Water quality criteria for European freshwater fish

Report on nitrite and freshwater fish

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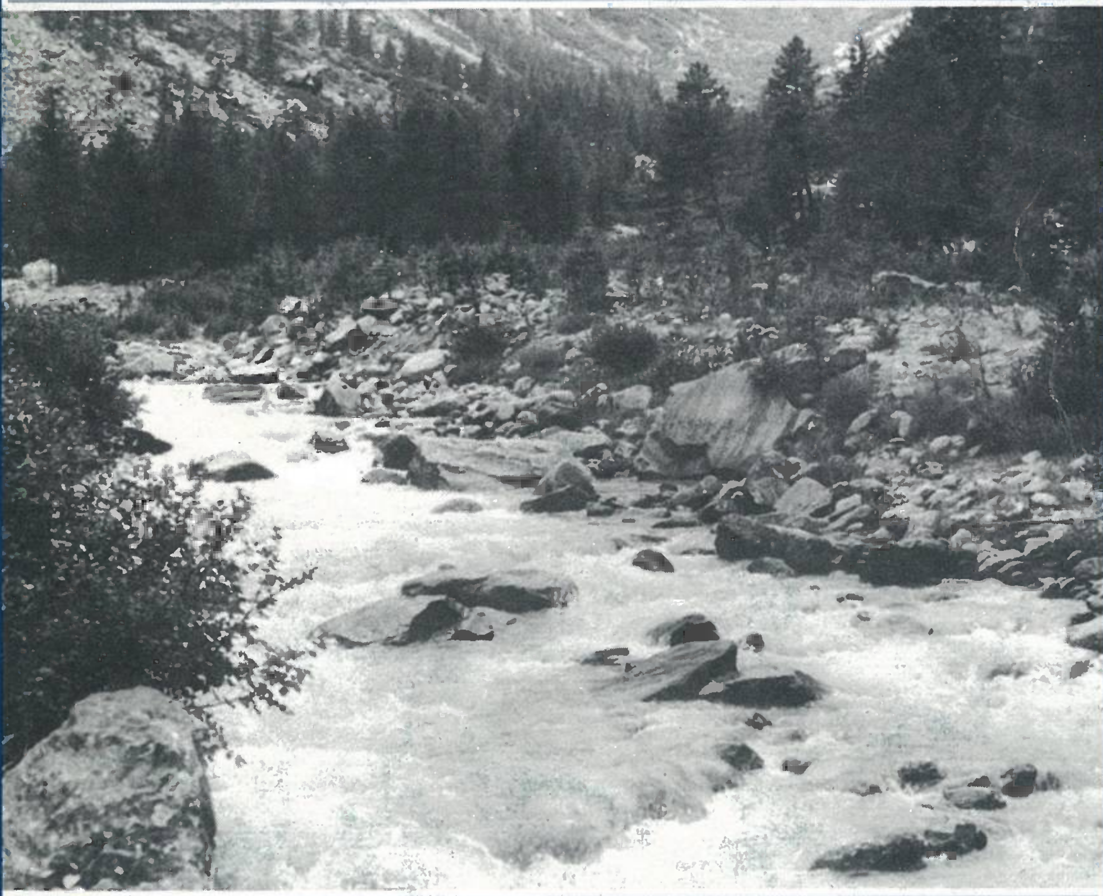

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Water quality criteria for European freshwater fish

Report on nitrite and freshwater fish

Prepared by the
EIFAC Working Party on Water Quality Criteria
for European Freshwater Fish

EUROPEAN INLAND FISHERIES
ADVISORY COMMISSION
(EIFAC)

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PREPARATION OF THIS DOCUMENT

This is the thirteenth review of the EIFAC Working Party on Water Quality Criteria for European Freshwater Fish.

For the preparation of this report, the following experts were appointed to the Working Party:

D. Calamari (Italy) - Convener

A. Arillo (Italy)

F.B. Eddy (United Kingdom)

R. Lloyd (United Kingdom)

J.F. de L.G. Solbé (United Kingdom)

FAO Secretariat:

D. Charbonnier - EIFAC Secretary

The Working Party used the same general basis for their work as that on which they had agreed for the preparation of their first report that:

"Water quality criteria for freshwater fish should ideally permit all stages in the life cycles to be successfully completed and, in addition, should not produce conditions in a river water which would either taint the flesh of the fish or cause them to avoid a stretch of river where they would otherwise be present, or give rise to accumulation of deleterious substances in fish to such a degree that they are potentially harmful when consumed. Indirect factors like those affecting fish-food organisms must also be considered should they prove to be important."

This report was prepared by F.B. Eddy, to be reviewed by the Working Party and to be presented to the Thirteenth Session of EIFAC (Aarhus, 23-30 May 1984) for approval.

Distribution:

Authors
EIFAC Mailing List
FAO Fisheries Department
FAO Regional Fishery Officers

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CONTENTS

1. INTRODUCTION

The harmful effects of nitrite to man and higher vertebrates have long been recognized, but it is only in recent years that their toxicity to fish has started to attract attention. Perhaps one of the most important reasons for this is the use of intensive methods for fish culture, which may rely upon recirculating water systems to remove waste products, particularly ammonia, from the water. In these systems nitrifying bacteria in the filtration system oxidise ammonia via nitrite to nitrate and, but where the oxidation of ammonia is incomplete, relatively high concentrations of nitrite can occur which may cause fish mortalities.

Nitrifying bacteria are of course present in most natural waters and together with denitrifying bacteria, which reduce nitrate to nitrite and nitrogen gas, form important links in the nitrogen cycle of ponds, lakes, rivers and other bodies of fresh water. Thus most unpolluted fresh waters contain nitrite, but only in minute amounts; although changes in environmental conditions such as introduction of nitrogenous wastes, including sewage, reduction of dissolved oxygen and changes in temperature, may increase concentrations, particularly of nitrite, in localized areas where there may be poor water flow and circulation or inadequate mixing of wastes with the diluting flow.

2. OCCURRENCE AND PRODUCTION OF NITRITE

2.1 Natural Production of Nitrite

In aquatic systems nitrogen gas may be "fixed" by certain bacteria and blue-green algae to form ammonia which is oxidized to nitrite, nitrate and nitrogenous compounds useful to plants. Atmospheric nitrogen may be converted to oxides of nitrogen by combustion or by lightning, these compounds forming nitrites and nitrates when dissolved in water. Throughout this report, concentration will be expressed as the mass of nitrogen in nitrite, i.e., mg N. NO_2 or µg N.NO_2 and, unless otherwise stated, nitrite will refer to both the nitrite ion (NO_2^-) and its conjugate acid (HNO_2), (Section 3.1).

