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Review Article

Ammonia toxicity and adaptive response in marine fishes – A review

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High environmental ammonia has become a universal problem for aquatic animals, especially in fish and induces a range of ecotoxicological effects. Perhaps waterborne ammonia is one of the most notorious pollutants in aquatic habitats. The effects of rising ammonia levels do not act in isolation; increasing human pressure including climate change (e.g. temperature, rising CO_2, hypoxia etc.) creates a variety of additional deleterious impacts on animals. The salinity gradient of some marine ecosystems has gradually reduced over the last few decades, which alters a suite of physiological and behavioural performance of marine fish with a severe threat on osmo-regulation. It has been documented that environmental salinity influences the ammonia toxicity in several marine species. Such interactive effect between ammonia toxicity and salinity challenge on differential physiological and metabolic compensatory responses has not been fully revealed in fish. It is essential to pay attention to the levels of salinity and ammonia in environment, and how the two interact, particularly when dealing with estuarine aquaculture.

[Keywords: Ammonia; Toxicity; Salinity; Tolerance.]

Introduction

Ammonia is a nitrogenous byproduct of amino acid catabolism that is often excreted by fish, (alongside urea) through the gill membrane[1]. However, a dilemma regarding ammonia continues to dodge biologists to date; the fact that it is both a by product of amino acid catabolism and a potent toxicant that causes convulsions and death in fish[1]. The toxicity of ammonia is often observed in environments with relatively higher levels of ammonia than the normal. The sources of this apparently high waterborne ammonia include sewage effluents, agricultural run-offs, industrial wastes and decomposition of biological matters.

Ammonia Chemistry and Aquatic Ecosystem

Ammonia exists in solution primarily as ionized form, NH_4^+ and unionized form, NH_3. The toxicity of ammonia to aquatic organisms has been attributed primarily to the unionized species NH_3, while the ionized species NH_4^+ is considered relatively less toxic or non-toxic[2]. However, knowledge of the concentration of NH_3 in any aqueous solution is mandatory to determine what proportion of total ammonia is toxic to aquatic life[2]. In marine environment, the proportion of NH_3 in the total ammonia is largely determined by ambient pH and temperature and, to a certain extent by salinity[3]. The concentration of NH_3 in sea water has been shown to increase with increase in ambient pH and temperature, while it decreases with increased ambient salinity[3]. Unfortunately, existing experimental methodologies do not permit the measurement of NH_3 and NH_4^+ ammonia entities separately. As such, analytical methods measure the percentage of total ammonia that is present as NH_3 or NH_4^+ only from calculations based on the ammonia-water equilibrium[2]. Often, aqueous ammonia solutions consist of un-ionized ammonia (NH_3) in equilibrium with ammonium ion (NH_4^+) and hydrogen ions (H^+) [2]. This equilibrium can be expressed by the equation[2]:

$$NH_{3\,(g)} + nH_2O_{\,(l)} \rightleftharpoons NH_3.nH_2O_{\,(aq)} \rightleftharpoons NH_4^+ + OH^- + (n\text{-}1)\,H_2O$$

From the above equation, it can be deduced that the dissolved ammonia exists in hydrated form since it is hydrogen bound to at least three water molecules. However, it is noteworthy that the concentration of NH_3 is dependent on multiple other factors, apart from the concentration of total ammonia. These other factors include temperature, pH, and ionic strength[2]. Studies have shown that increased temperatures and pH increase the concentration of NH_3. On the other

hand, increased ionic strength serves to decrease the concentration of NH_3 in saline waters like sea water [2]. Using pK values from derived data, the percentage of NH_3 in aqueous ammonia can be calculated with regards to temperature and pH, using a freshwater model as shown in the Table 1.

In addition, the percentage of NH_3 to total ammonia can also be deduced by this equation proposed by another author[4]:

$$\%NH_3 = 100/[1=10^{(\log K_1 - pH)}]$$

with

$$\log K_1 = -0.467 + 0.00113 \times S + 2,887.9 \times T^{-1}$$

where K1 is the dissociation constant, S (in g l^{-1}) the salinity, and T the temperature (°K).

