Chapter 1 Introduction

As part of a project conducted at Virginia Polytechnic Institute and State University to reevaluate the instream ammonia limit in the Chesapeake Bay watershed, this research focused on toxicity tests with additional saltwater organisms and the relationship between salinity and ammonia toxicity.

1.1 Background

Ammonia has been known to be toxic to aquatic organisms for a long time. When an organism is intoxicated, its acutely toxic symptoms include hyperventilation, hyperexcitability, convulsions, loss of equilibrium, coma and, finally, death [40, 43]. Chronic effects include reduction in growth and reproduction [48]. Sources of ammonia introduced into marine water include domestic sewage, agricultural runoff, industrial effluent, and animal metabolism [15, 37]. The national water quality criteria for ammonia in the marine environment were established to control ammonia discharge and protect aquatic organisms from toxicity of ammonia. Toxicity data for various marine organisms are essential to establish appropriate national criteria.

However, due to the lack of toxicity data with marine organisms, the instream limit for ammonia in saltwater is still a controversial issue. In some watersheds, like the Chesapeake Bay, ammonia discharges have not been regulated. The Virginia Department of Environmental Quality (DEQ) has proposed a tentative limit for the Chesapeake Bay watershed of approximately 1-2 mg/L for total ammonia. This 1-2 mg/L limit set is based on the national criteria for ammonia of 0.035 mg/L un-ionized ammonia, and is referred to as the Final Chronic Value (FCV) [11]. The FCV on the national criteria for ammonia was derived from both acute and chronic toxicity data. Seafood processing companies maintain that the 1-2 mg/L limit is questionable because some of the toxicity data used to determine this limit were based upon tests involving freshwater organisms. The same concentration of ammonia might have different affects on saltwater species since the

unique characteristics of saltwater will certainly influence the toxic mechanism. For these reasons, the limit has not officially been adopted by DEQ and this project was initiated to conduct toxicity tests with more marine species to determine whether this limit set by The Environmental Protection Agency (EPA) and DEQ is too stringent or too lenient.

In the first phase of this project, three commonly tested marine organisms, mysid (*Mysidopsis bahia*), sheepshead minnow (*Cyprinodon variegatus*), and grass shrimp (*Palaemonetes pugio*), were tested in acute tests (Hudgins, 1996). The 7-day chronic toxicity of ammonia was also evaluated for the sheepshead minnow. The test results were used to refine the national criteria.

1.2 Objectives of the Research

In this phase of the project, the acute and chronic toxicity of two additional marine species were performed. Since the effect of different salinity levels on ammonia toxicity is not well understood, various levels of salinity were included in this research to understand the relationship between salinity levels and ammonia toxicity.

The two saltwater species used in this research were the marine fish, Atlantic silverside (*Menidia menidia*), and the marine invertebrate, ghost shrimp (*Palaemonetes pugio*).

The objectives of this research were to:

1. determine the 48hr LC_{50} (lethal concentration for fifty percent mortality) values for the Atlantic silverside at three levels of salinity.
2. determine the 96hr LC_{50} for the ghost shrimp.
3. determine the no-observed-effect-concentration (NOEC) and lowest-observed effect-concentration (LOEC) of Atlantic silverside based on a 7-day chronic test.
4. determine the NOEC and LOEC of the ghost shrimp based on a 7-day chronic test.
5. Use the results generated to further refine the national criteria.

Throughout the report, all quantitative ammonia data have been expressed in terms of NH_3 mg/L for ease of comparison. The word, *ammonia, as* used in this report, represents the total amount of NH_3 and NH_4^+. While NH_3 refers to un-ionized ammonia, NH_4^+ refers to ionized ammonia or ammonium ion.

Chapter 2 Ammonia in Aquatic Environment

Ammonia toxicity is affected by many factors. The two forms of ammonia in aquatic system show different toxicity to aquatic organisms. The national criteria have been set at a basis of un-ionized ammonia. This chapter will deal with background information related to the water chemistry of ammonia, mechanisms of ammonia toxicity, and the national criteria for ammonia.

