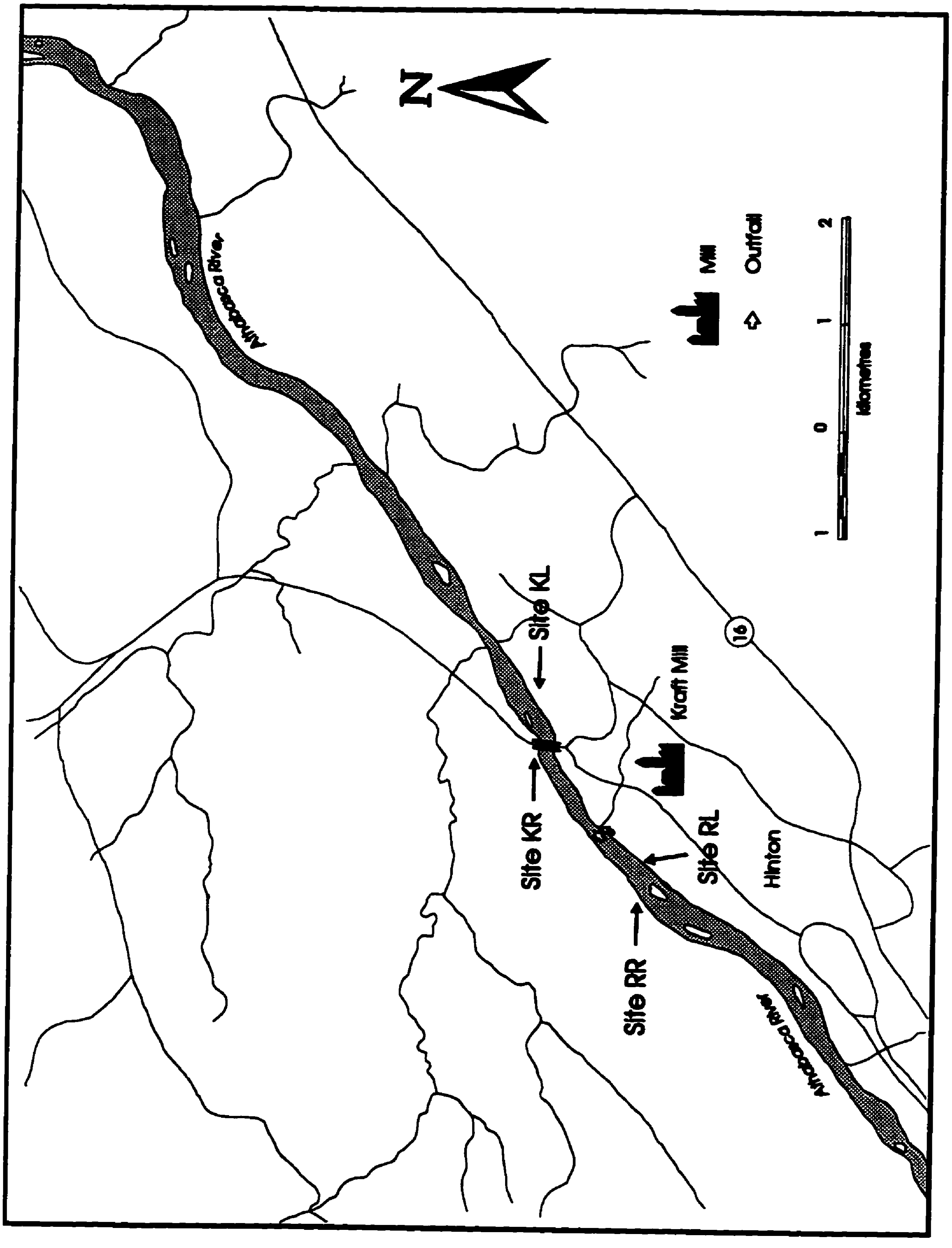

N
Athabasca River
Site KL
Site KR
Kraft Mill
Site RL
Site RR
Hinton
16
Athabasca River
Mill
Outfall
1
0
1
2
Kilometres

Figure A.2. Location of spoonhead sculpin (*Cottus ricei*) collection sites CL and CR (combined to site C) in the Hinton study area, Athabasca River, Alberta, 1995.