Ammonia Toxicity in Aquaculture

Ammonia naturally occurs in the environment, especially in water bodies. Ammonia in terrestrial environments is often not toxic because it is quickly destroyed by photolytic reactions[5]. Therefore, the main problem of toxicity lies in the aquatic exposure to ammonia, particularly in regions with high population densities; thus, human activity and large numbers of animals, especially pigs and cattle[5]. As earlier mentioned, the toxicity of ammonia is attributable to the NH_3 moiety. However, the toxicity of ammonia, expressed as total ammonia, increases as the level of pH in water rises[5].

Marine fish excrete ammonia across their gill membranes into water in the form of NH_3. This process requires facilitation by Rhesus (Rh)

Table 1 — Per cent NH_3 in aqueous ammonia at 0-30 °C and pH 6-10 [2]

Temp.	pH								
	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
0	0.00827	0.0261	0.0826	0.261	0.820	2.55	7.64	20.7	45.3
1	0.00899	0.0284	0.0898	0.284	0.891	2.77	8.25	22.1	47.3
2	0.00977	0.0309	0.0977	0.308	0.968	3.00	8.90	23.6	49.4
3	0.0106	0.0336	0.106	0.335	1.05	3.25	9.60	25.1	51.5
4	0.0115	0.0334	0.115	0.363	1.14	3.52	10.3	26.7	53.5
5	0.0125	0.0395	0.125	0.394	1.23	3.80	11.1	28.3	55.6
6	0.0136	0.0429	0.135	0.427	1.34	4.11	11.9	30.0	57.6
7	0.0147	0.0464	0.147	0.462	1.45	4.44	12.8	31.7	59.5
8	0.0159	0.0503	0.159	0.501	1.57	4.79	13.7	33.5	61.4
9	0.0172	0.0544	0.172	0.542	1.69	5.16	14.7	35.3	63.3
10	0.0186	0.0589	0.186	0.586	1.83	5.56	15.7	37.1	65.1
11	0.0201	0.0637	0.201	0.633	1.97	5.99	16.8	38.9	66.8
12	0.0218	0.0688	0.217	0.684	2.13	6.44	17.9	40.8	68.5
13	0.0235	0.07043	0.235	0.738	2.30	6.92	19.0	42.6	70.2
14	0.0254	0.0802	0.253	0.796	2.48	7.43	20.2	44.5	71.7
15	0.0274	0.0865	0.273	0.859	2.67	7.97	21.5	46.4	73.3
16	0.0295	0.0933	0.294	0.925	2.87	8.54	22.8	48.3	74.7
17	0.0318	0.101	0.317	0.996	3.08	9.14	24.1	50.2	76.1
18	0.0343	0.108	0.342	1.07	3.31	9.78	25.5	52.0	77.4
19	0.0369	0.117	0.368	1.15	3.56	10.5	27.0	53.9	78.7
20	0.0397	0.125	0.396	1.24	3.82	11.2	28.4	55.7	79.9
21	0.0427	0.135	0.425	1.33	4.10	11.9	29.9	57.5	81.0
22	0.0459	0.145	0.457	1.43	4.39	12.7	31.5	59.2	82.1
23	0.0493	0.156	0.491	1.54	4.70	13.5	33.0	60.9	83.2
24	0.0530	0.167	0.527	1.65	5.03	14.4	34.6	62.6	84.1
25	0.0569	0.180	0.566	1.77	5.38	15.3	36.3	64.3	85.1
26	0.0610	0.193	0.607	1.89	5.75	16.2	37.9	65.9	85.9
27	0.0654	0.207	0.651	2.03	6.15	17.2	39.6	67.4	86.8
28	0.0701	0.221	0.697	2.17	6.56	18.2	41.2	68.9	87.5
29	0.0752	0.237	0.747	2.32	7.00	19.2	42.9	70.4	88.3
30	0.0805	0.254	0.799	2.48	7.46	20.3	44.6	71.8	89.0

glycoprotein in addition to the active transport roles of the Na^+/H^+ exchanger, proton pump and Na^+/K^+ -ATPase[6]. However, when there are high levels of ammonia in the environment, the outward flux of ammonia excretion through the gills is reduced and a reverse inward flux occurs[6]. The result is that blood and tissue levels of ammonia in marine fish rises and the fish exhibit both chronic and acute symptoms of ammonia toxicity[6]. Often, the fish is still able to maintain the total ammonia concentration in their extracellular fluids below that of the external environment even after exposure to high levels of ambient ammonia[7]. This phenomenon is attributable to the active exchange of every NH_4^+ for external counter ion (Na^+ or H^+) that is sufficient enough to cancel out the passive inward diffusion of ammonia[7].

