

REVIEW PAPER

Ammonia in estuaries and effects on fish

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This review aims to explore the biological responses of fish in estuaries to increased levels of environmental ammonia. Results from laboratory and field studies on responses of fish to varying salinity and their responses increased ammonia will be evaluated, although studies which examine responses to ammonia, in relation to varying salinity, pH and temperature together are rare. In a survey of British estuaries the continuous measurement of total ammonia showed values that ranged from background levels increasing up to *c.* 10 mg N l⁻¹ although higher values have been noted sporadically. In outer estuaries pH values tended to stabilize towards sea water values (*e.g.* *c.* pH 8). Upper reaches of estuaries are influenced by the quality of their fresh waters sources which can show a wide range of pH and water quality values depending on geological, climatic and pollution conditions. In general the ammonia toxicity (96 h LC₅₀) to marine species (*e.g.* 0.09–3.35 mg l⁻¹ NH₃) appears to be roughly similar to freshwater species (*e.g.* 0.068–2.0 mg l⁻¹ NH₃). Ammonia toxicity is related to differences between species and pH rather than to the comparatively minor influences of salinity and temperature. In the marine environment the toxicity of ionized ammonia (NH₄⁺) should be considered. The water quality standard for freshwater salmonids of 21 µg l⁻¹ NH₃-N was considered to be protective for most marine fish and estuarine fish although the influence of cyclical changes in pH, salinity and temperature were not considered. During ammonia exposures, whether chronic or episodic, estuarine fish may be most at risk as larvae or juveniles, at elevated temperatures, if salinity is near the seawater value and if the pH value of the water is decreased. They are also likely to be at risk from ammonia intoxication in waters of low salinity, high pH and high ammonia levels. These conditions are likely to promote ammonia transfer from the environment into the fish, both as ionized and unionized ammonia, as well as promoting ammonia retention by the fish. Fish are more likely to be prone to ammonia toxicity if they are not feeding, are stressed and if they are active and swimming. Episodic or cycling exposures should also be considered in relation to the rate at which the animal is able to accumulate and excrete ammonia and the physiological processes involved in the transfer of ammonia.

In the complex environment of an estuary, evaluation of ammonia as a pollutant will involve field and laboratory experiments to determine the responses of fish to ammonia as salinity and temperature vary over a period of time. It will also be necessary to evaluate the responses of a variety of species including estuarine residents and migrants.

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Key words: Ammonia; estuaries; migratory fish; toxicity.

INTRODUCTION

BACKGROUND

Estuaries are among the most productive and varied of aquatic habitats and support a rich variety of plants and animals. Estuaries also support economic and recreational activities but many estuaries no longer have their full measure of biotic integrity because they receive wastewater discharges that may have depleted oxygen levels and contain pollutants such as ammonia, heavy metals, pesticides and herbicides. Whilst there may be information on the effects of such pollutants on fresh water and marine species, relatively little is known of their effects on estuarine species. Compared to the relative stability of marine or freshwater environments, estuaries are often considered to be more susceptible to the effects of pollutants. Since estuarine inhabitants experience daily changes in salinity and temperature do they experience additional stress on exposure to pollutants, compared freshwater or marine species? An understanding of the responses of estuarine animals to pollutants such as ammonia is required when recommending water quality criteria for environment management strategies.

Few studies have been concerned with the simultaneous measurement of pH, salinity and temperature, the prime information needed to understand effects of ammonia on estuarine fish. Some examples are shown in Table I that provides an indication of the range of conditions which may occur in estuaries.

Most studies on effects of ammonia on fish have been conducted in temperate climates and there is very little information that relates to effects in cold or near freezing conditions or in warmer waters *e.g.* c. 30° C. These areas merit further investigation.

There is little or no direct experimental work on the toxicity of ammonia to estuarine fish and probably the best that can be achieved is to extract material from the literature on the toxicity of ammonia to freshwater and seawater species and use this to estimate likely effects of ammonia on fish in estuarine waters. Recent work suggests that marine fish are more sensitive to ammonia compared to freshwater species but at present it is difficult to reach firm conclusions. This is because the literature for ammonia toxicity to marine species is limited and also because it is often difficult to make comparisons between studies. This information is used to explore possible responses and effects of ammonia on fish in waters of varying salinity with the aim of indicating areas for further research.

The literature on the occurrence of ammonia in the aquatic environment and its toxicity to fish has been extensively reviewed in recent years (Seager *et al.*, 1988; Eddy, 1999; USEPA, 1999; Ip *et al.*, 2001; McKenzie *et al.*, 2003). Information from these reviews and other recent literature has been used to make an informed assessment of the effects of ammonia on fish in estuaries.

AQUATIC CHEMISTRY OF AMMONIA

The aim of this section is to highlight relevant aspects of the aquatic chemistry of ammonia rather than to repeat detailed chemistry which has been presented on many previous occasions (Seager *et al.*, 1988; USEPA, 1999; Ip *et al.*, 2001).