2.1 Water Chemistry of Ammonia

In the aqueous environment, ammonia is present in two forms, ionized (NH_4^+) and un-ionized (NH_3), in accordance with the following equilibrium equation (2.1) [49]:

$$NH_4^+ + H_2O <==> NH_3 + H_3O^+ \quad (2.1)$$

The total ammonia concentration for a particular solution is the sum of NH_3 and NH_4^+ in the aqueous solution and the individual concentrations of NH_3 and NH_4^+ in the solution are governed by an ionization constant (25°C) given in the equation (2.2):

$$Ka = [NH_3]\,[H^+] / [\,NH_4^+] = 10^{-9.2} \quad (2.2)$$

The Ka for this reaction is a function of many physical factors, among which are pH, temperature, and salinity.

NH_3 is uncharged, lipid soluble and more easily absorbed by aquatic organisms than NH_4^+. The permeability of plasma membranes to the charged, hydrated ammonium ions (NH_4^+) is relatively low.

Because NH_3 is the major toxic form, knowing the exact amount of un-ionized NH_3 is important in determining the toxicity of total ammonia to aquatic animals. However, there is no direct way to assess the concentration of un-ionized NH_3. The concentration of un-ionized NH_3 is determined by indirect calculation based on the total ammonia concentration in a solution and other physical factors. Equation 2.3, developed by

Hampson (1977), is commonly used to calculate the percentage of un-ionized ammonia in saltwater [49]:

$$\% \text{ un-ionized } (NH_3) = 100[1+10(X+ 0.0324(298-T) + 0.0415\ P/T-pH]^{-1} \quad (2.3)$$

where

P = pressure, 1 atm ;

T = temperature, K;

X = pKsa or the stoichiometric acid hydrolysis constant of ammonium ions in saltwater based on I, which is given in the following equation (2.4):

$$I = 19.9273\ S\ (1000-1.005109\ S)^{-1} \quad (2.4)$$

where

I = molar ionic strength of the sea water;

S = salinity ppth (part per thousand);

It is apparent from formula 2.3 that three factors: pH, temperature, and salinity influence the fraction of NH_3 in a solution, consequently affecting the toxicity of ammonia in the aquatic environment. The concentration of un-ionized ammonia in the water is largely pH and temperature dependent, and to a lesser extent, salinity or ionic strength. According to this formula, pH and temperature are positively correlated with the concentration of NH_3, and salinity is inversely correlated. The following sections will discuss these three major factors and their influence on ammonia toxicity. Other factors will be briefly mentioned, as well.

2.1.1 pH

The pH is the most important factor which affects ammonia toxicity. According to equation 2.2, in a given solution, increase in pH indicates a decrease in hydrogen ion concentration, and, hence, leads to shifting of the equilibrium toward un-ionized ammonia. Therefore, theoretically, the higher the pH value, the more toxic ammonia will be to aquatic organisms, if other factors remain same.

Table 2.1 shows the relationship between pH and the percentage of un-ionized ammonia in a solution [6]. An increment of 0.5 in pH, from 7.8 to 8.3, results in approximately three times more un-ionized ammonia.

Table 2.1 The Relationship between pH and Percent of Un-ionized Ammonia (20°C, Salinity =20 ppth) [6]

pH	7.8	7.9	8.0	8.1	8.2	8.3
% of un-ionized ammonia	2.18	2.73	3.41	4.62	5.30	6.58

Most of test data from freshwater species support this type of trend but others do not, particularly test results with marine organism [12, 13, 44]. In the latter case, for example, the toxicity of ammonia to the marine species larval prawn (*Macrobachium rosenbergii*) exhibited a greater toxicity at lower pH [4].

2.1.2 Water Temperature

Temperature can affect ammonia toxicity to aquatic species, as well, and pKa is a function of temperature. As temperature increases, the pKa value will increase, resulting in an increased percentage of un-ionized ammonia. Table 2.2 shows about a two fold increase in the fraction of un-ionized ammonia for each 10°C rise in temperature, if other factors remain unchanged [6].

Therefore, for a given total ammonia concentration, a higher temperature leads to increases in concentration of un-ionized ammonia, which in turn increases the toxicity of ammonia. The temperature in a solution also can affect pH, organism resistance, and dissolved oxygen, which also alters the toxicity of ammonia to organisms [12, 13].

