Effects of pH and Hardness on Acute and Chronic Toxicity of Un-ionized Ammonia To *Ceriodaphnia dubia*

by

Camille G. Johnson

A Thesis

submitted in partial fulfillment of the

requirements for the degree

Master of Science

College of Natural Resources

University of Wisconsin

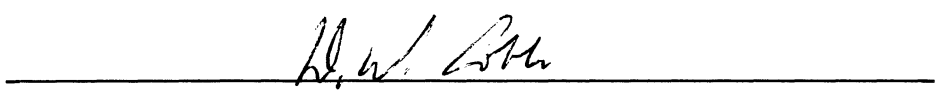
Stevens Point, Wisconsin

December 1995

Approved by Graduate Committee of:

Dr. Ronald L. Crunkilton, Committee Chair

Assistant Professor of Water Resources



Dr. Daniel W. Coble

Adjunct Professor of Fisheries

Dr. William H. LeGrande

Professor of Biology

ABSTRACT

Effects of pH and hardness on acute and chronic toxicity of un-ionized ammonia (NH_3) to *Ceriodaphnia dubia* were assessed. Effects of feeding on acute ammonia toxicity were also examined. Tests were performed at pH levels of about 6.5, 7, 8, and 9 in combination with hardness concentrations of about 40, 90 and 180 mg/L $CaCO_3$. Acute tests were static non-renewal 48-hour tests with a criterion-effect of mortality. Chronic tests were static renewal three-brood life cycle tests with criterion-effects of decreased reproduction and mortality. Generally, both acute and chronic toxicity of un-ionized ammonia decreased as hardness and pH increased.

C. dubia was highly sensitive to acute ammonia exposure. LC50 values for mortality ranged from 0.09 to 0.92 mg/L un-ionized ammonia-nitrogen (NH_3-N). Acute toxicity of NH_3 decreased as hardness increased. Un-ionized ammonia was least toxic in hard water (160-180 mg/L $CaCO_3$) at pH 6.5, 7 and 8. At pH 9, NH_3 was least toxic in medium hardness water (80-100 mg/L $CaCO_3$). Acute toxicity of NH_3 in soft (40-50 mg/L $CaCO_3$), medium, and hard water decreased as pH increased from 6.5 to 8. At all hardness levels tested, NH_3 was most toxic at pH 6.5. Feeding during acute tests significantly reduced toxicity of un-ionized ammonia.

C. dubia were also highly sensitive to chronic exposure of ammonia. Reproduction was significantly decreased at low levels, with 25% inhibition concentration (IC25) values ranging from 0.03 to 0.91 mg/L NH_3-N, and 50% inhibition concentration (IC50) values ranging from 0.07 to 1.01 mg/L NH_3-N. Chronic toxicity of NH_3 at the IC25 and IC50 generally seemed to decrease as hardness increased. Differences were not statistically significant based on the IC25, except at pH 8 where NH_3 was significantly more toxic in soft water than in medium hardness water. Based on the IC50, NH_3 was least toxic in hard water at pH 7, 8, and 9 whereas at pH 6.5 there was not a significant difference among hardness levels. Average chronic toxicity for NH_3 increased as pH decreased. For both IC25 and IC50, NH_3 was most toxic at pH 6.5.

ACKNOWLEDGEMENTS

I wish to thank the following funding sources for their contributions to this project: the University of Wisconsin - Stevens Point (UWSP), the UWSP College of Natural Resources, the Wisconsin Cooperative Fishery Research Unit, the UWSP Aquatic Toxicology Research Lab, and the International Womens Fishing Association.

Sincere thanks are extended to my advisor and committee chairman, Dr. Ron Crunkilton, for all of his assistance, guidance and for greatly expanding my knowledge of aquatic toxicology. I also wish to thank committee members Dr. Dan Coble and Dr. Bill LeGrande for their technical advice and review of the manuscript.

Special thanks are extended to the staff and students of the UWSP Aquatic Toxicology Research Lab for their moral and technical support. Deserving special mention are Laurie Niewolny and Kathy Weinfurter for their much needed encouragement.

I acknowledge with deep gratitude all the love and support from friends and family, especially my wonderful mother Emily, my special father Rheal, my caring brothers Ben, Chris, and Paul, and my dear in-laws Sue, Frank, and Paul.

I might never have completed a Masters degree if it weren't for my loving husband, Ted. You have been an extraordinary source of inspiration and

confidence from the very beginning. I greatly appreciate all the praise, encouragement, love, and assistance you have given me over the years. You are a wonderful, giving partner.

To my precious son, Bryce, who has been the motivation for completing this lengthy process, I dedicate this to you. It won't be long before you are realizing dreams and aspirations of your own.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Objectives

Objectives of this study were to document the sensitivity of *Ceriodaphnia dubia* to acute and chronic exposures of ammonia under standard test conditions and to determine effects of pH and hardness on the toxicity of un-ionized ammonia.

Background

Ammonia is a common toxicant in the aquatic environment and is highly toxic at low concentrations (Thurston et al. 1983, Mayes et al. 1986). Ammonia enters surface water from many sources, including: municipal, agricultural, fish-cultural, industrial, and as a natural degradation product of nitrogenous organic matter. The largest portion of ammonia input into surface water originates from publicly owned treatment works (American Petroleum Institute 1981). Un-ionized ammonia (the NH_3 form) is believed to be one of the two greatest threats to aquatic biota in the region of wastewater outfalls (Effler et al. 1990). Ammonia is a frequent cause of acute and chronic toxicity in aquatic organisms (Niederlehner and Cairns 1990).

Toxicity testing is used to determine lethal concentrations of chemicals and

length of exposure required to produce a specified effect. Effects vary with the kind of toxicity test, ranging from sublethal effects on behavior to mortality. More literature exists on toxicity of ammonia to fish than to invertebrates (Gersich and Hopkins 1986). Cladocerans studied have been primarily limited to *Daphnia magna*. Few studies have involved the tolerance of *C. dubia* to ammonia.

Importance of Cladocerans

D. magna, has been used extensively for toxicity testing because they are easily obtained, are sensitive to most chemicals, and play a vital role in aquatic ecosystems. However, *C. dubia* has been selected by the U.S. EPA as the standard cladoceran species for effluent and toxicity testing (U.S. EPA 1989). *C. dubia* are commonly found in waters throughout North America; they inhabit large streams, reservoirs, and lakes. They are valuable for testing effects of contaminants on reproduction because they are easily cultured and produce their first brood in 72 hours. *C. dubia* also is an important link in aquatic food chains (Norberg and Mount 1984).

Limited toxicity data exist for *C. dubia* because it is a relatively new test species. Although toxicity values for *D. magna* may apply to *C. dubia*, they may have different susceptibility to toxicants. Therefore, additional research to determine specific toxicity responses of *C. dubia* is needed.

Ammonia Chemistry

In aqueous ammonia solutions un-ionized ammonia (NH_3) exists in equilibrium with the ammonium ion (NH_4^+) and the hydroxide ion (OH^-). The equilibrium equation is:

$$NH_3(g) + nH_20(l) \rightleftharpoons NH_3 \cdot nH_2O(aq) \rightleftharpoons NH_4^+ + OH^- + (n\text{-}1)H_2O(l),$$

where g = gas l = liquid aq = aqueous

Total ammonia is the sum of the NH_3 and NH_4^+ forms. In accordance with the American Fisheries Society's recommendation, ammonia concentrations should be reported as elemental nitrogen whenever possible (Willingham et al. 1979). Therefore, ammonia is reported as mg/L NH_3-N for un-ionized ammonia-nitrogen, mg/L NH_4^+-N for ionized ammonia-nitrogen, and TAN for total ammonia-nitrogen.

Toxicity of aqueous ammonia solutions has been attributed primarily to un-ionized ammonia (NH_3), with the ionized form (NH_4^+) being less toxic (Thurston et al. 1981a). At low pH levels (i.e. pH 6.5), almost all the ammonia is in the ionized form and high concentrations of total ammonia have little or no toxicity (Williams et al. 1986).

The portion of total ammonia present in the un-ionized form (NH_3) depends mainly on the pH, temperature, and concentration of total ammonia. The amount present as NH_3 increases when pH increases. When pH increases, the hydroxide ion concentration is elevated, and the equilibrium shifts towards the NH_3 form.

Within a pH range normally acceptable to fish (i.e. pH 6-9), an increase of one pH unit will increase the concentration of NH_3 approximately 10 fold (Thurston et al. 1981a). Increase in temperature causes a decrease in the ammonia equilibrium constant, thereby increasing the concentration of NH_3. A temperature increase of 5 degrees Celsius increases the NH_3 concentration 40-50 percent (American Petroleum Institute 1981). From data on pH, temperature, and total ammonia-nitrogen concentrations, the amount of un-ionized ammonia present can be calculated by procedures of Emerson et al. (1975).

Toxicity Testing

Acute toxicity tests evaluate toxicity of a chemical to organisms over a short time period, i.e. 48 or 72 hours. The most common acute effect-criteria for invertebrates are mortality, immobility, and loss of equilibrium; for fish, mortality; and for algae, growth. Data are often expressed as LC50s, the concentration estimated to produce mortality in 50% of the test population over a specific period of time. A low LC50 value corresponds to high toxicity because it means that a smaller concentration of the toxicant killed 50% of the test organisms.