TABLE I. Values for pH, salinity, temperature and ammonia in estuaries. There appear to be few if any studies for estuaries where temperature, water pH, ammonia and status of fish stocks have been assessed together

Estuary	Temperature ° C	pH	Salinity ppt	Ammonia	Comments	Reference
Salt marshes, Gironde, France	5–28	7.2–7.4, daily cycle, lowest in early morning	10–25 Peak in Summer		Records over 1 year	Baudrimonta <i>et al.</i> (2005)
River Tweed, U.K.		Upper tidal reaches, up to pH 10. Lower reaches c. pH 8	Fresh water to sea water		Monthly measurements for 1 year	Howland <i>et al.</i> (2000)
River Humber, U.K.				0–23 mg L ⁻¹	Ammonia input mainly due to sewage and agriculture, measurement at many sites along estuary	Oguchi <i>et al.</i> (2000); Jarvie <i>et al.</i> (2000)
Rivers Tinto and Odeil, Spain		pH 2–3 in fresh water increasing in the mixing zone to pH 8 in the outer estuary	Fresh water to sea water		Sulphuric acid from metal sulphide processing. R. Tinto heavily polluted, pH remained low in most of mixing zone	Elbaz-Poulichet <i>et al.</i> (1999)
River Carron, Cornwall, U.K.		Low pH values in freshwater zone	Fresh water		Acid effluent from abandoned tin mines	Neal <i>et al.</i> (2005)
Mangrove estuary, North Queensland, Australia	25–30	pH lowered from c. 7.8 to 7.4 by effluent	25–30	Increased from c. 1–2 mg L ⁻¹ to 4–5 mg L ⁻¹ by effluent.	Effluent from shrimp ponds draining to estuary	Trott & Alongi (2000)

TABLE I. Continued

Estuary	Temperature ° C	pH	Salinity ppt	Ammonia	Comments	Reference
Estuarine waterway, Eastern Australia	Normally <i>c.</i> pH 6.5, lowered to <i>c.</i> pH 3 depending on rainfall and climatic conditions				Estuarine water draining soil with underlying sulphidic sediments.	Lin <i>et al.</i> (2004)
Thames, Hammersmith			Fresh water and tidal	7.04–9.36 mg L ⁻¹ total ammonia	Annual mean values. Similar values for other Thames stations and measurements on Humber, Forth and Tywi, 1989–1993. Class boundaries at 0.86 mg N l ⁻¹ (Class A/B); 4.7 mg N l ⁻¹ (Class B/C) and 8.6 mg N l ⁻¹ (Class C/D) using the upper 90% ile for estuaries in England and Wales	Nixon <i>et al.</i> (1995)

Ammonia (NH_3) is a gas which dissolves in water where it ionises to form the ammonium ion (NH_4^+). The pK (equilibrium constant) of the ammonia/ammonium reaction is *c.* 9.5 and varies with temperature, pressure and ionic concentration. These effects are minor compared to the effects of pH, however (*e.g.* increasing pH will increase the proportion of NH_3 compared to NH_4^+). Increasing the pH value by 1 unit (*e.g.* pH 7–8) at 10° C produces about a 10-fold increase in NH_3 concentration while increasing the temperature by 10° C (10–20° C) approximately doubles the NH_3 concentration. Increasing salinity from 0.5 to 32 ppt at 10° C reduces the NH_3 concentration by *c.* 15% (Table II).

Most biological membranes are permeable to unionized ammonia (NH_3) but relatively impermeable to ionized ammonia (ammonium ions, NH_4^+). In much of the literature toxicity is expressed in terms of the concentration of unionized ammonia [NH_3] (Seager *et al.*, 1988) although analysis of the toxicity data (USEPA, 1999) indicates that when ammonia is expressed as the concentration of total ammonia [$\text{NH}_3 + \text{NH}_4^+$] the toxicity is independent of temperature. Often ammonia concentrations are expressed as the nitrogen content of ammonia *e.g.* [$\text{NH}_3\text{-N}$], [$\text{NH}_4^+ - \text{N}$]. Toxicity increases at higher pH values since a greater proportion of the total ammonia is present as NH_3 , the species to which organisms are most permeable. Determination of the NH_3 concentration in the water is critically dependent upon accurate pH measurements. Questions remain about comparability of pH determinations, the values for the equilibrium constant and the calculated values for unionized ammonia concentrations in fresh water, brackish waters and sea water, (Seager *et al.*, 1988; Clegg & Whitfield, 1995; USEPA, 1999).

TOXICITY OF AMMONIA

Ammonia is present in terrestrial and aquatic environments. Plants and animals excrete ammonia, it is produced by the decomposition of organisms and by the activity of micro organisms (Prosser & Embley, 2002). Ammonia is continually volatilized from the earth's surface and enters the atmosphere where it is destroyed by phytolytic reactions. Some ammonia is returned to the earth's surface as wet or dry deposition. In general, exposure to terrestrial ammonia is a minor problem compared to exposure in the aquatic environment where ammonia is highly toxic. Anthropogenic activities produce increasing amounts of nitrogenous material and a significant proportion is in the form of ammonia or compounds which can yield ammonia. Significant amounts of ammonia are generated from the production and use of fertilizers, biomass burning, from animals, especially intensive animal husbandry (Alabaster & Lloyd, 1982; Bouwman, 1990; Jana, 1994), and thus ammonia enters fresh water from sources such as sewage, industry, animal wastes, and bacterial activity.