Table 2.2 The Relationship between Temperature and Percent of Un-ionized Ammonia (pH=8.2 Salinity =20 ppth) [6]

Temperature, °C	pKa	% of un-ionized ammonia
0	10.10	1.24
5	9.94	1.80
10	9.78	2.59
15	9.61	3.71
20	9.45	5.30
25	9.29	7.52

2.1.3 Salinity

Salinity or ionic strength has an effect on ammonia toxicity to a lesser extent than pH and temperature. The pKa is also salinity or ionic strength dependent. Theoretically, an increase in ionic strength leads to a decrease in percent of ammonia due to an increase in pKa, thereby lowering ammonia toxicity.

Table 2.3 The Relationship between Salinity and Percent of Un-ionized Ammonia (20°C, pH=8.2) [6]

Salinity (ppth)	Ionic strength	pKa	% of un-ionized ammonia
0	0	9.40	3.82
20	0.4	9.46	3.41
25	0.5	9.48	3.19
30	0.6	9.49	3.12
36	0.75	9.51	2.98

As shown in Table 2.3, a increase of 10 units in salinity results in a 9% decrease in fraction of un-ionized ammonia, and, therefore, less ammonia toxicity would be expected [6]. For example, Harader and Allen (1983) found that the 24hr LC_{50} of un-ionized ammonia was 0.36 mg/L in freshwater, and 2.2 mg/L in 9 ppth saltwater with Chinook

salmon. This study suggested that ammonia was more toxic in freshwater than in marine water due to a reduction in the fraction of un-ionized ammonia in saltwater. However, other studies did not support this hypothesis. Mill *et al*. (1990) reported that with exposure of inland silverside to two levels of salinity, the 96hr LC_{50} of ammonia was greater at 11ppth than that at 30ppth. These results indicated that ammonia toxicity increases as salinity increases. On the other hand, Hazel *et al.*(1971) found that salinity strengths has no effect on ammonia toxicity in experiments with stickleback.

A detailed table of the percent un-ionized ammonia as a function of pH, temperature, and salinity is presented in Appendix A [6].

2.1.4 Other Physical Factors

In addition to the three major factors mentioned above, factors such as dissolved oxygen and carbon dioxide also affect ammonia toxicity [1, 10].

2.2 Routes of Entry, Excretion, and Mechanisms

To understand ammonia toxicity, it is necessary to investigate routes of entry, excretion, and mechanisms. However, little is known about the toxic mechanisms of ammonia to aquatic organisms. Based on limited studies, some possible mechanisms have been proposed. This section will address routes of entry, excretion and several major postulated toxic mechanisms.

As mentioned early in section 2.1.1, the un-ionized form,(NH_3) can diffuse into cell membranes readily through the gills of both vertebrates and invertebrates, because it is un-charged and lipid soluble [6]. However, the ionized form, NH_4^+, may contribute towards toxicity, as well [14, 27]. Kristina and Lena (1993) suggested that NH_4^+ can be transported into cells by certain transport proteins, such as Na^+/K^+-ATPase, and by the carrier mediated, Na^+/NH_4^+ channel. For aquatic animals, particularly in teleosts, the majority of their nitrogenous waste is excreted by conversion of glutamine to glutamic acid and ammonia. This process of excretion depends mainly on gills [24]. Un-ionized ammonia is primarily the form excreted by gills [17], but ionized ammonia can also be

excreted through facilitated diffusion [17]. An increase in the ambient ammonia concentration can cause a reduction in excretion and a net gain of ammonia from the environment. Therefore, the accumulation of ammonia in the body may be deleterious to the aquatic animals. Several possible mechanisms for toxicity of ammonia are proposed:

1). Disturbance of electrochemical gradients.

2). Cerebral effects caused by a depletion of cerebral energy in the form of ATP.

3). Acid-base disturbance in the body of organisms.

Smart (1981), Arillo (1981), and Hawkins (1973) suggested that the toxic mechanism of ammonia is its effect on the electrochemical gradients of nerve cells. When present extracellularly, ammonia, like potassium, can increase the plasma potassium level which disrupts electrochemical gradients that normal cells maintain. Disturbance of electrochemical gradients decreases the resting transmembrane potential, therefore bringing the potential closer to the threshold for firing. This causes a general hyperexcitability and hyperventalition, and then results in convulsions.

