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# Ammonia toxicity in fish

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**Abstract**

Ammonia is present in the aquatic environment due to agricultural run-off and decomposition of biological waste. Ammonia is toxic to all vertebrates causing convulsions, coma and death, probably because elevated $NH_4^+$ displaces $K^+$ and depolarizes neurons, causing activation of NMDA type glutamate receptor, which leads to an influx of excessive $Ca^{2+}$ and subsequent cell death in the central nervous system.

Present ammonia criteria for aquatic systems are based on toxicity tests carried out on, starved, resting, non-stressed fish. This is doubly inappropriate. During exhaustive exercise and stress, fish increase ammonia production and are more sensitive to external ammonia. Present criteria do not protect swimming fish. Fish have strategies to protect them from the ammonia pulse following feeding, and this also protects them from increases in external ammonia, as a result starved fish are more sensitive to external ammonia than fed fish.

There are a number of fish species that can tolerate high environmental ammonia. Glutamine formation is an important ammonia detoxification strategy in the brain of fish, especially after feeding. Detoxification of ammonia to urea has also been observed in elasmobranches and some teleosts. Reduction in the rate of proteolysis and the rate of amino acid catabolism, which results in a decrease in ammonia production, may be another strategy to reduce ammonia toxicity. The weather loach volatilizes $NH_3$, and the mudskipper, *P. schlosseri*, utilizes yet another unique strategy, it actively pumps $NH_4^+$ out of the body.
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## 1. Introduction

Ammonia is found in the environment, especially in water. It is excreted by plants and animals, and produced as a result of the decomposition of organisms and sewage by micro-organisms, the release of fertilizers, industrial emissions, and volcanic activity. Ammonia in the environment is toxic. Terrestrial exposure is not usually a problem, even though there is continual volatilization from the earth's surface, as ammonia rises rapidly in the atmosphere and is destroyed by photolytic reactions. Some atmospheric ammonia is returned to the earth's surface as wet or dry deposition. Aquatic exposure, however, is a problem. Urban and agricultural run-off and most biological waste is released into rivers and oceans. Thus the major concern regarding ammonia toxicity is in aquatic systems, particularly in regions of high human habitation and/or large numbers of farm animals, such as pigs and cows.

Most biological membranes are permeable to ammonia but relatively impermeable to ammonium ions. Toxicity, expressed as total ammonia (the sum of $NH_3$ and $NH_4^+$) in the environment, increases with water pH, because ammonia enters organisms as $NH_3$ and the proportion of $NH_3$ increases with water pH. The pK of the ammonia/ammonium reaction is around 9.5 and varies with ionic strength, pressure and temperature. The effects of changes in pressure, temperature and ionic strength are minor compared with the effects of pH on the proportion of $NH_3$ and, therefore, the toxicity of ammonia to organisms.

## 2. The effect of water temperature, salinity and pH on ammonia toxicity

Ammonia toxicity, expressed as total ammonia ($[NH_3]^+$ $[NH_4^+]$, mg N/L), clearly increases with water pH (Fig. 1). Compiled normalized data on acute toxicity in various species of fish indicates that the effect of increased temperature on toxicity is minor between 3 and 30 °C in freshwater systems (USEPA, 1998). In general,

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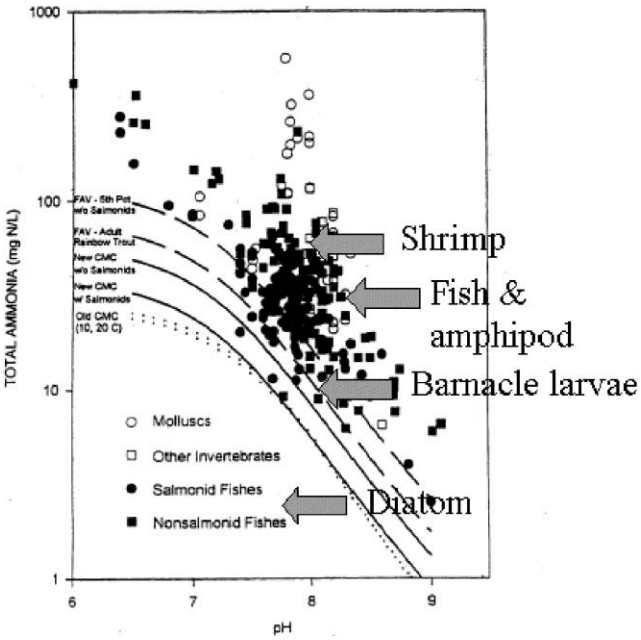



Fig. 1. Acute LC50s (US EPA, 1998) used to derive Final Acute Values (FAV) and Criterion Maximum Concentrations (CMCs) for ammonia. The green arrows indicate the results for some Hong Kong species (from Wu, 2001).

there is insufficient data in saltwater systems concerning the effects of temperature.

