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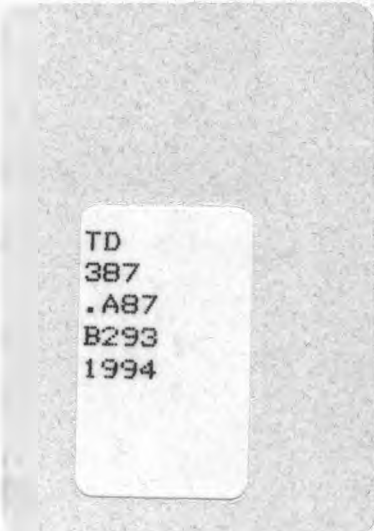
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# Northern River Basins Study



NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 29  
**DISSOLVED OXYGEN  
REQUIREMENTS FOR FISH  
OF THE PEACE, ATHABASCA  
AND SLAVE RIVER BASINS**





Prepared for the  
Northern River Basins Study  
under Project 3211-B1

by  
B.A. Barton and B.R. Taylor  
Environmental Management Associates

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REQUIREMENTS FOR FISH  
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Published by the  
Northern River Basins Study  
Edmonton, Alberta  
February, 1994

ATHABASCA UNIVERSITY

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## CANADIAN CATALOGUING IN PUBLICATION DATA

Barton, B. A.

Dissolved oxygen requirements for fish  
of the Peace, Athabasca and Slave River basins

(Northern River Basins Study project report,  
ISSN 1192-3571 ; no. 29)

Includes bibliographical references.

ISBN 0-662-22036-6

DSS cat. no. R71-49/3-29E

1. Water -- Dissolved oxygen.
  2. Water quality -- Alberta -- Athabasca River.
  3. Water quality -- Peace River (B.C. and Alta.)
  4. Water quality -- Slave River (Alta. and N.W.T.)
- I. Taylor, Barry R. (Barry Raymond)  
II. Northern River Basins Study (Canada)  
III. Title. IV. Series

TD387.A83B37 1994      553.7'8'097123      C94-980102-X

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## **PREFACE:**

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

It is explicit in the objectives of the Study to report the results of technical work regularly to the public. This objective is served by distributing project reports to an extensive network of libraries, agencies, organizations and interested individuals and by granting universal permission to reproduce the material.

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**NORTHERN RIVER BASINS STUDY  
PROJECT REPORT RELEASE FORM**

This publication may be cited as:

Barton, B.A., and Taylor, B.R., Environmental Management Associates,  
"Northern River Basins Study Project Report No. 29, Dissolved Oxygen  
Requirements for Fish of the Peace, Athabasca and Slave River Basins",  
Prepared for the Northern River Basins Study, Edmonton, Alberta,  
February, 1994.

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Northern River Basins Study and the terms of reference for that work are  
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28 Jan 1994  
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(Lucille Partington, Co-chair)

10 February 1994  
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(Date)





# DISSOLVED OXYGEN REQUIREMENTS FOR FISH OF THE PEACE, ATHABASCA AND SLAVE RIVER BASINS

## STUDY PERSPECTIVE

Dissolved oxygen is one of the most commonly monitored indicator of water quality and is a major factor affecting the distribution and health of fish communities within aquatic ecosystems. An immediate concern exists with respect to the impact of existing development effluent discharge contributing to diminished dissolved oxygen levels in the middle reaches of the Athabasca River (Hinton to Grand Rapids) and how this affects resident and transient fish populations.

Previous reviews have recommended studies to investigate the dissolved oxygen requirements of fish species resident to the Athabasca River. Currently little is known about the specific dissolved oxygen requirements of many of the fish, and their various life stages, found in the Peace-Athabasca-Slave river basins. Even less is known about how these dissolved oxygen requirements are affected by other factors, e.g., contaminants, flow regulation. A comprehensive project consisting of laboratory and field studies was proposed to address the information shortfall on dissolved oxygen.

This literature review was conducted to guide subsequent laboratory and field studies. Areas of insufficient information have been identified and candidate fish species were recommended for more detailed investigation because of their sensitivity to diminished dissolved oxygen values. Species included: bull trout, mountain whitefish, rainbow trout, burbot, longnose sucker. As a consequence of this review, subsequent laboratory work has focused on two fall spawning species, bull trout and mountain whitefish. These initial species were chosen because their eggs undergo development during ice cover when dissolved oxygen values are most diminished both naturally and by development impact.

### *Related Study Questions*

- 7) *What concentrations of dissolved oxygen are required seasonally to protect the various life stages of fish, and what factors control dissolved oxygen in the rivers?*
- 10) *How does and how could river flow regulation impact the aquatic ecosystem?*
- 13a) *What predictive tools are required to determine the cumulative effects of man made discharges on the water and aquatic environment?*
- 14) *What long term monitoring programs and predictive models are required to provide an ongoing assessment of the state of the aquatic ecosystems?*



## **EXECUTIVE SUMMARY**

Many factors affect the dissolved oxygen (DO) content of surface waters, including photosynthesis, respiration, temperature, ice cover, mixing and input of organic materials. In northern Alberta, the discharge of pulp mill wastes with a high biochemical oxygen demand into river systems can result in depletion of DO supplies in winter and increased concentrations of contaminants in the water. A need therefore exists to examine DO requirements of northern rivers fishes considering all life stages and interactions with other stressors, and to document the combined effects of low dissolved oxygen and contaminants on fish populations.

A number of major reviews have been conducted on the general subject of dissolved oxygen and fish. The goal of this project was to establish dissolved oxygen requirements for fish species found in northern Alberta rivers, concentrating on environmental conditions found there, particularly the presence of pulp mills and winter ice cover. Material for this review was compiled from an on-line computer search of major pertinent data bases and from printed literature reference services. The report is subdivided into a number of sections that discuss: (1) physiology of fish respiration, (2) limnology of dissolved oxygen in surface waters, (3) effects of adverse DO conditions on fish and invertebrates, (4) modifying effects of other environmental factors, and (5) individual species and life stage requirements of northern rivers fishes.

The primary site of gas exchange in teleost fish is the gill membrane; the diffusion of oxygen into the blood is aided by the high oxygen-binding capacity of hemoglobin. Gas exchange depends on the oxygen tension gradient, or partial pressure, between blood and water. Oxygen tension in the water, in turn, depends not only on the DO concentration, but on other physical conditions, particularly water temperature and atmospheric pressure. Large rivers generally maintain dissolved oxygen levels at or near saturation during open-water

periods by reaeration. Under winter ice cover, however, DO levels often decline as a result of decomposition of organic matter; DO sags increase with downstream distance in the river.

The detrimental effects of low dissolved oxygen on fish are well known. The fundamental effect at the cellular or tissue level is hypoxia, which occurs when the rate of oxygen delivery to the cells is less than that required to meet metabolic needs. Death results when insufficient oxygen delivery to the brain and other tissues results in cellular dysfunction. At the sublethal level, low environmental dissolved oxygen may cause alterations in physiological processes, blood chemistry and hematology, and result in histopathological damage. Moreover, exposure of fish to prolonged hypoxic conditions can lead to increased incidence of disease, reduced growth and decreased swimming performance, as well as altering predator avoidance, feeding, migration, reproduction and other behaviours. Just as important, adverse DO conditions may depress long-term production of fish populations by altering benthic invertebrate communities, especially food species.

The detrimental effects of suboptimal dissolved oxygen levels are exacerbated by the presence of contaminants in the environment, including those typically found in pulp mill effluent. This effect of contaminants may be direct, such as by interfering with normal oxygen uptake across the gill membrane, or indirect, by elevating metabolic rate, thereby increasing oxygen demand and reducing the fish's scope for activity. Fish exposed to reduced environmental dissolved oxygen display increased susceptibility to other toxicants and contaminants, notably those in pulp mill wastes. Increased gill ventilation in response to low DO further increases the rate of contaminant uptake.

All species and life stages of fish have both acute and chronic dissolved oxygen requirements. Acute requirements are the minimal levels of dissolved oxygen necessary to avoid short-term mortality. However, chronic requirements are more important ecologically as long-term exposure to suboptimal DO levels, even those well above levels necessary for survival, create stress and decrease growth and population production. Any reduction in the natural DO

content of surface waters is likely to have some detrimental effect on fish production; thus, the assumption of a "no effect" DO level below saturation is not valid.

Acutely lethal DO levels are relatively straightforward to measure and, based on available evidence on tolerances of adult fish, northern rivers species are grouped into categories of DO sensitivity as follows: (1) Sensitive (acute limit  $>2$  mg/L DO; includes all salmonids, longnose sucker and burbot); (2) Intermediate (acute limit 1-2 mg/L DO; includes all cyprinids except fathead minnow, walleye, white sucker, brook stickleback and goldeye); (3) Tolerant (acute limit  $<1$  mg/L DO; includes fathead minnow, northern pike and yellow perch); and (4) Unknown (insufficient information available; includes largescale sucker, ninespine stickleback, all sculpins and trout-perch).

The establishment of chronic dissolved oxygen requirements, which are more important to the long-term maintenance of healthy fish communities, is more problematic. Until more complete regional data are available, general DO criteria recommended in earlier reviews of this subject (e.g.,  $\geq 6$  mg/L for salmonids,  $\geq 5$  mg/L for nonsalmonids; Taylor and Barton 1992) are probably applicable to fish of the northern rivers of Alberta. Although considerable information exists on DO requirements for rainbow trout, and to a lesser extent for walleye, northern pike and fathead minnow, information on the requirements for the other northern river fish species is either very limited or lacking completely. Thus, it is recommended that future research efforts concentrate on these other species, as they are integral components of the fish community. Rainbow trout and bull trout (adults) and mountain whitefish (eggs and larvae) are recommended as sentinel species to monitor dissolved oxygen conditions. Burbot and longnose sucker might also be considered if their suspected DO sensitivities can be confirmed and they are sufficiently common in the northern rivers.

## **ACKNOWLEDGEMENTS**

Technical assistance for gathering and compiling the literature used in this review was provided by Mr. Kevin Egan and Mr. Dave Fernet provided valuable advice on ecology of northern Alberta fishes. Comments from three anonymous reviewers also substantially improved the final manuscript.

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## 1.0 INTRODUCTION

Historically, dissolved oxygen (DO) criteria for protection of aquatic life were applied as single numbers defining fixed limits to be met at all times without particular reference to individual species or duration of exposure (EPA 1976; IJC 1976). This approach was a reasonable first approximation, especially given the newness of the field and the level of knowledge of aquatic ecology at the time. In the past decade there have been large advances in our understanding of the dynamics of aquatic ecosystems and their component species. As early as 1975, it was realized that the traditional approach was deficient and that diverse criteria were necessary to protect different fish communities, specifically cold-water and warm-water fishes, and different life stages (Davis 1975).

Increasing human demands on waterbodies have required that protection criteria be defined more rigorously. Dissolved oxygen requirements of aquatic species vary according to season, age, health, and stage of the life cycle (EPA 1990). Therefore, any fixed, single-number criterion risks providing insufficient protection at one time, while being overly protective at another. Some life stages of fish, in particular embryos and newly hatched alevins, have higher DO requirements than adults (EPA 1990). For migratory fish species, such as most salmonids, the seasonal presence of the fish in any given reach is a further argument against fixed water quality criteria: it is inefficient and unnecessary to comply rigidly with a fixed water quality criterion when the species (or life-stage) it is designed to protect is not present. On the other hand, sustaining healthy fish populations in the long term requires that other non-migratory components of the biotic community such as benthic invertebrates also be protected.

Recent documents have established suites of criteria with varying DO limits according to fish family or temperature preference, life stage, and degree of risk to the population (EPA 1986; CCREM 1987). The most recent criteria from the U.S. Environmental Protection Agency (EPA), upon which the CCREM (1987) guidelines are based, proposed limits for

warm-water and cold-water fishes for the 1-day minimum, 7-day mean minimum, and 30-day mean DO concentrations (EPA 1986). The EPA guidelines are based on a thorough review of the literature up to that date and are presently the most advanced general criteria for DO levels.

General DO criteria need to be refined and adjusted to apply to particular species or river basins. The health of the populations, species composition, environmental conditions and the nature and magnitude of any other stressors to which fish are exposed need to be considered in the establishment of DO requirements specific to species, life stage or geographic area. Local DO criteria for selected sport fishes that are specific to both the species's life stages and the duration of exposure have been developed for Alberta (Taylor and Barton 1992). Although province-wide in scope, the criteria were developed for application to southern Alberta rivers where water quantity, not quality, is chiefly limiting to fishes. Similar DO criteria are required that are specific to the fish fauna in rivers of northern Alberta, particularly in those that receive effluents from pulp mills, sewage treatment facilities, or other point-sources of discharge. These waters support a variety of fish species from many different families, and with different habits and habitats; some species are not found elsewhere in the province.

Many factors cause changes in DO content of surface waters, including photosynthesis, respiration, temperature, ice cover, turbulent mixing, and input of organic materials requiring oxygen for aerobic decomposition. In northern Alberta rivers, the discharge of pulp mill wastewaters characterized by copious volume and high biochemical oxygen demand (BOD) can lead to both increased levels of contaminants in the water and sediments and to depletion of DO reserves under winter ice. Hence, there is a need to document the combined effects of low DO and contaminants on fish populations (Birtwell 1989), and to examine DO requirements for fishes in these rivers in the context of other disturbances acting on the fish and their various life stages.

The goal of this project therefore was to establish DO requirements for fishes found in the Northern River Basins Study (NRBS) area. Published data on the DO tolerances, requirements, and preferences for the fish species that occur in the Peace, Athabasca, and Slave rivers and their major tributaries were reviewed, along with general effects of DO variations on sensitive life stages of fishes under pristine and contaminated conditions. The available data were compiled and evaluated as to their applicability and completeness with respect to life stages and different biological functions. There have been a number of major reviews on the subject of DO and fish (Doudoroff and Shumway 1970; Davis 1975; Hughes 1981; Alabaster and Lloyd 1982; Chapman 1986); a thoroughgoing review of the subject is therefore unnecessary and beyond the scope of this report. Rather, the present report concentrates specifically on the fishes and environmental conditions found in the northern rivers of Alberta, and in particular on effects of pulp mill contaminants and winter ice cover.

To compile the material for this review, a number of on-line data bases were consulted using the CAN/OLE system, a computer literature-search service provided by subscription through the National Research Council. Data bases searched were BIOSIS, ASFA (Aquatic Sciences and Fisheries Abstracts) and NTIS (National Technical Information Service). In addition to on-line searches, the entire contents of Current References in Fisheries Research (1976 - 1992; covers about 320 journals) and Fisheries Review (formerly Sport Fisheries Abstracts) as far back as 1965 were reviewed for pertinent literature. Much of the material already reviewed for an earlier report on selected Alberta fishes (Taylor and Barton 1992) was incorporated into this report. Researchers throughout North America were contacted to identify and document research in progress that addresses DO requirements of species known in the NRBS area.

All references pertaining to the DO requirements of northern river fishes have been compiled in a computerized, cross-indexed, bibliographic data base, for which a user manual is provided. Scientific nomenclature of fishes has been updated to reflect the most recent

changes adopted by the American Fisheries Society for both North American and other species as described in Robins et al. (1991a,b).

This report is presented in separate sections. Background sections are provided to acquaint readers with the (1) anatomical features and physiological mechanisms associated with oxygen uptake (Section 2), and (2) limnological features that relate to oxygen concentration and saturation of surface waters (Section 3). Subsequent sections of the report describe (1) lethal and sublethal effects of reduced DO conditions on aquatic fauna (Section 4), (2) other environmental factors that modify organisms' responses to adverse DO (Section 5), and (3) DO requirements of individual species of northern rivers fishes and their life stages. (Section 6).

## **2.0 ANATOMY AND PHYSIOLOGY OF RESPIRATION IN FISH**

### **2.1 MECHANICS OF OXYGEN UPTAKE**

Teleost fishes in general have four pairs of gill arches. Four gill arches, one from each pair, are located in a common opening on each side of the head and covered by an operculum. Multiple pairs of gill filaments are anchored to each gill arch to form two rows, and numerous plate-like secondary lamellae are attached to each filament. Each row of filaments is called a hemibranch and two hemibranchs form a holobranch. Abductor and adductor muscles allow the fish to spread and collect hemibranchs on an individual gill arch (Stoskopf 1993). The primary site of gas exchange in salmonids is across the secondary lamellar surface. Within each lamella are specialized cells for gas exchange, afferent vessels leading to the site of gas exchange, and efferent vessels leading away. Detailed descriptions and diagrams of fish gill anatomy and function may be found in Randall (1970), Hughes (1984), and Moyle and Cech (1988).

The main function of the fish gill is to take in oxygen ( $O_2$ ); other functions are to eliminate waste and regulate hydromineral balance. In most temperate teleost fish, virtually all respiration, or gas exchange, takes place across the gill membranes; at least in trouts, no significant cutaneous respiration occurs (Nonnotte 1981), although it is not unknown in other species (Feder and Burggren 1985). Most teleost fish ventilate their gills actively and water movement is controlled by the buccal and branchial muscles. In a respiratory cycle, opening the mouth produces a small negative pressure in the buccal and branchial cavities. When the mouth closes, the resultant positive pressure in the buccal cavity forces water back into the branchial chamber and over the gills. The drop in pressure in the buccal cavity then allows water to enter when the mouth is opened again, thereby setting up a continuous pressure wave in one direction across the gills (Barton, in press).

Oxygen transfer across the gill is directly proportional to its surface area but inversely proportional to its thickness (Hughes 1984). Total gill area available for respiration varies among fishes and is usually related to their degree of activity. Active fishes generally have greater gill surface area per unit body weight than sedentary species. Only a portion of the gill lamellae are normally perfused with blood, but during conditions of increased activity or hypoxia, additional lamellae are "recruited" as required. Lamellar recruitment is apparently an adrenergic (adrenalin-mediated) response (Booth 1979).

Water contacts the lamellar surface only briefly (Randall and Daxboeck 1984) during which time gas exchange takes place by simple diffusion across the epithelial membrane, the water-blood barrier. Passive diffusion of  $O_2$  across the lamellar epithelium is governed by the partial pressure of  $O_2$  ( $PO_2$ ) in the blood relative to that in the water, i.e., the  $O_2$  tension gradient between the internal and external environments. Oxygen tension, or partial pressure, depends not only on the DO concentration in the water, but also on other physical and chemical properties of the freshwater environment, particularly water temperature and atmospheric pressure (see Section 3).

The exchange of gases across the lamellar epithelium is facilitated by a countercurrent arrangement of blood and water flow; that is, blood flow in the lamellae and water flow through the interlamellar spaces are in opposite directions (Piiper and Scheid 1984). Therefore, ventilatory volume, the amount of water passing over the gills, and cardiac output, which determines blood flow through the gills, both affect the rate of gas exchange. Ventilatory volume can be altered by changing either the frequency or the amplitude of ventilatory movements. Cardiac output can be altered by changing either the heart rate or the blood volume pumped per heartbeat (stroke volume).

To describe the various components of the pathway by which  $O_2$  molecules pass from the external medium to cellular mitochondria, Hughes (1964) used the concept of a respiratory chain. At each link in the chain, there is a resistance to  $O_2$  transfer and the driving force



to cross this resistance barrier is the difference in  $PO_2$ . The greatest difference in  $PO_2$  is about 50 Torr (1 Torr = 1 mm Hg = 133.3 Pa) at the water-blood barrier in the gills, which also contributes the greatest resistance to  $O_2$  transfer (Hughes 1981).

Diffusion of  $O_2$  across the gill membrane is assisted by the oxygen-binding capacity of hemoglobin. Hemoglobin can hold 10-20 times more  $O_2$  in bound form than can water. Because of hemoglobin's reversible  $O_2$ -binding characteristics, large quantities of  $O_2$  can be bound at the gills and released at metabolizing tissue sites with relatively small changes in blood  $PO_2$ . Teleost fish display hemoglobin polymorphism and in salmonids, for example, at least two types of hemoglobin with substantially different properties are present (Riggs 1970). Moreover, some species of salmonids may undergo ontogenetic shifts in hemoglobin types with age (Giles and Vanstone 1976). Multiple hemoglobins allow the fish to function efficiently in a range of DO regimes without appreciably affecting circulatory transport of  $O_2$ . Shifts in hemoglobin isomorph abundance have been observed in fish under changing environmental conditions, including hypoxia (Tun and Houston 1986; Marinsky et al. 1990).

The affinity of blood for  $O_2$  depends on the association-disassociation capacity of the hemoglobin molecule for  $O_2$ . A number of factors affect the binding of  $O_2$  with hemoglobin, particularly blood pH, partial pressure of  $CO_2$  ( $PCO_2$ ), and temperature. A fundamental property of hemoglobin is its ability to take up  $O_2$  at the gills and then unload  $O_2$  at the tissues as a result of its decreased affinity for oxygen in the presence of reduced blood pH caused by metabolic  $CO_2$  (the Bohr effect, see Riggs 1970; Satchell 1991). The dissociation of  $O_2$  from the hemoglobin molecule depends on both temperature and  $CO_2$  (Black et al. 1954; Cameron 1971). Whereas the Bohr effect is a pH-induced or  $PCO_2$ -induced shift in blood oxygen affinity, the Root effect is a shift in blood oxygen capacity; the latter is now considered an extreme Bohr shift (Moyle and Cech 1988). Unlike low- $O_2$  tolerant fish such as carp (*Cyprinus carpio*) that have a high blood affinity at low  $PO_2$ , fish that are physiologically efficient in well oxygenated environments, such as salmonids, have hemoglobin with relatively low blood  $O_2$  affinity.

In developing embryos, respiration is accomplished by diffusion of DO across the egg or chorion membrane. In most teleosts, initial post-hatching respiration is cutaneous as gill development is rudimentary. As the larvae develop, the O<sub>2</sub> requirement per unit weight declines with increasing body length. Body surface area per unit weight declines more quickly, however, so that cutaneous respiration is eventually insufficient to satisfy larval requirements. Thus, some extra O<sub>2</sub>-carrying capacity of the blood, via hemoglobin, is essential at this stage. Although the blood of many species's larvae does not contain much hemoglobin until later development, some hemoglobin is nonetheless present and the blood may appear pink at hatching (Blaxter 1988).