Most fish produce ammonia as an end product of protein metabolism and it is excreted via the gills to the surrounding water. Elevated levels of ammonia in the environment either impair ammonia excretion or result in a net uptake of ammonia from the environment. In many freshwater teleosts the concentration of ammonia in blood plasma is typically 0.15–0.3 mmol L⁻¹ and higher concentrations are associated with sublethal toxic effects. The increased ammonia burden in the body results in an imbalance of ionic regulation (Twitchen &

TABLE II. Total ammonia ($\text{NH}_3 + \text{NH}_4^+$) concentrations corresponding to an unionized ammonia value of $21 \mu\text{g L}^{-1}$ at four pH values for sea water, fresh water and an intermediate salinity (values calculated from Clegg & Whitfield, 1995)

Salinity ppt	Temp °C	pH	¹ Unionised ammonia		² CCC ($\text{NH}_3 + \text{NH}_4^+$ mg L^{-1})	² CMC salmonids ($\text{NH}_3 + \text{NH}_4^+$ mg L^{-1})	² CMC non-salmonids ($\text{NH}_3 + \text{NH}_4^+$ mg L^{-1})
			($\text{NH}_3 \mu\text{g L}^{-1}$)	Total ammonia ($\text{NH}_3 + \text{NH}_4^+$ mg L^{-1})			
32	20	8.0	21	0.67	1.27		
32	20	7.0	21	6.56			
32	20	6.5	21	21.0			
32	20	6.0	21	65.6			
15	20	8.0	21	0.60			
15	20	7.0	21	5.83			
15	20	6.5	21	17.5			
15	20	6.0	21	58.3			
1	20	8.0	21	0.55	1.63	7.35	8.4
1	20	7.0	21	5.36	3.8*		
1	20	6.5	21	16.8	4.47	41.7	62.7
1	20	6.0	21	53.8	5.7*		

¹It was suggested by Seager *et al.* (1988) that sensitive species such as salmonids, in fresh water or sea water, may be protected if the concentration of unionized ammonia was $\leq 21 \mu\text{g L}^{-1}$ for 90% of the time.

²Shown for comparison are values for criterion continuous concentration (CCC) and criterion maximum concentration (CMC) for total ammonia ($\text{NH}_3 + \text{NH}_4^+$ mg L^{-1}) from USEPA (1998) and Ip *et al.* (2001).

*values were estimated from Fig. 3, Ip *et al.* (2001).

Eddy, 1993, 1994; Eddy, 1999), leading to hyper-excitability and changes in behaviour, followed by convulsions and death. Increased ammonia levels in the water resulted in impairment of swimming performance (Beaumont *et al.*, 1995a, b), reduced feeding and slower growth. A likely cause of ammonia toxicity may be the depolarizing effect of NH_4^+ on neurones leading to excessive activation of NMDA receptors (N-methyl-D-aspartate receptors) and subsequent death of the cell (Ip *et al.*, 2001; Randall & Tsui, 2002; McKenzie *et al.*, 2003).

The permeability of the gills and body surface varies with temperature and other physical variables and generally the permeability of biological membranes increases by a factor of between 2 and 3 for a 10°C increase in temperature (Prosser, 1991). Thus during ammonia exposure an increase in temperature will not only increase the proportion of unionized ammonia in the water but may facilitate entry of ammonia into the fish through increased permeability of the body surface *e.g.* gill cell membranes. Further investigations are required to evaluate whether ammonia exposed fish at higher temperatures accumulate more ammonia than those at lower temperatures.

AMMONIA IN SEA WATER

There is a relatively small body of literature relating to the effects of ammonia on marine fish. There is some information on toxic effects of ammonia (USEPA, 1989) and several more recent studies relating mainly to effects of ammonia on cultured species (Handy & Poxton, 1993). As is the case for fresh water, values for unionized ammonia (NH_3) in sea water will depend on the temperature, salinity and pH values. It is likely that marine fish are susceptible to both ionized and unionized ammonia and the discussion below, whenever possible, indicates values for both species of ammonia.

The effects of ammonia and other nitrogenous compounds on marine fish have been reviewed by Handy & Poxton (1993). The toxicity of unionized ammonia (96 h LC_{50}) to freshwater fish is in the range $0.068\text{--}2.0\text{ mg l}^{-1}\text{ NH}_3\text{-N}$ (EIFAC, 1970; Seager *et al.*, 1988; Russo & Thurston, 1991). Acute toxicity for marine species is in the range $0.09\text{--}3.35\text{ mg l}^{-1}\text{ NH}_3\text{-N}$ depending on species, temperature and pH. Toxic effects are more difficult to predict when the fish are stressed or ammonia levels fluctuate *e.g.* following feeding (Handy & Poxton, 1993) and later in this review.