The second hypothesis suggests that ammonia toxicity depends on the depletion of cerebral energy of ATP by decreased α-ketoglutarate, which is a key component in the formation of ATP synthesis [3, 32, 39]. As ammonia concentration increases in cells, a depletion of α-ketoglutarate by amination to glutamic acid and then conversion to glutamine through using ATP and ammonia results in impairment of the reaction cycle of ATP formation, and later decrease in ATP (Figure 2.1). Stimulation in consumption of ATP and inhibition in formation of ATP leading to a depletion of ATP cause ammonia toxicity.

In addition, Twitchen and Eddy (1994) proposed that the mechanism of ammonia toxicity was caused by an acid-base disturbance. Disturbances in acid-base balance are known to be partly compensated by alterations in the differential diffusive efflux of Na^+ and Cl^- across the gills of fish. Increased Na^+ efflux and net Na^+ loss have been observed in their study due to increased Na^+ permeability of the gills, or partially due to carrier mediated Na^+/ NH_4^+ exchange.

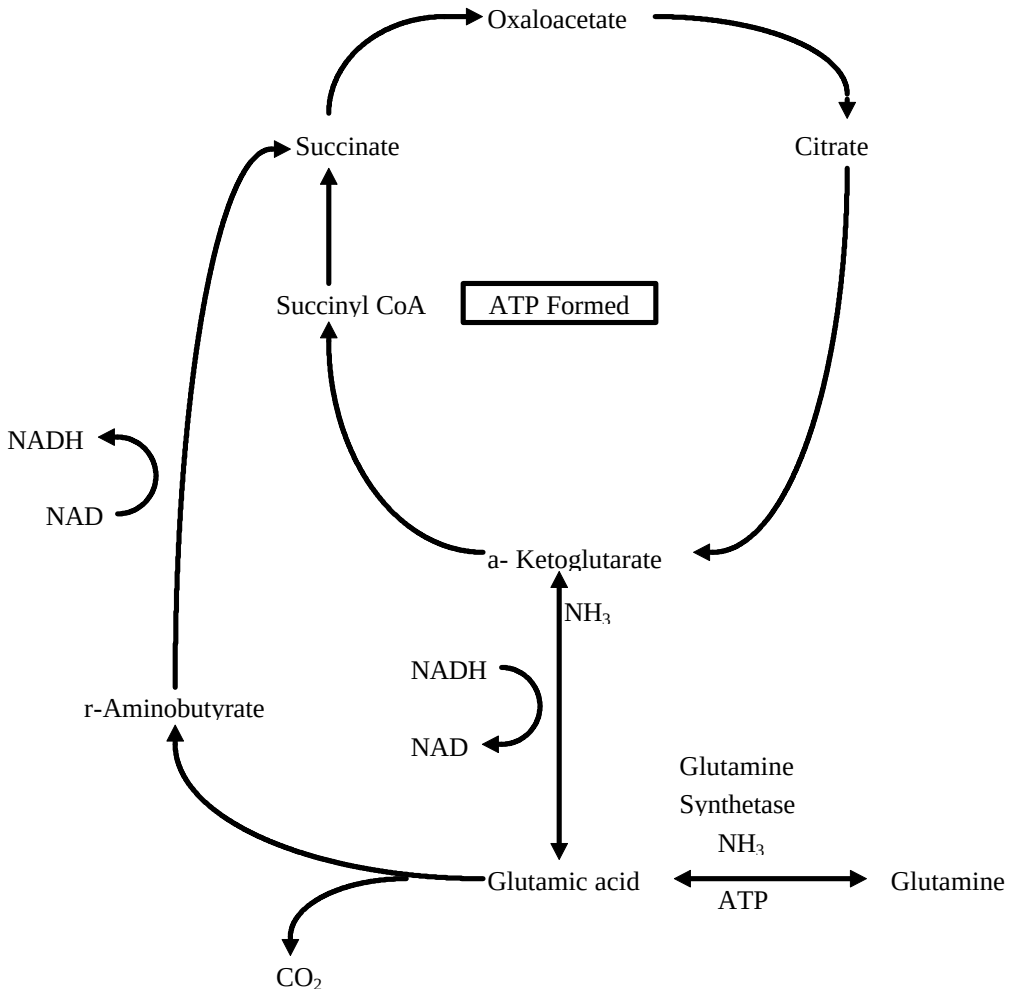

Figure 2.1 Postulated Biochemical Sites of Ammonia Toxicity [40]

