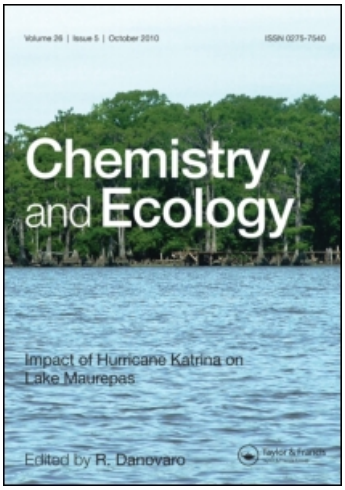

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Nitrite and Freshwater Fish

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Nitrite occurs naturally in fresh waters as a result of nitrification of ammonia and denitrification of nitrate, and its concentration can be enhanced by partial oxidation of ammoniacal discharges. Nitrite is toxic to vertebrates including fish and a principal effect is the conversion of haemoglobin to methaemoglobin which is incapable of oxygen transport although there are circulatory and tissue effects as well. The toxic species is the nitrite ion (NO_2^-) which is believed to enter the blood via the branchial chloride/bicarbonate exchange and fish such as salmonids with high chloride uptake rates are more susceptible than those with low chloride uptake rates, for example carp. Nitrite toxicity is strongly aleviated by chloride and the concentration ratio of these ions is of great importance in assessing toxicity. Short term and long term toxicity data for a variety of fish species are presented. There are no field data on fish populations in waters where nitrite was the only pollutant. However extensive field surveys indicated that, waters with a mean chloride concentration of 25 mg l^{-1} in good salmon fisheries were associated with concentrations of nitrite below 50 μg l^{-1} $N \cdot NO_2$, good coarse fisheries below 100 μg l^{-1} $N \cdot NO_2$.

KEY WORDS Fish; Nitrate; Freshwater

1 INTRODUCTION

The harmful effects of nitrite to man and higher vertebrates have long been recognized but it is only in recent years that its 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 water products, particularly ammonia, from the water. In these systems nitrifying bacteria in the filtration system oxidise ammonia via nitrite to nitrate, 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 water contains 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.

The occurrence, toxicity and physiology of nitrite in freshwater fish and other aquatic animals has been extensively reviewed by EIFAC (1984). A recent review by Lewis and Morris (1986) analysed nitrite toxicity to freshwater fish in some depth. More general reviews are by Russo (1985) and Colt and Armstrong (1981).

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 oxidised 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 nitrogen in nitrite, i.e., mg $N \cdot NO_2$ or μg $N \cdot NO_2$ 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 $\mu g\ l^{-1}$ $N \cdot NO_2$ 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 nitrite originates from reduction of nitrate through the activity of phytoplankton. The vertical distribution of nitrite 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 nitrite is formed by reduction of nitrate, though there are a few examples of lakes where nitrite 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 water contained only 1–2 $\mu g\ l^{-1}$ $N \cdot NO_2$, a narrow band of water approximately 1.5 m below the surface contained virtually no oxygen with a nitrite content of 45 $\mu g\ l^{-1}$ $N \cdot NO_2$ (Finlay, Span and Harman, 1983). A tropical lake in the Amazon Basin, Great Lake Jutai, temperature, 26–31°C contained in its surface water, nitrite concentrations of 0.5 $\mu g\ l^{-1}$, 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 $\mu g\ l^{-1}$ $N \cdot NO_2$ in the majority, 5–20 $\mu g\ l^{-1}$ $N \cdot NO_2$ in many others, while in a few cases values of over 20 $\mu g\ l^{-1}$ $N \cdot NO_2$ were observed (IRSA, 1980). The nitrite content of a number of North American Lakes was measured by McCoy (1972), who found little or no nitrite in open waters, 2.3 $mg\ l^{-1}$ $N \cdot NO_2$ in bays and backwaters, while shore sites containing decaying organic matter, chiefly plants and algae, commonly contained 2–18 $mg\ l^{-1}$ $N \cdot NO_2$, these sites occasionally containing as much as 180 $mg\ l^{-1}$ $N \cdot NO_2$ (Table I).

2.2 Nitrite levels in polluted waters

Concentrations of nitrite around 10 $\mu g\ l^{-1}$ $N \cdot NO_2$ in surface waters have long been regarded as indicating sewage contamination. Indeed, nitrogeneous 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, but significant amounts of ammonia are also 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