The chronic test is another important tool for estimating and evaluating

chemical hazards to aquatic organisms. In a chronic toxicity test more than one life stage of a test animal are exposed to a toxicant to assess thresholds for effects. Such tests are often used to determine concentrations of a chemicals that limit growth, development, survival, and reproductive potential. Data from chronic tests can be used to determine inhibition concentrations. An inhibition concentration (ICp) is the concentration of a chemical at which the test effect, e.g. reproduction, is inhibited by a certain percent (p) compared to a control. For example, an IC25 for a reproductive test is the concentration of toxicant at which reproduction was reduced by 25% compared to the control. Chronic data values in the literature have also been reported as MATC (maximum acceptable toxicant concentration) values. The MATC is a hypothetical concentration in a range bounded by the LOEC (lowest observable effect concentration) and the NOEC (no observed effect concentration), which is calculated from test results (NOEC < MATC < LOEC).

LITERATURE REVIEW

Due to the ubiquity and toxicity of ammonia in aquatic environments abundant literature exists on toxicity of ammonia to both fishes and invertebrates. Following is an examination of factors affecting un-ionized ammonia toxicity, a review of acute and chronic toxicity of ammonia, an explanation of possible physiological mechanisms of toxicity, and a discussion of current standards for ammonia.

Factors Affecting Toxicity of NH_3

Hardness, pH, dissolved oxygen, and temperature affect toxicity of un-ionized ammonia. Emerson et al. (1975) reported that an increase in water hardness decreased NH_3 concentration, but toxicity to fishes was not reduced unless hardness exceeded 200-300 mg/L $CaCO_3$. Tomasso et al. (1980) found that elevated $CaCO_3$ levels decreased NH_3-N toxicity to channel catfish (*Ictalurus punctatus*). They speculated that decreased ammonia toxicity was attributable to reduced gill permeability to NH_3 at high hardness levels.

Total ammonia is more toxic at high pH, but NH_3 is more toxic at low pH (Broderius et al. 1985, Robinette et al. 1988, Thurston et al. 1981b, and Tomasso et al. 1980). Toxicity may not be solely attributed to NH_3 for the crustacean,

Hyallela azteca. Monson et al. (1993) reported that NH_4^+ and NH_3 caused significant toxicity in waters with a hardness of 100 mg/L $CaCO_3$ or less. They also found that toxicity of ammonia to *H. azteca* decreased with increasing hardness.

Dissolved oxygen and temperature also affect NH_3 toxicity. Toxicity of NH_3 increased at lower dissolved oxygen concentrations for rainbow trout (*Oncorhynchus mykiss*) (Thurston et al. 1981b). Thurston and Russo (1983) found a decline in toxicity of un-ionized ammonia at higher temperatures within normal temperature ranges for fish.

Acute Toxicity of Ammonia

Freshwater Invertebrates

Although an extensive data base of acute ammonia toxicity values exists for invertebrates, few studies involve *C. dubia*. All the following literature on freshwater invertebrates attributed ammonia toxicity to the NH_3 form. LC50 values for acute ammonia toxicity have ranged from 0.53 to 22.8 mg/L NH_3-N for a variety of aquatic invertebrates (U.S. EPA 1985). For *C. dubia*, Cowgill and Milazzo (1991) reported a 48-hour LC50 value of about 0.87 mg/L NH_3-N

(estimated from a bar graph displaying total ammonia values) and a NOEC (no observed effect concentration) of 0.38 mg/L NH_3-N (a given value) at pH 8.3 and hardness 102 mg/L $CaCO_3$. Nimmo et al. (1989) reported a 48-hour LC50 of 1.06 mg/L NH_3-N for *C. dubia* at pH 7.8 in wastewater. They found *C. dubia*, fathead minnows (*Pimephales promelas*), johnny darters (*Etheostoma nigrum*), and white suckers (*Catostomus commersoni*) had comparable LC50 values.

In *D. magna*, 48-hour acute LC50s of ammonia have ranged from 0.53 to 4.94 mg/L NH_3-N under varied test conditions (Gerisch and Hopkins 1986, U.S. EPA 1985).

Geometric mean acute toxicity values of un-ionized ammonia in a study with nine invertebrate species varied from 1.10 to 18.3 mg/L NH_3-N (Arthur et al. 1987); Table 1. The fingernail clam (*Musculium transverum*) was the most sensitive test organism involved, and the crayfish (*Orconectes immunis*) was least sensitive. The cladoceran (*Simocephalus vetulus*), snails, amphipods, mayflies, isopods, and caddisflies fell in between.

Acute toxicity (96-hour LC50) of ammonia to 11 macroinvertebrate species tested in a continuous flow-through system ranged from 0.71 mg/L NH_3-N for *Planaria tenuis* (a flatworm) to 2.95 mg/L NH_3-N for *Hydropsyche augustipennis* (a caddisfly). The snail, *Lymnaea stagnalis*, was about 1.5 times more sensitive than another snail, *Physa fontinalis*, and both were more sensitive than the insects tested. The insects, *Leucfra inermis*, *Chironomus riparis*, *Baetis rhodani*, and

Table 1. Acute LC50 values for toxicity of ammonia to various invertebrates.

Organism	LC50 mg/L NH_3-N	pH	Hardness mg/L $CaCO_3$	Temp. degrees Celcius	Reference Cited
Class Bivalvia					
Musculium transversum	1.1	8.6	112-206	20.5	a
Class Crustacea					
Ceriodaphnia dubia	0.87	8.4-9	94-104	26	c
Ceriodaphnia dubia	1.06	7.8	-	25	e
Asellus aquaticus	1.92	7.8-8	98-106	11.5	f
Gammarus pulex	2.05	7.8-8	98-106	11.5	f
Daphnia magna	2.94	8.3-8.6	220-240	20.0	d
Artemia nauplii (marine)	5.65	7.5	-	27.0	b
Oronectes immunis	14.72	7.9	112-206	17.1	a
Class Gastropoda					
Lymnaea stagnalis	1.0	7.8-8	98-106	11.5	f
Physa fontinalis	1.7	7.8-8	98-106	11.5	f
Helisoma trivolis	2.04	7.9	112-206	22	a
Class Insecta					
Leucfra inermis	1.6	7.8-8	98-106	11.5	f
Baetis rhodani	1.65	7.8-8	98-106	11.5	f
Ephemerella ignita	1.7	7.8-8	98-106	11.5	f
Hydropsyche augustipennis	2.95	7.8-8	98-106	11.5	f
Philartcus quaeris	10.07	7.9	112-206	21.9	a
Class Oligochaeta					
Limnodrilus hoffmeisteri	1.85	7.8-8	98-106	11.5	f
Class Turbellaria					
Planaria tenuis	0.71	7.8-8	98-106	11.5	f

a = Arthur et al. 1987
b = Chen et al. 1989
c = Cowgill and Milazzo 1991
d = Gersich and Hopkins 1986
e = Nimmo et al. 1989
f = Williams et al. 1986

Ephemerella ignita were all intermediate in sensitivity while the oligochaete, *Limnodrilus hoffmeisteri*, the amphipod *Gammarus pulex*, and the isopod *Asellus aquaticus* were the most tolerant species (Williams et al. 1986); in Table 1.

In tests on the freshwater shrimp, *Macrobrachium rosenbergii*, post-larval, and juvenile shrimp had 48-hour LC50 values of about 2.5 mg/L NH_3-N at pH 9.0. When pH levels were increased to 9.5 the toxicity of un-ionized ammonia increased dramatically with LC50 values from 0.8 to 1.9 mg/L NH_3-N (Robinette et al. 1988).

Freshwater Fish

Toxicity of ammonia to fish has been studied in more depth than other aquatic organisms. Acute toxicity values for fish have ranged from 0.13 to 3.8 mg/L NH_3-N (U.S. EPA 1985); Table 2. Fishes tested by Arthur et al. (1987) resulted in LC50 values for acute toxicity that ranged from 0.53 to 2.17 mg/L NH_3-N. Rainbow trout were the most sensitive test organisms and fathead minnows were the least. Walleye (*Stizostedion vitreum*), channel catfish, and white suckers fell in between.

One of the most studied species, rainbow trout is among the most sensitive to ammonia. The acute LC50 values for rainbow trout have ranged from 0.13 to

Table 2. Acute LC50 ranges for commonly studied fish species.

Fish Species	LC50 Range mg/L NH_3-N	References
Rainbow Trout *Oncorhynchus mykiss*	0.13 - 1.1	U.S. EPA 1985
Fathead Minnow *Pimephales promelas*	0.25 - 2.55	Alexander et al. 1986, Mayes et al. 1986, Thurston et al. 1986, Arthur et al. 1987, Diamond et al. 1993
Walleye *Stizostedion vitreum*	0.51 - 1.10	Alexander et al. 1986, Mayes et al. 1986, Arthur et al. 1987
Smallmouth Bass *Micropterus dolomieui*	0.69 - 1.78	Broderius et al. 1985
Bluegill *Lepomis machrochirus*	0.69 - 1.06	Alexander et al. 1986, Mayes et al. 1986, Diamond et al. 1993
Largemouth Bass *Micropterus salmoides*	1.0 - 1.7	U.S. EPA 1985
Channel Catfish *Ictalurus punctatus*	1.4 - 3.8	Colt and Tchobanoglous 1976, Tomasso et al. 1980, Arthur et al. 1987

1.1 mg/L NH_3-N (Arthur et al. 1987, Thurston et al. 1981a and 1981b, and Thurston and Russo 1983). Rainbow trout fingerlings were found to be more sensitive to ammonia under low dissolved oxygen conditions at a pH of 7.8 and a temperature 12.5 degrees Celsius. The LC50s ranged from 0.32 mg/L NH_3-N at 2.0 mg/L D.O. to 0.81 mg/L NH_3-N at 8.6 mg/L D.O (Thurston et al. 1981a). Thurston et al. (1981b) investigated effects of pH on toxicity of un-ionized ammonia. At pH 8.3 ammonia was the least toxic, with toxicity increasing at pH levels above and below that level. Thurston and Russo (1983) performed 71 ammonia toxicity tests on rainbow trout at various life stages. The 96 hour acute values ranged from 0.16 to 1.1 mg/L NH_3-N. The authors concluded that tolerance of rainbow trout to ammonia increased as the fish develop through the larval stages, was greatest at juvenile and yearling stages, and decreased thereafter.