Although both acute and chronic ammonia toxicity effects have been widely studied for freshwater species, information on marine fish species is relatively scarce. LC_{50} data gathered from various studies have established safe levels of ammonia for growth of marine organism to range between 0.05 and 0.2 mgL^{-1} UnIonized Ammonia (UIA)-N [8]. These values are dependent on ambient temperature and pH, age and species of the organism [8]. In sea bass juvenile *Dicentrarchus labrax*, studies have established that the lethal concentration for 50 per cent of the population (96-h LC_{50}) was 1.7 mgL^{-1} UIA-N (40.0 mgL^{-1} total ammonia-nitrogen) [8]. Mortalities were recorded over 63 days of the experiment and the observed detrimental effects of ammonia aided to establish the LC_{50} values of ammonia in European sea bass [8]. Compared to other marine fish, the LC_{50} ammonia values for sea bass juveniles are relatively low, as shown in Table 2.

Table 2 — Comparative toxicity of ammonia to various marine fish

	Species	TA-N (mgL^{-1})	UIA-N (mgL^{-1})
4 -Day LC50	*Sea bass*	40	1.7
4 -Day LC50	Sea bream	57	2.5
4 -Day LC50	Turbot	59	2.6
4 -Day LC50	Cat fish	45	1.6
4 -Day LC50	Rainbow trout	22	0.3-0.6
8 -Day LC50	Sea bass	>22.3	>0.9
20 -Day LC50	*Sea bream*	15.7	0.89
28 -Day LC50	Turbot	38	1
20-Day EC50	Sea bream	15.7	0.89
28-Day EC50	Turbot	17-19	0.50-0.65
55-Day EC50	Turbot	17-21	0.60-0.75
55-Day EC50	Sea bass	22	0.9

LC50 = Lethal concentration for 50% of the population
EC50 = Concentration reducing growth by 50%

Effects of Ammonia Toxicity on Marine Fish Physiology and Behaviour

A study[9] in juvenile Atlantic Halibut established a direct correlation between growth pattern and feed intake in response to ammonia threat. A reduction in feed intake was observed following chronic exposure of fish to high (over 0.71 mgL^{-1}), medium (0.43, 0.53 and 0.64 mgL^{-1}) and even low (0.24 and 0.26 mgL^{-1}) ammonia concentration. This reduction of food intake by chronic exposure to various ammonia concentrations was, as shown in the experiment, an important factor that controlled growth reduction. This finding was in tune with prior studies, which have suggested that growth reduction due to exposure of fish to chronic sub-lethal levels of ammonia had close correlation to feed intake[9]. These findings are supported by Foss *et al*. (2009)[10] who reported that growth rates were reduced in Turbots (*Scophthalmus maximus*) exposed to increased ammonia levels (0.13 - 0.25 mgL^{-1}). The latter study, however, also took to account short daily peaks of ammonia that often occurred post-feeding. They noted that short daily peaks resulted in growth reduction equivalent to those recorded under chronic exposure. Additionally, they showed the rates of ammonia excretion to be higher (up to 3 times) immediately after feeding compared to starved fish[10]. The reduced growth is also shown to be a factor of reduced feed conversion rate as a result of chronic ammonia exposure. An exception to this general observation is a 1998 study by Linton *et al*[11], which suggested that chronic exposure to

Table 3 — Feed conversion efficiency (FCE), daily feeding rate (F %), and total feed consumption (CT g) of juvenile Turbot subjected to chronic and periodic un-ionized ammonia exposure10

Treatment	FCE	F (%)	CT (g)
Control	1.39 ± 0.04	1.62 ± 0.04	969.1 ± 88.9
Chronic High	1.35 ± 0.11	1.56 ± 0.08	864.8 ± 28.6
High Pulse	1.40 ± 0.03	1.55 ± 0.03	908.5 ± 13.6
Chronic Low	1.28 ± 0.11	1.58 ± 0.05	906.7 ± 16.1
Low Pulse	1.36 ± 0.13	1.57 ± 0.02	939.7 ± 12.2

low concentrations of ammonia produced a slight improvement in growth rates of fish, in spite of reduced feed intake.