TABLE I

Author	Location, system	NO_2 levels mg $N \cdot NO_2 l^{-1}$	
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.*, 1975a, 1975b	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 carp
Solbé, 1981a,b	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	
Westin, 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 minority	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
Cabrera, Toca, Diaze and Arambarri, 1984	Guadiamar River, Spain Mine and agricultural wastes	0.067 mean 0.82 max	Values varied at different sampling points
Kimoto, Dowley, Carre and Fiddler, 1981	Tap water, Philadelphia USA	0.001– 0.01	

are often used to fertilize fish ponds in extensive fish culture. Fish and other aquatic animals themselves produce ammonia which, as already 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 studied specifically on only a few occasions, and there is a marked lack of data on this subject, particularly regarding rivers. Up to $16.8\,mg\,l^{-1}\,N \cdot NO_2$ 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 nitrite levels were $0.01\,mg\,l^{-1}\,N \cdot NO_2$, 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 $30\,mg\,l^{-1}$. There is often seasonal variation in nitrite levels with traces, rarely exceeding $10\,\mu g\,l^{-1}\,N \cdot NO_2$, in winter while in summer higher values of around $50\,\mu g\,l^{-1}\,N \cdot NO_2$ have been recorded in relatively pollution free British rivers. In rivers associated with industry or intensive agriculture the corresponding values are higher, for example 0.03 and $0.24\,mg\,l^{-1}\,N \cdot NO_2$ in the river Nene, East Anglia. A detailed survey on the relationship between the nitrite content of waters and their fishery status was conducted by Solbé (1981a), and generally poor quality fisheries were associated with higher nitrite concentrations. Good salmonid fisheries were found in waters of low nitrite content, while poor ones occurred at nitrite concentrations from $60\,\mu g$ to $200\,\mu g\,l^{-1}\,N \cdot NO_2$. Coarse fisheries occurred in waters up to $0.3\,mg\,l^{-1}\,N \cdot NO_2$, but nitrite was not necessarily a critical factor in determining the quality of the coarse fishery. Additional data on the nitrite content of fish bearing waters are given in Table I.

2.3 Bacterial production of nitrite

Two groups of bacteria are principally responsible for nitrification or oxidation of ammonia to nitrate. The first, *Nitrosomonas*, oxidizes NH_3 to NO_2, while *Nitrobacter* oxidizes NO_2 to NO_3; in each case oxygen is required. In many instances the rate of nitrite oxidation is faster than its formation, and in sewerage systems,

where nitrification has been particularly well studied, the concentration of nitrite rarely exceeds 2 mg l^{-1} N · NO_2 in waste waters. Nitrification tends to be inhibited at low temperatures, particularly below 5°C and also in acid water (Collins *et al.*, 1975a), with a lower rate at pH 6 and complete inhibition at pH 5.5 in a recirculating water system containing channel catfish (*Ictalurus punctatus*). Antibacterial agents, such as erythromycin, also reduce nitrification rates (Collins *et al.*, 1975b), as does methylene blue when used as parasiticide, but formalin, malachite green in combination with copper sulphate and potassium permanganate are without effect at therapeutic levels (Collins *et al.*, 1975c).

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 reduction of nitrate may be utilized. 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).

2.4 Nitrates

Nitrates themselves are of low toxicity and are only likely to create health hazards when reduced to nitrites. The increasing use of nitrogenous fertilizers has led to elevated levels of nitrates in both British surface and ground waters in some areas approaching or exceeding 50 mg l^{-1} N · NO_3 close to the upper limit recommended by WHO and the European Community for drinking water (White, 1983).

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

$$HNO_2 \rightleftarrows H^+ + NO_2^-$$

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

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

at pH 7 and 12.5°C the pKa of nitrous acid is 3.337 (Weast, 1978), and therefore base/acid is 4603 i.e. 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 on 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 II, while variation of pKa HNO_2 with temperature, calculated according to Colt and Tchobanoglous (1976), are shown in Table IIb.

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

Water pH	$\frac{\text{base}}{\text{acid}}$ or $\frac{NO_2^-}{HNO_2}$	% HNO_2
10	4602566	0.00002
9	460257	0.0002
8	46030	0.0022
7	4603	0.022
6	460	0.22
5	46	2.17
4	4.6	21.7
3.3	1	50

TABLE IIb
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 temperature falls the dissociation constant for HNO_2 increases, so increasing the proportion of HNO_2. Thus, at a pH of 7.0 and 5°C there is 0.032% in the form of HNO_2, while at 25°C only 0.022% is HNO_2.

The relative hydrated ionic size of nitrite (compared to K^+) is 1.02 which compares with 0.96 for chloride, and 1.03 for nitrate, and the relative ability to penetrate whole cell membranes is $Br^- = SCN^- > Cl^- > NO_2^- > BF_4^- > I^- > NO_3^- > ClO_3^- > HCO_3^-$ (Araki, Ito and Oscarsson, 1961).

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, nitrite 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, 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 amounts of dimethyl-amine. Thus, for example

$$2\,HNO_2 \rightleftarrows N_2O_3 + H_2O$$

and

$$R_2NH_2 + N_2O_3 \rightleftarrows R_2N \cdot NO + HNO_2,$$

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. Nitrite inhibits oxygen uptake of mitochondrial cytochrome oxidase by interacting with the haem-site of the enzyme (Paitain, Markossian and Nalbandyan, 1985), and also oxidises myoglobin to metmyoglobin (Colpa-Boonstra and Minnaert, 1959; Lee and Cassens, 1976).

