



Environment  
Canada

Conservation  
& Protection

Environnement  
Canada

Conservation  
et Protection

Site Specific Water Quality Criteria  
for Fish and Aquatic Life  
in the Canadian Portion  
of the Flathead River Basin

NITRATE, NITRITE & AMMONIA

D.D. MACDONALD, L.E. FIDLER, D. VALIELA  
September, 1987

TD  
227  
B74  
FL87-1  
c.2

Inland Waters and Lands  
Pacific and Yukon Region  
Vancouver, B.C.



36 002 149

TD  
227  
B74  
FL87-1  
C,2

SITE SPECIFIC WATER QUALITY CRITERIA FOR FISH  
AND AQUATIC LIFE IN THE CANADIAN PORTION  
OF THE FLATHEAD RIVER BASIN:  
NITRATE, NITRITE AND AMMONIA

LIBRARY  
ENVIRONMENT CANADA  
PACIFIC REGION

D.D. MACDONALD<sup>1</sup>

L.E. FIDLER<sup>2</sup>

D. VALIELA<sup>1</sup>

<sup>1</sup>#502 - 1001 WEST PENDER STREET  
VANCOUVER, BRITISH COLUMBIA  
V6E 2M9

<sup>2</sup>UNIVERSITY OF BRITISH COLUMBIA  
DEPARTMENT OF ZOOLOGY

SEPTEMBER 1987

ID 312

## ABSTRACT

The Flathead River headwaters are in the southeastern corner of British Columbia; the river then crosses the International Boundary, eventually reaches Flathead Lake in Montana, and ultimately joins the Columbia River system. Concerns relative to proposed developments in the Canadian portion of the basin have led to management efforts to conserve Canadian interests while minimizing the potential for disputes over water quality in the basin. Management of the problem would be facilitated by defining water quality conditions to be pursued (water quality objectives). Water quality objectives require a basis in criteria for specified water uses, i.e. the site-specific water quality requirements to support agreed-to water uses. This report develops site-specific water quality criteria for toxic compounds of nitrogen for relevant fish and aquatic life species in the Canadian portion of the Flathead River.

Nitrogen chemistry in the aquatic environment is determined by the major nitrogen cycling processes; these include ammonification, direct assimilation, nitrification, and denitrification. A number of physical, chemical, and biological factors modify the nitrogen cycling processes in streams and rivers. Thus the relative proportions of various highly toxic and less toxic compounds of nitrogen will vary in a site-specific manner. Further, the particular species of fish present in the system, as well as their life stage and state of stress from interacting environmental factors, modify the toxicity of these compounds in a site-specific manner. These water quality and biotic factors were taken into consideration and used with laboratory toxicology information in development of site-specific water quality criteria for nitrate, nitrite, and ammonia.

Assuming the toxic effects of ammonia and nitrite are additive, where the two toxicants occur together, the following procedure should be used to calculate final criteria values. The concentration of one of the

compounds must be measured, and to comply with the criteria the sum of the ratios of ambient levels to individual criteria values (Tables 12 and 20) for the two compounds should not exceed 1.0. For example, if the concentration of  $\text{NH}_3$  is 60% of the maximum acceptable level of  $\text{NH}_3$  for a specific site in the Flathead basin (Table 20), then CMC (the Criterion Maximum Concentration)  $\text{NH}_3 = [\text{NH}_3]$  and CMC (Criterion Maximum Concentration)  $\text{NO}_2^-$  would be 40% of the maximum no-effect level of  $\text{NO}_2^-$  (Table 12).

## RESUME

Le cours supérieur de la rivière Flathead est situé à l'extrémité sud-est de la Colombie-Britannique; la rivière traverse la frontière internationale, se déverse ensuite dans le lac Flathead, situé au Montana, et rejoint finalement le bassin de la rivière Columbia. Certaines inquiétudes suscitées par des projets de développement dans la partie canadienne du bassin ont amené des plans d'aménagement visant à préserver les intérêts canadiens tout en minimisant les risques de conflit à propos de la qualité de l'eau du bassin. La mise en oeuvre de ces plans d'aménagement serait facilitée par la connaissance des conditions de qualité de l'eau à maintenir (des objectifs de qualité des eaux). Les objectifs doivent être basés sur des critères visant à permettre des usages spécifiques de l'eau, autrement dit, des exigences de qualité de l'eau spécifiques aux sites où de tels usages sont effectués. Le présent rapport élabore des critères de qualité des eaux spécifiques à des sites donnés, et ce pour des produits azotés toxiques envers les espèces importantes de poissons et de vie aquatique présentes dans la partie canadienne de la rivière Flathead.

La chimie de l'azote dans le milieu aquatique est déterminée par les processus principaux de recyclage de l'azote; ces processus sont l'ammonisation, l'assimilation directe, la nitrification et la dénitrification. De nombreux facteurs physiques, chimiques et biologiques influencent ces cycles dans les cours d'eau et les rivières. Par conséquent, les proportions relatives des divers composés plus ou moins toxiques peuvent varier entre des sites déterminés. De plus, les espèces particulières de poissons présentés dans le milieu, de même que leur stade de vie et le stress causé par les divers facteurs environnementaux, modifient la toxicité de ces composés de façon spécifique à chaque site. Ces facteurs chimique et biologiques ont été considérés et combinés avec des informations toxicologiques obtenues en

laboratoire afin d'élaborer des critères de qualité de l'eau spécifiques à chaque site, et ce pour le nitrate, le nitrite et l'ammoniac.

En supposant que les effets toxiques de l'ammoniac et du nitrite sont additifs lorsqu'ils sont tous deux présents, la procédure suivante peut être utilisée pour calculer les valeurs finales des critères. La concentration de l'un des deux composés doit être mesurée, et pour respecter les critères, la somme des rapports des niveaux ambiants aux critères individuels (tables 12 et 20) pour les deux composés ne doit pas excéder 1.0. Par exemple, si la concentration de l'ammoniac est à 60% du niveau maximum acceptable pour un site spécifique dans le bassin de la rivière Flathead (table 20), alors le CMC (Criterion Maximum Concentration, concentration maximum du critère) de l'ammoniac égale la concentration de celui-ci, et le CMC pour le nitrite est de 40% de son niveau maximum acceptable.

## TABLE OF CONTENTS

	PAGE NO.
ABSTRACT	i
RESUME	iii
TABLE OF CONTENTS	v
LIST OF FIGURES	viii
LIST OF TABLES	x
ACKNOWLEDGEMENTS	xii
1. INTRODUCTION	1
2. NITROGEN CHEMISTRY IN THE AQUATIC ENVIRONMENT	6
2.1 The Nitrogen Cycle	6
2.1.1 Ammonification	6
2.1.2 Direct Assimilation	8
2.1.3 Nitrification	9
2.1.4 Denitrification	10
2.2 Physical and Chemical Considerations	11
2.2.1 Ammonia Equilibrium	11
2.2.2 Aeration	14
2.2.3 Nitrification	16
2.2.3.1 Chemistry	16
2.2.3.2 Growth Rates	16
2.2.3.3 Oxidation Rates	17
2.2.3.4 Reaction Order	18
2.2.3.5 Dissolved Oxygen	19
2.2.3.6 Temperature	21
2.2.3.7 Water pH	24
2.2.3.8 Light Intensity	29
2.2.3.9 Stimulatory and Inhibitory Substances	29
2.2.4 Non-Steady State Effects	30
3. SITE-SPECIFIC WATER QUALITY CRITERIA DEVELOPMENT	35
3.1 Ambient Water Quality	35

## TABLE OF CONTENTS

	PAGE NO.
3.1.1 Background	35
3.1.2 Summary	36
3.1.2.1 Flathead River at the International Border	36
3.1.2.2 Howell Creek below Cabin Creek	37
3.2 Fisheries Interactions with Environmental Contamination	38
3.2.1 Bull Trout	38
3.2.2 Cutthroat Trout	39
3.2.3 Mountain Whitefish	42
3.3 Nitrogen Compounds	45
3.3.1 Nitrate	45
3.3.2 Nitrite	46
3.3.2.1 Fish	46
3.3.2.1.1 Mode of Toxic Action	46
3.3.2.1.2 Abiotic Factors Affecting Nitrite Toxicity	47
a) Hydrogen Ion Concentration	47
b) Chloride	51
c) Calcium	52
d) Dissolved Oxygen	55
e) Temperature	57
3.3.2.1.3 Biotic Factors Affecting Nitrite Toxicity	57
a) Species	57
b) Life History Stage	58
3.3.2.2 Invertebrates	58
3.3.2.3 Algae	59
3.3.2.4 Site-Specific Criteria for Nitrite	59
3.3.3 Ammonia	72
3.3.3.1 Fish	75
3.3.3.1.1 Mode of Toxic Action	75
3.3.3.1.2 Abiotic Factors Affecting Ammonia Toxicity	78
a) Dissolved Oxygen	78
b) Temperature	78
c) Hydrogen Ion Concentration	81
d) Carbon Dioxide	86
e) Fluctuating Exposures	87
3.3.3.1.3 Biotic Factors Affecting Ammonia Toxicity	87
a) Species	88
b) Life History Stage	88
c) Acclimation to Low Ammonia Concentrations	89



TABLE OF CONTENTS

	PAGE NO.
3.3.3.2 Invertebrates	92
3.3.3.3 Algae	92
3.3.3.4 Site-Specific Criteria for Un-ionized Ammonia	93
4. FINAL CRITERIA FOR NITROGEN COMPOUNDS	111
5. REFERENCES	114
6. APPENDIX 1 Existing Water Quality Conditions in the Flathead River at the International Boundary to 1982	124
7. APPENDIX 2 Existing Water Quality Conditions in Howell Creek to 1982	126

LIST OF FIGURES

		PAGE NO.
Figure 1	Flathead River Basin	2
Figure 2	Canadian Portion of the Flathead River Basin	3
Figure 3	The Nitrogen Cycle	7
Figure 4	The Effect of pH and Temperature on the Distribution of Ammonia and Ammonium Ions in Water	13
Figure 5	Effect of Temperature on the Half Saturation Constants of Nitrifying Bacteria	22
Figure 6	Effect of Temperature on the Maximum Growth Rates of Nitrifying Bacteria	23
Figure 7	Effect of Temperature on the Rate of Nitrification in Suspended and Attached Growth Systems	25
Figure 8	Effect of water pH on Nitrification Rate of Nitrosomonas	26
Figure 9	Effect of water pH on Nitrification Rate of Nitrobacter	27
Figure 10	Effect of pH on Nitrification Rate for a Variety of Growth Media	28
Figure 11	Bull Trout Life History Patterns in the Flathead System: Conceptual Model	40
Figure 12	Effect of pH on the Toxicity of Nitrite to Rainbow Trout	49
Figure 13	Effect of Chloride Concentration on the Toxicity of Nitrite to Rainbow Trout	53
Figure 14	Effect of Calcium Concentration on the Toxicity of Nitrite to Steelhead Trout	54
Figure 15	Relationship between 96 hr LC <sub>50</sub> for Nitrite and the Weight of Salmonid Fish	56
Figure 16	Calculated Final Acute Values of Nitrite for Bull Trout in the Flathead River System	64

LIST OF FIGURES

		PAGE NO.
Figure 17	Calculated Final Acute Values of Nitrite for Cutthroat Trout in the Flathead River System	67
Figure 18	Calculated Final Acute Value of Nitrite for Mountain Whitefish in the Flathead River System	70
Figure 19	Calculated No-Effect Levels of Nitrite in the Flathead River System	74
Figure 20	Effect of Dissolved Oxygen on the Toxicity of Ammonia to Rainbow Trout	80
Figure 21	Effect of Temperature on the Toxicity of Ammonia to Freshwater Fish	82
Figure 22	Effect of pH on the Toxicity of Ammonia to Rainbow Trout	84
Figure 23	Effect of pH on the Toxicity of Ammonia to Coho Salmon	85
Figure 24	Effect of Developmental Stage on the Toxicity of Ammonia to Pink Salmon	90
Figure 25	Effect of Fish Weight on the Toxicity of Ammonia to Salmonid Fish	91
Figure 26	Summary of Available Water Temperature Data for the Flathead River System (monthly means)	95
Figure 27	Summary of Available pH Data for the Flathead River System (monthly means)	96
Figure 28	Calculated Final Acute Values of Ammonia for Bull Trout in the Flathead River System	101
Figure 29	Calculated Final Acute Values of Ammonia for Cutthroat Trout in the Flathead River System	104
Figure 30	Calculated Final Acute Values of Ammonia for Mountain Whitefish in the Flathead System	107
Figure 31	Calculated No-Effect Levels of Ammonia in the Flathead River System	110

LIST OF TABLES

		PAGE NO.
Table 1	List of Substances Stimulatory to Nitrifier Growth	31
Table 2	List of Substances Inhibitory to Nitrifier Growth	32
Table 3	Summary of Life History Information for Flathead River Watershed Salmonid Fish Species	43
Table 4	Effect of pH on the Toxicity of Total Nitrite to Rainbow Trout	50
Table 5	Reference Final Acute Values of Nitrite for Key Life History Stages of Important Flathead River System Fish Species	61
Table 6	Calculated Final Acute Values and No-Effect Levels of Nitrite for Flathead River Bull Trout	62
Table 7	Calculated Final Acute Values and No-Effect Levels of Nitrite for Howell Creek Bull Trout	63
Table 8	Calculated Final Acute Values and No-Effect Levels of Nitrite for Flathead River Cutthroat Trout	65
Table 9	Calculated Final Acute Values and No-Effect Levels of Nitrite for Howell Creek Cutthroat Trout	66
Table 10	Calculated Final Acute Values and No-Effect Levels of Nitrite for Flathead River Mountain Whitefish	68
Table 11	Calculated Final Acute Values and No-Effect Levels of Nitrite for Howell Creek Mountain Whitefish	69
Table 12	Calculated No-Effect Levels of Nitrite in the Flathead River Watershed	73
Table 13	Reference Final Acute Values of Ammonia for Key Life History Stages of Important Flathead River System Fish Species	97

LIST OF TABLES

		PAGE NO.
Table 14	Calculated Final Acute Values and No-Effect Levels of Ammonia for Flathead River Bull Trout	99
Table 15	Calculated Final Acute Values and No-Effect Levels of Ammonia for Howell Creek Bull Trout	100
Table 16	Calculated Final Acute Values and No-Effect Levels of Ammonia for Flathead River Cutthroat Trout	102
Table 17	Calculated Final Acute Values and No-Effect Levels of Ammonia for Howell Creek Cutthroat Trout	103
Table 18	Calculated Final Acute Values and No-Effect Levels of Ammonia for Flathead River Mountain Whitefish	105
Table 19	Calculated Final Acute Values and No-Effect Levels of Ammonia for Howell Creek Mountain Whitefish	106
Table 20	Calculated No-Effect Levels of Ammonia in the Flathead River Watershed	109

## ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge those persons who contributed significantly to the production of this report. Dr. V. Thurston, Mr. T. Willingham, and Mr. C. Thorp provided unpublished data and relevant information that was unavailable from primary sources. Detailed reviews of various sections of this report were provided by Dr. A. Horpestad, Dr. P. McCart. Mr. L. Pommen, Dr. H. Mundie, Mr. T.W. Willingham, Dr. J. Stanford and Mr. C. Newcombe. Dr. R.N. Nordin kindly reviewed the final draft of this report. Figures were drafted by Mr. D. Boak and Mr. C. Tremewen. Word processing was provided by Ms. M.L. Haines.

## 1. INTRODUCTION

The Flathead River system drains approximately 1 582 km<sup>2</sup> of southeastern British Columbia, near the B.C.-Alberta-Montana borders. After flowing almost 70 km from its headwaters in the Rocky Mountains, the Flathead crosses the International Boundary, eventually reaching Flathead Lake in Montana, and ultimately joining the Columbia River system (Figure 1).

Developmental activities in the Canadian portion of the Flathead Basin (Figure 2), until recently, have been limited to small-scale utilization of forest resources and some mineral exploration. Recently, infestations of forest insects have induced clear-cutting activities throughout portions of the watershed to salvage affected timber. In addition, coal and oil deposit discoveries have resulted in increased activity in the area. Oil and gas developments are proposed, with the construction of a carbon dioxide purification facility and pipeline to transport extracted CO<sub>2</sub> gas. Coal deposits are considered extensive enough in the Cabin Creek area to sustain intensive mining activities for up to 20 years. These potential developments may affect the aquatic environment in Canada and in the United States (where the North Fork of the Flathead River flows along the western boundary of Glacier National Park, and has been designated a scenic and recreational river under the Wild and Scenic Rivers Act). Because the potential for impacts on aquatic resources may have international implications, there is clearly a federal concern in this water quality management issue. Among the most important uses of the river in the U.S.A. are those associated with recreational activities, particularly sport fishing.

**FIGURE 1**  
**FLATHEAD RIVER BASIN**

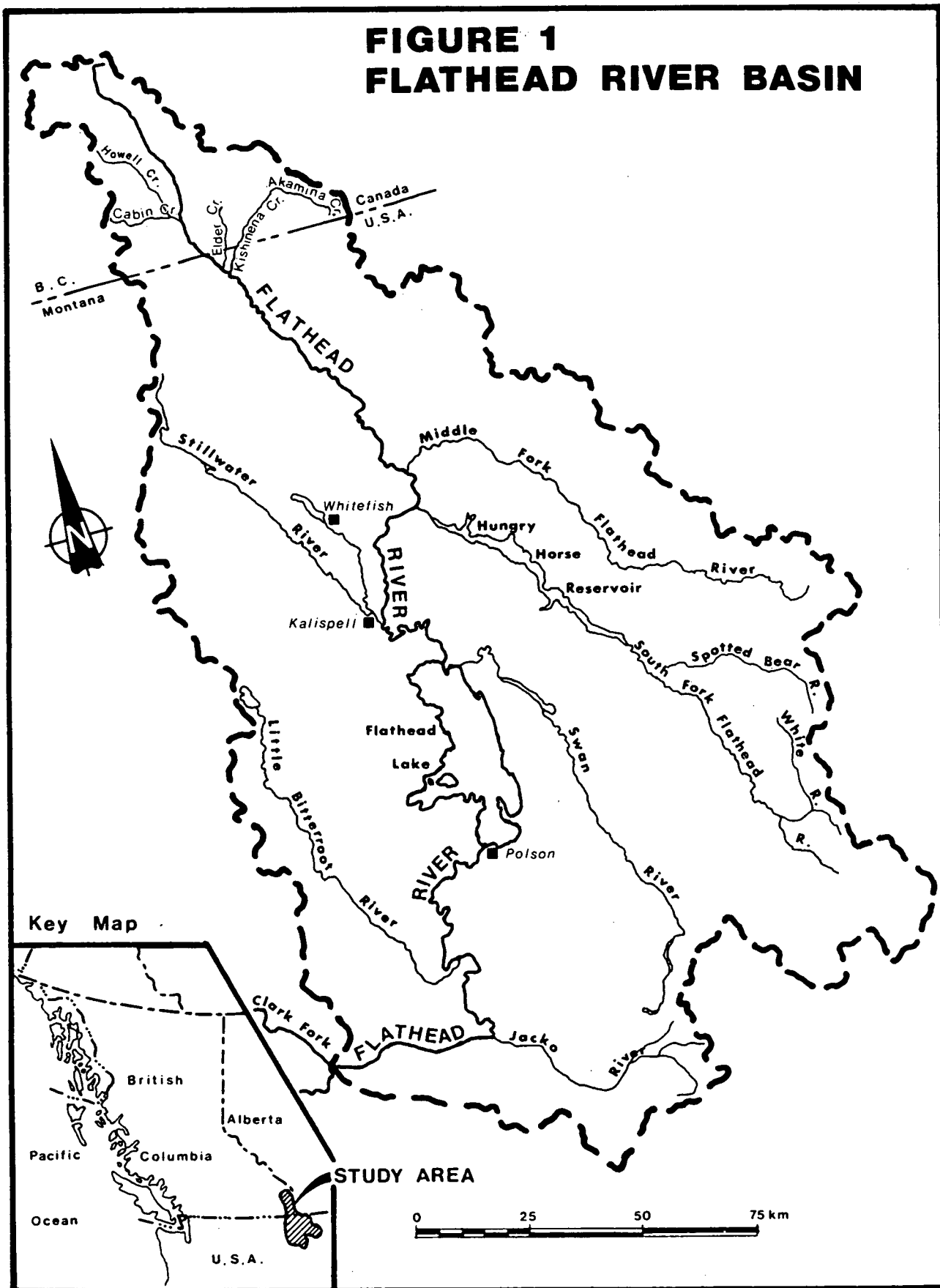
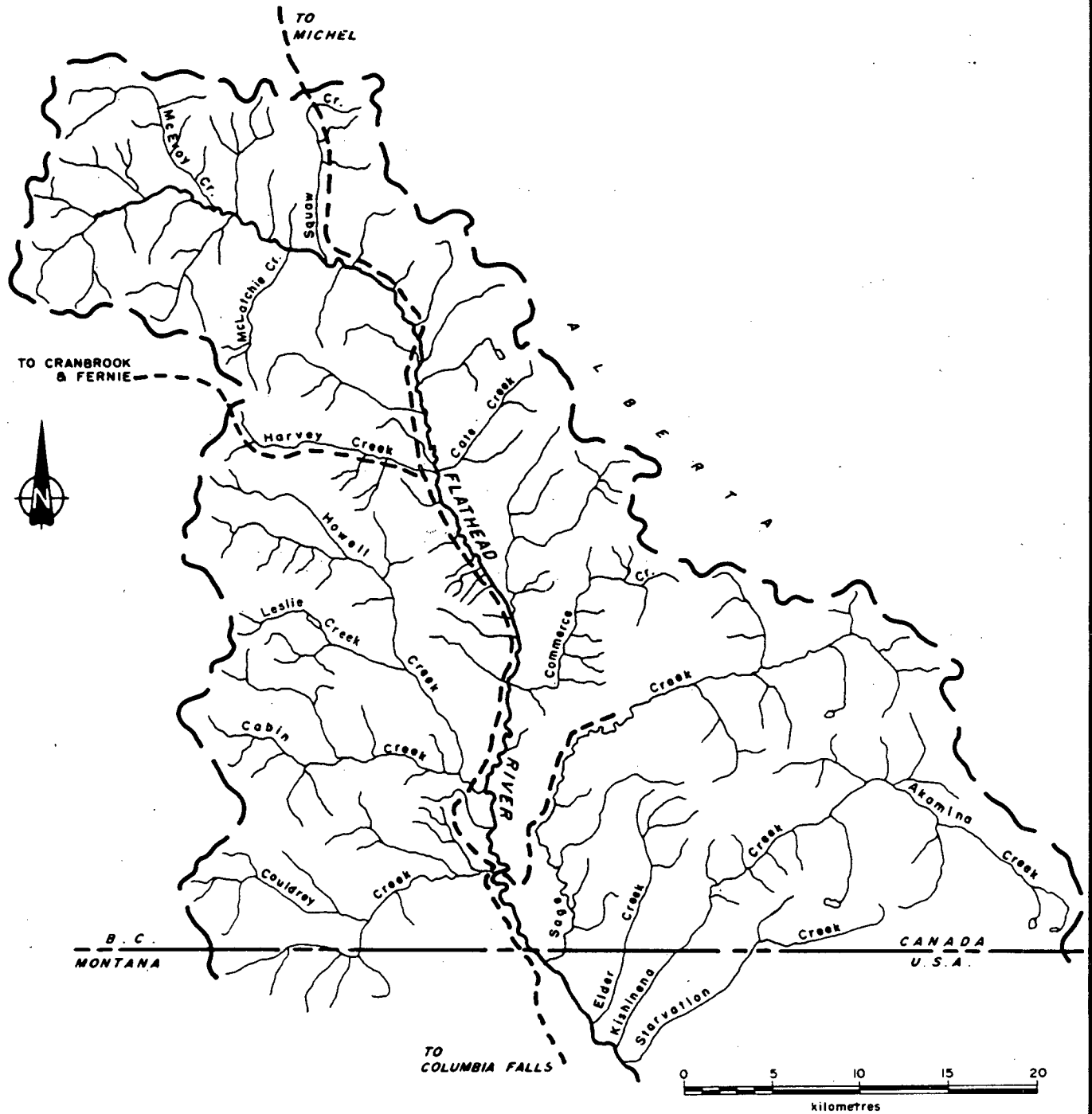




Figure 2 Canadian Portion of the Flathead River Basin



Inland Waters and Lands, Environment Canada will respond to these concerns by developing site-specific water quality criteria. These criteria are useful for the negotiation of water quality objectives for the Flathead system designed to conserve Canadian interests.

The sport fishery in Montana is primarily dependent on stocks of kokanee (Oncorhynchus nerka) salmon, which are produced entirely within the American portion of the basin. However, stocks of bull (Salvelinus confluentus) and cutthroat (Salmo clarki) trout are utilized extensively by sport fishermen both in the Flathead River and in Flathead Lake. Mountain whitefish (Prosopium williamsoni) are also utilized in the Flathead River during portions of the year. With the exception of kokanee, production of these important fish species is dependent, in part, on utilization of habitats within Canada for various stages of their life history cycles. In order to determine conditions required to support important sport fish species and fisheries, it is necessary to define the water quality requirements for these species during the critical life history stages that occur in the Canadian portions of the basin. In particular, Howell Creek and the Flathead River between Howell Creek and the International Boundary contain fish habitats of concern.

An examination of the effects of explosives used in surface coal mining on the quality of receiving waters (Pommen et al. 1982) indicated that significant increases in quantities of nitrogen compounds resulted from this activity. At Fording Coal Ltd. (on the Elk River system) approximately six percent of the nitrogen originally present in the explosives used was discharged to the Fording River, with nitrate, nitrite and ammonia accounting for most of that nitrogen. Wet blasting conditions, necessitating the use of slurry explosives, tended to increase the losses of these nitrogen compounds from the mine. Similarities in the climatic conditions and methods of coal extraction at the Fording and Howell Creek sites

suggest that considerable potential for nitrogen losses exists if developmental activities proceed in the Flathead River watershed. The loss of nitrogen compounds to the aquatic environment could result in elevated levels of those species which are considered to be toxic to fish and aquatic life. Water quality criteria, developed on a site-specific basis, provide a yardstick against which the effects of these elevated nitrogen levels can be measured. Therefore, formulation of site-specific water quality criteria, negotiation of water quality objectives, and implementation of a monitoring program to assess compliance with objectives for nitrates, nitrites, and ammonia will contribute to the fulfillment of Canadian obligations under the Boundary Waters Treaty, and reduce the potential for international disputes over water quality in this system.

In the context of this report, criteria are defined as constituent concentrations or levels of physical, chemical or biological characteristics of water, sediment or biota that represent a quality of water that supports a particular use. Specifically, a water quality criterion consists of three essential features:

1. The specific concentration or level of the water chemistry (physical) variable. Maximum and average criteria are determined where appropriate.
2. The frequency at which any criteria concentrations can be exceeded without impairing a specified water use, or without impairing it beyond predicted degrees.
3. The duration for which the criteria's concentration may be exceeded at the specified frequency without impairment of a specified water use.

Environmental Protection Agency (1985), Nordin and Pommen (1986) and Canadian Council of Resource and Environment Ministers (1987) provide recent and comprehensive information on general criteria for toxic compounds of nitrogen for fish and aquatic life. Much of this

information is used here in developing site-specific criteria for the Canadian portion of the Flathead River. In addition, the criteria formulated in this report take into consideration specific ambient water quality and biotic characteristics, including differences in susceptibilities of various life history stages and species in Howell Creek and in the Flathead River from Howell Creek to the International Boundary.

## 2. NITROGEN CHEMISTRY IN THE AQUATIC ENVIRONMENT

Nitrogen occurs in freshwater in a number of forms, including dissolved molecular nitrogen gas ( $N_2$ ), a large number of organic compounds, ammonia, nitrite and nitrate. The major nitrogen species in the environment are interrelated by a series of transformations that collectively comprise the nitrogen cycle (Figure 3). In aquatic systems, most of these transformations are mediated by biological processes, with abiotic processes (such as volatilization, sorption and sedimentation) important only under certain circumstances.

### 2.1 Nitrogen Cycle

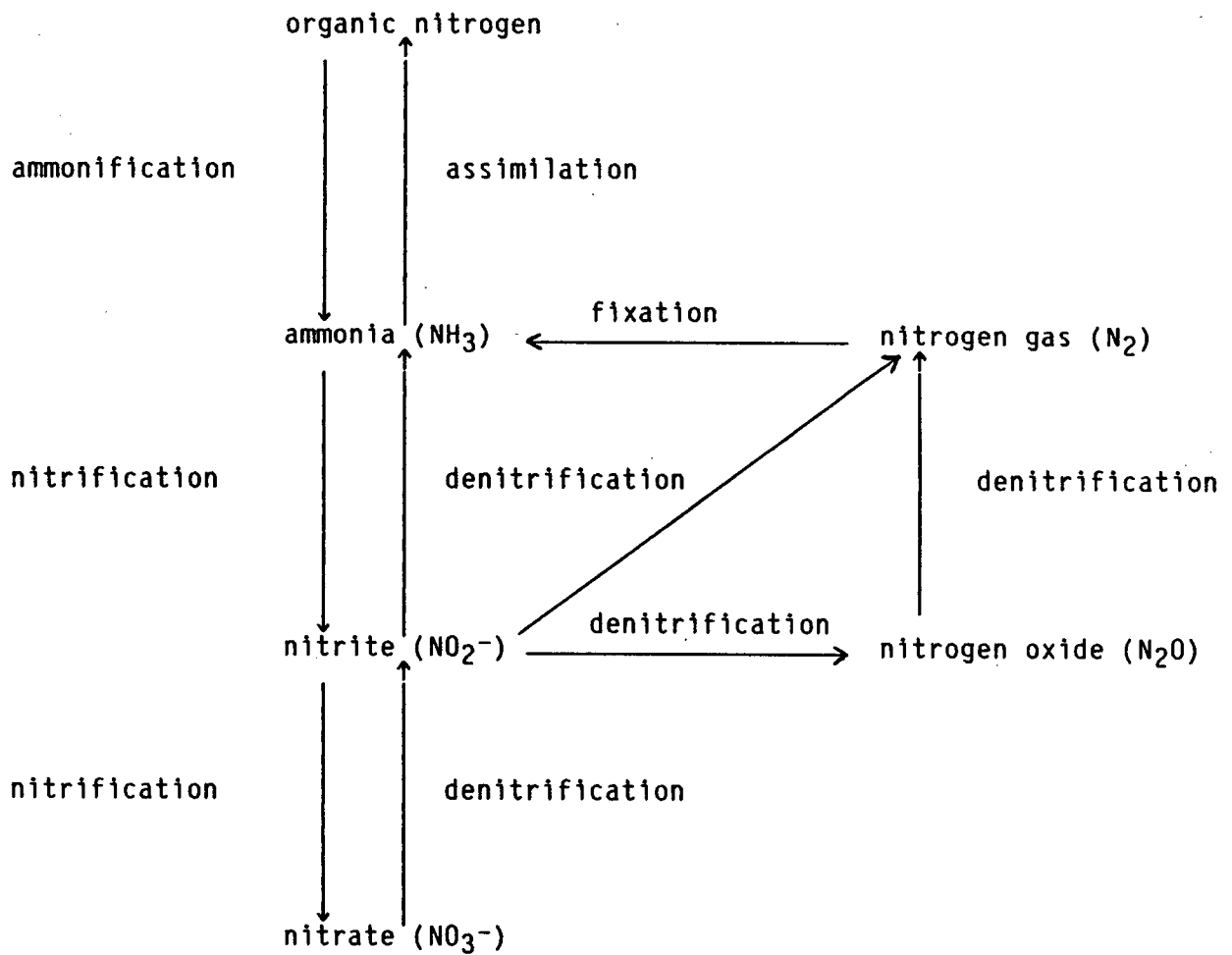
#### 2.1.1 Ammonification

As a result of metabolism, nitrogen is excreted by organisms (as ammonia, urea, urine, etc.). At death, inorganic compounds are formed as a result of cellular decomposition of tissues. This breakdown is carried on by heterotrophic bacteria in both the terrestrial and aquatic environments or through the process of proteolytic deamination (Painter 1970).

The net result is the production of ammonia from these organic nitrogen compounds. The ammonification process would not normally constitute a problem in a lake or free flowing stream, clear of man-made nitrogen inputs. However, when additional inorganic nitrogen is present, the primary production of an

FIGURE 3

THE NITROGEN CYCLE  
(from Nordin and Pommen, 1986)



aquatic system may increase dramatically if nitrogen is a limiting factor. The turnover of this elevated production can then lead to increased ammonification which, in turn, adds more nutrients to increase primary production in downstream areas (De Renzo 1978; Painter 1977; Raune and Krenkel 1975; Lopez-Bernal et al. 1977). This cycle (also known as eutrophication) in stream systems results in an increase in both the carbonaceous and nitrogenous oxygen demand on the system and ultimately may reduce oxygen content in a stream or lake to the point where it can no longer support many aquatic animals (De Renzo 1978; Painter 1977; Raune and Krenkel 1975; Lopez-Bernal et al. 1977).

#### 2.1.2 Direct Assimilation

With few exceptions, most phytoplankton and macrophytes containing chlorophyll are able to utilize  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  or  $\text{NO}_2^-$  directly for the synthesis of cellular material (Brezonik 1977; Brown et al. 1974; Forsberg 1977; Kolenbrander 1977). Fogg (1953) and Syrett (1962) report that when supplied together,  $\text{NH}_4^+$  is assimilated by most algae preferentially to  $\text{NO}_3^-$ , and that  $\text{NO}_3^-$  is assimilated only after most of the  $\text{NH}_4^+$  is exhausted. This result is understandable from the standpoint that the reduction of  $\text{NO}_3^-$  requires energy input (Vollenweider 1968). Examining the effects of inorganic nitrogen concentration on growth, Vollenweider (1968) concluded that the optimal concentration in planktonic algae cultures was between 0.3 and 1.3 mg/L. For green and blue-green algae the optimum was generally greater than 1 mg/L. Mulligan and Baronowski (1969), in a study of optimal inorganic nitrogen concentrations for growth of microphytes and macrophytes, found that the best growth of phytoplankton occurred at nitrogen concentrations above 1.0 mg/L, while vascular plants responded best to lower nitrogen concentrations.

Unfortunately little information is available regarding the rate of inorganic nitrogen turnover by aquatic vascular plants. It is known that the macrophyte Lemna minor (common duckweed) consumes  $\text{NH}_4^+$  and  $\text{NO}_3^-$  more effectively than  $\text{NO}_2^-$  (Simms et al. 1968; Sculthorpe 1967). Forsberg (1965) reported that Chara zetlanica can assimilate  $\text{NH}_4^+$  more readily than  $\text{NO}_3^-$ . In an analysis of the composition of water plants, Birge and Juday (1922) found that algae consisted of between 2.7% and 10% total nitrogen by dry weight. The corresponding phosphorus content ranged from 0.1% to 1.1%. The actual nitrogen content seems to vary with the species and the ability of the plant to accumulate nutrients during periods of good supply. The ability to store nutrients which can be used later by the cells to survive periods of low supply is called luxury uptake. Gerloff and Krombholz (1966) and Gerloff (1969) in a study of the critical nitrogen content of several macrophytes (Ceratophyllum demersum, Vallisneria americana, Heteranthera dubia, Elodea occidentalis, Najas flexilis and Zanichelia palustris) found the minimal nitrogen content needed to maintain the plant was 1.3% by dry weight.

In the Flathead River, it is known that an abundance of aquatic algal species inhabit various tributaries of the system seasonally. Therefore, it can be expected that direct assimilation of nitrogen compounds by these plants will play an important role in the fate of nitrogen introduced to this system.

#### 2.1.3 Nitrification

Under aerobic conditions, nitrifying bacteria of the genus Nitrosomonas oxidize  $\text{NH}_4^+$  to form  $\text{NO}_2^-$ . Similarly, the bacteria, of the genus Nitrobacter oxidize  $\text{NO}_2^-$  to form nitrate. Because the rate of growth of Nitrobacter is greater than that of Nitrosomonas,  $\text{NO}_2^-$  is a short lived compound in

most streams under steady state conditions (De Renzo 1978). Although the nitrification process can reduce concentrations of toxic ammonia and nitrite, it does so at the expense of oxygen. This will be described more fully in the section on physical and chemical considerations. The nitrification process has been reported as being responsible for depressed oxygen levels in several nitrogen polluted streams and lakes (Brezonik 1977; Raune and Krenkel 1977; Lopez-Bernal et al. 1977). In general, the nitrification process takes place in two distinct aqueous media. Nitrifying bacteria can be found in most streams as suspended organic matter or growing as a biological film attached to rocks, gravel and sand. Differences in the physical and chemical characteristics between the two media can result in differential rates of reaction. Since nitrifying bacteria occur in virtually all natural water courses, it can be expected that the process of nitrification will occupy a central role in the transformations of nitrogen in the Flathead River system.

#### 2.1.4 Denitrification

Under anaerobic conditions, a wide range of bacteria and fungi can reduce nitrite and nitrate to molecular nitrogen (Painter 1977; Forsberg 1977; De Renzo 1978). This process is particularly evident in poorly aerated rivers and lakes where most of the  $\text{NO}_3^-$  and  $\text{NO}_2^-$  conversions take place in the anaerobic silt layers at the river or lake bottom. It has been noted that in a mixed two phase river water-mud system, denitrification appears to take place only in the mud phase, which is anaerobic and not in the aerobic water phase (Edwards and Rolley 1965).