2.3 Background on the National Criteria for Ammonia

The Ambient Water Quality Criteria for Ammonia (Saltwater)-1989 was developed by the U.S Environmental Protection Agency (EPA), and is referred to as the national criteria. Two structures used in setting this criteria were: 1) the one-hour average concentration, which is related to acute tests, and 2) the four-day average concentration, which is related to chronic tests [42]. These concentrations cannot be exceeded more than once every three years. The national criteria are based on un-ionized ammonia and the total ammonia discharged for each site is determined in terms of the 90^{th} percentile pH and temperature, and average salinity for saltwater. Each site must meet the criteria for ammonia before the National Pollution Discharge Elimination System (NPDES) permit is granted [36].

The national criteria include an acute criterion and a chronic criterion. The procedures to derive both criteria are detailed in "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic organisms and Their uses" and Ambient Water Quality Criteria for Ammonia-1984 [42, 47].

The acute criterion for un-ionized ammonia concentration was established based on 21 marine species. The 21 species are listed in Table 3.2 in Chapter 3. The genus mean acute value (GMAV) for these 21 species were then calculated. Only the GMAVs for the top of four most sensitive species were used to calculate the final acute value (FAV) using the method developed by Stephan *et al.* [42]. The four most sensitive species were winter flounder (*Pseudopleuronectes americanus*), red durm (*Sciaenops ocellatus*), Sargassum shrimp (*Latreutes fucorum*), and prawn (*Macrobrachium rosenbergii*). The FAV of un-ionized ammonia was determined to be 0.465 mg/L. The acute criterion for un-ionized ammonia was 0.233 (FAV/2) mg/L [49].

The chronic criterion was calculated using acute-chronic ratios for two saltwater and four freshwater species. The four freshwater species were fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis macrochirud*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Salmo gairdneri*). The two marine species were mysid

(*Mysidopsis bahia*), and inland silversid (*Menidia beryllina*). Acute-chronic ratios were used to calculate the chronic criterion based on the hypothesis when EPA used to develop the chronic criterion for freshwater species. This hypothesis states "acute-chronic ratios for the most acutely and chronically sensitive species are technically more applicable when trying to define concentrations chronically acceptable to acutely sensitive species" [42]. The acute-chronic ratios of two marine species and four freshwater species were selected to determine the chronic criterion for ammonia because of insufficient chronic data for saltwater species. The geometric mean of these six acute-chronic ratios for mysid (7.2), inland silverside (21.3), fathead minnow (20), bluegill (12), channel catfish (10), and rainbow trout (14) was calculated and then divided into the FAV to yield the current chronic criterion of 0.035 un-ionized ammonia mg/L [49].

The potential instream limits for total ammonia can be assessed using the national saltwater criteria of un-ionized ammonia along with various pH, temperature, and salinity.

Chapter 3 Previous Ammonia Toxicity Testing

Ammonia has been known to be toxic to animals and humans for over a century. Toxicity of ammonia to aquatic animals was not studied rigorously until as early as 1934 [47, 49]. Since then, ammonia toxicity has attracted more and more attention and toxicity data have accumulated for freshwater and saltwater organisms. The national water criteria (in saltwater) for ammonia was established in 1985 and updated in 1989 in order to protect marine species. However, the criteria for marine species were established on the basis of both freshwater and marine species, not solely on marine species. In order to refine the nation criteria it is necessary to review previous ammonia toxicity works. This chapter will cover the following subjects:

3.1 Acute Toxicity Data in Freshwater and Marine Species

3.2 Chronic Toxicity Data in Freshwater and Marine Species

3.3 Summary of Comparisons with Freshwater and Marine Species

3.1 Acute Toxicity Data in Freshwater and Marine Species

Acute toxicity of ammonia has been more extensively studied with freshwater species than with marine species. About 60 freshwater species have so far been tested while only 21 marine species have been used in acute toxicity tests [8, 23, 49]. In the following sections, more attention is paid to marine species, but tests with freshwater species are disccussed briefly. Since vertebrates and invertebrates show different responses to ammonia toxicity, the comparison of toxicity values for these two in freshwater and saltwater are presented.