Channel catfish also have been a commonly tested species. The 96-hour LC50s have ranged from 1.4 to 3.8 mg/L NH_3-N (Colt and Tchobanoglous 1976, Tomasso et al. 1980 and Arthur et al. 1987). Tomasso et al. (1980) performed a 24-hour study with channel catfish at three pH levels. Effects of pH were similar to those reported by Thurston et al. (1981b) for rainbow trout. Toxicity was least for fish exposed to ammonia in soft water at pH 8.0 (LC50 of 1.82 mg/L NH_3-N), but at pH 7.0 (LC50 of 1.39 mg/L NH_3-N), and pH 9.0 (LC50 of 1.49 mg/L NH_3-N) toxicity was greater. Effect of hardness was also investigated at pH 7.0 in

waters with hardness of 40 mg/L $CaCO_3$ and 400 mg/L $CaCO_3$. LC50 values were not reported, but the authors stated that at higher hardness, toxicity of both NH_3-N and total ammonia was significantly decreased. They attributed decreased toxicity, to excess calcium which decreases gill permeability, or to an increased retention of sodium. Bader and Grizzle (1992) studied two ages of channel catfish at pH 8.2 and hardness of 260 mg/L $CaCO_3$. The data yielded no significant differences between 1-day-old and 7-day-old catfish. The 96-hour LC50s were 1.21 NH_3-N for 1-day-old and 1.61 NH_3-N for 7-day-old catfish.

Fathead minnow 96-hour LC50 values have ranged from 0.25 to 2.55 mg/L NH_3-N (Alexander et al. 1986, Mayes et al. 1986, Thurston et al. 1986, Arthur et al. 1987, and Diamond et al. 1993). Thurston et al. (1981b) found that fathead minnows are 1.5 to 3.0 times more tolerant of acutely toxic concentrations of ammonia than rainbow trout. At pH levels ranging from 6.5 to 9.0, they found a significant increase in toxicity of un-ionized ammonia above or below a pH of 8.5. At pH 6.5 the LC50 was 0.19, at pH 8.5 it was 1.38, and at pH 9.0 it was 1.21 mg/L NH_3-N. The authors speculated that the increase in H^+ at lower pH may increase toxicity of un-ionized ammonia. They did not attempt to explain the increase in toxicity above pH 8.5. Thurston et al. (1983) found a significant relation of ammonia toxicity to temperature in fathead minnows, but did not find a relation of ammonia toxicity to dissolved oxygen. In a previous study (Thurston et al. 1981a) under similar conditions, they found significant effects of dissolved

oxygen in rainbow trout; therefore, the authors concluded that species differences are important. Thurston et al. (1983) found that as temperature increased from 11 to 23 degrees Celsius the toxicity of un-ionized ammonia to fathead minnows decreased. They also tested fathead minnows from various sources and found that the source of the fish did not affect results.

The LC50 values for acute toxicity of ammonia have been measured for several other common sportfish including: walleye, 0.51 to 1.10 mg/L NH_3-N, (Alexander et al. 1986, Mayes et al. 1986, and Arthur et al. 1987), smallmouth bass (*Micropterus dolomieui*), 0.694 to 1.78 mg/L NH_3-N, (Broderius et al. 1985), bluegill (*Lepomis machrochirus*), 0.69 to 1.06 mg/L NH_3-N, (Alexander et al. 1986, Mayes et al. 1986, and Diamond et al. 1993), and largemouth bass, (*Micropterus salmoides*), 1.0 - 1.7 mg/L NH_3-N (U.S. EPA 1985); Table 2. Broderius et al. (1985) studied the effect of pH on ammonia toxicity in smallmouth bass. At pH levels from 6.5 to 8.7 ninety-six hour LC50s ranged from 0.69 mg/L NH_3-N to 1.78 mg/L NH_3-N. Toxicity of un-ionized ammonia increased as pH decreased.

Freshwater Invertebrates

Chronic toxicity studies have been limited to cladocerans, which have had maximum acceptable toxicant concentration (MATC) values ranging from 0.60 to 0.88 mg/L NH_3-N (Alexander at al. 1986, Gerish and Hopkins 1986, and Nimmo et al. 1989).

Gerisch and Hopkins (1986) tested 21-day survival, and reproduction of *D. magna* when exposed to ammonia. The MATC and LOEC were 0.60 and 0.87 mg/L NH_3-N. Mean total young per adult and mean number of broods both decreased as the concentration of ammonia increased. Alexander et al. (1986) reported MATC values based on mortality and reproductive endpoints such as reduction in brood size and total number of young per female for *D. magna* between 0.42 and 0.87 mg/L NH_3-N.

Cowgill and Milazzo (1991) studied chronic toxicity of fifteen compounds including ammonia, to *C. dubia*. The LC50 for the chronic ammonia test was about 0.95 mg/L NH_3-N (estimated from a bar graph in the paper). The NOEC was about 0.73 mg/L NH_3-N. Nimmo et al. (1989) studied ammonia toxicity to *C. dubia* using water from the St. Vrain River and wastewater discharged from Longmont, Colorado. The river water had chronic ammonia MATC values

ranging from 0.68 to 0.88 mg/L NH_3-N. MATC values for the wastewater were slightly more toxic than the river water, ranging from 0.62 to 0.70 mg/L NH_3-N.

Freshwater Fish

Chronic effects of ammonia on fish vary depending on the criteria, but the NOEC values have ranged from 0.04 to 0.61 mg/L NH_3-N and the LOEC values from 0.21 to 0.91 mg/L NH_3-N (Broderius et al. 1985, Mayes et al. 1986, Thurston et al. 1986, and Bader and Grizzle 1992). Mayes et al. (1986) studied effects of ammonia on mortality and deformities in fathead minnows. The MATC ranged from 0.17 to 0.26 mg/L NH_3-N. The LOEC for larval survival and for appearance of deformed larvae at hatching was 0.26 mg/L NH_3-N. Thurston et al. (1986) reported a NOEC value for parental growth and survival of 0.44 mg/L and 0.37 mg/L NH_3-N for egg production. The LOEC was 0.91 mg/L NH_3-N for parental growth, parental survival, and egg production. Toxicity based on the presence of brain lesions, a more sensitive indicator, was 0.11 mg/L NH_3-N for the NOEC and 0.21 mg/L NH_3-N for the LOEC and the chronic effects threshold was 0.15 mg/L NH_3-N. The chronic-effects threshold, based on survival, growth, and reproductive success, was estimated to be 0.27 mg/L NH_3-N.

Chronic effects on channel catfish were assessed by Colt and

Tchobanoglous (1978). Growth of juvenile catfish was reduced in a linear fashion during a 31-day test when the fish were exposed to concentrations of ammonia ranging from 0.05 to 0.99 mg/L NH_3-N. Mortality increased significantly beginning at 0.99 mg/L. Growth decreased by 50% at 0.52 mg/L NH_3-N, and no growth occurred at 0.97 mg/L NH_3-N. Bader and Grizzle (1992) studied effects of ammonia on 1-day-old and 7-day-old channel catfish and found that ammonia affected both ages of catfish equally with respect to growth, however the 1-day-old fish survived at a higher ammonia concentration than the 7-day-old fish (Table 3).

Table 3. Chronic toxicity of ammonia to 1-day old and 7-day old *Ictalurus punctatus* (data from Bader and Grizzle 1992).

Chronic value	1-day old growth test	7-day old growth test	1-day old survival test	7-day old survival test
NOEC	0.08	0.09	0.35	0.21
LOEC	0.23	0.21	0.49	0.34

Numbers represent un-ionized ammonia-nitrogen in milligrams per liter.

Broderius et al. (1985) tested smallmouth bass at four pH levels and found that growth decreased as pH decreased. Estimated 32-day NOECs ranged from 0.0437 at pH 6.6 to 0.612 mg/L NH_3-N at pH 8.68. Survival of organisms from embryos to 7-day-old larvae was unaffected by ammonia concentrations ranging from 0.236 to 0.865 mg/L NH_3-N at pH values from 6.60 to 8.68.

Physiological Effects of Ammonia

Despite the abundant evidence of acute and chronic toxicity, mechanisms of ammonia toxicity are poorly understood. Theories regarding toxic actions of ammonia range from impairment of cerebral energy metabolism to gill or other tissue damage. Ammonia may act in a variety of ways that differ depending on environmental factors. All studies were carried out on vertebrates, and may not provide information on toxic actions of ammonia on invertebrates.

In fish, Burrows (1964) postulated that toxicity of ammonia is manifested through damage to gill epithelium with subsequent respiratory impairment and eventual suffocation from chronic exposure. Fromm and Gillette (1968) found that un-ionized ammonia suppressed excretion of endogenous ammonia at the gill surface in rainbow trout. Mortality was attributed to neurological and cytological failure caused by un-ionized ammonia.

Flis (1968) showed that gills, skin, intestines, liver, and kidney can be damaged by ammonia. Gill damage was associated with reduction in the number of blood erythrocytes. Lloyd and Orr (1969) found that ammonia changes the permeability of rainbow trout and increases urine flow. Death can occur when permeability exceeds the maximum rate of urine production, and may be primarily due to kidney failure. They also found that fish can acclimate to sublethal levels of ammonia if they survive. At temperatures below 10 degrees Celsius, the ability

to excrete ammonia is reduced and may increase the susceptibility of the fish to ammonia poisoning.