Assimilation of nitrite and nitrate in these environments by bacteria such as Pseudomonas stutzeri has been documented by Hulme (1974) and Harvey (1955). The kinetics of the process,



however, have been described, for the most part, from information generated on sewage treatment systems (De Renzo 1978). From the analyses of data on denitrification in sewage treatment, sufficient information is available to make qualitative and in some cases quantitative assessments of denitrification rates (De Renzo 1978). In freely flowing, well-aerated streams and rivers, it is usually considered that denitrification is not an important process in the removal of inorganic nitrogen (Forsberg 1977; Painter 1977). This should be the case for most of the Flathead River.

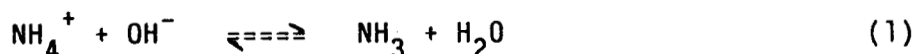
In summary, the central processes important in determining the fate of inorganic nitrogen in the Flathead River are the assimilation of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  by aquatic algal species, the removal of  $\text{NH}_3$  by aeration, and the nitrification of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  by bacteria. Because of the free flowing nature of the river, it should be aerated with sufficient oxygen to maintain denitrification to a low level. This does not imply that oxygen will not be depressed in the river, just that even low levels of oxygen are sufficient to halt the denitrification process (De Renzo 1978). If, as a result of inorganic nitrogen inputs to the Flathead River, the primary production of the system increases dramatically, the processes of ammonification and eutrophication may become important.

## 2.2 Physical and Chemical Considerations

### 2.2.1 Ammonia Equilibrium

Ammonia is present in freshwater systems in both the ionized ( $\text{NH}_4^+$ ) and un-ionized forms ( $\text{NH}_3$ ). The state of ammonia in water is largely controlled by pH, although ionic strength and temperature play roles as well. In general, an increase in pH

of one unit will produce a tenfold increase in the concentration of un-ionized ammonia concentration (Trussel 1972). At pH values below 7.2, less than 1% of the ammonia is present in the un-ionized form. Figure 4 shows the relationship between ionized and un-ionized ammonia as a function of water pH and temperature. The stoichiometry for the ionization of ammonia in water is given by:



The equilibrium constant,  $K_b'$ , for this reaction is approximately  $1.79 \times 10^5$  at infinite dilution and  $25^\circ$  Celsius where:

$$K_b' = [\text{NH}_4^+][\text{OH}^-]/[\text{NH}_3] \quad (2)$$

The variation of the equilibrium constant with ionic strength and temperature can be determined using standard methods in physical chemistry (Stumm and Morgan 1981). An important aspect of the kinetics of this reaction is that it occurs almost instantaneously in relation to other reactions of concern to the overall nitrogen pollution problem (Stumm and Morgan 1981). Other factors, which may affect the toxicity of ammonia at a given temperature and pH, are dissolved oxygen and carbon dioxide concentrations, as well as bicarbonate alkalinity.

In the removal of ammonia by aeration, assimilation and nitrification, equilibrium will be maintained at all water temperatures and pHs. That is, as  $\text{NH}_3$  is removed by aeration,  $\text{NH}_4^+$  will be converted to  $\text{NH}_3$  to maintain equilibrium. Likewise, as  $\text{NH}_4^+$  is consumed by aquatic plants and nitrifying bacteria,  $\text{NH}_3$  will be converted to  $\text{NH}_4^+$  to maintain equilibrium. In each case, the total ammonia of the system

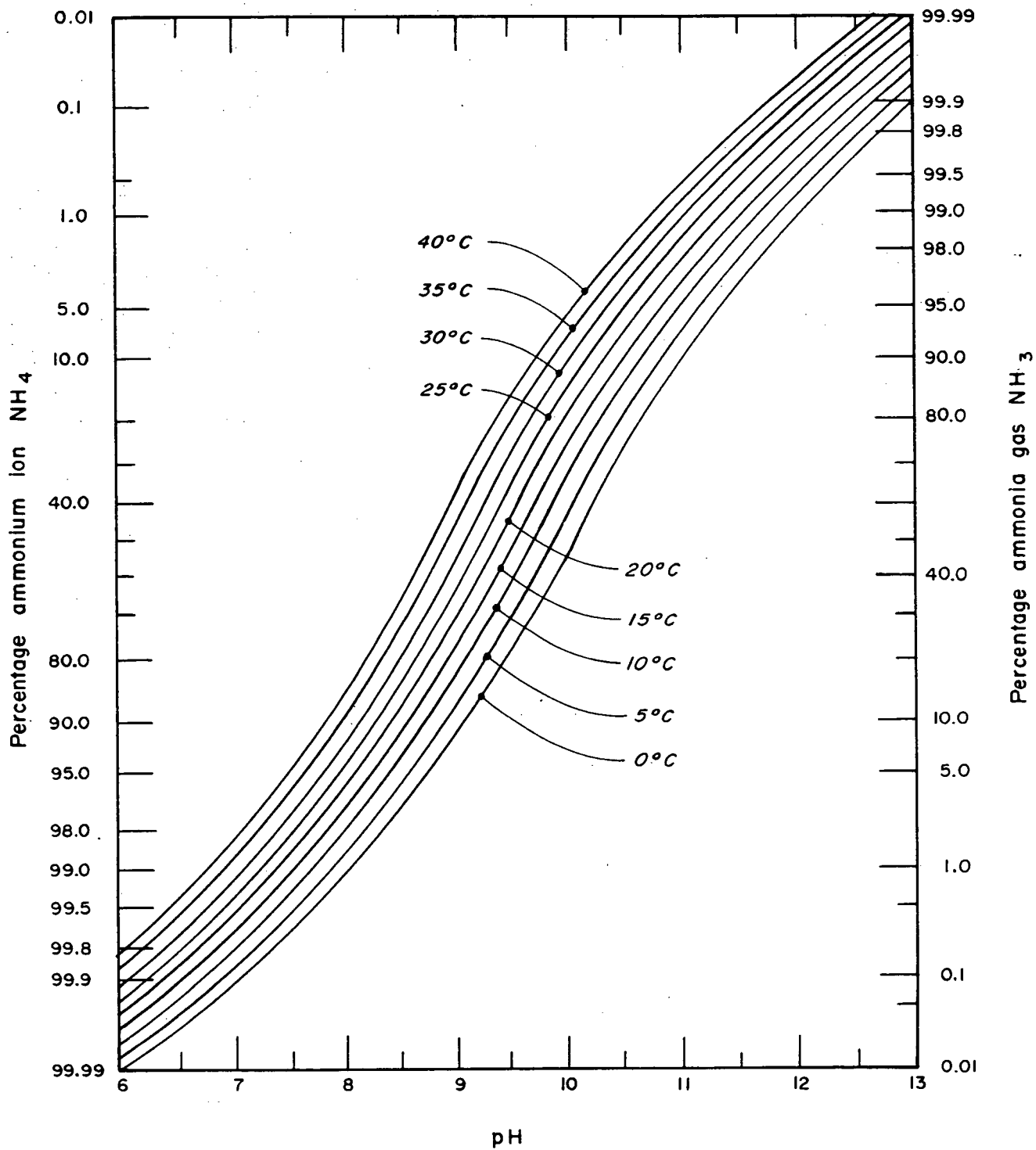


Figure 4 The effect of pH and temperature on the distribution of ammonia and ammonium ions in water. (After Mayo et. al. 1972)

will be reduced. Therefore, in modelling the various aquatic processes involving inorganic nitrogen, ammonia equilibrium will be an essential component.

### 2.2.2 Aeration

As mentioned earlier, ammonia concentrations in a stream may be reduced by the process of aeration. Ammonia in its un-ionized form is a gas which is soluble in water. Due to the absence of ammonia in the atmosphere surrounding a natural stream, a concentration gradient exists which drives ammonia out of solution into the atmosphere. It should be noted from Figure 4 that most of the ammonia in water is in the ionized form at pH values and temperatures of the Flathead River. Because of the low concentration of un-ionized ammonia in solution, the actual concentration gradients driving ammonia from solution by diffusion are small. This in turn means the rate of removal by simple diffusion will be very slow. On the other hand, if a stream is quite turbulent, convective mass transfer becomes important and the rate of ammonia removal can increase dramatically [see Welty et al. (1976) or Fidler (1983) for a discussion of turbulent mass transfer rates]. In addition to the removal of ammonia by aeration, aeration is an important consideration in the process of nitrification. As mentioned earlier, the oxidation of  $\text{NH}_4^+$  and  $\text{NO}_2^-$  by nitrification removes dissolved oxygen from the water. Unless this oxygen is replaced with that from the atmosphere, the nitrification process will be retarded (see section on dissolved oxygen). Fidler (1983 & 1985) reviewed the analytical methods available for predicting the mass transfer coefficients and rates of transfer of dissolved gases from aqueous solutions to and from the atmosphere. In order to apply these techniques to a river, detailed knowledge of water temperature, flow patterns, depth and levels of turbulence in the river must be known. In general, the rate at which ammonia is removed from a water

course will be enhanced by elevated water temperatures and pH, along with high levels of turbulence combined with shallow depth. The same conditions will apply to oxygen mass transfer, except that pH will have no bearing on mass transfer coefficients.

For an aeration process, the rate of change in concentration of a gaseous species in a one dimensional water flow can be expressed as:

$$Q \cdot (dC/dZ) = k_L \cdot A_L \cdot (C - C_S) \quad (3)$$

where: Q is the flowrate

C is the concentration of the gaseous species in solution

Z is the streamwise measure of distance

$k_L$  is the mass transfer coefficient

$A_L$  is a constant surface area per unit Z

$C_S$  is the equilibrium concentration of the gaseous species in solution

Integration of this equation (Fidler, 1983) yields:

$$C = C_0 - \exp(k_L \cdot A_L \cdot Z/Q) \quad (4)$$

where  $C_0$  is the initial concentration of the gaseous species at  $Z = 0$

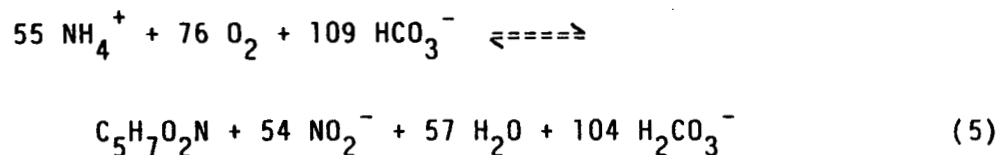
The assumption of one dimensional flow implies uniform mixing of the flow both laterally and vertically through the water column. For shallow turbulent streams, this condition is usually met. For deep, slow moving streams, there will be significant concentration variation both laterally and vertically, thus requiring a more complex two or even three dimensional set of equations.

### 2.2.3 Nitrification

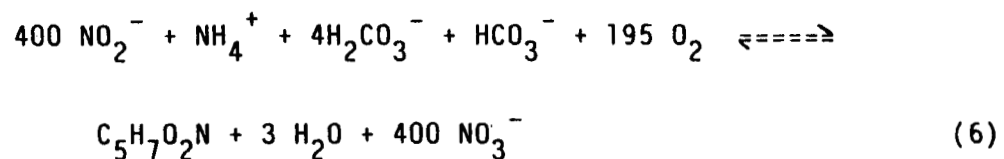
Nitrification involves the conversion of ionized ammonia,  $\text{NH}_4^+$ , first to nitrite and then to nitrate by bacterial action. This conversion is accomplished by the two groups of chemoautotrophic bacteria of the genera Nitrosomonas and Nitrobacter. They are termed chemoautotrophic because they derive their energy for growth from inorganic oxidation processes.

#### 2.2.3.1 Chemistry

Nitrosomonas oxidizes the ammonium ion ( $\text{NH}_4^+$ ) to nitrite ( $\text{NO}_2^-$ ) through the reaction:



The nitrite is further oxidized to nitrate ( $\text{NO}_3^-$ ) by Nitrobacter through the reaction:



The efficiency with which these bacteria carry out the oxidation of ammonia and nitrite is dependent on a number of factors, including the concentration of dissolved oxygen, temperature, pH, light intensity, nitrifier concentration, mass loading rate, and chemical stimulation and inhibition. The importance of these factors will be discussed below.

#### 2.2.3.2 Growth Rates

The kinetic equation proposed by Monod (1949) is most often used to describe the biological growth rate of either Nitrosomonas or Nitrobacter (Grady 1983; De Renzo 1978). The Monod growth relationship is defined by:

$$\mu = \mu_{\max} * A_o / (K_s + A_o) \quad (7)$$

where:  $\mu$  = specific growth rate of microorganisms/day  
 $\mu_{\max}$  = maximum growth rate/day<sup>-1</sup>  
 $A_o$  = growth limiting substrate concentration  
 $K_s$  = substrate concentration at  $1/2 \mu_{\max}$

Since the growth rate of Nitrobacter is considerably higher than the maximum growth rate of Nitrosomonas, Nitrosomonas growth usually becomes the limiting factor in ammonia oxidation. Further, this condition assures that under steady state conditions there is not an appreciable buildup of nitrite in the system. Non-steady state conditions do lead to some imbalance in the relative rates of Nitrosomonas and Nitrobacter mediated oxidations and an accumulation of nitrite can occur. For example, ammonia is toxic to Nitrobacter. Thus, until a population of Nitrosomonas is sufficient to convert most ammonia to nitrite, the development of Nitrobacter will be inhibited.

#### 2.2.3.3 Oxidation Rates

The ammonia oxidation rate constant ( $k_A$ ) can be related to the Nitrosomonas growth rate, as follows:

$$k_A = \mu / Y_A = k'_A * A / (k_s + A) \quad (8)$$

where:  $A$  = growth limiting substrate concentration  
 $(NH_4^+$  in mg/L)  
 $k'_A = \mu_{\max} / Y_A$  = maximum ammonia oxidation rate (in mg  $NH_4^+$  oxidized/mg VSS/day)  
 $k_s$  = half-saturation constant (in mg/L  $NH_4^+$ )  
 $Y_A$  = organism yield coefficient (in mg Nitrosomonas grown [VSS]/mg  $NH_4^+$  oxidized)  
VSS = volatile suspended solids (in mg/L)

This equation clearly indicates that the rate of ammonia removal is proportional to the growth rate of the organism. The values of  $\mu_{\max}$ ,  $k_s$  and  $Y_A$  vary considerably, depending on the environmental conditions for growth. Each of these parameters is influenced by dissolved oxygen concentration, pH, temperature, and the presence of growth inhibiting or enhancing substances.

The results obtained from Equation 8 are dependent on the mass of nitrifying bacteria present and are applicable to suspended growth environments. In the case of attached growth, the equation must be modified to account for an effective surface area for growth. It should be noted that in general, a gravel or sand stream bed will provide more specific surface area for microorganism growth than a silt or bedrock stream bed, and in turn an increased rate of ammonia oxidation (Curtis et al. 1975).

#### 2.2.3.4 Reaction Order

It has been found that the order of the kinetic reaction varies with the concentration of the limiting nutrient. For high levels of nutrients, nitrification processes are of zero order (Huang and Hopson 1974; Wild et al. 1971; Wong-Chong and Loehr 1975). In other words, the rate of ammonium removal by nitrification can be expressed as:

$$dA/dt = -k_A \quad (9)$$

where: A is the ammonium concentration and  
 $k_A$  is the rate constant for the reaction

For low levels of ammonia, the reactions are found to be first order with the rate of removal expressed as:

$$dA/dt = -A*k_A \quad (10)$$



For an attached film nitrification process, the rate equations must be coupled with the equations of mass transfer (Grady, 1983). Due to the oxidation of ammonia in the organic film layer, there will be a concentration gradient for both ammonia and oxygen from the bulk water to the film. As a result, a more complex set of equations is required to account for diffusion of ammonium and oxygen into the film layer and the reaction itself [see Welty et al. (1976) or Bird et al. (1960) for a complete description of the equations for convective mass transfer through a reacting boundary layer].

As mentioned earlier, if turbulent flow exists in the water next to the film, higher concentration gradients can be maintained (Grady 1983; Treyball 1980), and maximum oxidation rates achieved. Although high concentration gradients are important to the nitrification process, it should be noted that there will probably be a maximum velocity or turbulence level beyond which the reaction chemistry, and not flow conditions, becomes the limiting factor (Grady 1983). Turbulence and total volume of flow will also be important from the standpoint of assessing overall mixing and effective substrate concentrations. Under conditions of slow laminar flow, it can be expected that significant striations in substrate and dissolved oxygen concentration will be present. In this situation, the mass transfer and reaction equations can no longer be treated in a two dimensional form, but must involve their complete three dimensional forms.

#### 2.2.3.5 Dissolved Oxygen

The stoichiometric equations listed above (Equations 5 and 6) for combined oxidation - synthesis indicate that 3.31 mg  $O_2$  are required for each milligram of ammonium that is

completely oxidized to nitrate. The biological oxidation of ammonium in both a suspended and attached growth environment requires that ammonium and oxygen enter the biological film by the process of diffusion before they can be utilized by the bacteria. Haug and McCarty (1972) have shown that the diffusion coefficients of these two substances are very nearly equal ( $2 \times 10^{-5} \text{ cm}^2/\text{s}$ ) and that the stoichiometric balance of concentrations is essential to prevent the reaction from becoming rate limited. This is important since reaction kinetics show that oxygen becomes rate limiting before ammonium (Grady and Lim 1980). In order to correct the rate constant equations for the effects of oxygen concentration in a suspended growth environment, De Renzo (1978) applied the following correction factor:

$$\mu_N = \mu_{\max} * DO / (DO + k_o) \quad (11)$$

where:  $\mu_N$  is  $\mu_{\max}$  after correction for dissolved oxygen concentration

DO is the oxygen concentration (mg/L)

$k_o$  is the half saturation constant for oxygen (mg/L)

In the case of an attached growth system, the effects of oxygen concentration must be included in the combined mass transfer-reaction kinetics equations. For example, Lopez-Bernal et al. (1977) used the following one dimensional equation to describe oxygen concentration in several streams of the Tennessee Valley:

$$dC/dt = D_L * (d^2C/dx^2) - U * (dC/dx) + S \quad (12)$$

where: C is the concentration of oxygen (mg/L)  
 $D_L$  is the longitudinal mixing coefficient  
( $m^2/sec$ )  
S is a source or sink of oxygen (mg/L·sec)  
x is the distance along the stream (m)  
t is time (sec)  
U is stream velocity (m/sec)  
d indicates differentiation

The S term in the equation must account for the oxygen uptake due to nitrification, assimilation by aquatic plants and aeration effects. Each of these will in turn have a separate set of equations including reaction kinetics, uptake coefficients or mass transfer coefficients.

It should be noted that the oxygen requirements listed above are for stoichiometric conditions. In some cases, it has been found that additional oxygen demands are also present in a nitrifier culture. For example, Gigger and Speece (1979) found that in a nitrification filter application, the actual oxygen consumption was 225% of stoichiometric oxygen consumption, thus representing a nitrogenous oxygen demand in excess of  $7.4 \text{ mg } O_2 / \text{mg } NH_4^+$ . Since the source of the excess oxygen demand in these experiments could not be identified, it is not known if similar elevated demands would be present in a clear, free flowing stream.

#### 2.2.3.6 Temperature

Nitrification reactions are temperature dependent as defined by the van't Hoff - Arrhenius relations up to a temperature of  $30^{\circ}$  Celsius. In other words, as temperature increases, nitrification rates are accelerated (Wong-Chong and Loehr 1975; Sharma and Ahlert 1977). Figures 5 and 6 from De Renzo (1978) show the effect of temperature on the maximum

Figure 5. Effect of temperature on the half saturation constants [Ks] of nitrifying bacteria [from DeRenzo 1978].

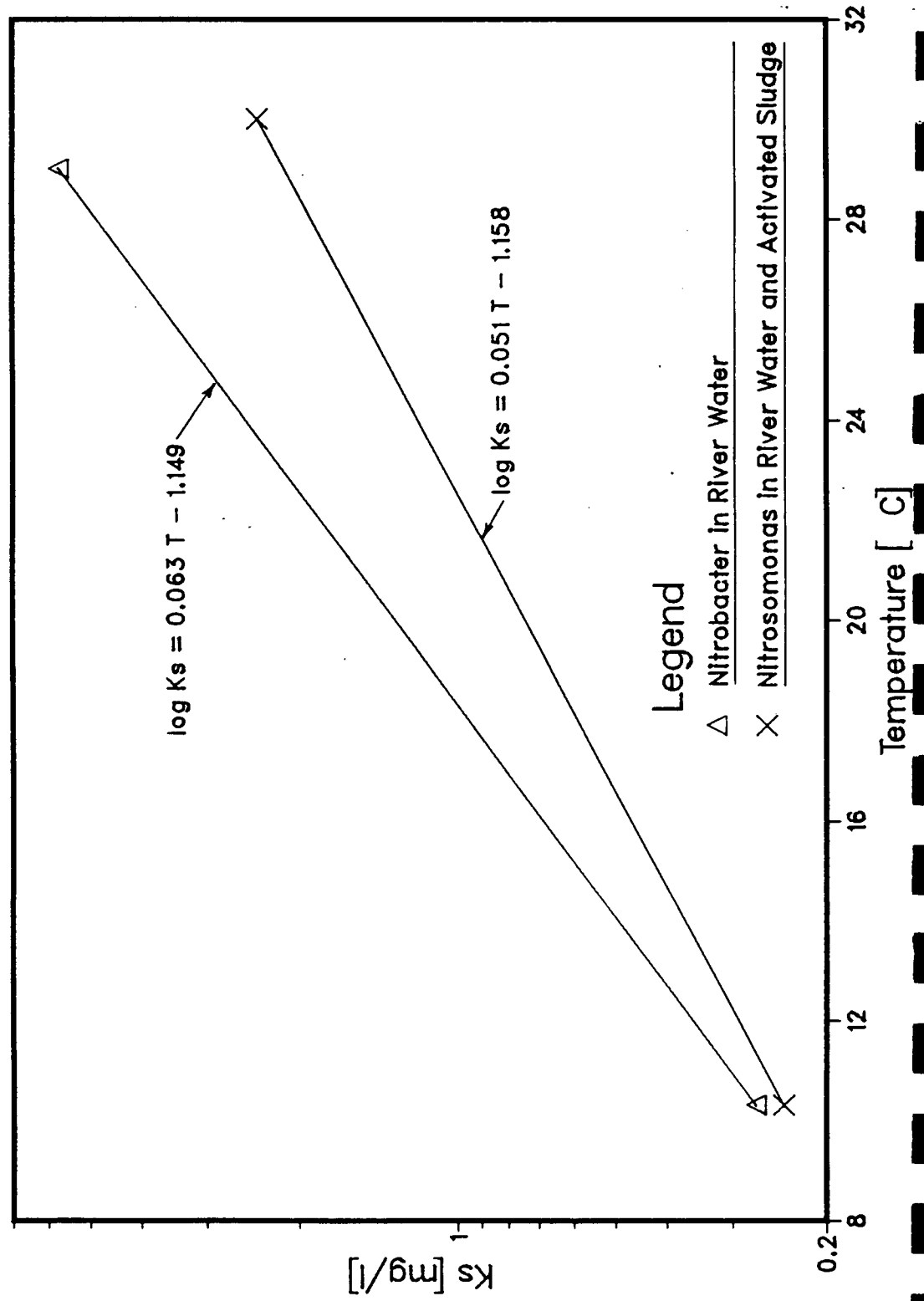
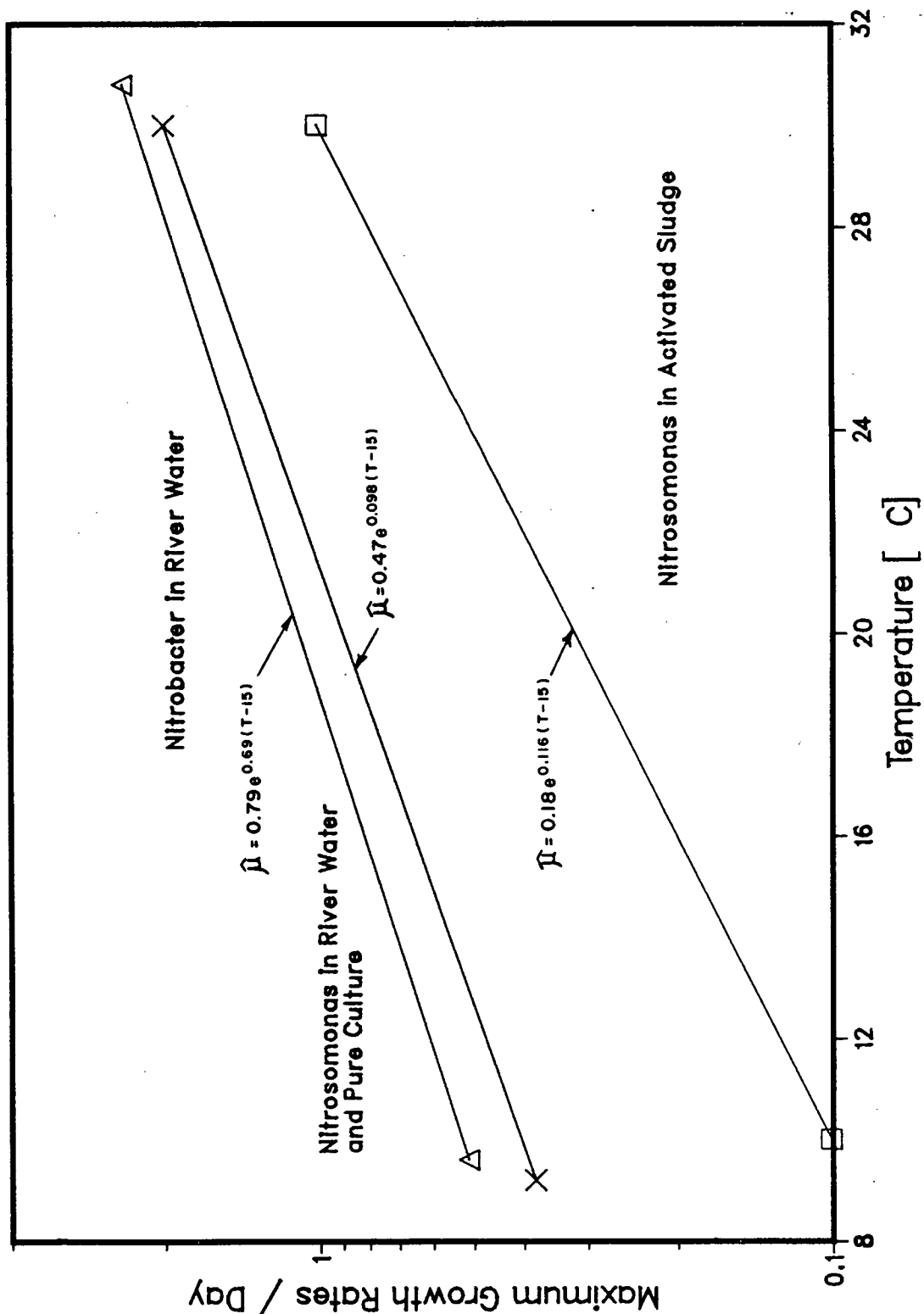


Figure 6. Effect of temperature on the maximum growth rates of nitrifying bacteria[from DeRenzo 1978].



growth rate and half saturation constants for nitrification in a suspended growth system. Figure 7 demonstrates the effect of temperature in an attached growth system. It is clear that significant variability exists in the data for attached growth systems. The source of this variability may be related to variations in other parameters that effect the nitrification rate, such as dissolved oxygen, pH or the presence of nitrifying inhibitors. It is also important to note that the ordinates in Figures 5 and 6 are logarithmic and that for low temperatures, both growth rates and half saturation constants decrease dramatically. In fact, Sharma and Ahlert (1977) found that nitrifier activity ceased completely at temperatures below 4° Celsius. This result will have special significance during the winter months in the Flathead River where ammonia and nitrite oxidation may cease and icing on a stream would not permit ammonia removal by aeration. In addition, low light levels and/or ice cover may limit ammonia assimilation by aquatic plants.

#### 2.2.3.7 Water pH

The concentration of hydrogen ions in solution also plays an important role in establishing the rate at which nitrification proceeds. Srinath et al. (1976) determined the nitrification rates for both Nitrosomonas and Nitrobacter as a function of water pH for wastewater. Figures 8 and 9 indicates that the optimum pH for nitrification lies between 7 and 8 and that the rate drops off significantly for pH values outside this range. Other investigators have found similar effects with variations in nitrification rates dependent on the environment in which the nitrifying bacteria are grown. Figure 10 from Sawyer et al. (1973) summarizes these results. Information from these sources indicates, collectively, that the optimum

Figure 7. Effect of temperature on the rate of nitrification in suspended and attached growth systems[from Huang and Hopson 1974].

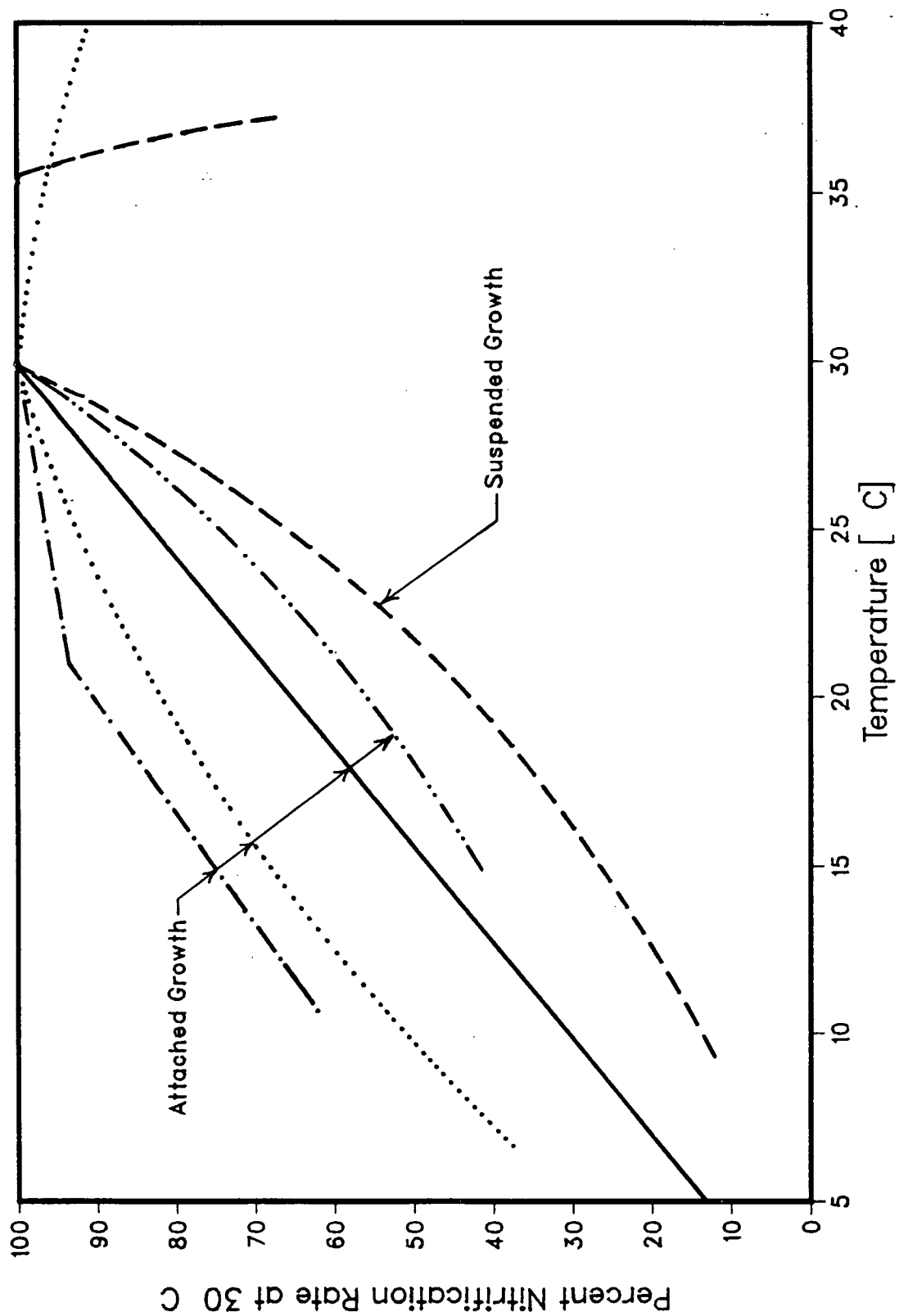


Figure 8. Effect of water pH on the nitrification rate of Nitrosomonas [from Srinath et al. 1976].

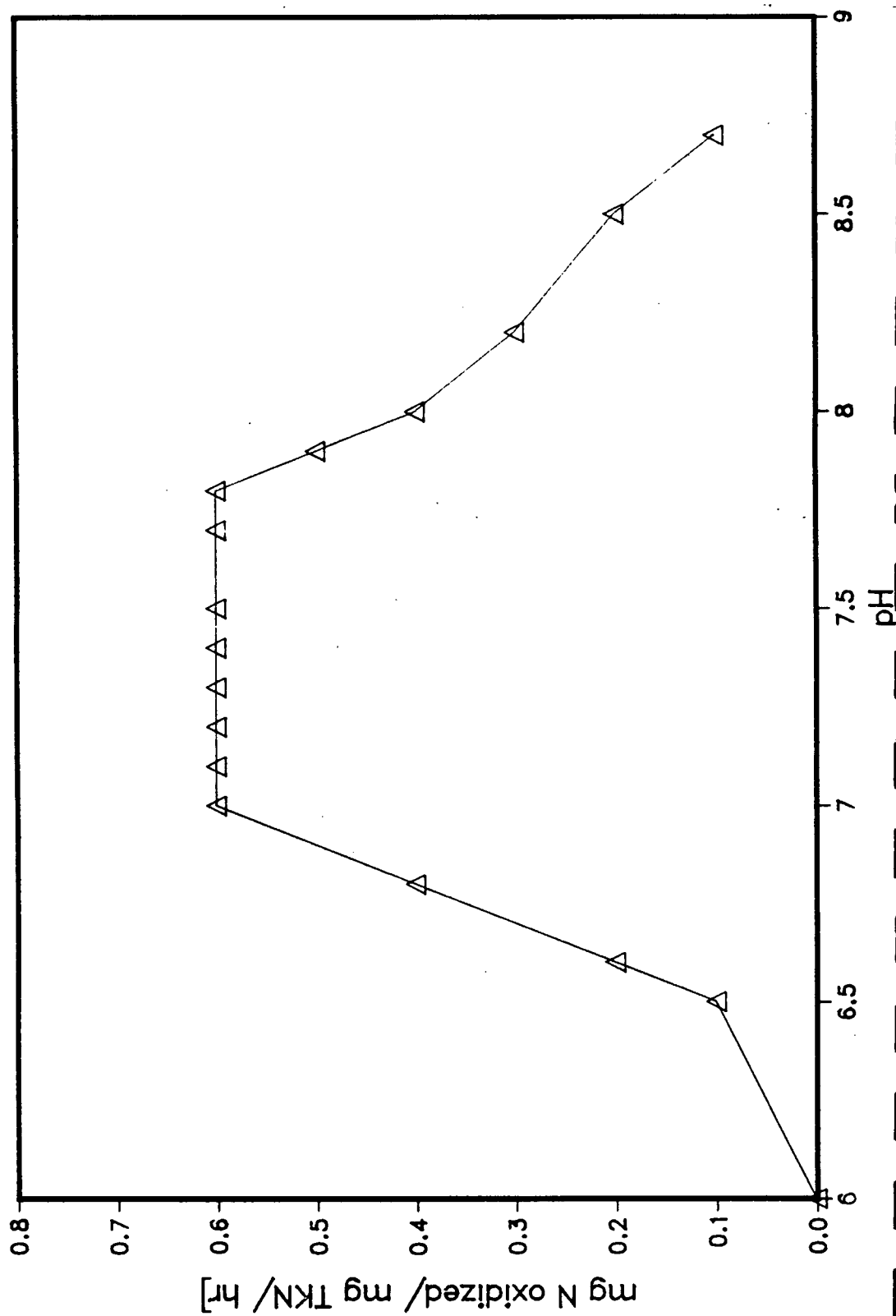




Figure 9. Effect of water pH on the nitrification rate of Nitrobacter [from Srinath et al. 1976].