Minute amounts of nitrite, up to about 2 µg 1^{-1}N.NO_2 occur in unpolluted surface waters of lakes, the concentration tending to vary seasonally with maximal concentrations in winter and minimal in summer. It is thought that most NO_2^- originates from reduction of NO_3^- through the activity of phytoplankton. The vertical distribution of NO_2^- in lakes is closely correlated with oxygen content with a maximum tending to occur between a well-oxygenated region rich in nitrate and a lower almost anaerobic region rich in ammonia. In most cases NO_2^- is formed by reduction of nitrate, though there are a few examples of lakes where NO_2^- is believed to result from the oxidation of ammonia (Hutchinson, 1957). A well-documented example is Priest Pot, a small (1-ha), lake in the English Lake District, typical of many throughout the world where the bottom periodically becomes anoxic. Although the surface waters contained only 1-2 µg 1^{-1} N.NO_2, a narrow band of water approximately 1.5 m below the surface contained virtually no oxygen with a NO_2^- content of 45 µg 1^{-1} N.NO_2^- (Finlay, Span and Harman, 1983). A tropical lake in the Amazon Basin, Great Lake Jutai, temperature of 26°-31°C contained in its surface water NO_2^- concentrations of 0.5 µg 1^{-1}, increasing to three times that value in the stagnant anoxic region at about 8 m. This region also contained the highest concentrations of carbon dioxide, nitrates, ammonia, iron and phosphorus, particularly during the dry season when the water level was at its lowest, the period most critical for fish life (Santos, 1980). In a survey of 65 unpolluted Italian lakes, concentrations were 1-5 µg 1^{-1} N.NO_2 in the majority, 5-20 µg 1^{-1} N.NO_2 in many others, while in a few cases values of over 20 µg 1^{-1} N.NO_2 were observed (IRSA, 1980). The NO_2^- content of a number of North American Lakes was measured by McCoy (1972), who found little or no NO_2^- in open waters, 2.3 mg 1 N.NO_2 in bays and backwaters, while shore sites containing decaying organic matter, chiefly plants and algae, commonly contained 2-18 mg 1 N.NO_2, these sites occasionally containing as much as 180 mg 1^{-1} N.NO_2 (Table 1).

2.2 Nitrite Levels in Polluted Waters

Concentrations of NO_2^- around 10 µg 1^{-1} N.NO_2 in surface waters have long been regarded as warnings of sewage contamination. Indeed, nitrogenous wastes from a variety of sources may contain nitrite and generally effluents containing ammonia are also likely to have a significant nitrite content. Probably, the commonest source of ammonia is sewage effluent, particularly if nitrification has been inhibited, and also significant amounts of ammonia

Table 1

Author	Location, system	NO_2^- levels mg $N.NO_2$ l^{-1}	
McCoy, 1972	Lake sites in Wisconsin, USA	0	Open waters
		2-3	Bays and backwaters
		10-70	Shore sites with organic matter
		180 +	Water associated with decaying algae and aquatic weed
Collins, *et al.*, 1975, 1975a	Recirculating systems	up to 0.5	Established systems
		up to 16	Newly started systems
Hollerman and Boyd, 1980	Channel catfish ponds	0.06	Unaerated
		0.2	Aerated
Perrone and Meade, 1977	Recirculating water system for salmonids	up to 1.06	
Smith and Williams, 1974	Recirculating water systems for salmonids	0.15	
Saeki, 1965	Water re-use systems for carp	1.8	Maximum concentration tolerated by carps
Solbé, 1981	British trout fisheries	0.012-0.2	
	British coarse fish fisheries	0.012-0.25	
Tucker and Schwedler, 1983	Channel catfish ponds	up to 3	
Weston, 1974	Recirculating water system for salmonids	0.12	
Hutchinson, 1957	Surface waters of lakes	up to 0.002	See text for details
Finlay, Span and Harman, 1983	Priest Pot, English Lake District		
	Surface waters	0.002	
	1.5 m below surface	0.045	
Santos, 1980	Great Lake Jutai Amazon Basin		
	Surface waters	0.0005	See text for details
	Anoxia region	0.0015	
IRSA, 1980	Survey of 65 unpolluted Italian lakes		
	Majority	0.001-0.005	
	Many others	0.005-0.02	
	Small minotiry	over 0.02	
Klinger, 1957	Rivers receiving effluent from metal works, etc.	up to 30	See text for details
Brown, Bellinger and Day, 1982	River Hulme, West Yorkshire		
	Minimum level	0.01	See text for details
	Maximum level, sewage, textile works	1.2	
Walsh, Bahner and Horning, 1980	River water in US polluted by textile mill effluents	up to 16.8	See text for details

are released in effluents associated with industries producing coal, gas, coke and fertilizers. Ammonia enters water systems from agriculture particularly from silage, manure and fertilizer although these substances are often used to fertilize fish ponds in extensive fish culture. Fish and other aquatic animals themselves produce ammonia which, as has already been mentioned, may be of considerable importance in intensive fish culture, particularly if bacterial nitrification is used in recirculating systems. The occurrence and effects of ammonia on freshwater fish have been reviewed by EIFAC (1970).

The nitrite content of fresh waters in relation to fish has been specifically studied on only a few occasions, and there is a marked lack of data on this subject, particularly regarding rivers. Up to 16.8 mg l^{-1} NO_2^- was reported from a variety of textile mill effluents in the United States (Walsh, Bahner and Horning, 1980). In the relatively unpolluted river Holme (West Yorkshire, UK), minimum NO_2^- levels were 0.01 mg l^{-1}, the average 0.16 mg l^{-1}, while maximum levels of 1.2 mg l^{-1} occurred in association with discharges from textile mills and sewage works (Brown, Bellinger and Day, 1982); minimum chloride levels were 30 mg l^{-1}. A detailed survey on the relationship between the NO_2^- content of waters and their fishery status was conducted by Solbé (1981), and generally poor quality fisheries were associated with higher NO_2^- concentrations. Good salmonid fisheries were found in waters of low NO_2^- content, while poor ones occurred at NO_2^- concentrations from 60 µg to 200 µg l^{-1} $N.NO_2^-$. Coarse fisheries occurred in waters up to 0.3 mg l^{-1} $N.NO_2^-$, but nitrite was not necessarily a critical factor in determining the quality of the coarse fishery. Additional data on the NO_2^- content of fish bearing waters are given in Table 1.

2.3 Bacterial Production of Nitrite

Two groups of bacteria are principally responsible for nitrification or oxidation of ammonia to nitrate. The first *Nitrosomonas* oxidize NH_3 to NO_2^- while *Nitrobacter* oxidize NO_2^- to NO_3^-, in each case oxygen is required. In many instances the rate of NO_2^- oxidation is faster than its formation and in sewage systems, where nitrification has been particularly well studied, the concentration of NO_2^- rarely exceeds 2 mg l^{-1} in waste waters. Nitrification tends to be inhibited at low temperatures, particularly below 5°C and also in acid waters (Collins, *et al.*, 1975), noting a lower rate of pH 6 and complete inhibition at pH 5.5 in a recirculating water system containing channel catfish. Antibacterial agents, such as erythromycin, reduced nitrification rates (Collins, *et al.*, 1975a), as did methylene blue when used as a parasiticide, but formalin, malachite green in combination with copper sulphate and potassium permanganate were without effect at therapeutic levels (Collins, *et al.*, 1975b).