TOXICITY OF TOTAL AMMONIA ($\text{NH}_3 + \text{NH}_4^+$) TO MARINE FISH

Differences in experimental conditions between various studies in which the toxicity of ammonia has been determined for marine fish tend to make comparison and interpretation difficult (Handy & Poxton, 1993). There are however, some features of interest. It has been reported that ammonia toxicity is higher when the pH value of sea water is lowered to *c.* 7, which is in contrast to fresh water where toxicity increases with increasing pH values. In some instances toxicity appeared to decrease in full strength sea water compared to brackish water although this was not always the case, see below (Hazel *et al.*, 1971; Bower & Bidwell, 1978; Alabaster *et al.*, 1979; Solderberg & Meade, 1991).

In many studies the toxicity of ammonia is described in terms of ambient unionized ammonia since this is the variable that correlates with toxicity (Alabaster & Lloyd, 1982; Thurston *et al.*, 1984). This criterion, however, is based almost exclusively on data from freshwater fish that are considered to be relatively impermeable to NH_4^+ . Since seawater fish apparently have enhanced permeability to NH_4^+ it may be inappropriate to refer to toxicity in terms of unionized ammonia alone (Wilson & Taylor, 1992; Wilkie, 1997). It may be for this reason that ammonia appears to be more toxic in sea water compared to fresh water. This is supported a recent analysis by Ip *et al.* (2001) and Randall & Tsui (2002) of the data presented by USEPA (1984, 1989) that indicated that the mean acute toxicity value for 32 freshwater species was *c.* 2.3 mg $\text{NH}_3\text{-N l}^{-1}$ compared with 1.5 mg $\text{NH}_3\text{-N l}^{-1}$ for 17 seawater species. For the five most sensitive species the values were 0.79 mg $\text{NH}_3\text{-N l}^{-1}$ and 0.68 mg $\text{NH}_3\text{-N l}^{-1}$ for fresh water and sea water respectively. Generally salmonids are amongst the most sensitive species and carp and cyprinids are amongst the least sensitive to ammonia (Seager *et al.*, 1988; USEPA, 1999). As previously mentioned, however, differences in ammonia sensitivity between species are much greater than differences related to salinity.

AMMONIA IN ESTUARIES

Estuaries represent an ever changing environment with respect to salinity, pH, temperature, oxygen and if present, pollutants. Resident species, including fish, may have evolved mechanisms to accommodate or avoid stressful aspects of an estuarine habitat *e.g.* migrating salmon smolts make a gradual entry to full sea water (Priede *et al.*, 1988; Arnesen *et al.*, 2003; Gowans *et al.*, 2003). Successful navigation of the estuary by migrating species could involve changes in physiological and behavioural systems so that there is less vulnerability to the stressful effects of ammonia and other pollutants.

The toxicity of ammonia to seawater organisms at different pH, temperature and salinity values was estimated by USEPA (1989) and acute toxicity for 21 species ranged between 0.23 and 43 mg l^{-1} total ammonia (pH 8, 20° C and 30 g l^{-1} salinity). Values for continuous exposure were *c.* 5–10% of the acute values. Temperature and salinity were considered to have minor effects on ammonia toxicity compared to pH. These values, however, are from tests where the ammonia concentration was held as constant as possible at known salinities and temperatures. These values may be of limited use when considering estuaries which are of variable morphology and present a complex environment with daily and seasonal changes in temperature, salinity and pH. Superimposed on these variables may be sustained or episodic inputs of relatively benign or toxic materials, including ammonia.

In considering the toxicity of ammonia in waters of different salinity the starting point has been to consider the water quality standards as applied to freshwater fish. Quality standards for ammonia in fresh waters requiring protection or improvement to support populations of fish were reviewed by Seager *et al.* (1988) whose recommendations were broadly similar to those indicated elsewhere (USEPA, 1984) (Table II). Salmonid and coarse fisheries are likely to be protected if unionised ammonia values are $\leq 21 \mu\text{g l}^{-1}$ $\text{NH}_3\text{-N}$ apart from

occasional 'minor peaks' *i.e.* that 95% of samples taken over a year satisfied the standard. Based on the few studies available a similar quality standard for ammonia was recommended for salmonids in the marine environment although it was noted that in some cases the toxicity of ammonia was greater in waters of intermediate salinity compared to fresh water or sea water (Seager *et al.*, 1988). It is not clear whether this standard would protect all stages of the life cycle of salmonids as well as other species.