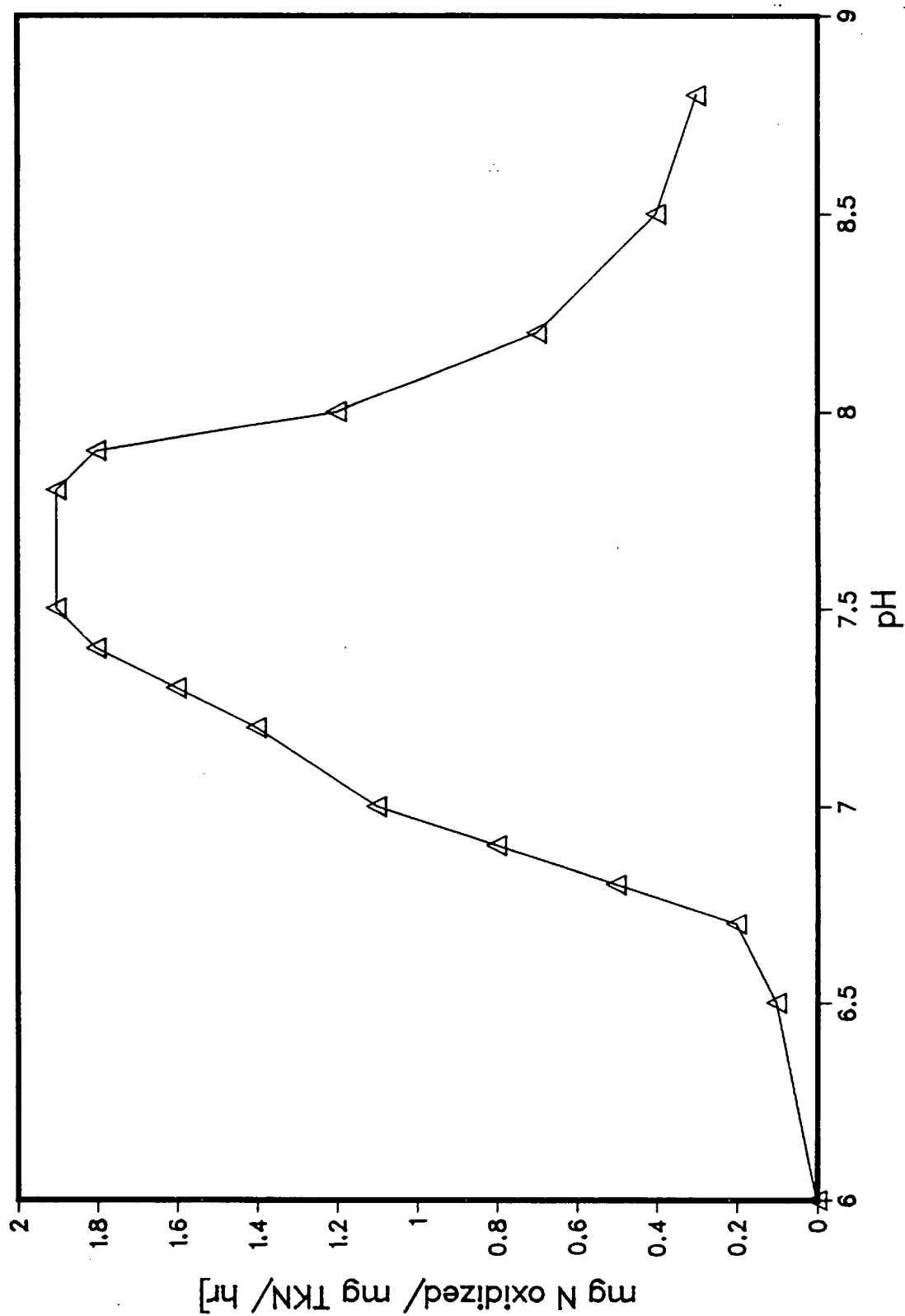
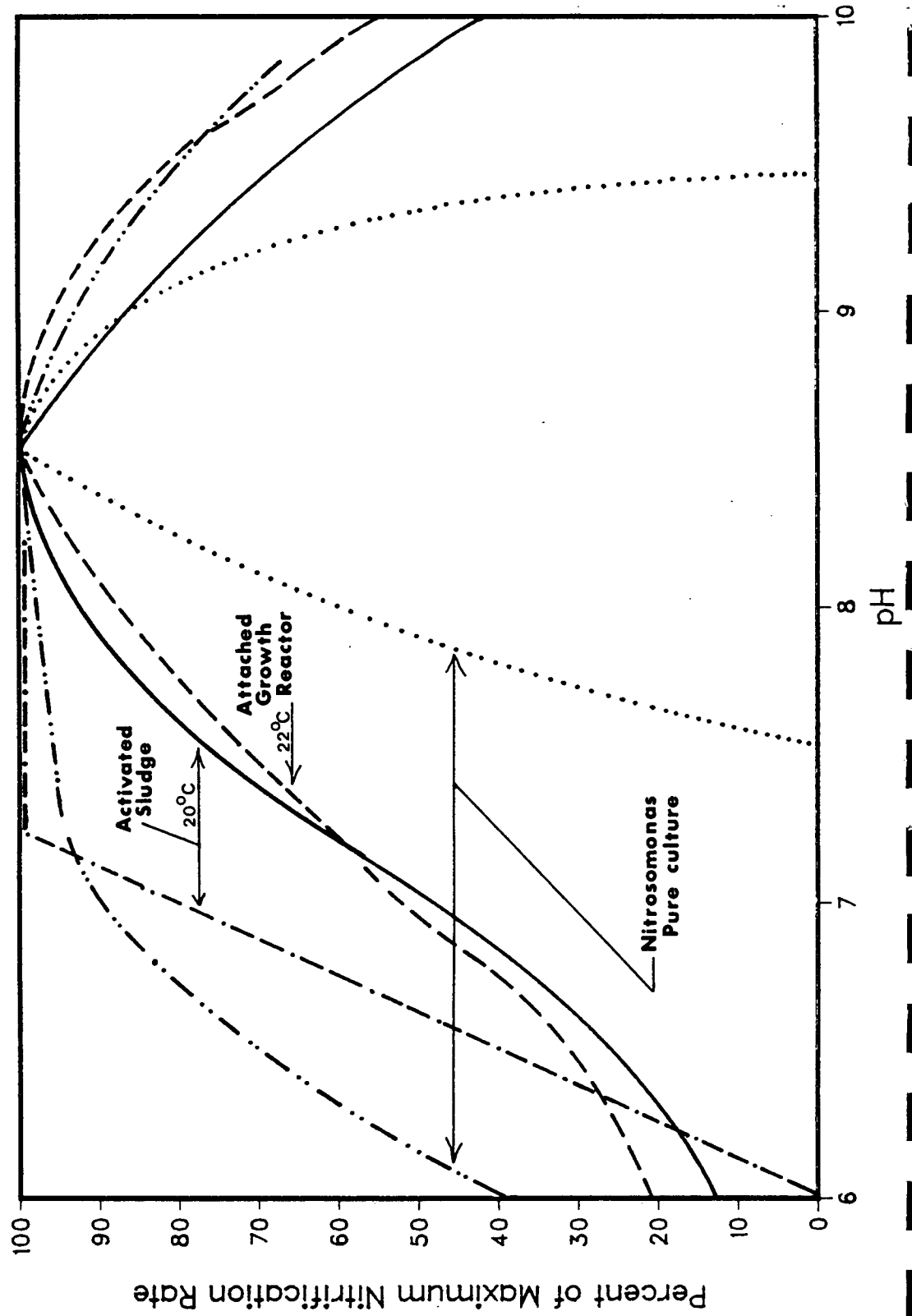


Figure 10. Effect of pH on the rate of nitrification for a variety of growth media [from Sawyer et al. 1973].



growth of nitrifying bacteria is realized between pH values of 7 and 9 and that growth rates are dramatically reduced at pH values outside that range.

Another important aspect of pH can be seen from re-examination of Equations 5 and 6 which describe the nitrification process. In the process of oxidizing one milligram of ammonium to nitrate, 7.14 mg of bicarbonate alkalinity are required. A small portion of this contributes to cell synthesis, while the rest is required to neutralize the hydrogen ions that are produced by the reactions (De Renzo 1978). Therefore, if the water source does not have sufficient alkalinity to buffer pH changes associated with the hydrogen ions produced by nitrification, the rate of nitrification will be reduced as water pH drops (Figure 10).

#### 2.2.3.8 Light Intensity

Sharma and Ahlert (1977) refer to several articles which demonstrate that nitrification reactions proceed at increased rates under conditions of darkness. One study (Hooper and Terry, 1973) reported complete inhibition of Nitrosomonas under a 200 watt bulb radiating at 420 lux. Thus, in a natural stream, it can be expected that nitrification will occur at increased rates at night and under cloudy skies. Low level winter light is also of benefit; however, low water temperatures, as mentioned above, may limit or arrest bacterial growth altogether at that time.

#### 2.2.3.9 Stimulatory and Inhibitory Substances

There appears to be considerable variability in the data reported from field studies on nitrification efficiency. This may, at least in part, be due to the presence of stimulatory or inhibitory substances in the water. Sharma and Ahlert (1977) have compiled a list of substances known

to stimulate the nitrification process (Table 1). Table 2 lists many of the substances known to be inhibitory to the growth of nitrifying bacteria. Also, as noted earlier, ammonia is toxic to Nitrobacter and affects the transient performance of the nitrification process. If the level of ammonia in a stream varies with time, it can be expected that ammonia, nitrite and nitrate concentrations will vary significantly both time wise and with location in the stream.

#### 2.2.4 Non-Steady State Effects

In an aquatic environment where inorganic nitrogen loadings, water temperature, streamflow, dissolved oxygen and pH are constant and have been so for a significant period of time, a steady state condition should exist. That is, the rate of assimilation of nitrogen by aquatic plants and the bacterial conversions of nitrogen should occur at a constant rate. In nature, however, the steady state condition is seldom, if ever, realized. For example, when ammonia is first introduced into a stream that was previously free of ammonia, there will be a transient period during which bacterial and aquatic plant populations will increase. In the case of nitrifying bacteria Nitrosomonas, as described earlier, will increase more rapidly than Nitrobacter. The period required for Nitrobacter to come into equilibrium with Nitrosomonas may take up to two months at 10° Celsius (D.P.M. Stechey. Canadian Aquaculture Systems. Windsor, Ontario, Personal communication, 1986). During this time, there will be higher levels of ammonia and nitrite downstream than would exist after an equilibrium is established. Similarly, for aquatic plants, there will be a period in which growth rates adjust to the available nutrients, and during that time higher levels of ammonia will be found downstream than will exist at equilibrium.

However, even after these adjustments have been made, equilibrium is seldom established because other factors will

TABLE 1  
LIST OF SUBSTANCES STIMULATORY TO NITRIFIER GROWTH (from Sharma and Ahlert, 1977)

Substance	Concentration*†	Effect‡	Reference
Phosphate	- 310 as P 5 as P	Reqd for Ns G & Nb G Reqd for Ns G & Nb G Reqd for Nb G	Lees (1955) Van Droogenbroeck & Laudelout (1967) Aleem (1959, cited in Painter, 1970)
Magnesium	- 5 10.5-50.5 (as $MgSO_4 \cdot 7H_2O$ ) 12.5-50 50-100	Reqd for Ns G & Nb G Reqd for Nb G No effect on Ns A Ns A + Slight Ns A -	Lees (1955) Aleem (1959, cited in Painter, 1970) Skinner & Walker (1961) Loveless & Painter (1968)
Molybdenum	- $10^{-9}M(0.0001)$ $10^{-2}M(1000)$	Nb A + 11-fold increase in Nb A and G Slight Nb A, G -	Aleem (1959, cited in Painter, 1970) Finstein & Delwiche (1965)
Iron	- 0.5-0.6 7	Reqd for Ns G & Nb G Ns G + Reqd for Nb G	Lees (1955) Skinner & Walker (1961) Aleem (1959, cited in Painter, 1970)
Calcium	- 0.5-10 10.5-50.5 (as $CaCl_2 \cdot 2H_2O$ )	Reqd for Nb G No effect by itself on Ns A; + in presence of 5 mg l <sup>-1</sup> EDTA No effect on Ns A	Lees (1955) Loveless & Painter (1968)
Copper	- 0-0.06 0.1	Reqd for Nb G Ns A + ; Added Ns A + along with 5 mg l <sup>-1</sup> EDTA Slight Ns A + ; With higher concentrations Ns A -	Skinner & Walker (1961) Lees (1955) Loveless & Painter (1968)
Sodium	0.1-0.5 0.6-1.5 1.5-7.0	Increasing Ns A Ns A + ; Ns G - Ns A - ; Ns G +	Skinner & Walker (1961) Loveless & Painter (1968)
Marine Salts	-	Reqd by some estuarine or littoral cultures of ammonia oxidizers	Finstein & Bitzky (1972)
Vitamins			
A-Palmitate	500,000 USP ml <sup>-1</sup>	Ns G + ; Nb G +	Pan (1971a)
Pantothenic Acid	0.05 mg ml <sup>-1</sup>	Nb G +	
	0.0025 µg ml <sup>-1</sup>	Ns A +	Gunderson (1955)
Nicotinic Acid	0.05 mg ml <sup>-1</sup>	Nb G +	Pan (1971a)
Ascorbic Acid	0.05 mg ml <sup>-1</sup>	Nb G +	
Biotin	0.-150 µg	2-4-fold Nb A + ; 100-1000-fold Nb G +	Krulwich & Funk (1965)
	2	Slight Ns A, G +	
Adenine Sulfate	0.05 mg ml <sup>-1</sup>	Nb G +	Clark & Schmidt (1967a) Pan (1971a)
Sodium Glutamate	1720 mg ml <sup>-1</sup>	Ns G + ; Nb G +	
Yeast Extract	2 mg ml <sup>-1</sup>	Nb G +	
L-Serine	4 µg ml <sup>-1</sup>	Ns A + ; Ns G +	Clark & Schmidt (1967a, b)
	1050 mg ml <sup>-1</sup>	Nb G +	Pan (1971a)
L-Glutamine	4 µg ml <sup>-1</sup>	Ns G + ; Ns A +	Clark & Schmidt (1967a)
	1450 mg ml <sup>-1</sup>	Nb G +	Pan (1971a)
L-Glutamic Acid	4 µg ml <sup>-1</sup>	Ns G + ; Ns A +	Clark & Schmidt (1967a)
L-Aspartic Acid	4 µg ml <sup>-1</sup>	Ns G + ; Ns A +	
Ash of corn steep liquor		Ns G +	Gunderson (1958)
Glucose, p-amino-benzoic acid	2-5	Ns A + ; Nb A + ; impure, mixed culture	Cooper & Catchpole (1973, cited in Painter, 1977, and in Stafford, 1974)

\*All results are for pure cultures unless indicated otherwise. †In mg l<sup>-1</sup> unless specified otherwise. ‡Ns = Nitrosomonas; Nb = Nitrobacter; G = Growth; A = Activity; + = Stimulation; - = Inhibition. e.g. Ns A + = stimulation of Nitrosomonas activity ≡ stimulation of nitrification.

TABLE 2  
LIST OF SUBSTANCES INHIBITORY TO NITRIFIER GROWTH (from Sharma and Ahlert, 1977)

Substance	Concentration†‡	Degree of Inhibition/Effect**	Reference
AM†† (2-amino-4-chloro-6 methyl-pyrimidine)	10 µg g <sup>-1</sup> soil	34% Ns & Nb A - after 5 days; none after 14 days††	Bremner & Bundy (1974)
ST§§ (sulfathiazole)	10 µg g <sup>-1</sup> soil	33% Ns & Nb A - after 5 days; none after 14 days††	Bremner & Bundy (1974)
Volatile sulfur Compounds CS <sub>2</sub>	10 µg g <sup>-1</sup> soil	97% Ns & Nb A - after 5 days; 95% after 14 days††	Bremner & Bundy (1974)
CH <sub>3</sub> SSCH <sub>3</sub> , CH <sub>3</sub> SH, CH <sub>3</sub> SCH <sub>3</sub> , H <sub>2</sub> S	Up to 50 µg g <sup>-1</sup> soil	Initial Ns & Nb A - ; almost negligible after 7-14 days	Bremner & Bundy (1974)
Disinfection/ chlorination	0.6-2 mg l <sup>-1</sup> Chlorine residual; contact time up to 60 min	Nitrifiers considerably more resistant than fecal streptococci; nitrifiers survive better than nitritifiers***	Strom et. al. (1976)
Pyridine or 4-methyl pyridine	100	50% Ns A - & 90% Ns A -, respectively. Almost complete Nb A - by both. Measured as O <sub>2</sub> uptake at 80 min. Act. Sludge.	Stafford (1974)
2-methyl pyridine	100	Very slight Nb A - ; 40% Ns A - ; at 80 min; activated sludge system	Stafford (1974)
3-methyl pyridine	100	Very slight Ns A and Nb A - ; activated sludge system	Stafford (1974)
Diethyldithio-carbamate	10 <sup>-5</sup> M	Complete inhibition of Ns A as measured by rate of nitrite formation after 20 min, relative to a control	Hooper & Terry (1973)
Ethyl xanthate	10 <sup>-4</sup> M		
3-Aminotriazole	10 <sup>-3</sup> M		
Methylene Blue	10 <sup>-4</sup> M		
Methanol	5 x 10 <sup>-3</sup> M	Complete inhibition of Ns A as measured by rate of nitrite formation after 20 min, relative to a control	Hooper & Terry (1973)
Ethanol	0.09 M		
n-Propanol	0.33 M		
n-Butanol	0.11 M		
Ethyl acetate	0.12 M		
Tannin and tannin derivatives phenolic acids and flavonoids	10 <sup>-6</sup> - 10 <sup>-8</sup> M	Very strong inhibitors	Rice & Pancholy (1974)

\*Also marketed by Hach Chemical Company, Ames, Iowa, as Formula-2533 nitrification inhibitor; and by Dow Chemical Co., Midland, Michigan. †All results are for pure cultures unless indicated otherwise. ‡In mg l<sup>-1</sup> unless indicated otherwise. §Degree of inhibition not reported. \*\*Ns = Nitrosomonas; Nb = Nitrobacter; A = Activity = nitrite-/nitratification; G = Growth; + = Stimulation; - = Inhibition ††Inhibition study by incubating soil samples in screw-cap bottles and measuring the amount of (nitrite + nitrate)-N formed. ‡‡Marketed by Toyo Koatsu Industries, Inc. Tokyo, Japan. §§Marketed by Sigma Chemical Co., St. Louis, Missouri. \*\*\*Lab and field study employing the MPN technique.

TABLE 2 (Continued)

Substance	Concentration†‡	Degree of Inhibition/Effect**	Reference
Halogen-substituted phenolic compounds	0	—§	Metcalf & Eddy (1973)
Halogenated solvents	0	—§	Metcalf & Eddy (1973)
Thiourea	0	—§	Metcalf & Eddy (1973)
	0.0003 M	Complete inhibition of nitrification until 16 days; mixed culture, soil perfusion expt	Quastel & Scholefield (1949)
	0.01 M	75% in O <sub>2</sub> uptake; mixed culture of soil nitrifiers	Quastel & Scholefield (1949)
Ethylurethane	0.1%	Normal nitrification after inhibition for 18 days; mixed culture, soil perfusion expt	Quastel & Scholefield (1949)
Heavy metals	10-20	—§	Metcalf & Eddy (1973)
Copper	0.05-0.56	Ns A -	Loveless & Painter (1968)
	4	75% Ns A - ; negligible for nitrifying activated sludge	Tomlinson et. al. (1966)
	150	75% nitrifying activated sludge	Tomlinson et. al. (1966)
Chromium, trivalent	>0.25	Ns G -	Skinner & Walker (1961)
	118	75% nitrifying activated sludge	Tomlinson et. al. (1966)
Nickel	>0.25	Ns G -	Skinner & Walker (1961)
Cyanides & all compounds from which hydrocyanic acid is liberated on acidification	20 mg l <sup>-1</sup>	—§	Metcalf & Eddy (1973)
Phenol and cresol	20 mg l <sup>-1</sup>	—§	Metcalf & Eddy (1973)
Phenol	100	Acclimatization by activated sludge if approached gradually	Stafford (1974)
Phenol, o-, m-, p-cresols	100	No effect on Nb A in activated sludge	Stafford (1974)
Vitamins			
Riboflavin; α-lipoic acid; B <sub>6</sub> pyridoxine	0.05 mg ml <sup>-1</sup>	Nb G -	Pan (1971a)
HCl; B <sub>12</sub>			
Panthothenic acid	0.05 mg ml <sup>-1</sup>	Nb G -	Pan (1971a)
Thiamine	5 µg ml <sup>-1</sup>	Ns A -	Gunderson (1955)
Amino Acids			
L-Lysine	4 µg ml <sup>-1</sup>	Ns A -, Ns G -	Clark & Schmidt (1967a)
	1460 mg ml <sup>-1</sup>	Ns G -	Pan (1971a)
L-Threonine	4 µg ml <sup>-1</sup>	Ns A -, Ns G -	Clark & Schmidt (1967a)
	1200 mg ml <sup>-1</sup>	Ns G -	Pan (1971a)
L-Histidine	100 µg ml <sup>-1</sup>	Ns A -	Gunderson (1955)
	4 µg ml <sup>-1</sup>	Ns A -, Ns G -	Clark & Schmidt (1967a)
L-Valine; L-Arginine	4 µg ml <sup>-1</sup>	Ns A -, Ns G -	Clark & Schmidt (1967a)
L-Methionine	4 µg ml <sup>-1</sup>	Ns A -, Ns G -	Clark & Schmidt (1967a)
	0.0005-0.01 M	Normal nitrification after inhibition for 16 days; mixed culture; soil perfusion expt	Quastel & Scholefield (1949)
Alanine; aspartate; glycine; glutamate; cysteine; methionine	0.01 M	Nitrification delayed but proceeds normally after adaptation	Quastel & Scholefield (1949)
Purines and pyrimidines			
cytosine, guanine	0.05 mg ml <sup>-1</sup>	Nb G -	Pan (1971a)
Pyruvate	0.4 mg ml <sup>-1</sup> as lithium pyruvate	Nb G -	Pan (1971a)
	5 x 10 <sup>-5</sup> M or greater	Nb G -	Pan (1971a)
Potassium chlorate	10 <sup>-5</sup> -10 <sup>-3</sup> M	Nb G - ; Nb A -	Lees & Quastel (1945); Voets et. al. (1975)
N-Serve*	10	Complete inhibition of nitrification	Voets et. al. (1975)
(2-chloro-6(tri-chloromethyl)-pyridine)	10 µg g <sup>-1</sup> soil	64% Ns & Nb A - after 5 days; 73% after 14 days††	(inhibition data not presented) Bremner & Bundy (1974)

constantly change the conditions for growth. As mentioned earlier, light plays an important role in the nitrification process as well as in the process of assimilation of nitrogen by aquatic plants. It can be expected that variations in available light will cause non-steady state nitrogen conditions on both a diurnal and a seasonal basis.

In northern latitudes, seasonal temperature changes lead to variations in bacterial populations, thereby resulting in potentially high rates of nitrification during the warmer months. However, as described in the temperature effects section above, winter may stop nitrification completely. Aquatic plants, depending on species, will respond to warmer water temperatures and increased light by increasing their biomass and, in so doing, by increasing their capacity to assimilate nitrogen compounds. During these seasonal changes there will be corresponding variations in ammonia, nitrite and nitrate levels throughout the system.

In addition to diurnal and seasonal changes affecting nitrogen concentrations in a river, there may also be variations in the rate of inorganic nitrogen loading. As nitrogen loading is increased, there will be a corresponding increase in aquatic plant and bacterial populations, with a lag phase as described above. As nitrogen loading decreases, the plant and bacterial populations will diminish until elevated loadings occur again.

From this discussion, it should be clear that the chemical state of inorganic nitrogen in a stream or river system will be in a non-steady state condition most of the time. Consequently, the modelling of inorganic nitrogen in a stream or river environment is complicated by the requirement to account for temporal effects of varying nitrogen loading as well as diurnal and seasonal variations in the chemical, physical, and biological processes that effect nitrogen dynamics.



### 3. SITE-SPECIFIC WATER QUALITY CRITERIA DEVELOPMENT

#### 3.1 Ambient Water Quality

##### 3.1.1 Background

Over the past ten years, the Water Quality Branch (IW/L) has characterized existing water quality conditions in the Flathead River basin. Water quality survey activities were initiated in December, 1975, to examine the water quality in the Canadian portion of the basin. This intensive water quality sampling program was completed in September, 1976, with follow-up surveys conducted in August, 1980 and July, 1982. Nutrients, metals and other chemical variables were measured at a total of nine sites in the watershed. Routine water quality monitoring activities commenced in July, 1979 and April, 1980, at the Flathead River at the International Border and on Sage Creek respectively. Sampling activities have been conducted at a variable frequency (6-10 times/year) with nutrients, metals and other variables measured in water only.

Recently, a second intensive survey was initiated (November, 1983) to assess the seasonal and yearly variability of high priority water quality variables. Nutrients, non-filterable residues and barium have been measured periodically throughout low flow periods and intensively during freshet. Measurements were routinely performed at the International Boundary, Howell Creek, and a site on the Flathead River upstream of the Howell Creek confluence with the mainstem. The results of this survey will be detailed in subsequent reports, and therefore only a brief overview of the water quality information required for nitrogen species criteria development will be included in this report.

### 3.1.2 Summary

A number of reports are currently available or in preparation [Sheehan et al. 1980; Sheehan et al. 1985; Water Quality Branch 1986; Thorp 1985; Valliela and MacDonald 1987a (in prep.); Valliela and MacDonald 1987b (in prep.)] that, collectively, provide a good indication of the existing water quality conditions in the Flathead River basin. Therefore, only a overview of these data will be provided in this report.

#### 3.1.2.1 Flathead River at the International Border

A statistical summary of existing data on water quality available as of 1982 for the Flathead River at the International Boundary is provided in Appendix 1.

The quality of surface waters is a function of the chemical properties of the precipitation that falls in the watershed, the physical, chemical and biological properties of the watershed itself, and the sum total of the anthropogenic inputs to the aquatic system within the watershed boundaries. Generally, precipitation has very low levels of nutrients, metals, major ions and non-filterable residues. Inputs of these variables are dependent on dissolution or suspension during the progression from moisture deposition to streamflow or on human activities.

In the Flathead River, changes in the concentrations of many of the water quality variables measured are closely linked to changes in streamflow.

Alkalinity, hardness, conductivity and pH exhibit distinct seasonal trends at the International Boundary station of the Flathead River. Measurements of all four variables remain relatively constant during the period from September to mid-March. Recorded values are highest for all of these

variables during this low flow period. However, with the onset of freshet, rapid snowmelt and precipitation events contribute a large volume of water to the Flathead River mainstem. At this time of the year, forest soils are saturated with water so that much of the runoff enters the river without percolating through the surface soil layers. Therefore, the runoff tends to be low in ionic content and in buffering potential. This dilution effect commences about mid-March, intensifies and reaches its peak near the end of May (corresponding to the peak in streamflow), and subsides during the period June 1 - August 31.

Suspended sediment, or non-filterable residue (NFR) levels in the Flathead River vary seasonally, and elevated levels generally correspond to spring freshet conditions. NFR levels remain relatively constant from July 15 - March 15, with concentrations generally falling in the 0 - 5 mg/L range. Extreme levels occur from mid March to mid July, with peak suspended sediment concentrations reaching 200 - 800 mg/L. Those variables, such as total metals, total phosphorus, etc., that are normally associated with suspended sediment tend to display seasonal distribution patterns that are very similar to that of NFR.

#### 3.1.2.2 Howell Creek below Cabin Creek

A statistical summary of existing data on water quality in Howell Creek below the confluence with Cabin Creek (to 1982) is provided in Appendix 2.

The seasonal variability patterns of the water quality variables measured in Howell Creek appear to be very similar to those measured in the Flathead River mainstem. The major differences between the two systems stem from the higher degree of groundwater input and lower discharge in Howell

Creek. These characteristics result in water that is somewhat harder, with better buffering potential and higher ionic content in Howell Creek as compared to the mainstem. In addition, values for NFR and related variables tend to be lower in Howell Creek.

### 3.2 Fisheries Interactions with Environmental Contamination

Environmental contaminants can be harmful to a fishery in at least five ways (Alabaster and Lloyd 1982):

- i) By acting directly on a fish swimming in the water, either by killing them or reducing their growth rate and resistance to disease;
- ii) By preventing the successful development of fish eggs and larvae;
- iii) By modifying natural movements and migrations of fish;
- iv) By reducing the abundance of food available to the fish;
- v) By affecting the efficiency of methods for catching fish.

It is therefore, important to detail life history patterns and identify the most sensitive developmental stages of recreationally important species in order to develop water quality criteria that will represent no-effect conditions for those species. The following information was extracted from MacDonald (1985), with contributions by A. Martin (personal communication, Fisheries Branch, Cranbrook, B.C., 1986).

#### 3.2.1 Bull Trout

Sexually mature adult bull trout leave Flathead Lake to begin their upstream migration in early spring, generally in April and May. Upstream migration takes place over a number of months, with the bull trout usually arriving at the mouths of their natal tributary streams from mid July to late August. The size and availability of these fish throughout the course of their upstream migration make them the target of a rather

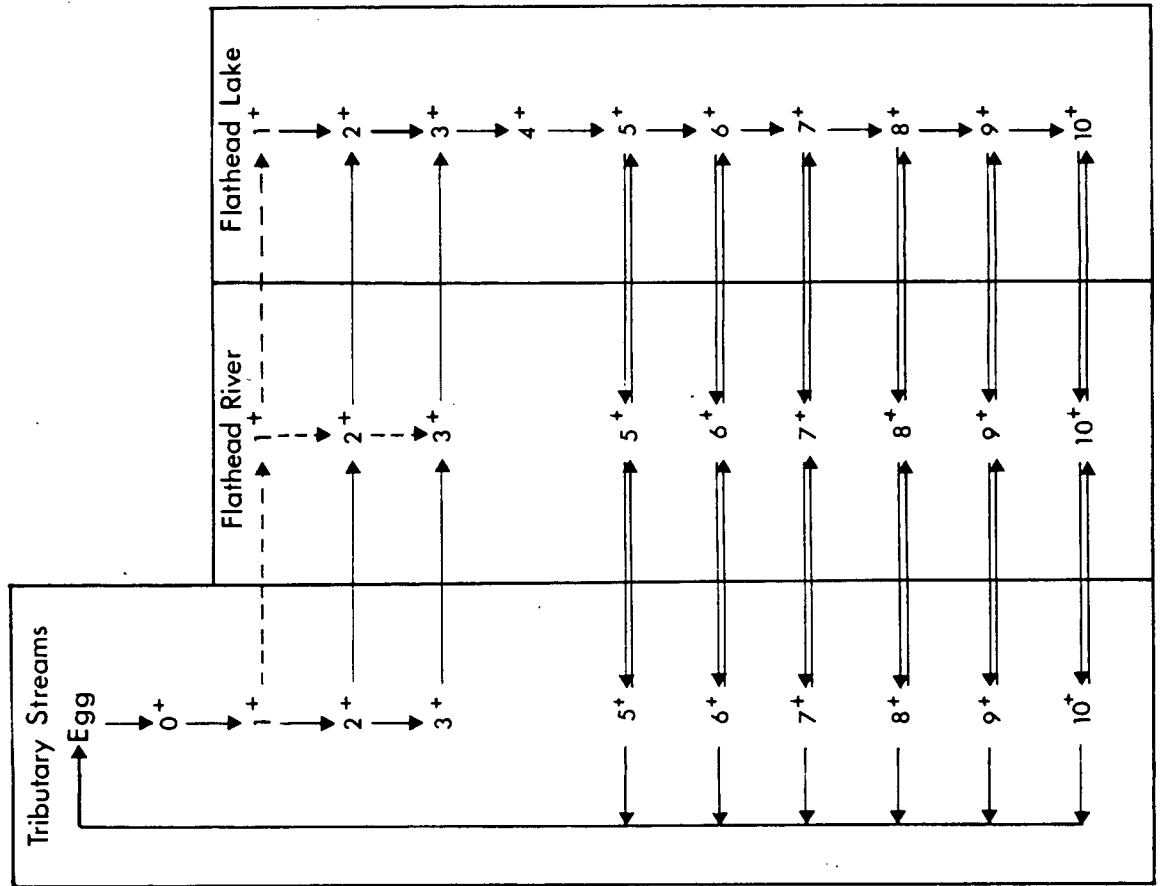
intense sport fishery in the Flathead River. The bull trout enter the tributary streams from early August to late September, and initiate spawning activities when water temperatures drop to approximately 8.0°C. The eggs are deposited in large redds that are normally constructed in areas with favourable gravel substrate characteristics and, frequently, upwelling groundwater flow.

Incubation proceeds through the autumn and winter months, with the peak of hatch generally occurring by mid January. Bull trout larvae remain in the gravel until yolk sac absorption is nearly complete. Peak of emergence of fry takes place by May 31 in most tributaries to the North Fork of the Flathead River, with the exact emergence timing dependent on spawning timing and water temperatures over the period of incubation. After rearing for 1-3 years in tributary streams, bull trout smolts migrate (June - August) to Flathead Lake. In the lake, an abundance of food items results in very rapid growth, with fish achieving trophy size by the age of 7+ or 8+. Substantial sport fishing effort is directed at these fish while in the lake, with the older year classes selected through gear choice and the minimum size limit (46.0 cm) on bull trout. Bull trout life history patterns in the Flathead system are diagrammed in Figure 11.

### 3.2.2 Cutthroat Trout

Cutthroat trout in the Flathead River watershed have developed a number of life history patterns that effectively utilize the habitat available in the system. A total of three forms, resident, fluvial and adfluvial, of westslope cutthroat trout exist in the Flathead system. The resident and migratory forms are indistinguishable as juveniles, and downstream emigration of smolts is the only means of differentiating the two forms. Adult (mature) cutthroat trout can be distinguished by size, with the largest being adfluvial, the intermediate sized fish

Figure 11. Bull trout life history patterns in the Flathead system: Conceptual model.



being fluvial, and the smallest being resident fish. A lack of temporal or spatial isolation during spawning indicates that there is probably a significant amount of transfer of genetic information between the three forms. Therefore, environmental rather than genetic factors may be responsible for the migratory behaviour of this species.

Cutthroat trout spawn in May and June in small to intermediate sized tributaries in the Flathead drainage. Development proceeds rapidly due to the increasing water temperatures over the incubation period. The peak of hatching usually takes place by mid July, with the alevins remaining in the stream-bed substrate until the yolk sac is nearly completely absorbed. Emergence takes place from late July to mid August with the exact timing mediated by water temperatures over the period of incubation. Juvenile cutthroat trout utilize pools and runs in the upper reaches of tributary streams for rearing activities. In general, fry densities decrease as stream order increases, indicating that the mainstem is not used extensively by juveniles for rearing activities. After 1 to 4 years (mostly 2 or 3) of rearing in tributary streams, juvenile adfluvial cutthroat trout migrate (June - July) to Flathead Lake. Juvenile fluvial cutthroat trout migrate (June - July) to the mainstem, while resident fish remain in tributary streams. It has also been postulated that some outmigration of cutthroat juveniles may occur in some tributaries in the fall. It is presumed that these movements are made to overwintering habitats, and may be related to the winter carrying capacity of the tributaries.

Upstream migration of sexually mature adfluvial cutthroat trout is initiated in mid January and continues through the month of April. These fish move into the tributaries during onset of freshet, with water temperatures and streamflows important in

the initiation of spawning movements and redd construction. Spawning often occurs during or just after the peak of freshet. Downstream movement of kelts begins shortly after spawning activities have been completed, and most of the spawners have left the tributaries by mid July.

### 3.2.3 Mountain Whitefish

Mountain whitefish is probably the most abundant fish species in fluvial habitats in the Flathead system. Whitefish utilize habitats within the Flathead River mainstem and the lower portions of tributary streams throughout their life history, undergoing seasonal movements associated with feeding, spawning, and overwintering.

Whitefish overwinter in deep pools of the mainstem, predominantly below Howell Creek in the Canadian portion of the basin. In the spring, these large overwintering populations gradually disperse upstream to take advantage of food resources available in the mainstem and the lower portions of tributary streams. Spring migrations are followed by gradual downstream migrations in the late summer and fall, when the fish begin to concentrate in pools. Sexually mature adults rapidly migrate upstream to spawning areas in late October. Spawning activities occur from mid October to mid to late November, with the eggs broadcast in riffle habitats over gravel and cobbles. Downstream migration takes place soon after spawning activities are completed.

Incubation of whitefish eggs and alevins proceeds over the winter months, with the emergence timing dependent on both spawning timing and water temperatures over the incubation period. The rearing of fry occurs primarily in the shallow riffle areas and backwaters of the Flathead River. A summary of life history information for Flathead River salmonid species is contained in Table 3.



TABLE 3

SUMMARY OF LIFE HISTORY INFORMATION FOR FLATHEAD RIVER  
WATERSHED SALMONID FISH SPECIES

Species	Period	Activity	Location
Bull Trout	Sept 1 - Jan 30	Incubation	Howell Creek
	Jan 1 - Jan 30	Hatching	Howell Creek
	Jan 1 - May 31	Alevin Development	Howell Creek
	May 15 - July 15	Early Rearing (to 1 g)	Howell Creek, Flathead River
	July 1 - June 30	Juvenile Rearing	Howell Creek, Flathead River
	June 1 - Aug 31	Smolt Outmigration	to Flathead Lake
	Apr 1 - July 30	Adult U/S Migration	to Tributary Mouth
	July 15 - Sept 15	Adult Entry into Tributaries	Howell Creek
	Sept 1 - Oct 15	Spawning	Howell Creek
	Sept 15 - Oct 30	Kelt Outmigration	to Flathead Lake
Cutthroat Trout (Adfluvial)	May 15 - July 20	Incubation	Howell Creek
	July 5 - July 20	Hatching	Howell Creek
	July 5 - Aug 15	Alevin Development	Howell Creek
	July 20 - Sept 20	Early Rearing (to 1 g)	Howell Creek
	Aug 15 - Aug 14	Juvenile Rearing	Howell Creek, Flathead River
	Aug 1 - Sept 30	Smolt Outmigration	to Flathead Lake
	Jan 15 - Apr 31	Adult U/S Migration	to Tributary Mouth
	Apr 15 - May 31	Adult Entry into Tributaries	Howell Creek
	May 15 - June 30	Spawning	Howell Creek
	June 1 - July 15	Kelt Outmigration	to Flathead Lake
Cutthroat Trout (Fluvial)	May 15 - July 20	Incubation	Howell Creek
	July 5 - July 20	Hatching	Howell Creek
	July 5 - Aug 15	Alevin Development	Howell Creek
	July 20 - Sept 20	Early Rearing (to 1 g)	Howell Creek
	Aug 15 - Aug 14	Juvenile Rearing	Howell Creek, Flathead River
	Aug 1 - Sept 30	Smolt Outmigration	to Flathead River
	Jan 15 - Apr 31	Adult U/S Migration	to Tributary Mouth
	Apr 15 - May 31	Adult Entry into Tributaries	Howell Creek
	May 15 - June 30	Spawning	Howell Creek
	June 1 - July 15	Kelt Outmigration	to Flathead River
Cutthroat Trout (Resident)	May 15 - July 20	Incubation	Howell Creek
	July 5 - July 20	Hatching	Howell Creek
	July 5 - Aug 15	Alevin Development	Howell Creek
	July 20 - Sept 20	Early Rearing (to 1 g)	Howell Creek
	Aug 15 - Aug 14	Juvenile Rearing	Howell Creek
	May 15 - June 30	Spawning	Howell Creek

TABLE 3 (Continued)

SUMMARY OF LIFE HISTORY INFORMATION FOR FLATHEAD RIVER  
WATERSHED SALMONID FISH SPECIES

Species	Period	Activity	Location
Mountain Whitefish	Oct 15 - Mar 15	Incubation	Howell Creek
	Mar 1 - Mar 30	Hatching	Howell Creek
	Mar 1 - May 30	Alevin Development	Howell Creek
	May 15 - June 15	Fry Outmigration	to Flathead River
	May 15 - June 20	Early Rearing (to 1 g)	Flathead River
	July 1 - June 30	Juvenile Rearing	Flathead River
	Oct 1 - Nov 1	Adult Migration into Tributaries	Howell Creek
	Oct 15 - Nov 15	Spawning	Howell Creek
	Nov 1 - Nov 30	Kelt Outmigration	to Flathead River

### 3.3 Nitrogen Compounds

Nitrogen can be present in the aquatic environment in a number of forms, each representing particular problems to freshwater fish species when present at high levels. While it is recognized that the potential exists for elevation of the levels of a wide variety of nitrogen compounds, only the effects of ammonia, nitrite and nitrate on freshwater fish will be considered in this document.