The average of the mean acute toxicity values for 32 freshwater species is 2.79 mg $NH_3$/l compared with 1.86 mg $NH_3$/l for 17 seawater species, taken from data reported in the USEPA (1984, 1989) reports, respectively. This comparison indicates that, in general, seawater species are slightly more sensitive to ammonia toxicity than freshwater species. Differences between species in either environment, however, are much greater than differences related to salinity. What is clear is that ammonia toxicity, expressed as total ammonia, in the aquatic environment varies with water pH, but the effects of temperatures and salinity are much less important.

## 3. Guidelines for toxicity tests

The USEPA (1998, 1999) revised its procedure for calculating acute and chronic ammonia criteria, using screened acute and chronic data converted to a uniform pH of 8.0 and a uniform expression as total ammonia-N. The acute and chronic ammonia criteria are then used to develop ammonia standards for the control of ammonia concentrations in aquatic systems. Australia and New Zealand (ANZECC and ARMCANZ, 2000) used the same general approach. Derived criteria are based on acute and chronic toxicity studies (Stephan et al., 1985). Acute toxicity studies follow standard guidelines, namely exposure of organisms to the toxicant under static conditions, using starved, resting unstressed animals. These standardized guidelines facilitate comparisons between studies. Internal ammonia levels in fish are increased following feeding, swimming and stress, all conditions that are avoided when acute toxicity tests are carried out using EPA guidelines. Thus present ammonia criteria are based on toxicity tests carried out on fish when they may be least sensitive to ammonia.

## 4. The effects of exercise on ammonia toxicity

Swimming fish have elevated internal ammonia levels when compared with resting fish. Mommsen and Hochachka (1988) reported that the ammonia level increased in the white muscle in rainbow trout following exercise, due to a breakdown of adenylates to inosine monophosphate (IMP) and $NH_4^+$. Beaumont et al. (1995) noted a correlation between plasma ammonia levels and decreased swimming performance in brown trout exposed to copper. Additionally, during the upstream migration of salmon, feeding ceases and the increase in structural protein catabolism results in a further increase in plasma ammonia (French et al., 1983). Both rainbow trout and coho salmon showed a significant linear decrease in critical swimming velocity with increasing water ammonia levels (Shingles et al., 2001; Wicks et al., 2002), probably due to an ammonia mediated decrease in muscle membrane potential and/or a change in muscle metabolism (Beaumont et al., 1995, 2000). Acute toxicity testing on swimming and resting rainbow trout revealed that the $LC_{50}$ level decreased from $207 \pm 21.99$ mg/L N in resting fish to $32.38 \pm 10.81$ in swimming fish. Thus, the $LC_{50}$ for resting fish was significantly higher than that for swimming fish. The acute value set forth by the USEPA at the same pH is 48.8 mg/L N and will not protect actively swimming salmonid fish and much lower concentrations of ammonia in water will impair swimming performance.

## 5. The effects of feeding on ammonia toxicity

Fed fish have plasma ammonia levels similar to those associated with death due to environmental ammonia exposure (Wicks and Randall, 2002a) and yet survive feeding. That is fish have mechanisms to protect them from potentially suicidal feeding bouts. What is special about fed fish? Firstly, if exposed to high external ammonia they stop feeding. Interestingly, although plasma ammonia levels increased with increasing ambient ammonia, at high external ammonia, plasma ammonia was lower in fed than unfed fish and the 24 h $LC_{50}$ ammonia concentration is higher for fed than unfed fish, that is fed fish are less sensitive to external ammonia than unfed fish. Feeding up-regulates muscle glutamine syn-

thetase activity allowing for increased production/storage of ammonia as glutamine, especially in muscle (Wicks and Randall, 2002b). Thus it appears that the up-regulation of glutamine synthetase in fed fish protects them from ambient ammonia toxicity by allowing for more rapid conversion of ammonia to glutamine. Thus the present ammonia criteria protect fed as well as unfed fish.