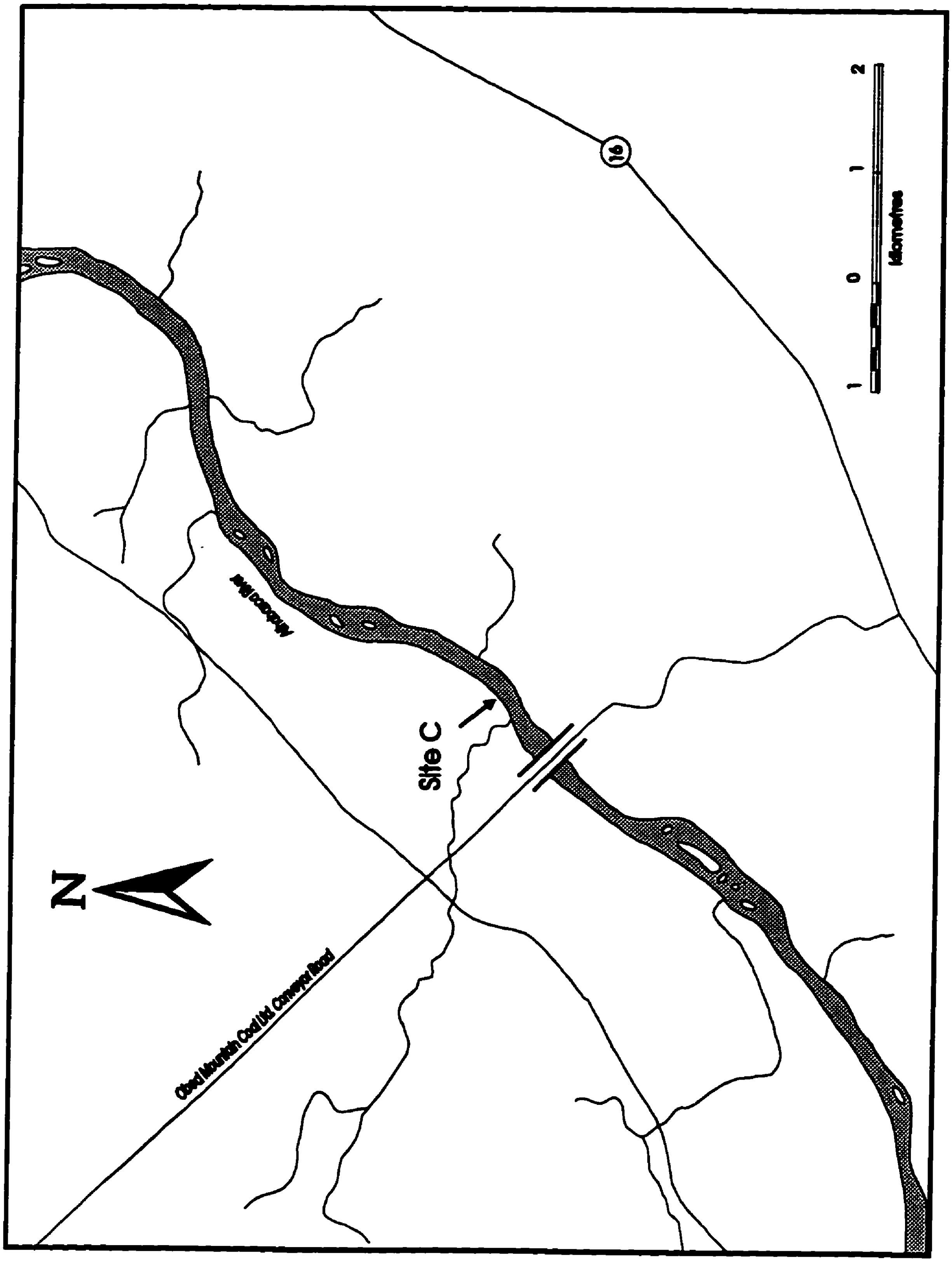

N
Site C
Obed Mountain Coal Ltd. Conveyor Road
16
1
0
1
2
Kilometres

Figure A.3. Location of the spoonhead sculpin (*Cottus ricei*) collection site E in the Hinton study area, Athabasca River, Alberta, 1995.