#### 3.3.1 Nitrate

Elevated levels of nitrate in surface waters appear to pose little or no threat to freshwater fish directly. Limited information on the effects of nitrate on fish suggest that acute toxicity is a problem only at very high concentrations. The 96 hour  $LC_{50}$  value for bluegills at 20°C was 12,000 and 3,000 mg/L for sodium nitrate and potassium nitrate respectively (Trama 1954). Subsequent studies on salmonid sensitivity to nitrate (Westin 1974) indicate that chinook salmon (1-10g) and rainbow trout (1-5g) are very resistant to nitrate poisoning with 96 hr  $LC_{50}$  (@13-17°C) values for sodium nitrate in freshwater being 5,800 and 6,000 mg/L respectively for the two species. In contrast, the eggs of coho salmon and rainbow trout are more sensitive to nitrate than other life history stages, with threshold toxic values in the 40-80 mg/L range (R. Nordin, B.C. Ministry of Environment and Parks. Victoria, B.C. Personal communication. 1987).

It is further anticipated that nitrate levels would be of concern only when they result in problematic levels of algal growth; therefore, no criteria will be proposed for nitrate in this document.

### 3.3.2 Nitrite

In water, nitrites are generally formed by oxidation of ammonia or by reduction of nitrates. These species ( $\text{NO}_2^-$ ,  $\text{HNO}_2$ , etc.) tend to be quickly oxidized to nitrates in surface waters, and are therefore rarely present in significant concentrations. However, discharges of large quantities of ammonia into receiving waters necessitates that the nitrification process proceed downstream from the discharge point. Discharges which result in levels of nitrite above ambient levels in the receiving water present a problem to the fish and aquatic life present (Russo and Thurston, 1975).

#### 3.3.2.1 Fish

##### 3.3.2.1.1 Mode of Toxic Action

Nitrite has been shown to be highly toxic to salmonids (Smith and Williams 1974; Cameron 1971; Thurston et al. 1978), however the exact mechanisms of toxicity are not well understood. It is well established that freshwater fish accumulate nitrite in the blood and in body tissues. Margiocco et al. (1983) indicated that rainbow trout accumulated nitrite in the blood to levels that were up to 60 times higher than environmental values after only a few hours of treatment. Cerebral and hepatic levels were up to 30 times higher than those in the treatment water. These data appear to indicate that nitrite is actively transported into fish blood.

High blood nitrite levels lead to a condition known as methaemoglobinemia (Brown and McLeay 1975; Smith and Russo 1975), which is due to the oxidation of haemoglobin (Hb) to methaemoglobin (Met-Hb) in erythrocytes. Methaemoglobin lacks the capacity to bind oxygen reversibly (Bodansky 1951), and therefore, an elevation of the level of

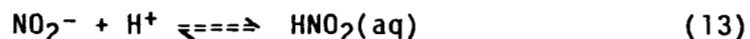
methaemoglobin in the blood can cause such pathological symptoms as cyanosis and tissue hypoxia (Kiese 1974). For this reason, some researchers (Wedemeyer and Yasutake 1978) consider the death of intoxicated animals to be a direct consequence of hypoxia generated by an impaired capacity of the blood to carry oxygen. Arillo et al. (1984) suggested that liver hypoxia is the root of the acute toxicity mechanism of nitrite in rainbow trout. Cerebral hypoxia, hypoglycemia, inhibition of several of the enzymes of Krebs Cycle and lysosomal damage have also been cited as acute problems associated with nitrite toxicity (Mensi et al. 1982; Arillo et al. 1984). It is likely that many or all of the above factors act together to cause mortality of nitrite affected fish.

#### 3.3.2.1.2 Abiotic Factors Affecting Nitrite Toxicity

The toxicity of nitrite to freshwater fish is modified by a number of factors that are present and measurable in the aquatic environment. These factors include, but are not necessarily limited to, ambient hydrogen ion, chloride and calcium levels in the surface water.

##### a. Hydrogen Ion Concentration

In aqueous solution, nitrite establishes the following equilibrium:



This equilibrium is affected by the concentration of hydrogen ions in solution; therefore the toxicity of the treatment water would also be affected by pH if the relative toxicity of the two nitrite species were different. Preliminary results of nitrite bioassays designed to test the influence of pH on nitrite toxicity (Russo and Thurston 1977) suggested that toxicity to rainbow trout was independent of pH over

the range 7.5 - 8.5, and that nitrite toxicity was correlated with  $\text{NO}_2^-$  concentration. However, subsequent studies (Russo et al. 1981) indicated that the toxicity of nitrite to rainbow trout is pH-dependent within the range considered acceptable to most freshwater aquatic life (pH 6.5 - 9.0). It was also found that both nitrite species are toxic, with the  $\text{HNO}_2$  species being much more toxic than the  $\text{NO}_2^-$  species (96 hour  $\text{LC}_{50}$  values:  $\approx 10$  ng/L  $\text{HNO}_2$  vs. 250,000 ng/L  $\text{NO}_2^-$  @ pH 8.0). The relative abundance of the two species (1 part  $\text{HNO}_2$ :  $10^4$ - $10^6$  parts  $\text{NO}_2^-$ ) within the acceptable range for pH means that both contribute significantly to the total toxicity. Figure 12 (Table 4) demonstrates the effect of pH on the toxicity of nitrite to rainbow trout. This exponential relationship can be linearized by plotting  $\ln \text{LC}_{50}$  against pH. Analysis of the available data indicates that the effect of pH on the toxicity of nitrite can be expressed by the equation:

$$\text{FAV (pH)} = \text{FAV ref.} \cdot e^{\frac{\text{SLP (pH-8.0)} + \text{LCR}}{\text{LCP}}} \quad (14)$$

where: FAV ref. is the Final Acute Value for the species determined under reference conditions. In this case, pH=8.0,

SLP is the slope of the curve, and is calculated directly from the  $\ln \text{LC}_{50}$  vs. pH plot,

LCR is the common reference point on the curve, and is calculated using:  
 $\text{LCR} = \ln \text{LC}_{50} (\text{pH}=8.0),$

and

LCP is the proportionality constant that permits the assessment of pH effects under non-reference conditions, and is calculated using:  $\text{LCP} = \text{LC}_{50} (\text{pH}=8.0).$

Figure 12. Effect of pH on the toxicity of nitrite to rainbow trout [Russo et al. 1981]

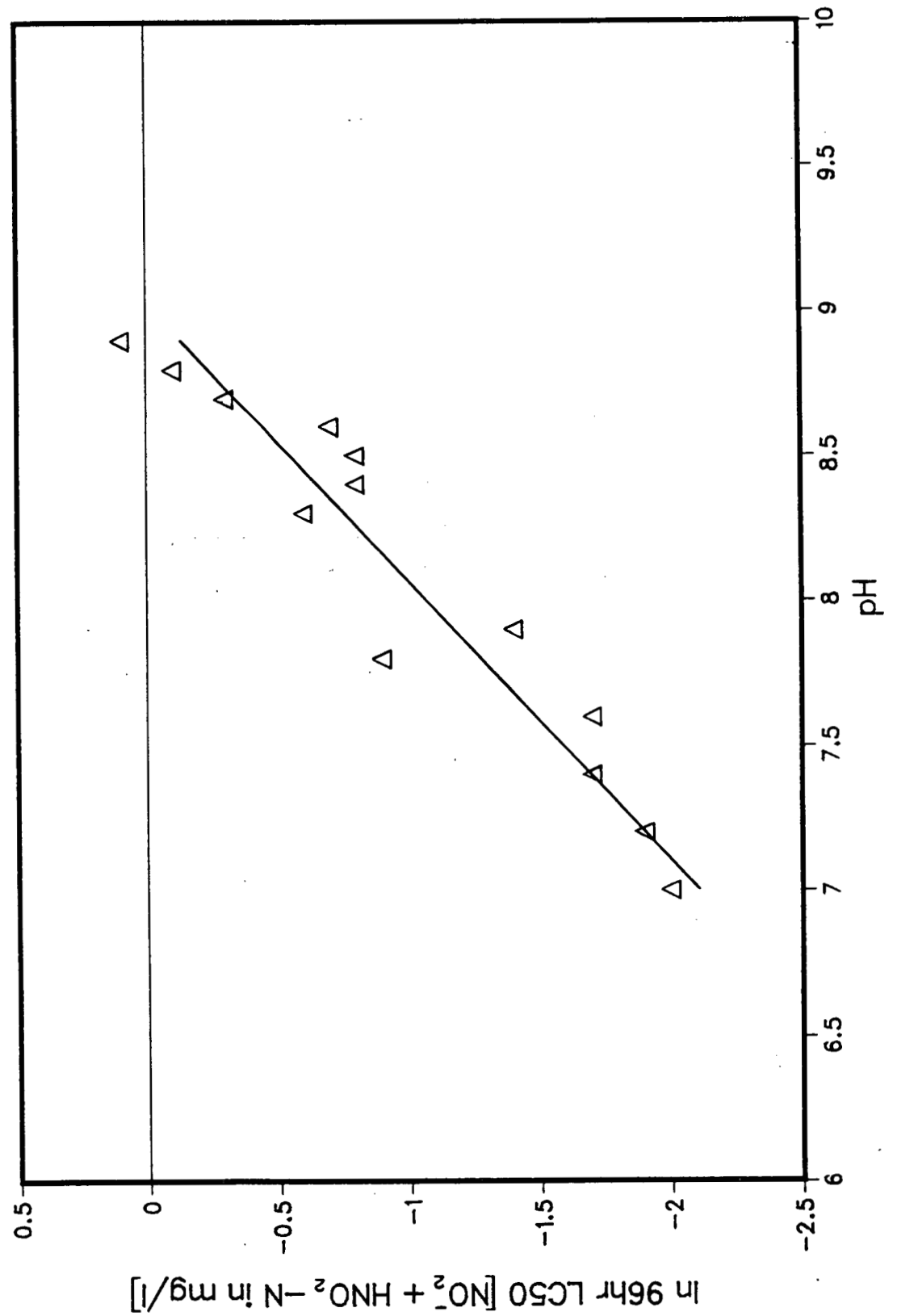


TABLE 4  
EFFECT OF pH ON THE TOXICITY OF TOTAL NITRITE  
TO RAINBOW TROUT (RUSSO et al. 1981).

pH	n	96 hr LC <sub>50</sub> (Range) <sup>a</sup>		
		NO <sub>2</sub> <sup>-</sup> + HNO <sub>2</sub> (mg/L)	NO <sub>2</sub> <sup>-</sup> (mg/L)	HNO <sub>2</sub> (ng/L)
7.0	2	0.13(0.11-0.14)	0.13(0.11-0.14)	30.2(25.9-34.4)
7.2	1	0.15	0.15	21.6
7.4	1	0.19	0.19	19.4
7.6	1	0.18	0.18	10.8
7.8	1	0.40	0.40	13.6
7.9	7	0.25(0.17-0.36)	0.25(0.17-0.36)	7.7(4.9-11.4)
8.3	1	0.54	0.54	7.2
8.4	1	0.46	0.46	4.3
8.5	1	0.45	0.45	3.6
8.6	1	0.50	0.50	3.0
8.7	1	0.71	0.71	3.5
8.8	2	0.89(0.61-1.17)	0.89(0.61-1.17)	3.3(2.2-4.3)
9.0	2	1.11(1.10-1.12)	1.11(1.10-1.12)	2.4(2.4-2.5)

a) ambient water chemistry during tests was D.O. = 6.6 - 9.3,  
NH<sub>3</sub> = 0.00-0.06, Cl = 0.00-0.47, Ca<sup>#</sup> = 46.8-61.3.



Analysis of pooled rainbow trout acute toxicity data provides estimates of 1.08, -1.10, and 0.33 for the three data set dependent variables in the equation, respectively. Therefore, the relationship between pH and nitrite toxicity can be quantified using the following equation:

$$\text{FAV (pH)} = \text{FAV ref.} \cdot e^{\frac{1.08(\text{pH}-8.0)-1.10}{0.33}} \quad (15)$$

b. Chloride

The suppression of nitrite toxicity in the presence of chloride has been reported for rainbow trout (Russo and Thurston 1977), coho salmon (Perrone and Meade 1977), and steelhead trout (Wedemeyer and Yasutake 1978). This amelioration of nitrite toxicity by chloride appears to be related to the mechanics of nitrite transport into the blood. It is likely that the gills are impermeable to  $\text{NO}_2^-$  but allow its conjugate, nitrous acid ( $\text{HNO}_2$ ), to diffuse freely into the blood, where it dissociates according to the blood pH value. Thus,  $\text{NO}_2^-$  will accumulate in the blood if it has a higher pH than the treatment water (Eddy et al. 1983). In addition, the movement of the  $\text{NO}_2^-$  species across the gill epithelia may be facilitated by the branchial anion exchange mechanism. Krous et al. (1982) speculated that if lamellar chloride cells are involved in active chloride uptake in freshwater, then it is possible that other anions use the same pathway for branchial entry into the blood. The direct correlation between plasma nitrite values and numbers of lamellar chloride cells discovered in rainbow trout acclimated to freshwater for various periods of time would seem to

substantiate this hypothesis. Russo and Thurston (1977) observed a linear relationship between nitrite toxicity (96 hr.  $LC_{50}$ ) and chloride concentration from 0 - 41 mg/L Cl (Figure 13). This relationship was expressed by the equation:

$$FAV (Cl^-) = FAV \text{ ref.} + 0.31 [Cl^-] \quad (16)$$

c. Calcium

The presence of calcium ions in surface waters appears to be an factor in the reduction of nitrite toxicity. Wedemeyer and Yasutake (1978) reported a 12 fold reduction of the toxicity of nitrite at 25 mg/L  $CaCl_2$  compared to a 2 fold decrease for that level of NaCl (Figure 14). These results indicate that considerable protection is afforded by increasing the concentration of  $Ca^{++}$ . Supportive evidence for this assertion is the seawater inhibition of nitrite toxicity to chinook salmon (Crawford and Allen 1977), in which the  $Ca^{++}$  ion was strongly implicated. Therefore, fish residing in hard water river systems with high levels of  $CaCO_3$  should be less sensitive to nitrite additions than fish exposed to nitrite under soft water conditions, although this relationship has not been definitively established.

Quantification of the effect of calcium on the toxicity of nitrite to freshwater salmonids is not a trivial process. Only a limited amount of data is available from literature sources, and much of this data is rendered unusable because researchers failed to fully report the water quality conditions under which the acute toxicity bioassays were conducted. However, standardization of those remaining data points to

Figure 13. Effect of chloride concentration on the toxicity of nitrite to rainbow trout [data from Russo and Thurston 1977]

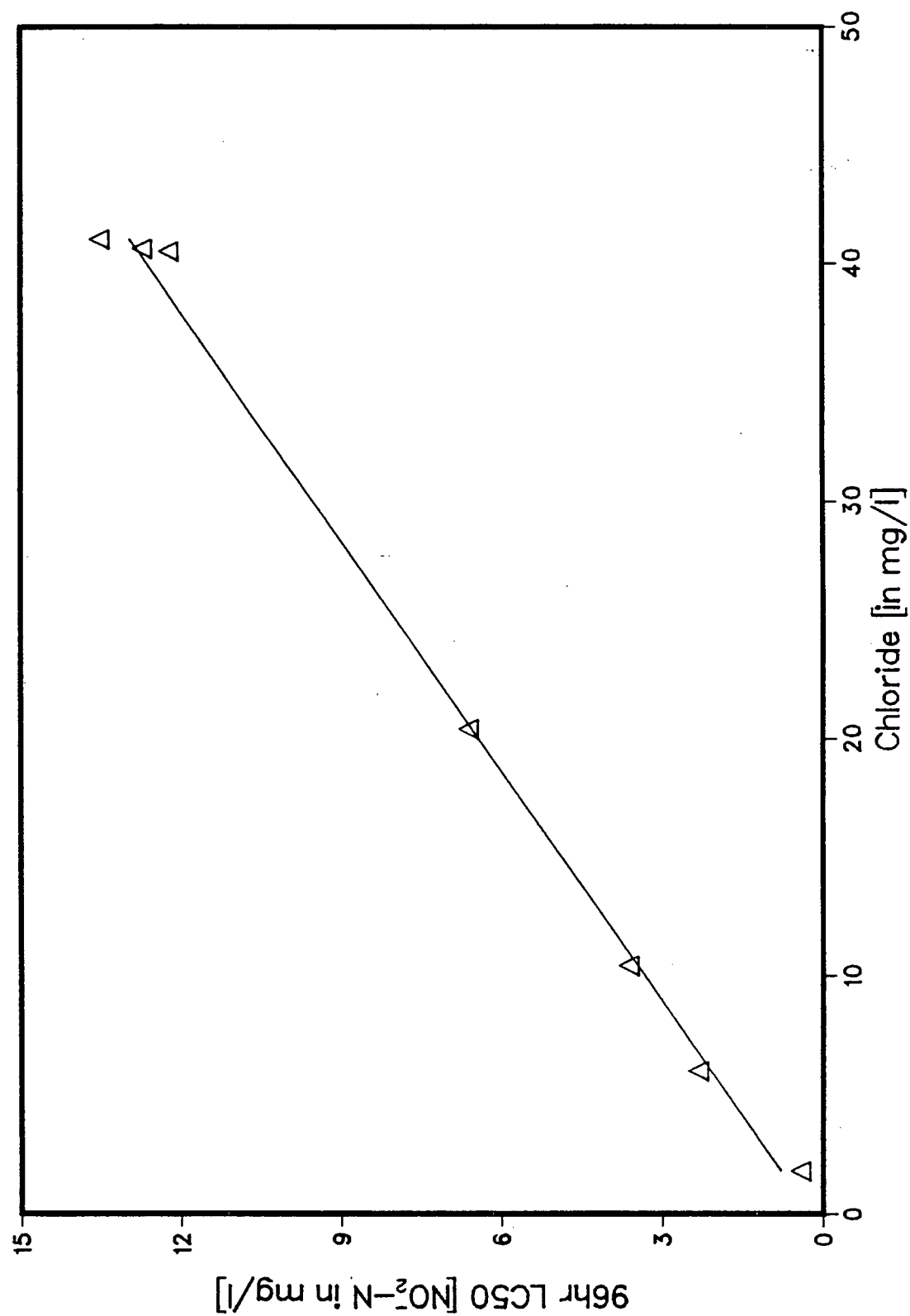
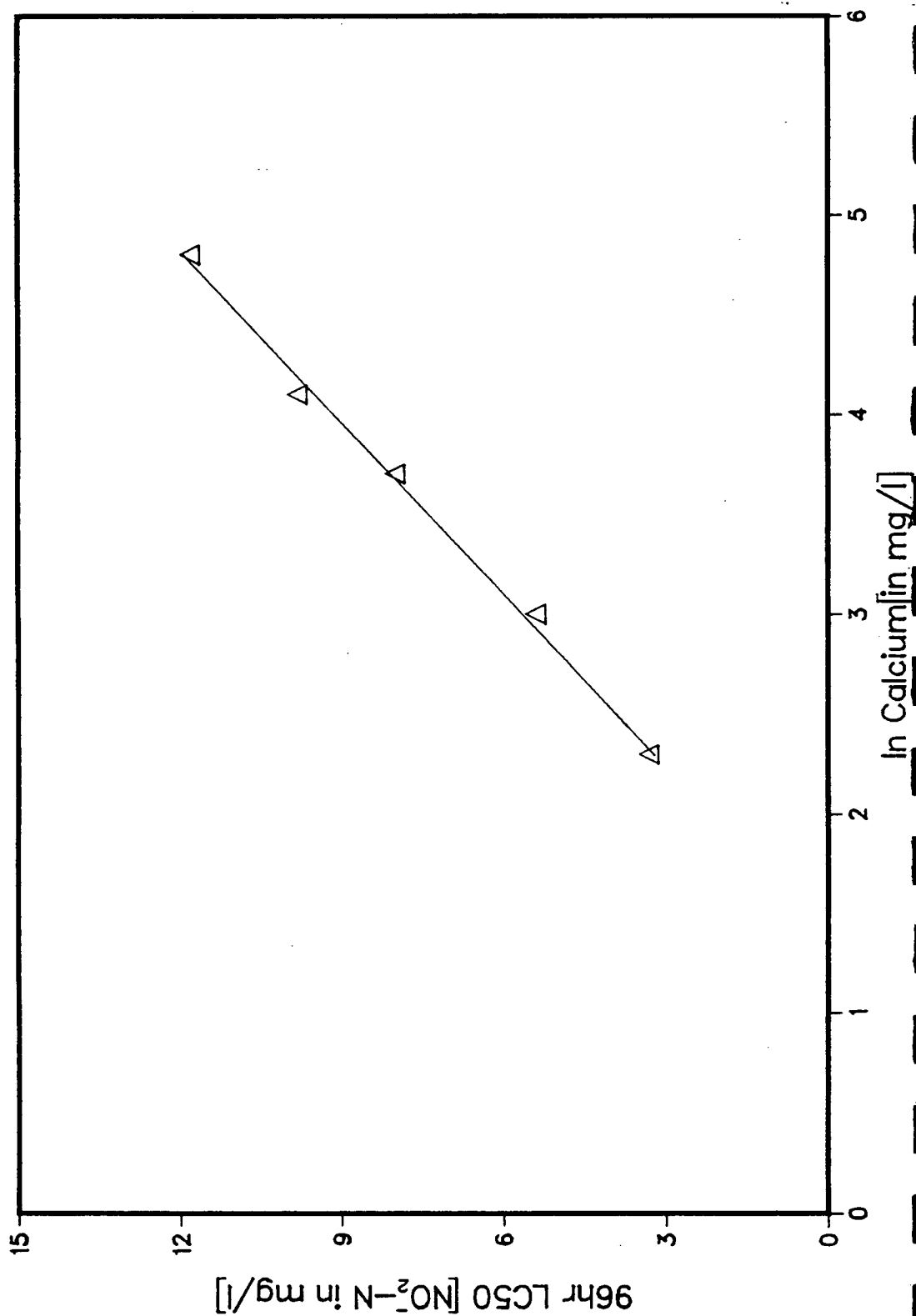


Figure 14. Effect of calcium concentration on the toxicity of nitrite to steelhead trout [data from Wedemeyer and Yasutake 1978].



reference conditions (pH=8.0,  $\text{Cl}^- = 0.35 \text{ mg/L}$ ) has facilitated a preliminary assessment of the influence of calcium on nitrite toxicity. The result of that assessment (Figure 15) is expressed by the following equation:

$$\text{FAV} (\text{Ca}^{++}) = \text{FAV ref.} \cdot \frac{\text{SLP} \ln [\text{Ca}^{++}] + \text{INT}}{\text{LCP}} \quad (17)$$

where SLP is the slope of the curve, and is calculated directly from the  $\text{LC}_{50}$  vs.  $\ln [\text{Ca}^{++}]$  plot.

INT is the y-axis intercept on the  $\text{LC}_{50}$  vs.  $\ln [\text{Ca}^{++}]$  plot.

and

LCP is the proportionality constant that permits the assessment of  $\text{Ca}^{++}$  effects under non-reference conditions, and is calculated using:

$$\text{LCP} = \text{LC}_{50} (\text{Ca}^{++} = 80.0 \text{ mg/L})$$

Analysis of rainbow trout acute toxicity bioassay data provides estimates of those three data set dependent variables, and results in the following relationship:

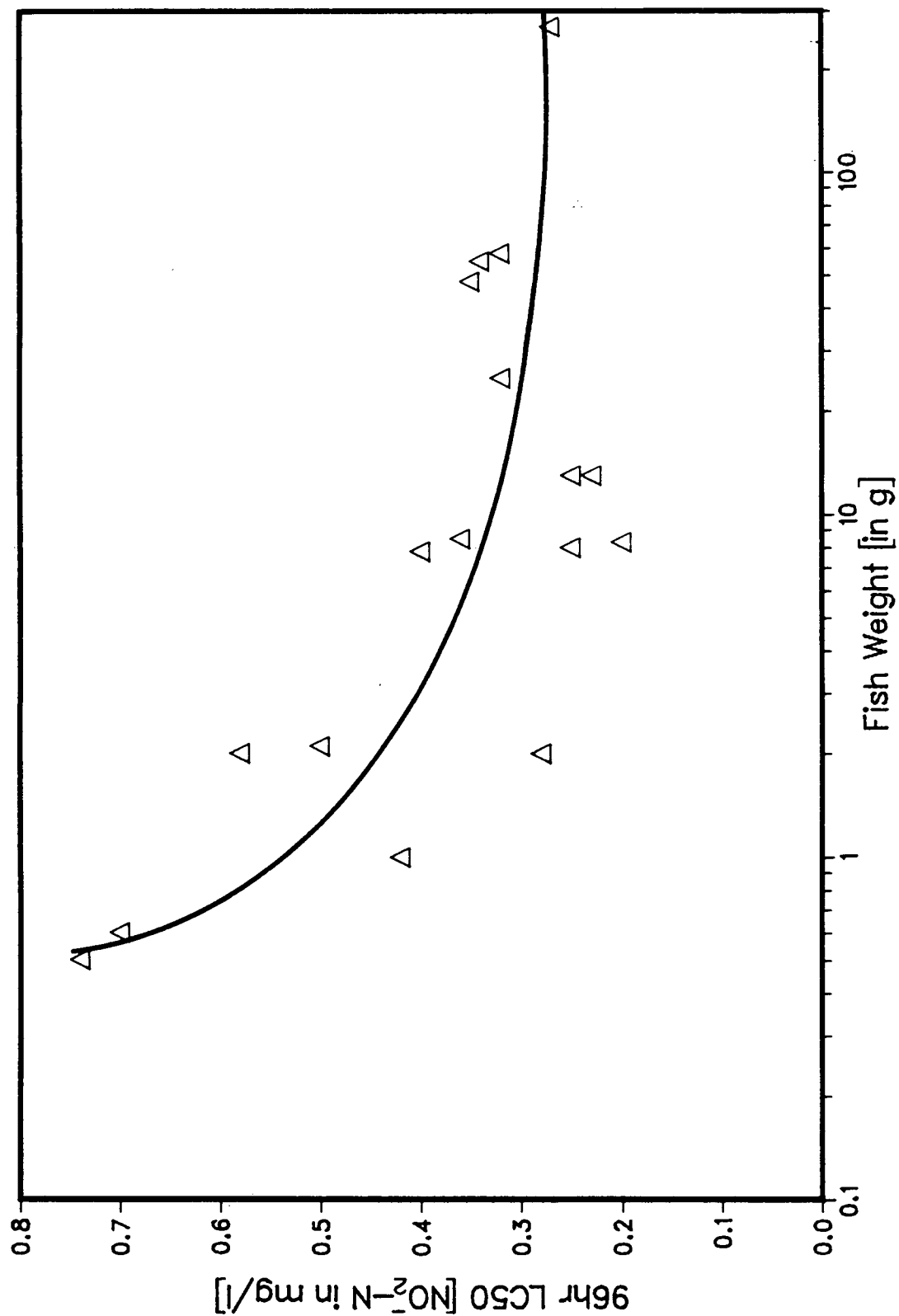
$$\text{FAV} (\text{Ca}^{++}) = \text{FAV ref.} \cdot \frac{4.0 \ln [\text{Ca}^{++}] - 6.8}{10.73} \quad (18)$$

This analysis assumes that the calcium will be associated, for the most part, with bicarbonate, and as such the influence of bicarbonate on the toxicity of nitrite is included in this equation.

d. Dissolved Oxygen

Little data is currently available on the influence of dissolved oxygen on the toxicity of nitrite to freshwater fish. However, given the mode of toxic action of nitrite it is certain that reductions of dissolved oxygen below saturation values would result in lower  $\text{LC}_{50}$  values for fish. A single published study (Bowser et al. 1983) on channel catfish would

Figure 15. Relationship between fish weight and the toxicity of nitrite to salmonid fish [data from Russo et al. 1974, Russo et al. 1981, and Thurston et al. 1978].



appear to substantiate the effect of low D.O. levels on nitrite toxicity. Therefore, the development of site-specific criteria for nitrite will require some judgement and interpolation when oxygen depressions are anticipated.

e. Temperature

To date, no information is available on the influence of temperature on nitrite toxicity. It is likely that temperature would affect nitrite uptake rates, and the equilibrium between the gaseous and ionic forms, and thereby alter its toxicity to freshwater fish. Additional research is required in this area before the influence of temperature can be considered in nitrite criteria development.

3.3.2.1.3 Biotic Factors Affecting Nitrite Toxicity

In addition to the physical characteristics of the water body under study, there are a number of biotic factors that affect the toxicity of nitrite to freshwater fish.

a. Species

Information from diverse sources (Gillette et al. 1952; Wallen et al. 1957; McCoy 1972; Smith and Williams 1974; Colt, 1974; Russo et al. 1974; Thurston et al. 1978; Tomasso, 1986) indicates differential sensitivities to water-borne nitrite depending on the species tested. Salmonids appear to be particularly sensitive, with reported 96 hr.  $LC_{50}$  values ranging from 0.11 - 12.6 mg/L  $NO_2^-$ -N in freshwater. Cyprinids, ictalurids and catostomids appear to be more resistant to the effects of nitrite, with 96 hr.  $LC_{50}$  values ranging from 1.5 - 100 mg/L, 7.5 - 40 mg/L, and to 100 mg/L  $NO_2^-$ -N, respectively. Within the family

salmonidae, the relative sensitivity seems to be rainbow trout  $\approx$  cutthroat trout  $\geq$  chinook salmon. No information is currently available on the relative sensitivity of Salvelinus or Prosopium to elevated levels of nitrite. To expedite criteria development it was be assumed that the three salmonid species identified in the Canadian portion of the Flathead River system have approximately the same sensitivity to nitrite as rainbow trout. This is a major assumption, but no better information is available at this time.

b. Life History Stage

Although the data are somewhat sketchy, there is some evidence that larger fish are more sensitive to high nitrite levels than are fry during early rearing. Russo et al. (1974) demonstrated a two-fold difference in the 96 hr.  $LC_{50}$  values between 2.0 g (0.39 mg/L  $NO_2-N$ ) and 235 g (0.20 mg/L  $NO_2-N$ ) rainbow trout (Figure 15). No information is currently available on the effects of high levels of water-borne nitrite on the eggs, alevins, or mature adults of any salmonid species. For the purpose of criteria development, it will be assumed that eggs and alevins are at least as sensitive to nitrite toxicity as are newly emergent fry, and that mature adults are approximately as sensitive as 235 g fish.

3.3.2.2 Invertebrates

Almost no research has been conducted on the toxicity of nitrite to freshwater invertebrates, and more on those species present in the Flathead basin. The results of a test on the freshwater prawn (Macrobrachium rosenbergii) suggest that this species is fairly resistant to the effects of nitrite (3-4 week  $LC_{50}$  = 15.4 mg/L; Wickens 1976). For



the purpose of defining criteria, it is assumed that invertebrates are at least as tolerant of elevated nitrite levels than are fish, and therefore criteria formulated for fish should be adequate to protect invertebrate species from harmful effects.

#### 3.3.2.3 Algae

Like ammonia and nitrate, nitrite can serve as a source of nitrogen for algal metabolism; however, at very high concentrations nitrite may inhibit the growth of some species (in laboratory studies; Lewin 1962). Very little information appears to be available on the toxicity of nitrite to algae. It is reasonable to assume that, given the high degree of resistance indicated by the limited laboratory studies, the algal community would not respond negatively to the criteria for nitrite developed for fish. Criteria for nitrite combined with ammonia and nitrate, that would prevent eutrophication of receiving water streams, will be reported in a document currently in preparation.

#### 3.3.2.4 Site-Specific Criteria for Nitrite

In order to develop site-specific criteria for nitrite it is necessary to integrate the factors that affect nitrite toxicity to fish with specific information on the ambient water quality conditions and life history patterns of target species in the watershed. The most important of these are pH, water hardness, chloride concentration, and life history stage.

The following procedure was utilized to calculate the final acute toxicity values (FAV's) for the three important sport fish species found in the Canadian portion of the Flathead River system:

- i. The available 96 hr.  $LC_{50}$  data for rainbow and cutthroat trout were pooled and adjusted to reference conditions ( $pH = 8.0$ ,  $Cl^- = 0.35$  mg/L) in accordance with the equations 13 - 18 listed previously. These data were then plotted against fish weight to provide estimates of sensitivity to nitrite by life history stage. The sensitivities of Prosopium and Salvelinus were assumed to be similar to those of Salmo.
- ii. Reference final acute values (FAV ref.) for each life history stage were interpolated directly from the fish weight-sensitivity plot (Table 5).
- iii. Final acute values (FAVs) were then calculated by integrating site-specific hydrogen ion ( $pH$ ), calcium and chloride concentration data with reference FAV information on a monthly basis in accordance with the following:

$$FAV(pH, Ca^{++}, Cl^-) = FAV \text{ ref.} \cdot FPH \cdot FCa + FCl \quad (19)$$

where:

$$FPH = \frac{e^{1.08(pH-8.0)-1.10}}{0.33} ; 6.5 < pH < 9.5 \quad (20)$$

$$FCa = \frac{4.0 \ln [Ca^{++}] - 6.8}{10.73} ; Ca^{++} < 150 \text{ mg/L} \quad (21)$$

$$FCl = 0.31 [Cl^-] ; 0.5 < Cl^- < 41 \text{ mg/L} \quad (22)$$

$$= 0 ; 0.5 \geq Cl^-$$

The final acute values for Flathead River mainstem and Howell Creek bull trout are presented in Tables 6 and 7 (Figure 16), respectively. FAVs for cutthroat trout (Tables 8 and 9, Figure 17) and mountain whitefish (Tables 10 and 11, Figure 18) have also been calculated using this methodology.

TABLE 5

REFERENCE FINAL ACUTE VALUES OF NITRITE FOR KEY LIFE HISTORY STAGES  
OF IMPORTANT FLATHEAD RIVER SYSTEM FISH SPECIES

Species	Dates	Life History Stage	FAV ref. (mg/L) <sup>a</sup>
Bull Trout	Sept. 1 - Jan. 30	Eggs	0.70
	Jan. 1 - Mar. 31	Early Alevins	0.70
	Mar. 15 - May 31	Late Alevins	0.70
	May 15 - June 15	Fry (to 0.25g)	0.70
	May 30 - June 30	Fry (to 0.50g)	0.70
	June 15 - July 15	Fry (to 1.00g)	0.70
	July 1 - June 30	Fry (to 10.0g)	0.40
	July 1 - June 30	Juveniles (to 50.0g)	0.25
	July 15 - Oct. 15	Adults	0.20
Cutthroat Trout	May 15 - July 20	Eggs	0.70
	July 5 - July 31	Early Alevins	0.70
	July 15 - Aug. 10	Late Alevins	0.70
	July 20 - Aug. 20	Fry (to 0.25g)	0.70
	July 31 - Aug. 31	Fry (to 0.50g)	0.70
	Aug. 15 - Sept. 20	Fry (to 1.00g)	0.70
	Sept. 1 - Aug. 31	Fry (to 10.0g)	0.40
	Sept. 1 - Aug. 31	Juveniles (to 50.0g)	0.25
	Sept. 1 - Aug. 31	Adults	0.20
Mountain Whitefish	Oct. 15 - Mar. 15	Eggs	0.70
	Mar. 1 - May 10	Early Alevins	0.70
	May 1 - May. 30	Late Alevins	0.70
	May 15 - June 15	Fry (to 0.25g)	0.70
	June 10 - June 30	Fry (to 0.50g)	0.70
	June 20 - July 20	Fry (to 1.00g)	0.70
	July 1 - June 30	Fry (to 10.0g)	0.40
	July 1 - June 30	Juveniles (to 50.0g)	0.25
	July 1 - June 30	Adults	0.20

a) Reference conditions: pH =  $8.0 \pm 0.2$ , T = 10°C, D.O.  $\geq 8.0$  mg/L  
 $\text{Ca}^{++}$  =  $80 \pm 5$  mg/L,  $\text{Cl}^-$  =  $0.5 \pm 0.5$  mg/L  
hardness =  $200 \pm 10$  mg/L as  $\text{CaCO}_3$ .