In a review paper about the effects of ammonia on the physiology of estuarine fish, Eddy (2005)[12] claims that the estuaries presented the most dynamic environment with daily changes in salinity, temperature, pH, oxygen and pollutants present. Because of these dynamics, resident inhabitants may have developed adaptation mechanisms to be able to survive in the stressful environment. For instance, migrating salmon smolts are known to make a gradual entry into full sea water[12]. The migration process is, therefore, thought to involve a series of physiological and behavioural alterations to lessen the vulnerability of the estuarine species to the stressful effects of pollutants such as ammonia[12].

In a study to investigate the gene expressions at different ambient ammonia concentrations in North Sea flounder (*Platichthys flesus L.*), tissue-specific gene expression showed that transcript levels were altered to adapt to the environmental conditions[13]. For instance, the study investigated expression of alpha and beta sub-units of haemoglobin in transplanted European flounder (*Platichthys flesus*) from the very saline North Sea environment and brackish Baltic Sea. These two groups showed clear differences in expression of these genes among different types of tissues. In the gills, gene expression was up-regulated as a result of salinity treatment[13]. In the liver, lower gene expression was recorded at simulated non-native salinity as compared to native salinities[13]. In the kidneys, stress response occurred with gene up-regulation seen in North Sea flounders transported to low salinity[13].

A study by Wicks and co-workers (2002)[14] aimed to investigate the hypothesis that swimming exacerbated ammonia toxicity in fish. Sub-lethal and acute toxicity tests were conducted with the fish at rest and swimming, respectively. Coho salmon (*Oncorhynchus kisutch*), which has both ocean and fresh water phases in their life cycle, showed a significant linear decrease in U_{crit} both with increasing water ammonia concentration and increasing plasma concentration of ammonia[14]. Data collected led the researchers to conclude that reduced swimming activity was both due to metabolic changes and depolarization of white muscle[14].

Toxicological symptoms and even death exhibited by the fish exposed to high levels of ambient ammonia emanate from the fact that elevated ammonia levels in the environment either impair the fish's ability to excrete ammonia or lead to a net uptake of ammonia from the environment[15]. The excretion of ammonia is not a common problem in environments with low pH. However, in high pH environment, the gradient for NH_3 diffusion is significantly reduced and there is a net build-up in ammonia within the fish tissues[15]. In acute cases, where this build-up is too rapid and toxic levels of ammonia are reached, there is an inhibition of Na^+ influx, and the fish experiences convulsion of white muscles and ultimately death[15]. Another cause of white muscle convulsions leading to death of fish could be the possibility of $NH4^+$ replacing K^+ in neural membrane transport systems disrupting action potential recovery or interfering with the metabolism of glutamate in the synapses[15].

On the other hand, chronic exposures to high ambient levels of ammonia causes retarded growth, altered metabolic processes, increased vulnerability to diseases and pathology to the gills[6], coupled with a series of physiological changes in the fish that causes them to adapt to the new environment.

Potential Mechanisms of Ammonia Excretion across Fish Gills

Marine fish and other teleosts excrete the bulk of their nitrogenous waste as ammonia. The bulk of this excretion results from clearance of ammonia from blood, which is then excreted through the gills. Under normal environmental ammonia concentrations, the trans-brachial ammonia gradient is positive and the net flow is from the gills to water[7]. However, alterations of ambient ammonia concentration may change the course of flow of ammonia leading to accumulation of ammonia in fish blood and hence ammonia toxicity. Most fish species are, however, able to adapt their excretion of ammonia in varied environmental conditions. This is because the trans-brachial ammonia excretion occurs in a number of pathways that augment each other. These pathways include: Trans-cellular and/or paracellular diffusion of NH_3, paracellular diffusion of NH_4^+, apical or basolateral NH_4^+/Na^+ exchange, and basolateral $NH_4^+/Na^+/2Cl^-$ co-transport[7].

Experimental studies have established that when the positive ammonia gradient is reversed by exposing fish to high ambient ammonia concentration, marine teleosts and fresh water fish species still maintain the

total ammonia concentration in their extracellular fluids below that of the external environment[7]. This phenomenon is largely attributed to the active exchange of every NH_4^+ for external cation (Na^+ or H^+) sufficient to cancel out the passive inward diffusion of ammonia[7]. However, blood analysis for respiratory acid-base status after a 24 hour exposure of both fresh water and marine fish to different salinities reveals an acid load in fresh water trout and a base load in sea water trout. This indicates that the active exchange of NH^+ predominantly occurred through the NH_4^+/H^+ transporter in fresh water and NH_4^+/Na^+ transporter in sea water, respectively[7].