Smart (1976) studied gill structure in relation to short- and long-term exposure of ammonia in rainbow trout. He had observed earlier that reduced environmental oxygen concentration enhanced toxicity of ammonia to a greater extent than any other pollutants; therefore he thought that ammonia acted primarily on the respiratory system. After short term exposure, macroscopic and microscopic examination of the gills revealed only minimal changes. Blood spaces of the secondary lamellae were dilated, and branchial vascular resistance was decreased. Heart rate and blood pressure increased, suggesting increased flow of blood through the gills. Impaired oxygen uptake due to damage of the gills was not found to be a primary acute toxic action of ammonia. However, with long term exposure many deaths occurred between 14 and 21 days. Most fish were diseased with fin and tail rot believed to have been caused by stress from ammonia. Gill structure changes may have greatly reduced oxygen diffusing capacity with chronic exposure, which may cause death unless the fish are able to compensate with cardiovascular and respiratory changes. Gills of fish not showing signs of disease had increased mucus production, thickened lamellar epithelium, swollen and rounded secondary lamella, and some ruptured epithelium and hemorrhaging. In most cases the pillar cell system was completely broken down.

Acute toxicity of ammonia may be from impairment of cerebral energy

metabolism in fish (Smart 1978). Smart (1978) studied gas exchange changes in relation to ammonia toxicity in rainbow trout and found increased heart rate, respiratory frequency and buccal pressure following ammonia exposure, but erythrocyte count, hematocrit, and haemoglobin concentration did not change. Dorsal aortic blood oxygen tension dropped more than 50 percent when the fish were exposed to ammonia, but the drop in blood tension was not believed to be due to the gills' ability to transfer oxygen. Instead, the decrease in blood tension may have been due to poor uptake by the blood due to increased gill ventilation, increased perfusion rates, branchial vasodilation, and highly deoxygenated venous blood. The author concluded that ammonia disturbs cellular metabolism, causing increased need for oxygen. Schenker et al. 1967 suggested in studies of mammals that un-ionized ammonia impairs the cerebral energy metabolism causing a depletion of high energy compounds in the brain. Symptoms of ammonia poisoning in fish and mammals are similar: hyperexcitability, coma, convulsions, and hyperventilation. Therefore, Smart postulated that the primary acute action is the same for fish and mammals, and that ammonia impairs cerebral energy metabolism.

Tomasso et al. (1980) found that elevated calcium levels increased tolerance of fish to ammonia. They postulated that calcium may decrease gill permeability to ammonia by allowing tighter packing of membrane molecules. Increased calcium levels might also stabilize the sodium/ammonium exchange operating in

chloride cells of fish. The elevated calcium could cause increased influx of sodium, which would help slow plasma sodium depletion thereby increasing a fish's tolerance of ammonia (Maetz and Garcia-Romeu 1964).

Ruffier et al. (1981) concluded that the acute toxicity of ammonia-bearing waste waters resulted from a number of factors including mean ammonia concentration, the amount of fluctuation in ammonia concentration, and acclimation abilities of the test species. They found that at high concentrations of ammonia bluegill sunfish exhibited very few symptoms of stress before death. Initially they behaved normally, then they went into a coma and died. However, at low concentrations of ammonia there were signs of stress in both the bluegill and the rainbow trout. Fish lost coordination and muscular control. Fish placed in clean water after exposure normally survived. The authors also found that bluegill were able to acclimate to low levels of ammonia.

Szumski et al. (1982) reviewed studies involving pH and NH_3 toxicity. The studies involved four fish species (two cold water and two warm water) and two invertebrates (one marine and one freshwater). They found that all the studies reported decreasing acute toxicity of NH_3 with increasing pH. The authors did not address why these species were more tolerant of NH_3 at higher pH except in fish which they theorized had a reduced concentration of un-ionized ammonia at the gill surface caused by the pH shift in the gill chamber.

Thurston et al. (1986) found brain lesions which may indicate important

effects of ammonia toxicity that could lead to organ dysfunction, early death, and greater susceptibility to predation in the natural environment. Fathead minnows in the highest concentration were significantly shorter than those at lower concentrations. At 0.42 mg/L NH_3-N and higher, the authors found lesions on heads of minnows, and proliferated tissue was found completely surrounding the brain in the highest concentrations. If tissue damage were used as the chronic-effects criterion, the threshold value derived from their study would be 0.15 mg/L NH_3-N. If typical criteria, e.g. survival, growth, or reproduction, were used instead; the threshold value would be higher, 0.27 mg/L NH_3-N. In addition, Thurston (1986) reported no effects on survival, growth, reproduction, egg viability, or egg hatching of rainbow trout at 0.074 mg/L NH_3-N (the highest concentration run). Yet, brain lesions were found with exposures of only 0.044 mg/L NH_3-N.

Wajsbrot et al. (1991) studied a marine species, juvenile seabream *Sparus aurata* under low dissolved oxygen concentrations and found responses similar to most freshwater fish. Ammonia toxicity increased with decreasing dissolved oxygen; the 96-hour LC50 values ranged from 0.82 to 1.33 mg/L NH_3-N. At the lowest oxygen levels, about 15% saturation, toxicity was attributed to low dissolved oxygen alone. Response to ammonia was rapid with mortality occurring within two hours after exposure. Histopathological examination showed no evidence of changes in either the gills, liver, or kidneys.

Ammonia Standards

The U.S. EPA (1985) published national standards for ammonia based on pH, temperature, and sensitivity of fish. Theoretically, the water body should not be detrimentally affected if either the one-hour average concentration of un-ionized ammonia does not exceed the standard value more than once every three years, or the 4-day average concentration of un-ionized ammonia does not exceed the standard value more than once every three years. For sensitive species one-hour average limits range from 0.0091 to 0.26 mg/L NH_3-N (0.58 - 35.0 mg/L TAN) and four-day average limits, from 0.0007 to 0.035 mg/L NH_3-N (0.08 - 2.5 mg/L TAN) depending on pH and temperature. For areas where sensitive species are absent, the one-hour average limits range from 0.0091 to 0.37 mg/L NH_3-N (0.82 - 35.0 mg/L TAN) and four-day average limits, from 0.0007 to 0.05 mg/L NH_3-N (0.11 - 2.5 mg/L TAN). The three year time period was based on EPA's best judgement of the amount of time a system would need to recover from a pollution event.

National criteria are meant as guidelines, and each state may address the problem differently. An alternative to national or state criteria is the development of site-specific criteria. Site-specific tests can be used to create standards or to verify the validity of current standards. Site-specific standards may be easier to justify and enforce than general guidelines. In addition, by considering resident

species and water characteristics in the region, biota in a stream may be protected without creating standards which are either under or over protective. Characteristics of the water (i.e. hardness) can also be mimicked in dilution water used for tests, or the site water itself can be used.

Diamond et al. (1993) conducted a site-specific study on a wooded stream on the Delmarva Peninsula in Virginia, Maryland where site water and resident species were used in acute and chronic tests. The site water had no effect on un-ionized ammonia toxicity. They found much lower chronic sensitivity of resident species to un-ionized ammonia than previous laboratory testing with the same species, although acute sensitivity was similar to other studies. The data for this stream suggested that the standards developed by the EPA for warm-water streams may be over protective in this case. In a study comparing site water from the St. Vrain River in Colorado to laboratory water, results with site water were not significantly different from those with laboratory water (Nimmo et al. 1989). Results of acute and chronic tests verified that the pre-existing ammonia criteria for the river were adequate. In Michigan, on the Tittabawassee River, chronic and acute tests were run with site water and typical tests species, water fleas (*D. magna*), fathead minnows, bluegill, and walleye. The Michigan DNR used the results from these tests to develop site-specific acute and chronic water-quality criteria, as well as estimated assimilative capacity for the section of the river studied. A portion of the assimilative capacity was then allocated to each

discharger and effluent limitations were set (Alexander et al. 1986).

MATERIALS AND METHODS

Test Treatment

For each treatment, five concentrations of ammonium sulfate $(NH_4)_2SO_4$, oven dried at 100 degrees Celsius for two hours, were prepared with appropriate by diluting stock solution with the appropriate dilution water. This stock solution differed for each treatment based on pH level being tested. Test concentrations were 100, 60, 36, 21.9, and 12.96% of the stock solution.

Range-finding tests were used to determine the appropriate ammonia concentration range for each treatment. Treatments were pH of about 6.5, 7, 8, and 9 in combination with hardness levels of 40-50 mg/L $CaCO_3$ - soft, 80-100 mg/L $CaCO_3$ - medium, and 160-180 mg/L $CaCO_3$ - hard, yielding a total of twenty-four acute and chronic tests (Table 4).

Table 4. Hardness and pH combinations used in acute and chronic tests.

Hardness, mg/L $CaCO_3$	pH			
Soft, 40-50	6.5	7	8	9
Medium, 80-100	6.5	7	8	9
Hard, 160-180	6.5	7	8	9

Test Organism and Diet

The cladoceran, *Ceriodaphnia dubia* was cultured from a single adult that was killed and mounted on a microscope slide for positive identification of species. Each female used for neonate production was isolated in a 30 ml culture cup to monitor reproduction. Females that produced less than 15 young in 3 broods (usually 7-8 days) were culled from culture. Young from second or subsequent broods that were 8 hours old or younger and which had a minimum of 8 neonates in the brood were used for testing.

C. dubia (except during most acute tests) were fed the algae, *Selenastrum capricornutum,* from a solution with a cell count of $3.5x10^7$ cells/ml at a rate of 50 ul per 15 ml of test or culture solution and Yeast-Cerophyl-Trout Chow Food (YCTF) with a solids count of 1800 mg/L at a rate of 100 ul per 15 ml of test or culture solution (U.S. EPA 1991). *C. dubia* were acclimated to the water hardness of their test solution for at least five generations before the start of any test. Control organisms were always transferred to test solutions first, followed by the lowest concentration and subsequent concentrations to avoid any cross-contamination.