3.1.1 Freshwater Fish and Invertebrates

Table 3.1 gives 96hr LC_{50} values of various freshwater vertebrates and invertebrates. The most sensitive fish is Atlantic salmon with a 96hr LC_{50} of 0.09 NH_3 mg/L [16]. Fathead minnow, bluegill, and channel catfish are less sensitive than Atlantic salmon [37]. White sucker is the least sensitive fish with a 96hr LC_{50} of 1.8 NH_3 mg/L

[46]. Of freshwater invertebrates, crayfish and clam represent the most tolerant and the most sensitive species, respectively [5, 21]. As indicated in Table 3.1, the 96hr LC_{50} values of the two invertebrates are approximately ten fold greater than those of the fish. Other invertebrates, also have higher 96hr LC_{50} values than fish do although the difference is not ten fold. In general, the 96hr LC_{50} values of freshwater invertebrates are greater than those of fish by a factor of 2-3; in other words, freshwater fish are more acutely sensitive to ammonia than invertebrates. As a result, regulations are set based on acute fish data because these data are sufficient to protect invertebrates.

Table 3.1 96-hr LC_{50} values for Several Freshwater Fish and Invertebrates

	Vertebrates	96hr LC_{50} NH_3 mg/L	Invertebrates	96hr LC_{50} NH_3 mg/L
Freshwater Species	Atlantic salmon [16] (*Salmon gairdnerli*)	0.09	clam [21] (*Muscullum transversum*)	1.10
	fathead minnow [37] (*Pimephales promelas*)	0.95	snail [46] (*Helisoma trivolvis*)	2.76
	bluegill sunfish [41] (*Lepomis macrochirud*)	1.06	cladoceran [2] (*Daphnia magna*)	2.94
	channel catfish [9] (*Ictalurus punctatus*)	1.2	amphipod [5] (*Crangonyx pseudogracilis*)	3.12
	white sucker [46] (*Morone americana*)	1.8	crayfish [5] (*Orconnectes immunis*)	18.3

In addition, many researchers suggested that several factors, such as the life stage of organism, pH, and temperature, can affect the ammonia toxicity in freshwater. Arillo *et al.*(1981), for example, reported that larval rainbow trouts (48hr LC_{50} of 0. 58 NH_3 mg/L) were more sensitive than adults (48hr LC_{50} of 0.92 NH_3 mg/L).

3.1.2 Marine Fish and Invertebrates

The acute toxicity of ammonia to marine species has been studied in crustaceans, bivalve mollusks, and fish. The acute values for 21 marine species, belonging to 18 genera, are summarized in Table 3.2 and ranked in order of their sensitivity to ammonia [49]. The species mean acute values **(**SMAV) in the table include both 48hr and 96hr acute data. Table 3.3 gives 96hr LC_{50} values of representative marine species.

Winter flounder is the most sensitive fish, while three-spined stickleback is the least sensitive fish [49]. Other fish like sheepshead minnow, inland silverside and striped bass are in the middle, with 96hr LC_{50} values are in the range of 0.97-2.23 NH_3 mg/L [19, 22, 44]. For invertebrates, sargassum shrimp is the most sensitive species and eastern oyster is the most resistant organism. The 96hr LC_{50} values of other invertebrates, such as copepod, amphipod, and mysid, range from 0.82 to 1.54 NH_3 mg/L [4, 8, 23]. Both invertebrates and vertebrates have similar 96hr LC_{50} values in marine waters. Hence, there is no evident trend for the acute values of marine fish and invertebrates. This result indicates that both fish and veterbrates need to be conducted on toxicity test in saltwater. However, if the most sensitive and the most tolerant species are removed from both the invertebrates and vertebrates, it appears that marine invertebrates are more acutely sensitive than marine fish. This suggests that more invertebrate data should be used to calculate the SMAV. As in freshwater, life stages of organisms in saltwater can also affect ammonia toxicity, and mature stages show a higher degree of tolerance [33].