TABLE 6

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF NITRITE FOR FLATHEAD RIVER BULL TROUT.  
(See Text for Definition of Symbols)

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Most Sensitive Life History Stage	Juvenile	Juvenile	Juvenile	Adult	Adult	Adult	Adult	Adult	Juvenile	Adult	Juvenile	Juvenile
FAV ref. (mg/L)	0.25	0.25	0.25	0.20	0.20	0.20	0.20	0.20	0.25	0.20	0.25	0.25
pH <sup>a</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Cl <sup>-a</sup>	0.33	0.30	0.30	0.30	0.25	0.27	0.20	0.30	0.30	0.25	0.37	0.35 <sup>b</sup>
Ca <sup>++a</sup>	41.2	42.7	42.3	33.6	29.5	28.8	38.2	40.1	41.3	40.9	43.7	42.0 <sup>b</sup>
FPH	1.25	1.40	1.47	0.91	1.07	1.19	1.40	1.56	1.32	1.25	1.32	1.40
FCI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FCa	0.75	0.77	0.76	0.68	0.63	0.62	0.72	0.74	0.75	0.75	0.77	0.76
FAV(mg/L)	0.23	0.27	0.28	0.12	0.13	0.15	0.20	0.23	0.25	0.19	0.25	0.27
[Maximum] <sup>c</sup>	0.12	0.14	0.14	0.06	0.07	0.08	0.10	0.12	0.13	0.10	0.13	0.14
[96 hr $\bar{x}$ ]	0.023	0.027	0.028	0.012	0.013	0.015	0.020	0.023	0.025	0.019	0.025	0.027

a) From Thorp (1985) and Water Quality Branch unpublished data (1982-83).

b) Estimated values.

c) No-effect levels of nitrite are reported as maximum and 96 hr arithmetic mean concentrations.

TABLE 7

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF NITRITE FOR HOWELL CREEK BULL TROUT.  
(See Text for Definition of Symbols)

Most Sensitive Life History Stage	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Juvenile	Juvenile	Juvenile	Juvenile	Juvenile	Juvenile	Juvenile	Juvenile	Adult	Adult	Juvenile	Juvenile
FAV ref. (mg/L)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.20	0.20	0.25	0.25
pH <sup>a</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Cl <sup>a</sup> (mg/L)	0.40 <sup>b</sup>	0.30	0.40 <sup>b</sup>	0.40	0.33	0.25	0.20	0.25	0.33	0.40 <sup>b</sup>	0.40 <sup>b</sup>	0.60
Ca <sup>++a</sup> (mg/L)	42.0 <sup>b</sup>	43.9	42.0 <sup>b</sup>	35.8	30.6	32.5	40.0	41.2	41.3	42.0 <sup>b</sup>	42.0 <sup>b</sup>	42.6
FPH	1.25	1.40	1.47	0.91	1.07	1.19	1.40	1.56	1.32	1.25	1.32	1.40
FCI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FCa	0.76	0.78	0.76	0.70	0.64	0.66	0.74	0.75	0.75	0.76	0.76	0.76
FAV(mg/L)	0.24	0.27	0.28	0.16	0.17	0.20	0.26	0.29	0.20	0.19	0.25	0.27
[Maximum] <sup>c</sup>	0.12	0.14	0.14	0.08	0.09	0.10	0.13	0.15	0.10	0.10	0.13	0.14
[96 hr $\bar{x}$ ]	0.024	0.027	0.028	0.016	0.017	0.020	0.026	0.029	0.020	0.019	0.025	0.027

a) From Sheehan et al. 1985.

b) Estimated values.

c) No-effect levels of nitrite are reported as maximum and 96 hr arithmetic mean concentrations.

Figure 16. Calculated Final Acute Values of Nitrite for Bull Trout in the Flathead River System.

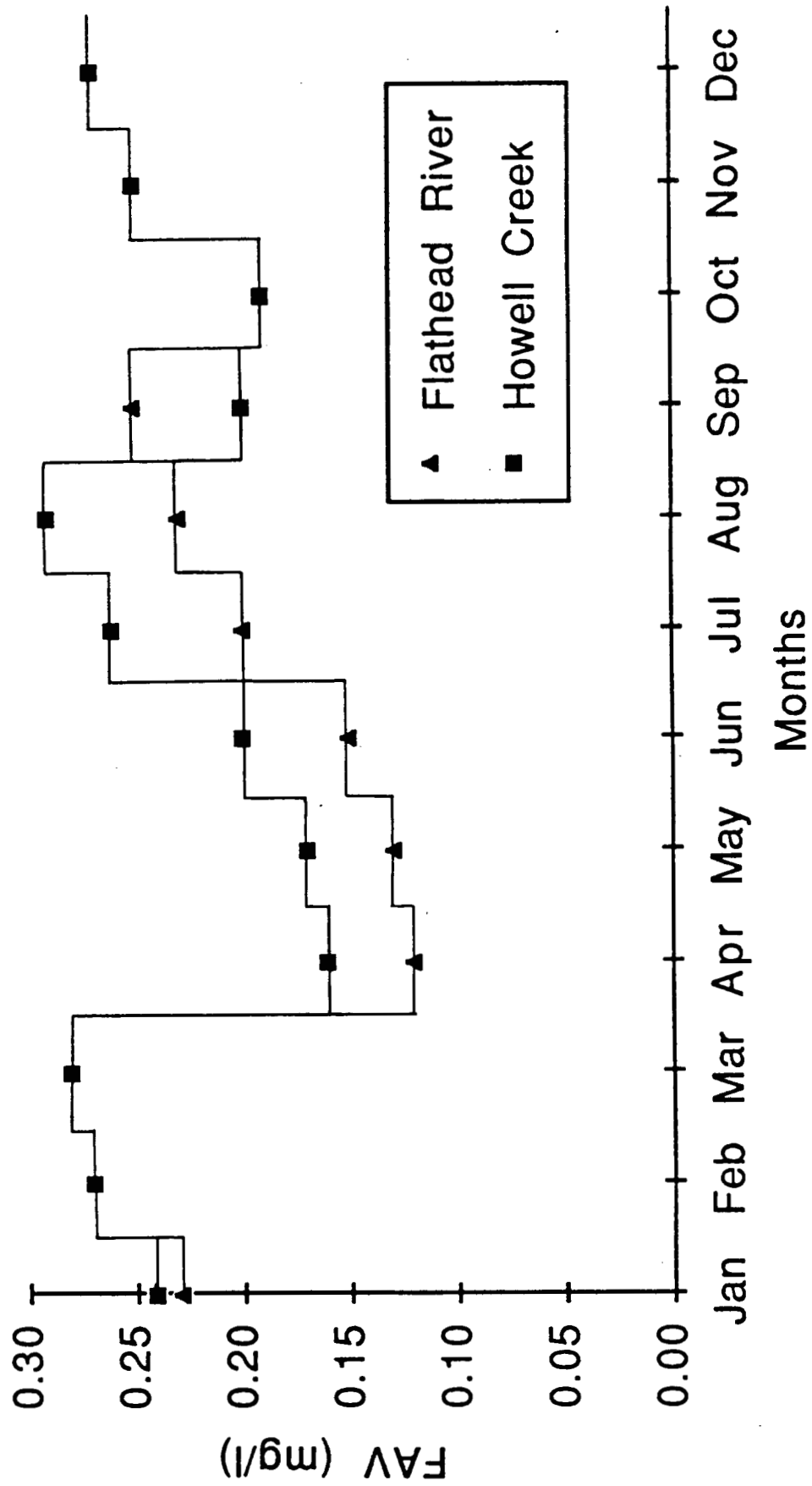


TABLE 8

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF NITRITE FOR FLATHEAD RIVER CUTTHROAT TROUT  
(See Text for Definition of Symbols)

Most Sensitive Life History Stage	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Adult	Adult	Adult	Adult	Juvenile	Juvenile	Adult	Adult	Adult	Adult	Adult	Adult
FAV ref. (mg/L)	0.20	0.20	0.20	0.20	0.25	0.25	0.20	0.20	0.20	0.20	0.20	0.20
pH <sup>a</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Cl <sup>-a</sup>	0.33	0.30	0.30	0.30	0.25	0.27	0.20	0.30	0.30	0.25	0.37	0.35 <sup>b</sup>
Ca <sup>++a</sup>	41.2	42.7	42.3	33.6	29.5	28.8	38.2	40.1	41.3	40.9	43.7	42.0 <sup>b</sup>
FPH	1.25	1.40	1.47	0.91	1.07	1.19	1.40	1.56	1.32	1.25	1.32	1.40
FCI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FCa	0.75	0.77	0.76	0.68	0.63	0.62	0.72	0.74	0.75	0.75	0.77	0.76
FAV(mg/L)	0.19	0.22	0.22	0.12	0.17	0.18	0.20	0.23	0.20	0.19	0.20	0.21
[Maximum] <sup>c</sup>	0.10	0.11	0.11	0.06	0.09	0.09	0.10	0.12	0.10	0.10	0.10	0.11
[96 hr $\bar{x}$ ]	0.019	0.022	0.022	0.012	0.017	0.018	0.020	0.023	0.020	0.019	0.020	0.021

a) From Thorp (1985) and Water Quality Branch unpublished data (1982-83).

b) Estimated values.

c) No-effect levels of nitrite are reported as maximum and 96-hour arithmetic mean concentrations.

TABLE 9

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF NITRITE FOR HOWELL CREEK CUTTHROAT TROUT  
(See Text for Definition of Symbols)

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Most Sensitive Life History Stage	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
FAV ref. (mg/L)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
pH <sup>a</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Cl <sup>a</sup> (mg/L)	0.40 <sup>b</sup>	0.30	0.40 <sup>b</sup>	0.40	0.33	0.25	0.20	0.25	0.33	0.40 <sup>b</sup>	0.40 <sup>b</sup>	0.60
Ca <sup>++a</sup> (mg/L)	42.0 <sup>b</sup>	43.9	42.0 <sup>b</sup>	35.8	30.6	32.5	40.0	41.2	41.3	42.0 <sup>b</sup>	42.0 <sup>b</sup>	42.6
FPH	1.25	1.40	1.47	0.91	1.07	1.19	1.40	1.56	1.32	1.25	1.32	1.40
FCI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FCa	0.76	0.78	0.76	0.70	0.64	0.66	0.74	0.75	0.75	0.76	0.76	0.76
FAV(mg/L)	0.19	0.22	0.22	0.13	0.14	0.16	0.21	0.23	0.20	0.19	0.20	0.21
[Maximum] <sup>c</sup>	0.10	0.11	0.11	0.07	0.07	0.08	0.11	0.12	0.10	0.10	0.10	0.11
[96 hr $\bar{x}$ ]	0.019	0.022	0.022	0.013	0.014	0.016	0.021	0.023	0.020	0.019	0.020	0.021

a) From Sheehan et al. 1985.

b) Estimated values.

c) No-effect levels of nitrite are reported as maximum and 96-hour arithmetic mean concentrations.



Figure 17. Calculated Final Acute Values of Nitrite for Cutthroat Trout in the Flathead River System.

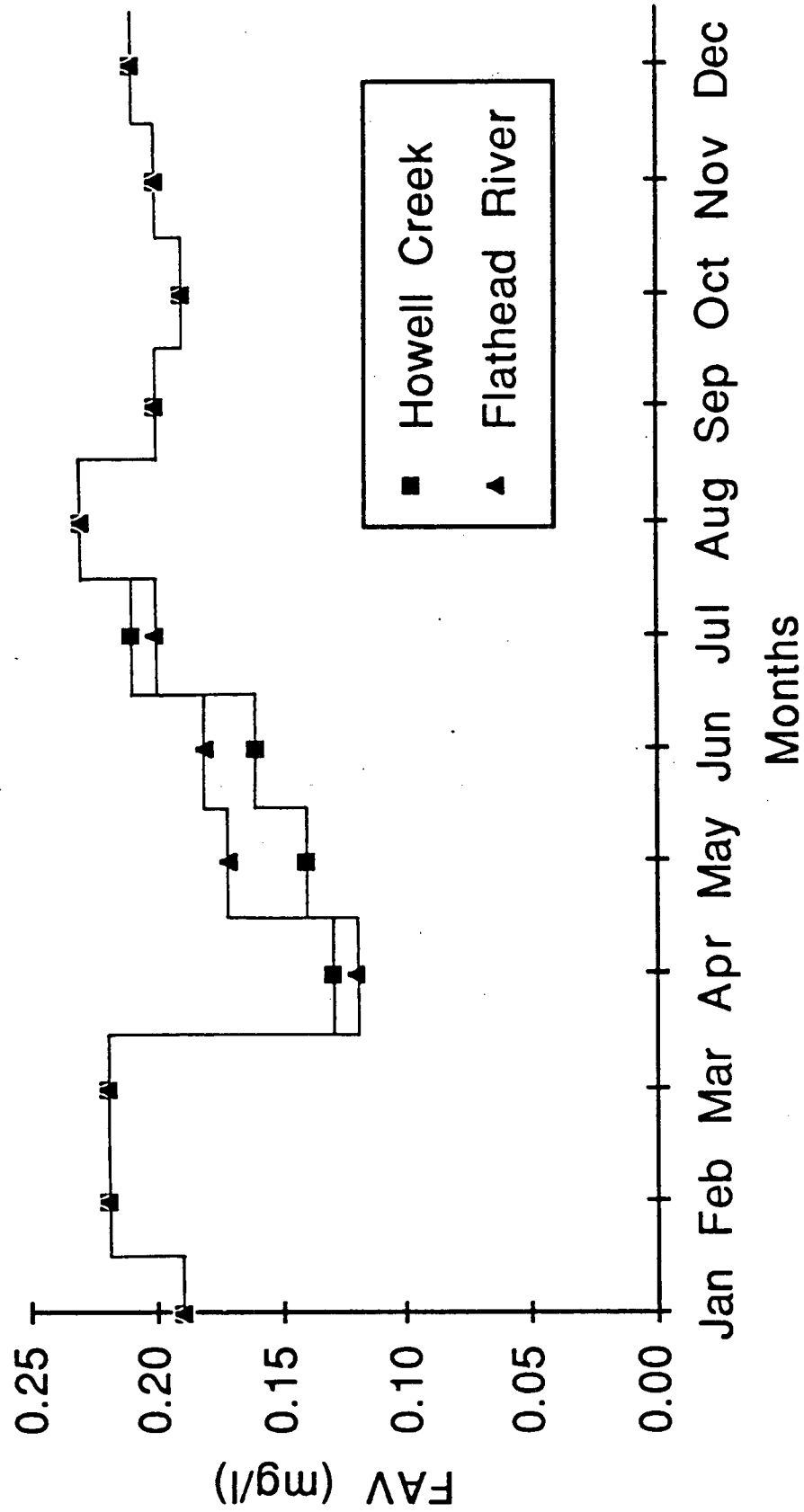


TABLE 10

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF NITRITE FOR FLATHEAD RIVER MOUNTAIN WHITEFISH  
(See Text for Definition of Symbols)

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Most Sensitive Life History Stage	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult
FAV ref. (mg/L)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
pH <sup>a</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Cl <sup>-a</sup>	0.33	0.30	0.30	0.30	0.25	0.27	0.20	0.30	0.30	0.25	0.37	0.35 <sup>b</sup>
Ca <sup>++a</sup>	41.2	42.7	42.3	33.6	29.5	28.8	38.2	40.1	41.3	40.9	43.7	42.0 <sup>b</sup>
FPH	1.25	1.40	1.47	0.91	1.07	1.19	1.40	1.56	1.32	1.25	1.32	1.40
FCI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FCa	0.75	0.77	0.76	0.68	0.63	0.62	0.72	0.74	0.75	0.75	0.77	0.76
FAV(mg/L)	0.19	0.22	0.22	0.12	0.13	0.15	0.20	0.23	0.20	0.19	0.20	0.21
[Maximum] <sup>c</sup>	0.10	0.11	0.11	0.06	0.07	0.08	0.10	0.12	0.10	0.10	0.10	0.11
[96 hr $\bar{x}$ ]	0.019	0.022	0.022	0.012	0.013	0.015	0.020	0.023	0.020	0.019	0.020	0.021

a) From Thorp (1985) and Water Quality Branch unpublished data (1982-83).

b) Estimated values.

c) No-effect levels of nitrite are reported as maximum and 96-hour arithmetic mean concentrations.

TABLE 11

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF NITRITE FOR HOWELL CREEK MOUNTAIN WHITEFISH  
(See Text for Definition of Symbols)

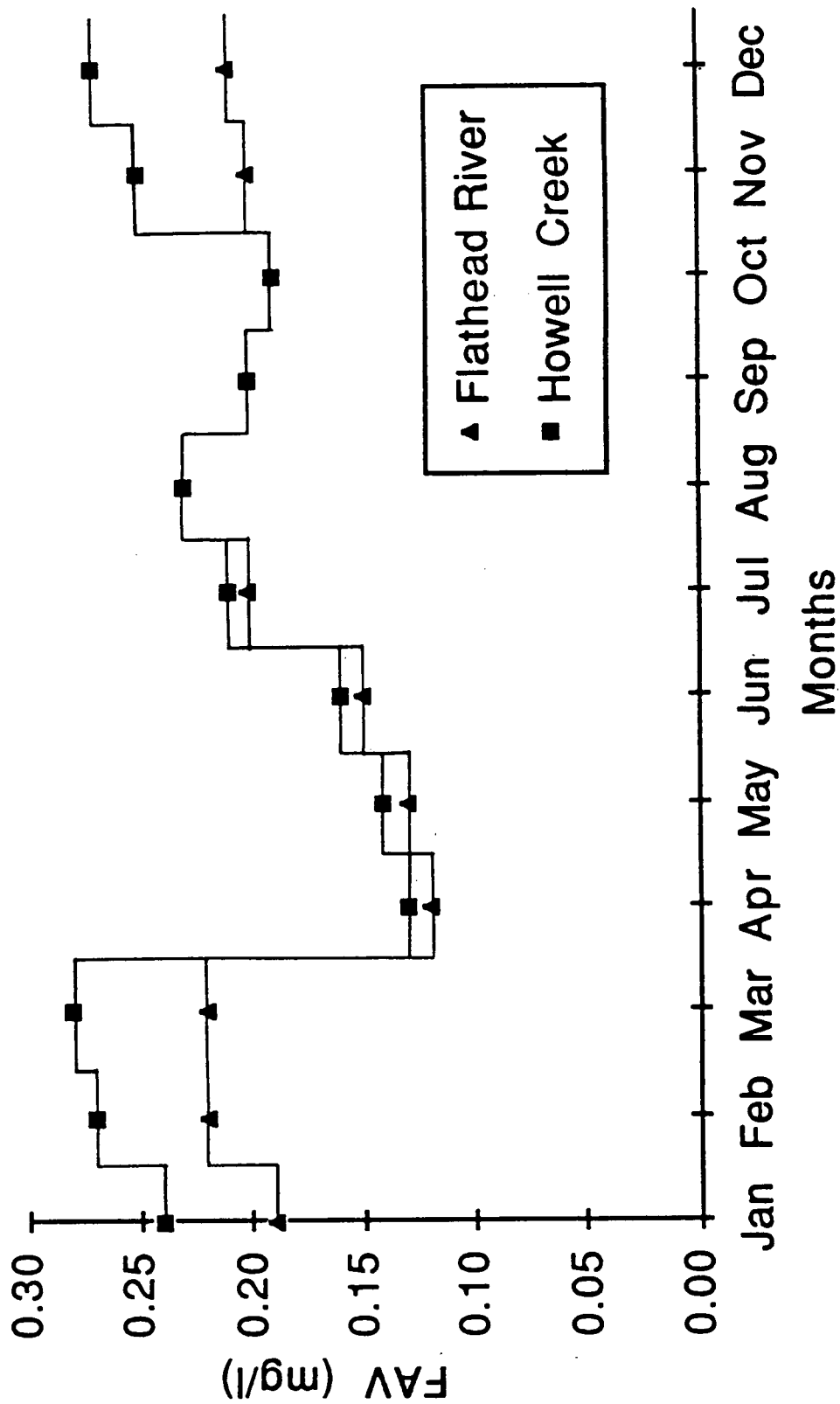
Most Sensitive Life History Stage	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Juvenile	Juvenile	Juvenile	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Juvenile	Juvenile
FAV ref. (mg/L)	0.25	0.25	0.25	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.25	0.25
pH <sup>a</sup>	8.20	8.30	8.35	7.90	8.05	8.30	8.30	8.40	8.25	8.20	8.25	8.30
Cl-a (mg/L)	0.40 <sup>b</sup>	0.30	0.40 <sup>b</sup>	0.40	0.33	0.20	0.20	0.25	0.33	0.40 <sup>b</sup>	0.40 <sup>b</sup>	0.60
Ca <sup>++a</sup> (mg/L)	42.0 <sup>b</sup>	43.9	42.0 <sup>b</sup>	35.8	30.6	40.0	40.0	41.2	41.3	42.0 <sup>b</sup>	42.0 <sup>b</sup>	42.6
FPH	1.25	1.40	1.47	0.91	1.07	1.40	1.40	1.56	1.32	1.25	1.32	1.40
FCI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FCa	0.76	0.78	0.76	0.70	0.64	0.66	0.74	0.75	0.75	0.75	0.76	0.76
FAV(mg/L)	0.24	0.27	0.28	0.13	0.14	0.16	0.21	0.23	0.20	0.19	0.25	0.27
[Maximum] <sup>c</sup>	0.12	0.14	0.14	0.07	0.07	0.08	0.11	0.12	0.10	0.10	0.13	0.14
[96 hr $\bar{x}$ ]	0.024	0.027	0.028	0.013	0.014	0.016	0.021	0.023	0.020	0.019	0.025	0.027

a) From Sheehan et. al. 1985.

b) Estimated values.

c) No-effect levels of nitrite are reported as maximum and 96-hour arithmetic mean concentrations.

Figure 18. Calculated Final Acute Value of Nitrite for Mountain Whitefish in the Flathead River System.



Development of water quality criteria for nitrite also requires detailed information on biotic responses to sub-lethal doses in order to predict no effect levels for a particular watershed. A review of the relevant literature on acute and chronic toxicity of nitrite to rainbow (Perrone and Meade 1977) and cutthroat trout (Thurston et al. 1978) suggests an acute:chronic ratio of approximately two. Long-term  $LC_{50}$  values (36 days) were similar to those reported for 5 to 7 day periods, indicating that resistance might begin to develop within fish after that period of time (Lewis and Morris 1986). Information available on sub-lethal effects, such as growth suppression and tissue damage, indicates that there is, as yet, no evidence to suggest that nitrite concentrations equal to or less than 10% of the 96 hr  $LC_{50}$  would be detrimental to freshwater fishes (Lewis and Morris, 1986).

No-effect levels of nitrite-nitrogen were then derived using the monthly site-specific FAV's calculated for the three important sportfish species together with information available on acute:chronic ratios and sub-lethal effects. Lewis and Morris (1986) reported that the ratio of 24 hr  $LC_{50}$  values to 96 hr  $LC_{50}$  values has a median value of 2.0 and is fairly uniform across species. These data suggest that, while freshwater fish species exhibit a wide range of sensitivities, the general pattern of response to elevated levels of nitrite is similar. Therefore, the same approach to criteria development was used for each of the three species of salmonid. Specifically, the maximum levels of nitrite-nitrogen thought to result in no negative effects to fish and aquatic life resources were established using the guidelines proposed by the

U.S. Environmental Protection Agency by calculating the level of 0.5 of the monthly FAV (Stephan et al. 1985). The average 96 hr concentration thought to result in no negative effects to fish and aquatic life resources was tentatively set at 0.1 of the monthly FAV to take into account potential sub-lethal effects and to provide a reasonable margin of safety since acute:safe ratios have not been established definitively. The site-specific water quality criteria so developed are designed to minimize the potential for extended exposures to chronically toxic or unsafe levels of nitrite. No-effect levels of  $\text{NO}_2^-$ -N in Howell Creek and the Flathead River mainstem are presented in Table 12 and Figure 19.

### 3.3.3 Ammonia

Under natural conditions, ammonia is formed upon decomposition of nitrogenous organic matter. Most naturally occurring ammonia is produced by bacterial action on amino acids or polypeptide chains. In solution, a dynamic equilibrium is established between the  $\text{NH}_3$  (un-ionized ammonia) and  $\text{NH}_4^+$  (ionized ammonium) species. The equilibrium is dependent on both pH and temperature and, as such, these two factors primarily determine the relative proportions of each of the two forms (Thurston et al. 1974). The relationship is expressed by the equation:

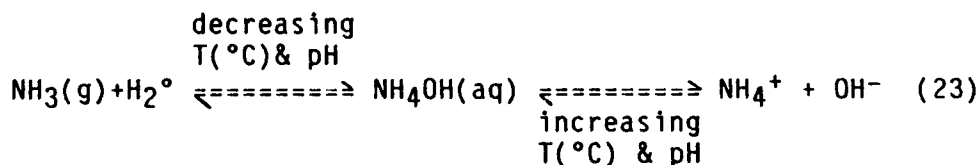
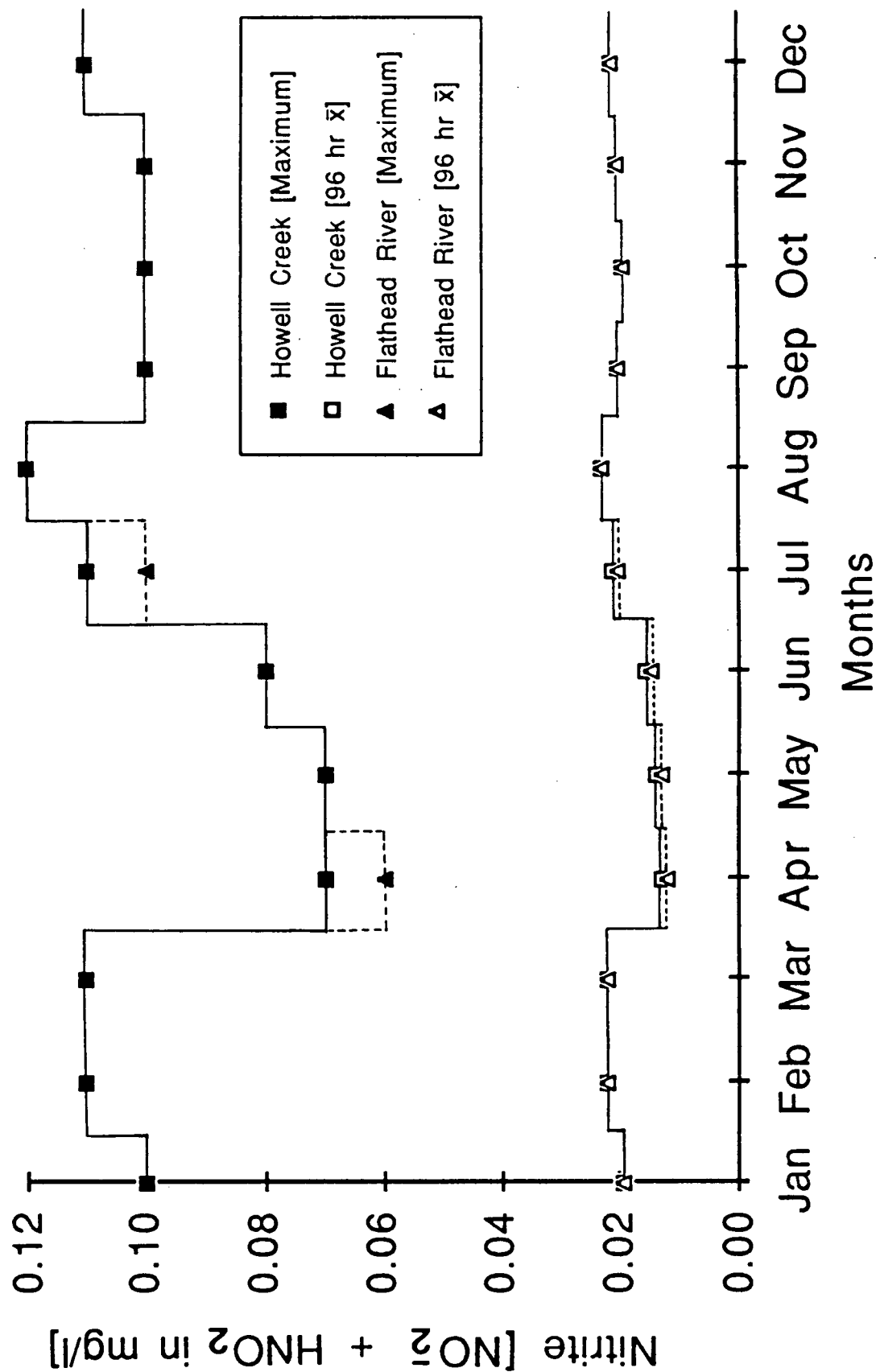


TABLE 12  
CALCULATED NO-EFFECT LEVELS OF  
NITRITE IN THE FLATHEAD RIVER WATERSHED

Month	Acceptable Levels of Nitrite ( $\text{NO}_2^- + \text{HNO}_2$ in mg/L)			
	Flathead River		Howell Creek	
	[Maximum]	[96 hr $\bar{x}$ ]	[Maximum]	[96 hr $\bar{x}$ ]
January	0.10	0.019	0.10	0.019
February	0.11	0.022	0.11	0.022
March	0.11	0.022	0.11	0.022
April	0.06	0.012	0.07	0.013
May	0.07	0.013	0.07	0.014
June	0.08	0.015	0.08	0.016
July	0.10	0.020	0.11	0.021
August	0.12	0.023	0.12	0.023
September	0.10	0.020	0.10	0.020
October	0.10	0.019	0.10	0.019
November	0.10	0.020	0.10	0.020
December	0.11	0.021	0.11	0.021

Figure 19. Calculated No Effect Levels of Nitrite in the Flathead River System.





### 3.3.3.1 Fish

#### 3.3.3.1.1 Mode of Toxic Action

The symptoms of acute ammonia toxicity in fish include hyperventilation and violent, erratic movements with convulsions, followed by coma and finally death (Smart 1975). Chronic exposures to elevated levels of environmental ammonia result in reduced growth rates (Brockway 1950), reduced stamina, increased susceptibility to bacterial gill infections, and proliferation (Burrows 1964) and thickening of gill lamellae (Larmoyeaux and Piper 1973). In addition, blood vessel lesions and tissue disintegration (Flis 1968), reduced haemoglobin (Buckley 1978) and dorsal aortic blood oxygen levels (Smart 1978), osmoregulatory problems (Lloyd and Orr 1969), and electron transport chain and Krebs cycle impairment (Ariello et al. 1981) have been reported in fish exposed to sub-lethal levels of ammonia.

The major problems associated with elevated levels of ammonia seem to stem from two sources. First, unprotonated ammonia ( $\text{NH}_3$ ) molecules 'seek' to complete their more stable, octet configuration (ie. tetrahydral form) by 'picking up' a hydrogen ion. The removal of a proton from solution causes localized alkalinity or, in other words, an increase in pH. Alkaline conditions tend to decrease membrane stability (and therefore gas and metabolic waste product transport and ionic balance maintenance efficiency), alter the calcium dynamics in the sacroplasmic reticulum (thereby generating locomotory problems), and impair oxidative metabolism (Hochachka and Somero 1973). Second, high ammonia levels exert a direct effect on the kinetics of the ornithine cycle, Krebs cycle and the electron transport series, and thereby disrupt cellular energy metabolism.

Disruption of membrane stability with increasing ammonia concentrations can increase the permeability of freshwater fish to water (Lloyd and Orr 1969). Increased absorption of water affects osmoregulatory processes, imposes stress on the kidneys, and thereby results in problems with the blood system and tissues.

Efficient and coordinated contraction of skeletal muscles in vertebrates is dependent on the tight regulation of  $\text{Ca}^{++}$  ion (which triggers muscle contractions) concentrations in the myoplasm. This regulation is the result of a process whereby  $\text{Ca}^{++}$  ions are sequestered in the sarcoplasmic reticulum (SR) until released in response to electrical neural impulses. An efficient calcium pump then returns the  $\text{Ca}^{++}$  ions to the SR against a strong concentration gradient (Hoar 1975). It is likely that high levels of  $\text{NH}_3$  affect the stability of the SR membrane, rendering it 'leaky' to  $\text{Ca}^{++}$  ions, and, therefore, result in the erratic, uncoordinated muscle contractions associated with convulsions.

Disruption of oxidative metabolism due to high un-ionized ammonia levels is the end result of decreased oxygen delivery and cellular utilization efficiency. Brockway (1950) and Ellis (1937) reported severe reductions in blood oxygen levels of trout exposed to high levels of ammonia, and suggested that ammonia may inhibit the binding of oxygen to haemoglobin. Decreases in the number of circulating erythrocytes in trout (Reichenbach-Klinke 1967) and haemolysis in carp (Danecker 1964) have also been reported after exposures to ammonia. Reduced oxygen delivery to the tissues also results from  $\text{NH}_3$  induced pH increases in the capillaries. Under normal conditions, metabolically generated  $\text{CO}_2$  creates more acidic conditions in the tissues than in the gills where the oxygen is 'loaded' onto the Hb

molecule. 'Unloading' of oxygen in the tissues is then facilitated because the affinity of Hb for oxygen and the oxygen carrying capacity of Hb drop with decreasing pH (Bohr and Root effects, respectively). Elevated levels of  $\text{NH}_3$  in the tissues markedly disrupt the normal pH shift and thereby interfere with the unloading of oxygen.

At the cellular level, high levels of  $\text{NH}_3$  affect energy metabolism, both in the cytoplasm and in the mitochondria. Within the mitochondria, electron transport chain-linked ATP synthesis is reduced as a consequence of NADH depletion due to: (i) excess glutamate production, (ii) malate-aspartate shuttle inhibition, and (iii) Krebs cycle impairment (Arillo et al. 1981). Within the cytoplasm, glutamine production from glutamate further reduces available ATP reserves. ATP depletion stimulates the glycolytic breakdown of storage polysaccharides to pyruvate and finally lactate (Campbell 1973). An increased cytosolic lactate: pyruvate ratio is a clear indication of oxygen debt and anoxic stress. It is likely that impairment of energy metabolic processes is the primary mode of toxic action of ammonia, with the other factors contributing to bring about mortality.

While it is clear that elevated blood ammonia levels contribute strongly to the overall toxicity of  $\text{NH}_3$ , there is still some question about how high environmental ammonia levels affect blood  $\text{NH}_3$  levels. Many researchers (Smart 1975; Wuhrmann and Woker 1948; Hillaby and Randall 1979) have suggested that environmental ammonia moves freely across the gill surface and enters the blood at that point. Other researchers (V. Thurston, Department of Chemistry, Montana State University, Bozeman, Montana personal communication) have indicated that high concentrations of ammonia in the water result in reduced rates of excretion of

metabolically produced ammonia due to the elimination of the substantial concentration gradient that is normally in evidence. This, in turn, leads to elevated blood, cytosolic and mitochondrial  $\text{NH}_3$  levels. It is likely that both of these mechanisms are important under various environmental conditions. Increases in environmental  $\text{NH}_3$  levels above those levels evident in the blood would result diffusatory movements of  $\text{NH}_3$  into the blood until the levels in the water and the blood equilibrated. Additional  $\text{NH}_3$  in blood would subsequently result from metabolic sources (Cameron 1986). Less substantial increases in environmental  $\text{NH}_3$  would reduce concentration gradients and, therefore, result in high metabolically produced blood  $\text{NH}_3$  levels.

### 3.3.3.1.2. Abiotic Factors Affecting Ammonia Toxicity

The toxicity of ammonia to fish can largely be attributed to the un-ionized ( $\text{NH}_3$ ) form (Downing and Merckens 1955). The fraction of the total ammonia that is present in the  $\text{NH}_3$  form is dependent on pH and temperature. Emerson et al. (1975) quantified the relationship according to the following equations:

$$\text{pKa} = 0.0901821 + \frac{2729.92}{T(^{\circ}\text{C}) + 273.16} \quad (24)$$

$$f = \frac{1}{[10(\text{pKa} - \text{pH}) + 1]} \quad (25)$$

$$\text{NH}_3 = f [\text{total ammonia}] \quad (26)$$

where  $f$  is the fraction of un-ionized ammonia,  
 $\text{pKa}$  is the negative log of the ionization constant,  
 $T$  is temperature in degrees Celcius.