Dilution Water

Dilution water was reconstituted water prepared from guidelines for moderately hard (160-180 mg/L $CaCO_3$) reconstituted water found in APHA Standard Methods (1989). Base water was type 1 reagent-grade deionized water from a Barnstead Nanopure (D4741) system (Dubuque, IA). Reagent grade salts from Fisher Scientific -- KCl, 0.008 g/L; $MgSO_4$, 0.120 g/L; $NaHCO_3$, 0.192 g/L; $CaSO_4$ $2H_2O$, 0.120 g/L -- were added to a 20 liter carboy of base water to make hard reconstituted water. Sodium selenate (Na_2SeO_4) was added to the prepared reconstituted water at a rate of 2 ug/L to improve *C. dubia* reproduction (Cowgill and Milazzo 1991). Soft and medium dilution water was made by diluting this hard water with the correct proportion of deionized water. The mixture was then aerated on a stir plate for at least an hour before preparation of test solutions. All mixtures of dilution water were prepared immediately before use.

Glassware

Glassware used in toxicity tests was cleaned with alconox detergent, 10% HCL acid wash, and acetone. New beakers were soaked in a 20% sulfuric acid solution overnight before cleaning. Disposable 30 ml polystyrene cups used in

chronic tests were rinsed with distilled water before use. Cups were replaced twice during chronic tests when the test solutions were renewed. Disposable plastic pipets were used for transfer of organisms.

Analytical Methods

During entire study relevant quality control practices including standard operating procedures were always followed. Total ammonia-nitrogen (TAN), pH and temperature were measured with an Orion model 920A Ion Analyzer fitted with Orion ammonia, pH and automatic temperature compensation (ATC) probes. Dissolved oxygen was measured with a YSI model 58 oxygen meter. Hardness was measured by the titrimetric method of APHA Standard methods (1989). To avoid disturbing test organisms, dissolved oxygen, pH, and ammonia measurements were performed in duplicate beakers that did not contain organisms. To verify accuracy of duplicate beakers, periodic measurements were made in test beakers.

During 48-hour acute tests ammonia, pH, temperature, and dissolved oxygen were measured at the beginning and end of the tests. Temperature, dissolved oxygen, and pH were also measured at 24 hours. Hardness of the dilution water was measured at the beginning of the test.

During chronic 7-day tests, ammonia was measured at the beginning of the test and on the two renewal days, usually days 3 and 6. Dissolved oxygen, pH, and temperature were measured daily. The hardness of the dilution water was measured at the beginning of the test and for each new mixture of dilution water.

Un-ionized Ammonia Calculations

The pH, temperature, and total ammonia-nitrogen measurements were used to calculate un-ionized ammonia-nitrogen concentrations following Emerson et al. (1975). These equations are:

$$pK_a = 0.0901821 + 2729.92/T,$$

where pK_a = negative log of the acid dissociation constant of NH_4^+ and T = temperature in degrees Kelvin.

This pK_a value was then used to calculate the fraction of ammonia in the NH_3 form or (f), where

$$f = 1/(10^{pKa-pH} + 1), \text{ and}$$

$$f \times \text{Total Ammonia} = [NH_3]$$

Average temperature during tests was used for un-ionized ammonia calculations. Hydrogen ion concentrations were calculated for each pH data point, these

concentrations were averaged and converted back to pH, to derive average pH values. Average pH and average total ammonia-nitrogen measurements at each concentration during tests were used to determine the un-ionized ammonia concentration. The calculated un-ionized values were then used to determine LC50s, IC25s, and IC50s.

pH Manipulation

Chemical buffers for pH control were found unsatisfactory because of magnesium precipitation and buffer related mortality of *C. dubia*. Therefore, carbon dioxide was used to maintain pH 6.5 and 7 at all hardness levels, and pH 8 in medium and hard water following the method of Mount and Mount (1992). Test beakers filled with test solutions were placed in a sealed glass container, 40.6 centimeters long by 20.3 centimeters wide by 25.4 centimeters high, into which a precalculated amount of carbon dioxide was injected. The increased level of carbon dioxide caused the test solution pH to fall in proportion to the quantity of carbon dioxide injected. After two hours the sealed container was opened to check pH. If the pH was within $\pm$ 0.2 pH units of the desired level, *C. dubia* were added to the beakers, the container was resealed and the same quantity of carbon dioxide was injected. Whenever the container was opened to check for mortality,

to feed, or to renew test solutions, it was re-injected with the appropriate amount of CO_2. Carbon dioxide concentrations of up to 10% of the container headspace are reported to be safe for *C. dubia* (Mount and Mount 1992) and were not exceeded in these tests. The pH levels achieved by this method were not exactly 6.5, 7.0, and 8.0, but average values were within 0.1 to 0.2 pH units. Therefore, to facilitate a clear discussion of results I refer to pH levels as pH 6.5, 7, and 8, although the true value may differ by $\pm$ 0.2 pH units.

The pH was increased without use of buffers in all the tests at pH 9 and at pH 8 in soft water by removal of carbon dioxide from the sealed test chamber with a 5% solution of sodium carbonate (Na_2CO_3). Soft dilution water had a pH of 7.2-7.4; therefore the pH had be increased for tests at pH 8. The sodium carbonate solution stripped carbon dioxide from the air, causing a rise in the test solution pH. Tests were carried out in the same containers as those with CO_2 except two pieces of aquarium air hose were run through sealed holes in test container lid. A 115 volt aquarium pump was placed outside the test container. The sodium carbonate solution was put into a plastic bottle inside the container and one of the hoses led from the outflow of the pump through the lid and into the solution. The other hose led from open space near the bottom of the test container out through the lid and into the intake valve of the pump (Figure 1). Air was drawn out of the test container and pumped back into the sodium carbonate solution in a continuous loop. The hose in the sodium solution had an airstone on the end to decrease

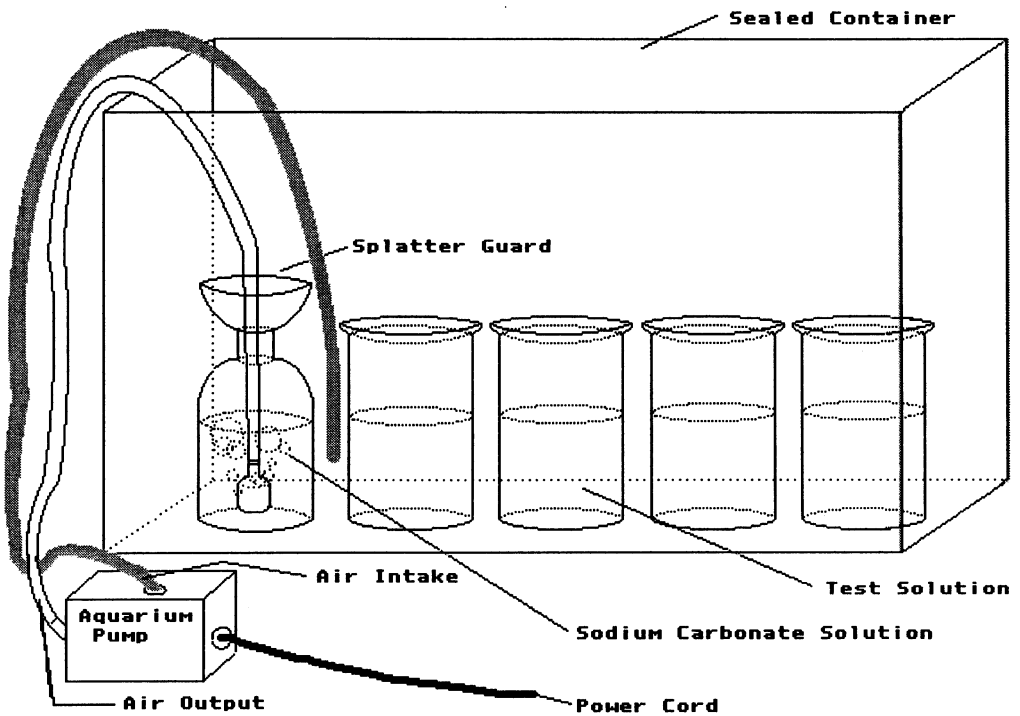


Figure 1. Diagram of carbon dioxide removal system used for tests run at pH 9 for all hardness levels and at pH 8 in soft water only.

bubble size. The pump had to be run overnight to yield a pH of about 9, at which time test organisms were added to the beakers. For pH 8, the pump was run for 5-8 hours before *C. dubia* were added to test beakers. After test organisms were added, a fresh solution of sodium carbonate was placed in the container, the container was re-sealed, and the pump was turned back on. The pump was usually run throughout tests to maintain the pH. The pH levels attained by this method were within 0.1 pH units of pH 8.0, but average values for pH 9 ranged from pH 8.6 for soft water to pH 8.8 for hard water. For ease of discussion, I refer to pH levels attained as pH 8 and pH 9 although exact values may differ slightly.

Acute Toxicity Test

Static, non-renewal toxicity tests with a range of ammonia concentrations were conducted at each of the pH and water hardness levels. Test procedures were those of U.S. EPA (1991) for *C. dubia* (Table 5). Ammonia, pH, and water hardness levels were manipulated for desired test ranges. The endpoint was mortality. If 90% or greater survival in the controls was not attained, results were discarded. Tests were conducted in 150 ml beakers with 100 ml of test solution, 10 organisms per beaker, and 2 beakers per concentration. Organisms were less than 8 hours old at the start of a test and were fed before toxicity testing. Each test was run for 48 hours with no feeding, with the exception of the tests at pH

Table 5. Summary of acute test methods.