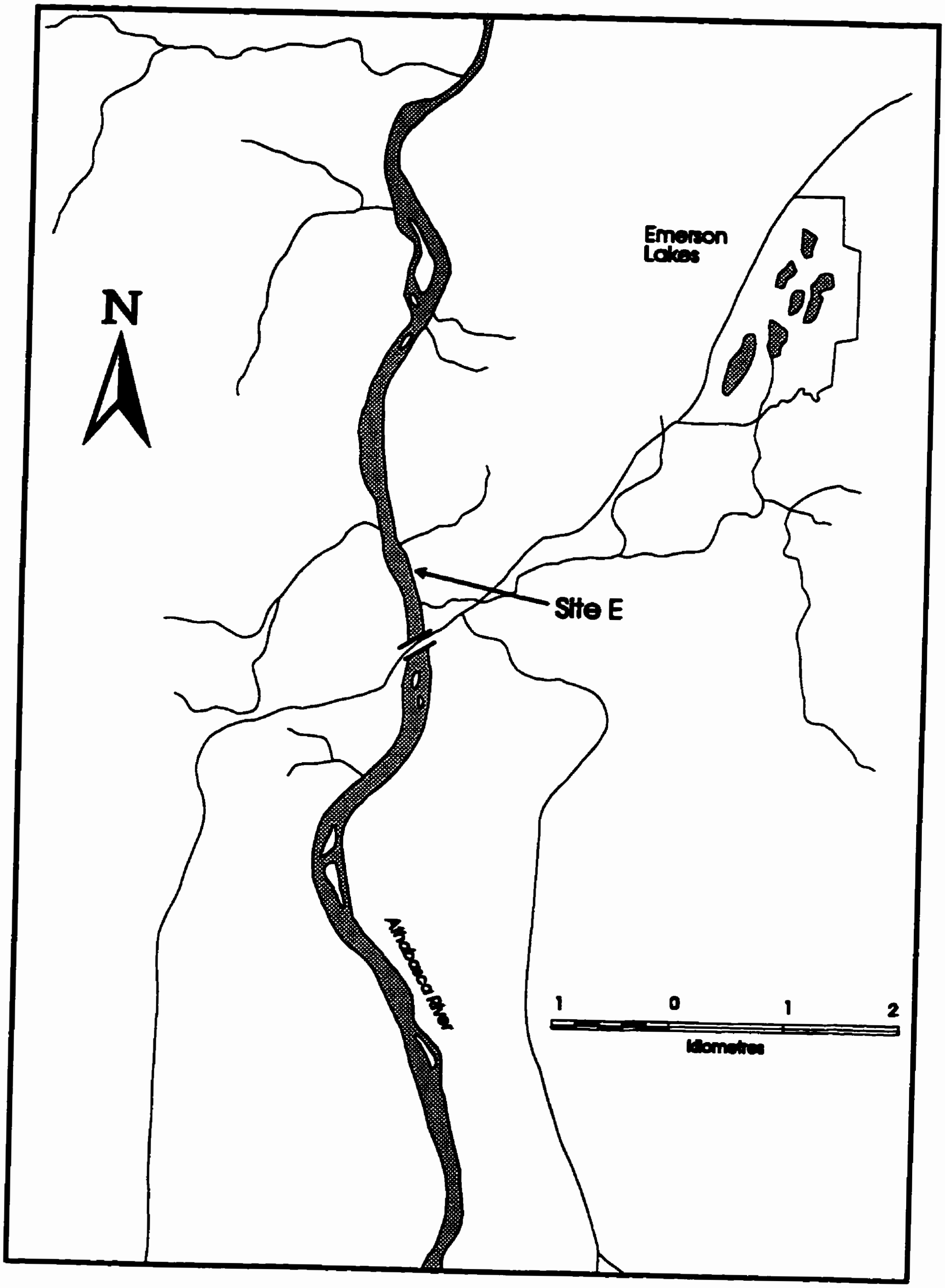

Emerson
Lakes
N
Site E
Athabasca River
1 0 1 2
kilometres

Figure A.4. Location of the lake chub (*Couesius plumbeus*) collection site WF in the Whitecourt study area, Athabasca River, Alberta, 1994.