The toxicity of this  $\text{NH}_3$  (ammonia) fraction to fish is further modified by the physical and chemical characteristics of the water body under study.

a) Dissolved Oxygen

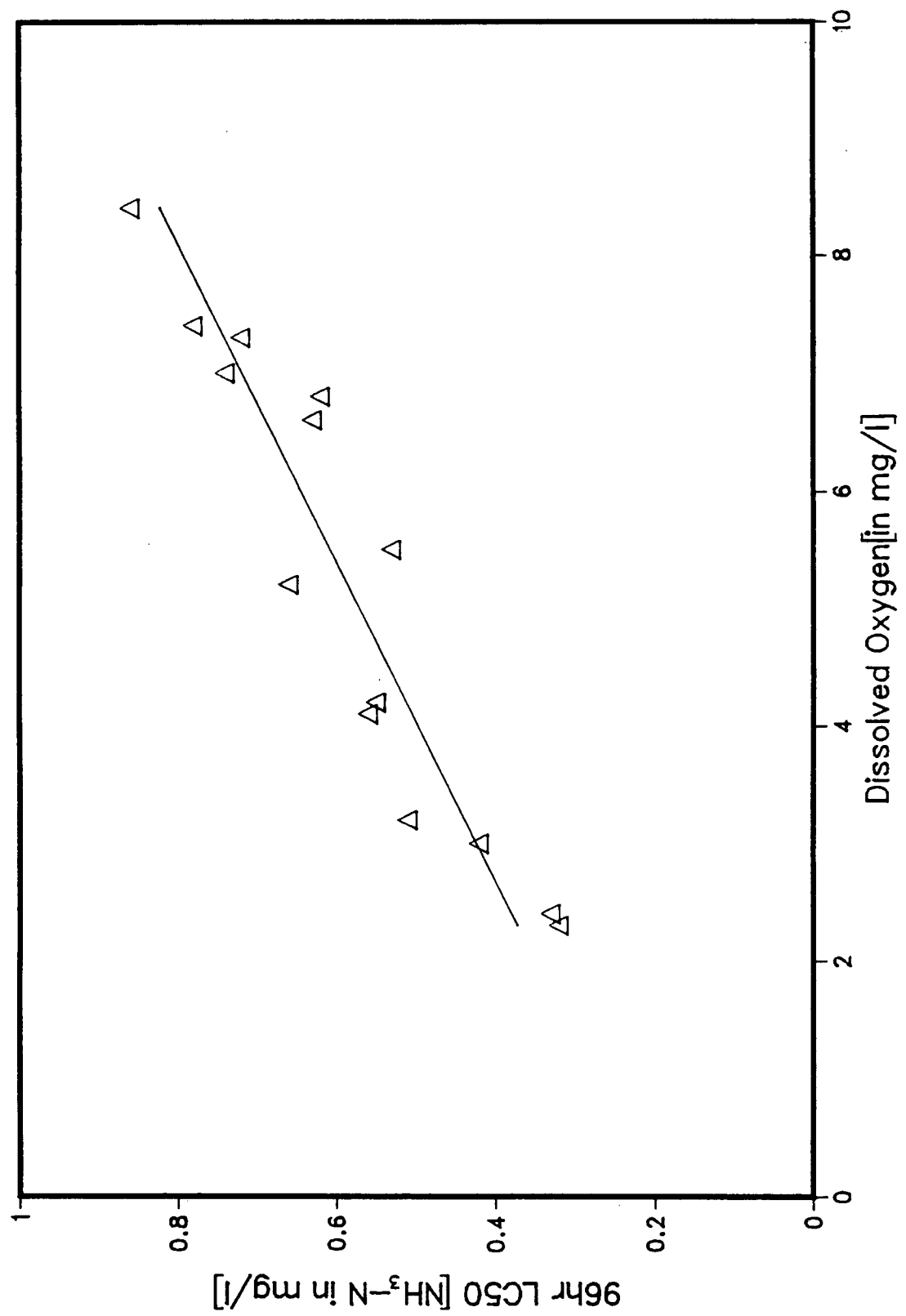
As stated earlier, elevated levels of un-ionized ammonia are disruptive to oxidative metabolic processes in fish, both in terms of delivery and utilization. It therefore follows that the toxicity of  $\text{NH}_3$  would be increased at low dissolved oxygen (D.O.) concentrations. Downing and Merkens (1955) examined the influence of D.O. on survival times of rainbow trout exposed to toxic concentrations of un-ionized ammonia. In that study, a reduction of D.O. in the water to 50% saturation reduced the survival time of rainbow trout by two-thirds. Lloyd (1961) demonstrated that low oxygen tensions (40% saturation) increased the toxicity of  $\text{NH}_3$  to trout by up to 2.5 times. Similarly, Danecker (1964) reported a rapid increase in the toxicity of un-ionized ammonia when dissolved oxygen dropped below two-thirds of the saturation value. A detailed study of the toxicity of  $\text{NH}_3$  on rainbow trout fingerlings (Thurston et al. 1981b) indicated that 96 hr  $\text{LC}_{50}$ s were strongly, linearly correlated to dissolved oxygen levels over the 2.5 - 9.0 mg/L D.O. range (Figure 20). The relationship was described by the following equation:

$$\text{LC}_{50}(\text{D.O.}) = \text{LC}_{50}(\text{D.O.}=8.0 \text{ mg/L}) - 0.067(8.0\text{mg/L}-\text{D.O.}) \quad (27)$$

b) Temperature

The toxicity of ammonia to freshwater fish is affected by temperature in at least two ways. Firstly, the relative proportion of the more toxic  $\text{NH}_3$  fraction changes readily in response to changes in water temperature in accordance with the equations (24-26) presented above. Secondly, the toxicity of the  $\text{NH}_3$

Figure 20. Effect of dissolved oxygen on the toxicity of ammonia to rainbow trout [Thurston et al. 1981]



fraction tends to increase as the water temperature decreases (Burrows 1964). Brown (1968) suggested a two-fold increase in the threshold  $LC_{50}$  of un-ionized ammonia for rainbow trout when the water temperature was increased from 3° to 10°C. Similar results were obtained by Thurston and Russo (1983) with rainbow trout in the 7 - 12 g range, with the 96 hr.  $LC_{50}$  increasing approximately three-fold over the temperature range of 10° to 19°C. Recent analysis of available data (Erickson, 1985) indicated that the effect of temperature on the toxicity of un-ionized ammonia to different species of fish is very similar. The equations:

$$LC_{50}(T^{\circ}C) = LC_{50}(10^{\circ}C) \cdot 10^{0.03(T^{\circ}C-10)}; T=0-10^{\circ}C \quad (27)$$

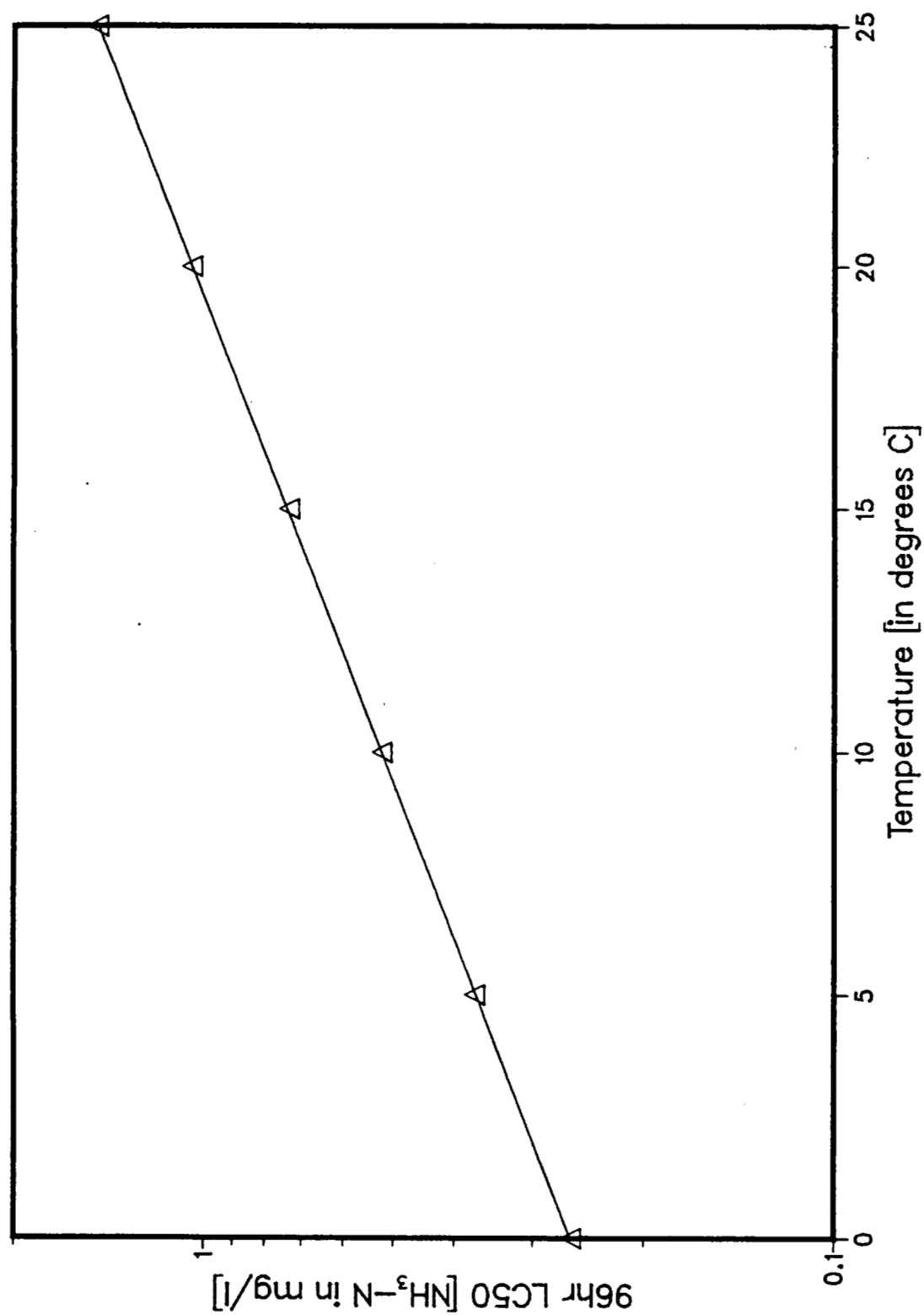
$$= LC_{50}(10^{\circ}C) \cdot 10^{0.03(10-T^{\circ}C)}; T=10-20^{\circ}C \quad (28)$$

describe the temperature dependence of ammonia ( $NH_3$ ) toxicity for freshwater fishes (Figure 21).

c) Hydrogen Ion Concentration

The effect of pH on the toxicity of ammonia to freshwater fish is substantial, with  $LC_{50}$ s declining by as much as an order of magnitude per unit pH increase (Environmental Protection Agency 1985). The bulk of this variation is due to the effect of pH on the  $NH_3 - NH_4^+$  equilibrium, with increases in pH favouring the more toxic  $NH_3$  form. However, when the toxicity is expressed on the basis of un-ionized ammonia it becomes evident that some residual pH dependence exists. In general,  $LC_{50}$ 's for  $NH_3$  increase with increasing pH, with the curve leveling off at high pH values (Thurston et al. 1981c).

Figure 21. Effect of temperature on the toxicity of ammonia to freshwater fish [Erickson 1985].





Regression analysis of pooled data sets for Daphnia sp., rainbow trout (Figure 22), coho salmon (Figure 23), and fathead minnow (Erickson, 1985) resulted in the following empirical model to describe pH dependence of  $\text{NH}_3$  toxicity:

$$\text{LC}_{50}(\text{pH}) = \text{LIM} / [1 + 10^{\text{SLP}(\text{PHT}-\text{pH})}] \quad (13)$$

where LIM is the asymptotic  $\text{LC}_{50}$  at high pH.  
 SLP is the asymptotic slope at low pH.  
 PHT is a transition pH that contributes to the definition of the curve.

Common values for SLP(1.03) and PHT(7.32), obtained from the pooled data analysis, yielded good results for regression analysis of individual data sets. Therefore, calculation of  $\text{LC}_{50}$  values of  $\text{NH}_3$  for a given pH required the input of only one data set dependent variable (LIM). However, this model did not incorporate indications in some data sets that  $\text{LC}_{50}$ 's decline as the pH increases over 8.5 (Environmental Protection Agency 1985). Therefore, calculation of FAVs for water systems with high ambient pH values could require some degree of interpolation. Modification of this model to account for the joint toxicity of  $\text{NH}_3$  and  $\text{NH}_4^+$  (ie. assumed SLP=1.0) resulted in the following:

$$\text{LC}_{50}(\text{pH}) = \text{LC}_{50}(\text{pH}=8.0) \quad ; \text{pH} \geq 8.0 \quad (29)$$

$$\text{LC}_{50}(\text{pH}) = \text{LC}_{50}(\text{pH}=8.0) \cdot \frac{1+10^{\text{PHT}-8}}{1+10^{\text{PHT}-\text{pH}}} \quad ; \text{pH} < 8.0 \quad (30)$$

Figure 22. Effect of pH on the toxicity of ammonia to rainbow trout [Environmental Protection Agency 1985].

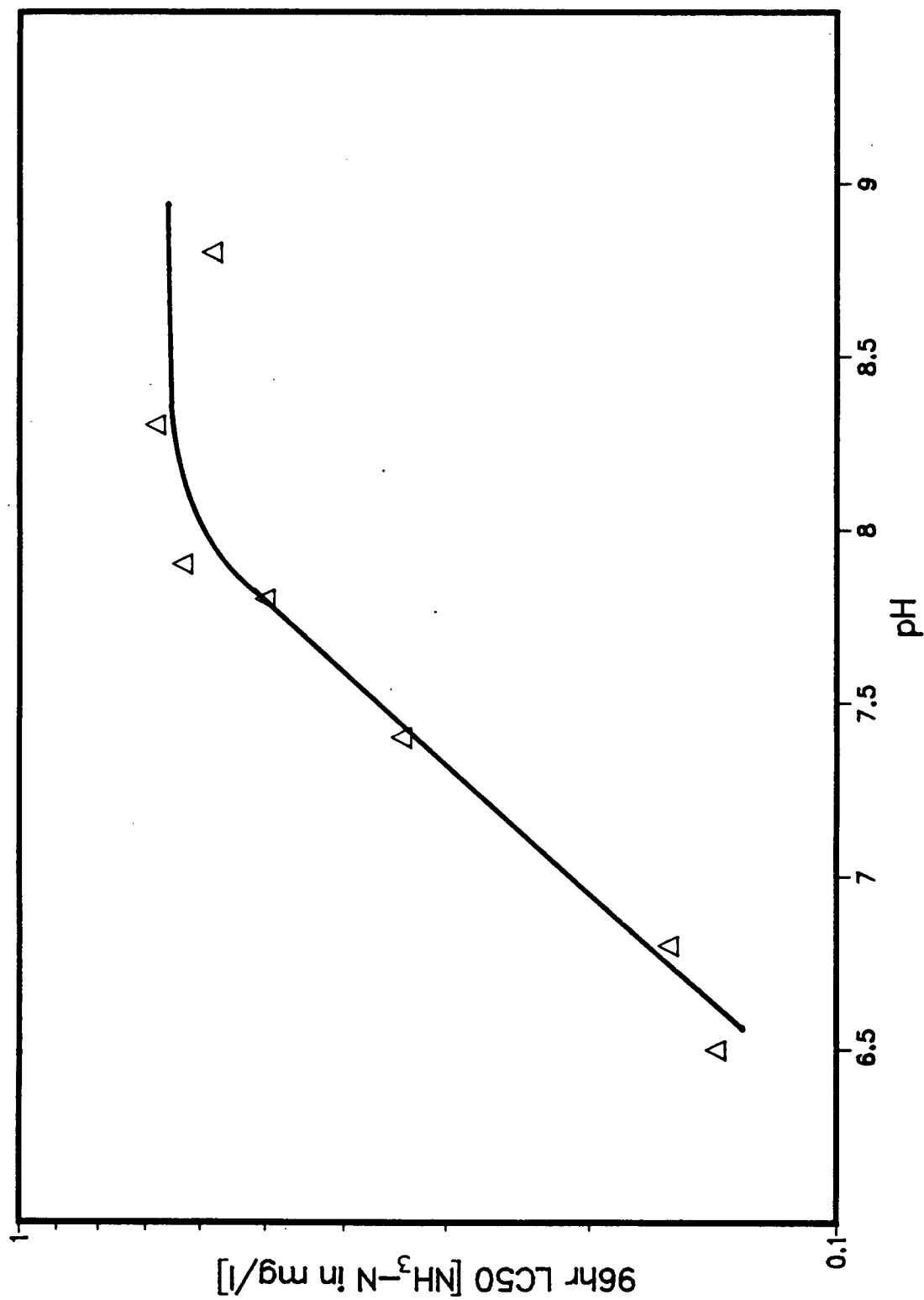
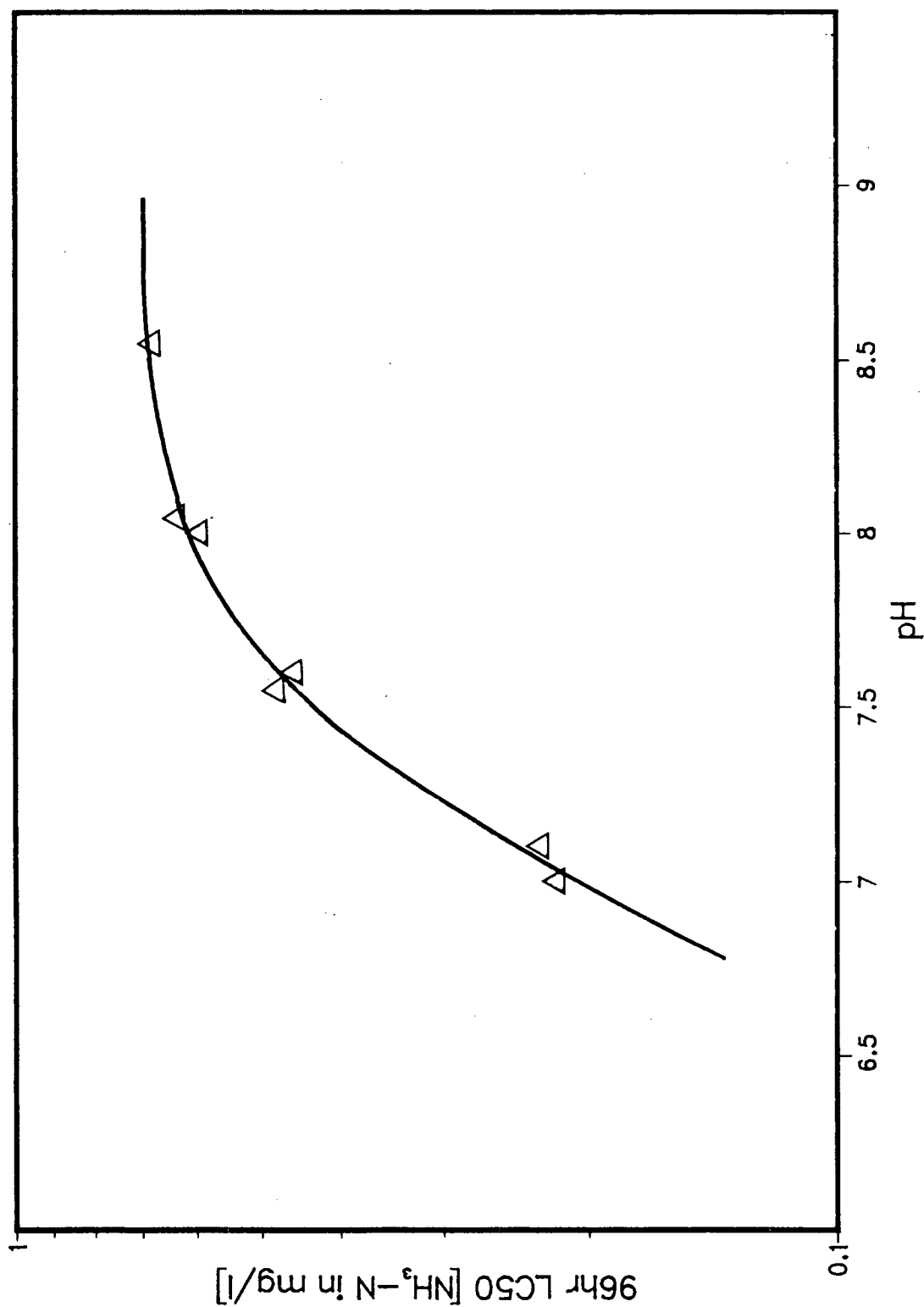


Figure 23. Effect of pH on the toxicity of ammonia to coho salmon [Environmental Protection Agency 1985].



Using the modified model, regression analysis of pooled data sets resulted in an estimate of 7.4 for the parameter PHT (Environmental Protection Agency 1985). Simplification of the model represented above results in the final relationship:

$$LC_{50}(pH) = LC_{50}(pH=8.0) \quad ; pH \geq 8.0 \quad (31)$$

$$LC_{50}(pH) = \frac{LC_{50}(pH=8.0) \cdot 1.25}{1 + 10^{7.4 - pH}} \quad ; pH < 8.0 \quad (32)$$

d) Carbon Dioxide

Alabaster and Herbert (1954) demonstrated that the toxicity of total ammonia to freshwater fish is reduced by increasing the level of free carbon dioxide ( $CO_2$ ) in the water. This effect results primarily from the influence of high levels of  $CO_2$  on pH. Decreases in pH result in decreases in the proportion of un-ionized ammonia in solution, and therefore reduced toxicity. The significance of metabolic  $CO_2$  excretion in terms of mitigating the effects of high  $NH_3$  levels remains in question. Broderius et al. (1977) suggest that the uncatalyzed  $CO_2$  hydration reaction is too slow, within normal temperature and pH ranges, to produce significant numbers of protons while that water is in contact with the gill lamellae. Szumski et al. (1982) hypothesized that  $CO_2$  is excreted as  $H^+$  and  $HCO_3^-$  at the gill epithelium, thereby making protons immediately available to ionize  $NH_3$  molecules. Recent studies (L. Fidler, unpublished data) on the mechanisms of  $CO_2$  excretion in fish have identified the presence of carbonic anhydrase in the mucous of the skin and gill epithelia. The levels of this carbonic anhydrase (which catalyzes the  $CO_2$  hydration reaction) are fairly

low, however, indicating that simple diffusion probably provides the mechanism of excretion for the bulk of the metabolically produced  $\text{CO}_2$  (Perry 1986). Still, metabolic processes likely provide a limited number of protons to partially mediate the effects of high levels of  $\text{NH}_3$ . However, at low fish densities, characteristic of natural freshwater systems, no additional protection against  $\text{NH}_3$  is anticipated due to metabolically produced  $\text{CO}_2$ , and therefore, this factor will not be used in criteria development.

e) Fluctuating Exposures

The influence of fluctuating levels of un-ionized ammonia on toxicity to freshwater fish is an important consideration when releases of nitrogen compounds to the aquatic environment are anticipated to be episodic in nature. To assess the significance of short-term cyclic fluctuations of ammonia, Thurston et al. (1981a) conducted acute toxicity tests on rainbow and cutthroat trout. Companion tests were also run using constant concentrations of ammonia. When  $\text{LC}_{50}$  values for constant and fluctuating levels of un-ionized ammonia were compared, based on total dose exposure, it was found that fish were more tolerant of constant concentrations. The magnitude of the effect of fluctuating exposures on toxicity is difficult to assess. However, Brown et al. (1969) reported that the mean survival time of rainbow trout could be reduced by as much as 50% when fish are subjected to fluctuating rather than constant levels of  $\text{NH}_3$ .

3.3.3.1.3 Biotic Factors Affecting Ammonia Toxicity

It is likely that a number of biotic factors act in concert to modify the effects of elevated levels of environmental

ammonia ( $\text{NH}_3$ ) on freshwater fish. The most significant of these factors are species, life history stage and acclimation periods.

a) Species

An extensive review of ammonia literature (Environmental Protection Agency 1985) indicated acute toxicity to freshwater organisms at concentrations (uncorrected for pH) ranging from 0.083 to 4.60 mg/L  $\text{NH}_3$ -N for 23 fish species from nine families. Among fish species, salmonids were most sensitive with 96 hr  $\text{LC}_{50}$  values ranging from 0.083 to 1.09 mg/L  $\text{NH}_3$ -N. Data on nine freshwater fish species (from five families) indicated chronic effects of exposure to ammonia at concentrations ranging from 0.0017 to 0.612 mg/L  $\text{NH}_3$ -N, with salmonids again being the most sensitive. These data suggest that salmonid fish are likely the most sensitive ecosystem component with respect to  $\text{NH}_3$  toxicity. Within the family salmonidae, there is some evidence to suggest that Oncorhynchus is more sensitive than Salmo. Limited relevant information on the relative sensitivity of Salvelinus and Prosopium (Thurston and Meyn 1984) indicates that chars may be slightly more resistant than, and whitefish approximately as resistant as, trout to  $\text{NH}_3$ .

b) Life History Stage

Incubating salmonid eggs appear to be relatively resistant to exposures to un-ionized ammonia. Burkhalter and Kaya (1977) exposed rainbow trout eggs to concentrations of  $\text{NH}_3$ -N in the 0.05 - 0.37 mg/L range from fertilization to hatching without affecting survival rates. Similar results were obtained with rainbow trout eggs continuously exposed to 0.00 - 0.077

mg/L  $\text{NH}_3\text{-N}$  (Thurston et al. 1984). Short-term exposure (96 hr) of pink salmon (O. gorbuscha) eggs to levels of  $\text{NH}_3\text{-N}$  in excess of 1.5 mg/L (Rice and Bailey, 1980) also had little effect on survival. In contrast, Rankin (1979) demonstrated that sockeye salmon (O. nerka) eggs cannot tolerate un-ionized ammonia concentrations greater than 0.403 mg/L  $\text{NH}_3\text{-N}$  for extended periods (ie. 60 days). Salmonid alevins are, very likely, the most sensitive life history stage with respect to ammonia toxicity. Rice and Bailey (1980) reported 96 hr  $\text{LC}_{50}$  values for early and late alevins to be 0.33 and 0.083 mg/L  $\text{NH}_3\text{-N}$  respectively. Sensitivity to elevated levels of  $\text{NH}_3$  decreased after emergence (Figure 24). General inhibition of development and failure to absorb the yolk-sac was observed in rainbow trout exposed to  $\text{NH}_3\text{-N}$  levels of 0.19 mg/L or higher. Thurston and Russo (1983) conducted a total of 71 acute toxicity tests on rainbow trout to delineate the relationship between fish weight and 96 hr  $\text{LC}_{50}$  value. The results of that study indicate that resistance to exposures to  $\text{NH}_3$  increases from emergence to a maximum at the 1.0-10.0 g size. Resistance decreases thereafter as fish weight increases. Figure 25 details the relationship between sensitivity to un-ionized ammonia and fish weight.

c) Acclimation to Low Ammonia Concentrations

Acclimation of fish to low levels of un-ionized ammonia is important primarily because it might enable the fish to survive exposures to  $\text{NH}_3$  that would otherwise be acutely lethal. Lloyd and Orr (1969) conducted acclimation experiments on rainbow trout and concluded that resistance to  $\text{NH}_3$  is maintained for at least one day after exposure, but is lost after three days.

Figure 24. Effect of developmental stage on the toxicity of ammonia to pink salmon [Rice and Bailey 1980]

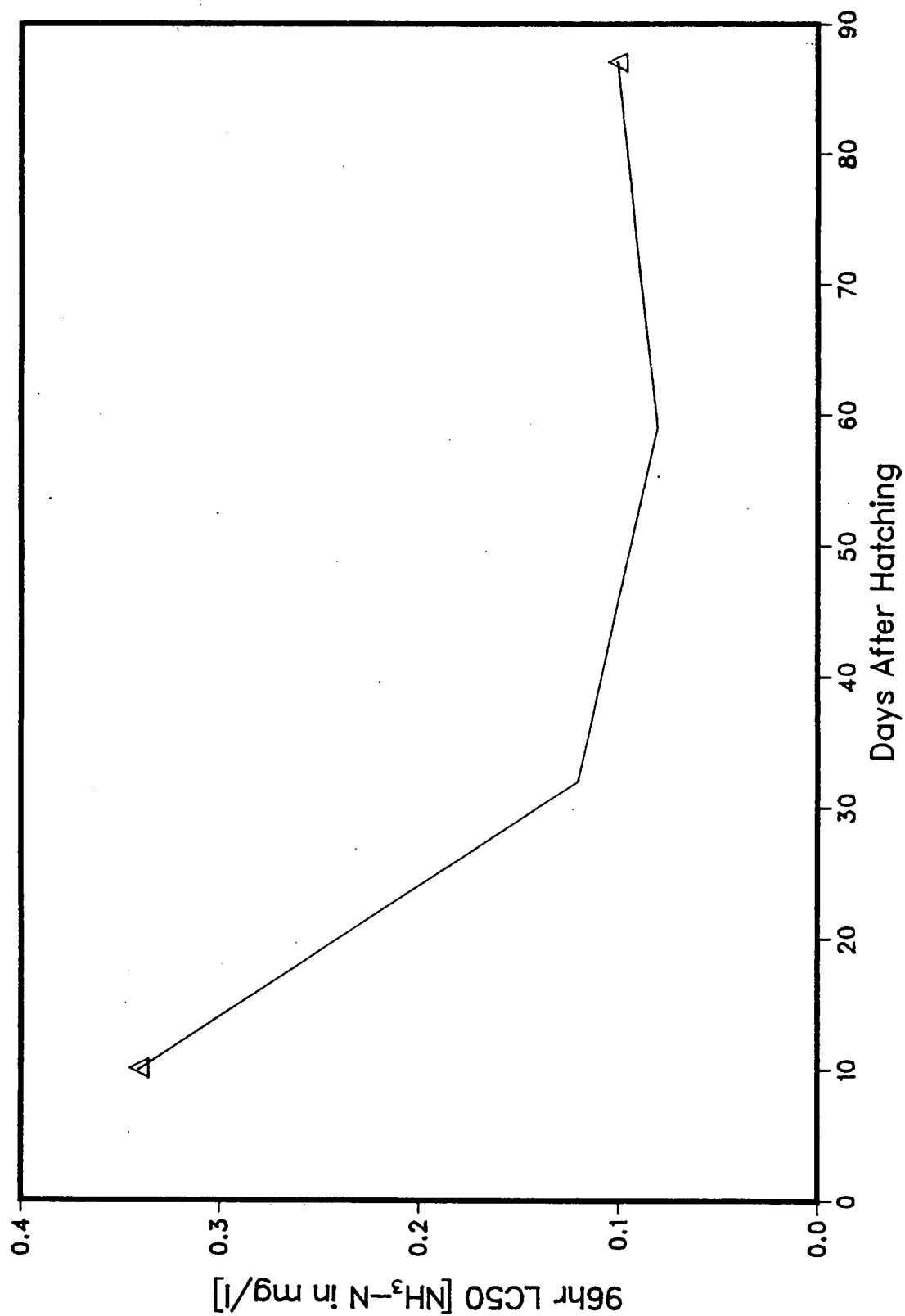
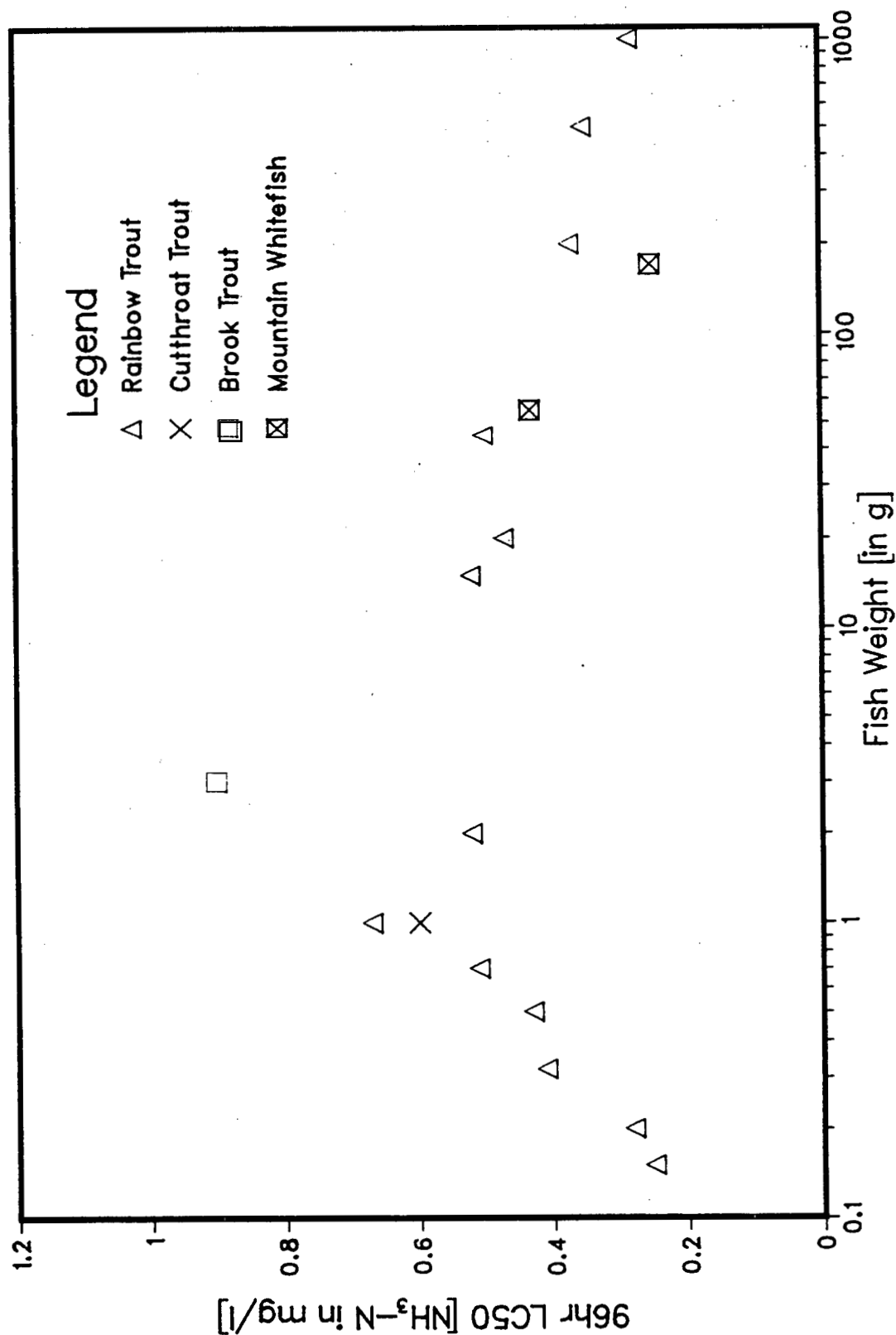




Figure 25. Effect of fish weight on the toxicity of ammonia to salmonid fish [data from various sources standardized to reference conditions: pH=8.0, T=10 °C, DO=8.0 mg/l].



Schulze-Wiehenbrauck (1976) acclimated juvenile rainbow trout to approximately one-third of the 8.5 hr  $LC_{50}$  value (0.45 mg/L  $NH_3$ -N). When test fish were subsequently exposed to 0.45 mg/L  $NH_3$ -N for 8.5 hrs 100 percent of the fish survived. In contrast, control fish had only 50 percent survival at that concentration of un-ionized ammonia. Atlantic salmon smolts acclimated to ammonia (Alabaster et al. 1979) also acquired increased resistance to high ammonia levels. Acclimated fish survived exposures to toxic levels of  $NH_3$  better than control fish, with 24 hr  $LC_{50}$  values 38-79% higher than those for fish without prior ammonia acclimation. It is, therefore, evident that salmonids acquire some degree of resistance to high levels of environmental  $NH_3$  through prior exposure to sub-lethal levels, with the protection afforded being only temporary in nature.

#### 3.3.3.2 Invertebrates

Several studies on the toxicity of ammonia indicate that freshwater invertebrates are generally less sensitive than fish, with 96 hr  $LC_{50}$  values ranging from 0.53 to 8.00 mg/L for invertebrate species representing nine families (Environmental Protection Agency 1985). Fish should be the focus of criteria development (Nordin and Pommen, 1986). Thus, criteria for freshwater invertebrates for ammonia toxicity have not been developed. The criteria developed for freshwater fish are considered to indicate no effect conditions for freshwater invertebrates as well.

#### 3.3.3.3 Algae

With few exceptions, algae can use ammonia (as  $NH_4^+$ ) directly for the synthesis of cellular material (Brown et al. 1974; Brezonik 1977). Fogg (1953) and Syrett (1962)

reported that, when  $\text{NO}_3^-$  and  $\text{NH}_4^+$  are supplied together,  $\text{NH}_4^+$  is assimilated by most algae preferentially to  $\text{NO}_3^-$ , and that  $\text{NO}_3^-$  is used only after all the  $\text{NH}_4^+$  is exhausted. This is understandable because the reduction of  $\text{NO}_3^-$  requires an expenditure of metabolic energy (Vollenweider 1968). Therefore, additions of ammonia can be expected to provide the periphyton community with a highly available nitrogen source and, therefore, to result in increases in growth rates and in biomass if other environmental factors are not limiting.

There has been little work on the toxicity of ammonia to freshwater algae. A review (Nordin and Pommen, 1986) indicates that, although some depression of photo-assimilation rates can occur at fairly low levels of ammonia (0.6 mg/L), in general algae are fairly resistant to ammonia toxicity. Since the criteria proposed for fish are adequate to protect algal species, no additional criteria specifically for algae are developed. Criteria for ammonia relevant to eutrophication of affected streams are proposed in a report on algal nutrients currently in preparation.

#### 3.3.3.4 Site-Specific Criteria for Un-ionized Ammonia

There is a great deal more to ammonia toxicity than can be related by a single criterion value. It is necessary to integrate the abiotic and biotic factors that modify  $\text{NH}_3$  toxicity with specific information about ambient environmental conditions in the watershed in order to develop site-specific ammonia criteria.

The most important of these factors in the Flathead system are likely to be temperature, pH, D.O., species and life history stage affected. In the process of site-specific criteria development, it will be assumed that carbon dioxide

levels will remain low and that the effects of fluctuating exposures and acclimation will be roughly equal, and therefore cancel each other out. Temperature and pH profiles for the Flathead River and Howell Creek are presented in Figure 26 and 27, respectively. In the absence of site-specific information to the contrary, it will be assumed that dissolved gas levels are at or near saturation values in surface water throughout most of the year. The effect of dissolved oxygen concentration on ammonia toxicity will be included in the calculation of final acute values when the results of on-site measurements become available.