Test Chemical	$(NH_4)_2SO_4$
Test Species	*Ceriodaphnia dubia*
Test Type	Static, Non-renewal
Duration	48 Hours
Endpoint	Mortality
pH Levels	6.5, 7, 8, 9
Hardness Levels	40-50 mg/L $CaCO_3$ 80-100 mg/L $CaCO_3$ 160-180 mg/L $CaCO_3$
pH Manipulation	CO_2 - 6.5, 7, 8 in medium and hard water Na_2CO_3 - 8 in soft water, 9
Feeding	pH 7, 8, 9 - none pH 6.5 - 0.75 ml YCTF & 0.75 ml *S.cap.*
Photoperiod	16h light:8h dark
Temperature	24.5$\pm$1.5 Degrees Celsius
# of Concentrations	5
Dilution	40% dilution water, each concentration 60% of higher concentrationm
Beaker Size	150 ml
Solution Volume	100 ml
# Organisms/beaker	10
# Replicates	2
# Controls	2
Control Survival Required	90% or greater

6.5, which were fed 0.75 ml of YCTF and *S. capricornutum* daily. Tests at pH 6.5 without feeding failed because of control organism mortality; with feeding at pH 6.5, 90% survival of control organisms was attained. Due to inability to achieve control survival at pH 6.5 as well as discrepancies between acute and chronic test results the effect of feeding on acute toxicity of un-ionized ammonia to *C. dubia* was also investigated. Toxicity of ammonia at pH 6.5 in medium water was compared between organisms which were fed and those which were not fed. All beakers were inspected for dead organisms at 24 and 48 hours. Each test container was kept in an environmental chamber at 24.5 $\pm$ 1.5 degrees Celsius and constant photoperiod (16h light : 8h dark).

Chronic Toxicity Test

A three-brood life cycle test with *C. dubia* was conducted at each of the pH and water hardness levels to determine effects of ammonia on mortality and reproduction (U.S. EPA 1989); Table 6. Four modified brood boards, each with 18 randomly assigned polystyrene 30 ml cups were stacked in a 5.5-gallon glass container. Each level had pieces of styrofoam at both ends to keep at least one inch of space between levels. A glass lid affixed with sealant assured an airtight seal. Two holes were drilled in each glass lid for CO_2 injection and recirculation

Table 6. Summary of chronic test methods.

Test Chemical	$(NH_4)_2SO_4$
Test Species	*Ceriodaphnia dubia*
Test Type	Static, renewal
Duration	7-9 days (end when 60% controls have 3 broods)
Endpoint	Reproduction and mortality
pH Levels	6.5, 7, 8, 9
Hardness Levels	40-50 mg/L $CaCO_3$ 80-100 mg/L $CaCO_3$ 160-180 mg/L $CaCO_3$
pH Manipulation	CO_2 - pH 6.5, 7, 8 in medium and hard water Na_2CO_3 - pH 8 in soft water, 9
Feeding	100 ul YCTF & 50 ul *S.cap* per day
Photoperiod	16h light:8h dark
Temperature	24.5$\pm$1.5 Degrees Celsius
# of Concentrations	5
Dilution	40% dilution water, each concentration 60% of higher concentration
Beaker Size	30 ml
Solution Volume	15 ml
# Organisms/beaker	1
# Replicates	10
# Controls	10
Control Survival Required	90% or greater

of air through the sodium carbonate solution. Rubber stoppers were used to close the lid holes when not in use.

One *C. dubia* was placed in each test cup with 15 ml of test solution and there were 10 cups and 10 *C. dubia* per concentration. Test solutions were renewed on days 3 and 6 and the test was terminated when 60% of the surviving controls had three broods of young (usually on day 8). Ninety percent survival was required in the controls for a valid test. The organisms were fed YCTF and *S. capricornutum* daily. The test container was kept in an environmental chamber at a constant temperature and photoperiod. The number of young and dead adults were counted in each test cup daily. Any males present were also identified by microscopic examination and excluded from results.

Statistical Analysis

The LC50 estimates were calculated with Probit Analysis (Gulley and West Inc. 1994) when model assumptions were met. The assumptions of Probit Analysis are: a geometric series of concentrations, each organism acts independently, and must have two concentrations which have at least partial mortality, one concentration above 50% mortality and one below 50% mortality. Several of the tests on effects of feeding failed to meet the assumptions necessary to use Probit Analysis and therefore, data for Figure 8 were generated by

Spearman Karber Analysis (Gulley and West Inc. 1994). For chronic test results, the Linear Interpolation Method for Sublethal Toxicity, the Inhibition Concentration (ICp) Approach, version 2.0 (U.S. EPA 1993) was used to determine 25% and 50% inhibition concentrations for reproduction and corresponding confidence limits. The confidence limits are not always symmetrical about the data point because they are calculated by the bootstrap method which resamples the data a minimum of 80 times to generate multiple ICp values. The standard error of the ICp is estimated by the standard deviation of the individual ICp estimates and confidence intervals are derived from the quantiles of the ICp empirical distribution.

RESULTS and DISCUSSION

Acute Toxicity

Total ammonia is the sum of the ionized (NH_4^+) and un-ionized (NH_3) forms of ammonia. The equilibrium reaction is pH dependent. Low pH favors the ionized form, and high pH favors the un-ionized form. For most organisms tested, toxicity primarily depends on concentration of un-ionized ammonia (Tabata 1962, U.S. EPA 1985). Ionized ammonia is 300 to 400 times less toxic than un-ionized ammonia in *P. promelas* and *O. mykiss* (Thurston et al. 1981b).

Un-ionized ammonia (NH_3) was most toxic to *C. dubia* at pH 6.5 in soft water (0.09 mg/L NH_3-N), and least toxic at pH 8 in hard water (0.92 mg/L NH_3-N) (Table 7). Total ammonia-nitrogen (TAN) was most toxic at pH 9 in soft water (2.6 mg/L TAN), and least toxic at pH 6.5 in hard water (150 mg/L TAN) (Table 7).

Total Ammonia

To determine if NH_3 was the main toxicant in our tests, the LC50s were graphed in terms of TAN (Table 7, Figure 2). Total ammonia should be least

Table 7. Acute toxicty of ammonia to *C. dubia* at pH levels of about 6.5, 7, 8 and 9 and hardness concentrations of about 40, 90, and 180 mg/L $CaCO_3$. Values reported for temperature, dissolved oxygen, hardness, and pH are mean values of measurements made in reserve beakers (one at each ammonia concentration). Ranges for pH and temperature are reported in parentheses following the mean values.

pH	Hardness mg/L $CaCO_3$	Dissolved Oxygen mg/L	Temperature degrees Celcius	48h LC50 - mg/L NH_3-N (95% CI)[a]	48h LC50 - mg/L Total Ammonia-Nitrogen (95% CI)
6.44 (6.36-6.50)	44.0	7.8	23.3 (23.1-23.4)	0.09 (0.08-0.10)	64 (55-74)
6.46 (6.41-6.51)	88.0	6.9	23.4 (23.3-23.5)	0.10 (0.07-0.13)	70 (52-89)
6.55 (6.52-6.59)	172	8.1	22.9 (22.8-23.0)	0.25 (0.21-0.29)	150 (130-170)
6.99 (6.87-7.05)	40.0	7.0	22.7 (22.5-23.0)	0.25 (0.19-0.30)	48 (37-58)
6.88 (6.67-6.99)	92.0	7.0	24.3 (23.9-25.2)	0.37 (0.31-0.42)	100 (82-120)
7.04 (6.93-7.10)	180	6.9	24.9 (24.4-25.5)	0.74 (0.64-0.84)	140 (110-160)
7.95 (7.83-8.02)	48.0	8.5	23.3 (23.2-23.4)	0.64 (0.52-0.75)	16 (13-20)
7.81 (7.71-7.88)	92.0	7.2	24.8 (24.1-25.6)	0.48 (0.40-0.55)	15 (12-18)
7.95 (7.85-8.02)	184	7.4	24.7 (24.3-25.1)	0.92 (0.79-1.05)	22 (19-26)
8.56 (8.39-8.73)	40.0	7.1	23.3 (23.1-23.6)	0.31 (0.24-0.37)	2.6 (1.9-3.2)
8.66 (8.50-8.76)	88.0	8.3	23.3 (23.0-23.6)	0.86 (0.75-0.97)	5.4 (4.5-6.2)
8.79 (8.67-8.85)	180	7.2	23.2 (23.0-23.6)	0.61 (0.49-0.74)	3.1 (2.3-3.9)

[a] CI = confidence interval.