## 2.2. RELATIONSHIP BETWEEN OXYGEN CONSUMPTION AND METABOLISM

Metabolic rates of fish are usually measured indirectly and expressed as O<sub>2</sub> consumption rate (mg O<sub>2</sub>.kg<sup>-1</sup>.h<sup>-1</sup>) (Cech 1990). Metabolic rate is influenced mainly by temperature. The Q<sub>10</sub> for teleosts in general is about 2.3 (Brett and Groves 1979); that is, for every 10°C rise in temperature, metabolic rate increases about 2.3 times. In salmonids the Q<sub>10</sub> may be somewhat lower (Wedemeyer, in press). Although other factors affect metabolic rate, such as DO levels and diurnal periodicity (Brett and Groves 1979), one factor of concern to biologists is stress from both physical and chemical disturbances. For example, Barton and Schreck (1987) showed that even brief handling was sufficient to double the metabolic rate of steelhead (*Oncorhynchus mykiss*), as measured by O<sub>2</sub> consumption. Similarly, exposing sockeye salmon (*Oncorhynchus nerka*) to dilutions of kraft mill effluent (KME) resulted in an increase in metabolic rate by up to two times (Webb and Brett 1972; Davis 1973).

Nutritional status also affects the rate of O<sub>2</sub> consumption because of the additional metabolic demand placed on the fish to digest food. Metabolic rate in fish is much higher just after food has been consumed than after food has been digested and assimilated. The period of post-feeding elevated metabolic rate may last as long as 48 h, but in general it is about 24 h (Beamish 1978; Brett and Groves 1979).

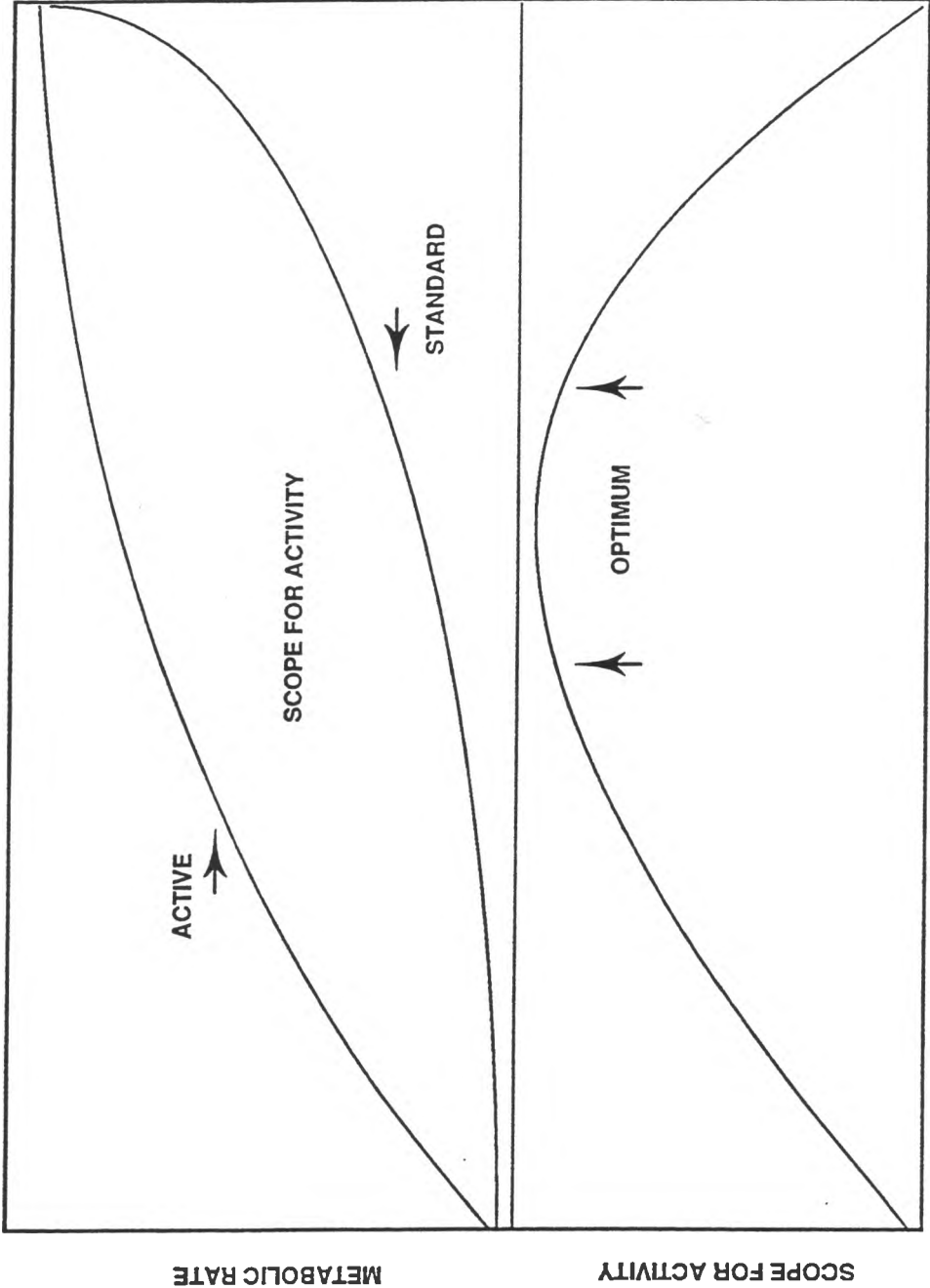
In simple terms, bioenergetics is the fate of digested food. The energy budget of a fish is described by the formula:

$$I = E + M + G$$

where I = energy ingested or ration, E = energy lost through excretion, M = energy for metabolism, and G = energy used for growth (Brett and Groves 1979). Growth includes both somatic (production of body tissue) and gametic (development of sexual products) growth.

Energy for standard metabolism is that required by the fish to maintain basic metabolic functions without activity. As standard metabolism is difficult to measure in practice, this value is usually estimated from O<sub>2</sub> consumption measurements made of fish at various levels of activity, with the resultant plot extrapolated back to zero activity. Most direct metabolic rate measurements made on "inactive" fish are more appropriately called routine metabolism, which includes spontaneous activity (Cech 1990). Values for weight-specific standard metabolism determined for salmonids fed normal rations range from about 80 to 120 mg O<sub>2</sub>.kg<sup>-1</sup>.h<sup>-1</sup> (Brett and Groves 1979). Routine metabolic rates are about 1.5 to 2 times higher than standard rates. The difference between the active metabolic rate (i.e., that at maximum sustained activity) and standard metabolic rate, as determined by O<sub>2</sub> consumption, is known as the scope for activity (Fry 1947, 1971). Both active and standard metabolic rates vary independently with temperature. The maximum scope for activity occurs, therefore, at that temperature where the difference between active and standard metabolic rates is greatest (Figure 1).

Unlike the response to temperature, standard metabolism remains more or less constant as DO levels increase, while active metabolism increases, up to DO saturation. Consequently the scope for activity also increases with increasing DO concentration. Because of the combined influences of temperature and DO on active metabolic rate, low DO tensions may



**RANGE FOR ACTIVITY**

Figure 1. Diagrammatic representation of the change in scope for activity over the range of an environmental condition (e.g. temperature) for activity. The optimum is the range where scope for activity is greatest.

limit the fish's scope for activity, with the effect becoming progressively greater as temperature rises (Neill and Bryan 1991).

The gross rate of metabolism ( $\text{mg O}_2 \cdot \text{h}^{-1}$ ) in fish increases with increasing size according to a power function with an exponent of about 0.86 in teleosts (Brett and Groves 1979). The weight-specific metabolic rate ( $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ), however, decreases as fish grow larger; that is, small fish consume more  $\text{O}_2$  per unit weight than do large fish (Cech 1990). When other energy requirements have been satisfied, the amount of energy remaining in the energy budget is used for somatic or gametic growth. Brett and Groves (1979) calculated that for carnivorous fish the energy available for growth is about 29% of the total energy intake.

Growth may be defined as the elaboration of tissue. Brett (1979) described growth as the net outcome of a series of behavioural and physiological processes that terminate with the deposition of animal substance. Numerous environmental factors affect growth, including DO, temperature, age, size, season, migratory and spawning activity, interspecific and intraspecific interactions, and availability of adequate food (Brett 1979).

Growth and other aspects of metabolism compete for the net energy available to fish after fixed costs have been satisfied (Fry 1947). Any factor that diverts energy toward maintenance, or that controls the rate of active metabolism, such as DO level, will limit the energy potentially available for other performance components, including growth. Thus, either increased maintenance metabolism or significantly decreased  $\text{O}_2$  tension, all other things being equal, will result in a relative decrease in rates of growth and production (Brett and Groves 1979).

In his review, Hughes (1981) described the difference between independent and dependent respiration during conditions of increasing hypoxia or falling DO tension in the water. Laboratory experiments with a number of species have shown that  $\text{O}_2$  consumption remains constant (independent) in spite of decreasing DO up to a threshold or critical level, below

which O<sub>2</sub> consumption declines (dependent) in parallel with declining O<sub>2</sub> tension (Springer and Neill 1988). The critical O<sub>2</sub> tension, or  $T_c$ , for rainbow trout (*Oncorhynchus mykiss*) ranged from 22 to 112 Torr depending on the physiological measurement considered, but for "normal life", was considered about 80-90 Torr (numerous papers cited in Hughes 1981); normal life was defined as the O<sub>2</sub> level that does not interfere with normal growth or reproduction. Davis (1975) determined that the critical O<sub>2</sub> tension, or  $T_c$ , for salmonids was 120 Torr (about 76-82% saturation) whereas that for non-salmonids was 95 Torr (about 61-65% saturation). It follows that once DO levels drop below the  $T_c$  level, growth declines as insufficient environmental O<sub>2</sub> is available to satisfy all metabolic requirements. Whereas several researchers have reported increases in  $T_c$  with increasing temperature, this notion was questioned by Ott et al. (1980) who found little temperature effect in trout, suggesting that discrepancies may have been due to environmental design. For example, the critical  $T_c$  for rainbow trout was 21, 22 and 27 Torr at 10, 15 and 20°C, respectively, a very small increase and one that those authors apparently considered insignificant (Ott et al. 1980). However,  $T_c$  rose from 22 to 34 Torr for rainbow trout when pH was dropped from 8 to 4 (Ultsch et al. 1980).

### 3.0 LIMNOLOGY OF DISSOLVED OXYGEN IN NORTHERN RIVERS

Gas exchange across the fish gill depends on the  $O_2$  tension gradient between the internal environment (blood) and the external environment (water) (see Section 2.1). Oxygen tension,  $PO_2$ , depends not only on the concentration of  $O_2$  in the water, but also other physical and chemical properties of the environment, particularly water temperature and atmospheric pressure. The capacity of water to hold DO decreases with temperature and increases with atmospheric pressure. Therefore, for water with any fixed DO concentration, the saturation level (i.e. per cent saturation) will increase with temperature and will also increase slightly with altitude. The significance of these physical relationships is that with increased temperature or altitude, the quantity of oxygen available to the fish will be less for a given level of saturation.

Field measurements of DO are almost always expressed as concentration, as are most published water quality criteria. The conversion of DO concentrations, expressed as mg/L, to  $PO_2$ , expressed as Torr, is a relatively straightforward process because the parameters covary. For example, using altitudes representative of the high and low ends of the range for northern rivers, 200 m (Peace-Athabasca Delta) and 1000 m (upper Athabasca River), Table 1 compares DO concentrations and  $PO_2$  values for five temperatures, based on the formula in Davis (1975) with atmospheric pressure corrected for altitude.

Because the difference between minimum and maximum altitudes in the northern rivers area is not large (about 800 m), temperature exerts the larger effect on oxygen solubility. Effects of temperature can be addressed by considering changes in saturation. The representative saturation values in Table 2 derived for different temperatures and altitudes at a DO concentration of 5 mg/L are similar to those of Davis (1975) (medium protection level for salmonids). Chapman (1986) concluded that DO criteria expressed as percent saturation are complex and may result in unnecessarily stringent criteria during cold months and potentially unprotective criteria during periods of high ambient temperatures or at high

**TABLE 1**

**DISSOLVED OXYGEN IN WATER AT 100% SATURATION EXPRESSED AS  
CONCENTRATION (mg/L) AND PARTIAL PRESSURE (TORR) AT 200 AND 1000 m  
ABOVE SEA LEVEL**

TEMPERATURE °C	AT 200m		AT 1000m	
	mg/L	TORR	mg/L	TORR
0	14.3	157.1	13.0	149.9
5	12.5	156.7	11.4	149.5
10	11.1	153.5	10.0	148.9
15	9.9	152.7	9.0	148.2
20	8.9	151.7	8.1	147.2



**TABLE 2**

**PERCENT SATURATION OF OXYGEN IN WATER AT REPRESENTATIVE TEMPERATURES AND ALTITUDES IN ALBERTA WHEN THE MEASURED OXYGEN CONCENTRATION IS 5 mg/L (DERIVED FROM FORMULA IN APHA 1985).**

TEMPERATURE °C	ALTITUDE (m)		
	500	750	1000
5	50	51	53
10	56	58	60
15	63	65	67
20	70	72	74
25	77	79	82

elevations. Unfortunately, literature data on fish and DO are expressed variously as absolute concentrations, partial pressures or percentages of saturation, and the ancillary information necessary to translate these to a common system, preferably milligrams per litre, are seldom given.

Large rivers have a characteristic dissolved oxygen regime, determined by the balance of atmospheric reaeration and photosynthesis against community respiration. Photosynthetic oxygen production may be significant in shallow, warm, slow-flowing rivers, but in the northern rivers of western Canada neither rooted plants nor plankton make a significant contribution to the oxygen regime. Deep water, high spring discharges (scouring flows), turbidity and especially the harsh climate of the region all minimize in-river plant production. Consequently atmospheric reaeration is the only significant source of oxygen in these rivers. In the open-water seasons, reaeration is sufficient to keep DO concentrations at or near saturation all along the rivers, except immediately below wastewater discharges (McCart et al. 1976; Noton et al. 1989; Shaw et al. 1990). With only minimal plant respiration diurnal cycles are insignificant; on the Athabasca River, for example, diurnal variation in DO is typically <1 mg/L (Hamilton et al. 1985).

Quite a different situation occurs in winter, when ice cover effectively prevents atmospheric reaeration. In lakes and standing water generally the wintertime supply of dissolved oxygen is limited to the quantity contained at the time of ice formation. Because of the inverse relationship between temperature and oxygen solubility, the saturated water contains >13 mg/L of DO at ice-up. Decomposition of organic matter produced during the growing season leads to a progressive decline in DO content of the water over the winter. In rivers the same process occurs, with the important difference that because of the unidirectional movement of river water, time and distance are correlated. Hence, DO concentrations in ice-covered rivers tend to decline temporally throughout winter, and spatially from upstream to downstream. Lowest concentrations are reached far downstream in March, just before spring melt.

Wintertime dissolved oxygen levels in the northern rivers of Alberta conform to this pattern. Highest concentrations are always observed in the mountain foothills, which are ultimately fed by turbulent streams that never freeze over. The rate of downstream decline may be very slow, but over the hundreds of kilometres of river length, a significant decline in DO may still occur. In March, DO concentrations in the Smoky River declined from 11 mg/L at the Wapiti River confluence to 9.3 mg/L at the junction with the Peace River, a distance of about 120 km (Noton et al. 1989). Similar declines have been observed in some of the smaller tributaries of the Peace River (Shaw et al. 1990).

Historically, wintertime DO sags in northern rivers have been small, despite their great lengths and the six-month duration of ice cover, because the natural oxygen demand for decomposition was small. Biochemical oxygen demand (BOD) in all the rivers is naturally very low, invariably <2 mg/L (Hamilton et al. 1985; Noton et al. 1989). Elevated oxygen demands occur below pulp mills and other waste discharges, however, and the decomposition of these organic wastes can reduce oxygen concentrations far downstream. For example, effluent from the Proctor and Gamble mill on the Wapiti River (BOD 35-66 mg/L) in 1982 was seen as largely responsible for the 2 mg/L decline in DO along the length of the Smoky River in that winter (Noton et al. 1989). Pulp mills discharge large quantities of organic matter, which tends to accumulate on the sediment below the mills, creating elevated sediment oxygen demand (SOD) (Macdonald and Hamilton 1989). The SOD tends to increase over winter until spring high flows scour the sediments (Casey 1990).

The most pronounced DO sags have been observed in the Athabasca River. In late winter of 1988 and 1989, the DO concentration in the headwaters above Hinton was 11.5 mg/L, but declined to 10 mg/L by Whitecourt, because of the combined effluent from Hinton's municipal effluent and the Weldwood pulp mill. By the town of Smith, 200 km farther downstream, concentrations had declined to 8.3-9.1 mg/L in 1988 and 6.0-7.7 mg/L in 1989 (after the Millar-Western mill at Whitecourt began production). The decline continued

downstream until concentrations were reset to near saturation by inflow of oxygenated water from the Lesser Slave River, and by reaeration at Grand Rapids. Dissolved oxygen below Smith has reached 7 mg/L and occasionally dips below 6 mg/L (Noton and Shaw 1989).

The Athabasca River example illustrates two important points. First, decomposition of organic matter in flowing waters under ice continues to reduce DO levels for long distances downstream. As treatment of pulp mill wastes has improved, and the proportion of recalcitrant material in final effluent has increased, DO sags have been displaced farther downstream. Second, open water leads and rapids are critical sources of oxygen in wintertime. The effect of the chutes at Grand Rapids on the Athabasca River water is dramatic (Macdonald and Hamilton 1989). Open water in the mountains maintains the initially high DO of river water. In the Athabasca River, the DO concentration at Hinton (11.5 mg/L) already shows a decline from saturation (13 mg/L) from decay of native organic matter in the headwater reach (Noton and Shaw 1989). Reaeration is less in cold winters because the area of open water recedes. In the Alberta reach of the Peace River, wintertime DO sags have always been negligibly small, because of the low BOD, reaeration at two major rapids (Vermillion Chutes and Boyer Rapids) and a very long reach of open water extending 200 km below the W.A.C. Bennett Dam (Shaw et al. 1990).

## **4.0 EFFECTS OF ADVERSE DISSOLVED OXYGEN CONDITIONS ON FISH AND INVERTEBRATES**

### **4.1 FISH RESPONSES TO HYPOXIA**

The most usual adverse effect of low DO concentrations on fish is that of hypoxia as a result of a reduction in their O<sub>2</sub> supply. Hypoxia may be defined as any condition in which the amount of O<sub>2</sub> is below normal (Heath 1987). Cellular hypoxia can develop at any point along the respiratory chain (Hughes 1981). When a cell becomes hypoxic, the rate of O<sub>2</sub> delivery is less than that required for oxidative metabolism to supply the energy needs of the cell (Dunn and Hochachka 1986). Death from asphyxiation occurs when insufficient O<sub>2</sub> delivery to the brain and other organs or tissues results in cellular dysfunction. Hughes (1973) classified different types of hypoxia based on their cause. The types of hypoxia in fish of major concern to northern river fisheries managers are: (1) environmental hypoxia caused by a reduction in PO<sub>2</sub> (oxygen tension or partial pressure) of ambient water, and (2) hypoxia resulting from factors, such as pollutants, that affect the normal gas diffusion exchange at the gills.

#### **4.1.1 Mortality**

Severe hypoxia can reduce survival, particularly in less tolerant species such as salmonids. Such species may have a limited capacity to adjust to hypoxic conditions and, therefore, safeguard essential gas transport (Jensen et al. 1993). Mortality is the most obvious and most easily measured response to environmental hypoxia, but it is probably relatively rare in nature compared with various sublethal effects. Threshold or incipient lethal levels of DO in surface waters have been investigated for a number of fish species. Dourdoroff and Shumway (1970) documented lethal thresholds of DO from published literature after 1937 (see Section 6). In general, Doudoroff and Shumway (1970) found from their review that mortality of most adult fish becomes apparent at DO concentrations between 1 and 3 mg/L.

Little evidence since that review suggests that these general values are inaccurate. Most fish exposed to lethal hypoxia survive for 1 to 3 hours.

Lethal DO thresholds are not entirely fixed. Shepard (1955) found that by reducing the ambient DO, the threshold level of DO that young brook trout (*Salvelinus fontinalis*) were able to withstand was also lowered. For example, at 100% saturation, the incipient lethal DO level was 1.58 mg/L, but this could be lowered to 1.05 mg/L through acclimation of the fish to reduced ambient DO. However, acclimation did not enhance the ability of fish to withstand total O<sub>2</sub> deprivation. Ultsch et al. (1978) found that natural summertime DO concentrations of 3.3 mg/L were as much as 1.0-1.7 mg/L less than the threshold lethal DO concentrations for six darter species determined in the wintertime. The fish compensated for this by a metabolic adjustment that lowered the threshold DO level in summer, allowing them to occupy habitats from which they would normally be excluded. This ability does not appear to be general in fish other than darters (Ultsch et al. 1978).

Genetic differences apparently exist within species of fish in their ability to cope with low DO. Klar et al. (1979a,b) reported differences among phenotypes of rainbow trout in their swimming performance, O<sub>2</sub> consumption and mortality when subjected to acute hypoxia of 2 mg/L DO. Adaptation to hypoxia also appears to be age dependent. Strel'tsova (1964) found that after 22 d of exposure to 20-29% DO saturation at 1-2°C, the O<sub>2</sub> consumption rate in two-year-old rainbow trout dropped by half and the lethal threshold dropped from 1.07 to 0.84 mg/L. However, in fingerling rainbow trout using these criteria, acclimation to low DO occurred at day 27, and the lethal threshold declined from 0.95 to 0.67 mg/L. At 15°C, acclimation to low DO was more rapid than at 1-2°C, but occurred faster in the fingerlings than in the two-year-old fish (Strel'tsova 1964).