The following procedure was used to calculate the final acute toxicity value (FAV) for the three important species of sportfish found in the Canadian portion of the Flathead River system:

- i. Available 96 hr  $LC_{50}$  data for Salmo, Salvelinus and Prosopium were adjusted to reference conditions (pH=8.0, T=10°C, D.O.=8.0 mg/L) in accordance with equations 8-17 listed previously. These data were then plotted against fish weight to provide an estimate of sensitivity by life history stage. It was assumed that Prosopium and Salvelinus would demonstrate a similar life history sensitivity pattern to that of Salmo, and therefore missing data points for those species were interpolated (Figure 25).
- ii. Reference final acute values (FAVref.) for each life history stage were estimated directly from the sensitivity plots generated for each species (Table 13).
- iii. Final acute values were then calculated by integrating FAVref. information with site-specific temperature, pH

Figure 26. Summary of Available Water Temperature Data for the Flathead River System (Monthly Means).

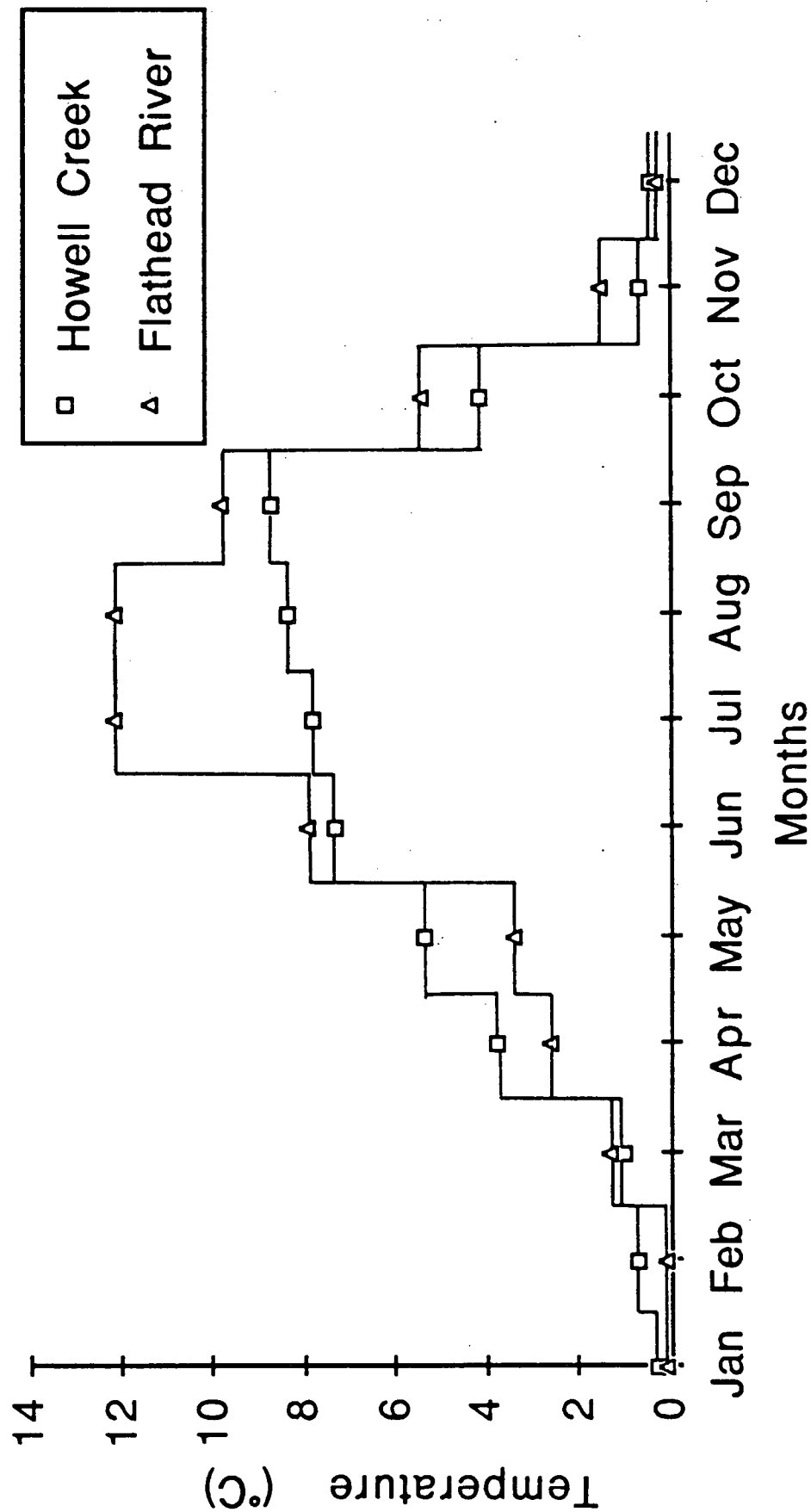


Figure 27. Summary of Available pH Data for the Flathead River System (Monthly Means).

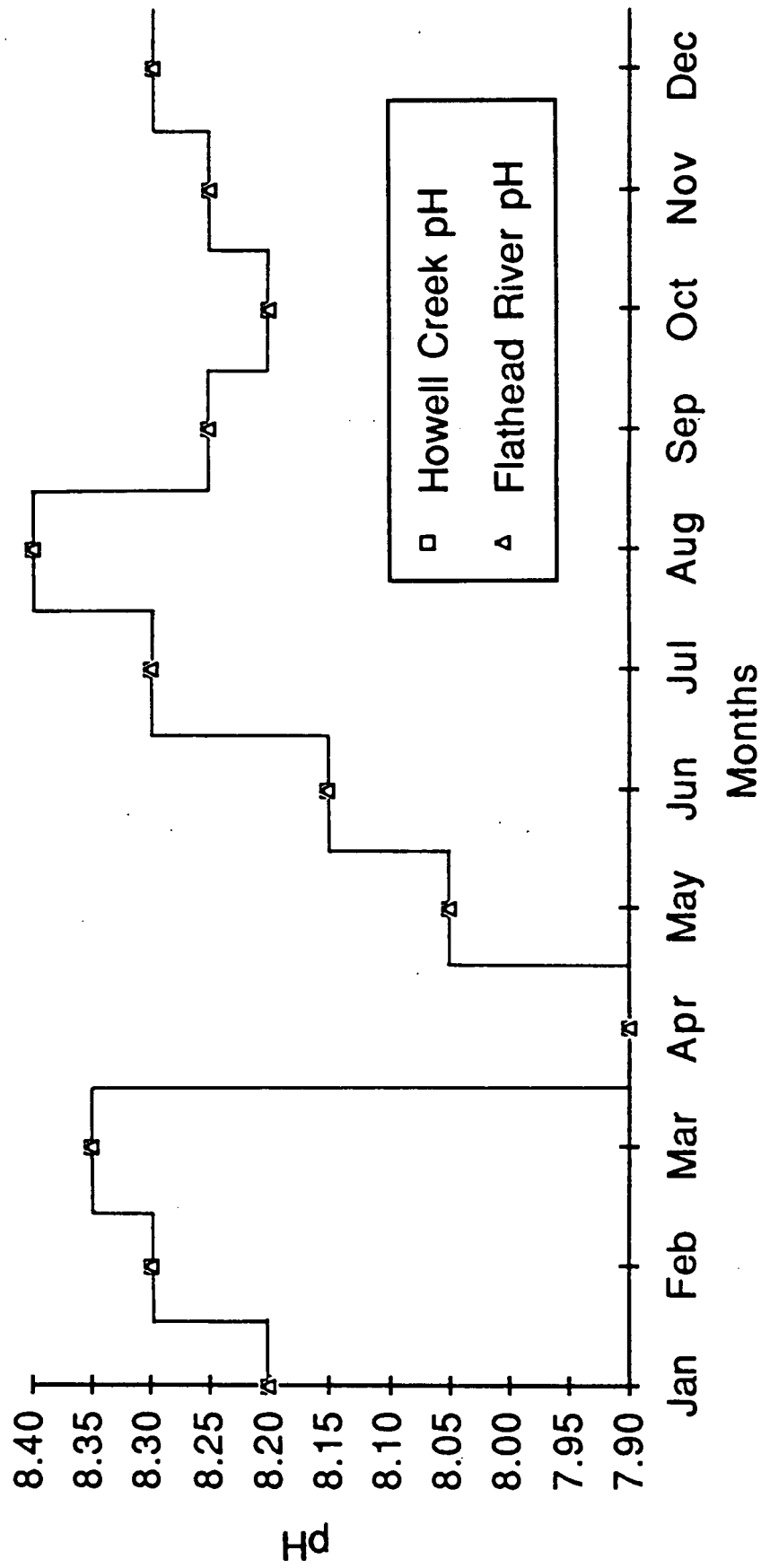


TABLE 13

REFERENCE FINAL ACUTE VALUES OF AMMONIA FOR KEY LIFE HISTORY STAGES  
OF IMPORTANT FLATHEAD RIVER SYSTEM FISH SPECIES

Species	Dates	Life History Stage	FAV ref. (mg/L) <sup>a)</sup>
Bull Trout	Sept. 1 - Jan. 30	Eggs	0.40
	Jan. 1 - Mar. 31	Early Alevins	0.30
	Mar. 15 - May 31	Late Alevins	0.20
	May 15 - June 15	Fry (to 0.25g)	0.40
	May 30 - June 30	Fry (to 0.50g)	0.65
	June 15 - July 15	Fry (to 1.00g)	0.80
	July 1 - June 30	Fry (to 10.0g)	0.80
	July 1 - June 30	Juveniles (to 50.0g)	0.70
	July 15 - Oct. 15	Adults	0.40
Cutthroat Trout	May 15 - July 20	Eggs	0.40
	July 5 - July 31	Early Alevins	0.30
	July 15 - Aug. 10	Late Alevins	0.20
	July 20 - Aug. 20	Fry (to 0.25g)	0.25
	July 31 - Aug. 31	Fry (to 0.50g)	0.35
	Aug. 15 - Sept. 20	Fry (to 1.00g)	0.50
	Sept. 1 - Aug. 31	Fry (to 10.0g)	0.55
	Sept. 1 - Aug. 31	Juveniles (to 50.0g)	0.50
	Sept. 1 - Aug. 31	Adults	0.35
Mountain Whitefish	Oct. 15 - Mar. 15	Eggs	0.40
	Mar. 1 - May 10	Early Alevins	0.30
	May 1 - May 30	Late Alevins	0.20
	May 15 - June 15	Fry (to 0.25g)	0.20
	June 15 - July 15	Fry (to 0.50g)	0.30
	July 1 - July 30	Fry (to 1.00g)	0.45
	July 1 - June 30	Fry (to 10.0g)	0.50
	July 1 - June 30	Juveniles (to 50.0g)	0.45
	July 1 - June 30	Adults	0.30

a) Reference conditions: pH=8.0, T=10°C, D.O.=8.0 mg/L

and dissolved oxygen data on a monthly basis in accordance with the following:

$$FAV(pH, T, D.O.) = [FAV_{ref.} \cdot FT \cdot FPH] - FDO \quad (33)$$

$$\text{where: } FT = 10.0^{0.03(T-10)} ; T=0-10^{\circ}C \quad (34)$$

$$= 10.0^{0.03(10-T)} ; T=10-20^{\circ}C \quad (35)$$

$$FPH = \frac{1.0}{1+10.0^{7.4-pH}} ; pH \geq 8.0 \quad (36)$$

$$= \frac{1.25}{1+10.0^{7.4-pH}} ; pH < 8.0 \quad (37)$$

$$FDO = 0.067(8.0-D.O.) ; D.O. < 10.0 \text{ mg/L} \quad (38)$$

$$= -0.134 ; D.O. > 10.0 \text{ mg/L} \quad (39)$$

Final acute values for bull trout are presented in Tables 14 and 15 for the Flathead River mainstem and Howell Creek respectively, and represented graphically in Figure 28. FAVs for cutthroat trout (Tables 16 and 17; Figure 29) and mountain whitefish (Tables 20 and 21; Figure 30) have also been calculated using this methodology. No effect levels of  $NH_3$ -N are reported as maximum and 96 hr arithmetic mean concentrations in Tables 14-19.

A review of the relevant literature on acute and chronic toxicity of ammonia to rainbow trout (Environmental Protection Agency 1985) indicated an acute to chronic ratio of approximately 14. Acute: chronic ratios for cutthroat and bull trout, and mountain whitefish were assumed to be similar to that of rainbow trout. Acceptable levels of un-ionized ammonia were, then, derived by applying Environmental Protection Agency (1985) safety factors for ammonia-nitrogen toxicants to the site-specific FAVs for the



TABLE 14

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF AMMONIA FOR FLATHEAD RIVER BULL TROUT<sup>a</sup>  
(See Text for Definition of Symbols)

Most Sensitive Life History Stage	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Juvenile	Juvenile	Juvenile	Adult	Adult	Adult	Adult	Adult	Juvenile	Adult	Adult	Juvenile
FAV <sub>ref</sub> (mg/L) <sup>b</sup>	0.70	0.70	0.70	0.40	0.40	0.40	0.40	0.40	0.70	0.40	0.40	0.70
pH <sup>c</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Temperature (°C) <sup>d</sup>	0.1	0.1	1.3	2.6	3.4	7.9	12.1	12.1	9.8	5.4	1.5	0.3
Dissolved Oxygen (mg/L)	No site-specific data currently available											
FPH	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FT	0.50	0.50	0.55	0.60	0.63	0.86	1.16	1.16	0.99	0.73	0.56	0.51
FDO	-	-	-	-	-	-	-	-	-	-	-	-
FAV(mg/L)	0.35	0.35	0.39	0.23	0.25	0.34	0.46	0.46	0.69	0.29	0.22	0.36
[Maximum] <sup>e</sup>	0.18	0.18	0.20	0.12	0.13	0.17	0.23	0.23	0.35	0.15	0.11	0.18
[96 hr $\bar{x}$ ]	0.018	0.018	0.020	0.012	0.013	0.017	0.023	0.023	0.035	0.015	0.011	0.018

a) Assumes negligible bull trout spawning in Flathead River below Howell Creek.

b) FAV<sub>ref</sub> was calculated by calibrating acute bioassay data on brook trout (Environmental Protection Agency, 1985) to standard reference conditions: pH=8.0, T=10°C, and D.O.=8.0 mg/L.

c) Mean monthly pH: from Sheehan et al. 1985.

d) Mean monthly temperature: from Environment Canada, 1977; Environment Canada, 1985.

e) No-effect levels of ammonia are reported as maximum and 96 hr arithmetic mean concentrations.

TABLE 15

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF AMMONIA FOR HOWELL CREEK BULL TROUT  
(See Text for Definition of Symbols)

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Most Sensitive Life History Stage	Early Alevin	Early Alevin	Early Alevin	Late Alevin	Late Alevin	Fry (to 0.25)	Juvenile	Juvenile	Adult	Adult	Egg	Egg
FAV <sub>ref</sub> (mg/L) <sup>a</sup>	0.30	0.30	0.30	0.20	0.20	0.40	0.70	0.70	0.40	0.40	0.40	0.40
pH <sup>b</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Temperature (°C) <sup>c</sup>	0.3	0.7	1.0	3.7	5.3	7.3	7.8	8.3	8.7	4.1	0.6	0.4
Dissolved Oxygen (mg/L)	No site-specific data currently available											
FPH	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FT	0.51	0.53	0.54	0.65	0.72	0.83	0.86	0.89	0.91	0.67	0.52	0.52
FDO	-	-	-	-	-	-	-	-	-	-	-	-
FAV(mg/L)	0.15	0.16	0.16	0.13	0.14	0.33	0.60	0.62	0.36	0.27	0.21	0.21
[Maximum] <sup>d</sup>	0.08	0.08	0.08	0.07	0.07	0.17	0.30	0.31	0.18	0.14	0.11	0.11
[96 hr $\bar{x}$ ]	0.008	0.008	0.008	0.007	0.007	0.017	0.030	0.031	0.018	0.014	0.011	0.011

a) FAV<sub>ref</sub> was calculated by calibrating acute bioassay data on brook trout (Environmental Protection Agency, 1985) to standard reference conditions: pH=8.0, T=10°C, and D.O.=8.0 mg/L.

b) Mean monthly pH: from Sheehan et al. 1985.

c) Mean monthly temperature: from Environment Canada, 1985.

d) No-effect levels of ammonia are reported as maximum and 96 hr arithmetic mean concentrations.

Figure 28. Calculated Final Acute Values of Ammonia for Bull Trout in the Flathead River System.

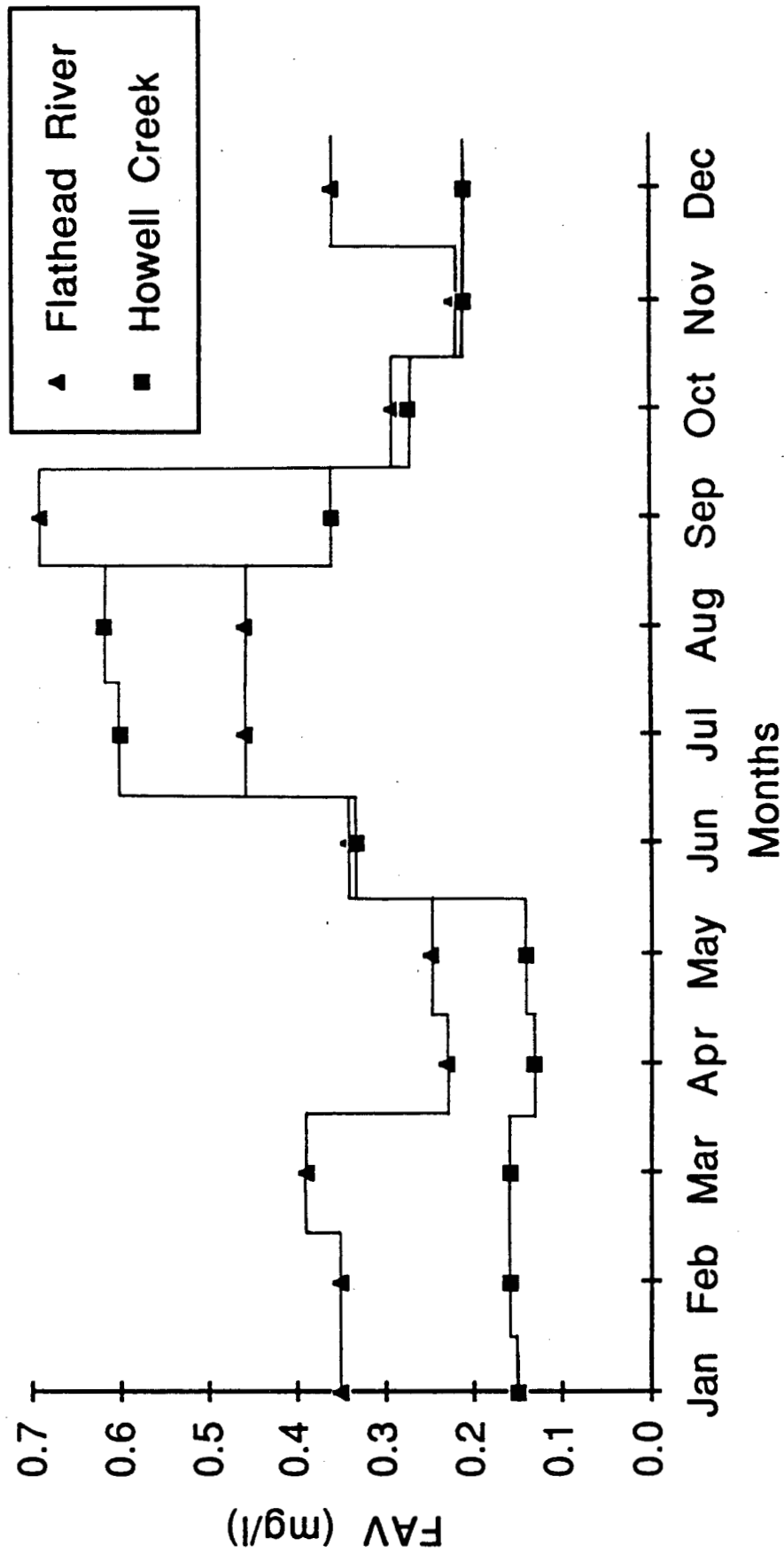


TABLE 16

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF AMMONIA FOR FLATHEAD RIVER CUTTHROAT TROUT<sup>a</sup>  
(See Text for Definition of Symbols)

Most Sensitive Life History Stage	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Adult	Adult	Adult	Adult	Juvenile	Juvenile	Adult	Adult	Adult	Adult	Adult	Adult
FAVref (mg/L) <sup>b</sup>	0.35	0.35	0.35	0.35	0.50	0.50	0.35	0.35	0.35	0.35	0.35	0.35
pH <sup>c</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Temperature (°C) <sup>d</sup>	0.1	0.1	1.3	2.6	3.4	7.9	12.1	12.1	9.8	5.4	1.5	0.3
Dissolved Oxygen (mg/L)	No site-specific data currently available											
FPH	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FT	0.50	0.50	0.55	0.60	0.63	0.86	1.16	1.16	0.99	0.73	0.56	0.51
F00	-	-	-	-	-	-	-	-	-	-	-	-
FAV(mg/L)	0.18	0.18	0.19	0.20	0.32	0.43	0.41	0.41	0.35	0.26	0.20	0.18
[Maximum] <sup>e</sup>	0.09	0.09	0.10	0.10	0.16	0.22	0.21	0.21	0.18	0.13	0.10	0.09
[96 hr x]	0.009	0.009	0.010	0.010	0.016	0.022	0.021	0.021	0.018	0.013	0.010	0.009

- a) Assumes negligible cutthroat trout spawning in Flathead River below Howell Creek.  
b) FAVref was calculated by calibrating acute bioassay data (Environmental Protection Agency, 1985) to standard reference conditions: pH=8.0, T=10°C, and D.O.=8.0 mg/L.  
c) Mean monthly pH: from Sheehan et al. 1985.  
d) Mean monthly temperature: from Environment Canada, 1977; Environment Canada, 1985.  
e) No-effect levels of ammonia are reported as maximum and 96 hr arithmetic mean concentrations.

TABLE 17  
CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF AMMONIA FOR HOWELL CREEK CUTTHROAT TROUT  
(See Text for Definition of Symbols)

Most Sensitive Life History Stage	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Adult	Adult	Adult	Adult	Adult	Adult	Late Alevin	Late Alevin	Adult	Adult	Adult	Adult
FAV <sub>ref</sub> (mg/L) <sup>a</sup>	0.35	0.35	0.35	0.35	0.35	0.35	0.20	0.20	0.35	0.35	0.35	0.35
pH <sup>b</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Temperature (°C) <sup>c</sup>	0.3	0.7	1.0	3.7	5.3	7.3	7.8	8.3	8.7	4.1	0.6	0.4
Dissolved Oxygen (mg/L)	No site-specific data currently available											
FPH	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FT	0.51	0.53	0.54	0.65	0.72	0.83	0.86	0.89	0.91	0.67	0.52	0.52
FDO	-	-	-	-	-	-	-	-	-	-	-	-
FAV(mg/L)	0.18	0.19	0.19	0.23	0.25	0.29	0.17	0.18	0.32	0.23	0.18	0.18
[Maximum] <sup>d</sup>	0.09	0.10	0.10	0.12	0.13	0.15	0.09	0.09	0.16	0.12	0.09	0.09
[96 hr $\bar{x}$ ]	0.009	0.010	0.010	0.012	0.013	0.015	0.009	0.009	0.016	0.012	0.009	0.009

- a) FAV<sub>ref</sub> was calculated by calibrating acute bioassay data (Environmental Protection Agency, 1985) to standard reference conditions: pH=8.0, T=10°C, and D.O.=8.0 mg/L.
- b) Mean monthly pH: from Sheehan et al. 1985.
- c) Mean monthly temperature: from Environment Canada, 1985.
- d) No-effect levels of ammonia are reported as maximum and 96 hr arithmetic mean concentrations.

Figure 29. Calculated Final Acute Values of Ammonia for Cutthroat Trout in the Flathead River System.

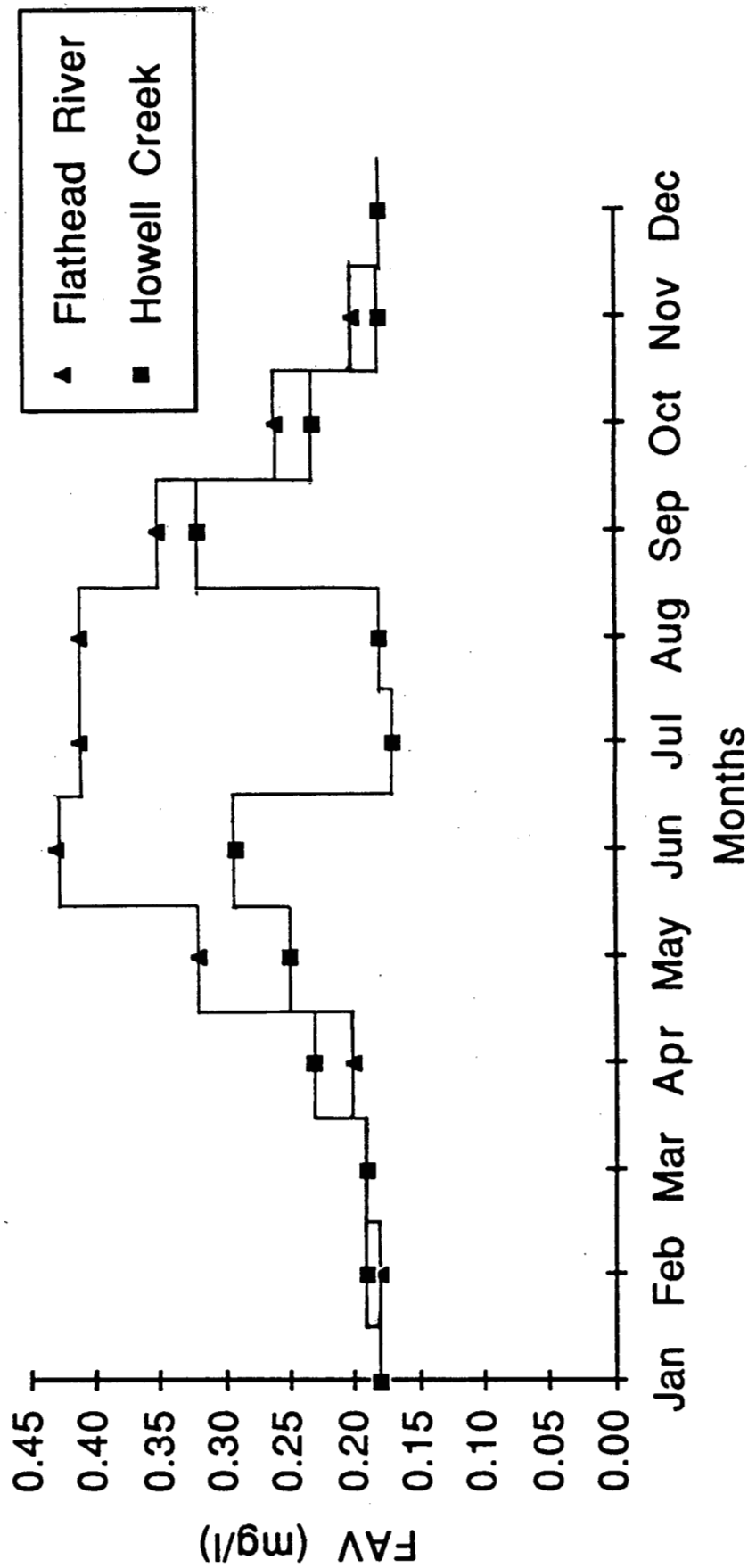


TABLE 18

CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF AMMONIA FOR FLATHEAD RIVER MOUNTAIN WHITEFISH<sup>a</sup>  
(See Text for Definition of Symbols)

Most Sensitive Life History Stage	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
	Adult	Adult	Adult	Early Alevin	Late Alevin	Fry (to 0.25g)	Adult	Adult	Adult	Adult	Adult	Adult
FAV <sub>ref</sub> (mg/L) <sup>b</sup>	0.30	0.30	0.30	0.30	0.20	0.20	0.30	0.30	0.30	0.30	0.30	0.30
pH <sup>c</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Temperature (°C) <sup>d</sup>	0.1	0.1	1.3	2.6	3.4	7.9	12.1	12.1	9.8	5.4	1.5	0.3
Dissolved Oxygen (mg/L)	No site-specific data currently available											
FPH	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FT	0.50	0.50	0.55	0.60	0.63	0.86	1.16	1.16	0.99	0.73	0.56	0.51
FDO	-	-	-	-	-	-	-	-	-	-	-	-
FAV(mg/L)	0.15	0.15	0.17	0.18	0.13	0.19	0.35	0.35	0.30	0.22	0.17	0.15
[Maximum] <sup>e</sup>	0.08	0.08	0.09	0.09	0.07	0.10	0.18	0.18	0.15	0.11	0.09	0.08
[96 hr $\bar{x}$ ]	0.008	0.008	0.009	0.009	0.007	0.010	0.018	0.018	0.015	0.011	0.009	0.008

- a) Assumes significant spawning activity by mountain whitefish in the Flathead River below Howell Creek.  
b) FAV<sub>ref</sub> was calculated by calibrating acute bioassay data (Environmental Protection Agency, 1985) to standard reference conditions: pH=8.0, T=10°C, and D.O.=8.0 mg/L.  
c) Mean monthly pH: from Sheehan et al. 1985.  
d) Mean monthly temperature: from Environment Canada 1977; Environment Canada 1985.  
e) No-effect levels of ammonia are reported as maximum and 96 hr arithmetic mean concentrations.

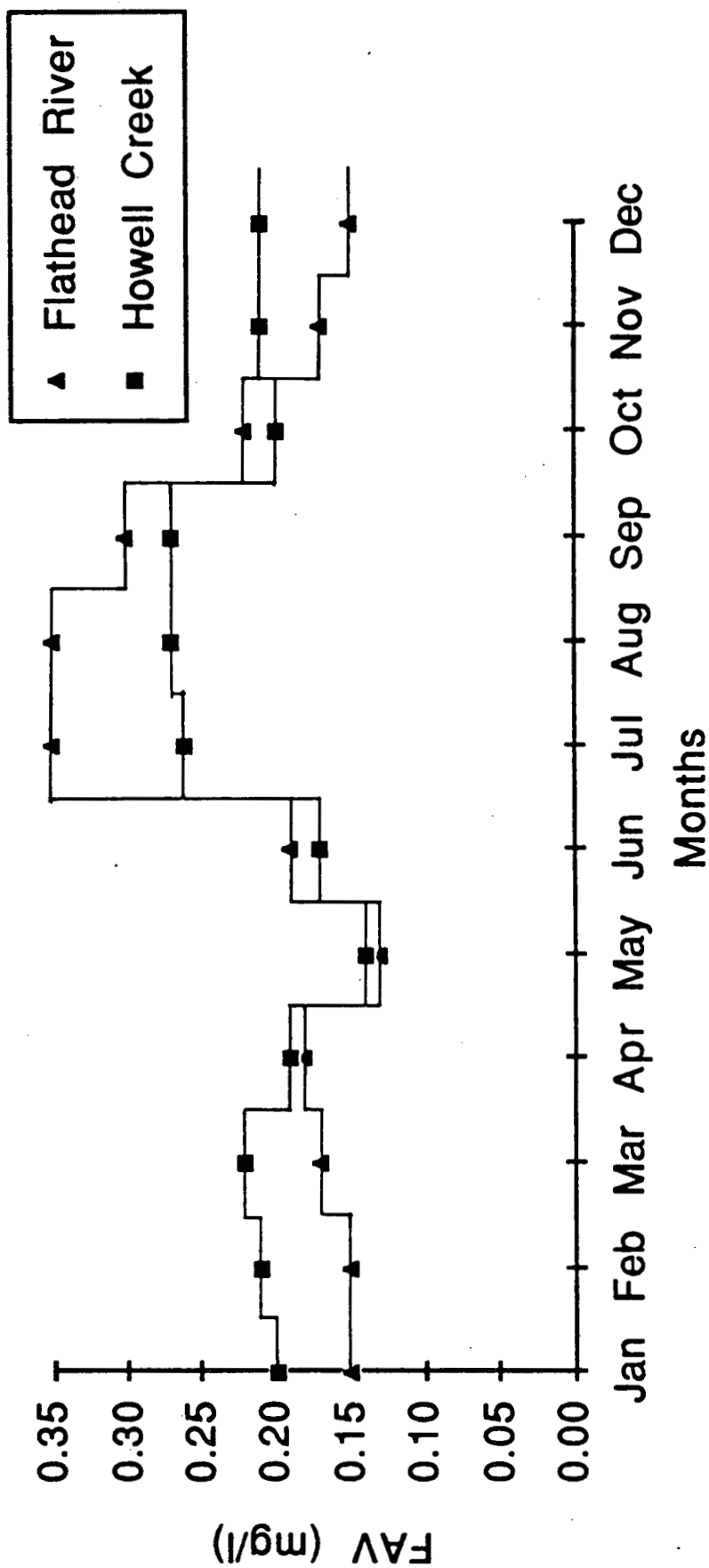
TABLE 19  
CALCULATED FINAL ACUTE VALUES AND NO-EFFECT LEVELS OF AMMONIA FOR HOWELL CREEK MOUNTAIN WHITEFISH  
(See Text for Definition of Symbols)

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Most Sensitive Life History Stage <sup>a</sup>	Egg	Egg	Egg	Early Alevin	Late Alevin	Fry (to 0.25g)	Adult	Adult	Adult	Adult	Egg	Egg
FAV <sub>ref</sub> (mg/L) <sup>b</sup>	0.30	0.30	0.30	0.30	0.20	0.20	0.30	0.30	0.30	0.30	0.40	0.40
pH <sup>c</sup>	8.20	8.30	8.35	7.90	8.05	8.15	8.30	8.40	8.25	8.20	8.25	8.30
Temperature (°C) <sup>d</sup>	0.3	0.7	1.0	3.7	5.3	7.3	7.8	8.3	8.7	4.1	0.6	0.4
Dissolved Oxygen (mg/L)	No site-specific data currently available											
FPH	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FT	0.51	0.53	0.54	0.65	0.72	0.83	0.86	0.89	0.91	0.67	0.52	0.52
FDO	-	-	-	-	-	-	-	-	-	-	-	-
FAV(mg/L)	0.20	0.21	0.22	0.19	0.14	0.17	0.26	0.27	0.27	0.20	0.21	0.21
[Maximum] <sup>e</sup>	0.10	0.11	0.11	0.10	0.07	0.09	0.13	0.14	0.14	0.10	0.11	0.11
[96 hr $\bar{x}$ ]	0.010	0.011	0.011	0.010	0.007	0.009	0.013	0.014	0.014	0.010	0.011	0.011

- a) Assumes mountain whitefish adults do not overwinter in tributary streams.  
b) FAV<sub>ref</sub> was calculated by calibrating acute bioassay data (Environmental Protection Agency, 1985) to standard reference conditions: pH=8.0, T=10°C, and D.O.=8.0 mg/L.  
c) Mean monthly pH: from Sheehan et al. 1985.  
d) Mean monthly temperature: from Environment Canada, 1985.  
e) No-effect levels of ammonia are reported as maximum and 96 hr arithmetic mean concentrations.



Figure 30. Calculated Final Acute Values of Ammonia for Mountain Whitefish in the Flathead River System.



three species of fish indigenous to the system. This approach to criteria development suggests that the level of 0.50 of the 96 hr  $LC_{50}$  (or FAV) value should not be exceeded at any time in the receiving water after mixing, and the average 96 hr concentration should not exceed 0.05 of the 96 hr  $LC_{50}$  (or FAV) value. The criteria so developed are designed to minimize the potential for extended exposures to chronically toxic levels of  $NH_3$ -N. Acceptable levels of un-ionized ammonia, presented in Table 20 and Figure 31, were calculated by integrating all of the species-specific FAVs by month to produce system-specific FAVs, and then multiplying by the appropriate application factor.