The first stage in denitrification is the reduction of NO_3^- to NO_2^- and the second step is reduction of NO_2^- to nitrogen gas or N_2O and each step can be carried out by several species of bacteria. The reaction is usually aerobic, but under anaerobic conditions oxygen from nitrate may be utilized, i.e., reduction of NO_3^-. Thus, in fresh waters supporting fish denitrification is capable not only of producing nitrite, but also of reducing the dissolved oxygen content of the water.

It is not the purpose of this review to give a detailed account of nitrification and denitrification, but such reviews are to be found in Focht and Chang (1976), and Henze-Christensen and Harremoes (1976).

3. CHEMISTRY AND ANALYSIS

3.1 Chemistry

In aqueous solution the nitrite ion exists in equilibrium with its conjugate acid and the concentration of each species is determined by pH and temperature.

Thus $$HNO_2 \rightleftharpoons H^+ + NO_2^-$$

and an increase in pH will favour an increase in the amount of NO_2^-. The relative amounts of NO_2^- and HNO_2 can be calculated with knowledge of the pH value of the water, its temperature and the equilibrium constant or pKa for HNO_2.

Thus from the expression

$$\text{antilog (pH - pKa)} = \frac{\text{base}}{\text{acid}}$$

at pH 7 and 12.5°C the pKa of nitrous acid is 3.337 (Weast, 1978), and therefore base/acid is 4603 or 99.978% exists as NO_2^-. A decrease of one pH unit in the water will decrease base/acid by 10 times to 457 or 99.781% as NO_2^-. Temperature variation of pKa for HNO_2 is given by Colt and Tchobanoglous (1976), as

$$\frac{655.586}{T + 273.16} + 1.148$$

where T is the water temperature, however, the dissociation constant of nitrous acid with respect to changes in the temperature and pH of water are not well-documented. Some examples of the proportion of NO_2^- relative to HNO_2 at different water pH values are shown in Table 2a, while variation of pKa NHO_2 with temperature calculated according to Colt and Tchobanoglous (1976), are shown in Table 2b.

Table 2a

Equilibrium of nitrite and nitrous acid at different water pH values Temperature 12.5°C

Water pH	$\frac{\text{base}}{\text{acid}}$ or $\frac{NO_2^-}{HNO_2}$	% NHO_3
10	4 602 566	0.00002
9	460 257	0.0002
8	46 030	0.0022
7	4 603	0.022
6	460	0.22
5	46	2.17
4	4.6	21.7
3.3	1	50

Table 2b

Variation of pKa HNO_2 with temperature (Colt and Tchobanoglous, 1976)

Temperature	pKa HNO_2
5	3.505
10	3.463
15	3.423
20	3.384
25	3.347
30	3.311
35	3.275

As the water is cooled the dissociation constant for HNO_3^- increases, so increasing the proportion of HNO_2. Thus at a pH of 7.0 and at 5°C there is 0.032% in the form of HNO_2 while at 25°C only 0.022% is HNO_2.

3.2 Reactivity of Nitrite with Some Molecules of Relevant Biological Interest

3.2.1 Formation of nitroso-compounds

In aqueous solutions and particularly in acid conditions, such as in the stomach where the pH value may approach the pKa value of nitrous acid, this substance is converted to a variety of active nitrosating agents, e.g., nitrous anhydride N_2O_3, nitrosyl thiocyanate ON-NCS, nitrosyl halide NOX, nitrous acidium ion $H_2NO_2^+$. These substances can actively nitrosate various classes of compounds such as amines, amides, thiols, peptides, sugar-aminoacids, etc., to form nitroso-compounds, many of which are toxic, mutagenic and/or carcinogenic (Natake *et al.*, 1979; IARC, 1982). Nitrosation of secondary amines is of a particular interest because fish contain relatively large amount of dimethyl-amine. Thus, for example:

$$2\ HNO_2 \rightleftharpoons N_2O_3 + H_2O$$

and $$R_2NH_2 + N_2O_3 \rightarrow R_2N.NO + HNO_2$$

the rate of reaction being largely dependent upon the nitrous acid concentration (Mirvish, 1975).

3.2.2 Formation of nitric oxide complexes

Nitrite can react, especially in anaerobic conditions, with several haemoproteins other than haemoglobin to produce, for example, a mitochondrial NO- cytochrome (Walters and Taylor, 1965), and a microsomal NO- cytochrome P-450 complex (Kahl, Wulff and Netter, 1978; Duthu and Shertzer, 1979); the latter may seriously impair the microsomal metabolism of several hazardous compounds.

3.3 Analysis

Nitrite is conveniently analysed spectrophotometrically using a variety of methods based on the reaction of nitrite with sulphanilic acid to form a diazonium salt which couples with a napthylamine derivative (e.g., 1-napthylamine 7-sulphinic acid), to form a red colour (Wood, Armstrong and Richards, 1967; Shechter, Gruener and Shuval, 1972). Such methods are suitable for most work involving water samples, but a more sensitive method using chemiluminesence can be employed (Walters, et al., 1980). There are a number of problems associated with determination of nitrite in tissues and other biological material. Strongly acidic media should be avoided (N HCl or greater), since this may cause denitrosation of some nitrose compounds leading to an apparent increase in free nitrite concentration, while in less acidic media some biological compounds such as NADH may cause a loss of colour development. When assaying such samples, it is recommended that interference effects should initially be assessed by checking for recovery of added nitrite standards (Arillo, personal communication). Polymers and ion exchange resins selective for anions including NO_2^- may prove to be useful analytical tools after further development (Chiou, et al, 1981), as may gas/liquid chromatographic methods. Nitrous oxide electrodes are useful in that an instantaneous result is obtained, but sensitivity is limited at 6 mg l^{-1} NO_2^- (Krous, Blazer and Meade, 1982).

4. TOXIC ACTION AND LETHAL EFFECTS OF NITRITE

Nitrite is a highly toxic substance and small amounts entering the body, either from the diet or from the aquatic environment, may prove harmful to fish. This section reviews the research which has been carried out on various aspects of its mode of action.

4.1 Dietary Nitrite

Most of the data on the effects of dietary nitrite relate man and laboratory mammals and the main points are summarized here because they may be of relevance, at least in a broad sense, to fish.

Nitrite may be ingested in the diet or may be formed in the gut as a result of bacterial reduction of nitrate. In man, the principal source of dietary nitrite is cured meats and fish, where the nitrite has an antibacterial activity as well as imparting favourable flavour and colour. A limit of 200 mg kg^{-1} of $NaNO_2$ has been imposed on cured meats with dietary intake of NO_2^- recommended at not greater than 0.4 mg kg^{-1} per day in USA (Wolff and Wasserman, 1972), while the World Health Organization recommends that dietary intake should not be greater than 1 mg l^{-1} as $N.NO_2^-$ (Dean and Lund, 1981; WHO, 1978).