This idea is explored further in Table II where the unionized ammonia concentration is held at $21 \mu\text{g l}^{-1} \text{NH}_3$ and the variations in total ammonia concentration over a range of pH and salinity values at 20°C are displayed. A decrease in the pH value by 1 pH unit increases the total ammonia concentration approximately 10-fold, and as previously mentioned, changes in pH have a far greater influence than changes in salinity or temperature. The value of $21 \mu\text{g l}^{-1} \text{NH}_3\text{-N}$ (at pH 8, 32 ppt salinity at 20°C) corresponds to a total ammonia concentration of *c.* 0.67 mg l^{-1} (Table I). This is *c.* 20% of the total ammonia level for no growth in marine fish and <5% the acute toxicity levels for swimming fish. At this level, over the greater part of a year, there could be sublethal or behavioural effects of ammonia though any detrimental effects to the overall well-being of an actively swimming, non-feeding and contaminant exposed population have yet to be determined. Obviously at temperatures $<20^\circ \text{C}$ the risk will be decreased.

There could be significant problems for ammonia exposed fish at values below pH 8 in sea water. Holding the value of $21 \mu\text{g l}^{-1} \text{NH}_3\text{-N}$, at pH 7 and 20°C the total ammonia concentration is about 6.5 mg l^{-1} (Table I). This exceeds the values for no growth and is *c.* 20% of the acute toxic value for swimming fish. Also there is the possibility of increased ammonia accumulation through increased permeability of the gills to NH_4^+ . Thus, under these conditions an actively swimming and non-feeding population may be at risk of ammonia intoxication. At values below pH 7 the total ammonia concentration increases greatly when unionized ammonia is $21 \mu\text{g L}^{-1} \text{NH}_3$ *e.g.* at pH 6 the total ammonia is *c.* 60 mg l^{-1} which would be toxic to most organisms. This is in agreement with the criterion continuous concentration (CCC) and criterion maximum concentration (CMC) from USEPA (1998) and Ip *et al.* (2001), (Table I). These criteria are derived from toxicity data for a variety of invertebrate and vertebrate species and are intended to be protective to sensitive species.

AVOIDANCE OR TOLERANCE OF AMMONIA TOXICITY

In habitats where excretion of ammonia by diffusion is difficult *e.g.* on land, in waters with high pH values or high ambient ammonia levels, then internal accumulation of ammonia is likely. The fish will need to respond by the detoxification of endogenously produced ammonia as well as ammonia penetrating the body from the exterior. Ammonia toxicity may be ameliorated by decreasing production, enhancement of ammonia excretion and converting ammonia to less toxic compounds for storage or excretion. These topics are reviewed by Ip *et al.* (2001) and Randall & Tsui (2002).

When exposed to elevated ambient ammonia levels, the synthesis of glutamine from glutamate and NH_4^+ occurs in many fish species *e.g.* common carp

(*Cyprinus carpio* L., goldfish *Carassius auratus* L., mudskippers *e.g.* *Periophthalmodon schlosseri* Pallas). This occurs mainly in ammonia sensitive organs such as the brain. Glutamine can be stored in tissues and then used as an oxidative substrate upon return to normal, but a disadvantage is energetic cost of ammonia detoxification. Excretion of ammonia by diffusion from the body requires less energy than synthesis of less toxic nitrogen products such as urea. The ornithine-urea pathway is absent in most fish but occurs in tilapia (*Oreochromis alcalicus* Grahame) (from Lake Magadi where the water is very alkaline, *c.* pH 10) and impairs diffusion of ammonia from the body. During air exposure, urea is produced by the African catfish (*Clarias mossambicus* Peters) (Eddy *et al.*, 1980) and by the Indian air breathing fish (*Heteropneusties fossilis* Bloch) (Saha *et al.*, 2001). An active NH_4^+ ornithine-urea cycle is present in many fish embryos as protection against the high rate of ammonia production occurring during utilization of yolk (Wright *et al.*, 1995). Reduction in ammonia excretion is a strategy employed by some species *e.g.* mudskippers (*P. schlosseri*) exposed to air (Lim *et al.*, 2001), rainbow trout (*Oncorhynchus mykiss* Walbaum) exposed to water of pH 10 (Wilson *et al.*, 1998).

The weather loach (*Misgurnus anguillicaudatus* Cantor), an air-breather which uses the gut for gas exchange, can volatilize unionized ammonia from this organ (Tsui *et al.*, 2002). It has been suggested that in either sea water or fresh water the rainbow trout could actively excrete NH_4^+ in exchange for H^+ (Wilson & Taylor, 1992), while the mudskipper (*P. schlosseri*) is able to survive high ambient ammonia levels by active excretion of the ammonium ion (Randall *et al.*, 1999) as is the case for euryhaline crustaceans (Weihrauch *et al.*, 2004).

EFFECTS OF EXERCISE, FEEDING AND STRESS ON AMMONIA TOXICITY

Acute toxicity tests for ammonia have tended to follow standard guidelines *i.e.* exposure of the organism to the toxicant under static conditions using starved, rested and unstressed animals so facilitating comparisons between studies. Following feeding, swimming or stress, internal ammonia levels in fish are elevated and they are more sensitive to external ammonia than would be indicated by standard toxicity tests.