TABLE 20

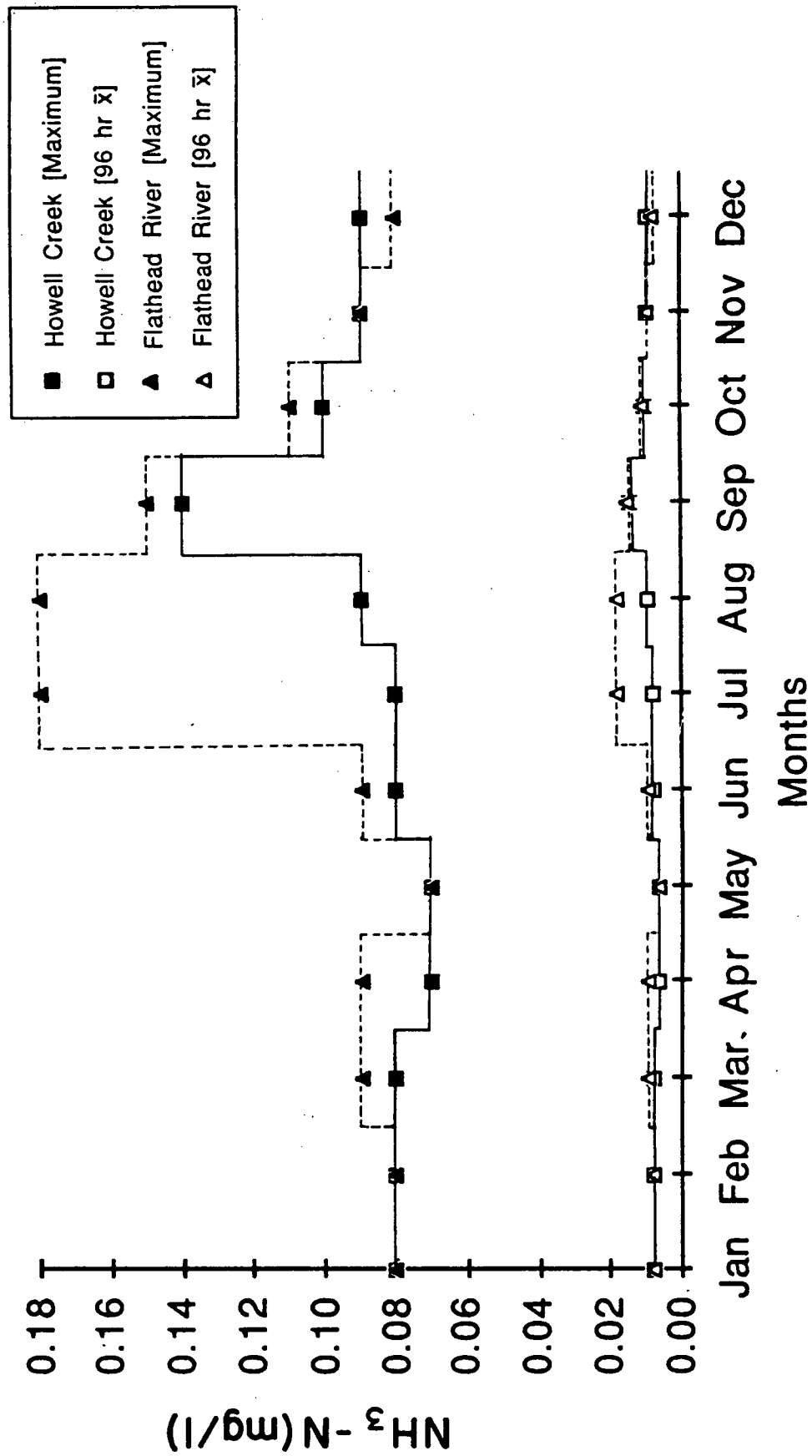
CALCULATED NO-EFFECT LEVELS OF AMMONIA IN THE  
FLATHEAD RIVER WATERSHED<sup>a</sup>

Month	Ammonia (NH <sub>3</sub> -N in mg/L)			
	Flathead River		Howell Creek	
	[Maximum]	[96 hr. $\bar{x}$ ] <sup>b</sup>	[Maximum]	[96 hr. $\bar{x}$ ] <sup>b</sup>
January	0.08	0.008	0.08	0.008
February	0.08	0.008	0.08	0.008
March	0.09	0.009	0.08	0.008
April	0.09	0.009	0.07	0.007
May	0.07	0.007	0.07	0.007
June	0.09	0.009	0.08	0.008
July	0.18	0.018	0.08	0.008
August	0.18	0.018	0.09	0.009
September	0.15	0.015	0.14	0.014
October	0.11	0.011	0.10	0.010
November	0.09	0.009	0.09	0.009
December	0.08	0.008	0.09	0.009

a) See text for method used for calculation of acceptable levels of NH<sub>3</sub>.

b) Denotes maximum 96 hr mean concentration

Figure 31. Calculated No Effect Levels of Ammonia in the Flathead River System.



#### 4. FINAL CRITERIA FOR NITROGEN COMPOUNDS

The final criteria for toxic nitrogen compounds are expressed as maximum (Criterion Maximum Concentration - CMC) and long-term arithmetic mean (Criterion Continuous Concentration - CCC) concentrations on a monthly basis. For this report, the CMC and CCC are defined as follows:

- 1) Criterion Maximum Concentration - This criterion is intended to provide an estimate of the level of a toxicant that, if maintained for short periods of time (i.e., <24 hrs) only, will cause no adverse short-term effects in the receiving water system, provided the long-term average criterion (CCC) is complied with.
- 2) Criterion Continuous Concentration - This criterion is intended to provide an estimate of the safe level (i.e., the level that if maintained continuously, will cause no adverse long-term effects) of a toxicant in a receiving water system. The concentration of a pollutant may exceed the CCC providing, a) the magnitude and duration of the excursions above the CCC are limited, and, b) there are recovery periods of time during which the concentration is below the CCC. The higher the concentration is above the CCC, the shorter period of time it can be tolerated (Stephan et al. 1985).

The water quality criteria for ammonia and nitrite incorporated the results of laboratory bioassays reported in the literature. These tests provided the response of single test organisms to high levels of a single toxicant. However, the toxic effects of ammonia and nitrite may be at least additive (V. Thurston, Montana State University. Bozeman, Montana. Personal communication 1986). Therefore, criteria developed for ammonia or nitrite alone are non-protective when the two occur together. Individual criteria

values then require modification to account for the probable additive nature of the effects of the two toxicants. Additivity of the toxic effects of ammonia and nitrite is only a guess based on their similar modes of toxic action. An alternative assumption would be synergism but no information exists to support this hypothesis.

Assuming the toxic effects of ammonia and nitrite are additive, where the two toxicants occur together the following procedure should be used to calculate final criteria values. The concentration of one of the compounds must be measured, and to comply with the criteria the sum of the ratios of ambient levels to individual criteria values (Tables 12 and 20) for the two compounds should not exceed 1.0. For example, if the concentration of  $\text{NH}_3$  is 60% of the maximum acceptable level of  $\text{NH}_3$  for a specified site in the Flathead basin (Table 20), then  $\text{CMC}(\text{NH}_3) = [\text{NH}_3]$  and  $\text{CMC}(\text{NO}_2^-)$  should be 40% of the maximum acceptable level of  $\text{NO}_2^-$  (Table 12). The detailed procedure is as follows:

- 1) Measure temperature, pH and total ammonia concentration in the water body on site (frequency of sampling should be dictated by proximity of ambient levels to criteria values).
- 2) Using equations 24-26 in the Ammonia section calculate the concentration of  $\text{NH}_3\text{-N}$  in solution.
- 3) Calculate ratios  $R_1$  and  $R_2$  where:

$$R_1 = \frac{[\text{NH}_3\text{-N}]}{\text{Maximum No-effect } [\text{NH}_3\text{-N}]} ; \text{ from Table 20} \quad (40)$$

$$R_2 = \frac{(\sum_{i=1}^n [\text{NH}_3\text{-N}]_i)/n}{\text{No-effect 96 hr } \bar{x} [\text{NH}_3\text{-N}]} ; n = \text{sample size} \quad (41)$$

4. Calculate final criteria values where:

a) If  $R_1 \leq 1$  then,

$$CMC (NH_3) = [NH_3-N] \quad (42)$$

$$CMC (NO_2^-) = (1-R_1) \cdot \text{Maximum No-effect } [NO_2^- - N];$$

$$\text{from Table 12} \quad (43)$$

b) If  $R_2 \leq 1$  then,

$$CCC(NH_3)^- = \left( \sum_{i=1}^n [NH_3-N]_i \right) / n \quad (44)$$

$$CCC(NO_2^-)^- = (1-R_2) \cdot \text{Acceptable 96 hr } \bar{x} [NO_2^- - N];$$

$$\text{from Table 12} \quad (47)$$

## 5. REFERENCES

- Alabaster, J.S. and D.M.W. Herbert. 1954. Influence of carbon dioxide on ammonia. *Nature* 174:404-405.
- Alabaster, J.S., D.G. Shurben, and G. Knowles. 1979. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, Salmo salar L. *Journal of Fish Biology* 15(6):705-712.
- Alabaster, J.S. and R. Lloyd. 1982. Water quality criteria for freshwater fish. 2nd Edition. F.A.O. Butterworth Scientific. London. 360 pp.
- Arillo, A., C. Margiocco, F. Melodia, P. Mensi and G. Schenone. 1981. Ammonia toxicity mechanism in fish: studies on rainbow trout (Salmo gairdneri Rich.). *Ecotoxicology and Environmental Safety* 5:316-328.
- Arillo, A., E. Gaiino, C. Margiocco, P. Mensi, and G. Schenone. 1984. Biochemical and ultrastructural effects of nitrite in rainbow trout: liver hypoxia as the root of the acute toxicity mechanisms. *Environmental Research* 34:135-154.
- Bird, R.B., W.E. Stewart and E.N. Lightfoot. 1960. Transport phenomena. John Wiley & Sons. New York.
- Birge, E.A. and C. Juday. 1922. The inland lakes of Wisconsin: The plankton, its quality and chemical composition. Wisconsin Natural History Survey Bulletin, Volume 64.
- Bodansky, O. 1951. Methemoglobin and methemoglobin-producing compounds. *Pharmacological Review* 3:144-196.
- Bowser, P.R., W.W. Falls, J. VanZandt, N. Collier, and J.D. Phillips. 1983. Methemoglobinemia in channel catfish: methods of prevention. *Progressive Fish Culturist* 45:154-158.
- Brezonik, P.L. 1977. Dentrification in natural waters. In: S.H. Jenkins (ed.), Nitrogen as a Water Pollutant. Progress in Water Technology, Volume 8. Pergamon Press. Oxford, England.
- Brockway, D.R. 1950. Metabolic products and their effects. *Progressive Fish Culturist* 12(3):127-129.
- Broderius, S.S., L.L. Smith, Jr., and D.T. Lind. 1977. Relative toxicity of free cyanide and dissolved sulphide forms to the fathead minnow. Pimephales promelas. *Journal of Fisheries Research Board of Canada* 34(12):2323-2332.



- Brown, D.A. and D.J. McLeay. 1975. Effect of nitrite on methemoglobin and total hemoglobin of juvenile rainbow trout. *Progressive Fish Culturist* 37:36-38.
- Brown, C.M., D.S. MacDonald-Brown and J.L. Meers. 1974. Physiological aspects of microbial inorganic nitrogen metabolism. *Advances in Microbial Physiology* 11:1-52.
- Brown, V.M. 1968. The calculation of the acute toxicity of mixtures of poisons to rainbow trout. *Water Research* 2(10):723-733.
- Brown, V.M., D.H.M. Jordon and B.A. Tiller. 1969. The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. *Journal of Fish Biology* 1(1):1-9
- Buckley, J.A. 1978. Acute toxicity of un-ionized ammonia to fingerling coho salmon. *Progressive Fish Culturist* 40(1):30-32.
- Burkhalter, D.E. and C.M. Kaya. 1977. Effects of prolonged exposure to ammonia on fertilized eggs and sac fry of rainbow trout (Salmo gairdneri). *Transactions of American Fisheries Society* 106(5):470-475.
- Burrows, R.E. 1964. Effects of acclimated excretory products on hatchery-reared salmonids. Fish and Wildlife Service. Research Report 66:1-2. U.S. Department of the Interior. Washington, D.C. 12 pp.
- Cameron, J.N. 1971. Methemoglobin in erythrocytes of rainbow trout. *Comparative Biochemistry and Physiology* 40A:743-749.
- Cameron, J.N. 1986. Responses to reversed  $\text{NH}_3$  and  $\text{NH}_4^+$  gradients in a teleost (Ictalurus punctatus), an elasmobranch (Raja erinacea), and a crustacean (Callinectes sapidus): Evidence for  $\text{NH}_4^+/\text{H}^+$  exchange in the teleost and the elasmobranch. *Journal of Experimental Zoology* 239:183-195.
- Campbell, J.W. 1973. Nitrogen Excretion. In: C.L. Prosser (ed.), *Comparative Animal Physiology*. W.B. Saunders Co. Toronto.
- Canadian Council of Resource and Environment Ministers. 1987. Canadian water quality guidelines. Task Force on Water Quality Guidelines. Ottawa, Canada.
- Colt, J.E. 1974. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish. Unpublished Ph.D. thesis. University of California. Davis, California. 94 pp.
- Crawford, R.E. and G.H. Allen. 1977. Sea water inhibition of nitrite toxicity to chinook salmon. *Transactions of American Fisheries Society* 106(1):105-109.

- Curtis, E.J., K. Durrant and M.M.I. Harman. 1975. Nitrification in rivers in the Trent basin. *Water Research* 9(3):255-268.
- De Renzo, D.J. (ed.) 1978. Nitrogen control and phosphorus removal in sewage treatment. Noyes Data Corporation, USA.
- Danecker, E. 1964. Die Jauchevergiftung von Fischen--eine Ammoniakvergiftung. (The jauche poisoning.) *Osterr. Fischerei.* 3/4:55-68. (In English translation).
- Downing, K.M. and J.C. Merkens. 1955. The influence of dissolved oxygen concentration on the toxicity on un-ionized ammonia to rainbow trout (Salmo gairdneri Richardson). *Annals of Applied Biology* 43(2):243-246.
- Eddy, F.B., P.A. Kunzlik and R.N. Bath. 1983. Uptake and loss of nitrite from the blood of rainbow trout, Salmo gairdneri Rich., and Atlantic salmon, Salmo salar L. in fresh water and in dilute sea water. *Journal of Fish Biology* 23:105-116.
- Edwards, R.W. and H.L.J. Rolley. 1965. Oxygen consumption in river muds. *Journal of Ecology* 53:1-19.
- Ellis, M.M. 1937. Detection and measurement of stream pollution. *Bulletin of U.S. Bureau Fisheries* 48(22):364-437.
- Emerson, K., R.C. Russo, R.E. Lund and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *Journal of Fisheries Research Board of Canada* 32(12): 2379-2383.
- Environment Canada. 1977. Water Temperatures - British Columbia and Yukon Territory. Volume 2. Inland Waters Directorate, Pacific and Yukon Region. 507 pp.
- Environment Canada. 1985. Unpublished water temperature information for Howell Creek and the Flathead River. Inland Waters Directorate. Pacific and Yukon Region.
- Environmental Protection Agency. 1985. Ambient Water Quality Criteria for Ammonia - 1984. Office of Water Regulations and Standards, Criteria and Standards Division. EPA 440/5-85-001. Washington, D.C. 217 pp.
- Erickson, R.J. 1985. An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms. *Water Research* 19(8):1047-1058.
- Fidler, L.E. 1983. Analysis Methods Applicable to the Prediction of Hatchery Water Conditioning System Characteristics. Penny Applied Sciences Ltd. A report to the Department of Fisheries and Oceans. Vancouver, B.C.

- Fidler, L.E. 1985. Design and analysis procedure applicable to hatchery aeration systems. Penny Applied Sciences Ltd. A report to the Department of Fisheries and Oceans. Vancouver, B.C.
- Flis, J. 1968. Anatomicohistopathological changes induces in carp (Cyprinus carpio L) by ammonia water. Part II. Effects of subtoxic concentrations. *Acta Hydrobiologica* 10:225-238.
- Fogg, G.E. 1953. The metabolism of algae. London, U.K.
- Forsberg, C. 1965. Nutritional studies of Chara in axenic cultures. *Physiology of Plant* 18:275-290.
- Forsberg, C. 1977. Nitrogen as growth factor to fresh water. *Progress in Water Technology* 8:275-290.
- Gerloff, G.C. and P.H. Kromholz. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperme aquatic plants. *Limnology and Oceanography* 11:529-537.
- Gerloff, G.C. 1969. Evaluating nutrient supplies for the growth of aquatic plants in natural waters. In: *Eutrophication: Causes, Consequences and Correctives. International Symposium on Eutrophication.* National Academy of Sciences. Washington, D.C.
- Gigger, R.P. and R.E. Speece. 1979. In: Miller, G.E. and Libey, G.S. (1984) Evaluation of a trickle filter in a recirculating aquaculture system containing channel catfish. *Aquacultural Engineering* 3:39-57.
- Gillette, L.A., D.L. Miller, and H.E. Redman. 1952. Appraisal of a chemical waste problem by fish toxicity tests. *Sewage and Industrial Wastes* 24(11): 1397-1401.
- Grady, C.P.L. 1983. Modeling of biological fixed films - a state of the art review. In: C.W. Yeun and E.D. Smith (ed.) *Fixed film biological processes for wastewater treatment.* Noyes Data Corporation. U.S.A.
- Grady, C.P.L. and H.C. Lim. 1980. *Biological waste water treatment: Theory and applications.* Marcel Dekker, Inc. New York, N.Y.
- Harvey, H.W. 1955. *The chemistry and fertility of sea waters.* Cambridge University Press. Cambridge, U.K.
- Haug, R.T. and P.L. McCarty. 1972. Nitrification with submerged filters. *Journal of Water Pollution Control Federation* 44(11):2086-2102.
- Hillaby, B.A. and D.J. Randall. 1979. Acute ammonia toxicity and ammonia excretion in rainbow trout (Salmo gairdneri). *Journal of Fisheries Research Board of Canada* 35:621-629.

- Hoar, W.S. 1975. General and Comparative Physiology. Second Edition. Prentice-Hall Inc. Englewood Cliffs, New Jersey.
- Hochachka, P.W. and G.N. Somero. 1973. Strategies of Biochemical Adaptation. W.B. Saunders Co. Toronto.
- Hooper, A.B. and K.R. Terry. 1973. Specific inhibitors of ammonia oxidation in Nitrosomonas. Journal of Bacteriology 115:480-485.
- Huang, C.S. and N.E. Hopson. 1974. Nitrification rate of biological processes. Journal of Sanitary Engineering Division of the American Society of Civil Engineers. 100(EE2):409-422.
- Hulme, W. 1974. The mechanism of denitrification. Transactions of Chemical Society 105:623-632.
- Kiese, M. 1974. Methemoglobinemia: A comprehensive treatise. CRC Press. Cleveland, Ohio. 260 pp.
- Kolenbrander, G.J. 1977. Nitrogen in organic matter and fertilizer as a source of pollution. Progress in Water Technology 8:67-84.
- Krous, S.R., V.S. Blazer and T.L. Meade. 1982. Effect of acclimation time on nitrite movement across the gill epithelia of rainbow trout: The role of 'chloride cells'. Progressive Fish Culturist 44(3):126-130.
- Larmoyeaux, J.D. and R.D. Piper. 1973. Effects of water reuse on rainbow trout in hatcheries. Progressive Fish Culturist 35(1):2-8.
- Lewin, R.A. 1962. Physiology and Biochemistry of Algae. Academic Press. New York, New York. 929 pp.
- Lewis, W.M., Jr. and D.P. Morris. 1986. Toxicology of nitrite to fish: A review. Transactions of American Fisheries Society 115(2):183-195.
- Lloyd, R. 1961. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (Salmo gairdneri Richardson). Journal of Experimental Biology 38(2):447-455.
- Lloyd, R. and L.D. Orr. 1969. The diuretic response by rainbow trout to sub-lethal concentrations of ammonia. Water Research 3(5):335-344.
- Lopez-Bernal, F.F., P.A. Krenkel and R.J. Raune. 1977. Nitrification in free flowing streams. Progress in Water Technology 9:821-832.
- MacDonald, D.D. (ed.) 1985. Proceedings of the Flathead River basin bull trout biology and population dynamics modelling information exchange. Fisheries Branch. British Columbia Ministry of Environment. Cranbrook, British Columbia.

- McCoy, E.F. 1972. Role of bacteria in the nitrogen cycle in lakes. Water Pollution Control Research Series 16010-EHR-03172. Office of Research and Monitoring. Environmental Protection Agency. Washington, D.C. 23 pp.
- Margiocco, C., A. Arillo, P. Mensi, and G. Schenone. 1983. Nitrite bioaccumulation in Salmo gairdneri Rich. and hematological consequences. Aquatic Toxicology 3:261-270.
- Mayo, R.D., P.B. Piao and W.G. Williams. 1972. A study for the development of fish hatchery water treatment systems. Contract Number DACW68-71-0059. Report for the Army Corps of Engineers. Seattle, Washington.
- Mensi, P., A. Arillo, C. Margiocco and G. Schenone. 1982. Lysosomal damage under nitrite intoxication in rainbow trout (Salmo gairdneri Rich.). Comparative Biochemistry and Physiology 73C(1):161-165.
- Monod, J. 1949. The growth of bacterial cultures. Ann. Rev. Microbiology 3:371-394.
- Mulligan, H.F. and A. Baranowski. 1969. Growth of phytoplankton and vascular aquatic plants at different nutrient levels. Verh. Int. Ver. Limnol. 17:802-810.
- Nordin, R.N. and L.W. Pommen. 1986. Water quality criteria for nitrogen (nitrate, nitrite and ammonia). Technical Appendix. B.C. Ministry of Environment and Parks. Victoria, B.C.
- Painter, L.A. 1970. A review of literature of inorganic metabolism in micro-organisms. Water Research 4:393-450.
- Painter, H.A. 1977. Microbial transformations of inorganic nitrogen. Progress in Water Technology 8:3-29.
- Perrone, S.J. and T.L. Meade. 1977. Protective effect of chloride on nitrite toxicity to coho salmon (Oncorhynchus kisutch). Journal of Fisheries Research Board of Canada 34:486-492.
- Perry, S.F. 1986. Carbon dioxide excretion in fishes. Canadian Journal of Zoology 64:565-572.
- Pommen, L.W., R.N. Nordin and N.K. Nagpal. 1982. The effect on water quality of explosives use in surface mining. Presented at 49th Annual Pacific Northwest Pollution Control Association meeting and 1982 Annual B.C. Water and Waste Association Conference. Vancouver, B.C.
- Rankin, D.P. 1979. The influence of un-ionized ammonia on the long-term survival of sockeye salmon eggs. Fisheries Marine Service Technical Report 912. 17 pp.

- Raune, R.J. and P.A. Krenkel. 1975. Nitrification and other factors affecting nitrogen in the Holston River. Proceedings of IAEPR Conference on Nitrogen as a Water Pollutant. Copenhagen, Denmark.
- Reichenbach-Klinke, H.-H. 1967. Investigations on the influence of the ammonia content on the fish organism. Archir. für Fischereiwissenschaft 17:122-132.
- Rice, S.D. and J.E. Bailey. 1980. Survival, size, and emergence of pink salmon, Oncorhynchus gorbuscha, alevins after short and long-term exposures to ammonia. Fisheries Bulletin 78(3):641-648.
- Russo, R.C., C.E. Smith, and R.V. Thurston. 1974. Acute toxicity of nitrite to rainbow trout (Salmo gairdneri). Journal of Fisheries Research Board of Canada 31(10):1653-1655.
- Russo, R.C. and R.V. Thurston. 1975. Acute toxicity of nitrite to cutthroat trout (Salmo clarki). Technical Report No. 75-3. Fisheries Bioassay Laboratory. Montana State University. Bozeman, Montana.
- Russo, R.C. and R.V. Thurston. 1977. Acute toxicity of nitrite to fishes. In: Tubb, R.A. (ed.) Recent Advances in Fish Toxicology. Environmental Protection Agency Ecological Research Series EPA-600/3-77-085. Environmental Protection Agency, Corvallis, Oregon.
- Russo, R.C., R.V. Thurston, and K. Emerson. 1981. Acute toxicity of nitrite to rainbow trout (Salmo gairdneri); Effects of pH, nitrite species and anion species. Canadian Journal of Fisheries Aquatic Sciences 38:387-393.
- Sawyer, C.N., H.E. Wild and T.C. McMahon. 1973. Nitrification and denitrification facilities, wastewater treatment. Report prepared for the Environmental Protection Agency technology transfer program. Athens, Georgia.
- Schulze-Wiehenbrauck, H. 1976. Effects of sub-lethal ammonia concentrations on metabolism of juvenile rainbow trout (Salmo gairdneri) Richardson). Der. Dtsch. Wiss. Komm. Meeresforsch, 24(4):234-250.
- Sculthorpe, C.D. 1967. The biology of aquatic vascular plants. London, U.K.
- Sharma, B. and R.C. Ahlert. 1977. Nitrification and nitrogen removal. Water Research 11:897-925.
- Sheehan, S.W., G.L. Ennis and R.L. Hallam. 1980. A water quality study of the Flathead River basin in British Columbia prior to proposed coal mining. Water Quality Branch. Environment Canada, Vancouver, B.C. 137 pp.

- Sheehan, S.W., G.L. Ennis, R.L. Hallam, A.L. Smith, and T.M. Tuominen. 1985. A water quality study of the Flathead River basin in British Columbia prior to proposed coal mining (Data Report). Water Quality Branch. Environment Canada, Vancouver, B.C. 260 pp.
- Simms, A.P., B.F. Folkes and A.H. Bussey. 1968. Mechanisms involved in the regulation of nitrogen assimilation in microorganisms and plants. In: E.J. Hewitt and C.V. Cutting (ed.). Recent aspects of nitrogen metabolism in plants. London, U.K.
- Smart, G. 1975. The acute toxic mechanisms of ammonia to rainbow trout (Salmo gairdneri). Ph.D. Thesis. University of Bristol. U.K.
- Smart, G. 1978. Investigations of the toxic mechanisms of ammonia to fish-gas exchange in rainbow trout (Salmo gairdneri) exposed to acutely lethal concentrations. Journal of Fish Biology 12(1):93-104.
- Smith, C.E. and W.G. Williams. 1974. Experimental nitrite toxicity in rainbow trout and chinook salmon. Transactions of American Fisheries Society 103:389-390.
- Smith, C.E. and R.C. Russo. 1975. Nitrite-induced methemoglobinemia in rainbow trout. Progressive Fish Culturist 37(3):150-152.
- Srinath, E.G., R.C. Loehr and T.B.S. Prakasam. 1976. Nitrifying organism concentration and activity. Journal of Environmental Engineering Division ASCE 102: 449-463.
- Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency. Duluth, Minnesota. 98 pp.
- Stumm, W. and J.J. Morgan. 1981. Aquatic chemistry: An introduction emphasizing chemical equilibria in natural waters. 2nd Edition Wiley-Interscience. Toronto, Ontario. 583 pp.
- Syrett, P.J. 1962. Nitrogen assimilation. In: R.A. Lewin (ed.). Physiology and biochemistry of algae. New York, N.Y..
- Szumski, D.S., D.A. Barton, H.D. Putman and R.C. Polta. 1982. Evaluation of EPA un-ionized ammonia toxicity criteria. Journal of Water Pollution Control Federation 54(3):281-291.
- Thorp, A.C. 1985. Detailed surface water quality data, British Columbia. 1979-1981. Water Quality Branch. Environment Canada. Vancouver, B.C. 58 pp.
- Thurston, R.V., R.C. Russo and K. Emerson. 1974. Aqueous ammonia equilibrium calculations. Technical Report 74-1. Fisheries Bioassay Laboratory. Montana State University. Bozeman, Montana. 18 pp.

- Thurston, R.V., R.C. Russo, and C.E. Smith. 1978. Acute toxicity of ammonia and nitrite to cutthroat trout fry. Transactions of American Fisheries Society 107(2):361-368.
- Thurston, R.V., C. Chakoumakos, and R.C. Russo. 1981a. Effect of fluctuating exposures on the acute toxicity of ammonia to rainbow trout (Salmo gairdneri) and cutthroat trout (Salmo clarki). Water Research 15(7):911-917.
- Thurston, R.V., G.R. Phillips, R.C. Russo and S.M. Hinkins. 1981b. Increased toxicity of ammonia to rainbow trout (Salmo gairdneri) resulting from reduced concentrations of dissolved oxygen. Canadian Journal of Fisheries Aquatic Sciences 38(8):983-988.
- Thurston, R.V., R.C. Russo, and G.A. Vinogradov. 1981c. Ammonia toxicity to fishes: effect of pH on the toxicity of the un-ionized ammonia species. Environmental Science and Technology 15(7):837-840.
- Thurston, R.V. and R.C. Russo. 1983. Acute toxicity of ammonia to rainbow trout. Transactions of American Fisheries Society 112:696-704.
- Thurston, R.V. and E.L. Meyn. 1984. Acute toxicity of ammonia to five fish species from the northwest United States. Technical Report 84-4. Fisheries Bioassay Laboratory, Montana State University, Bozeman, Montana 13 pp.
- Thurston, R.V., R.C. Russo, R.J. Luedtke, C.E. Smith, E.L. Meyn, C. Chakoumakos, K.C. Wang and C.J.D. Brown. 1984. Chronic toxicity of ammonia to rainbow trout. Transactions of American Fisheries Society 113:56-73.
- Tomasso, J.R. 1986. Comparative toxicity of nitrite to freshwater fish. Aquatic Toxicology 8:129-137.
- Trama, F.B. 1954. The acute toxicity of some common salts of sodium, potassium and calcium to the common bluegill (Lepomis macrochirus Rafinesque). Proceedings of the National Academy of Sciences Philadelphia 106:185-205.
- Treybal, R.E. 1980. Mass Transfer Operations. McGraw Hill Book Company. New York, N.Y.
- Trussel, R.P. 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. Journal of Fisheries Research Board of Canada 29:1505-1507.
- Valiela, D. and D.D. MacDonald. 1987a. Nutrients, sediments and barium in Howell Creek and the mainstem of the Flathead River. Water Quality Branch. Environment Canada. Vancouver, B.C. (in prep.).



- Valiela, D. and D.D. MacDonald. 1987b. Water quality at the International Border station of the Flathead River. Water Quality Branch. Environment Canada. Vancouver, B.C. (in prep).
- Vollenweider, R.A. 1968. Scientific fundamentals of the eutrophication of lakes and flowing waters with particular reference to nitrogen and phosphorus as factors in eutrophication. Organization for Economic Cooperation and Development. Paris, France.
- Wallen, I.E., W.C. Greer, and R. Lasater. 1957. Toxicity to Gambusia affinis of certain pure chemicals in turbid waters. Sewage and Industrial Wastes 29(6):695-711.
- Water Quality Branch. 1986. Unpublished water quality data. Environment Canada. Vancouver, B.C.
- Wedemeyer, G.A. and W.T. Yasutake. 1978. Prevention and treatment of nitrite toxicity in juvenile steelhead trout (Salmo gairdneri). Journal of Fisheries Research Board of Canada 35:822-827.
- Welty, J.R., C.E. Wicks and R.E. Wilson. 1976. Fundamentals of Momentum, Head and Mass Transfer. 2nd Edition John Wiley and Sons. New York, N.Y.
- Westin, D.T. 1974. Nitrate and nitrite toxicity to salmonid fishes. Progressive Fish Culturist 36(2):86-89.
- Wickens, J.F. 1976. The tolerance of warm water prawns to recirculated water. Aquaculture 9:19-37.
- Wild, H.E., C.N. Sawyer, and T.C. McMahon. 1971. Factors affecting nitrification kinetics. Journal of Water Pollution Control Federation 43:1845-1854.
- Wong-Chong, G.M. and R.C. Loehr. 1975. The kinetics of microbial nitrification. Water Research 9:1099-1106.
- Wuhrmann, K. and H. Woker. 1948. Beitrage zur Toxikologie der Fische. II. Experimentelle Untersuchungen uber die Ammoniak-und Blausaurevergiftung. Schweiz Z. Hydrol. 11:210-244.

6. APPENDIX 1

EXISTING WATER QUALITY CONDITIONS IN THE FLATHEAD RIVER  
AT THE INTERNATIONAL BOUNDARY TO 1982  
(00BC08NP0003)

Variable <sup>a</sup>	n (replicates)	Mean <sup>a</sup>	Range <sup>a</sup>		Standard Deviation
Alkalinity, total	20	112.6	80.4	- 148.0	20.5
Conductivity (µS/cm)	20	215.0	157.0	- 288.0	37.7
Hardness, as CaCO <sub>3</sub>	20	116.6	83.9	- 146.0	21.5
pH (units)	20	8.2	8.0	- 8.6	0.2
Calcium	20	34.4	25.4	- 47.0	6.3
Chloride	20	0.3	< 0.2	- 0.5	0.1
Fluoride	19	0.08	< 0.05	- 0.12	0.02
Magnesium	20	7.45	5.0	- 11.2	1.6
Potassium	20	0.3	0.2	- 0.4	0.1
Silica	20	4.7	4.0	- 5.3	0.3
Sodium	20	0.7	0.3	- 0.9	0.1
Sulphate	20	4.3	2.6	- 7.1	1.1
Phosphorus, total dissolved	14(3)	0.007	0.002	- 0.022	0.003
Phosphorus, total	19(6)	0.076	0.002	- 4.155	0.171
Nitrogen, dissolved NO <sub>2</sub> +NO <sub>3</sub>	19(6)	0.021	< 0.0002	- 0.110	0.020
Nitrogen, dissolved ammonia	19(6)	0.015	< 0.001	- 0.300	0.025
Nitrogen, dissolved	17(6)	0.083	0.03	- 0.55	0.05
Nitrogen, particulate	3(3)	0.010	0.004	- 0.018	0.007
Carbon, total organic	19(6)	2.6	< 1.0	- 8.2	1.9
Carbon, total inorganic	19(6)	27.0	19.3	- 34.8	5.2
Carbon, particulate	2(3)	0.146	0.113	- 0.165	0.037

<sup>a</sup>in mg/L unless otherwise indicated

APPENDIX 1 (cont)

EXISTING WATER QUALITY CONDITIONS IN THE FLATHEAD RIVER  
AT THE INTERNATIONAL BOUNDARY TO 1982  
(00BC08NP0003)

Variable <sup>ab</sup>	n (replicates)	Mean	Range		Standard Deviation
Arsenic	17(1)	0.0004	0.0001	- 0.0010	0.0002
Barium	17(1)	0.28	0.11	- 1.60	0.35
Cadmium	18(1)	0.0007	< 0.0002	- 0.0050	0.0001
Cobalt	16(1)	< 0.001	< 0.001	- 0.001	0.000
Copper	19(1)	0.001	< 0.001	- 0.004	0.001
Iron	19(1)	0.165	0.006	- 0.630	0.205
Lead	19(1)	< 0.001	< 0.001	- 0.002	0.000
Manganese	19(1)	0.02	< 0.01	- 0.05	0.01
Mercury	18(1)	< 0.00005	< 0.00005	- 0.00008	0.00001
Nickel	17(1)	< 0.001	< 0.001	- 0.002	0.000
Selenium	17(1)	0.0001	< 0.0001	- 0.0002	0.0000
Zinc	19(1)	0.004	< 0.001	- 0.050	0.011

<sup>a</sup>in mg/L unless otherwise indicated

<sup>b</sup>extractable values reported for metals levels

7. APPENDIX 2

EXISTING WATER QUALITY CONDITIONS IN HOWELL CREEK TO 1982  
(00BC08NP0008)

Variable <sup>a</sup>	n (replicates)	Mean	Range	Standard Deviation
Alkalinity, total	15(1)	120.6	92.5 - 147.0	17.3
Conductivity (µS/cm)	11(1)	246.8	180.0 - 310.0	47.8
Hardness, as CaCO <sub>3</sub>	15(1)	126.4	98.1 - 160.0	18.6
pH (units)	15(1)	8.2	8.1 - 8.4	0.09
Calcium	15(1)	37.6	28.9 - 43.9	5.1
Chloride	15(1)	0.3	0.2 - 0.6	0.1
Fluoride	14(1)	0.09	< 0.05 - 0.14	0.02
Magnesium	15(1)	7.9	5.9 - 12.2	1.7
Potassium	15(1)	0.36	0.2 - 0.5	0.1
Silica	15(1)	4.8	4.4 - 5.4	0.3
Sodium	15(1)	0.7	0.6 - 0.8	0.1
Sulphate	15(1)	5.3	3.7 - 8.9	1.5
Phosphorus, total dissolved	7(3)	0.011	0.004 - 0.018	0.004
Phosphorus, total	14(6)	0.021	0.009 - 0.062	0.017
Nitrogen, dissolved NO <sub>2</sub> +NO <sub>3</sub>	14(6)	0.025	0.002 - 0.082	0.022
Nitrogen, dissolved ammonia	14(6)	0.008	0.002 - 0.012	0.003
Nitrogen, dissolved	13(6)	0.08	0.05 - 0.15	0.03
Nitrogen, particulate	1(3)	0.012	0.010 - 0.015	
Carbon, total organic	14(6)	3.1	< 1.0 - 7.0	2.1
Carbon, total inorganic	14(6)	28.3	21.5 - 35.0	4.7
Carbon, particulate	1(3)	0.184	0.171 - 0.200	0.015

<sup>a</sup>in mg/L unless otherwise indicated

APPENDIX 2 (cont)

EXISTING WATER QUALITY CONDITIONS IN HOWELL CREEK TO 1982  
(00BC08NP0008)

Variable <sup>ab</sup>	n (replicates)	Mean	Range	Standard Deviation
Arsenic	13(1)	0.0005	0.0003 - 0.0010	0.0002
Barium	11(1)	0.30	0.12 - 1.50	0.40
Cadmium	11(1)	0.0011	< 0.0002 - 0.0100	0.0030
Cobalt	12(1)	< 0.001	-	0.000
Copper	12(1)	< 0.001	< 0.001 - 0.001	0.000
Iron	12(1)	0.060	0.009 - 0.200	0.064
Lead	12(1)	< 0.001	< 0.001 - 0.001	0.000
Manganese	12(1)	0.01	0.01 - 0.02	0.004
Mercury	10(1)	< 0.00005	-	0.000
Nickel	12(1)	< 0.001	< 0.0001 - 0.001	0.000
Selenium	13(1)	0.0002	0.0001 - 0.0003	0.0001
Zinc	12(1)	< 0.001	< 0.001 - 0.001	0.000

<sup>a</sup>in mg/L unless otherwise indicated

<sup>b</sup>extractable values reported for metals levels