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-napthyl-ethylenediamine), to form a red colour (Wood, Armstrong and Richards, 1967; Shechter, Gruener and Shuval, 1972). These methods give higher values when compared with values obtained by differential pulsed polarography (Kimoto *et al.*, 1981). Such methods are suitable for most work involving water samples, but a more sensitive method using chemiluminescence 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 nitroso 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 be assessed initially by checking for recovery of added nitrite standards (Arillo, personal communication). Polymers and

ion exchange resins selective for anions including nitrite 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). High pressure liquid chromatography methods are described by Kuchniki, Sarna and Webster (1985), while second derivative spectroscopy techniques are discussed by Nagashima, Matsumoto and Suzuki (1985).

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 to 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 sodium nitrite has been imposed on cured meats with dietary intake of nitrite recommended at not greater than $0.4\,mg\,kg^{-1}\,d^{-1}$ 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 $N \cdot NO_2$ (Dean and Lund, 1981; WHO, 1978). Fatalities in man occurred upon a single ingestion of about 1 g of nitrite (McQuiston, 1936).

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. Possible links between ingested nitrates and methaemoglobinaemia (Super *et al.*, 1981) and gastric cancers (Forman, Al-Dabbagh and Doll, 1985; Tannenbaum and Correa, 1985) have been discussed.

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, 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, although in some marine teleosts higher levels have been recorded (Graham and Fletcher, 1986). In human blood four pathways exist for the reduction of methaemoglobin to haemoglobin which in order of importance are NADH methaemoglobin reductase, ascorbic acid, GSH (reduced glutathione) and NADPH methaemoglobin reductase (Scott, Duncan and Erkstand, 1965). NADH reductase activity has been found in fish blood (Freeman, Beitinger and Huey, 1983; Huey and Beitinger, 1982a) and its activity in rainbow trout red cells is comparable to that of the human red cell (Scott and Harrington 1985), requiring the presence of glucose and lactate (Gruca and Grigg, 1980; Jaffé, 1981). The presence of nitrite in the water rapidly induced 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. Recovery from severe methaemoglobinaemia occurred within 24 hours both in freshwater

and marine fish (Eddy, Kunzlick and Bath, 1983; Huey, Simco and Criswell, 1980; Scarano and Saroglia, 1984), although longer exposure resulted in haemolytic anaemia (Scarano *et al.*, 1984).

Fish are able to accommodate relatively high levels of methaemoglobin, for example, 25–30% is regarded as a safe level for channel catfish (Tucker and Schwedler, 1983), while with over 70% methaemoglobin both rainbow trout (*Salmo gairderi*) and chinook salmon (*Oncorhynchus tschawytscha*) were stressed, although 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 hydrocholoride, or replacement of blood with plasma or saline. It was shown that apart from an increase in cardiac stroke volume, resting 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 nitrite entering the blood plasma and the rate of methaemoglobin formation is closely correlated to the concentrations of nitrite 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 (see section 4.2). 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). Rainbow trout chronically exposed for two weeks to 1.4 mg l^{-1} N · NO_2 showed a twofold increase in chloride cell numbers (T. Cock, personal communication).

A further interesting feature is the ability of gills to concentrate nitrite in blood and tissue to many times the external level. In rainbow trout exposed for 24 hours 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 nitrite (0.45 mg l^{-1} N · NO_2) for 72 hours 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 a little below the blood level and with much lower concentrations occurring in muscle. Accumulation of nitrite in the blood appears to be species dependent with fish such as salmonids, tilapia (*Tilapia aurea*) and channel catfish showing fairly rapid uptake while plasma nitrite increased only at very high external levels (50–100 mg l^{-1} N · NO_2) in largemouth bass (*Micropterus salmoides*) (Palachek and Tomasso, 1984a), smallmouth bass (*Micropterus dolomieui*) and green sunfish (*Lepomis cyanellus*) (Tomasso, 1986).

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 chloride/bicarbonate 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. There is evidence to support this hypothesis since blood nitrite levels exceed those in the water (Bath and Eddy, 1980; Eddy, Kunzlick and Bath, 1983). Chloride uptake by rainbow trout gills is saturable, has a slightly higher affinity for chloride than for nitrite, which behaves as a competitive inhibitor. Perch (*Perca fluviatilis*) resemble trout in this respect while carp (*Cyprinus carpio*), tench (*Tinca tinca*) and eels (*Anguilla anguilla*) are characterised by very much lower chloride uptake rates which limit the rate of nitrite entry into the body so delaying the onset of toxic effects (Williams and Eddy, 1986). The largemouth bass may

also have a very low chloride uptake rate and nitrite levels in the blood plasma may remain low except at high external nitrite concentrations (Palachek and Tomasso, 1984a; Tomasso, 1986).