EFFECTS OF EXERCISE

Internal ammonia levels are elevated in swimming fish compared to resting fish (Mommensen & Hochachka, 1988). Elevated plasma ammonia levels correlated with decreased swimming performance in brown trout (*Salmo trutta* L.) exposed to copper (Beaumont *et al.*, 1995*a, b, c*). In rainbow trout and coho salmon there was a decrease in critical swimming velocity with increasing water ammonia levels (Shingles *et al.*, 2001; Wicks *et al.*, 2002; McKenzie *et al.*, 2003). The LC_{50} for total ammonia N was $207 \pm 21.99 \text{ mg l}^{-1}$ for resting fish decreasing to $32.38 \pm 10.81 \text{ mg l}^{-1}$ for swimming fish. The acute value indicated by USEPA (1999) at the same pH is 48.8 mg l N . This will not protect swimming salmonid fish and much lower levels of ammonia in the water will impair swimming performance (Randall & Tsui, 2002).

EFFECTS OF AMMONIA ON FEEDING AND GROWTH

Recently fed rainbow trout (*O. mykiss*) in fresh water have blood plasma ammonia (c. 1800 μM , Kaushik *et al.*, 1983) approaching the levels seen in unfed fish nearing morbidity from exposure to high environmental ammonia (Wicks & Randall, 2002a). When exposed to high external ammonia, fed fish ceased feeding and even though blood plasma levels of ammonia increased, the concentration remained lower than the values for similarly exposed unfed fish. The 24 h LC_{50} value for fed fish was higher than for unfed fish but the protective effect of feeding was not evident after longer exposures (Wicks & Randall, 2002a). Feeding up-regulates glutamine synthase, mainly in white muscle, allowing for increased production or storage of ammonia in glutamine (Wicks & Randall, 2002b). Thus fish have mechanisms to protect them from potentially suicidal feeding bouts. Non-feeding fish are vulnerable to high ambient ammonia levels, however, especially if they are swimming (Wicks *et al.*, 2002). Salmon (*Oncorhynchus nerka* Walbaum) actively swimming during the upstream migration do not feed, have elevated blood ammonia levels (French *et al.*, 1983) and are likely to be sensitive to ambient ammonia. Low levels of ambient ammonia served as a growth stimulant without altering consumption in freshwater rainbow trout (Wood, 2004).

Effects of ambient ammonia on turbot (*Scophthalmus maximus* L.) and a variety of marine species are discussed by Person-Le Ruyet *et al.* (1995, 1997a, b, c, 2003). For juvenile turbot, seabass (*Dicentrarchus labrax* L.) and seabream (*Sparus auratus* L.) The 96 h LC_{50} values were in the range of 1.3–2.6 mg L^{-1} $\text{NH}_3\text{-N}$, (35–70 mg L^{-1} total ammonia-N) in sea water, pH 8.15 and salinity of 34.5 ppt (Person-Le Ruyet *et al.*, 1995, 1997a, c). Blood plasma levels of total ammonia-N increased from control levels of about 2.5 mg l^{-1} to about 45 mg l^{-1} (3.2 μM) within 3 h, and were not accompanied by marked changes in osmoregulatory physiology. The removal of ambient ammonia resulted in unloading of ammonia from the blood in c. 3 h (Person-Le Ruyet *et al.*, 1997a, b). Turbot exposed to 0.34, 0.73 or 0.88 mg l^{-1} $\text{NH}_3\text{-N}$ (9.1, 19.7 and 23.8 mg l^{-1} total ammonia-N) had low growth rates and elevated plasma cortisol levels over 84 days, compared to control fish. Atlantic salmon (*Salmo salar* L.) exposed to up to 112 $\mu\text{g l}^{-1}$ $\text{NH}_3\text{-N}$ (12.5 mg L^{-1} total ammonia-N) were unaffected after 14 days. Salmon exposed to 225 $\mu\text{g l}^{-1}$ $\text{NH}_3\text{-N}$ (25.3 mg l^{-1} total ammonia-N), however, had osmoregulatory imbalance and mortality within 6 days (Knoph & Thorud, 1996). In general, similar results have been obtained for other marine species including wolfish (*Anarhichas minor* Olafsen) (Foss *et al.*, 2003) and grey mullet (*Mugil platanus* Risso) (Sampaio *et al.*, 2002). In aquaculture systems one of the main considerations has been the effect of ambient ammonia on growth. Ammonia levels of c. 100 $\mu\text{g L}^{-1}$ as $\text{NH}_3\text{-N}$ (3.2 mg L^{-1} total ammonia at pH 8) should be considered as an upper limit since this was the threshold for no growth in turbot and sole (*Solea solea* L.) (Alderson, 1970).

EFFECTS OF STRESS

Stress tends to increase ammonia production in fish and generally stressed fish are more sensitive to external ammonia. Stress also results in increased cortisol

levels, however, which up-regulates glutamine synthase so that repeated stress may protect fish during exposure to increased levels of ammonia during the post stress period (Randall & Tsui, 2002).