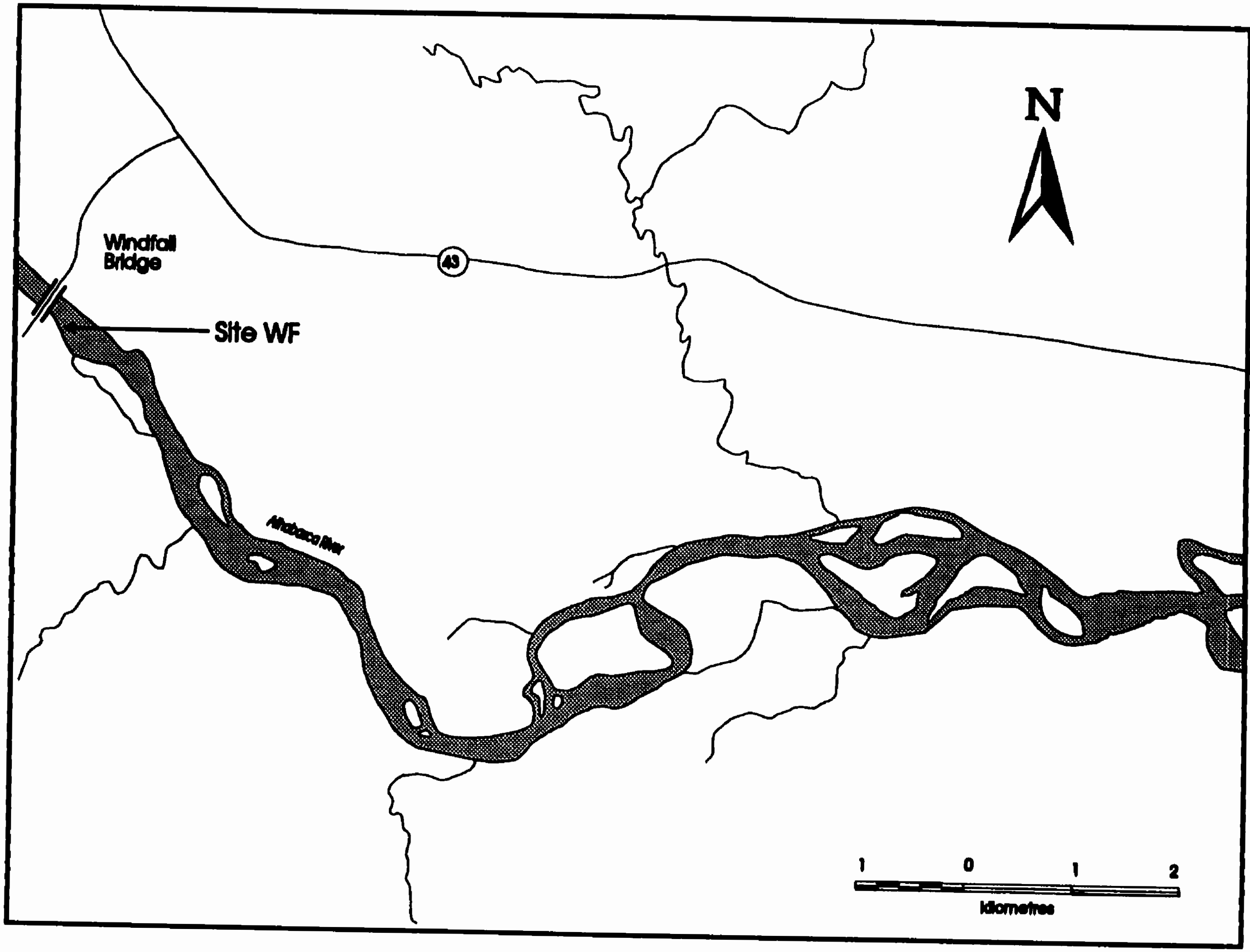

N
Windfall
Bridge
Site WF
43
Athabasca River
1
0
1
2
kilometres

Figure A.5. Location of lake chub (*Couesius plumbeus*) collection sites R2 and TMP in the Whitecourt study area, Athabasca River, Alberta, 1994.