Table 3.2 Acute Toxicity of Ammonia to Saltwater Animals*

Rank	Species	Mean Acute Value (mg/L NH_3)	Range of pH	Range of Temp °C	Salinity (ppth)
1	Winter flounder (*Pseudop-leuronectes americanus*)	0.492	7.9-8.1	7.5	31
2	Red drum (*Sciaenops ocellatus*)	0.545	8.0-8.2	25-26	28-30
3	Sargassum shrimp (*Latreutes fucorum*)	0.773	8.07	23.4	28
4	Prawn (*Macrobrachium rosenbergii*)	0.777	6.8-8.3	28	12
5	Copepod (*Eucalanus pileatus*)	0.793	8.2	20.5	34
6	Planehead filefish (*Monocanthus hispidus*)	0.826	8.07	23.4	28
7	Copepod (*Eucalanus elongatus*)	0.867	8.0	20.3	34
8	White perch (*Morone americana*)	2.130	8.0	16	14
9	Striped bass (*Morone saxatiois*)	2.481	7.2-8.2	15-23	5-34
10	Mysid (*Mysidopsis bahia*)	1.021	7.0-9.2	19-26	10-31
11	Spot (*Leiostomus xanthurus*)	1.040	7.92	20.4	9.3
12	Atlantic silverside (*Menidia menidia*)	1.050	7.0-9.0	11-25	10-30
13	Inland silverside (*Menidia beryllina*)	1.317	7.1-9.1	18-26	11-33
14	Striped mullet (*Mugil cephalus*)	1.544	7.99	21	10
15	Grass shrimp (*Palaemonetes pugio*)	1.651	7.9-8.1	19-20	10-28
16	American lobster (*Homarus americanus*)	2.210	8.1	21.9	33.4
17	Sheepshead minnow (*Cyprinodon variegatus*)	2.737	7.6-8.1	10-33	10-33
18	Three-spined stickleback (*Gasterosteus aculeatus*)	2.932	7.6	15-23	11-34
19	Brackish water clam (*Bangia cuneata*)	3.080	7.95	20.2	9.2
20	Quahog clam (*Mercenaria mercenaria*)	5.360	7.7-8.2	20	27
21	Eastern oyster (*Crassostrea virginica*)	19.102	8.0	20	27

* taken from reference[49].

Table 3.3 96-hr LC_{50} values for Several Marine Vertebrates and Invertebrates

	Vertebrates	96hr LC_{50} NH_3 mg/L	Invertebrates	96hr LC_{50} NH_3 mg/L
Marine species	winter flunder [49] (*Pseudopleuronectes americanus*)	0.52	sargassum shrimp [5] (*Latreutes fucorum*)	0.64
	inland silverside [11] (*Menidia beryllina*)	0.98	copepod [21] (*Eucalanus pileatus*)	0.82
	sheephead minnow [22] (*Cyprinodon variegatus*)	2.70	amphipod [23] (*Cranodoyx macacius*)	0.91
	striped bass [19] (*Morone saxatiois*)	2.23	mysid [30] (*Mysidopsis bahia*)	1.54
	three-spine stickleback[19] (*Gasterosteus aculeatus*)	2.93	eastern oyster [49] (*Crassostrea virginica*)	11.8

Furthermore, as compared to the work with freshwater species, the relationships between ammonia toxicity and pH, temperature, and salinity were poorly understood in marine species. With limited data, there appears an evident trend between ammonia toxicity and pH. However, the results based upon different temperature and salinity are not consistent. Salinity is an important factor which should be considered during ammonia toxicity tests with marine species. Atlantic salmon and Chinook salmon were found to be less sensitive to ammonia in saltwater than in freshwater [1, 18]. However, the effect of different salinity levels on ammonia toxicity is not well known. Therefore, it is necessary to further investigate the relationship of ammonia toxicity and salinity. The influence of pH, temperature, and salinity on ammonia toxicity were discussed in section 2.2.