OSMOREGULATION AND RESPIRATION

OSMOREGULATION

The main osmoregulatory processes in fish are ionic exchanges across the gills and water balance, (Fuentes & Eddy, 1997). How ammonia influences these processes in the estuarine environment has received little or no experimental attention although it is possible to suggest some possible responses. Effects of ammonia on ionic balance and acid base balance have been extensively studied in recent years (McKenzie *et al.*, 2003). Unionized ammonia entering the fish or retained by the fish acts as a base as it ionizes in the body fluids and thereby affects sodium balance. In addition, because external ammonium ions are competitive inhibitors of active sodium uptake in the gills the fish experience a net loss of sodium due to partial inhibition of sodium uptake resulting in an increased passive loss of sodium (Twitchen & Eddy, 1994). As euryhaline fish such as salmonids or flounders (*Platichthys flesus* L.) progress from fresh water to sea water they experience osmoregulatory challenges of salt gain and dehydration. The challenge of dehydration is addressed by drinking and absorbing sea water *via* the gut, but in doing so a salt load is acquired. Salt balance is achieved by excreting excess sodium and chloride *via* the 'chloride cells' in the gills while divalent ions are excreted *via* the gut and kidney. In progressing into sea water the fish become increasingly permeable to both water and ions and significant passive exchanges of water, sodium and chloride occur between the body fluids and the medium *via* the gills (*c.* 20% of the body ionic content per hour). These passive ionic fluxes account for a small diffusive gain of sodium and chloride and a small diffusive loss of water by the fish. As in freshwater fish, the gills of marine fish absorb sodium and chloride apparently as part of the mechanism for acid base regulation (Potts & Eddy, 1973; Potts *et al.*, 1989; Talbot *et al.*, 1989; Evans, 1993).

How is ammonia likely to influence any or all of these osmoregulatory processes? First, ammonia excretion in most marine fish is similar to that in freshwater fish and exposure to external ammonia will lead to increased ammonia levels in the blood and body fluids where it could interfere with sodium absorption and acid base balance, as is the case in freshwater fish. Second, in sea water, ammonium ions could compete with sodium ions in passive cationic diffusion across the gills, as mentioned above. In most situations, however, competition by ammonium ions would be minimal since their concentration would be very low compared to the concentration of sodium ions (*e.g.* <0.2%). Third, as in freshwater fish, ammonium ions could compete with sodium ions for absorption *via* the gills but again this would not be favoured because of the high concentration of sodium ions compared to ammonium ions. In dilute sea water, however, the concentration of ammonium ions relative to sodium ions would increase so increasing the possibility of uptake of ammonium ions. There is very little experimental work in this area though it is interesting to

note that ammonia was apparently more toxic to salmonids in environments of intermediate salinity (Herbert & Shurben, 1965; Alabaster *et al.*, 1979; Harader & Allen, 1983).

It is also possible that ammonia exposure could influence water balance in fish although salt and water balance are likely to be closely linked. Exposure of rainbow trout (*O. mykiss*) to ammonia in fresh water resulted in increased urine production presumably as a result of increased permeability of the gills to water or to increased drinking (Lloyd & Orr, 1969; Best *et al.*, 2003). It is not known if exposure of euryhaline or marine fish to ammonia results in similar responses.

EFFECTS OF DISSOLVED OXYGEN AND RESPIRATION

The information summarized in Alabaster & Lloyd (1982) and the results on Atlantic salmon (*S. salar*) from Alabaster *et al.* (1983) suggest that a reduction in the level of dissolved oxygen in the water increases the toxicity of ammonia to fish. Less clear cut results were obtained, however, for rainbow trout (*O. mykiss*) (Thurston & Russo, 1983) and fathead minnows (*Pimephales promelas* Rafinesque) (Thurston *et al.*, 1983). Respiration by the fish will reduce dissolved oxygen and increase carbon dioxide levels which will decrease the pH value of the water. These events have been discussed in relation to effects of ammonia on physiological and ionic exchange events at the level of the microclimate and surface of the gill (Alabaster & Lloyd, 1982; Ip *et al.*, 2001). Effects of ammonia on fish should also be considered in relation to diurnal events in the estuary. If plants or algae are present then photosynthesis will increase oxygen levels and removal of carbon dioxide will increase the pH value of the water. At night, respiration by these organisms will deplete oxygen, increase carbon dioxide and increase acidity.