#### 4.1.2 Physiological Effects

Numerous studies have documented physiological mechanisms by which fish adjust to nonlethal environmental hypoxia; these were reviewed recently by Jensen et al. (1993). These adaptations may include any number of the following: bradycardia (decreased heart rate but possibly higher stroke volume), tachycardia (increased heart rate), increased ventilatory rate, reduction of peripheral resistance (i.e., in gills), and recruitment of additional secondary lamellae, all of which serve to maintain an adequate transfer of O<sub>2</sub> in spite of an overall reduction in PO<sub>2</sub> (Hughes 1973, 1981). During environmental hypoxia, erythrocytes (red blood cells) may undergo swelling. Soivio and Nikinmaa (1981) concluded from their experiments with rainbow trout that erythrocyte swelling may increase the oxygen affinity of blood in such instances, suggesting a further mechanism in fish for coping with hypoxia stress.

Numerous studies have documented the increase in respiratory volume and metabolic rate in fish subjected to hypoxia. For example, low DO levels can increase respiratory volumes in resting fish by as much as 30 times (Saunders 1962). In addition to the reduction in DO resulting in reduced O<sub>2</sub> availability, fish must increase their metabolic rate to satisfy their elevated ventilatory flow rate that must, of necessity, increase to bring sufficient amounts of DO in contact with the gill (Jensen et al. 1993). In a study of Great Plains fishes, many of which are found in northern Alberta, Gee et al. (1978) documented the sequence of behavioural events in response to progressive hypoxia. Initially, all species increased frequency of opercular movements. Maximum movement was reached just before fish lost coordination or started moving to the surface to gulp air. In the latter stages of hypoxia, most of the species became less active or reduced the frequency of opercular movements.

Farrell et al. (1989) determined that both reduced PO<sub>2</sub> and increased heart rate reduced *in vitro* performance of the rainbow trout heart, arguing that stroke volume is decreased with the increase in heart rate. Some, if not all, of the physiological strategies used by fish to

cope with low DO involve the action of catecholamine hormones (Booth 1979; Pettersson and Johansen 1982; Boutilier et al. 1988). Under conditions of increased functional surface area resulting from catecholamine-induced secondary lamellar recruitment, there may be an accompanying trade-off of increased ion loss (Gonzalez and McDonald 1992).

Kakuta et al. (1992) reported a remarkable decline in urinary volume in carp exposed to hypoxia. Volume of flow decreased in close parallel with heart rate, to a minimum of 5% of the normal rate. The low urinary rate reduced ion losses in urine to roughly 10% of normal losses, despite lowered efficiency of ion resorption in the kidneys. The change in urinary volume could be an adaptation to compensate for augmented ion losses through the gills during hypoxia.

Northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) exposed to successively lower DO conditions from 4 to 0.25 mg/L over a 5-d period increased their gill ventilation rates; rates doubled for yellow perch and quadrupled for northern pike (Petrosky and Magnuson 1973). Fathead minnow (*Pimephales promelas*), brook stickleback (*Culea inconstans*) and central mudminnow (*Umbra limi*) exposed to DO concentrations from 4.0 down to 0.25 mg/L all rapidly increased gill ventilation rates to a maximum of about 70 beats per minute. The DO concentration at which the maximum rate was attained varied according to the DO requirements of each species (Klinger et al. 1982). When ambient DO was rapidly decreased from 11 to 2.5 mg/L, frequency of respiratory movements in Baikal grayling (*Thymallus arcticus baicalensis*) increased up to a maximum value in the first hour of hypoxia (Karpovich and Korlupayev 1980). A long period of exposure resulted in cardiac arrhythmia in most of the fish. Ontogenetic differences have also been observed in whether fish display bradycardia or tachycardia in response to hypoxia (Holeton 1971).

Under extreme hypoxic conditions, many fish species can use anaerobic metabolism (lactate/pyruvate pathway) as an adaptive strategy to maintain energy required for survival (Heath et al. 1980). The threshold  $PO_2$  for anaerobic metabolism varies among species, but



for rainbow trout at 15°C, it was about 80 Torr (Heath et al. 1980). Lowering the temperature to 5°C reduced the critical  $PO_2$  to 40 Torr. Dunn and Hochachka (1987) observed a more than seven-fold increase in lactate turnover rate in rainbow trout subjected to 3 h of hypoxia at 30 Torr, arguing that this response was indicative of the activation of the anaerobic metabolic pathway under such conditions. The major source of lactate for anaerobic metabolism appears to be white muscle through the glycolytic pathway (Dunn and Hochachka 1986). Boutilier et al. (1988) reported that rainbow trout tolerated an acute hypoxia of 30 Torr by increasing their reliance on anaerobic metabolism and also reducing their metabolic rate. Oxygen consumption declined by 30% when ambient DO was reduced from 155 Torr (normoxic) to 80 Torr, but did not change when the reduction was only to 120 Torr.

However, such adaptive responses cannot continue indefinitely without some metabolic cost to the fish at DO levels less than the critical  $O_2$  tension ( $T_c$ ). Reduced  $O_2$  consumption will decrease the fish's scope for activity, i.e., the total metabolic energy through respiration available for other performance components such as somatic and gametic growth (Fry 1947). The reduced scope for activity is a result of an increase in metabolic rate to meet the energy demand of both respiratory and cardiac pumps during hypoxia, in order to satisfy  $O_2$  requirements at a time of reduced external DO (Hughes 1981). This additional energy demand will have the effect of reducing available energy within the fish's scope for activity even further. At the sublethal level, the reduction in metabolic scope will result in a decrease or cessation of individual growth or population production. At the lethal threshold, where the fish is respiring at the maximum rate, insufficient DO can be taken up to meet cellular and metabolic demands, and death eventually ensues (Shepard 1955).

An important effect of hypoxia is the upset of acid-base balance in fish resulting in changes in arterial pH (Hughes 1981). Presumably blood pH is altered as a result of increased concentrations of lactic acid (pH decreases) or hyperventilation associated with  $CO_2$  elimination (pH increases). Increases in blood lactic acid levels of ten-fold or more can

result from severe hypoxia (Heath and Pritchard 1965). In rainbow trout, plasma acidosis was only evident at an environmental  $PO_2$  of 30 Torr (Boutilier et al. 1988). When trout (unspecified species) were subjected to acute environmental hypoxia of 25 Torr, metabolic acidosis became apparent when their arterial  $O_2$  tension ( $PaO_2$ ) dropped to about 15 Torr (Claireaux et al. 1988). Those authors also noticed an increased affinity of blood for  $O_2$  in the fish with acidosis, which was partly explained by a 20% increase in blood cells in circulation as well as biochemical changes within the erythrocytes themselves.

#### 4.1.3 Biochemical Changes

Fish exposed to hypoxic conditions showed significant declines in white muscle adenosine triphosphate (ATP) levels, which were reflected in reductions in total adenylate concentrations (Vetter and Hodson 1982). Vetter and Hodson (1982) suggested that measurements of total adenylate concentration and adenylate energy charge would be useful as hypoxic stress indicators. Hypoxia caused a reduction in erythrocyte ATP levels but not in hemoglobin components of rainbow trout and Mozambique tilapia (*Tilapia mossambica*), leading Smit and Hattingh (1981) to conclude that energy substrate (i.e., ATP) levels in erythrocytes and not conformational changes were responsible for change in  $O_2$  affinity of hemoglobins under hypoxic conditions.

Prolonged exposure (10 wk) to DO at 5 mg/L led to decreased concentrations of free amino acids involved in gluconeogenesis (production of glucose from protein substrates) and of the proteins alanine, histidine and aspartic acid in plasma, muscle or liver compared with fish held at 8 mg  $O_2$ /L (Medale et al. 1987). The pattern of biochemical changes was consistent with a shift away from tissue building (anabolism) in favour of energy production to meet metabolic needs. Continuation of the new balance of energy production versus energy storage would lead to the reduced growth often observed in fish exposed to hypoxia for long periods (see below). A sudden change in DO levels from 8-9 mg/L to 2-3 mg/L caused

changes in the intestinal uptake of the amino acid glycine in rainbow trout (Boge et al. 1980), suggesting that hypoxia may alter normal digestive functions.

#### 4.1.4 Alterations in Blood Chemistry

Bouck and Ball (1965) found that when two sunfishes (*Centrarchidae*) were subjected to 3 mg/L DO for 8 h/d, for 9 d, serum protein patterns were significantly altered in a manner indicative of stress. Long-term chronic exposure to such conditions, while not necessarily being lethal, may compromise those fishes's ability to compete with more tolerant species in a low-DO environment.

Plasma corticosteroids are very sensitive indicators of most forms of environmental stress (Barton and Iwama 1991; Brown 1993). In channel catfish (*Ictalurus punctatus*), Tomasso et al. (1981) showed that plasma corticosteroid levels rose dramatically in response to both acute (<1 h) and chronic (3 d) hypoxia events. Swift (1981) found rainbow trout exposed to  $\leq 3$  mg/L DO showed elevated levels of plasma cortisol, as well as increased blood glucose, decreased plasma chloride and increased hematocrit. River lampreys (*Lampetra fluviatilis*) subjected to 7 d hypoxia exhibited significant hyperglycemia (Hardisty et al. 1976). Similarly, channel catfish subjected to prolonged sublethal hypoxia displayed increases in plasma glucose and lactic acid as well as in hemoglobin (Scott and Rogers 1981). Indeed, numerous studies have demonstrated that an increased level of lactic acid in circulation is a typical response to environmental hypoxia and may reflect the shift toward anaerobic metabolism in such instances (e.g., Heath and Pritchard 1965; Holeyton and Randall 1967; Caillouet 1968; Hunn 1969; Burton 1971; Burggren and Cameron 1980). Mazeaud et al. (1977) also determined that the stress associated with prolonged hypoxia can cause increases in glucose and free fatty acids in fish.

#### 4.1.5 Changes in Hematology

Hematological changes in fish subjected to low DO include increases in hemoglobin, blood cell count, hematocrit, and associated blood parameters (e.g., Scott and Rogers 1981; Tun and Houston 1986; Lochmiller et al. 1989; Marinsky et al. 1990). In fathead minnows, hematocrit rose by 1.4% for each 1 mg/L decrease in DO level (MacLeod and Smith 1966). Increases in these hematological features may be caused by water loss concentrating the cellular fraction, or recruitment of additional blood cells into circulation from storage organs, such as the spleen (Swift 1981). Transient hypoxia was shown to increase the number of immature erythrocytes in circulation, suggesting increased erythropoiesis or release of erythrocytes in storage to increase blood oxygen-carrying capacity (Murad et al. 1990). Similarly, long-term chronic exposure of ninespine sticklebacks (*Pungitius pungitius*) to low DO led to an initial release of erythrocytes into circulation from storage, followed by anemia at 4.5-5 months, and then an increase in hemopoiesis and rise in immature erythrocytes in the blood (Zhiteneva 1971). Exposure of rainbow trout to hypoxia in the range of 55-60 Torr altered the blood O<sub>2</sub>-dissociation curve, increased erythrocyte volume, decreased intracellular hemoglobin and ATP, and increased intraerythrocytic pH; these processes were all functionally related (Nikinmaa and Soivio 1982).

#### 4.1.6 Histopathological Effects

Histopathological damage in fish in response to hypoxia has been little studied. Channel catfish exposed to acutely low DO displayed a number of histopathological effects in various tissues including the gills (Plumb et al. 1976; Scott and Rogers 1980). In brown trout (*Salmo trutta*) sampled after death, evidence of gill damage from acute hypoxia was only evident at the electron-microscopic level (Drewett and Abel 1983). Walters and Plumb (1980) found that channel catfish held at low DO levels had a much higher mortality rate and incidence of histopathological damage after being exposed to a disease organism (*Aeromonas hydrophila*) when they were also exposed to adverse levels of NH<sub>3</sub> and CO<sub>2</sub>. Results

indicated that the additional poor water quality stressors exacerbated the stressful effect of hypoxic exposure.

#### 4.1.7 Changes in Fish Performance

Effects of hypoxia stress on aspects of individual fish performance such as disease resistance ultimately affect the entire population (Jensen et al. 1993). Grizzle (1981) noticed that rainbow, brown and brook trout, and yellow perch had higher incidences of bacterial isolations in waters with the lowest DO in a tailwater receiving hypolimnetic discharge from a reservoir, suggesting the possibility of a higher susceptibility to pathogen invasion in low O<sub>2</sub>-stressed fish. Giles (1987) found that threespine sticklebacks (*Gasterosteus aculeatus*) infected with a parasite reacted to progressive hypoxia by employing aquatic-surface respiration (movement to the air-water boundary) more quickly than non-infected fish. Although Giles's (1987) work was a behavioural study, the results suggested that the added stressor of the parasite elevated the stickleback's metabolic requirements.

Rainbow trout subjected to brief anoxia (combined with handling) showed increased susceptibility to a virulent bacterium (*Aeromonas salmonicida*) as well as a decreased chemiluminescence response of kidney phagocytes, which indicates a possible reduction in phagocytic activity (Angelidis et al. 1987). While such findings do not constitute definitive proof that hypoxia directly reduces a fish's resistance to disease, it is well known that disease incidence in both cultured and wild fish populations can increase when fish are subjected to most types of environmental stressors (reviewed in Wedemeyer 1970, Snieszko 1974 and Pickering 1987).

An important index of fish performance is growth. Under conditions of hypoxia, growth rates are reduced (Brett and Blackburn 1981). Chapman (1986) reported reductions in growth rates of salmonids at DO levels as high as 7 mg/L (Table 3). Adelman and Smith (1970) observed a progressive decrease in growth rate of northern pike as oxygen declined

**TABLE 3**

**PERCENT REDUCTION IN GROWTH RATE OF SALMONIDS AT VARIOUS DISSOLVED OXYGEN CONCENTRATIONS EXPRESSED AS THE MEDIAN VALUE FROM N TESTS WITH EACH SPECIES (FROM CHAPMAN 1986)**

DISSOLVED OXYGEN (mg/L)	SPECIES (NUMBER OF TESTS)					
	CHINOOK SALMON (6)	COHO SALMON (12)	SOCKEYE SALMON (1)	RAINBOW TROUT (2)	BROWN TROUT (1)	LAKE TROUT (2)
9	0	0	0	0	0	0
8	0	0	0	1	0	0
7	1	1	2	5	1	2
6	7	4	6	9	6	7
5	16	11	12	17	13	16
4	29	21	22	25	23	29
3	47	37	33	37	36	47
MEDIAN TEMP. (°C)	15	18	15	12	12	12

to 3-4 mg/L, then a rapid decrease below that threshold level. Those authors attributed that response to decreased food consumption and conversion efficiency.

Other authors have seen similar growth-DO curves in different species, but with different threshold levels (Herrman et al. 1962; Stewart et al. 1967); in coho salmon (*Oncorhynchus kisutch*), the threshold was about 4-5 mg/L (Herrman et al. 1962). Similarly, under controlled laboratory conditions, growth and food conversion efficiency of juvenile coho and sockeye salmon at 15°C for a 6-8-wk period were not limited above critical DO levels of 4.0 and 4.5 mg/L (Brett and Blackburn 1981). When DO was reduced from 8 to 4 mg/L in a production system, DO consumption of trout (unspecified species) declined by 17% but growth performance declined by 34% (Keesen et al. 1981). Juvenile channel catfish exposed to a combined stressor of low DO (40% saturation) and disease infection showed reduced growth as measured by RNA:DNA ratios (Paterson and Brown-Paterson 1992); although length did not change, weight decreased. However, those authors suggested that the disease, not low DO, may have been the main factor in the growth reduction.

A number of studies have shown a decrease in swimming speed associated with hypoxia in rainbow trout (Jones 1971; Bushnell et al. 1984) and other species (Dahlberg et al. 1968; Kutty 1968). There appears to be a critical DO level at which point fish stop swimming, which has been determined for selected species challenged in a swimming performance test (Kutty and Saunders 1973). The critical level for rainbow trout is 2.0 mg/L compared with 4.5 mg/L for Atlantic salmon (*Salmo salar*). Such information is useful for evaluating oxygen demands relative to fish requirements in flowing waters at a time when spawning migrations are underway (Kutty and Saunders 1973). Dahlberg et al. (1968), however, observed that swimming performance of juvenile coho salmon, as measured by maximum sustained swimming speed at 20°C, was reduced with any appreciable decline in DO below 9 mg/L, the level of complete saturation.

#### 4.1.8 Behavioural Changes

The effects of environmental hypoxia on fish behaviour are numerous, and the ecological implications of behavioural changes are profound and incompletely understood. Fish may display four classes of behavioural response to compensate for reduced DO conditions: (1) change in activity, (2) change in habitat (vertical and horizontal), (3) increase air-breathing, and (4) increase aquatic surface breathing (Kramer 1987).

Changes in activity include short-term responses to acute low-DO episodes, and long-term adjustments to chronic hypoxia, such as occur below winter ice. Reactions of fish to hypoxia in the laboratory are variable and appear to be species-specific. Shepard (1955) observed that exposure to low DO conditions caused hyperactive behaviour in brook trout followed by loss of equilibrium, then cessation of respiratory movement shortly thereafter. Hyperactivity is common in fish under hypoxic conditions, and it may be an escape behaviour (Kramer 1987). Other species, however, show a marked reduction in activity when DO declines, perhaps an adaptation to offset the increasing metabolic demand for respiration (Klinger et al. 1982). Metabolic rates of seven California stream fishes all declined under low DO, and the decrease was greater following a rapid 5°C increase in temperature (Cech et al. 1990).

Avoidance, where possible, is the simplest and most often used strategy used by fish to cope with low environmental DO (Jensen et al. 1993). Other changes in activity may be more subtle. There is a tendency for many species to move to the surface to take advantage of more favourable conditions there (see below). Escape behaviour or upward movements render fish more susceptible to predation by other fish and by birds (Kramer 1987). Eurasian minnows (*Phoxinus phoxinus*) remained within their home range in an experimental tank with continuously decreasing DO concentration until DO reached about 1.2 mg/L, about 2.5-3 h after the experiment began (Stott and Buckley 1979). At this point, the minnows moved out of their home range to avoid the low DO and returned when DO levels were



again increased. The critical avoidance limit for salmonids is expectedly higher than for many other species, such as cyprinids. Waters with DO <7-8 mg/L were typically avoided by juvenile chinook salmon (*Oncorhynchus tshawytscha*) (Birtwell and Kruzynski 1989). A similar avoidance response threshold of 8.3 mg/L was observed for brook trout (Dandy 1970).

Predator avoidance, feeding efficiency and reproduction may all be impaired by low DO levels. Although Birtwell's (1989) studies showed that salmonids will make forays into waters with reduced DO to feed, they return to water with near-saturation DO very quickly. Walleye (*Stizostedion vitreum*), which often inhabit murky water and feed at dawn and dusk, are negatively phototactic. Under conditions of DO depletion, however, the negative response to light was almost completely obliterated (Scherer 1971), suggesting a dysfunction of the avoidance response and possibly impaired feeding efficiency under hypoxia. Depressed DO levels resulting from high-BOD effluent from a pulp mill in early fall impeded or completely blocked upstream-migrating Pacific salmon (Birtwell 1989). Numerous studies, in fact, have documented the movement of salmonids through zones of reduced DO (reviewed in Birtwell 1989). Although the data are variable, likely as a result of varying environmental conditions, it appears that migrating salmonids avoid DO levels below about 3.5 to 5 mg/L, depending on conditions, or if exposed to levels below that limit during their migration, they become fatigued. Effects of this kind have immediate relevance to the northern rivers.

Changes in activity or habitat are perhaps of greatest concern for northern river species, particularly those of the sportfish groups (e.g., salmonids, esocids, percids) as these strategies may affect population production and community structure over time. There is increasing evidence that many fish will avoid areas of low DO if other habitats are available, and that normally preferred habitats will be vacated if DO levels decline (Kramer 1987, Pihl et al. 1991). Many fish migrate out of anoxic, ice-covered lakes into tributary streams where DO is higher (Klinger et al. 1982). Birtwell (1989) reported that DO was the most significant

factor influencing the vertical distribution of juvenile chinook salmon in the water column; the fish generally avoided waters with <40% saturation. Laboratory studies of DO avoidance (Birtwell and Kruzynski 1989) confirmed earlier field observations (Birtwell et al. 1983) that most adult salmon migrating through the zone of influence of a pulp mill discharge were found in the upper 2.5 m of water where DO ranged from 65% to 95% saturation.

Schurmann et al. (1991) observed that final preferred temperatures of rainbow trout under hypoxia were lower than at normal DO concentrations. Several physiological advantages could be realized by moving to cooler water: (1) reduction of metabolic rate at lower temperature allows for decreased DO consumption, (2) solubility of O<sub>2</sub> in water is increased at lower temperature, thereby offsetting the decrease in O<sub>2</sub> content between inspired and expired water, and (3) blood affinity for O<sub>2</sub> is higher at lower temperature (Schurmann et al. 1991). Plains minnow (*Hybognathus placitus*) temperature preference also declined from 30 to 17°C when DO was reduced from 4.8 to 2 mg/L (Bryan et al. 1984). The fish evidently select the temperature at which they can operate within the zone of respiratory independence. Similarly, upper lethal temperatures of three intermittent-stream fishes were significantly reduced under hypoxia (1.2 mg O<sub>2</sub>/L) compared with a normal DO concentration (7 mg/L). Moreover, lethal temperatures under hypoxia were lower in fish prevented from swimming to the surface to take advantage of aquatic surface respiration (Rutledge and Beitinger 1989). Thermal stress significantly elevates the oxygen demand of fish, and as a result, ventilatory rates also increase (Heath 1973).

Aquatic surface respiration is a strategy used by a variety of species under severe hypoxia to take advantage of the O<sub>2</sub>-rich layer of surface film water, usually only the uppermost millimetre (Gee et al. 1978; Kramer and Mehegan 1981). Movement of fish to surface water is a general, if not universal response to hypoxia (Dandy 1970; Petrosky and Magnasun 1973; Klinger et al. 1982; Birtwell and Kruzynski 1989; Rutledge and Beitinger 1989; and references therein). The time at which fish began using aquatic surface respiration was

considered by Gee et al. (1978) as an indicator of the incipient lethal limit, as this may represent the fish's "last resort for survival". The behavioural criterion results in PO<sub>2</sub> values that are lower, in some cases by two-fold, than what would be considered approximate lethal limits based on observations of the decline in opercular movement or activity (Table 4). Aquatic surface respiration was not observed in the salmonids or walleye. Aquatic surface respiration is not limited to open-water seasons; in winter fish evidently make use of oxygen trapped in air bubbles beneath the ice or diffusing through cracks (Klinger et al. 1982).