## 6. The effects of stress on ammonia toxicity

Stress results in an increase in cortisol levels in fish (Wendelaar Bonga, 1997), stimulating both glycogenesis and gluconeogenesis, as well as an increase in protein catabolism (Mommsen et al., 1999) and ammonia production. Both stress, in the form of an increase in fish density, and cortisol injection exacerbated ammonia toxicity in rainbow trout (Wicks, unpublished data). Thus stressed fish are more sensitive to external ammonia than unstressed fish. Stressed fish up-regulate glutamine synthetase, so repeated stress may protect fish during exposure to increased levels of environmental ammonia in the post stress period. It appears that cortisol plays a role in the up-regulation of glutamine synthetase (Walsh and Milligan, 1995), however, glutamate and/or ammonia may also play some role as the pattern of glutamine synthetase up-regulation varies with the nature of the stress and ammonia exposure.

## 7. The nature of ammonia toxicity

Elevated levels of ammonia in the body have a large number of deleterious effects (Ip et al., 2001a). Acute toxicity of ammonia is mainly due to its effect on the central nervous system of vertebrates. Following acute ammonia intoxication, convulsions and death soon follow in all vertebrates. There is evidence indicating that high ammonia levels in the brain lead to high levels of extracellular glutamate by increasing glutamate release or/and decreasing glutamate synaptic reuptake (Rao et al., 1992; Bosman et al., 1992; Schimdt et al., 1993). It has also been proposed that ammonia toxicity is mediated by excessive activation of NMDA type glutamate receptors (Marcaida et al., 1992). Excessive activation of NMDA receptors leads to influx of $Ca^{2+}$ and $Na^{+}$. The increased intracellular $Ca^{2+}$ activates $Ca^{2+}$-dependent enzymes and a cascade of reactions takes place that eventually results in cell death. Blocking NMDA receptors in rats by antagonists such as MK-801 significantly reduced death rate induced by ammonia intoxication. Fish death due to ammonia intoxication is also associated with convulsions, and the NMDA blocker, MK-801, had a similar protective effect in the loach, *Misugurnus anguillicaudatus*, (Tsui, unpublished observations). It is therefore tempting to speculate that the primary cause of ammonia toxicity in all vertebrates is due to an increase in brain extracellular glutamate level, which causes excessive activation of NMDA receptors and subsequently neuronal cell death. However, recent evidence shows that this may not be the case. Hermenegildo et al. (2000) showed that activation of NMDA receptors preceded the increase in extracellular glutamate levels. Blocking NMDA receptors with MK-801 was also shown to prevent increases in extracellular glutamate levels. They proposed that the initial activation of NMDA receptors was due to a depolarization effect of $NH_4^+$ on neurons. It has already been suggested much earlier that $NH_4^+$ can substitute for $K^+$ and affect the membrane potential in squid giant neuron (Binstock and Lecar, 1969); Beaumont et al. (2000) reported measured levels of depolarisation of muscle fibres in trout with elevated levels of ammonia in their tissues (from −87 to −52 mV), that matched the effect predicted on the basis of the measured gradient for ammonia across the cell membranes. One of the consequences of excessive NMDA receptor activation is ATP depletion, which reverses the sodium-dependent glutamate uptake mechanism. The increase in extracellular glutamate is thus a consequence, and not a cause, of activation of NMDA receptors. Therefore, the primary cause of ammonia toxicity may be the depolarization effect of $NH_4^+$ on neurons, leading to excessive activation of NMDA receptors and subsequent death of the cell.

## 8. Responses of fish to elevated environmental ammonia levels

Elevated ammonia levels in the environment either impair ammonia excretion or cause a net uptake of ammonia from the environment. The end result is an elevation in body ammonia levels, leading to convulsions and death. Most fish species cannot tolerate high environmental ammonia levels but some species are ammonia-tolerant and have a variety of strategies to avoid ammonia toxicity. Maintaining excretion (Randall et al., 1999) and/or converting ammonia to other less toxic substances (Levi et al., 1974; Dabrowska and Wlasow, 1986; Randall et al., 1989; Mommsen and Walsh, 1992; Peng et al., 1998) are some strategies that have been described in the literature (Fig. 2).