CYCLING AND EPISODES

Compared to freshwater or marine species, those that inhabit estuaries experience daily changes in salinity and temperature and they may experience additional stresses of cyclical exposure to pollutants such as ammonia. Exposure of fish to ammonia will result in a time and concentration dependent accumulation of ammonia in the tissues whilst return to ammonia free water will result in unloading of ammonia from the body. The rate of ammonia loading and unloading from the body is of critical importance in understanding the animal's responses during episodic exposures. Rainbow trout (*O. mykiss*) acclimated to fresh water, dilute or full strength sea water were exposed to ambient ammonia (1 mmol l^{-1} , pH 7.9) resulting in increased total ammonia levels in the blood plasma which began to stabilize after 8–10 h at *c.* 0.4 mmol l^{-1} (Wilson & Taylor, 1992). Exposure of juvenile turbot (*S. maximus*) to ammonia in sea water resulted in blood plasma levels of *c.* 45 mg l^{-1} total ammonia N within 3 h and removal of the ambient ammonia resulted in ammonia unloading from the blood in *c.* 3 h (Person-Le Ruyet *et al.*, 1997a, b). Whilst ammonia may load and unload from the blood within a few hours these processes may be much slower in other tissues. In salmon alevins (*S. salar*) exposed to 15.8 mmol L^{-1} total ammonia in fresh water, pH 7, the ammonia burden in the body after 12 h

was *c.* $6 \mu\text{mol g}^{-1}$. When the ammonia loaded alevins were placed in ammonia free fresh water, the accumulated ammonia was unloaded in *c.* 10 h but body sodium levels took longer to resume normal levels (Paley *et al.*, 1993). Thus, following exposure to an ammonia episode, the rate of unloading accumulated ammonia from the body will be of critical importance in determining the animal's response to the next episode. If this occurs before ammonia unloading is substantially complete then the animal is likely to accumulate a greater, and potentially more damaging ammonia burden compared to the first exposure. An additional approach is further development of mathematical models for episodic exposures, using rates of gain, loss and accumulation of ammonia (Lumbers & Wishart, 1989). In devising such models it would be necessary to include the perturbations of ionic balance resulting from episodic ammonia exposures (Paley *et al.*, 1993).

CONCLUSIONS

Estuaries are individual and complex environments and their inhabitants, whether migratory or resident, are exposed to daily and seasonal changes in salinity and temperature. Superimposed on these challenges may be the stress of pollutants such as ammonia. However management of water quality in estuaries requires a better understanding of how fish respond to ammonia when the water varies in salinity, pH and temperature.

Most biological membranes are permeable to unionized ammonia (NH_3) but relatively impermeable to ionized ammonia (ammonium ions, NH_4^+). In much of the literature toxicity is expressed in terms of the concentration of unionized ammonia (NH_3) (Seager *et al.*, 1988) although analysis of the toxicity data (USEPA, 1999) indicates that when ammonia is expressed as the concentration of total ammonia [$\text{NH}_3 + \text{NH}_4^+$] the toxicity is independent of temperature. The toxicity of ammonia is strongly influenced by differences between species and pH rather than by the comparatively minor influences of salinity and temperature.

The toxicity of ammonia (96 h LC_{50}) to freshwater fish species is in the range $0.068\text{--}2.0 \text{ mg l}^{-1} \text{ NH}_3$, similar to the range for marine species, $0.09\text{--}3.35 \text{ mg l}^{-1} \text{ NH}_3$, and it is likely that the toxicity of ammonia to estuarine fish falls within these ranges. For freshwater salmonids the protective standard of $21 \mu\text{g l}^{-1} \text{ NH}_3\text{-N}$ was suggested to be protective for most marine and estuarine fish as well, although the influence of cyclical changes in pH, salinity and temperature were not considered. In marine and estuarine fish the toxicity of ionized ammonia (NH_4^+) should also be considered since the gills show some permeability to this ion.

During ammonia exposure, estuarine fish are likely to be most at risk when they are larvae or juveniles, if the temperature is elevated, if salinity is near the sea water value and if the pH value decreases below pH 7. They are also likely to be at risk in waters of low salinity, high pH and high ammonia levels. These conditions favour transfer of ammonia from the environment into the fish, as both ionized and unionized ammonia, and retention of ammonia by the fish is likely. Since ammonia interferes with nervous function there may be impairment of activity and behaviour. Fish will be further at risk from ammonia toxicity if they are not feeding, if they are stressed and if they are active and swimming.

Episodic or cycling exposures should also be considered in relation to the rate at which the animal is able to accumulate and excrete ammonia and the effects of ammonia ionic regulatory and acid-base processes in the gill. The rate of unloading the accumulated ammonia from the body will be of critical importance in determining the response to the next episode. If this occurs before ammonia unloading is substantially complete then a larger and potentially more damaging burden of ammonia may accumulate with possible disruption of ionic regulatory processes. Modelling may be useful in developing an understanding of these aspects of ammonia toxicity to fish.

Evaluation of the effects of ammonia in estuaries will involve devising field and laboratory experiments to determine responses of fish to ammonia as salinity and temperature vary diurnally and over longer periods of time. It will also be necessary to devise ways to evaluate the responses of estuarine residents and of migrants. Behavioural experiments may reveal how far estuarine fish are able to detect and avoid polluted areas. If entry to an ammonia polluted area is unavoidable then the responses of the fish will be determined by its developmental stage, condition, activity level and its physiological capacity to respond to ammonia levels in the estuarine environment over a period of time.

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