#### **4.1.9 Population and Community Effects**

The response of fish populations and communities integrate and sum all the biochemical, physiological and behavioural changes induced by hypoxia, and it is at this level that the long-term effects of suboptimal DO regimes will be most evident. Numbers of all species, numbers of sportfish species (e.g., esocids, percids, centrachids), and percent sport fish in the fish community were greater in areas of the Wisconsin River where average summer DO levels exceeded 5 mg/L than at sites where DO was <5 mg/L (Coble 1982). This empirical observation supports the notion that the 5-mg/L criterion represents a point of departure between good and poor fish populations. Total density of fish increased below a reservoir releasing partially deoxygenated hypolimnetic water, but the decrease in DO from 8.7 mg/L upstream to 4.1 mg/L downstream was sufficient to eliminate both rainbow trout and brown trout, and to profoundly alter the proportions of remaining species. Thus, a change in DO regime caused not only loss of sensitive species, but a profound restructuring of the fish community (Camargo 1991). Matthews and Styron (1981) found that minnow (Cyprinidae) and darter (Percidae) species from headwater streams, where DO oscillates widely, were more tolerant of low DO conditions than those from mainstem streams, where conditions are more predictable. Intraspecifically, fantail darters (*Etheostoma flabellare*) from intermittent headwaters were more tolerant of low DO than their conspecifics in mainstems, supporting the view that populations exposed to more rigorous environmental conditions are more resistant to abrupt physical changes than populations from more hospitable habitats.

TABLE 4

ESTIMATED INCIPIENT LETHAL ENVIRONMENTAL O<sub>2</sub> TENSIONS (ILL) OF SELECTED NORTHERN RIVERS SPECIES BASED ON EITHER AQUATIC-SURFACE BREATHING BEHAVIOUR OR INITIATION OF THE DECLINE OF OPERCULAR MOVEMENT AND ACTIVITY (MEAN OF BOTH MODIFIED FROM GEE ET AL. 1978)

SPECIES	ESTIMATED ILL (TORR) (aquatic-surface respiration)	ESTIMATED ILL (TORR) (decline in opercular movement and activity)
Rainbow trout	N/A <sup>1</sup>	42.8
Lake whitefish	N/A <sup>1</sup>	23.7 <sup>2</sup>
Northern pike	6.3	42.1 <sup>3</sup>
Northern redbelly dace	9.5	18.2
Brassy minnow	11.3	32.2
Emerald shiner	15.6	21.8
Spottail shiner	17.2	19.5 <sup>2</sup>
Fathead minnow	13.7	13.7 <sup>2</sup>
Longnose dace	8.0	16.9
Pearl dace	7.9	13.3
White sucker	13.6	19.9
Brook stickleback	18.7	34.1
Ninespine stickleback	49.9	58.7 <sup>2</sup>
Yellow perch	11.7	29.1
Walleye	N/A <sup>1</sup>	32.8
Iowa darter	12.2	38.6 <sup>3</sup>

<sup>1</sup> Aquatic-surface respiration not evident in these species

<sup>2</sup> Decline in activity only

<sup>3</sup> Decline in opercular movement only

## 4.2 DISSOLVED OXYGEN REQUIREMENTS OF AQUATIC INVERTEBRATES

Dissolved oxygen requirements for the maintenance of healthy fish communities must consider not only the physiological needs of the fish themselves, but also the physical and biological environment, and especially food species, that are necessary to sustain healthy fish populations (Chapman 1986). Other than fish, the most important and oxygen-sensitive ecosystem component is the benthic invertebrate community. Invertebrates are important to the maintenance of fish both indirectly, through their functions in organic matter processing and nutrient cycling, and directly, as a food source. Bottom-dwelling or drifting insects and other invertebrates are the major food source for many fishes in rivers, and a key link in the aquatic food chain (Carpenter et al. 1985). Even strictly piscivorous top predators such as northern pike are ultimately dependent on benthic invertebrate populations to maintain their supply of forage fishes.

Dissolved oxygen is one of the central environmental factors structuring benthic invertebrate communities. Other things being equal, the most diverse assemblages are always found in areas such as riffles that have the highest DO levels. Recognizing this, the EPA acute DO criterion for salmonids is set at 4.0 mg/L to protect invertebrate food species, many of which are more sensitive to low DO levels than are the salmonids themselves (EPA 1986). For example, Gaufin (1973) and Nebeker (1972) together provide 96-hour  $LC_{50}$  values of DO concentrations for 26 species of aquatic insects, including Plecoptera, Ephemeroptera, Trichoptera and Diptera (Table 5). Test temperatures were 6.4°C (Gaufin 1973) and 10.5°C (Nebeker 1972). The  $LC_{50}$  values varied from <0.6 mg/L for the midge, *Tanytarsus dissimilis*, to >5 mg/L for the fast-water mayfly, *Ephemerella doddsi*. Significantly, the  $LC_{50}$  for half of the species tested was 3-4 mg/L (Table 5), indicating that at least 4 mg/L of DO is needed for populations to survive (Chapman 1986).

TABLE 5

**ACUTELY LETHAL CONCENTRATIONS OF DISSOLVED OXYGEN  
TO AQUATIC INSECTS  
(FROM CHAPMAN 1986)**

SPECIES	96-h LC <sub>50</sub> (mg/L)	SOURCE <sup>1</sup>
Stonefly		
<i>Acroneuria pacifica</i>	1.6 (H) <sup>2</sup>	G
<i>Acroneuria lycorias</i>	3.6	N
<i>Acynopteryx aurea</i>	3.3 (H)	G
<i>Arcynopteryx parallela</i>	<2 (H)	G
<i>Diura knowltoni</i>	3.6 (L)	G
<i>Nemoura cinctipes</i>	3.3 (H)	G
<i>Pteronarcys californica</i>	3.9 (L)	G
<i>Pteronarcys californica</i>	3.2 (H)	G
<i>Pteronarcys dorsata</i>	2.2	N
<i>Pteronarcella badia</i>	2.4 (H)	G
Mayfly		
<i>Baetisca laurentina</i>	3.5	N
<i>Callibaetis montanus</i>	4.4 (L)	G
<i>Ephemerella doddsi</i>	5.2 (L)	G
<i>Ephemerella grandis</i>	3.0 (H)	G
<i>Emphemerella subvaria</i>	3.9	N
<i>Hexagenia limbata</i>	1.8 (H)	G
<i>Hexagenia limbata</i>	1.4	N
<i>Leptophlebia nebulosa</i>	2.2	N

TABLE 5 CONTINUED

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SPECIES	96-h LC <sub>50</sub> (mg/L)	SOURCE <sup>1</sup>
Caddisfly		
<i>Brachycentrus occidentalis</i>	<2 (L)	G
<i>Drusus</i> sp	1.8 (H)	G
<i>Hydropsyche</i> sp.	3.6 (L)	G
<i>Hydropsyche betteria</i>	2.9 (21°C)	N
<i>Hydropsyche betteria</i>	2.6	N
<i>Hydropsyche betteria</i>	2.3 (17°C)	N
<i>Hydropsyche betteria</i>	1.0 (10°C)	N
<i>Lepidostoma</i> sp.	<3 (H)	G
<i>Limnophilus ornatus</i>	3.4 (L)	G
<i>Neophylax</i> sp.	3.8 (L)	G
<i>Neothremma alicia</i>	1.7 (L)	G
Diptera		
<i>Simulium vittatum</i>	3.2 (L)	G
<i>Tanytarsus dissimilis</i>	<0.6	N

<sup>1</sup> G = Gaufin (1973) -- all test at 6.4°C

N = Nebeker (1972) -- all tests at 18.5°C except as noted; flow 125 mL/min

<sup>2</sup> H = high flow (1000 mL/min)

L = low flow (500 mL/min)

Lethal DO concentrations for a variety of European species at 12°C spanned an extremely wide range, from near 1.1 mg/L for the stonefly *Nemoura cinerea* to >8.0 mg/L for riffle mayflies (*Epeorus sylvicola*, *Baetis alpinus*). With a few exceptions, DO requirements of the 22 species tested ranged from 10% to 80% of saturation (Jacob et al. 1984). Dissolved oxygen requirements were strongly temperature-dependent; for example, the LC<sub>50</sub> for *Nemoura cinerea* increased from 10.5% saturation at 12°C to 32.6% at 25°C, and the LC<sub>50</sub> for *Epeorus sylvicola* increased from 75% saturation at 12°C to 96.5% at 20°C (Jacob et al. 1984). Of course the increase is exaggerated because the same DO concentration is a higher percentage of saturation at the higher temperatures. In the context of the northern rivers, this temperature-dependence is fortunate because the DO demands of the invertebrate community is least in winter when river DO is also lowest.

DO requirements of benthic invertebrates also vary with stage of development. At 8°C, the DO level ensuring 95% survival indefinitely was 2.2 mg/L for the mayfly *Cloëon dipterum*, 5.1 mg/L for the stonefly *Taeniopteryx nebulosa* and 3.1 mg/L for the predatory stonefly *Diura nanseni*, one month before emergence. Just before emergence, however, DO requirements of the last two species increased to 7.1 mg/L and 5.3 mg/L, respectively, as the insects prepared to transform into adults (Nagell and Larshammer 1981).

The results outlined above imply that a minimum DO of 4 mg/L as recommended by the EPA (Chapman 1986) would not necessarily protect all species of benthic invertebrates; many species, especially coolwater mayflies, have LC<sub>50</sub> values well above that limit (Gaufin 1973; Jacob et al. 1984). Dissolved oxygen levels above 4 mg/L are sufficient to maintain a healthy invertebrate community, but the species composition may change.

Low DO concentrations will also affect feeding opportunities for fish because many insects, especially mayflies, are more prone to drift or migrate under hypoxia (Nagell 1977; Jacob et al. 1984), which renders them more susceptible to fish predation. In laboratory experiments, mayfly nymphs (*Callibaetis montanus*) became more active and migrated



upward from the substratum when benthic DO was lowered to about 1.5 mg/L, but to a lesser extent in the presence of fish predators (brook trout and brown trout). Nevertheless, mortality from predation was sharply higher under hypoxia because mayflies that left the stressful benthic conditions were more exposed to fish predators (Rahel and Kolar 1990). Given that the range in hypoxia tolerance of freshwater benthic invertebrates varies widely, this interaction could powerfully influence both the structure of benthic invertebrate communities and the feeding choices of fish in winter. In the northern rivers, hypoxia is likely to be most severe along the bottom because of sediment oxygen demand.

A similar change in feeding choices by predatory fishes has been observed in the estuary of Chesapeake Bay (Pihl et al. 1992). Periods of anoxia forced many otherwise burrowing organisms to move to the surface or enter the water column, where they were efficiently exploited by feeding fish. Both the average size of prey and the range of species selected changed after periods of hypoxia, and it appears the fish were able to optimally exploit moribund or slowly recovering benthos. Hence, the increased vulnerability of benthic organisms to fish predation during and after hypoxia episodes has important consequences for both the structure of benthic communities and the pattern of ecosystem energy flow (Pihl et al. 1992).

## 5.0 INTERACTIONS OF DISSOLVED OXYGEN WITH POLLUTANTS AND CONTAMINANTS

Reduced concentrations of dissolved oxygen increase the toxicity of most contaminants. This interaction has been repeatedly observed with a wide variety of fish and toxic substances. As an example, Figure 2 (from Thurston et al. 1981) illustrates the change in toxicity of un-ionized ammonia to rainbow trout as a function of DO. The relationship is strictly linear and leads to a doubling of toxicity ( $LC_{50}$  reduced by half) as DO decreases from 9 to 3 mg/L.

The above example illustrates that the effect of low DO on toxicity can be marked. The same interaction of effects extends to chronic toxicity as well. Carlson (1987) used a survival and growth rate bioassay with fathead minnows to test the toxicity of 1,2,4-trichlorobenzene at differing DO levels. Trichlorobenzene is an intermediary in pesticide manufacture and is similar to the chlorinated organics in pulp mill effluents. At the high DO concentration (8.1 mg/L), mean survival after 32 d was 37.5% less and mean weight of surviving larvae was 28.7% less for fish exposed to 0.920 mg/L trichlorobenzene than for controls. But at 4.5 mg/L of DO and the identical toxicant concentration, mean survivorship decreased 88.5% and mean weight by 60.5%. The dramatic increase in toxicity occurred despite the fact that the lower DO concentration is far above the lethal concentration for this species, and no effect could be attributed to lowered DO alone (Carlson 1987).

The sensitivity of toxicity to DO level implies that only a moderate decline in DO from optimum levels is sufficient to substantially increase the threat to aquatic life from dissolved contaminants. In a recent review of this interaction, Sprague (1985) suggested that a DO reduction from 100% to 80% of saturation could increase the incidence of mortality of fish exposed to contaminants. The increase in toxicity under hypoxia extends to pulp mill effluent and its components: exposure increases the respiratory rate and susceptibility to normally nonfatal hypoxia (Hicks and DeWitt 1971). Birtwell (1989) reviewed the literature on contamination from pulp mills and other sources, and concluded that DO requirements

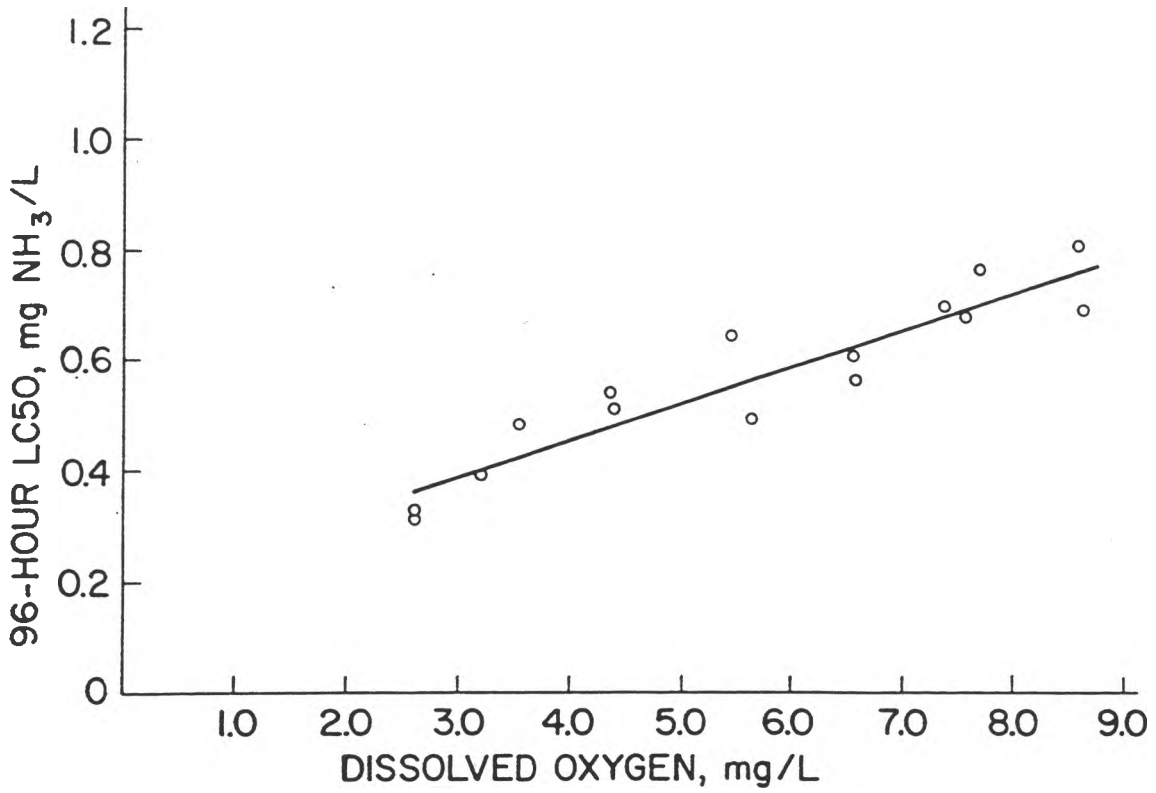


Figure 2. Effect of dissolved oxygen on the acute toxicity of ammonia to rainbow trout (From Thurston et al. 1981)

of aquatic organisms are likely to be higher in contaminated than in pristine habitats. The interaction of toxicity and DO bears directly on the situation in the northern rivers, where aquatic biota are exposed to lowered DO levels and pulp mill contaminants simultaneously in winter.

Resin acids, the principal toxicant in effluent from mills pulping softwoods (Leach and Thakore 1977), become decidedly more toxic as DO concentrations decline (Taylor et al. 1988). Resin acids interfere with normal O<sub>2</sub> uptake by the gill lamellae and induce O<sub>2</sub> starvation. Intoxicated fish show signs of respiratory distress, i.e., "coughing" and increased gill ventilation. Since oxygen cannot be taken up with the usual efficiency, a low O<sub>2</sub> tension that normally would be tolerated may be lethal. Sockeye salmon exposed to a normally sublethal concentration of dehydroabietic acid, the commonest resin acid, at DO levels of 75% saturation experienced 100% mortality, while fish exposed under identical conditions in well-oxygenated water (90% saturation) experienced only 5% mortality (Kruzyński 1979). The mortality rate in hypoxic water began to decline once fish deaths reduced O<sub>2</sub> consumption in the aquaria and raised the DO level to about 85% saturation.

Water-borne contaminants can affect gas exchange mechanisms at all levels of the respiratory chain. A major compounding effect of environmental contaminants on cellular hypoxia is the physical damage caused to gill tissues, which compromises O<sub>2</sub> uptake. Thus, fish can suffer hypoxic effects with contaminant exposure even though ambient DO levels may be otherwise adequate. At the gill level, contaminants can restrict gas transfer, affect ventilatory rhythm by irritation ("coughing" response), precipitate onto gill surfaces, and interfere with O<sub>2</sub> diffusion into the blood (Hughes 1981). For example, exposure of rainbow trout to zinc caused a reduction in the diffusive capacity of the gills, which in turn initiated a cascade of responses similar to those that occur with reductions in environmental oxygen (Skidmore 1970; Hughes and Adeney 1977). Thus, contaminants that interfere with gill function, such as zinc and resin acids, create a positive feedback: impairment of O<sub>2</sub> transport

by the toxicant induces increased gill ventilation, which in turn further accelerates toxicant uptake because more contaminated water passes over the gills (Taylor et al. 1988).

Interference with gas transfer at the gills reduces the level of O<sub>2</sub> in blood circulating to the brain, which may also trigger nervous responses at the respiratory centre (Ballintijn and Bamford 1975). In addition to nervous responses, hormonal responses such as catecholamine release may occur (Hughes 1981). While some hormonal response will be due to the exposure to the contaminant itself, secondary responses may also be related to the resultant hypoxia. In a study with zinc-exposed rainbow trout, Hughes (1981) found that both ventilatory frequency and amplitude showed greater increases during hypoxic conditions than in control fish subjected to hypoxia alone. The limited evidence led Hughes (1981) to conclude that sublethal zinc concentrations may affect the ability of fish to respond to changes in DO and, under some conditions, reduce their chances of survival.

A number of studies have examined the combined effect on fish of low DO and exposure to kraft pulp mill effluent (KME). The major concern is that KME discharges have a high BOD and also contain toxic substances, so the toxic effect of the compounds could be exacerbated by the additional stress associated with low DO in the zone of influence below the discharge. For example, the effluent BOD load from the Weldwood mill at Hinton is about 2000 kg/day; the Millar-Western CTMP mill releases 6000 kg/day (Noton et al. 1989).

Hicks and DeWitt (1971) showed that the toxicity of KME to juvenile coho salmon increased with progressively reduced levels of DO. The LC<sub>50</sub> dropped from 10.1% (as percent of effluent strength) at 7.5 mg O<sub>2</sub>/L to 8.5% at 4.1 mg/L. Median survival time in 33% effluent declined from 27 to 11 h at DO concentrations of 6.6 and 3.4 mg/L. Figure 3 illustrates the effect of DO on effluent toxicity. Again, the level of DO itself was never low enough to be directly lethal. Hicks and DeWitt (1971) concluded that it would be hazardous to estimate "safe" levels of effluent discharge for aquatic life based on water saturated with DO.

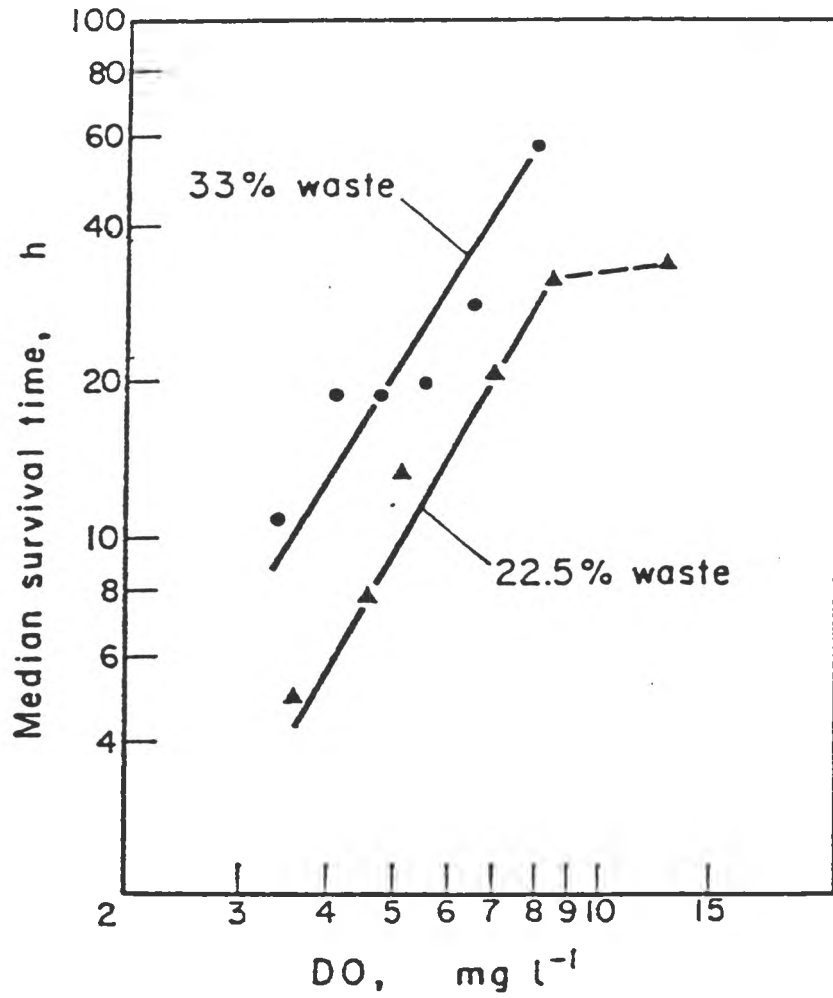


Figure 3. Regressions of median survival time of juvenile coho salmon against concentration of Kraft pulp mill effluent (33% and 22.5% dilutions) (Source: Hicks and DeWitt 1971)

Bleached KME is toxic to early life stages of fish often at concentrations well below those that are deleterious to adults. Eggs of northern pike, a common species in the northern rivers region, suffered reduced hatching success when incubated for 18 days in various dilutions of bleached KME in Finland (Tana and Nikunen 1986). The median effective concentration was <4% of full strength effluent, and the threshold of effects was near 0.5%. Similarly, brown trout eggs incubated in bleached KME suffered lower hatching success in 2% effluent (Vuorinen and Vuorinen 1987). In this species, at least, the just-hatched alevins were more sensitive than the eggs, and exhibited retarded growth and development and increased mortality at effluent concentrations down to 0.5 %. Mortality of sac-fry incubated in 2% effluent was 100% (Vuorinen and Vuorinen 1987). If lowered DO tensions further increase the potency of KME to incubating eggs, as seems reasonable, then impairment of reproduction could be felt at extremely low effluent dilutions and for long distances downstream from mill effluent outfalls, if DO is significantly depressed.