Dietary nitrate is not itself toxic, but only becomes so when the gut environment favours microbes which can reduce nitrate to nitrite. Thus, the relatively alkaline gut of infants, particularly those under four months old, favours production of nitrite which may, in extreme cases, result in methaemoglobinaemia and mortalities. Also decomposing vegetation such as silage may favour nitrate reduction which, when ingested, may induce methaemoglobinaemia in livestock.

Dietary aspects of nitrate and nitrite metabolism in fish have been little studied and experiments should be conducted to determine whether nitrate is reduced in the fish gut, particularly in herbivorous species.

4.2 Methaemoglobin

Transport of oxygen from the respiratory surface to the tissues depends upon the reversible combination of haemoglobin with oxygen. Functional haemoglobin contains iron in the divalent (Fe^{2+}) form, but oxidation to the trivalent (Fe^{3+}) form, which can be achieved by a number of substances including nitrite, results in the characteristically brown-coloured pigment, methaemoglobin which is no longer able to react reversibly with oxygen.

In both mammals and fish, a small amount of the haemoglobin, usually 5% or less (Meade and Perrone, 1980; Eddy, Kunzlick and Bath, 1983), exists as methaemoglobin and the red cells contain a reductase enzyme which keeps this oxidized form to minimal levels. The presence of nitrite in the water rapidly induces methaemoglobinaemia in freshwater fish, the amount of methaemoglobin produced depending upon the nitrite concentration, the chloride concentration (see Section 5.1), the species of fish and the exposure time.

Fish are able to accommodate relatively high levels of methaemoglobin, e.g., 25-30% is regarded as a safe level for channel catfish (Ictalurus punctatus)(Tucker and Schwedler, 1983), while with over 70% methaemoglobin both rainbow trout (Salmo gairdneri) and chinook salmon (Oncorhynchus tschawytscha) were stressed, but mortalities did not occur (Smith and Williams, 1974; Brown and McLeay, 1975). Fish are able to survive with considerably less than their normal complement of functional haemoglobin, as has been demonstrated with carbon monoxide (Anthony, 1961) injection of drugs, such as phenylhydrozine hydrochloride or replacement of blood with plasma or saline. It was shown that apart from an increase in cardiac stroke volume, rainbow trout with haemotocrit values of 2-5% showed more or less normal respiratory patterns (Cameron and Davis, 1970). Certain Antarctic fish possess no haemoglobin at all, the blood plasma alone fulfilling respiratory requirements mainly because at near freezing temperatures its dissolved oxygen content is substantially increased (Holeton, 1970, 1971).

4.3 Nitrite Uptake into Blood and Tissues

Appearance of methaemoglobin is associated with NO_2^- entering the blood plasma and the rate of methaemoglobin formation is closely correlated to the concentrations of NO_2^- in the blood, as is the disappearance of methaemoglobin when fish are returned to clean water (Eddy, Kunzlick and Bath, 1983). Rapid recovery of channel catfish with nitrite-induced methaemoglobinaemia was shown to be associated with the presence of a methaemoglobin reductase enzyme in the red blood cells (Huey and Beitinger, 1982; Freeman, Beitinger and Huey, 1983). Nitrite enters the blood via the gills and the chloride cells seem to be involved since they become hypertrophic and increase in number in the gills of nitrite exposed rainbow trout (Gaino, Arillo and Mensi, 1984). Similar changes were seen in fish kept in deionized water where chloride cells developed in the gill secondary lamellae rather than in the primary epithelium (Laurent and Dunel, 1980), while a correlation between levels of nitrite in the blood plasma and the number of lamellar chloride cells was noted by Krous, Blazer and Meade (1982).

A further interesting feature is the ability of gills to concentrate nitrite in blood and tissues to many times the external level. In rainbow trout exposed for 24 h to 10 mg l^{-1} $N.NO_2^-$, the blood concentration was 100-140 mg l^{-1} $N.NO_2^-$, a concentration gradient of 10 (Bath and Eddy, 1980; Eddy, Kunzlick and Bath, 1983), while exposure of free swimming trout to a much lower level of NO_2^- (0.45 mg l^{-1} $N.NO_2^-$) for 72 h resulted in levels of 19 mg l^{-1} $N.NO_2^-$ in blood. However, in fish which had overturned, much higher blood levels of up to 34 mg l^{-1} $N.NO_2$ were noted (Margiocco, et al., 1983), which represents a concentration gradient of about 70. These authors also showed that nitrite penetrated the tissues with the concentration in gills, liver and brain being below the blood level with much lower concentrations occurring in muscle.

The mechanism by which nitrite enters the gills and its concentration in blood and tissues is not completely understood, but it has been suggested that the branchial Cl^-/HCO_3^- exchange mechanism (Maetz, 1971), which is believed to be responsible for chloride uptake in freshwater fish, has an affinity not only for chloride but also for nitrite. If this was the case, then nitrite should be absorbed at a predictable rate in the absence of chloride while inhibitors of chloride uptake should also arrest nitrite uptake. There is some evidence to support this hypothesis (Bath and Eddy, 1980; Eddy, Kunzlick and Bath, 1983). A second possibility is that the gills are permeable to HNO_2 (the conjugate acid of NO_2^-), but not to nitrite itself, and this theory predicts that when the water is more acidic than the blood, NHO_2^- will diffuse across the gills and ionize in the blood to form NO_2^-. Thus, the toxicity of nitrite should be influenced by the water pH value and in alkaline waters nitrite should be least toxic. Evidence for this theory is not strong since it does not explain the protective effect of Cl^- (see Section 5.1), but the toxicity data of Russo, Thurston and Emerson (1981) suggest that both NO_2^- and HNO_2 are toxic species.

4.4 Physiological Effects

It has been reported that tricaine methanesulphonate (MS 222), ameliorates nitrite-induced methaemoglobinaemia in channel catfish possibly through partial inhibition of the branchial chloride uptake mechanism (Huey and Beitinger, 1982a). Dietary ascorbic acid (approximately 200 mg kg^{-1}), increased tolerance of steelhead trout to nitrite (Blanco and Meade, 1980); these authors also noted in normal animals an increased methaemoglobinaemia at warmer temperatures. When investigating the response of channel catfish to either ammonia or nitrite, Tomasso, Davis and Simco (1981) noted increases in the concentration of blood plasma corticosteroids. With ammonia, a peak occurred after 8 h, declining to near normal levels after 24 h, but with 5 mg 1^{-1} nitrite (1.5 mg 1^{-1} $N.NO_2$) there was a build-up of corticosteroids, reaching 10 times the control value at 24 h, and it was suggested that catfish were able to adapt more easily to ammonia than to nitrite. Rainbow trout exposed to 0.45 mg 1^{-1} $N.NO_2^-$ for up to 72 h showed inhibition of liver lysosomal proteolytic activity, as well as increased fragility of lysosomal membranes (Mensi, et al., 1982). Nitrite intoxication in rainbow trout exposed to 0.45 mg 1^{-1} $N.NO_2^-$, particularly evident in overturning fish, was attributed to liver damage caused by anaemic hypoxia (i.e., when the blood oxygen carrying capacity is reduced, such as in methaemoglobinaemia in contrast to hypoxic hypoxia which occurs when the environmental oxygen concentration is reduced). Thus, nitrite caused structural and biochemical damage in hepatocytes and in liver mitochondria, which led to decreases in glycogen and ATP and increases in lactate and succinate, paralleled by an uncoupling-like effect in biochemical respiration. Similar tests on brain suggested that it was less susceptible than liver to elevated nitrite concentrations (Arillo, et al., 1984).