3.1.3 Marine Versus Freshwater

In order to explore the general trends in ammonia toxicity for freshwater and marine species, the acute test data given in Tables 3.1 and 3.2 will be compared for vertebrates and invertebrates separately.

In fish, it appears that freshwater species are generally more sensitive than marine fish. With exclusion of the most sensitive and the most tolerant fish, the majority of freshwater fish have lower 96hr LC_{50} values values for ammonia than those of their marine counterparts. This phenomenon can be partly explained by the reduction of the un-ionized ammonia fraction in saltwater.

Fewer marine invertebrates were acutely tested than freshwater invertebrates. With little data on marine inveterbrates, it appears that marine invertebrates exhibit higher sensitivity to ammonia than freshwater invertebrates because higher 96hr LC_{50} values for ammonia were found in freshwater than in saltwater. This evidence suggests that the test results of freshwater fish should not be used to protect marine invertebrates.

3.2 Chronic Toxicity Data in Freshwater and Marine Species

The chronic ammonia toxicity to aquatic organisms has been less extensively studied in marine water than in freshwater. At present, 15 freshwater species were used in chronic tests, but only two marine species were assessed in chronic tests [47, 49]. In this section, chronic data chosen for several representative freshwater species are compared with those of two marine species.

Table 3.4 Chronic Values for Several Freshwater and Two Marine Species

Freshwater species	Chronic value NH_3 mg/L	Marine species	Chronic value NH_3 mg/L
cladoceran [49] (*Daphnia magna*)	1.2	mysid [30] (*Mysidopsis bahia*)	0.23
channel catfish [9] (*Ictalurus punctatus*)	0.25	inland silverside [30] (*Menidia beryllina*)	0.06
fathead minnow [11] (*Pimephales promelas*)	0.13		
bluegill sunfish [41] (*Lepomis macrochirud*)	0.09		
rainbow trout (*Salmo gairdneri*)[37]	0.03		

Table 3.4 summarizes the chronic values for several representative freshwater species and two marine species. In freshwater, it appears that fish exhibit higher sensitivity to ammonia than invertebrates. A similar pattern was also noted in acute test with freshwater species. *Daphnia* represents the most tolerant and rainbow trout is the most sensitive. The other three fish are intermediate between these two extremes, i.e., channel catfish, fathead minnow, and bluegill. In fact, all of the three species were used to establish the saltwater criteria as they represent typical values. In saltwater, the chronic value for mysid determined by Mill *et al.* (1990) was 0.23 NH_3 mg/L during a 32-day life cycle. The chronic value of Inland silverside (0.06 NH_3 mg/L) was lower than that of the mysid. No general trend can be made for the comparison of chronic values for freshwater and marine species due to the very limited data for marine species.

3.3 Summary of Comparisons of Freshwater and Marine Species

The comparisons in the previous section are summarized below:

1) In freshwater, fish are more acutely sensitive than invertebrates. As a result, regulations were established for freshwater species under the assumption that the use of acute fish data were sufficient to protect invertebrates.

2) In saltwater, it is difficult to find an evident trend for the acute values of marine fish and invertebrates. From the majority of tested species, it appears that marine invertebrates are more acutely sensitive than marine fish, if the most sensitive and the most tolerent species are excluded. These results indicate that both fish and inveterbrates need to be assessed in ammonia toxicity tests.

3) For both freshwater and marine species, the life stages of organisms can affect ammonia toxicity, and it may be generalized that the more mature the species is, the more tolerant it is.

4) For marine species, the relationships between ammonia toxicity and water quality were poorly studied when compared to freshwater species. The effect

of different salinity strengths on ammonia toxicity is not well understood and further studies are needed.

5) With respect to marine versus freshwater species: it appears that marine fish are generally more acutely tolerant than freshwater fish. This phenomenon can be partly explained by the reduction of the un-ionized ammonia fraction in saltwater.

6) However, marine invertebrates exhibit a higher sensitivity than freshwater invertebrates. This evidence suggests that the use of freshwater fish data is not sufficient to protect marine invertebrates.

7) No general trend is apparent for the comparison of chronic values between for freshwater and marine species due to the limited data available for marine species.