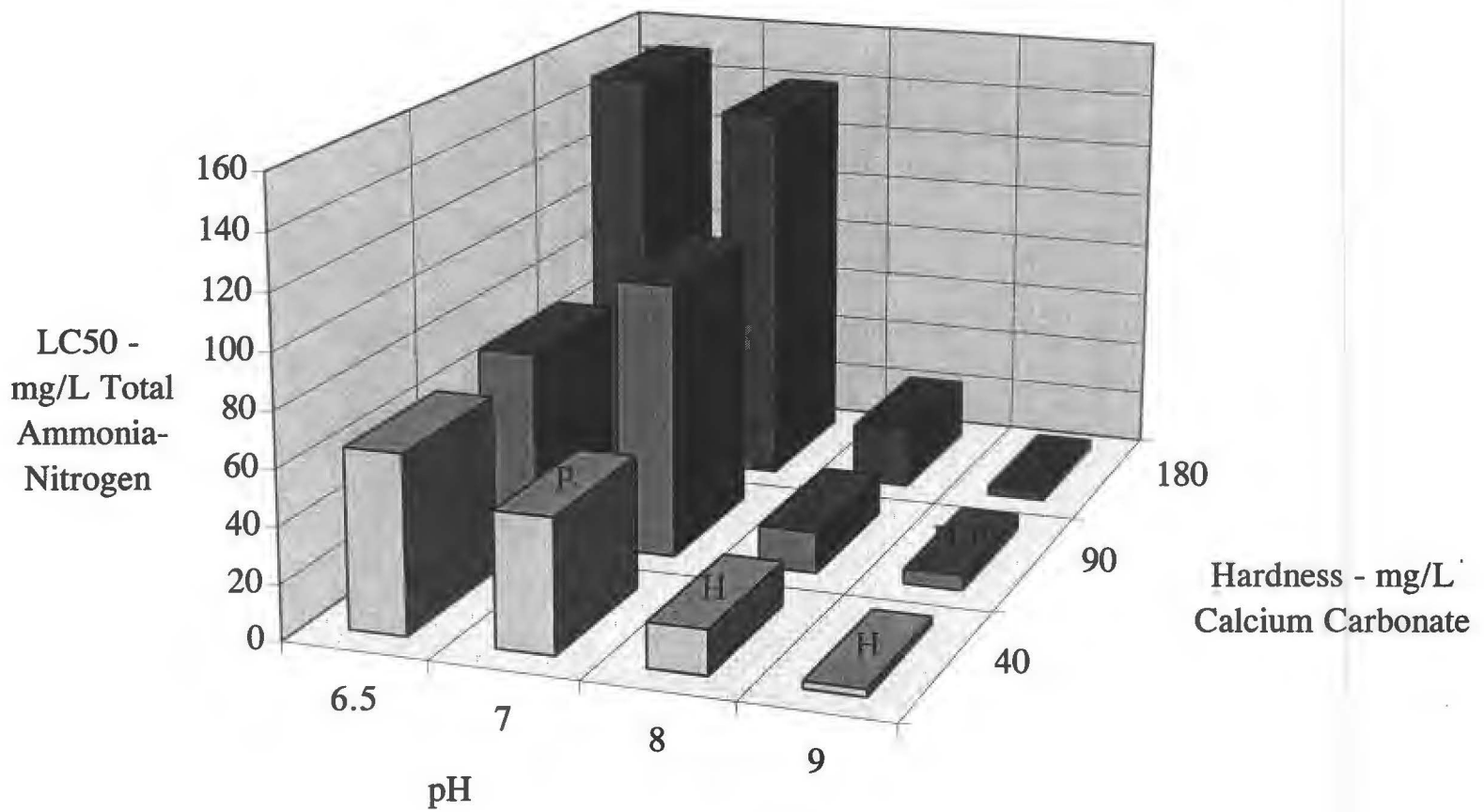


Figure 2. Acute toxicity (LC50) of total ammonia to *C. dubia* at pH levels of about 6.5, 7, 8, and 9 and hardness levels of about 40, 90, and 180 mg/L $CaCO_3$. Bars with "P" above them are significantly different ($p < 0.05$) from others at the same pH. Bars with "H" above them are significantly different ($p < 0.05$) from others at the same hardness.

toxic at low pH levels if NH_3 is responsible for the toxic effects. The inverse relation between the pH and LC50 values suggests that NH_3 was indeed causing toxicity to *C. dubia*. At pH 6.5 in hard water, total ammonia was least toxic with a LC50 of 150 mg/L TAN while at pH 9 in soft water total ammonia was most toxic with a LC50 of only 2.6 mg/L TAN. Nearly identical results were found in a study with rainbow trout which had a 96 hour LC50 of 161 mg/L TAN at pH 6.5, while at pH 9 it was 2.53 mg/L TAN (Thurston et al. 1981b).

An exception to this trend was apparent when total ammonia was more toxic at pH 6.5 than at pH 7 in medium hardness water. The exception is probably more apparent than real because data for pH 6.5 and 7 were not significantly different ($p > 0.05$) from each other at any hardness level. Although NH_3 probably is the most toxic form, it is possible that NH_4^+ contributed to toxicity at the lowest pH levels due to the high concentration of ionized ammonia.

Hardness Effects

Although acute toxicity of un-ionized ammonia to *C. dubia* appeared to decrease as hardness increased, the data (mean LC50 values for four pH levels at each hardness concentration) were not significantly different.

Effects of hardness on acute NH_3 toxicity are not the same at each pH level

(Figure 3). At pH 6.5, NH_3 was least toxic in hard water, but toxicity was greater in both soft and medium water -- which were not significantly different from one-another. At pH 7, NH_3 was least toxic in hard water and most toxic in soft water. At pH 8, NH_3 was also least toxic in hard water, but soft and medium water were more toxic -- although not significantly different from each other. At pH 9, NH_3 was least toxic in medium water, most toxic in soft water, and hard water was intermediate. With the exception of pH 9, these results are similar to those of Monson et al. (1993) who found toxicity of NH_3 to *Hyallela azteca* decreased as water hardness increased.

Un-ionized ammonia was least toxic in hard water at pH 6.5, 7, and 8, but at pH 9 NH_3 was least toxic in medium water. At both pH 7 and 9, NH_3 was most toxic in soft water, but at pH 6.5 and 8 toxicity in soft and medium water was not significantly different.

Increased ionic strength at higher hardness levels can slightly reduce the concentration of NH_3, thereby decreasing toxicity (Thurston et al. 1979). Increased calcium concentrations were also associated with a decrease in ammonia toxicity to channel catfish but mechanisms of protection were unclear (Tomasso et al. 1980).

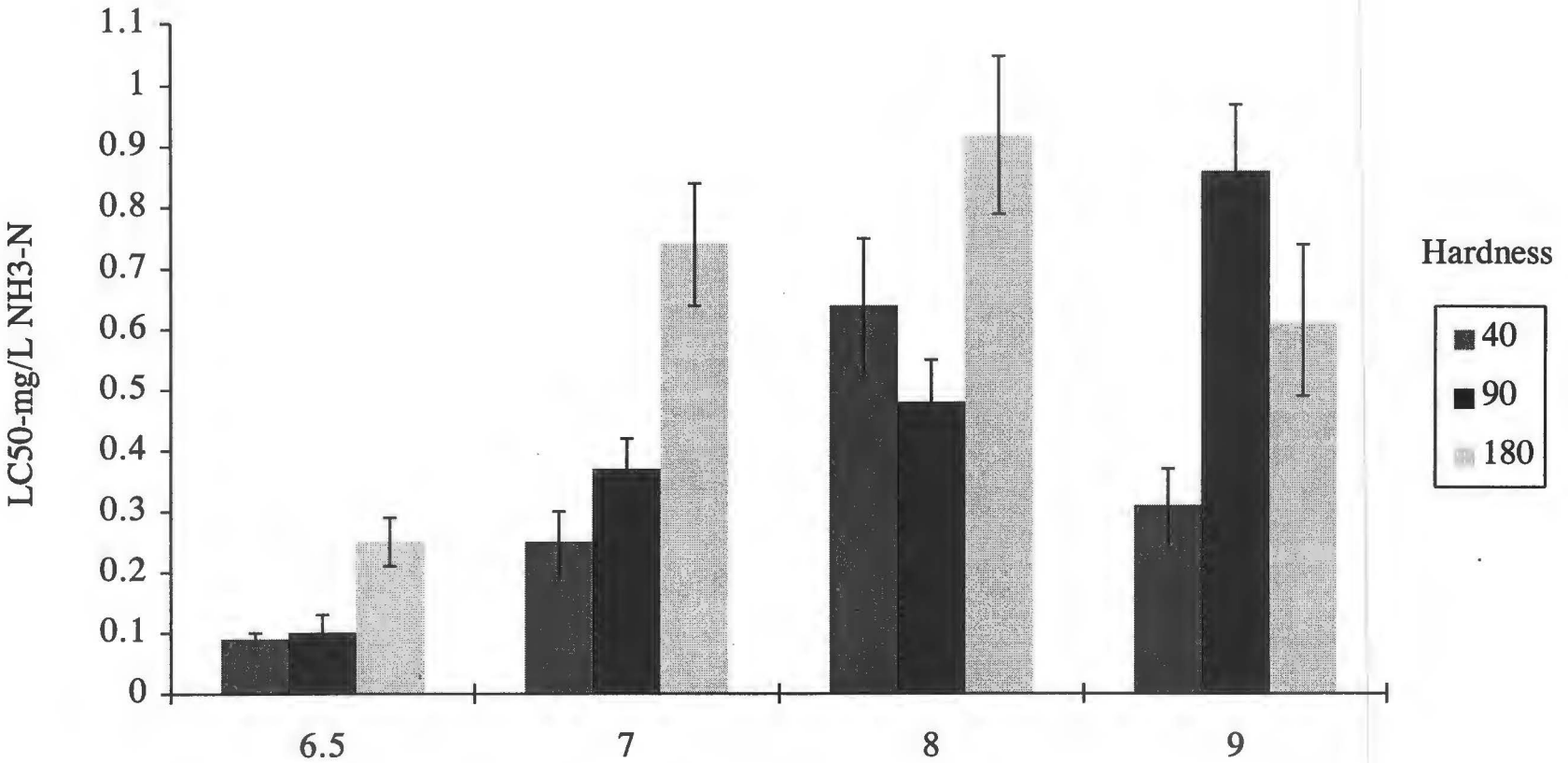


Figure 3. Effects of hardness on acute toxicity (LC50) of un-ionized ammonia (NH_3-N) to *C. dubia* at pH levels of about 6.5, 7, 8, and 9. Error bars represent 95% confidence intervals.