Nitrite has been used therapeutically in man as a vasodilator and blood-pressure depressant and this effect also occurs in fish as well (Bath, 1980; Windholz, 1976).

4.5 N-Nitroso Compounds

Nitrite reacts with some classes of amines and other compounds to form N-nitrosamines and related N-nitrosamides, many of which have been shown to have carcinogenic or mutagenic properties, e.g., a single dose of 5 ppm N-nitrosodimethylamine fed to rats induced tumours in more than 70% of the animals (Wolff and Wasserman, 1972; and Section 3.2).

Such studies in fish are rare, but De Flora and Arillo (1984) noted mutations in *Salmonella typhimurium* treated with muscle extracts from rainbow trout which had been exposed to 450 µg 1^{-1} $N.NO_2^-$.

Waters containing nitrite, especially those receiving discharges from sewage effluent, are likely to contain a number of amines which are potential sources of nitrosamines. The formation of such compounds is increased by the degradation products of pesticides and especially by diethylanolamine, a common constituent of detergents, and other consumer products (Yordy and Alexander, 1981). The effects on fish life of such precursors of nitroso compounds in combination with nitrite are unknown.

5. PHYSICOCHEMICAL FACTORS AFFECTING LETHAL CONCENTRATION

5.1 Chloride

The main environmental factor which affects nitrite toxicity is undoubtedly chloride. Early studies on nitrite toxicity to fish produced widely varying results even with the same species, and these difficulties were not resolved until it was shown that chloride ions in the external medium strongly counteracted nitrite toxicity to coho salmon (Oncorhynchus kisutch)(Perrone and Meade, 1977). Confirmatory results were obtained for rainbow trout (Wedemeger and Yasutake, 1978; Bath, 1980; Bath and Eddy, 1980; Russo, Thurston and Emerson, 1981), and for channel catfish (Tomasso, Simco and Davis, 1979). Thus, the concentration of chloride relative to that of nitrite (expressed by weight, i.e., mg Cl^-/mg $N.NO_2^-$ l^{-1}) is of critical importance when considering water quality and toxicity to fish.

The $Cl^-/N.NO_2^-$ ratio giving maximum protection to rainbow trout was about 15 (Bath and Eddy, 1980), about 18 for coho salmon (Perrone and Meade, 1977), and 41 for channel catfish (Tomasso, Simco and Davis, 1979), while a ratio of 10 was sufficient to prevent mortalities in channel catfish held in ponds, although the fish had methaemoglobin levels of 25-30% (Tucker and Schwedler, 1983).

5.2 Other Inorganic Ions

The efficacy of a number of inorganic ions in reducing the acute toxicity of nitrite has been tested and a variety of anions only one bromide showed the same potency as chloride with bicarbonate showing moderate potency for rainbow trout (Eddy, Kunzlick and Bath, 1983), and also for channel catfish (Huey, Simco and Oriswell, 1980). Addition of calcium reduced mortality, but not methaemoglobin in chinook salmon (Crawford and Allen, 1977), and also in steelhead trout (Salmo gairdneri)(Wedemeger and Yasutake, 1978), these authors demonstrated an alleviating effect of methylene blue. Tomasso et al. (1980), noted that chloride added as the calcium salt rather than as the sodium salt at a concentration of 60 mg l^{-1}, resulted in a small increase in the 24 h LC_{50} to channel catfish (91 and 98 mg l^{-1} NO_2^-, respectively)(Table 3). There was an insignificant difference in protection offered to rainbow trout exposed to 9.8 mg l^{-1} $N.NO_2^-$ between chloride added either as the calcium or the sodium salt (Eddy, Kunzlick and Bath, 1983), but considerable superiority in the protective effect of calcium chloride to steelhead trout, in some instances six times as great as sodium chloride, was noted by Wedemeger and Yasutake (1978).

5.3 pH

A number of reports suggest that nitrite (NO_2^- + HNO_2) toxicity decreases with increasing pH. The 96-h LC_{50} for 10 g steelhead trout was 1.4 mg l^{-1} $N.NO_2^-$ at pH 6 increasing to 3.6 mg l^{-1} $N.NO_2^-$ at pH 8 (Wedemeger and Yasutake, 1978). The 48-h LC_{50} for bluegills was substantially increased at pH 7.2 compared with pH 4 (Huey and Beitinger, 1982), while coho salmon exposed to 3 mg l^{-1} $N.NO_2^-$ showed higher levels of blood plasma nitrite at pH 8 compared to pH 6.5 (Meade and Perrone, 1980).

In the pH range of 7.5-8.5, unless HCl was added, there was no difference in toxicity of NO_2^- (Russo and Thurston, 1977). A similar result was noted by Bath (1980) in rainbow trout exposed for 96 h to 10 mg l^{-1} $N.NO_2^-$ where there was no difference in mortalities at intermediate pH values, but there was enhanced survival at pH 8.8 and 10 and an increased mortality at 4.6, this pH range giving a 10^5 increase in HNO_2 concentration, which was not significantly linked to survival. However, Russo, Thurston and Emerson (1981), from an extensive series of toxicity tests on rainbow trout in the pH range of 6.4-9.1, found that as pH increased, the toxicity of NO_2^- decreased while that of HNO_2 increased suggesting that both forms - acid and anion - are toxic. Thus, second only to chloride, the pH of the water appears to be an important variable when considering nitrite toxicity, but the available data do not allow firm conclusions, particularly at intermediate pH values.

6. LONG-TERM LETHAL CONCENTRATIONS AND SUBLETHAL EFFECTS

Compared with work on acute lethal concentrations, there have been few laboratory studies on the long-term effects of nitrite. A number of studies have shown that fish of various species are tolerant of moderately high levels of nitrite-induced methaemoglobinaemia (see Section 4.1), one example being that coho salmon apparently tolerated up to 80% methaemoglobin without stress (Perrone and Meade, 1977). It seems likely that blood methaemoglobin levels may be of critical importance in waters of reduced oxygen content and during periods of activity, but as yet there is no information on these points.