Graves et al. (1981) examined the interactive effects of low DO and KME on all stages of the life cycle of sheepshead minnows (*Cyprinodon variegatus*). Those authors found a significant interactive effect of low DO on survival of juvenile fish exposed to effluent, but embryo survival was affected by the effluent only and fry survival was affected by low DO only. Adult fish were not affected by either DO or KME, alone or combined. This example serves to illustrate the complexities of effects that are possible, given the life-cycle variation in both sensitivity to contaminants and metabolic O<sub>2</sub> demands.

As discussed in Section 3, much of the dissolved oxygen demand of pulp mill effluents is transferred to the sediments through settling of decomposing organic matter (Macdonald and Hamilton 1989). Historically, the problem was wood fibres released as byproducts of debarking or pulping that settled out of the effluent plume for many kilometres downstream of the effluent outfall (Colby and Smith 1967). With the advent of primary and secondary wastewater treatment, raw wood fibres have been removed in the settling tanks, but the final effluent still contributes particulate organic matter, consisting of bacterial flocs, lignin and

semidigested wood tissue. This material settles to form jell-like benthic mats that coat the river bottom and exert a powerful oxygen demand: up to  $0.44 \text{ g.m}^{-2}.\text{d}^{-1}$  below the Weldwood Kraft mill at Hinton, and up to  $0.61 \text{ g.m}^{-2}.\text{d}^{-1}$  below the Millar-Western CTMP mill at Whitecourt (Macdonald and Hamilton 1989).

Early research demonstrated the considerable impact of benthic sludge mats on fish reproduction. A wood-fibre sludge mat in Rainy River, Minnesota, created a steep vertical gradient of DO concentration right at the sludge-water boundary, from near saturation to sometimes  $<1 \text{ mg/L}$  over a distance of 20 mm (Colby and Smith 1967). Hatching success of walleye eggs was lower in trays on the sludge mats than in trays 30 cm above them, or on mineral substrates. Direct contact with the sludge mats was lethal to walleye eggs and fry, as well as to the benthic crustacean *Gammarus pseudolimnaeus*. The direct cause of death could have been either hypoxia itself or elevated levels of sulphide exuded from the mats. If the latter, it was still the low DO above the mats that permitted sulphide to persist without being immediately oxidized (Colby and Smith 1967).

Wood fibres in pulpmill effluent may also interfere with fish before the fibres settle out. In fathead minnows, fibre suspensions of 100 to 800 mg/L decreased active metabolism, reduced swimming endurance, increased maintenance energy requirements, and increased blood hematocrit (MacLeod and Smith 1966). All these responses were exaggerated in the presence of low DO levels. In short, the fish exhibited a generalized stress response to both low DO and wood fibres, that would in the long term reduce the survival and production of the population (MacLeod and Smith 1966). Although pulp mill effluents do subject fish to suspended organic matter and low DO at the same time, the results of MacLeod and Smith (1966) may not be applicable to modern mills in which the concentration of suspended material is much lower. Fish are relatively insensitive to suspended solids unless concentrations are very high (McLeay et al. 1987). More important is the observation that low DO and physical and chemical contamination each produce stress in exposed fish and that the stresses are cumulative.



A more subtle, but potentially more far-reaching effect of pulp mills on fish reproduction arises from the high nitrogen and phosphorus content of their effluents. Reproductive success of whitefish (unspecified species) in European lakes is negatively correlated with trophic state, as predicted by phosphorus concentrations at spring overturn (Müller 1992). The oxygen-demanding organic sediments of the more eutrophic lakes deprive whitefish eggs of the high DO concentrations necessary for embryo development. A similar effect is certainly possible in riverine systems, through deposition of organic detritus or stimulation of benthic algal growth, but it may not be an issue in the northern rivers because of the dilution of effluents in large river volumes, and because of the frequency of scouring flows.

Sublethal effects of pulp mill effluent have been reviewed by Davis (1976), Kovacs (1986) and McLeay and Associates (1986). Observed sublethal responses include elevated frequency of "coughing", reduced O<sub>2</sub> saturation in arterial blood, changes in blood chemistry (especially increases in plasma glucose and lactate), depression of liver glycogen storage, increased ventilation, less vigorous feeding, decreased growth and food conversion efficiency, and avoidance behaviour. The increases in plasma glucose and lactate, and concomitant decline in glycogen storage are indicative of an elevated metabolic demand, which in turn leads to reduced growth. The similarity of responses to pulp mill effluent to those for hypoxia generally (see Section 4) suggest that a general stress response is being manifested.

The effect of KME exposure will manifest itself in a variety of sublethal responses that relate to respiratory capacity and metabolic scope. Davis (1973) found that the threshold level of KME that caused respiratory responses in sockeye salmon was 20% of the effluent's LC<sub>50</sub>; respiratory responses examined included increased ventilatory volume, increased cough frequency, and increased buccal pressure. Kraft mill effluent also caused an elevation in O<sub>2</sub> uptake. Exposure to KME caused arterial O<sub>2</sub> tension to decline from 90-95% to 55-80% of saturation. The failure of blood to become saturated with O<sub>2</sub> in spite of increased respiratory activity appeared to be related to an increase in diffusion distance across the gill membrane due to elaboration of mucus. Davis's (1973) results supported earlier

observations of KME causing "respiratory stress" in salmonids (Alderdice and Brett 1957; Walden et al. 1970). Using cough frequency as an indicator, Walden et al. (1970) found that threshold concentrations of KME for eliciting the minimum respiratory response was 4% of the effluent.

One area of fish performance related directly to scope for activity is growth (see Section 2.2). Webb and Brett (1972) found that exposure to 25% concentration of KME (and possibly 10%) caused a significant reduction in sockeye salmon growth rate. Standard metabolism was elevated by a factor of two in fish exposed to a KME concentration of 1% of the 24-h  $LC_{50}$ . They concluded that decreased growth caused by KME exposure was associated with raised maintenance energy cost, thereby reducing that energy available for growth. Similarly, Whittle and Flood (1977) demonstrated that the 18-d growth rate of rainbow trout fry was significantly reduced in 6% KME; lower concentrations caused no growth impairment. Fish held in 6 and 10% KME showed erratic respiratory activity after 7 d of exposure.

Effects of KME exposure are also evident at the population level. Kelso (1977) documented behavioural avoidance by radio-tagged suckers (*Catostomus* sp.) exposed to low concentrations of pulp mill effluent and disorientation when exposed to high concentrations (>15% of effluent). Conditions near the effluent discharge caused species dominance in the fish community to switch from yellow perch to suckers at the mill site. Böhling et al. (1991) documented disruption in natural year-class patterns of perch (*Perca fluviatilis*) populations in Baltic Sea areas affected by pulp mill discharges, but did not state what component of the effluent caused the recruitment impairment.

The preceding examples serve to demonstrate that both hypoxia and pulp mill effluents produce a general stress response in fish, which is characterized by physiological upset and diversion of energy toward maintenance that would otherwise go to growth and production. Moreover, it appears that the effects of such stresses are cumulative. Stress effects on

individual fish will translate into effects at the population and community levels (Adams 1990). Fish populations respond in a predictable way to multiple stressors: mean age increases but condition factor and fecundity both decrease (Munkittrick and Dixon 1989). A response of this kind, varying in degree according to the severity of the stressors, can be expected in the northern rivers if pulp mill effluents produce further lowering of wintertime dissolved oxygen levels.

## **6.0 SPECIES AND LIFE STAGE REQUIREMENTS**

### **6.1 GENERAL ASPECTS OF DISSOLVED OXYGEN REQUIREMENTS**

Fish have both acute and chronic DO requirements. The acute requirement is the minimum level of DO necessary to avoid short-term, potentially fatal hypoxia. Most fish can withstand brief exposures to DO concentrations far below their optimum; but long-term exposures to suboptimal DO levels, even at concentrations well above those necessary for mere survival, create stress and decrease growth and population production (see Section 4). Establishment of acutely lethal DO levels is relatively straightforward and can be accomplished in the laboratory. In theory, an LC<sub>50</sub> value (the DO concentration quickly lethal to 50% of the test organisms) can be established in the same manner as for other toxicants. In practice this approach is seldom used and lethal DO levels must be inferred from mortality rates of fish exposed to gradually diminishing oxygen concentrations (Doudoroff and Shumway 1970).

Acutely lethal DO minima are relatively easily defined, but somewhat higher DO levels may still be detrimental to fish health and production in the long term. Critical DO levels for growth, the best general index of health, are determined by finding the concentration at which growth (or related physiological functions) become oxygen dependent. Above the critical concentrations, the functions in question remain independent of DO level (Hughes 1981; Pederson 1987); below the critical level, the physiological rates increase or decrease in tandem with the external DO concentration. The critical O<sub>2</sub> concentration is that below which growth impairment may be expected, becoming increasingly severe as the mean DO level declines (see Section 2).

Determination of long-term dissolved oxygen requirements, even where critical DO levels for growth or other functions are known, is more difficult. Doudoroff and Shumway (1970) pointed out that, based on many studies on many species, there is evidently no concentration level by which the DO level of natural waters can be reduced without risking some adverse

effects on the growth or reproduction of fishes inhabiting those waters. In other words, the DO level, like other environmental conditions, acts as a limiting factor on fish growth and population production (Fry 1971). Because DO must be acquired through expending energy, and because the concentration of DO limits activity and production of fishes (see Section 2.2), oxygen can be considered a limiting resource, in the ecological sense, analogous to food or nutrients (Kramer 1987). Hence, any reduction of DO levels may proportionately reduce production by fish and other aquatic organisms.

Yet DO levels well below full saturation, as often occur naturally, may still permit the long-term persistence of a healthy fish population. Therefore, long-term depression of DO concentrations below their natural level in a waterbody will be manifested more as a reduction of fish growth and production than as mortality (Kramer 1987). The depression of production is greatest when temperatures are high and food is abundant, and hence both metabolic demand and growth potential are greatest (Chapman 1986). There are parallel increases, as DO concentration declines, in susceptibility to stress, predation, toxicants and disease. Because of this continuum of effects of DO level, requirements cannot be predicated upon a level of "no effect" as is often done for toxic substances. Rather, the approach has been to define families of criteria or equations that correspond to a given level of risk or protection of the fish population (Doudoroff and Shumway 1970; Davis 1975). The classes or levels of protection are usually defined qualitatively, because the exact degree of effect will vary according to the fish species, climate, food supply and many other uncontrolled factors. Chapman (1986) has drawn on this earlier work and more recent data to define four levels of protection, corresponding with severe, moderate, slight or no production impairment. The no-impairment class requires very high DO levels and is probably only realized in pristine waterbodies; still, large areas of the northern rivers do, or until recently did, maintain DO concentrations at the no-impairment level.

In addition to specific differences in dissolved oxygen requirements and those for both acute and chronic exposures, there are substantial differences in requirements for different stages

of the life cycle. Generally, larval stages are far less tolerant of low DO than adult fish (Davis 1975). While Doudoroff and Shumway (1970) determined that deformities in embryonic fish only occurred when DO levels decreased below 2-3 mg/L, they concluded that any reduction in O<sub>2</sub> saturation could result in hatching delays and smaller than normal fry. Further, the review in Section 5 illustrates that low DO magnifies toxic effects of pulp mill effluent.

Consideration of these factors led to the set of DO criteria proposed by Taylor and Barton (1992) for trouts, whitefishes, and non-salmonids (i.e., walleye and sauger [*Stizostedion canadense*]) in Alberta. The DO criteria they proposed were modified from those developed by Chapman (1986) for the EPA, the most complete and recent criteria available at the time. Chapman (1986) incorporates or considers a number of earlier reviews and criteria documents (e.g., Doudoroff and Shumway 1970; Davis 1975; EPA 1976; IJC 1976; Alabaster and Lloyd 1982) as well as more recent literature.

The EPA criteria include four periods of averaging DO concentrations, from one day to one month. The 30-day mean limit (compressed to a 7-day mean in Taylor and Barton [1992]) is intended to protect against stress and reduced production and growth from prolonged exposure to sub-optimal DO levels. The same end is accomplished by a 7-day mean limit for eggs and larvae, which persist for such short periods that a 30-day average is not sufficiently protective. The remaining criteria are a 7-day mean minimum (that is, the mean of the nightly minimum DO values each week) applied to adult fish, and a 1-day minimum applied to all life stages.

The complexity of dissolved oxygen requirements, and lack of information for many species, makes establishment of DO criteria for each individual fish species in the northern rivers impractical and probably unnecessary. Rather, in the following sections information for families and subfamilies (within the Salmonidae) is compiled, with a view toward defining classes of fish with similar DO requirements. Where exact data are not forthcoming, DO

requirements have been inferred from the general habits and habitats of the species. Using this classification, in concert with the earlier review of interactions between DO and pollution stresses, it should be possible to establish a workable array of criteria that will ensure reasonable protection of all fishes within the northern rivers.

## 6.2 REQUIREMENTS OF SALMONINAE

Rainbow Trout	<i>Oncorhynchus mykiss</i>
Kokanee	<i>Oncorhynchus nerka</i>
Bull Trout	<i>Salvelinus confluentus</i>

Most of the research on DO requirements of salmonines, upon which the EPA criteria (Chapman 1986) are based, has been done with rainbow trout (e.g., see references in Davis 1975). Based on a number of papers cited in their synopsis, Raleigh et al. (1984) concluded that optimal DO levels for rainbow trout were  $\geq 7$  mg/L at temperatures  $\leq 15^\circ\text{C}$ , and  $\geq 9$  mg/L at  $> 15^\circ\text{C}$ ; the latter represents full saturation at 1000 m. Comparative available data suggest that all trout (with the possible exception of brown trout, which is not found in the northern rivers) have quite similar DO requirements. Salmonids generally have a higher  $\text{O}_2$  demand than many other fish groups, but oxygen tolerances and requirements are similar for most salmonine species. Acute mortality of adult fish is apparent at DO between 1 and 3 mg/L (Doudoroff and Shumway 1970). Davis (1975) also reported the highest acutely lethal DO level is near 3.0 mg/L. Brown trout exposed to acutely lethal DO levels survived an average of <50 min at 1.0 mg/L, and 1.5 h at 1.5 mg/L (Drewett and Abel 1983); mean survival time of brook trout exposed to DO at 1.7 mg/L was 130 min (Dandy 1970).

Dissolved oxygen concentrations substantially higher than acutely lethal levels affect behaviour, growth and swimming performance. For example, in trout at  $15^\circ\text{C}$ , the DO concentration controls food consumption below 6 mg/L, and growth and food conversion

efficiency below 7 mg/L (Pederson 1987). Morrison and Piper (1986) found that below a DO concentration of 5.7 mg/L, growth rate, food conversion efficiency, and survivorship (to 140 d) of young brown trout all declined in direct proportion with DO levels.

There is evidence from diverse sources that salmonines actively avoid areas of low DO concentration. In their review, Hickman and Raleigh (1982) stated that optimal DO levels for cutthroat trout (*Oncorhynchus clarki*) appear to be >7 mg/L at temperatures  $\leq 15^{\circ}\text{C}$  and higher for warmer temperatures, and that this species avoided concentrations <5 mg/L. Brook trout raised in water with 11.3 mg/L DO became increasingly active at lower DO concentrations, with a response threshold near 8.0 mg/L (Dandy 1970). Similarly, Birtwell and Kruzynski (1989) found chum (*Oncorhynchus keta*) and coho salmon exposed to oxygen gradients in estuaries off Vancouver Island began migrating out of hypoxic waters when the DO concentration was still 7-8 mg/L. Thus, any DO concentration below about 7 mg/L would appear to be suboptimal for most salmonines. The EPA has recommended DO criteria of 4.0 mg/L as an acute lower limit to prevent mortality and 6.5 mg/L as a chronic limit to protect natural populations from production impairment (Chapman 1986; CCREM 1987).

Extensive data on DO requirements are lacking for salmonines other than rainbow trout; the substantial amount of experimental work on tolerance limits that has been conducted with rainbow trout has not been repeated with most of the species of concern in northern Alberta. However, much of the preceding general discussion should apply to kokanee and bull trout as well as rainbow trout. No information on DO requirements is available specifically on kokanee; however, as kokanee are landlocked sockeye salmon and the same species as their anadromous counterparts, their DO requirements are likely the same. One study listed in Doudoroff and Shumway (1970) indicates a lethal DO concentration of 2.3-2.7 mg/L, confirming that this species is similar to other salmonines. Sockeye salmon eggs require at least 5.0 mg O<sub>2</sub>/L for successful incubation. At 15°C, DO levels >4.2 mg/L did



not affect growth of juvenile sockeye salmon but growth ceased at 2.6 mg/L or less (Brett and Blackburn 1981). Presumably, these requirements apply to kokanee as well.

Similarly, information on oxygen tolerances specifically for bull trout is lacking, but other reports document sublethal effects of low DO on other charrs. At an environmental PO<sub>2</sub> of about 120 Torr, which represented about two-thirds saturation, active respiration in lake trout (*Salvelinus namaycush*) switched from independent to dependent (Gibson and Fry 1954). DO uptake approached zero at 40 Torr at 10°C and at 60 Torr at 22°C (Gibson and Fry 1954).

Klyashtorin (1975) determined critical ( $T_c$ ) and lethal threshold levels of PO<sub>2</sub> for fry-sized Arctic char (*Salvelinus alpinus*), coho salmon, chinook salmon, and sockeye salmon. Critical PO<sub>2</sub> values were similar at 10°C, ranging 33-35 Torr. Threshold PO<sub>2</sub> levels were also similar at 10.8-19.5 Torr (Klyashtorin 1975).

Salmonine embryos have very high DO requirements that change with their development. A concentration of 5 mg/L DO is the lethal limit for (rainbow) trout embryos, and survival drops off sharply below 6 mg/L (Sowden and Power 1985). Recent research has shown that for rainbow trout, DO requirements of the embryo increase through development to a peak just before hatching (Rombough 1988). Greatest required DO levels were: 7.5 mg/L at 6°C, 8.9 mg/L at 9°C, and 9.6 mg/L at 12°C. A minimum DO of 6 mg/L is recommended for eggs to the eyed stage and up to 11 mg/L (at 10°C) from the eyed stage to hatching (DFO 1983). For post-hatch stages, Davis (1975) recommended a DO level of 7.8 mg/L for the maximum level of protection.

For trout eggs and alevins, which live within the stream-bottom gravel, the oxygen environment around the eggs could be quite different from that in the overlying water. A minimum difference of 3 mg/L in DO level between interstitial water and the water column has been reported from the few direct measurements in incubating redds (Chapman 1986).

The DO concentration gradient between the water column and interstitial water is dependent on water depth and velocity as well as substrate type. Pore water DO levels in fine sand substrates on the Pembina and South Saskatchewan Rivers were >3 mg/L below those in the water column (P. Chambers, personal communication). Therefore, maintenance of at least 6 mg/L in interstitial gravels requires minimum water-column DO levels of 9 mg/L.

For trout, the early life stage ends when the alevins complete yolk-sac absorption, leave the protection of the redd, and begin feeding; at this point, they are known as fry. Dissolved oxygen requirements of fry are higher than those of adults because the fish are growing quickly and have not yet developed tolerance of extreme conditions. On the other hand, when young trout leave the protection of the bottom gravel after swim-up, the requirement of maintaining high interstitial DO levels would no longer be necessary. From that point onward, fish have access directly to the DO in the water column.

### 6.3 REQUIREMENTS OF COREGONINAE

Mountain Whitefish	<i>Prosopium williamsoni</i>
Round Whitefish	<i>Prosopium cylindraceum</i>
Lake Whitefish	<i>Coregonus clupeaformis</i>

Given the similarity of ecological requirements among salmonids generally, DO requirements of trouts are assumed to apply to whitefishes as well. One possible exception is the DO requirement for whitefish fry. Davis (1975) and Chapman (1986) apply the early-life-stage criteria for salmonids to embryos and all juveniles up to one month after hatching. This is reasonable for trout, but Davies and Thompson (1976) found newly emerged mountain whitefish fry inhabiting shallow, still backwaters that presumably had significantly lower DO concentrations than the main channel. Thus, the DO requirements for early life stages of these fish may be lower than that for trouts.