### *8.1. Conversion of ammonia to less toxic substances*

#### *8.1.1. Glutamine*

Many fish detoxify ammonia to glutamine when exposed to elevated environmental ammonia. Brain glutamine levels showed a linear correlation with ambient ammonia levels when goldfish were exposed to up to 0.75 mM $NH_4Cl$ (Levi et al., 1974). When exposed to

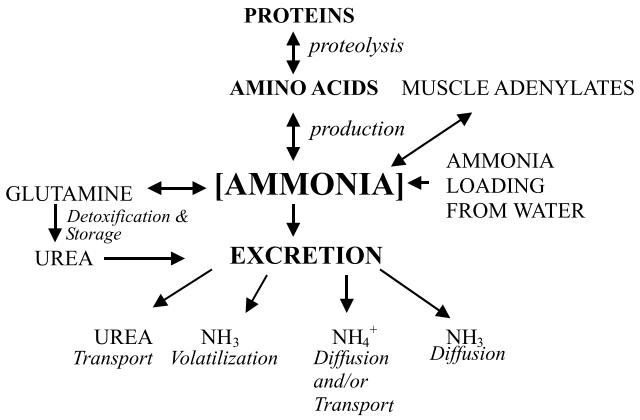



Fig. 2. Summary of strategies utilized by fish to ameliorate ammonia toxicity (adapted from Ip et al., 2001a,b).

0.75 mM $NH_4Cl$ for 24–48 h, the goldfish brain glutamine increased 10-fold. The common carp, *Cyprinus carpio*, the Lake Magadi tilapia, *Oreochromis alcalicus grahamii*, and the mudskippers, *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*, are some other fish species that have been reported to increase cerebral glutamine levels upon exposure to ammonia (Dabrowska and Wlasow, 1986; Mommsen and Walsh, 1992; Peng et al., 1998). During aerial exposure, the sleeper, *Bostrichthys sinensis* (Ip et al., 2001a,b), and the marble goby, *Oxyeleotris marmoratus* (Jow et al., 1999), have also been reported to detoxify ammonia to glutamine.

Glutamine is formed from glutamate and $NH_4^+$ by the enzyme glutamine synthetase. Glutamate is in turn produced from α-ketoglutarate and $NH_4^+$ by glutamate dehydrogenase. Therefore, starting with α-ketoglutarate, formation of 1 mole of glutamine will detoxify 2 moles of $NH_4^+$ (Ip et al., 2001b). The generally high glutamine synthetase activities observed in brain tissues are thought to be the ammonia detoxification mechanism that protects this ammonia-sensitive organ in both mammals (Cooper and Plum, 1987) and fish (Ip et al., 2001b). It is possible that the major selective force in the development of this strategy was the need to deal with the ammonia pulse following feeding, especially in carnivorous fishes. An advantage of this strategy is that glutamine can be stored in the tissues and is readily utilized as an oxidative substrate upon return to normal conditions. However, one drawback is that for every mole of ammonia detoxified, two moles of ATP equivalent are hydrolyzed.

#### *8.1.2. Urea*

Some fish have the capacity to convert ammonia to the less toxic urea via the ornithine urea cycle, but this mechanism is suppressed in most teleosts because diffusion of ammonia into the environment is usually sufficient and requires less energy than making urea. The marine toadfish, *Opsanus beta*, is unusual in that it makes urea via the ornithine urea cycle and releases it in a pulsatile manner (Wood et al., 1995), probably to reduce predation. The Lake Magadi tilapia excretes all nitrogenous waste as urea produced via the ornithine urea cycle (Randall et al., 1989). The high pH of Lake Magadi (about 10) impairs ammonia excretion, leading to ammonia accumulation and death in other fish, including closely related tilapia species. The Lake Magadi tilapia survives because it converts ammonia to urea, thereby avoiding ammonia toxicity. The Indian air breathing fish (*Heteropneustes fossilis*) can produce urea to avoid ammonia toxicity during air exposure (Saha et al., 2001). Fish embryos utilize yolk proteins at a high rate during development and this results in a high rate of ammonia production during periods when diffusive loss is impaired because the circulation and gills are still developing. Many fish embryos have an active ornithine urea cycle and convert ammonia to urea to avoid ammonia toxicity during the early stages of development (Wright et al., 1995). Thus urea formation is used by several fish species during development or under certain environmental conditions, such as air exposure or alkaline water pH, to avoid problems of ammonia accumulation and toxicity.