Table 3

Summary of nitrite toxicity data for a variety of fish species
Tests are static unless indicated by * which indicates use of a flow through system

Fish species	Weight (g)	Temp. (°C)	pH value	Ca hardness as mg l^{-1} $CaCO_3$	Cl^- mg l^{-1}	LC_{50} in mg l^{-1} $N.NO_2$ or other response	Exposure period days	Author and additional information
Steelhead trout (*Salmo gairdneri*)	5	10	6.8	25	1	0.5		
			8.4	300	8.4	10.3		Wedemeger and Yasutake,
	10		6.8	25	1	0.9	4	1978
			8.4	300	8.4	12.1		
Rainbow trout (*Salmo gairdneri*)	10-25	10	6.8	8 ppm Ca^{2+}	7	3.9	4	Bath, 1980
							indefinite survival	Eddy, Kunzlick and Bath, 1983
Coho salmon* (*Oncorhynchus kisutch*)	13	11	7.2	32.3	19.6	50% mort. 9.2+	1	
	22				148	0% mort. 8.9+	3	Perrone and Meade, 1977
	Fry				32.2	0% mort. 8.9+	3	
Rainbow trout* (*Salmo gairdneri*)	70	10	7.92	199	1.2	0.46	4	Russo and Thurston, 1977
	99		7.74		41	12.2		
Rainbow trout* (*Salmo gairdneri*)	28	10	7.5	174	10.9	3.74	4	Russo, Thurston and Emerson, 1981
	79		7.9	177	10.4	3.54		
	147		8.5	188	10.6	4.35		Similar results for fish weight 25-341 g also results at intermediate pH values.
	244		8.6	184	10.5	5.34		
	9-15	10	7.0	178-209	0-0.47	0.14	4	0.47 mg C^{-1} chloride used to calculate ratio
			7.9			0.21		
			9.0			1.12		

Table 3 (continued)

Fish species	Weight (g)	Temp. (^oC)	pH value	Ca hardness as mg l^{-1} $CaCO_3$	Cl^- mg l^{-1}	LC_{50} in mg $l^{-1}N.NO_2$ or other response	Exposure period days	Author and additional information
Channel catfish (Ictalurus punctatus)	7-13 cm	21-24	7.0	40 mg l^{-1}	4 60 as NaCl 60 as $CaCl_2$	4.99 98 91	1 1 1	Tomasso, et al., 1980
Channel catfish (Ictalurus punctatus)	7-13 cm	22-25	7.0		61-306	No significant MeHb formation	1	Tomasso, Simco and Davis, 1979
Fathead *minnow** (Pimephales promelas)	2.3	13	8.05	199	-	2.3-2.99 3.9-5.5	4 1	Russo and Thurston, 1977
Mottled sculpin* (Cottus bardi)	5.2	14	8.08	199	-	60	6	
Carp* (Cyprinus carpio)	5.6 cm	14	7.6	260	19	40	4	Solbé, et al., 1981
Roach* (Rutilus rutilus)	6.9 cm	16	7.4	261	20	12	4	Cooper and Solbé, 1980

One of the few long-term studies is that of Wedemeger and Yasutake (1978) on steelhead trout. In soft water of low chloride content, fish exposed to a range of nitrite levels with maximum of 0.06 mg l^{-1} $N.NO_2^-$ for up to six months showed no detectable physiological changes apart from mild methaemoglobinaemia (about 5%), while growth was normal and there were no mortalities. During the initial four-week exposure period, a few secondary lamellar epithelial cells showed hypertrophy followed by hypertrophy of almost the entire secondary lamellar epithelium. After seven weeks, the changes were seen less frequently, suggesting adaptation was occurring, and after 28 weeks the fish had recovered showing little or no lamellar change. Further evidence that fish may acclimate to nitrite is given by Tucker and Schwedler (1983), showing that channel catfish previously kept in low levels of nitrite (0.01 mg l^{-1} $N.NO_2^-$) and then exposed to about 8.2 mg l^{-1} $N.NO_2^-$ developed significantly less methaemoglobin than similarly tested catfish which had previously not been exposed to nitrite.

The medium lethal concentration of nitrite after two-three weeks exposure was 21.8-26.4 mg l^{-1} $N.NO_2^-$ for carp (Cyprinus carpio) and 9.0-11.2 mg l^{-1} $N.NO_2^-$ for roach Rutilus rutilus (95% confidence limits); the chloride level in each case was about 20 mg l^{-1}, giving $Cl^-/N.NO_2$ ratios of 0.8 and 2, respectively (Solbé, 1981). This compares with 96-h LC_{50} values of 40 mg l^{-1} and 12 mg l^{-1} found by this author for the same two species (Table 3). The 42-day nitrite LC_{50} for brown trout (Salmo trutta) was 1.0 mg l^{-1} $N.NO_2$ in well-aerated water with a chloride concentration of 20 mg l^{-1} and total hardness 271 mg l^{-1} as $CaCO_3$. For brown trout exposed to water of gradually reducing oxygen content from around 100% air saturation to 40% air saturation over an eight-day period and held at the lower level for a further seven days before exposure to nitrite, the 84-day LC_{50} value was 0.72 mg l^{-1} $N.NO_2$ (Willis, personal communication).

7. FIELD DATA

A survey has been carried out by Solbé (1981) to obtain a relationship between the status of fisheries (salmonid and coarse) and the mean concentrations of nitrite and chloride found in UK waters. These data are shown in Figure 1. The correlation between fisheries, nitrite and chloride may be masked at higher chloride concentrations if these are associated with sewage effluent discharges containing other pollutants, in particular ammonia to which both coarse and salmonid fish are in general equally sensitive. Nevertheless, the data show that even in the presence of other associated pollutants, in waters with a mean chloride concentration of up to 25 mg l^{-1}, good salmonid fisheries were associated with concentrations of nitrite below 50 µg l^{-1} $N.NO_2$. Values for 95% percentiles were found to be three times the mean nitrite concentration, that is, 300 and 450 µg l^{-1} $N.NO_2$, respectively (Figure 1).

8. SUMMARY OF TOXICITY DATA ON FISH

Toxicity data for nitrite relating to freshwater fish can be correctly assessed only if other water quality values are known, the most important being chloride, although pH and calcium are of some importance as well (see Sections 5.1, 5.2 and 5.3). Thus, Table 3 has been assembled making use of those reports offering reasonable complete data on mortalities as well as water quality. Most of these studies are on salmonids, particularly rainbow trout, while a few relate to channel catfish and, as yet, there has been very little work on coarse fish.