After a 24 hour exposure to different salinities, the total plasma ammonia load is also shown to increase with salinity[7]. These differences cannot be attributed to differences in prevailing trans-brachial ammonia gradient solely because at high salinities, environmental ammonia gradient is relatively lower. As such, it can be presumed that the differences emanate from increased permeability to NH_4^+ with increased ambient salinity[7]. Moreover, in contrast to gills of freshwater fish, many marine teleost gills are characterized by relatively shallow tight junctions. The high branchial cation permeance conferred by these shallow tight junctions promotes passive NH_4^+ loss. Additionally, unlike in freshwater teleosts, gill boundary layer acidification is probably not involved in marine teleosts because of the increased buffering capacity of seawater and the lack of apical proton pumps[7].

Therefore, the excretion of NH_4^+ is a more important process in marine teleosts than in fresh water teleosts[7]. Marine teleosts have also been shown to have a favourable trans-epithelial potential[7], an observation that supports the former argument. In brief, ammonia excretion in sea water occurs by both passive NH_3 and NH_4^+ diffusion via transcellular pathways, and 'leakier' paracellular routes. However, the increased permeability to NH_4^+ by marine teleosts' gill membranes insinuates that they would be more severely affected than fresh water teleosts in case of ammonia toxicity.

To mitigate this danger of ammonia toxicity, a majority of sea water teleosts such as the European sea bass, has developed mechanisms to convert ammonia into less toxic end products as shown in Figure 1. The suitability of which end product of ammonia is excreted largely depends on species-dependent physiological processes and their demands. For instance, uric acid, whose excretion requires little water, is the ammonia by product often excreted by birds, reptiles and many terrestrial invertebrates[16]. However, it does not seem to be excreted in significant amounts by fishes. Fish, therefore, alternatively excrete ammonia as urea. Urea excretion seem to be favoured by this fish species because, although its bipolarity accords it almost similar solubility to ammonia, it is almost insoluble in lipids, making its membrane permeability almost two-folds less than that of ammonia[16].

Salinity Challenge

Salinity can be defined as a measure of the saltiness of a solution in that high saline solutions have higher saltiness and low saline solutions are low in salt content. Salinity has been shown to be a major mitigating or exacerbating factor in pollutant toxicity in most aquatic environments. For instance, environmental salinity has been demonstrated to influence the toxicity of ammonia-Nitrogen (NH_3-N) and nitrite-Nitrogen (NO_2-N) in most marine species[6]. The toxicological action of high ambient ammonia levels is thought to be accentuated by salinity alteration[6]. Increased ambient salinity is thought to reduce the level of toxicity of ammonia since it serves to reduce the proportion of gaseous ammonia NH_3, which is the most toxic form of ammonia, to aquatic culture[17]. NH_3 has been shown to exist in equilibrium in water with NH_4^+. This equilibrium is largely affected by pH, temperature and salinity so that as pH rises the proportion of NH_3 in the solution increases while a rise in salinity significantly reduces the proportion of NH_3 in the solution[17]. Nitrites, which penetrate fish mucous membranes to oxidise haemoglobin to met-haemoglobin, the latter having markedly reduced ability to bind oxygen, are also

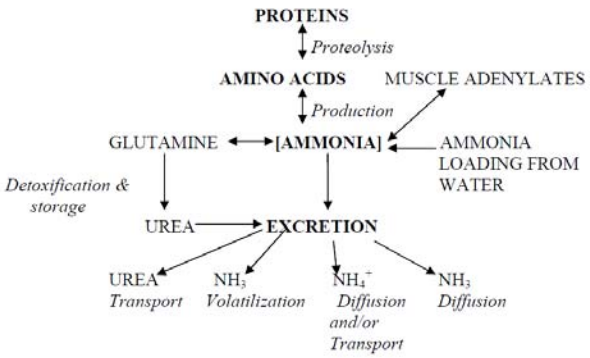

Fig 1 — Ammonia excretion via end products to minimize toxicity[16]

greatly inhibited by increased ambient salinity[17]. Salinity has also been shown to help better maintain the ionic influx and gill membrane his to chemistry in the face of gill diseases such as Amoeba Gill Disease (AGD)[18].