Other life stages appear to be similar to trout in their DO requirements. Siefert et al. (1974) found that survival of mountain whitefish eggs and larvae incubated at 4°C was the same at DO concentrations of 12 or 6.5 mg/L, and growth rates were similar. Incubation time was prolonged by two weeks at the lower DO tension. At a DO level of 4.6 mg/L, however, survival and growth rate 100 days after hatching were significantly reduced. At DO levels of 3.3 mg/L or less, hatching success, growth and survival declined drastically (Siefert et al. 1974). Augmentation of criteria to account for interstitial DO concentrations, such as was done for trout (Taylor and Barton 1992), is not required for mountain whitefish, which are broadcast spawners. Notwithstanding, because of the strong DO gradient at the sediment-water boundary in rivers enriched with settleable organic matter, it would be prudent to maintain water-column DO levels above the minima to ensure embryos are well-supplied (Müller 1992).

There are few data available on specific dissolved oxygen requirements for lake whitefish or round whitefish. Doudoroff and Shumway (1970) reported lethal levels of generally <3 mg/L for a number of European and Asian whitefishes, which are again similar to those reported for other salmonids. Coregonine fishes were not included in the extensive review by Davis (1975) of oxygen requirements for Canadian species. In the absence of experimental data on DO requirements of coregonine fishes, those for salmonines should be assumed to apply.

#### 6.4 REQUIREMENTS OF THYMALLINAE

Arctic Grayling

*Thymallus arcticus*

In sealed-jar assays, Arctic grayling acclimated to 15°C and tested at 20°C died at a DO concentration of 2.0 mg/L (McLeay et al. 1987). The lethal DO level declined to 1.5 mg/L for fish acclimated to 5°C and tested at 10°C. Median times to death were 147 and 158 min in duplicate assays. Residual oxygen assays probably underestimate the lethal DO

concentration because the distressed fish continue to deplete the O<sub>2</sub> supply until they die. Nevertheless, the lethal DO concentration for Arctic grayling is similar to those for underyearling rainbow trout and coho salmon held under identical test conditions (McLeay et al. 1987). Minimum threshold lethal DO levels ranged from 2.2 mg/L at 19°C to 1.7 mg/L at 6°C in Arctic grayling acclimated to 13°C (Hubert et al. 1985). Threshold DO minima for fry were lower, ranging from 1.8 mg/L at 20°C to 1.4 mg/L at 8°C (Feldmuth and Eriksen 1978).

Some anecdotal evidence suggests, however, that Arctic grayling may be more tolerant to reduced DO conditions than other salmonids (Eriksen 1975). Dissolved oxygen levels of 0.6 to 4.8 mg/L have been observed in overwintering areas (Alt and Furniss 1976 and Bendorck 1980, as cited *in* Hubert et al. 1985). On the other hand, West and Smith (1992) found that Arctic grayling in northern Alaska overwintered exclusively in spring areas, deep lakes or river pools, the latter often spring-fed and open. No low DO tolerance would be required to survive in these habitats. Dissolved oxygen requirements of Arctic grayling eggs and alevins have apparently not been tested.

## 6.5 REQUIREMENTS OF CYPRINIDAE

Longnose Dace	<i>Rhinichthys cataractae</i>
Finescale Dace	<i>Phoxinus neogaeus</i> (formerly <i>Chrosomus</i> )
Northern Redbelly Dace	<i>Phoxinus eos</i> (formerly <i>Chrosomus</i> )
Pearl Dace	<i>Margariscus margarita</i> (formerly <i>Semotilus</i> )
Flathead Chub	<i>Platygobio gracilis</i> (formerly <i>Couesius plumbeus</i> )
Northern Squawfish	<i>Ptychocheilus oregonensis</i>
Redside Shiner	<i>Richardsonius balteatus</i>
Emerald Shiner	<i>Notropus atherinoides</i>
Spottail Shiner	<i>Notropus hudsonius</i>
Fathead Minnow	<i>Pimephales promelas</i>

Little information on tolerance limits of cyprinid fishes is available beyond what was already reviewed by Doudoroff and Shumway (1970). In that review, only five of the cyprinids tested are found in northern rivers. Those species, their lethal DO levels, and reference (as cited in Doudoroff and Shumway 1970) are described in Table 6.

Mean time to loss of equilibrium in four cyprinid species ranged from 60 to 119 min at 1.2 to 1.5 mg/L DO (Matthews and Maness 1979). Species differences were considerable with plains minnow being the most DO tolerant, indicating that generalizations about tolerances at the family level may not be appropriate. Of the four species investigated, emerald shiner was the only one native to northern rivers, and it was found to be the least DO tolerant; the mean time to loss of equilibrium at this low DO was 60 min (Matthews and Maness 1979).

Eurasian minnows have similar low DO tolerance (Stott and Buckley 1979); in avoidance tests, minnows did not leave their home range until DO levels reached 1.2 mg/L. Wares and Ingram (1979) noted that fathead minnows appeared to make the transition between regulating (independent) and conforming (dependent) respiration at about 5 mg/L DO. Their results suggested that fathead minnows were neither regulators nor conformers, but partial regulators or conformers depending on the ambient DO level. Under extreme hypoxia, fathead minnows are able to effectively use aquatic surface respiration by rapidly adjusting swimbladder gas through air-gulping (Gee and Ratynski 1988).

Even among cyprinids, fathead minnows appear to be particularly well-adapted to low DO. Lethal thresholds for fathead minnows exposed to gradually declining DO tensions averaged 0.50 mg/L, with a range of 0.4 - 0.7 mg/L (Castleberry, and Cech, 1992). In a frozen Wisconsin lake, fathead minnows survived DO < 0.5 mg/L longer than either brook sticklebacks or central mudminnows. Further, fathead minnows were the only species that did not reduce locomotor activity at low DO, even at 0.25 mg/L, and were the last to begin aquatic surface respiration beneath air bubbles. Brungs (1971) reported no effect on fecundity in fathead minnows after chronic (11-month) exposure to DO levels of  $\geq 3$  mg/L;

**TABLE 6****LETHAL DO LIMITS OF SELECTED CYPRINIDS  
(AS CITED IN DOUDOROFF AND SHUMWAY 1970)**

<b>SPECIES</b>	<b>LETHAL DO (mg/L)</b>	<b>TEMPERATURE (°C)</b>	<b>REFERENCE</b>
Northern redbelly dace	< 2.0	20	Black et al. 1954
Finescale dace	< 1.0	18 - 21	Black et al. 1954
Pearl dace	< 2.0	18 - 19	Black et al. 1954
Fathead minnow	1.0 - 2.0	18 - 26	Black et al. 1954; Whitworth and Irwin 1961
Northern squawfish	1.4	23	Chapman 1940

but at 2 mg/L, the number of eggs per female was reduced, and at 1 mg/L, no eggs were laid. Fathead minnow fry growth was lower in all hypoxic conditions than in the control DO of 7.9 mg/L. Hatching time increased with decreasing DO levels, and fry survival was reduced at  $\leq 4$  mg/L DO with 18% of the survivors at 4 mg/L being deformed (Brungs 1971).

## 6.6 REQUIREMENTS OF ESOCIDAE

Northern Pike

*Esox lucius*

Northern pike appears to be very tolerant of low DO. Lethal DO levels for northern pike cited from early reports in Doudoroff and Shumway (1970) ranged from 3.1 mg/L to as low as 0.2-0.5 mg/L; the high lethal levels were reported from fish held in cages submerged in a lake, whereas the lethal levels given in the range of 0.2 to 1.6 mg/L appear to be from laboratory studies, although methods are not clearly described. Based on tolerance of severe hypoxia in ice-covered lakes, northern pike appear to have lower DO requirements during winter than either yellow perch or walleye (Inskip 1982). Metabolic oxygen requirements increase with temperature, but the incipient lethal concentration at 28°C is still  $< 1.5$  mg/L (Inskip 1982).

In a laboratory simulation of under-ice conditions, most northern pike survived successively lower DO, going from 4 to 0.25 mg/L over 5 d, by altering their behaviour (Petrosky and Magnuson 1973). Pike were able to move upwards in the water column to take advantage of higher  $PO_2$  in the surface layer just under the ice. Still, prolonged periods with dissolved oxygen  $< 1$  mg/L may cause partial or complete winterkill (Inskip 1982).

Lower threshold DO for incubating northern pike eggs was about 1.8 mg/L (Gulidov 1969). Rates of embryonic development varied according to  $PO_2$ , leading the author to conclude that when DO in water is reduced below "natural saturation", embryogenesis is retarded and time to hatching increased. At DO concentrations of 5.4 and 4.1 mg/L, a higher incidence

of abnormal development was observed than at the control DO level of 8.1 mg/L (Gulidov 1969). In larval northern pike, ambient DO at 50% saturation (4.5 mg/L) appeared to be adequate to allow hatching survival and development to occur up to the time of first feeding (Siefert et al. 1973). However, DO tensions of about 33% saturation (3.2 mg/L) were insufficient for proper survival, particularly after the initiation of feeding.

As with many other species, DO concentrations for optimal growth and production of northern pike are much higher than the acute lethal limits. Growth rates of juvenile northern pike declined in parallel with lowered DO concentrations, with an effect threshold of 7 mg/L. The decline was gradual down to DO tensions of 3-4 mg/L but steep at levels below 3 mg/L (Adelman and Smith 1970). Young-of-the-year northern pike showed signs of stress when held at DO concentrations <6 mg/L at 21-22°C (Inskip 1982); feeding ceased at 2 mg/L and 100% mortality ensued at 1 mg/L.

### 6.7 REQUIREMENTS OF PERCIDAE

Walleye	<i>Stizostedion vitreum</i>
Yellow Perch	<i>Perca flavescens</i>

Early field studies indicated that the greatest abundance of adult walleye occurs where minimum DO levels are >3-5 mg/L (Dendy 1948). The limited data available on oxygen requirements of walleye are compiled in Table 7. Hoff and Chittenden (1969) report an acute lethal concentration for adult walleye of 1.2 mg/L (range 1.1-1.6 mg/L) at 24°C. Colby et al. (1979), as part of a comprehensive synopsis of the biology of walleye, indicated that 2 mg/L may be tolerated by adults in the laboratory, but cite no experimental work. Scherer (1971) found adult walleye could tolerate 2 mg/L DO for a short time, but behavioural disruption and eventual loss of equilibrium occurred at DO levels ≤1.5 mg/L. Perturbation of normal behavioural patterns at low DO levels could seriously impair feeding performance



TABLE 7

## SUMMARY OF LOW DISSOLVED OXYGEN EFFECTS ON WALLEYE

LIFE STAGE	DO LEVEL (mg/L)	EFFECT	SOURCE
adult	2	tolerance limit in laboratory	Colby et al. 1979
populations	>3	"most abundant in natural waters"	Colby et al. 1979
adult (1+)	1.5 - 2 <1.5	loss of light-avoiding behaviour loss of equilibrium (probably fatal)	Scherer 1971
adult	1.1 - 1.6	mortality	Hoff and Chittenden 1969
embryo	5 - 6 <5 3	optimum for incubation, 12 - 13°C reduced hatching success severely reduced hatching success; 10 - 13% shorter larvae than at 7 mg/L	Oseid and Smith 1971
embryo/larva	5.0 3.4 2.5 5.7 - 7.3  <3 5.3	95% survival after 20 days 40% survival no survivors at 24°C, considered optimum for hatcheries 1 hour exposure, >25% mortality no mortality after 1 day	Siefert and Spoor 1974

of walleye as these fish feed at night or early morning when DO levels would be at their daily minimum.

The early life stage of walleye includes the period when the newly hatched larvae leave the spawning grounds and disperse throughout the lake or river (Scott and Crossman 1973); this time is relatively short as the yolk sac is absorbed quickly (Nickum 1986). In non-salmonid fish generally, newly hatched larvae are considerably more sensitive to low DO levels than older fish (Doudoroff and Shumway 1970), and walleye larvae in particular, being initially too small to fight currents (Walburg 1972), cannot actively avoid episodes of low DO.

Scherer (1971) noticed that under declining DO conditions, behavioural responses were evident when DO was <6 mg/L. At DO levels progressively lower than 6 mg/L, walleye displayed increasing mobility and less time remaining under shelter. At very low DO (1-2 mg/L), the fish remained outside of the shelter, which corresponds with their observation of the loss of avoidance response to light at such levels (Scherer 1971).

Except during very earliest life, walleye are opportunistic piscivores, feeding predominantly on species of smaller fish that are available (Scott and Crossman 1973; Lyons 1987). Those benthic invertebrates that are taken are generally tolerant, large-river forms such as chironomids, amphipods, molluscs and burrowing mayflies (Craig and Smiley 1986; Ritchie and Colby 1988; Fox 1989) that do not require high DO levels. The only exception is newly hatched larvae, which feed on zooplankton and other invertebrates for the first 1-2 months, until they are large enough to consume fish (Walburg 1972; Raisanen and Applegate 1983). Hence, to maintain a healthy walleye population, a healthy and diverse population of forage fishes must be maintained.

More data are available on DO requirements of young walleye (Table 7). From experimental experience, it appears that concentrations much below 3.5 mg/L are likely to lead to reduced survival of embryos and young larvae. For example, larval survival to 20 d

at 17°C was 42%, 39%, 15% and none, at DO saturations of 100%, 50%, 35% and  $\leq 25\%$ , respectively (Siefert and Spoor 1974); these saturation values (at 0 m altitude) are equivalent to about 9.7, 4.9, 3.4 and  $\leq 2.4$  mg/L. Similarly, DO levels  $\leq 3.4$  mg/L can cause delayed hatching as well as a reduction in embryo size at hatching (Colby and Smith 1967; Siefert and Spoor 1974; see Table 7). In experiments incubating walleye eggs at DO levels ranging between 2 and 7 mg/L and temperatures of either 12-13°C or 4-5°C, Oseid and Smith (1971) found that the eggs incubated at the lower DO levels required one to four days longer to hatch.

Oseid and Smith (1971) recommended that optimal DO levels for walleye egg incubation be not less than 5-6 mg/L. Criteria for DO optima to protect walleye populations in Alberta have been recommended at 5.0 mg/L as a 1-day minimum and 6.0 mg/L as a 7-day mean (Taylor and Barton 1992). It is important to ensure these criteria are sufficiently protective, because survival of earliest life stages is seen as a key control of walleye populations (Noble 1972; Scott and Crossman 1973).

Yellow perch appear to be relatively tolerant of low DO concentrations. Doudoroff and Shumway (1970) cite studies that describe lethal DO levels for this species ranging from 3.1 mg/L to as low as 0.5-0.8 mg/L. As with northern pike, the highest lethal value is from caged fish held in a lake, whereas lethal levels  $< 2.0$  mg/L appear to be mainly from more rigorous experiments and thus may be more valid. For example, lethal DO levels for European perch, a closely related species, were all  $< 2$  mg/L with many  $< 1$  mg/L. Krieger et al. (1983) cite studies of wintertime yellow perch survival claiming DO levels from 0.2 to 1.5 mg/L were lethal. At a summer temperature of 26°C, DO concentrations  $< 3.1$  mg/L were lethal (Krieger et al. 1983). However, the studies reviewed in Krieger et al. (1983) used relatively short ( $< 5$  d) exposures, leading the authors to conclude that the lower optimum DO limit for yellow perch would be 5 mg/L.

In a laboratory simulation of under-ice conditions, most yellow perch survived successively lower DO, going from 4 to 0.25 mg/L over 5 d, by altering their behaviour (Petrosky and Magnuson 1973). Perch were able to move upwards in the water column to take advantage of possible microhabitat conditions of higher PO<sub>2</sub> in the surface layer of water just under the ice.

## 6.8 REQUIREMENTS OF CATOSTOMIDAE

Longnose Sucker	<i>Catostomus catostomus</i>
White Sucker	<i>Catostomus commersoni</i>
Largescale Sucker	<i>Catostomus macrocheilus</i>

Dissolved oxygen requirements of suckers have not been widely researched. In the study of hypoxia responses of Gee et al. (1978), estimated incipient lethal levels for white sucker were similar to those of most cyprinids, although lower than sticklebacks, percids and salmonids (Table 4). Doudoroff and Shumway (1970) cite one study which found a lethal DO of <2 mg/L, again similar to many cyprinids. White suckers are reported to avoid areas in a reservoir where the DO was  $\leq 2.4$  mg/L, but specific information on their physiological tolerances is lacking (Twomey et al. 1984). White sucker embryos reportedly cannot survive DO <1.2 mg/L and growth of fry is reduced at DO levels <2.5 mg/L (Siefert and Spoor 1974).

Even less is known about DO requirements of other suckers. Longnose suckers, a species of fast, clean rivers and cold, clear, oligotrophic lakes, evidently has a higher DO requirement than white suckers. While lethal DO levels for this species have not been determined, Edwards (1983) cites a number of early studies showing that habitat supporting longnose suckers had DO concentrations of 5.6-10 mg/L. The strong preference of this species for very clear, clean water (Edwards 1983) implies that its DO requirement is probably high.

Eggs of longnose sucker are released at the base of riffles, where DO levels are always at saturation. This suggests that embryos and larvae are probably also very intolerant of low DO. No information is available on the DO requirements of largescale sucker, although they may be similar to white sucker as the two species appear to be biologically similar (Scott and Crossman 1973) and are known to hybridize (Nelson and Paetz 1992).

## 6.9 REQUIREMENTS OF GASTEROSTEIDAE

Brook Stickleback	<i>Culaea inconstans</i>
Ninespine Stickleback	<i>Pungitius pungitius</i>

The limited evidence suggests that among nonsalmonid fishes, stickleback are a relatively sensitive group. Doudoroff and Shumway (1970) cite one study for this family, listing a lethal DO level for brook stickleback of <2.0 mg/L at 20-23°C. Giles (1987) found that the median threshold tolerance level of threespine sticklebacks was about 1.7 mg/L; the threshold level was that which caused the fish to show at least 10 seconds of continuous aquatic surface respiration. In a frozen bog lake, brook stickleback was less tolerant of hypoxia than either fathead minnow or central mudminnow and was the first to expire at DO concentrations < 1.0 mg/L (Klinger et al. 1982). Sticklebacks also increased gill ventilation at higher DO concentration (1.0 mg/L) than the other species (0.25-0.5 mg/L), and was the first to move to the surface and begin aquatic surface respiration around gas bubbles.

Control of swimbladder lift is important for efficient performance of aquatic surface respiration, especially in physoclistic fish that are unable to gulp air to offset gas loss. Gee and Ratynski (1988) note that brook sticklebacks were more effective at rapidly regulating buoyancy than ninespine sticklebacks, suggesting that the former species is better adapted for using aquatic surface respiration. Whereas brook sticklebacks were able to maintain

swimbladder lift, a measure of buoyancy, in abrupt hypoxia of 5% saturation for 1 h, ninespine stickleback were unable to do so at 10% DO saturation (Gee and Ratynski 1988).

## 6.10 REQUIREMENTS OF OTHER SPECIES

### Cottidae

Slimy sculpin                      *Cottus cognatus*

Spoonhead sculpin              *Cottus ricei*

Prickly sculpin                    *Cottus asper*

### Hiodontidae

Goldeye                              *Hiodon alosoides*

### Gadidae

Burbot                                *Lota lota*

### Percopsidae

Trout-Perch                        *Percopsis omiscomaycus*

Information on DO requirements for other northern rivers species is extremely limited. Two studies cited in Doudoroff and Shumway (1970) give lethal DO concentrations for burbot as <2.0 mg/L at 12-18°C and 1.4-3.2 mg/L at 0°C, respectively. Hart (1968) determined lethal DO concentrations for goldeye as being 0.7-1.6 mg/L at 5°C and 1.2-1.5 mg/L at 15°C. Data do not appear to be readily available for trout-perch or the sculpin species found in the northern rivers. However, for the reticulate sculpin (*Cottus perplexus*), found mainly in coastal Oregon streams (Lee et al. 1980), Davison et al. (1959) found that the DO level resulting in 80% mortality after a 5-d exposure was 1.4 mg/L; no mortality was reported at 1.6 mg/L.

## 6.11 SUMMARY OF DO REQUIREMENTS

On the basis of the information summarized in the Sections 6.2 to 6.10, the fish species living in the northern rivers study area may be tentatively classified into one of four groups (Table 8). It is emphasized, however, that this grouping is based on very limited information for most of the species of concern. The classification is based on acute tolerances of adults. To the extent that they are known, requirements of embryos, fry and juveniles appear to follow approximately the same ranking, but the requirements of early life stages are invariably greater than those of adults. Dissolved oxygen requirements to sustain long-term health of the population, and to avoid depression of growth, development or reproduction, are substantially higher than acute lethal limits, and are also much more similar among species.

All salmonids are classed as sensitive to DO tensions, in agreement with other reviews (Doudoroff and Shumway 1970; Davis 1975; Alabaster and Lloyd 1982; Chapman 1986). There is no evidence that subfamilies or individual species within the Salmonidae differ significantly in their DO requirements, but data for many of the lesser known species, in particular the whitefishes and Arctic grayling, are very incomplete. For the northern rivers, longnose suckers and burbot have also been tentatively classified as sensitive; longnose suckers appear to be very selective of highly oxygenated habitats at all stages of their life cycle, and the range of acute DO thresholds for burbot is similar to that for salmonids. More definitive information on the DO requirements of these species should therefore be a priority.

The intermediate category captures the largest number of species, being those that are not as demanding as salmonids but which nevertheless cannot tolerate severe hypoxia. The inclusion of all cyprinids except fathead minnow in the intermediate category is to some degree speculative because most of the species indigenous to the northern rivers have not been studied in detail. Moreover, the data on those cyprinids that have been tested suggest

**TABLE 8**

**TENTATIVE ACUTE DISSOLVED OXYGEN SENSITIVITY CLASSIFICATIONS  
FOR NORTHERN RIVERS FISHES**

SPECIES	SENSITIVITY CLASSIFICATION
All salmonids including whitefishes Longnose sucker Burbot	Sensitive; acute lethal limit >2 mg/L
All cyprinids except fathead minnow Walleye White sucker Brook stickleback Goldeye	Intermediate; acute lethal limit 1-2 mg/L
Fathead minnow Northern pike Yellow perch	Tolerant; acute lethal limit <1 mg/L
Largescale sucker Ninespine stickleback Trout-perch All sculpins	Unknown; insufficient information to establish tentative DO sensitivity



a considerable range of variation in DO requirements. Notwithstanding, there are no laboratory or field observations to suggest that any cyprinids are sensitive, and a misclassification of tolerant species errs on the side of caution. Walleye and white sucker are both intermediate, although the preferred DO level for long-term health of walleye (6 mg/L) is perhaps higher than for other members of the class. Classification of brook stickleback and goldeye in this category is tentative, for want of better information.