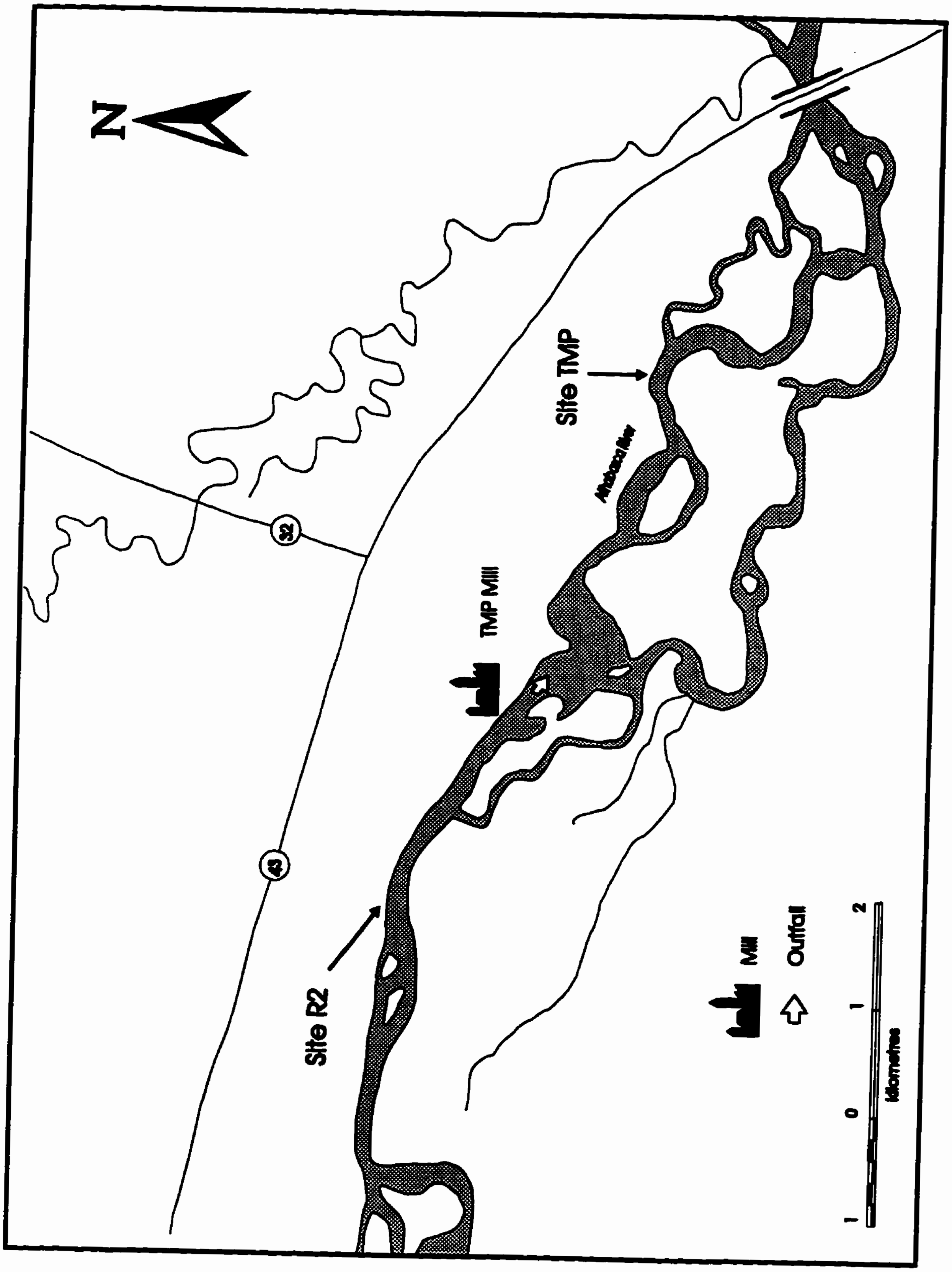
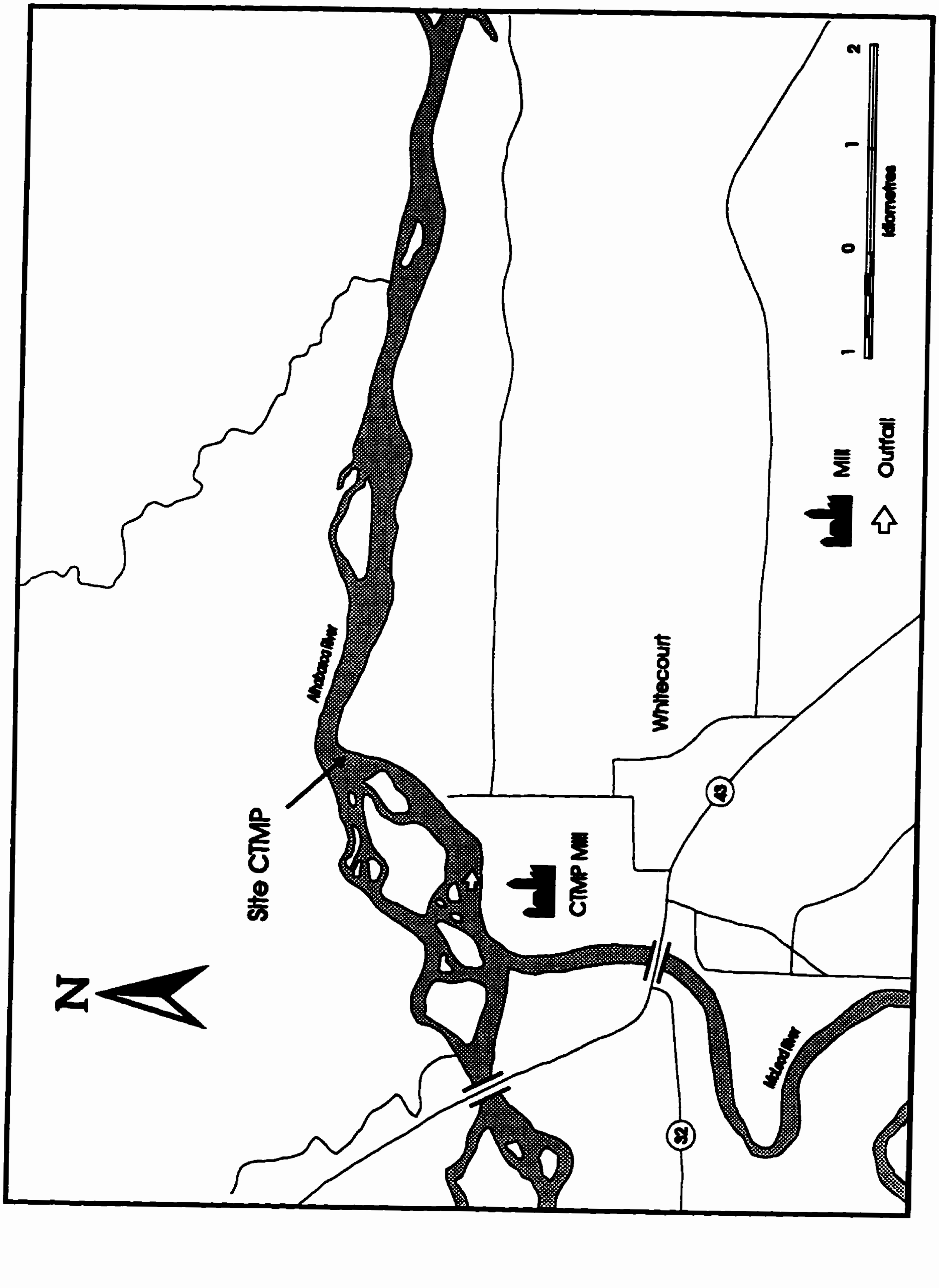