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, HNO_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 chloride (see section 5.1), but the toxicity data of Russo, Thurston and Emerson (1981) suggest that both HNO_2 and NO_2^- are toxic species. This subject is discussed in great detail by Lewis and Morris (1986) who conclude that the effect of pH on nitrite toxicity appears small (see section 5.3).

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, 1982b). Dietary ascorbic acid (approximately 200 mg kg^{-1}), increased tolerance of steelhead trout (*Salmo gairdneri*) to nitrite (Blanco and Meade, 1980). These authors also noted an increased methaemoglobinaemia at warmer temperatures, subsequently confirmed for channel catfish; this point was elaborated by Huey, Beitinger and Wooten (1984). However, an inverse relationship was found between temperature tolerance and nitrite-induced methaemoglobinaemia (Watenpaugh, Beitinger and Huey 1985). 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 hours, declining to near normal levels after 24 hours, but with 5 mg l^{-1} nitrite (1.5 mg l^{-1} N · NO_2) there was a build-up of corticosteroids, reaching 10 times the control value at 24 hours, and it was suggested that catfish were able to adapt more easily to ammonia than to nitrite. The rates of nitrite uptake to the blood plasma and oxidation of haemoglobin are reduced in the presence of adrenaline, a factor

which may protect acutely stressed fish (Williams and Eddy, 1987). Nitrite-induced methaemoglobinaemia decreased tolerance to hypoxia in channel catfish (Bowser *et al.*, 1983), decreased metabolic rate as measured by oxygen uptake in fathead minnows (*Pimephales promelas*) (Watenpaugh and Beitinger, 1985) and swimming performance in channel catfish (Watenpaugh, Beitinger and Huey, 1985).

Rainbow trout exposed to 0.45 mg l^{-1} $N \cdot NO_2$ for up to 72 hours showed inhibition of liver lysosomal proteolytic activity, as well as increased fragility of lysosomal membranes (Mensi, *et al.*, 1982). Nitrite intoxication in these fish, particularly evident in overturning fish, was attributed to liver damage caused by anaemic hypoxia (i.e., the blood oxygen carrying capacity is reduced 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 mitochrondria, which led to decreases in glycogen and ATP and to increases in lactate and succinate, paralleled by an uncoupling-like effect on biochemical respiration (Arillo *et al.*, 1984). Similar tests on brain suggested that it was less susceptible than liver to elevated nitrite concentrations (Arillo, Mensi and Pirozzi, 1984).

Nitrite has been used therapeutically in man as a vasodilator and blood-pressure depressant and this effect also occurs in fish (Bath, 1980; Windholz, 1976). The vasodilatory effect of nitrite is mediated through guanylate cyclase (Mittal and Murad, 1982).

In a variety of mammals, blood levels of nitrite are 30 μg l^{-1} $N \cdot NO_2$ and of nitrate 0.3 mg l^{-1} $N \cdot NO_2$ (Rath and Krantz, 1942), arising principally through drinking water and dietary intake (see section 4.1), although there is also evidence for a mammalian nitrate biosynthetic pathway (Stuehr and Marletta, 1985). Loss of blood nitrates and nitrites is mainly via the urine with smaller losses via the colon to the faeces (Witter, Gatley and Balish, 1979; Thayer *et al.*, 1982). A small but important route for nitrate excretion is via the saliva (Fritsch and Saint Blanquat, 1985), which upon entering the digestive tract may be reduced to nitrite by oral or intestinal bacteria. Orally produced nitrite enters the stomach where under acidic conditions potentially carcinogenic nitrosamines may be produced, possibly linking high nitrate intake and gastric

cancer (Tannenbaum *et al.*, 1981) (see sections 3.2.1 and 4.5). Nitrite can also enter the blood directly from the stomach and, together with intestinally produced nitrite, enters the circulation where oxidation of haemoglobin occurs with simultaneous generation of nitrate (Kosaka *et al.*, 1979), although some nitrite may be oxidised by the liver (Thayer *et al.*, 1982). Thus ingested nitrate may follow a complex cyclic pathway involving digestive tract, blood circulation and salivary glands. The fate of nitrates entering the fish body is unknown.

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, for example, a single dose of $5\,mg\,l^{-1}$ *N*-nitrosodimethylamine fed to rats induced tumours in more that 70% of the animals (Wolff and Wasserman, 1972; and section 3.2).

Such studies in fish are rare, but De Flora and Arillo (1983) noted mutations in *Salmonella typhimurium* treated with muscle extracts from rainbow trout which had been exposed to $450\,\mu g\,l^{-1}\,N \cdot NO_2$.