The pronounced tolerance of fathead minnows, at least to short periods of severe hypoxia, distinguishes this species from the other cyprinids. Again however, DO concentrations of 3-4 mg/L or more are believed necessary for long-term protection of the species. Yellow perch and northern pike have in common that they both frequent beds of aquatic macrophytes along riverbanks and lakeshores, where diurnal fluctuations in DO are apt to be severe. It is perhaps surprising, therefore, that brook stickleback are not equally tolerant of low DO as they often occupy similar habitat. For northern pike at least, much higher DO levels must be maintained to ensure survival of eggs and juveniles.

Largescale sucker and ninespine stickleback have been classed as unknown because there have been no measurements of their DO requirements, and there is such variation within their respective families that data for one species cannot be generalized to others. No data are available for sculpins or trout-perch; it is likely that DO requirements of sculpins vary significantly among species.

The information compiled in previous sections leads to two firm conclusions. First, dissolved oxygen is a limiting resource to aquatic biota. Any reduction in the natural DO content of northern rivers is detrimental to a greater or lesser degree to fish populations that live there; it is fallacious to assume that a limited reduction, so long as it remains above critical levels, will have no effect. This is not to suggest that healthy fish populations cannot persist in waters with DO levels below saturation, but rather that, like temperature and food supply, the adequacy of dissolved oxygen supplies governs the ultimate productivity, diversity and

robustness of the fish community. Therefore, any reduction in DO from its historical level imposes a new, lowered limit on the productive capacity of the water body in proportion with the magnitude of the DO reduction. The goal of sensible DO criteria thus should be to ensure that such reductions in DO content are minimized.

Second, depressed DO in natural waters produces stress on fish at the individual and population levels. Contaminants from pulp mills and other industrial or municipal sources are a second source of stress for fish in northern rivers. The combined effect of these two stressors on fish is cumulative. Therefore, the additional stress from industrial effluents, and from any other environmental factors, should be considered in the formulation of DO criteria for northern rivers fish.

To fully protect a population of a given species of fish, it is necessary to also protect the species with which it interacts, especially other links in the food web. Thus, forage fishes and invertebrate populations should be afforded adequate protection if populations of top predators are to be sustained. It is also necessary to ensure that essential behaviours (feeding, migration) and biological functions (reproduction) are unimpeded, and that the normal dynamics of trophic interactions are maintained. To achieve this end, DO criteria should be based on protection of fish communities rather than individual species. In the northern rivers, summertime low DO and violations of thresholds of acute lethality are unlikely to pose problems, except very locally; exposure to sub-optimal DO levels over the long term (months) under winter ice is the more significant source of stress for fish communities. Criteria for dissolved oxygen to be applied to the northern rivers must guard against long-term erosion of the naturally high DO concentrations in winter if the diverse assemblages of fish living in these waters are to be fully protected.

## 7.0 INFORMATION NEEDS

The most conspicuous, and the most easily remedied, deficiency in our knowledge of dissolved oxygen requirements of northern rivers fishes, and therefore the first priority for research, is the dearth of information on acute low oxygen tolerances for many species. This is apparent in the large number of species in the "Unknown" category in Table 8, and the tentativeness of the categorization generally. Without information on dissolved oxygen requirements of more species, the consequences of DO reductions in the northern rivers cannot be fully understood, and there will always be a risk that an untested species is more sensitive than anticipated. Management options for the rivers will also be restricted by planning without full knowledge of the needs of the biota. Add to this that acute DO tolerance limits are among the simplest data to obtain, and the rationale for assigning this work first priority is apparent. Some selectivity, however, should be used in deciding which species to test.

No additional research on the dissolved oxygen requirements for Salmonidae is justifiable given that a vast amount of information on rainbow trout is available and that salmonid requirements appear to be similar. An exception may be made for bull trout, as no information on this species exists despite its importance as one of the few native salmonids in Alberta and the present concern for the wellbeing of the species throughout its distribution. Similarly, information on DO requirements of mountain whitefish and Arctic grayling is very limited, and is even more so for the other whitefishes. Further consideration should also be extended to examining embryo and larval tolerances in bull trout and the whitefishes as they are fall spawners and eggs are susceptible to winterkill.

For the non-salmonids, a reasonable amount of information is available for northern pike and walleye, for both adult fish and embryos. Information is limited, however, on DO requirements of cyprinids except for fathead minnow, suckers, sticklebacks and yellow perch.

Moreover, information is either very limited or virtually lacking for the sculpins, burbot, goldeye and trout-perch.

Thus, future research efforts should be directed towards those northern rivers species for which little or no information is currently available. While most of these species may not be considered important in a recreational or commercial context, they are important ecologically as components of ecosystems and representatives of the biological diversity of the region.

The first priority should be to obtain rigorous estimates of acute DO tolerances (i.e., lethal limits) for adults of all the untested species. These data are fundamental descriptors of a species and are invaluable for inter-species comparisons, setting water quality objectives and guiding research. The second, and more difficult, task is then to extend these measurements to other life stages, particularly eggs and alevins or larvae. These life stages tend to be more sensitive to dissolved oxygen levels than adults, are less mobile and therefore less able to avoid inhospitable habitats, and their survival rate is often limiting to the growth and stability of the population. Wherever possible, research should expose the fish to conditions emulating those in the northern rivers; but determination of effects of low oxygen alone should precede examination of interactions with other factors.

Efforts in early-life-stage work would be best expended by again focussing on species outside of the well-studied salmonidae, with two exceptions. First, as mentioned above, more information on the DO requirements of all life stages of bull trout could be justified, given the paucity of direct data on this species and its precarious status in the province. Second, direct measurements of DO concentrations within trout redds, and the oxygen gradient between the redds and overlying water, would be invaluable. Chapman (1986) points out that only two studies are extant that directly measured DO within natural redds, and the species studied were coho salmon and brook trout. These studies want for confirmation.

Beyond those two exceptions, early-life stage studies would better focus on broadcast spawners such as the various species of whitefish. Mountain whitefish is the logical candidate for research because it is widespread both throughout the northern rivers and Alberta generally. Research needs pertaining to eggs include measurements of DO gradients between the water column and the sediment surface, the effect of different sediment types (and their oxygen demand) on dissolved oxygen levels and embryo survival, and the frequency and effects of burial beneath moving sediments or organic debris from pulp mill effluents. It would be worthwhile as well to confirm with field studies exactly where whitefish spawn and the downstream distance that newly released eggs travel before settling.

Information on dissolved oxygen requirements of larval and young whitefish, and indeed their general biology, including habitat preferences and migration patterns, is markedly deficient. Beyond one or two studies in southern Alberta and a few anecdotal observations, the biology of early life stages of whitefish is largely speculation. Research here should concentrate on not only determining the DO requirements of young fish, but establishing the DO regime in preferred habitats. If larval whitefish congregate in shallow riparian pools (Taylor and Barton 1992), they may be subjected to extremes of DO concentration quite different from those in the main channel. Again, a case could be made for similar research on early life stages of other non-salmonid species, particularly burbot and longnose sucker, whose DO requirements appear to be high, and the various species in the Unknown category, if tests on adults reveal any of them to be especially sensitive.

The third research priority (in terms of the best order of pursuit, rather than significance) is to determine the long-term effects of dissolved oxygen on population growth and maintenance for the various species in the northern rivers. That work would naturally include examination of the effects of contaminants, particularly those from pulp mill effluents, and the interactions of contaminants with dissolved oxygen. Two research questions are paramount: first, what are the effects of prolonged low DO episodes in winter on toxicity of pulp mill contaminants, and second, what effect does low DO have on fish

energy budgets and the efficiency of metabolic functions related to detoxification and resistance. Chronic toxicity and effects at the population level are the key features that research should seek to illuminate. A natural corollary to all of this work is to find correlations, if they exist, between acute lethal limits and long-term needs of the various fish species; such correlations would be very useful for setting long-term DO objectives where only acute tolerance data are available. Of course, all these studies should concentrate on wintertime conditions, because it is in winter that fish and other aquatic biota are exposed to low dissolved oxygen levels in the northern rivers.

## 7.1 SENTINEL SPECIES

The purpose of sentinel species is to provide an early warning of deterioration of environmental conditions so that appropriate action can be taken before the situation worsens. Sentinel species, therefore, should be the most sensitive species or life stages inhabiting a reach of river in a given season. In the northern rivers, sentinel species of dissolved oxygen decline need only be identified for winter because DO levels remain near their naturally high levels in the ice-free seasons. Unfortunately, it is not possible to make firm recommendations of sentinel species for the northern rivers because even the most basic information on dissolved oxygen requirements is lacking for many species; the following list thus should be considered tentative and interim.

Among the relatively well studied species, salmonids appear to be the most sensitive. Adult rainbow trout should be considered as sentinels because they are native to the northern rivers and are by far the most widely studied salmonid, so their dissolved oxygen requirements are well known. The other salmonids are either less thoroughly studied, less widespread in distribution, or both, but it appears they all have DO requirements similar to those of rainbow trout. However, native rainbow trout in Alberta are spring spawners; thus eggs and alevins are not present in winter when DO levels may be depressed. Mountain whitefish, a fall-spawning species, would be a better sentinel for eggs and larvae.

A third salmonid that should be considered is bull trout. While DO requirements of bull trout are not well studied, they are probably similar to those of other trout. Bull trout may be somewhat more numerous than rainbow trout in the northern rivers, and this population is vitally important because of the distressingly low population densities elsewhere in its range. Hence, a decline in bull trout in the northern rivers would be of interest directly, and as a sentinel of deteriorating environmental conditions.

Bull trout, rainbow trout and mountain whitefish are widely distributed in the northern rivers, but because of seasonal migrations and habitat preferences they may not be present in every river reach in winter. Some thought should be given to appointing other sentinel species as alternatives. Burbot and longnose sucker are the obvious choice because they have different habits and habitats than the salmonids, and the incomplete data collected so far suggests they have high DO requirements. Longnose sucker in particular may be confined to very well oxygenated habitats. Confirmation of quantitative oxygen tolerances would be required, however, before use of these species as sentinels can be sanctioned.





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## **APPENDIX I**

### **GLOSSARY**



## GLOSSARY

**active metabolism:** rate of metabolism (measured as oxygen consumption) during maximum sustained activity.

**acute:** referring to an event or a response that has a sudden onset or which lasts only a short time (an *acute exposure*, an *acute response*). In toxicology, acute events are usually taken as those that last <96 hours (4 days). The acute response (to exposure to a toxicant or high temperature or low DO tensions) of interest is usually death, but coughing, or avoidance behaviour, or metabolic changes, can all be acute responses.

**adrenergic:** involves stimulation by the peptide hormone, adrenalin, or related hormones; often a localized sympathetic response.

**alevin:** post-hatch fish with yolk sac still attached; sac fry.

**anoxia:** deficiency of oxygen; usually refers to body tissues, but also refers to complete lack of oxygen in the environment.

**arrythmia:** irregular rhythm of heart beat.

**chronic:** referring to an event or a response that is lingering or persists for a long time (a *chronic exposure*, a *chronic response*). Chronic exposures are usually taken as occurring over a significant part of the life cycle of the organism, usually weeks or months. A chronic response is often subtle and persistent, such as elevated blood enzyme levels or slowed growth, but death can also result from a chronic stress.

**criterion:** here, a dissolved oxygen concentration specified to protect the species of interest from death or physiological harm. *Acute criteria* protect the animals against brief exposures; *chronic criteria* protect against exposures of weeks or months. The

criterion is the lowest) temperature DO tension that with reasonable confidence can be said to cause no harm to the animals concerned.

**critical:** a general term here referring to any temperature or dissolved oxygen tension that (a) exceeds an acute lethal limit, or (b) approaches an acute or chronic physiological limit closely enough that the animals experience special metabolic demands to deal with it. The critical level of dissolved oxygen, for example, is the level below which the function of interest (usually growth or respiration efficiency) depends on the DO level.

**digestive state:** refers to the status of food still contained in the organism's digestive tract. A post-digestive state, for example, would be one where all food material in the gut has been digested and waste products eliminated.

**disturbance:** an extreme event severe enough to cause substantial mortality.

**embryo:** egg up to the time of hatching.

**erythropoiesis:** process of red blood cell (erythrocyte) production; see also *hemopoiesis*.

**exceedence:** any temperature or dissolved oxygen level that violates a criterion.

**fatal:** see *lethal*.

**fry:** early post-stage of fish after yolk sac has been absorbed; swim-up fry.

**hemopoiesis:** process of blood cell production; unlike bone marrow in higher vertebrates, the major hemopoietic tissues in fish are the head kidney and spleen.

**hypoxia:** reduced levels of oxygen; can refer to either conditions at the cellular or tissue level, or in the external environment.



**incipient lethal limit:** the maximum (minimum) temperature (DO concentration) tolerated indefinitely by 50% of the animals tested.

**juvenile:** generally considered as immature fish with one season of growth up to the age of first maturity.

**lethal:** capable of causing death; fatal. A lethal response (i.e., death) may result from an acute or chronic exposure to a toxicant or other stressor such as low DO concentrations. Strictly, lethality refers to the external agent causing death (a lethal chemical), whereas fatal refers to the response leading to death (a fatal overdose).

**limit:** used here in the ordinary sense of a boundary or edge. The *lethal limit* for temperature is the highest temperature that an organism can withstand without dying. If the exposure is brief, then the temperature is the *acute lethal limit*. The lethal limit is sometimes termed the *tolerance threshold*.

**normoxia:** normal (i.e., fully saturated) oxygen conditions; usually refers to the environment.

**performance capacity:** in the context of energy availability, the potential to carry out activities beyond the minimum required for survival. For example, the energetic capacity to capture prey, escape predators, resist disease, etc.

**Q<sub>10</sub>:** the factor by which metabolic rate increases for a temperature increase of 10°C; a generally accepted Q<sub>10</sub> value for teleost fish is 2.3.

**routine metabolism:** rate of metabolism (measured as oxygen consumption) of an organism in a normal, thermally acclimated, postabsorptive (post-digestive) state; includes normal spontaneous activity.

**scope for activity:** range of metabolism available for all activities; defined as the difference between active and standard metabolism.

**standard metabolism:** theoretical minimum rate of metabolism (measured as oxygen consumption) of a thermally acclimated intact organism in a postabsorptive (post-digestive) state at zero level of activity; difficult to measure in practice.

**stochastic:** pertaining to chance; random; unpredictable.

**stress:** a change in biological condition or state, beyond the normal range of resting conditions, caused by an environmental stimulus that challenges homeostasis and, thus, represents a threat to the fish's well-being.

**stress response:** a biological response to stress in order to regain a normal resting or homeostatic condition.

**stressor:** any environmental condition sufficiently outside the preferred range of an organism to evoke a biological response in order to cope with the stimulus and maintain homeostasis. Typical stressors, or stress factors, are chemical (pH, pollutants, low DO), physical (handling), thermal or behavioural in nature.

**Torr:** a unit of gas pressure equivalent to 1 mm mercury (Hg) or 133.32 pascals (Pa).

**APPENDIX II**

**RESEARCH IN NORTH AMERICA RELATED TO OXYGEN  
REQUIREMENTS OF FISHES**



## APPENDIX II

### RESEARCH IN NORTH AMERICA RELATED TO OXYGEN REQUIREMENTS OF FISHES (RESPONSE TO MAIL SURVEY)

INVESTIGATOR	CURRENT RESEARCH AREAS
<b>CANADA</b>	
<p>Dr. J.M. Casselman Ontario Ministry of Natural Resources Fisheries Branch RR#4 Picton, Ontario K0K 2T0</p>	<ul style="list-style-type: none"> <li>• low DO effects on survival and development of lake trout embryos and fry</li> <li>• oxygen requirements of northern pike</li> </ul>
<p>Dr. A. Farell Department of Biological Science Simon Fraser University Burnaby, B.C. V5A 1S6</p>	<ul style="list-style-type: none"> <li>• oxygen uptake and swimming performance of salmonids and BC resident freshwater fishes at various temperatures</li> </ul>
<p>Dr. M.A. Giles Department of Fisheries and Oceans Freshwater Institute Winnipeg, Manitoba R3T 2N6</p>	<ul style="list-style-type: none"> <li>• oxygen consumption of char species relative to temperature, age, and size.</li> <li>• effects of contaminants on oxygen consumption</li> </ul>
<p>Dr. M.S. Graham Vancouver Public Aquarium P.O. Box 3232 Vancouver, B.C. V6B 3X8</p>	<ul style="list-style-type: none"> <li>• oxygen requirements of arctic marine fishes</li> </ul>
<p>Dr. D.L. Kramer Department of Biology McGill University Montreal, PQ H3A 1B1</p>	<ul style="list-style-type: none"> <li>• effects of DO on behaviour and habitat selection</li> </ul>
<p>Dr. T.W. Moon Department of Biology University of Ottawa Ottawa, Ontario K1N 6N5</p>	<ul style="list-style-type: none"> <li>• endocrine stress and metabolic responses to environmental stressors such as alterations in oxygen availability</li> </ul>

APPENDIX II CONTINUED

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INVESTIGATOR	CURRENT RESEARCH AREAS
<p>Dr. S.F. Perry                      Department of Biology                      University of Ottawa                      Ottawa, Ontario K1N 6N5</p>	<ul style="list-style-type: none"> <li>• Interactive effects of environmental stress on hypoxia tolerance</li> <li>• acclimation temperature effect on tolerance of fish to hypoxia</li> <li>• consequence of chloride cell proliferation on ability of trout to withstand hypoxia</li> </ul>
<p>Dr. D.J. Randall                      Department of Zoology                      University of British Columbia                      Vancouver B.C. V6T 1Z4</p>	<ul style="list-style-type: none"> <li>• effects of diet on response to hypoxia</li> <li>• role of adenosine in hypoxia tolerance in fish</li> <li>• influence of carbonic anhydrase on blood oxygen content</li> <li>• physiological mechanisms of fish respiration</li> </ul>
<b>UNITED STATES</b>	
<p>Dr. T.L. Beitinger                      Department of Biological Sciences                      University of North Texas                      Denton, TX 76203</p>	<ul style="list-style-type: none"> <li>• comparison of oxygen tolerance among stream fishes</li> <li>• effects of toxicants on oxygen tolerance</li> <li>• effects of DO content on critical thermal maxima and minima in fish</li> </ul>
<p>Dr. H. Bergman                      Department of Zoology and Physiology                      University of Wyoming                      Laramie, WY 82071</p>	<ul style="list-style-type: none"> <li>• low DO tolerance of several warmwater fish species</li> </ul>
<p>D.C. Miller                      U.S. Environmental Protection Agency                      Environmental Research Laboratory                      Narragansett, RI 02882</p>	<ul style="list-style-type: none"> <li>• tolerance and growth effects of hypoxia on saltwater fishes and invertebrates</li> <li>• development of DO criteria for regional marine species</li> </ul>

APPENDIX II CONTINUED

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INVESTIGATOR	CURRENT RESEARCH AREAS
Dr. W.H. Neill Department of Wildlife and Fisheries Sciences Texas A & M University College Station, TX 77843	<ul style="list-style-type: none"><li>• critical oxygen tension for various species</li><li>• relationships between temperature and respiration in fish</li><li>• effects of temperature - oxygen interaction on metabolic scope</li></ul>
<b>OTHER SUGGESTED CONTACTS (NON-RESPONDENTS)</b>	
Dr. P.J. Rombough Department of Biology Brandon, University Brandon, Manitoba R7A 6A9	
Dr. J.J. Cech, Jr. Department of Wildlife and Fisheries Biology University of California Davis, CA 95616	





**APPENDIX III**  
**TERMS OF REFERENCE**



**NORTHERN RIVER BASINS STUDY  
SCHEDULE A - TERMS OF REFERENCE**

Page 1 of 4

**PROJECT 321 - DISSOLVED OXYGEN REQUIREMENTS OF NORTHERN RIVER  
BASINS FISH**

**SUB-PROJECT 3211 - B1 LITERATURE REVIEW**

**Description**

A consultant will be retained to examine the existing literature for information on the dissolved oxygen needs of various life stages of Northern River Basins fish species. Information relevant and valid for use in the NRBS will be documented. A list of on-going research relevant to NRBS will be documented.

The information will be used to design field and laboratory programs. The information will be provided in a stand alone electronic database designed to facilitate updating and enlargement.

**Objectives**

- A. To determine if published data on the dissolved oxygen requirements for all the fish species that occur in the Peace, Athabasca and Slave rivers, and their major tributaries (NRB fish) is suitable and applicable. Focus is on the requirements of each species life stage (e.g., eggs, fry, sub-adults, adults) for different functions (e.g., spawning, incubation). Review available literature on the oxygen tolerances, requirements, and preferences of those life stages.
- B. To report on the effects of dissolved oxygen variations on sensitive life history stages of NRB fish under pristine and contaminated conditions, with special regard to the effects of temperature and atmospheric pressure.
- C. To identify deficiencies for describing the variation in dissolved oxygen needs of NRB fish for various temperature and water qualities.
- D. To produce a computerized cross-indexed bibliographic database and user manual of all references pertaining to the dissolved oxygen requirements of NRB fish.
- E. To identify research presently under way that is dealing with dissolved oxygen needs of species known to the NRB, noting the area of study, its availability to NRB and its significance to addressing NRB needs.

**Study requirements:**

**The contractor will:**

- 1. Review, assess and document published data pertaining to the dissolved oxygen requirements of fish species found in the Northern River Basins study area, inclusive of life stage and functional needs. This is to include information relating to tolerances, requirements and preferences.