The second part of this section briefly reviews other studies on nitrite toxicity to freshwater fish. Klinger (1957) investigated the effect of nitrite on minnows (Phoxinus laevis), noting that at sublethal concentrations the fish responded by decreasing activity and often becoming motionless on the bottom - an observation noted by subsequent workers using a variety of species. Weber (1966) noted the ameliorating effect of calcium on guppies (Lebistes reticulatus) exposed to nitrite, while Wallen, Greer and Lasater (1957) examined the toxicity of a variety of separate chemicals to Gambusia affinis, finding nitrite to be second only to cyanide in toxicity reporting a 48-h LC_{50} value of 7.5 mg l^{-1}. The toxicity of 13 species of North American freshwater fish was assessed by McCoy (1972) who found that perch (Perca carpoides) were amongst the most sensitive surviving less than 3 h in 5 mg l^{-1} $N.NO_2^-$. However, carp (Cyprinus carpio) and black bullhead (Ictalurus melas) survived 40 mg l^{-1} $N.NO_2^-$ for at least 48 h - the duration of the test, while common suckers (Catostomus commersoni) survived at least 48 h in 100 mg l^{-1} $N.NO_2^-$. The size of the fish tested were described as "fingerling" or "minnow", while the concentrations of nitrite used in tests were similar to those found in the field. Russo and Thurston (1977)

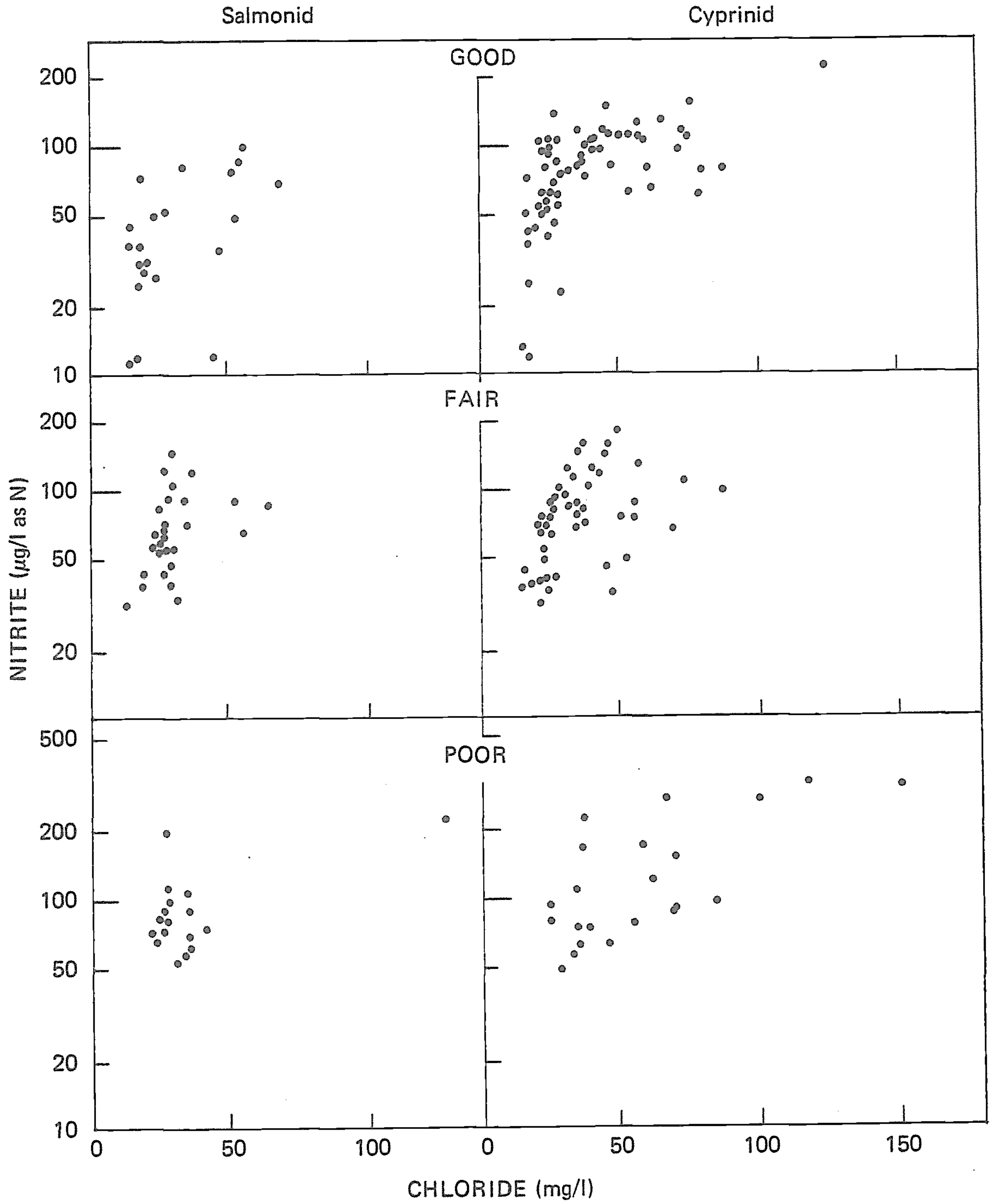

Fig. 1. Relationships between the mean concentrations of chloride and nitrite in each type of fishery (Solbé, 1981)

found fathead minnows (Pimephales promelas) about an order of magnitude less sensitive than rainbow trout (and cut-throat trout), while mottled sculpins (Cottus bardi) survived the highest concentrations of NO_2^- tested, 67 mg 1^{-1}, without mortality.

Studies on salmonids not included in Table 3 are those of Weston (1974) who noted a 96-h median tolerance limit of about 3 mg 1^{-1} NO_2^- (0.91 mg 1^{-1} $N.NO_2$) for chinook salmon fingerlings, while Smith and Williams (1974) found the 24-h LC_{50} value for rainbow trout fingerlings to be 1.6 mg 1^{-1} $N.NO_2^-$ considerably higher than a value of 0.96 mg 1^{-1} $N.NO_2^-$ for larger fish. The finding that fry and fingerling stages of salmonids are more tolerant of nitrite than later stages was also noted by Perrone and Meade (1977), Russo, Smith and Thurston (1974) and Russo and Thurston (1977). Toxicity of nitrite to seawater adapted rainbow trout and Atlantic salmon (Salmo salar) was studied by Eddy, Kunzlick and Bath (1983), while Crawford and Allen (1977) noted that in seawater adapted chinook salmon methaemoglobinaemia developed even at low external nitrite levels, i.e., the high external concentration of chloride was not exerting the expected protective effect.

Toxicity studies for catfish (Ictalurus punctatus), not listed in Table 3, include those of Konikoff (1975) who noted a 96-h LC_{50} value of 7.4 mg l^{-1} $N.NO_2$, and Collins, et al., (1975a) who noted mortalities after about 10 days when the nitrite concentration reached around 15 mg l^{-1} $N.NO_2$ in a newly started water recirculating system.

Bearing in mind the important effects of chloride, two general conclusions can be drawn from the toxicity data:

(i) coarse fish, particularly bottom feeding types such as carp and bullheads, are much more resistant to nitrite than salmonids and other related species;

(ii) fry and fingerlings of salmonid species are more tolerant of nitrite than larger fish (Perrone and Meade, 1977; Russo, Smith and Thurston, 1974; and Russo and Thurston, 1977).