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 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

TABLE III

Summary of nitrite toxicity data for a variety of fish species. Tests were 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} $N \cdot NO_2$ or other responses	Exposure period days	Author and additional information
Steelhead trout	5	10	6.8	25	1	0.5	4	Wedemeyer and
(*Salmo gairdneri*)	10		8.4	300	8.4	10.3		Yasutake,
			6.8	25	1	0.9		1978
			8.4	300	8.4	12.1		
Rainbow trout	10–25	10	6.8	8 ppm Ca^{2+}	7	3.9	4 indefinite survival	Bath, 1980 Eddy, Kunzlick and Bath, 1983
Coho salmon*	13	11	7.2	32.3	19.6	50% mort. 9.2 +	1	Perrone and
(*Oncorhynchus*	22				148	0% mort. 8.9 +	3	Meade, 1977
kisutch)	Fry				32.2	0% mort. 8.9 +	3	
Rainbow trout	70	10	7.92	199	1.2	0.46	4	Russo and
(*Salmo gairdneri*)	99		7.74		41	12.2		Thurston 1977
Coho salmon*	13	11	7.2	32.3	19.6	50% mort. 9.2 +	1	Perrone and
(*Oncorhynchus*	22				148	0% mort. 8.9 +	3	Meade, 1977
kisutch)	Fry				32.2	0% mort. 8.9 +	3	
Rainbow trout	70	10	7.92	199	1.2	0.46	4	Russo and
(*Salmo gairdneri*	99		7.74		41	12.2		Thurston 1977
Rainbow trout	28	10	7.5	174	10.9	3.74	4	Russo, Thurston
(*Salmo gairdneri*)	79		7.9	177	10.4	3.54		and Emerson,
	147		8.5	188	10.6	4.35		1981
	244		8.6	184	10.5	5.34		Similar results
	9–15	10	7.0	178–209	0–0.47	0.14	4	for fish weight
			7.9			0.21		25–341 g also
			9.0			1.12		results at intermediate pH

								values. 0.47 mg l^{-1} chloride used to calculate ratio.
Cutthroat trout (*Salmo clarki*), fry	1–3	12	8.0	176	0.44	0.52	4	Thurston, Russo and Smith, 1978
Channel catfish (*Ictalurus punctatus*)	7.13 cm	21–24	7.0	40 mg l^{-1}	4	4.99	1	Tomasso, *et al.*, 1980
					60 as NaCl	98	1	
					60 as $CaCl_2$	91	1	
Channel catfish (*Ictalurus punctatus*)	7–13 cm	22–25	7.0		61–306	no significant MetHb formation	1	Tomasso, Simco and Davis, 1979
Channel catfish (*Ictalurus punctatus*)	3	23	7.7–8.1	190	22	7.1	4	Palachek and Tomasso, 1984a
Fathead minnow (*Pinephales promelas*)	0.3–0.8	23	7.2	310	22	70	4	Palachek and Tomasso, 1984b
	0.9–3.3					45	4	
Fathead minnow (*Pinephales promelas*)	2.3	13	8.05	199	0.35	2.2–2.99	4	Thurston and Russo, 1977
						3.9–5.5	1	
Mottled sculpin* (*Cottus bardi*)	5.2	14	8.08	199	0.35	60	6	
Carp* (*Cyprinus carpio*)	5.6 cm	14	7.6	260	19	40	4	Solbé, Cooper, Willis and Mallet, 1985
Roach* (*Rutilus rutilus*)	6.9 cm	16	7.4	261	20	12	4	as above

TABLE III

Fish species	Weight (g)	Temp. (°C)	pH value	Ca hardness as $mg\,l^{-1}$ $CaCO_3$	Cl^- $mg\,l^{-1}$	LC_{50} in $mg\,l^{-1}$ $N \cdot NO_2$ or other responses	Exposure period days	Author and additional information
Bluegill (*Lepomis macrochirus*)	7–17	30	7.2 7.2		60	211 4.6	2 2	Huey, Wooten, Freeman and Beitinger, 1982. Similar experiments at pH 4 as well
Largemouth bass (*Micropterus salmoides*)	2.8	23	7.7–8.1	190	22	140	4	as above
Tilapia (*Tilapia aurea*)	3.4	23	7.9	190	22	16	4	as above
Carp (*Cyprinus carpio*)	0.22 −0.29	28	7.7	50	1 5 10.5 27.5 45	2.6 5.8 14.4 27.3 48.7	4 4 4 4 4	Hasan and McIntosh, 1986
Perch (*Perca fluviatilis*)	20–40	10	6.8	8 ppm Ca^{2+}	7	17	1	Williams and Eddy, 1986
Carp (*Cyprinus carpio*)	2–78					490	1	as above
Tench (*Tinca tinca*)	113–168					700	1	as above
Eel (*Anguilla anguilla*)	59–138					1120	1	as above