2. Document the dissolved oxygen requirements for NRB fish species under various temperature and water quality conditions, principally those associated with the dissolved and particulate portions of pulp mill effluents.
3. From a review of existing published material, report on fish behaviour and residence under various conditions in effluent mixing zones, especially under winter flow conditions.
4. Document and report on the specific and general behavioural, physiological and developmental effects of acute and chronic exposures of sub-optimal oxygen levels on fish life stages. Acute and chronic effects could include among other others: blood chemistry, histopathology, response to challenges (eg.,disease, temperature variation),reproductive potential, metabolism.
5. Document where insufficient information is available to identify the dissolved oxygen requirements of NRB fish species at different life stages, life function and under various temperature and contaminant conditions.
6. Produce a computerized bibliography of all references pertaining to the dissolved oxygen requirements of fish identified in Schedule A. Unless otherwise approved by the Study Office, the Pro-Cite Data-base software is the preferred computerized program (Personal Bibliographic Software Inc. of Ann Arbor, Michigan). The bibliography should be cross-indexed by fish species, life stage, life function, effect type, temperature and contaminant.
7. Provide a user guide for handling and actively interacting with the database.
8. With supporting documentation make a recommendation on NRB fish species most suited to being used as sentinel species.
9. The contractor will provide an electronic copy of the final report material as a Wordperfect file.

**Reporting Requirements:**

1. Provide ten copies of the DRAFT FINAL REPORT to the Department's representative by March 31, 1993.
2. Three weeks after receipt of review comments on the draft, provide the department's representative with five cerlox bound copies and two unbound, camera ready copies of the final report. At the same time, provide to the Department's representative an electronic copy, in WordPerfect 5.1 format, (3½ inch 2HD floppy disks) of the final report. Data for any tables, figures or appendices in the report are also to be submitted in DBase IV format on 3½ inch floppy disks. The final report will include an executive summary and Terms of Reference as an appendice.

## NORTHERN RIVER BASINS STUDY

PROJECT 321 - DISSOLVED OXYGEN REQUIREMENTS OF NORTHERN RIVER  
BASINS FISH

## SUB-PROJECT 3211 - B1 LITERATURE REVIEW

FISH SPECIES - PEACE, ATHABASCA AND SLAVE RIVERS

<u>SPECIES</u>	<u>SCIENTIFIC NAME</u>
Artic Grayling	<i>Thymallus Arcticus (Pallas)</i>
Mountain Whitefish	<i>Prosopium Williamsoni (Girard)</i>
Lake Whitefish	<i>Coregonus Clupeaformis (Mitchill)</i>
Bull Trout	<i>Salvelinus Confluentus</i>
Rainbow Trout	<i>Oncorhynchus Mykiss (Richardson)</i>
Kokanee	<i>Oncorhynchus Nerka (Walbaum)</i>
Northern Pike	<i>Esox Lucius Linnaeus</i>
Yellow Perch	<i>Perca Flavescens (Mitchill)</i>
Walleye	<i>Stizostedion Vitreum (Mitchill)</i>
Goldeye	<i>Hiodon Alosoides (Rafinesque)</i>
Burbot	<i>Lota Lota (Linnaeus)</i>
Longnose Sucker	<i>Catostomus Catostomus (Forster)</i>
White Sucker	<i>Catostomus Commersoni (Lapédède)</i>
Largescale Sucker	<i>Catostomus Macrochelilus (Girard)</i>
Flathead Chub	<i>Couesius Plumbeus (Agassiz)</i>
Longnose Dace	<i>Rhinichthys Cataractae (Valenciennes)</i>
Northern Squawfish	<i>Ptychocheilus Oregonensis (Richardson)</i>
Redside Shiner	<i>Richardsonius Balteatus (Richardson)</i>
Emerald Shiner	<i>Notropis Atherinoides (Rafinesque)</i>
Spottail Shiner	<i>Notropis Hudsonius (Clinton)</i>

SPECIESSCIENTIFIC NAME

Fathead Minnow	<i>Pimephales Promelas (Rafinesque)</i>
Trout-Perch	<i>Percopsis Omiscomaycus (Walbaum)</i>
Brook Stickleback	<i>Culaea Inconstans (Kirtland)</i>
Slimy Sculpin	<i>Cottus Congatus (Richardson)</i>
Spoonhead Sculpin	<i>Cottus Ricei (Nelson)</i>
Prickly Sculpin	<i>Cottus Asper (Richardson)</i>
Round Whitefish	<i>Prosopium Cylndraceum</i>
Finescale Dace	<i>Chrosomus Neogaeus</i>
Northern Redbelly Dace	<i>Chrosomus Eos</i>
Pearl Dace	<i>Semotilus Margarita</i>
Ninespine Stickleback	<i>Pungitius Pngitius</i>

## **APPENDIX IV**

### **A MANUAL FOR USING THE DISSOLVED OXYGEN BIBLIOGRAPHIC DATABASE**





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## 1.0 INTRODUCTION

In November 1992, the Northern River Basins Study retained Environmental Management Associates to conduct a review of the literature on dissolved oxygen (DO) requirements of fishes found in the Northern River Basins Study (NRBS) area. Published data on DO tolerances, requirements, and preferences for the fish species that occur in the Peace, Athabasca, and Slave rivers and their major tributaries were reviewed, along with general effects of DO variations on sensitive life stages of fish under pristine and contaminated conditions. The data were compiled and evaluated as to their applicability and completeness with respect to life stages and different biological functions (EMA 1993).

All references pertaining to the DO requirements of northern river fishes have been compiled in a computerized, cross-indexed, bibliographic data base. This bibliographic data base is called "DO<sub>2</sub>" and was developed and compiled using the Pro-Cite® bibliographic software package. The data base includes a keyword index to assist in searching for relevant literature.

The Pro-Cite software system is a simple and versatile tool for the manipulation of the bibliographic data base. This manual is intended to provide support for people using the DO<sub>2</sub> data base and provide information on how to perform the various functions in data base inquiry and manipulation using the Pro-Cite software. This manual does not replace the Pro-Cite software user's manual and the user is referred to specific sections of the manual to conduct more complex operations.

This manual has been separated into six sections, each referring to one of the main functions which will be employed in order to effectively use the DO<sub>2</sub> data base. These sections are:

1. Installation of the software
2. Setting defaults and options for using the DO<sub>2</sub> data base
3. Viewing the DO<sub>2</sub> data base
4. Making changes to the DO<sub>2</sub> data base
5. Creating a bibliography for the data base
6. Performing a data base search for selected parameters

## 2.0 INSTALLING AND ACTIVATING THE PROCITE SOFTWARE

Hardware and software requirements for using the software are outlined in the Pro-Cite manual. The fundamental requirements are an IBM compatible computer, a hard disk drive (the Pro-Cite software requires approximately 1.7 MB of disk space), and DOS 3.0 or higher. The installation procedure outlined assumes that you will be installing the program using the *a:* drive which could be a 3.5" or 5.25" floppy disk drive.

In order to install the program, conduct the following steps:

1. Make a directory on your hard drive named Pro-Cite [*mkdir Procite*]
2. Move to that directory [*cd Procite*]
3. Use the Pro-Cite install program [*a:install*]

In order to copy the DO<sub>2</sub> data base system to your computer, conduct the following steps:

1. Make a subdirectory with Pro-Cite called <DO> [*mkdir DO*]
2. Move to that directory [*cd DO*]
3. Copy the contents of the disk supplied to that directory [*copy a:\*. \**]
4. Type *install*
5. Return to the Pro-Cite main directory [*cd ...*]

To start the Pro-Cite software simply type [*procite*] at the DOS prompt (ie. *c:\procite>*).

## 2.0 INSTALLING AND ACTIVATING THE PROCITE SOFTWARE

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2. Move to that directory [*cd DO*]
4. Copy the contents of the disk supplied to that directory [*copy a:\*. \**]
5. Return to the Pro-Cite main directory [*cd ...*]

To start the Pro-Cite software simply type [*procite*] at the DOS prompt (ie. *c:\procite>*).

### 3.0 SETTING DEFAULTS AND OPTIONS FOR THE DO, DATA BASE

To ensure that Pro-Cite is searching for data bases and authority lists (lists of keywords, authors, journals, etc.) in the correct directory you must set the correct default drives. To set defaults for using the DO, data base conduct the following steps:

1. At the main menu choose Customize *[C]*
2. Select Default directories *[D]*
3. Within this menu select the default directory applicable to your computer directory setup. Ensure that the authority list and data base directories correspond with the directory where the DO, data base system has been saved (i.e., c:\procite\DO).
4. Other default directories should remain as c:\procite unless certain applications which you are using (such as import\export or manuscript search) require the use of other directories.
5. Select Open authority list *[O]* to provide a list of the authority lists available. Select "DESCRIPT" to use the list of descriptors and keywords for the DO, data base.
6. Press *[Esc] [Esc] [Esc]* to return to the main menu

#### 4.0 VIEWING THE DO<sub>2</sub> DATA BASE

If you have not already done so, select the DO<sub>2</sub> data base as your active data base file. To do this conduct the following steps:

1. Select Data bases *[D]* from the main menu
2. Select Open data bases *[O]* from the Data bases menu
3. Select the DO<sub>2</sub> Data base (DO) from the list of Pro-Cite data bases
4. Press *[Esc]* *[Esc]* to return to the main menu

To view the DO<sub>2</sub> data base select Browse *[B]* from the main menu. The browse function allows you to view an abbreviated version of the records in the data base. The browse screen enables you to view the record where you are presently located in the data base (the active record), as well as the previous and succeeding records in the data base. Within the browse screen, no changes to the data base entries are possible. To see the entire data base entry for the active record, select viEw *[E]* from the browse menu.

It is possible to move throughout the data base by using the Next *[N]* or Previous *[P]* commands in the browse main screen. If you wish to move quickly to a specific area of the data base, select Jump *[J]*. You will be prompted to identify the location in the data base where you would like to "jump" to. The Select/deSelect *[S]* command enables you to toggle between selecting or deselecting the active data base record to conduct operations on a subset of the data base records.

To return to the main menu select eXit *[X]* from the browse main menu or press the *[Esc]* key.

## 5.0 MAKING CHANGES TO THE DO, DATA BASE

### 5.1 Record Entries, Workforms, and the Record Display Screen

From the main menu select Edit, insert, view [E] to provide a full display of the active record. The field names are displayed along the left side of the screen and the corresponding entries for each field are presented. A definition and description for each of the fields within a record is provided in Appendix B of the Pro-Cite user's manual.

Each record in the DO, data base is entered on what Pro-Cite calls a workform. There are twenty different types of workforms, corresponding to the type of presentation format which each reference source represents. In addition, users can develop their own workforms for reference sources that do not conform to any of the predefined workforms. Of the twenty predefined workforms which Pro-Cite provides, three were used in the DO, data base to enable the entry of the different types of presentation formats. The three workform types used were:

1. **Journal (Short)** - This workform was used for the entry of all types of journal articles.
2. **Book (Short)** - This workform was used for the entry of "stand alone" reference materials such as text books and reports.
3. **Book (Long)** - This workform was used for the entry of reference materials which compose part of a larger reference source. This includes a chapter from a book, or one article from a symposium.

On the display screen the workform used for the active record is displayed on the left hand side of the screen in the status line which is located immediately above the menu selections. The centre of the status line, indicates if the active record has been selected. On the right hand side of the screen the status line indicates the record number of the active entry.



## 5.2 Editing Records in the Data base

From the main menu select Edit, insert, view *[E]* to provide a full display of the active record. To alter the contents of the active record select Edit *[E]* from the menu selections. This command invokes what Pro-Cite calls the "Edit mode"; this mode enables you to alter the record entry by typing over the existing entry. While in the edit mode the function keys (F1 to F10) have specific functions specific to editing which are intended to speed up record alterations. The functions of these keys are listed on the display across the bottom of the edit mode screen.

Pressing the *[Esc]* key takes the program out of the edit mode and returns it to the record display menu. If you have made changes to the active record while in edit mode, you will be prompted to answer if you would like the changes made to be saved to the DO<sub>2</sub> data base. Answer yes *[Y]* or no *[N]* to this prompt indicating whether the changes made were intentional and correct.

## 5.3 Deleting Records from the DO<sub>2</sub> Data base

From the main menu select Edit, insert, view *[E]* to provide a full display of the active record. If you wish to delete the active record from the data base type *[D]* from the record display menu. You will be prompted to answer if you wish this record to be deleted. Answering yes *[Y]* will remove the active record from the DO<sub>2</sub> data base.

**If you wish to delete several records from the data base:** Select the records you wish to delete using the Select *[S]* command in the record editing menu. Selecting or deselecting all records is discussed in the section on searching data bases (Page 7). Once you have selected the records you would like to delete, select the Global *[G]* command from the display menu. You can then deLete the records which you do not need from the data base by selecting *[L]* from the global menu. You will then be prompted to answer if you would like to delete the selected records. Only answer yes *[Y]* if you are certain that you do not require these records and you have a backup copy of the DO<sub>2</sub> data base on a separate disk.

#### 5.4 Inserting Records into the Data base

If you wish to add records to the DO<sub>2</sub> data base you should first select the workform type that corresponds to the presentation format of the reference source material to be added to the DO<sub>2</sub> data base. Select "set Workform" [*W*] from the record display menu. This will display the twenty predefined workforms which Pro-Cite provides. Select the workform applicable to the format of the new record entry.

Once you have set the workform for the new entry, select Insert [*I*] from the display menu. A blank workform will appear on the display screen in edit mode. Complete the fields applicable to the new record entry (Definitions of each field are provided in Appendix B of the Pro-Cite user's manual).

When you have completed entering the information for the new record, press [*Esc*] and you will be prompted to answer if you would like to save the changes (additions) you have made. Answering yes [*Y*] to this prompt will place the new record into the data base in alphabetical order within the list of records. Note however, that the record number of the new entry reflects that it was the last record entered.

## 6.0 GENERATING A DATA BASE BIBLIOGRAPHY

Pro-Cite has the capability to prepare a bibliography (a listing), summarizing the entries in the DO<sub>2</sub> data base. A bibliography can be generated from the Print command in either the record display or main menu screens. A more comprehensive list of the bibliography generation options are available via the main menu. The following discussion assumes that bibliography generation will be conducted using the main menu options.

If you are presently in the record display screen, return to the main menu by pressing the *[Esc]* key. Once in the main menu select Print *[P]* from the menu options. Within the Print menu there are several format parameters for generating the bibliography. The most important ones are located in the central portion of the Print menu.

The bibliography can be prepared in a number of formats and can include a variety of information from the field entries. Format, style, and punctuation can be set using the set pUnctuation command *[U]* in the Print menu. Depressing *[U]* provides a listing of the punctuation files which are available in Pro-Cite. Examples of each punctuation style are provided in Appendix F of the Pro-Cite manual. The bibliography for the DO<sub>2</sub> data base (Appendix A of this manual) was prepared using the "ANSI standard" Pro-Cite punctuation format.

The output format can also be set by selecting output *[O]* from the print menu. Pro-Cite has the capability to produce an output in the following formats:

- a. Screen preview
- b. Sent directly to local printer
- c. ASCII text files
- d. Wordperfect format
- e. Microsoft Word format

If the bibliography is generated in ASCII, WordPerfect, or Word format it is saved to a file. You will be prompted to provide a name for the bibliography file when you initiate printing.

Other output parameters can also be specified to customize the bibliography output by selecting Output *[O]* from the print menu. Options for these parameters are discussed in section 16 of the Pro-Cite users manual. Once you have specified the output parameters and are ready to generate the bibliography, initiate printing by pressing *[P]*.

## 7.0 PERFORMING DATA BASE SEARCHES

Performing data base searches is expected to be one of the most frequent applications which will be conducted with the DO<sub>2</sub> data base. Following the procedures outlined below will enable searches to be done quickly and efficiently. The information outlined below will provide the user with a basic understanding of how a Pro-Cite data base search is conducted; however, if more comprehensive searches are required, the user should refer to section 9 of the Pro-Cite user manual.

Before performing a data base search you will need to customize the searching defaults for the DO<sub>2</sub> data base. From the main menu, select customize [C] to reveal the customization options for the data base.

### 7.1 Quick Searches

Within Pro-Cite, several types of searches can be conducted. The fastest type of search is the quick search. A quick search scans specific fields in the workforms of the data base for a specified parameter (expression) which the user defines. To conduct a quick search you must first specify the fields to be searched.

Select Quick search fields [Q] from the customize menu. A listing of the forty-five field names from the workforms will be displayed. By default, Pro-Cite searches ten of these fields in a quick search scan. These ten fields are Author, Title, Place of Publishing, Publisher, Date, ISBN, Notes, Abstract, Call number, and Descriptors.

These default quick search fields should be sufficient for any of the quick searches which are likely to be conducted on the DO<sub>2</sub> data base. If the user wishes to perform a search on a field not scanned by default, this field may be added to the list of fields to scan. From the select quick search fields display press [Esc] [Esc] to return to the main menu. From the main menu, select Search data base [S] to reveal the search data base menu. Within this menu you can select the portion of the data base that you wish to search. For most

applications, the user will want to search the entire DO<sub>2</sub> data base. If this is the case, choose select All [A] from the search data base options. To specify the data base search parameters select Search selected records [S] from the menu options. This will activate the search display screen.

Before specifying your search expression, ensure that the quick search function has been enabled. The quick search mode indicator is located at the bottom-centre of the search expression box in the centre of the screen. If quick search is off, press F7 to enable this function.

The key to conducting an efficient search is to provide sufficient information in the search expression to select all relevant information sources. The search expression specified can be a subject, author, keyword, or any other "string" that can be searched for in the quick search fields.

Example: In performing a search for records prepared or co-authored by F.E.J. Fry you could conduct a quick search using the string "fry". The results of this search would be seven selected records. Of the seven records selected, four would have been prepared or coauthored by F.E.J. Fry, and three other records simply contained the word fry in the title.

## 7.2 Searching by Field

One way to avoid this problem of selecting irrelevant records, and also to speed up the search process, is to specify the search field in the search expression. To specify the field to search, you can take advantage of the list field display (F2) and provide a specific field and string to be used in the search.

Example: In performing a search for records prepared or co-authored by F.E.J. Fry. Press [F2] to reveal the field names for the workforms. Select the field "author" to be searched [1] [return]. Type in the search expression [Fry] and press return to initiate the search.

A search conducted in this fashion reveals that three records were selected. This technique speeds up the searching process considerably, and reduces the problems of selecting unrelated records. However, this example also identifies one problem of conducting a search by field. There are actually four records in the data base which were authored by F.E.J. Fry: three were entered as journals and one was recorded on a book (short) workform. The principal authors in a book are listed as the Monograph Author (field #7), while the principal authors of a journal article are listed as the Analytical Authors (field #1). As a result, one of the records was not selected by the search. This problem is identified so that the user of the DO<sub>2</sub> data base is aware of some of the considerations when conducting a search by field.

### 7.3 Searching by Keywords

One of the quickest and most efficient methods to conduct a search on the DO<sub>2</sub> data base is to use the keywords (descriptors) included in each record of the data base. Field #45 of the workform is the descriptors field . By using this field indicator and the list of descriptors for the DO<sub>2</sub> data base, it is possible to conduct a fast search of the data base.

Example: To conduct a search of records which contain dissolved oxygen information on charr (*Salvelinus*), conduct the following steps:

1. From the main menu select Search data base [S]
2. Ensure that the search will be conducted on all records by selecting select All [A] from the search menu
3. Go to the search data base screen by selecting search selected records [S]
4. Select the field to search [F2] [45] [return]
5. Call up the list of descriptors for the DO<sub>2</sub> data base [shift + F4]
6. Select the descriptor to search for by highlighting "Salvelinus" [return]
7. Initiate the search [return]

#### 7.4 Viewing the Results of a Search

Once a search has been completed the results can be viewed by using the browse function. Return to the main menu *[Esc]* *[Esc]* and select Browse *[B]*. The items selected during the search will appear in bold text. The selected records can be viewed by typing *[T]* to view the next selected item in the list, or *[V]* to view the previously selected record. Type *[X]* to exit to the main menu.

If you would like to save the results of a particular search to a separate file, select Utilities *[U]* from the main menu. To copy selected records and workforms to a separate file select *[C]*. You will then give the subset of the data base a New destination by pressing *[N]* and then specifying a file name at the prompt.

The results of your search have now been saved in a separate file for which you can generate a bibliography by following the procedure outlined in section 6.0. Press *[spacebar]* to return to the utilities menu, and *[Esc]* to return to the main menu.

#### 7.5 Performing Searches With the Use of Operators

In certain applications the user may wish to conduct more specific searches using a number of relevant keywords. These types of searches may require the use of more than one descriptor in order to select only the records which are relevant to a certain application. "Operators" are characters that Pro-Cite interprets as a special searching instruction, and are used in the formation of a search expression. There are two basic types of operators: relational and logical. Relational operators are used to express a relationship between multiple search terms. Pro-Cite has five relational operators: = (equal); < (less than); > (greater than); <= (less than or equal to); >= (greater than or equal to). Pro-Cite has three logical operators: and; or; not. Using these two types of operators the user can conduct comprehensive data base searches for a specific application. For details on how to use operators in the application of data base searches please see section 9 of the Pro-Cite users manual.



Example: To select records which provide relevant information on the development of oxygen criteria for northern pike (*Esox lucius*), the user could conduct the following steps:

1. From the main menu select the search data base option *[S]*
2. Ensure that all records in the data base are included in the search *[A]*
3. Go to the search data base screen *[S]*
4. Clear any search expressions from the present search criteria *[F8]*
5. Call up the list of descriptors by pressing *[shift + F4]*, highlight "Esox" and press *[return]*.
6. Call up the list of operators to use in the search *[F3]*, highlight "AND" and press *[return]*.
7. Call up the list of descriptors *[shift + F4]* and highlight "criteria" press *[return]*
8. Hit *[return]* to initiate the search.
9. Return to the main menu *[space] [Esc] [Esc]*.

The program will search for all records containing both the keywords "Esox" and "criteria".



**APPENDIX A DISSOLVED OXYGEN BIBLIOGRAPHY**



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## **APPENDIX B PRO-CITE DATA FILES**

The disk comprising this Appendix contains three files: 384,235 bytes.

1. D02.EXE; being 121,173 bytes in size.
2. INSTALL.BAT; being 70 bytes in size.
3. DISCLAIM.TXT; being 500 bytes in size.

Following the instructions for installing the database will result in 10 files 384,735 bytes; these files pertain to 212 bibliographic references.

**There is no warranty expressed or implied for the use of this database; the Northern River Basins Study does not guarantee the accuracy of the data. The NRBS does not assume any liability for actions or consequences resulting from the use of the data; individuals using this database do so entirely at their own risk. The NRBS will not update the data except as deemed necessary for its own purposes.**

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