However, care should be taken when rearing fish in estuarine environment, where major fluctuations in salinity are commonly experienced[17]. This is because these fluctuations may cause with them increases in ambient pH and temperature, exacerbating the toxicity of ammonia and nitrites. Daily changes in oxygen gradient and pollutant concentration are also common phenomena in estuarine environment[12]. To be able to survive in this dynamic environment, it is thought that residents of estuaries have developed adaptation mechanisms. For instance, when salmon smolts migrate, they gradually enter the full sea water to allow sufficient time for physiological and behavioural alterations to lessen their vulnerability to the stressful effects present in the estuarine environment[12].

The dynamics in estuarine environment are perhaps best manifested by the recent increased melting of glaciers, ice caps and ice sheets in this environment. This melting of glaciers, thought to be a result of global warming, contributes to major changes in global sea levels[18]. It has been shown to be on a linear trend since the twentieth century and the Arctic sea ice is expected to reduce by 10 per cent by the year 2050[18]. Of significance to the living condition of aquatic organism, however, is the fact that the increased melting of glaciers has a diluting effect on the sea, thereby reducing the salinity of marine water[18]. This factor, coupled with the dynamics of most estuarine environments and increased industrial and sewage effluents into these environments, favours the toxicological effects of pollutants such as ammonia[19]. Moreover, salinity is a key factor in the rearing of the European sea bass, a culture which has been on the rise in the Mediterranean region.

Tolerance to High Ambient Ammonia

Studies to investigate acute toxicity of ammonia to juvenile Sea bass revealed that toxicity was augmented by increased concentration of NH_3 in the environment[20], exhibited by massive convulsions and mortality. However, chronic exposure did not produce any mortality past seven days of exposure to high ambient levels of ammonia[8]. This phenomenon is largely attributable to the fact that Sea bass is tolerant to prolonged exposure to ammonia and nitrites[6]. Juvenile Sea bass are also shown to have the capacity to recover to normal feeding behaviour within 24 hours of being exposed to handling stress[21]. After 2 hours of transportation, ammonia nitrogen excretion rate shot to 4 times the levels in sea water while ammonia concentration peaked 10 hours post-stocking[21]. This level returned to normal level 24 hour post-stocking which was in line with post-feeding ammonia excretion trends, except in the latter, peak level was achieved within 6 hours [21]. However, perhaps the most remarkable attribute of the sea bass is its ability to convert ammonia into less toxic substances such as Urea[5].

Conclusion

Ambient ammonia concentration and salinity act interactively in determining the level of pollutant toxicity. Concentration of ammonia, especially the NH_3 moiety, largely determined the gradient of diffusion of ammonia in and out of the fish gills. A negative gradient caused by high ambient ammonia concentration causes ammonia to accumulate within the fish tissues causing toxic effects. On the other hand, salinity is the key determinant of the proportion of the NH_3 moiety in this high ambient ammonia concentration. It has been shown that NH_3 is responsible for the toxicological action of ammonia since it readily diffuses through tissues into blood. Increased salinities have been shown to minimize the proportion of NH_3 in ambient ammonia concentrations, thereby markedly reducing its toxicological effect. It is, therefore, imaginable how much stress load fish living in an environment with high ambient ammonia concentration and low salinity, accompanied with high pH and temperatures may be going through. Hence it is essential to pay attention to the levels of salinity and ammonia in environment, and how the two interact, particularly when dealing with estuarine aquaculture.

References

1 Wright P.A. and Wood C.M., Seven things fish know about ammonia and we don't. *Respir Physiol Neurobiol*., 184, (2012) 231-240

2 Emerson K., Russo R.C., Lund R.E. and Thurston R.V., Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Board Can*., 32, (1975) 2379-2383

3 Bowen C.E. and Bidwell J.P., Ionization of Ammonia in Seawater: Effects of Temperature, pH, and Salinity. *J. Fish Res. Board Can*., 35, (1978) 1012-1016

4 Johansson O. and Wedborg M., The Ammonia-ammonium equilibrium in sea water at temperatures between 5 and 25°C. *J. Solution Chem.,* 9, (1980) 37-44