Goldfish (*Carassius auratus*)	13	23	7.7–8.4	191–268	22	175	4	Tomasso, 1986
Smallmouth bass (*Micropterus dolomieui*)	3					525	4	as above
Bluegill (*Lepomis macrochirus*)	8					350	4	as above
Green sunfish (*Lepomis cyanellus*)	7					550	4	as above
Sea bass	12–15 cm	17	8.1–8.4	sea water		274	4	Saroglia, Scarano
(*Dicentrarchus*		23		36 g l^{-1}		220		and Tibaldi,
labrax)		27				154		1981
European eel	7 cm	20	8.1	salinity	0* g l^{-1}	86	4	Saroglia, Scarano
(*Anguilla*					6	223	4	and Tibaldi,
anguilla)					12	302	4	1981
elvers					24	563	4	as above
					36	1140	4	as above

these difficulties were not resolved until it was shown the 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 (Wedemeyer 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 (hereafter expressed by weight, i.e., mg Cl^-/mg $N \cdot NO_2 l^{-1}$) is of critical importance when considering water quality and toxicity to fish.

The chloride/nitrite 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). A ratio of 5 prevented mortality in carp fry but higher ratios may be necessary for complete protection in fish farms (Hasan and Macintosh 1986).

5.2 Other inorganic ions

The efficacy of the halides in reducing the acute toxicity of nitrite has been tested, the relative potency being $Br^- \geq Cl^- > I^- > F^-$ (Bath, 1980). Bicarbonate showed moderate potency for rainbow trout (Eddy, Kunzlick and Bath, 1983), and also for channel catfish (Huey, Simco and Criswell, 1980). Addition of calcium reduced mortality, but not methaemoglobin, in chinook salmon (Crawford and Allen, 1977); and in steelhead trout Wedemeyer and Yasutake (1978) 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} $N \cdot NO_2$, respectively) (Table III).. There was an insignificant difference in protection offered to rainbow trout exposed to 9.8 mg l^{-1} $N \cdot NO_2$ by chloride added as the calcium or the sodium salt (Eddy, Kunzlick and Bath, 1983), but the protective effect of calcium chloride to the closely related steelhead trout was in some instances six times greater than that of sodium chloride (Wedemeyer 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} $N \cdot NO_2$ at pH 6 increasing to 3.6 mg l^{-1} $N \cdot NO_2$ at pH 8 (Wedemeyer and Yasutake, 1978). The 48-h LC_{50} for bluegills (*Lepomis macrochirus*) was substantially increased at pH 7.2 compared with pH 4 (Huey *et al.*, 1982), while coho salmon exposed to 3 mg l^{-1} $N \cdot NO_2$ 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 nitrite to rainbow trout (Russo and Thurston, 1977). A similar result was noted by Bath (1980) in rainbow trout exposed for 96 h to 10 mg l^{-1} $N \cdot NO_2$. Bath (1980) found no difference in mortalities at intermediate pH values, but 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 nitrite decreased while that of HNO_2 increased suggesting that both acid and anion forms are toxic. Thus, second only to chloride, the pH of the water appears to be an important variable when considering nitrite toxicity even though the available data do not allow firm conclusions, particularly at intermediate pH values (see also section 4.3 and Lewis and Morris, 1986).

6 LONG-TERM LETHAL CONCENTRATIONS AND SUBLETHAL EFFECTS

6.1 Adult and juvenile fish

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 4.2), one example being coho salmon which 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 little information on these points (see section 4.4).

One of the few long-term studies is that of Wedemeyer and Yasutake (1978) on steelhead trout. In soft water of low chloride content, fish exposed to a range of nitrite levels with maximum of $0.06\,mg\,l^{-1}\,N \cdot NO_2$ 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, hypertrophy was found in a few secondary lamellar epithelial cells followed by hypertrophy of almost the entire secondary lamellar epithelium. After seven weeks, the changes were seen less frequently, suggesting that 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), who showed that channel catfish previously kept in low levels of nitrite ($0.01\,mg\,l^{-1}\,N \cdot NO_2$) and then exposed to about $8.2\,mg\,l^{-1}\,N \cdot NO_2$ developed significantly less methaemoglobin than similarly tested catfish which had not previously been exposed to nitrite.

The nitrite LC_{50} value for cut-throat trout (*Salmo clarki*) (1–3g) at five days was $0.52\,mg\,l^{-1}\,N \cdot NO_2$ decreasing slightly to $0.27\,mg\,l^{-1}\,N \cdot NO_2$ after thirty-six days (Thurston, Russo and Smith, 1978). The median lethal concentration of nitrite after two to three weeks exposure was $21.8–26.4\,mg\,l^{-1}\,N \cdot NO_2$ for carp and $9.0–11.2\,mg\,l^{-1}\,N \cdot NO_2$ for roach (*Rutilus rutilus*) (95% confidence limits); the chloride level in each case was about $20\,mg\,l^{-1}$, giving chloride/nitrite ratios of 0.8 and 2, respectively (Solbé 1981a,b). 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 III). The 42-day nitrite LC_{50} for brown trout (*Salmo trutta*) was $1.0\,mg\,l^{-1}\,N \cdot NO_2$ 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 $0.72\,mg\,l^{-1}\,N \cdot NO_2$ (Willis, personal communication).