Elasmobranch fish use urea produced from ammonia via the ornithine-urea cycle to increase their body osmolarity; they also excrete most of their nitrogenous waste as urea across their gills. Elasmobranchs have high concentrations of urea in their bodies, which they prevent from leaking into the environment by having very low permeability to urea in their gill epithelia (Part et al., 1998). This low permeability is due to a high cholesterol composition of the gill membrane, coupled to a urea/sodium antiporter in the basolateral membrane that transports urea back into the blood (Fines et al., 2001). The elasmobranch kidney also functions to conserve urea (Schimidt-Nielsen et al., 1972).

### *8.2. Reduction in ammonia production*

Reduction in ammonia production as a strategy to ameliorate the problem of ammonia toxicity has been observed in several fish when exposed to high pH (Wilson et al., 1998) and aerial conditions (Lim et al., 2001). Although there is no report on this strategy being utilized by fish exposed to high environmental ammonia, it probably occurs under this condition as well.

When exposed to aerial conditions in constant darkness, the mudskippers, *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*, significantly reduced their ammonia and urea excretion rate (Lim et al., 2001). There was no accumulation of urea in the muscle and the liver, and the accumulation of ammonia in these tissues was not enough to account for the reduction of ammonia excretion. Total free amino acid levels were also found to have decreased significantly in the liver of

*P. schlosseri* and in the muscle of *B. boddaerti*. The data indicates that there was reduction in the rate of both proteolysis and amino acid catabolism in the two mudskippers. Since there was reduction in total free amino acid levels, the reduction in the rate of proteolysis might be greater than the reduction in the rate of amino acid catabolism (Lim et al., 2001).

Wilson et al. (1998) also concluded that the rainbow trout, *Oncorhynchus mykiss*, reduced ammonia production when exposed to pH 10. They observed that there was a significant decrease in ammonia excretion, and the calculated accumulation of ammonia, urea and glutamine in the body could not account for the reduction in ammonia excretion. They concluded, therefore, that rainbow trout ameliorated ammonia accumulation when exposed to high pH water by reducing ammonia production.

### 8.3. $NH_3$ volatilization

The weather loach, *Misgurnus anguillicaudatus*, volatilizes ammonia as $NH_3$ gas during aerial exposure and ammonia loading (Tsui et al., 2002). It can accumulate and tolerate ammonia in its body to an extent much higher than other fishes (Chew et al., 2001). Accumulation of ammonia may facilitate ammonia efflux and, thus, volatilization. The pH of the skin surface increased from a control value of $6.65 \pm 0.09$ to $8.23 \pm 0.18$ after 48 h of aerial exposure. Thus the skin is a possible site of $NH_3$ volatilization. However, in its natural environment, the loach buries itself into the mud during drought (aerial exposure). The skin would then not be a suitable site for $NH_3$ volatilization. Furthermore, when it was exposed to high ammonia concentrations in water, it acidified the surrounding ammonia solution, and this would tend to reduce any volatilization from the water surface. Tsui et al. (2002) found that the pH of the mucosal surface of the anterior part of its gut significantly increased during both ammonia and aerial exposure. Since this loach uses its gut for gaseous exchange (it is a facultative air breather), the anterior part of the gut may be the site of ammonia volatilization. When it gulps air through the mouth, $NH_3$ can then escape together with the "exhaled" air via the vent. In fact, when the fish was prevented from coming to the water surface to breathe air, $NH_3$ volatilization was halted.

### 8.4. Active excretion of ammonium ions

Wilson and Taylor (1992) found that the rainbow trout could excrete part of an ammonia load against high ambient levels in both freshwater and seawater. They suggested that $NH_4^+$ may be actively exchanged for $H^+$ in freshwater and $Na^+$ in seawater. It appears that the mudskipper, *Periophthalmodon schlosseri,* is able to survive both high ammonia levels in the water and terrestrial exposure by actively excreting ammonium ions (Randall et al., 1999). This animal uses its buccal cavity to breath air and will die if denied access to air. The gills are a complex structure with large blood to environment diffusion distances that limit gas transfer across the gill epithelium. The gill epithelium contains a large number of chloride cells, probably involved in ammonium ion excretion. There are high levels of an ammonium ion sensitive $Na^+/K^+$ ATPase and a $Na^+/K^+/2Cl^-$ cotransporter on the basolateral membrane, both of which could move ammonium ions into the cell. It is suggested that ammonium ions are moved across the apical membrane in exchange for sodium; both the NHE2 and NHE3-like isoforms of the $Na^+/H^+$ ($NH_4^+$) exchanger are present in the apical membrane of the chloride cell (Wilson et al., 2000) of this mudskipper (Fig. 3).