5 Randall D.J. and Tsui T.K.N., Ammonia toxicity in fish. *Marine Pollution Bulletin,* 45, (2002) 17-23

6 Weirich C.R. and Riche M.A., Tolerance of juvenile black sea *bass Centropristis striata* to acute ammonia and nitrite exposure at various salinities. *Fisheries Science*, 72, (2006) 915-921

7 Wilson B.W. and Taylor A.W., Transbranchial Ammonia Gradients and Acid-Base Responses to High External Ammonia Concentration in Rainbow Trout (*Oncorhynchus mykiss*) Acclimated to Different Salinities. *Journal of Experimental Biology*, 166, (1992) 95-112

8 Lemarie´ G., Dosdat A., Cove `s D., Dutto G., Gasset E. and Person-Le Ruyet, J., Effect of chronic ammonia exposure on growth of European seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture,* 229, (2004) 479-491

9 Paust L.O., Foss A. and Imsland A.K., Effects of chronic and periodic exposure to ammonia on growth, food conversion efficiency and blood physiology in juvenile Atlantic halibut (*Hippoglossus hippoglossus L.*). *Aquaculture*, 315, (2011) 400-406

10 Foss A., Imsland A.K., Roth B., Schram E. and Stefansson S.O., Effects of chronic and periodic exposure to ammonia on growth and blood physiology in juvenile turbot (*Scophthalmus maximus*). *Aquaculture,* 296, (2009) 45-50

11 Linton T.K., Morgan I.J., Walsh P.J. and Wood C.M., Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth, and metabolism. *Can. J. Fish Aquat. Sci.,* 55, (1998) 576-586

12 Eddy F.B., Ammonia in estuaries and effects on fish. *J. Fish Biol*., 67, (2005) 1495-1513

13 Larsen P.F., Nielsen E.E., Hansen M.M., Wang T., Meier K., Pertoldi C. and Loeschcke V., Tissue specific haemoglobin gene expression suggests adaptation to local marine conditions in North Sea flounder (*Platichthys flesus L.). Genes & Genomics*, 35, (2013) 541-547

14 Wicks B.J., Joensen R., Tang Q. and Randall D.J., Swimming and ammonia toxicity in salmonids: the effects of sub lethal ammonia exposure on the swimming performance of coho salmon and the acute toxicity of ammonia in swimming and resting rainbow trout. *Aquatic Toxicology,* 59, (2002) 55-69

15 Ip Y.K. and Chew S.F., Ammonia production, excretion, toxicity, and defense in fish: a review. *Frontiers in Physiology*, 1, (2010) 134

16 Wilkie M.P., Ammonia Excretion and Urea Handling by Fish Gills: Present Understanding and Future Research Challenges. *Journal of Experimental Zoology*, 293, (2002) 284-301

17 Sampaio L.A., Wasielesky W. and Miranda-Filho K.C., Effect of Salinity on Acute Toxicity of Ammonia and Nitrite to Juvenile *Mugil platanus. Bull. Environ. Contam. Toxicol.,* 68, (2002) 668-674

18 Roberts S.D. and Powell M.D., Comparative ionic flux and gill mucous cell histochemistry: effects of salinity and disease status in Atlantic salmon (*Salmo salar L.). Comparative Biochemistry and Physiology* Part A, 134, (2003) 525-537

19 Timmermann R. and Hellmer H.H., Southern Ocean warming and increased ice shelf basal melting in the twenty-first and twenty-second centuries based on coupled ice-ocean finite-element modelling. *Ocean Dynamics,* 63, (2013) 1011-1026

20 Sprague J.B., Measurement of Pollutant Toxicity to Fish Bioassay Methods for Acute Toxicity: A Review. *Water Research Pergamon Press,* 3, (1969) 793-821

21 Tudor M., Katavic, I. and Marsic-Lucic J., Acute toxicity of ammonia to juvenile Seabass (*Dicentrarchus labrax L.*) at different aeration levels. *Aquaculture,* 128, (1994) 89-95

22 Kayali B., Yigit M. and Bulut M., Evaluation of the Recovery Time of Sea Bass (*Dicentrarchus Labrax* Linnaeus, 1758) Juveniles from Transport and Handling Stress: Using Ammonia Nitrogen Excretion Rates as a Stress Indicator. *Journal of Marine Science and Technology,* 19 (6), (2011) 681-685