N
Site TMP
TMP Mill
Site R2
32
43
Mill
Outfall
1
0
1
2
Kilometres

Figure A.6. Location of the lake chub (*Couesius plumbeus*) collection site CTMP in the Whitecourt study area, Athabasca River, Alberta, 1994.


N
Site CTMP
CTMP Mill
Athabasca River
Whitecourt
McLeod River
43
32
Mill
Outfall
1 0 1 2
Kilometres

APPENDIX B

Table B.1. Mean and SE (n) of age, body size, organ metrics, EROD activity and *in vitro* sex hormone production for trout-perch (*Percopsis omiscomaycus*) collected from the exposure site (MTDS) on the Mattagami River, fall 1995, Moose River system, Ontario.

Sex	Mattagami River MTDS
Male	
Fork Length (cm)	6.2 ± 0.1 (33)
Body Weight (g)	2.89 ± 0.20 (33)
Carcass Weight (g)	2.66 ● 0.19 (30)
Age (y)	3.2 ● 0.2 (32)
K	1.10 ± 0.02 (30)
GSI (%)	2.17 ± 0.10 (30)
LSI (%)	1.67 ± 0.06 (30)
EROD (pmol/mg protein/min)	1.93 ● 0.14 (29)
Testosterone (pg/20mg tissue)	20.96 ● 2.22 (15)
Female	
Fork Length (cm)	6.2 ● 0.1 (30)
Body Weight (g)	2.91 ± 0.16 (30)
Carcass Weight (g)	2.63 ± 0.14 (28)
Age (y)	3.3 ● 0.1 (29)
K	1.08 ● 0.01 (28)
GSI (%)	4.28 ± 0.24 (26)
LSI (%)	2.34 ± 0.08 (28)
EROD (pmol/mg protein/min)	1.89 ± 0.22 (29)
Testosterone (pg/20 follicles	14.76 ± 1.21 (15)
Estradiol (pg/20 follicles)	13.50 ● 2.33 (14)