9. EFFECT OF NITRITE ON OTHER GROUPS OF AQUATIC ANIMALS

9.1 Invertebrates

One of the few freshwater invertebrates studied is the crayfish (Procambarus simulans) (Beitinger and Huey, 1981). At an external chloride concentration of 5 mg l^{-1}, the 96-h LC_{50} value was 6.1 mg l^{-1} NO_2^- (1.9 mg l^{-1} $N.NO_2^-$), but when the external chloride was increased to 300 mg l^{-1}, there were few mortalities. At lower pH values (pH 5.6 compared with 7.0), resistance times decreased slightly and the protective effect of chloride was reduced. There has been some work on marine invertebrates, particularly those species such as prawns useful in aquaculture and often reared in recirculating seawater systems (Wickins, 1981). Two observations from this work seem relevant. One is that relatively low levels of nitrite in sea water can cause mortalities, e.g., the 3-4 week LC_{50} value for juvenile Macrobranchium rosenbergii was 15.4 mg l^{-1} $N.NO_2^-$, while growth of other species was reduced at much lower levels and the expected protective effect of the high chloride content of sea water is apparently lacking. A second observation is that species having the respiratory pigment haemocyanin are apparently more susceptible to nitrite than those species without it (Wickins, 1982).

9.2 Amphibia

The 96-h LC_{50} value for larval salamanders (Amblystoma texanum) was 1.09 mg l^{-1} $N.NO_2^-$ at an external chloride concentration of 5 mg l^{-1} ($Cl/N.NO_2$ = 4.6), but there were no mortalities when the external chloride was increased to 300 mg l^{-1} (Huey and Baitinger, 1980). Tadpoles of Rana catesbiana developed methaemoglobinaemia in response to nitrite of up to 50 mg l^{-1} NO_2^- (15.2 mg l^{-1} $N.NO_2^-$ at an external chloride level of 5 mg l^{-1} ($Cl/N.NO_2$ = 0.31), but no methaemoglobin was produced when the chloride level was 50 mg l^{-1} ($Cl/N.NO_2$ = 3.3)(Huey and Beitinger, 1980a). A green frog of unknown species lived for four weeks in water of 100 mg l^{-1} $N.NO_2$ (McCoy, 1972).

It can be concluded that larval amphibians respond to nitrite in a manner similar to that for fish.

10. SUMMARY AND CONCLUSIONS

(i) Nitrite occurs naturally in lakes and rivers as a result of nitrification of ammonia and denitrification of nitrate, normal values being about 2-10 µg $^{-1}$ $N.NO_2$ in surface waters. In stagnant lakes and ponds much higher NO_2^- levels occur around anoxic regions (2.1).

(ii) Natural nitrite concentrations can be enhanced by discharge of effluents containing nitrite and by the partial oxidation of ammoniacal discharges as indicated by values in excess of 10 µg l^{-1} $N.NO_2$ (2.2).

(iii) Water reuse systems which depend upon bacterial nitrification of ammonia produced by fish can, under some conditions, achieve only partial oxidation leading to a build-up of NO_2^- (2.3).

(iv) Nitrite is toxic to vertebrates including fish and a principal effect is conversion of haemoglobin to the brown-coloured methaemoglobin which is incapable of transporting oxygen. This is not necessarily the prime toxic action since fish are moderately tolerant of 50% or more methaemoglobin in the blood, and it has been suggested that death may occur through effects on tissues or on the circulating system (4.1, 4.2, 4.3).

(v) The main toxic species is believed to be NO_2^- which enters the blood via the branchial Cl^-/HCO_3^- uptake exchange (4.3).

(vi) Nitrite toxicity is strongly alleviated by chloride ions in the water, and in waters where nitrite occurs and is likely to be a hazard to fish, it is recommended that both NO_2^- and Cl^- concentrations be measured to determine their weight ratio. For maximum protection a weight ratio (mg Cl^- l^{-1}/mg $N.NO_2$ l^{-1}) of about 17 is required for rainbow trout and about 8 for coarse fish (5, 6, 7).

(vii) In short-term exposures, LC_{50} values for several species of fish ranged from 0.1 to 1 mg l^{-1} $N.NO_2$ where very low concentrations occurred. Under other conditions, short-term LC_{50} values are in the range of 1-10 mg l^{-1} $N.NO_2$ for salmonids and up to 100 mg l^{-1} $N.NO_2$ for channel catfish (8 and Table 3).

(viii) The few data available on long-term studies indicate that in soft water of low chloride content, steelhead trout grew normally when exposed for six months to a range of nitrite levels, the maximum being 0.06 mg l^{-1} $N.NO_2$ (6).

(ix) There are no field data on fish populations in waters where nitrite was the only pollutant. However, an extensive field survey showed that in waters with a mean chloride concentration of up to 25 mg l^{-1}, good salmonid fisheries were associated with concentrations of nitrite below 50 µg l^{-1} $N.NO_2$ and good coarse fisheries below 100 µg l^{-1} $N.NO_2$. Values for 95% percentiles were found to be three times the mean nitrite concentration, that is 300 and 450 µg l^{-1} $N.NO_2$, respectively (7 and Figure 1).

(x) (a) Tentative water quality criteria

It is clear that nitrite is most toxic to fish in water of low chloride content. It is proposed that for salmonid waters the average nitrite concentration should not exceed 0.01 mg l^{-1} $N.NO_2$ where the chloride concentration is 1 mg l^{-1}. Standards derived from waters of higher chloride content are derived from an analysis of laboratory and field data. For coarse fisheries the proposed standard for nitrite is twice that for salmonids, and these proposed criteria are shown in the following Table. These standards apply to the above concentrations of nitrite and experience has shown that at the higher nitrite levels other pollutants may be present in significant concentrations to affect the fish populations present.

Chloride mg l^{-1}	Nitrite criteria as mg l^{-1} NO_2-N			
	Salmonid		Coarse fish	
	Average	95 percentile[a]	Average	95 percentile[a]
1	0.01	0.03	0.02	0.06
5	0.05	0.15	0.10	0.30
10	0.09	0.27	0.18	0.54
20	0.12	0.36	0.24	0.72
40	0.15	0.45	0.30	0.90

a/ Based on ratio of average: 95 percentile of 1:3

These tentative criteria are applicable to situations where there are only moderate fluctuations in nitrite concentrations. There may be other situations, for example in intensive aquaculture with water recirculation through a biological filter, where sporadic high nitrite concentrations may occur for short periods which, although not causing the annual average concentration to exceed the tentative criteria, may be sufficiently high to cause mortality of fish.

(b) Protection of human health

Reference has been made in this report to the possible role of nitrite and nitrate in the formation of mutagenic and carcinogenic compounds in fish tissues. There is no information on the importance of such compounds in fish for human consumption, but it should be made clear that the water quality criteria proposed in the preceding paragraph apply to the maintenance of healthy populations of fish only and do not include public health considerations.

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