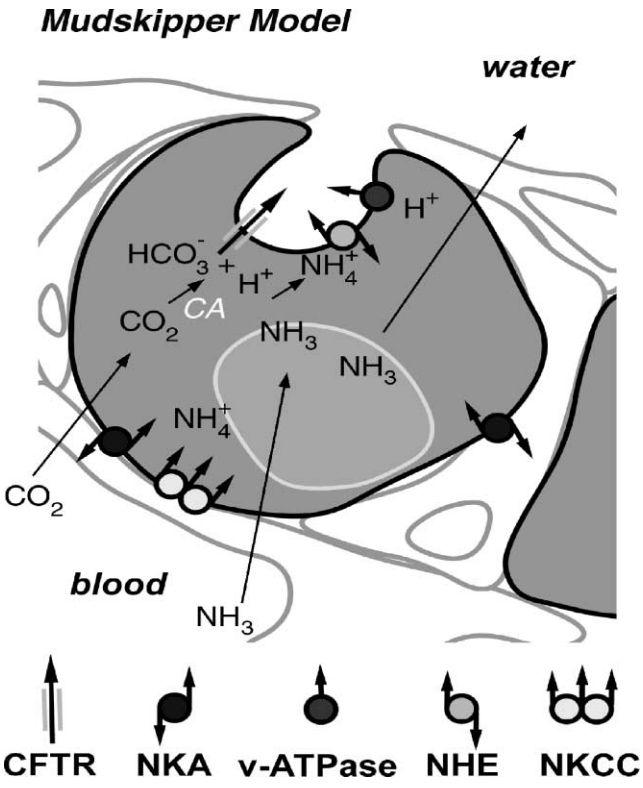



Fig. 3. Active ammonium ion elimination probably occurs through the mitochondrial rich (chloride) cell of the mudskipper (Periophthalmodon schlossei) gill epithelium. Ammonium ions are carried into the cell via either $Na^+/K^+$ ATPase (NKA) or $Na^+/K^+/2Cl^-$ cotransporter (NKCC) on the basolateral membrane. The apical membrane contains a proton (v-type) ATPase and a $Na^+/H^+$ exchange (NHE)-like carrier, as well as a cystic fibrosis transmembrane regulator, CFTR-like anion channel. The cell also contains high levels of carbonic anhydrase (CA) associated with the apical membrane. Ammonium ions can leave the cell via the NHE in exchange for sodium. CA will supply protons to both the proton pump and $NH_3$ protonation. Bicarbonate formed can leave the cell via the anion channel, accounting for the pH neutral excretion of ammonia (Wilson et al., 2000).

## 9. Conclusion

Ammonia is an environmental toxicant that is especially problematic for aquatic organisms. Its level in aquatic systems can rise due to agricultural run-off and decomposition of biological waste. The nature of ammonia toxicity seems to be similar in fish and mammals. Convulsion, coma and death take place in both groups of animals soon after ammonia intoxication. Current data suggests that elevated $NH_4^+$ displaces $K^+$ and depolarizes neurons, causing excessive activation of NMDA type glutamate receptor, which leads to influx of excessive $Ca^{2+}$ and subsequent cell death in the central nervous system.

Present ammonia criteria for aquatic systems are based on toxicity tests carried out on, starved, resting, non-stressed fish. This is doubly inappropriate. During exhaustive exercise and stress, fish increase ammonia production and are more sensitive to external ammonia. Present criteria do not protect swimming fish. Fish have strategies to protect them from the ammonia pulse following feeding, and this also protects them from increases in external ammonia. So starved fish are more sensitive to external ammonia than fed fish.

There are fish species that are tolerant to high environmental ammonia. They utilize different strategies in order to ameliorate the problem of ammonia toxicity. The glutamine synthetase activities in brain tissues of fish are generally high. Glutamine formation is thus an important ammonia detoxification strategy in the brain of fish. Detoxification of ammonia to urea has also been observed in elasmobranches and some teleosts. Reduction in the rate of proteolysis and the rate of amino acid catabolism, which results in a reduction of ammoniagenesis, may be another strategy to avoid ammonia toxicity. The weather loach volatilizes $NH_3$ which reduces the accumulation of ammonia within the body. The mudskipper, *P. schlosseri*, utilizes yet another unique strategy, it actively pumps $NH_4^+$ out of the body.

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