6.2 Eggs and larvae

In a variety of marine teleost larvae (Brownell, 1980) the 24 hour LC_{50} value was between 1200–2400 mg l^{-1} N · NO_2, while marine red drum eggs (*Sciaenops ocellatus*) were unaffected by exposure to 500 mg l^{-1} N · NO_2 for two weeks, although four days after hatching only 14% survived compared to 100% survival of the unexposed controls (Holt and Arnold, 1983). Atlantic salmon eggs (*Salmo salar*) were unaffected by exposure to 140 mg l^{-1} N · NO_2 (chloride

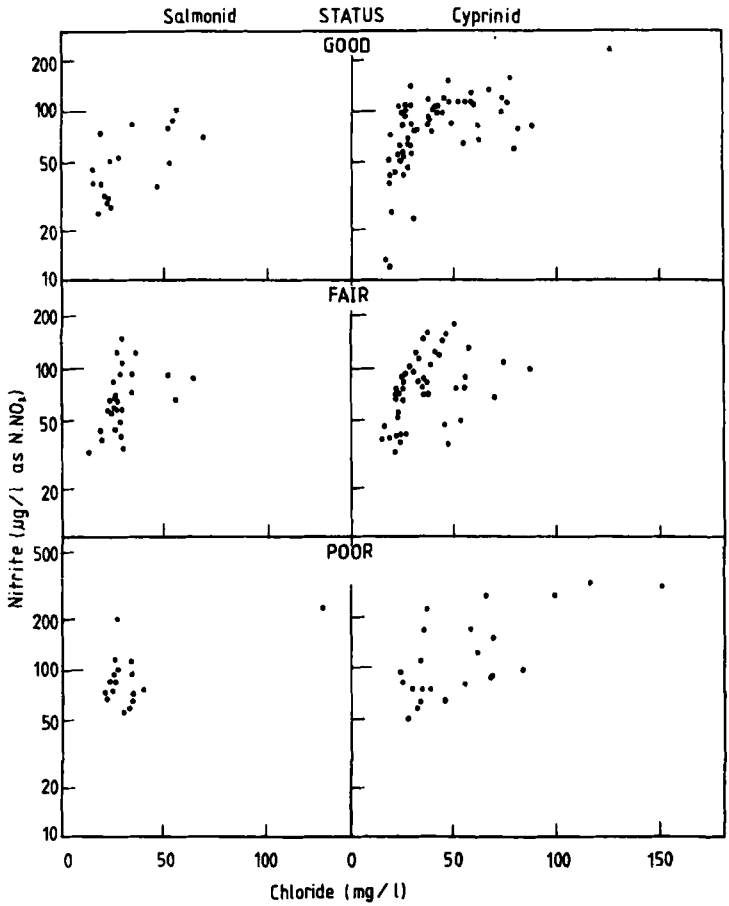

FIGURE 1 Relationships between the mean concentrations of chloride and nitrite in salmonid and cyprinid fisheries (Solbé 1981a,b).

0.6 mg l^{-1}) for four weeks and upon hatching, which was delayed, tolerance of yolk-sac larvae, first-feeding alevins and fry to nitrite declined with age and development (E. M. Williams, personal communication).

7 FIELD DATA

A survey has been carried out by Solbé (1981a,b) to obtain a relationship between the status of fisheries (salmonid and coarse) and the mean concentration 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 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 concentrations, that is, 300 and 450 μg l^{-1} N · NO_2, respectively (Figure 1) (Cooper and Solbé, 1980; Solbé *et al.*, 1985).

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, 5.3). Thus, Table III has been assembled making use of those reports offering reasonably complete data on mortalities as well as water quality. Most of these studies are on salmonids, particularly rainbow trout; as yet, there has been less work on coarse fish although a few studies relate to channel catfish.

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 96-h LC_{50} value of $1.5\,mg\,l^{-1}\,N \cdot NO_2$. 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 $5\,mg\,l^{-1}\,N \cdot NO_2$. However, carp and black bullhead (*Ictalurus melas*) survived $40\,mg\,l^{-1}\,N \cdot NO_2$ for at least 48 h—the duration of the test—while common suckers (*Catostomus commersoni*) survived at least 48 h in $100\,mg\,l^{-1}\,N \cdot NO_2$. The size of the fish tested was 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) found fathead minnows about an order of magnitude less sensitive than rainbow trout (and cut-throat trout), while mottled sculpins (*Cottus bardi*) survived the highest concentrations of nitrite tested, $67\,mg\,l^{-1}\,N \cdot NO_2$, without mortality.

Studies on salmonids not included in Table III are those of Westin (1974) who noted a 96-h median tolerance limit of about $3\,mg\,l^{-1}\,NO_2$ ($0.91\,mg\,l^{-1}\,N \cdot NO_2$) for chinook salmon fingerlings, while Smith and Williams (1974) found the 24-h LC_{50} value for rainbow trout fingerlings to be $1.6\,mg\,l^{-1}\,N \cdot NO_2$, considerably higher than a value of $0.96\,mg\,l^{-1}\,N \cdot NO_2$ for larger fish. The observation that fry and fingerling stages of salmonids are more tolerant of nitrite than later stages was confirmed by Perrone and Meade (1977), Russo, Smith and Thurston (1974) and Russo and Thurston (1977), the same being true for fathead minnows (Palachek and Tomasso, 1984b). Toxicity of nitrite to sea water adapted rainbow trout and Atlantic salmon (*Salmo salar*) was studied by Eddy, Kunzlick and Bath (1983), while Crawford and Allen (1977) noted that in sea water adapted chinook salmon, methaemoglobinaemia developed even at low external nitrite levels; in this case the high external concentration of chloride was not exerting the expected protective effect (Table III).

The toxicity studies for channel catfish, not listed in Table III,

include those of Konikoff (1975) who noted a 96-h LC_{50} value of 7.4 mg l^{-1} $N \cdot NO_2$, and Collins, *et al.* (1975b) who noted mortalities after about 10 days when the nitrite concentration reached around 15 mg l^{-1} $N \cdot NO_2$ 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.

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 1.9 mg l^{-1} $N \cdot NO_2$, 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. Crayfish (*Procambarus clarkii*) have been observed to concentrate nitrite in their haemolymph to over 274 mg l^{-1} $N \cdot NO_2$ when exposed to 12 mg l^{-1} $N \cdot NO_2$ nitrite for 24 hours (Grutzmer and Tomasso, 1985).

There has been some work on marine invertebrates, the 24, 96 and 192 hour LC_{50} values for larvae of the giant Malaysian prawn (*Macrobrachium rosenbergii*) at 28°C were 130, 8.6 and 4.5 mg l^{-1} $N \cdot NO_2$ respectively and the highest concentration in which no mortalities occurred was 1.4 mg l^{-1} $N \cdot NO_2$ over a 168 hour period. Nitrite exposure resulted in a significantly slower growth rate (Armstrong, Stephenson and Knight, 1976). The LC_{50} values for nitrite for the clam (*Mercenaria mercenaria*) and the

oyster (*Crassostrea virginica*) at 20–27°C are 756 and 532 mg l^{-1} N · NO_2 respectively (Epifiano and Srna 1975). Wickens (1981) studied the effects of nitrite on marine invertebrates particularly those species such as prawns useful in aquaculture and often reared in recirculating sea water systems, and 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 to 4 week LC_{50} value for juvenile *Macrobrachium rosenbergii* was 15.4 mg l^{-1} N · NO_2, while growth of other species is 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 (Wickens, 1982).

9.2 Amphibia

The 96 hour LC_{50} value of 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 Beitinger, 1980). Tadpoles of *Rana catesbiana* developed methaemoglobinaemia in response to nitrite of up to 15.2 mg l^{-1} N · NO_2 at an external chloride level of 5 mg l^{-1} (chloride/nitrite ratio = 0.31), but no methaemoglobin was produced when the chloride level was 50 mg l^{-1} (chloride/nitrite ratio = 3.3) (Huey and Beitinger, 1980). A green frog of a unreported species lived for four weeks in water of 100 mg l^{-1} N · NO_2 (McCoy, 1972). Larval amphibia (*Rana temporaria*) are unusual in being relatively resistant to nitrite with a 24 hour LC_{50} of 112 mg l^{-1} N · NO_2 while possessing a high affinity and uptake rate for chloride (Williams and Eddy, 1986).

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 nitrite levels occur in 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 $\mu g\, l^{-1}$ N · NO_2 (2.2).

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

iv) Nitrite is toxic to vertebrates including fish; a principal effect is conversion of haemoglobin to the brown-coloured methaemoglobin which is unable to transport 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 circulatory system (4.1, 4.2, 4.3).

v) The main toxic species is believed to be nitrite which enters the blood via the branchial chloride/bicarbonate 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 nitrite and chloride 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 (5.1, 6.1, 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 chloride 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 grow normally when exposed for six months to a range of nitrite levels, the maximum being 0.06 mg l^{-1} N · NO_2 (6.1).

ix) There are no field data on fish populations in waters where nitrite is 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 are associated with concentrations of nitrite below 50 $\mu g\, l^{-1}$ N · NO_2 and good coarse fisheries below 100 $\mu g\, l^{-1}$ N · NO_2. Values for 95% percentiles were found to be three times the mean nitrite concentration, that is 300 and 450 $\mu g\, l^{-1}$ N · NO_2, respectively (7 and Figure 1).

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