Effects of pH

Although acute toxicity of NH_3 to *C. dubia* appeared to decrease as pH increased from 6.5 to 8, the data (mean LC50 values for the three hardness concentrations at each pH level) for pH 7, 8, and 9 were not significantly different (Figure 4). Only toxicity at pH 6.5 was significantly different from other pH levels.

Un-ionized ammonia was most acutely toxic at pH 6.5 at all three hardness concentrations but the effects of pH were not uniform at higher levels of pH (Figure 5). In soft water, NH_3 was most toxic at pH 6.5, and least toxic at pH 8. In medium water, NH_3 also was most toxic at pH 6.5, but it was least toxic at pH 9. Acute toxicity values at pH 7 and 8 were intermediate and were not significantly different from one-another. In hard water, NH_3 was again most toxic at pH 6.5 but pH 7 and 8, as well as 7 and 9 were not significantly different from each other. Toxicity at pH 9 was significantly greater than at pH 8.

These results are comparable to another investigator, Tabata (1962) who found a steady decrease in toxicity of un-ionized ammonia to *D. magna* as pH increased from 6.0 to 8.0. Thurston et al (1981b) reported a similar pattern for fathead minnows and rainbow trout in which NH_3 toxicity decreased from pH 6.5 to 8.3, with a subsequent increase in toxicity at pH levels above 8.3 (Thurston et al. 1981b). In acute tests with channel catfish, Tomasso et al. (1980) found the

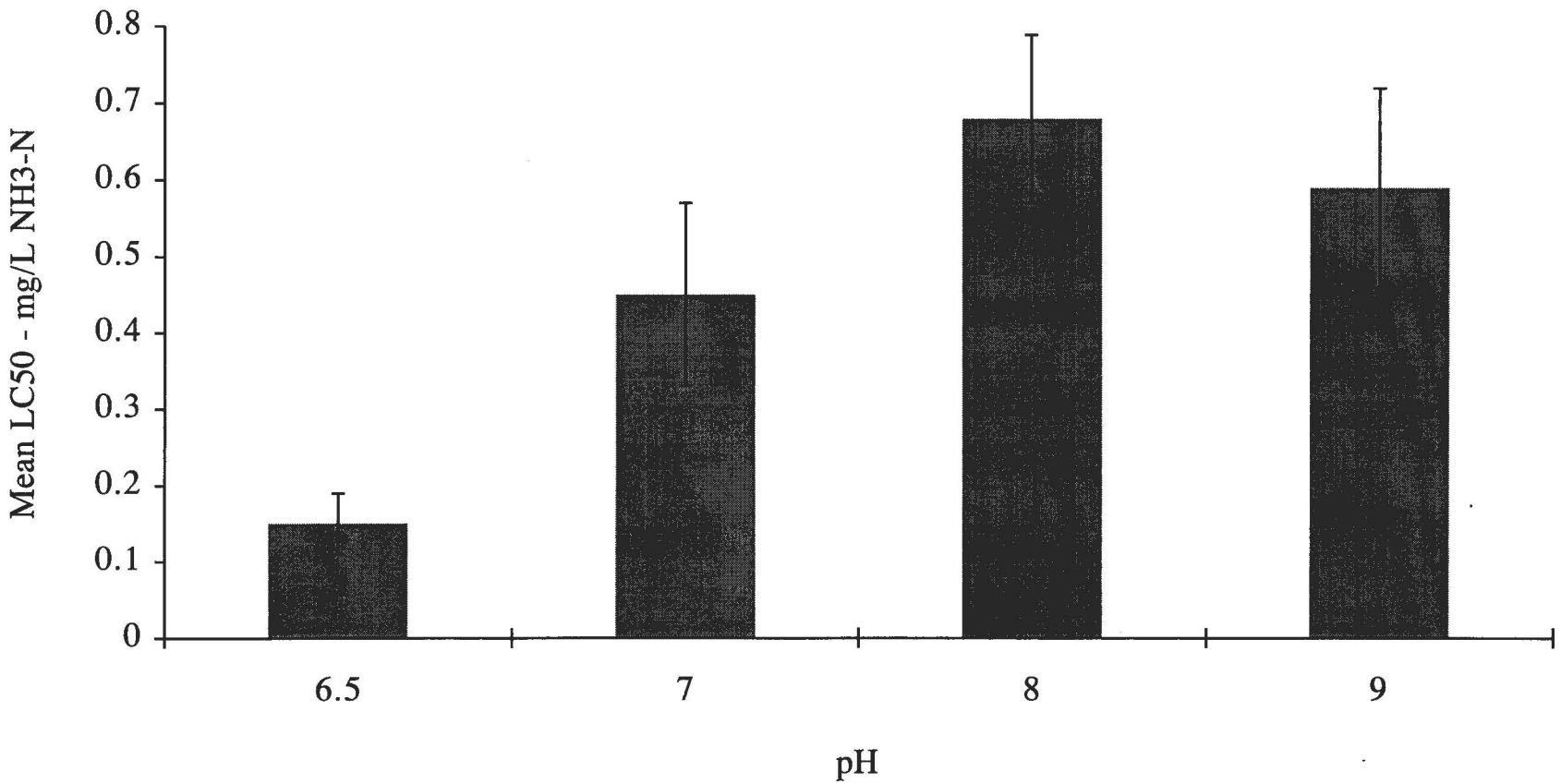


Figure 4. Average effect of pH on acute toxicity (LC50) of un-ionized ammonia (NH_3-N) to *C. dubia*. Histogram represents mean LC50 values for toxicity tests at hardness levels of about 40, 90, and 180 mg/L $CaCO_3$ at each pH. Error bars represent one standard error.

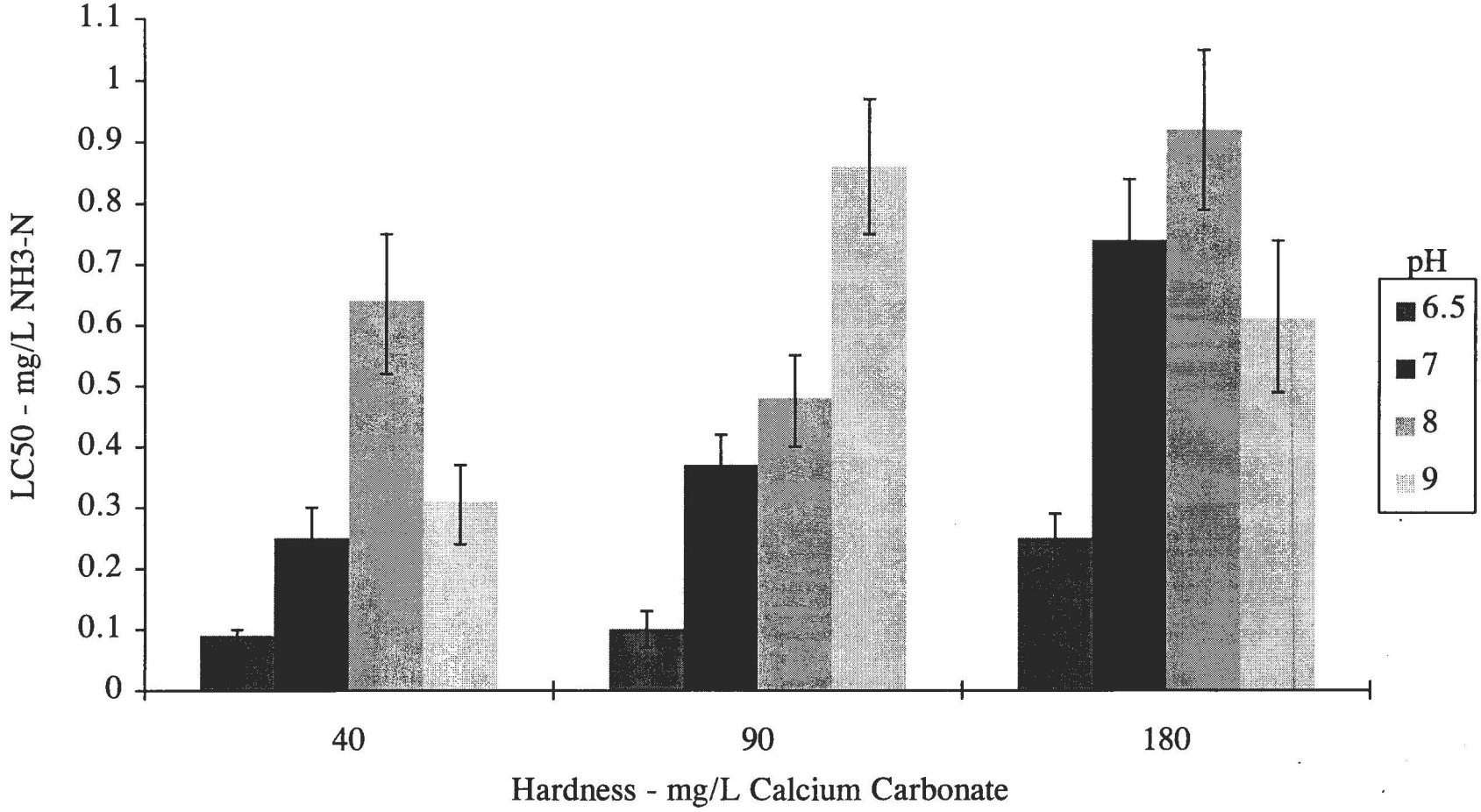


Figure 5. Effects of pH on acute toxicity (LC50) of un-ionized ammonia (NH_3-N) to *C. dubia* at hardness levels of about 40, 90, and 180 mg/L $CaCO_3$. Error bars represent 95% confidence intervals.

same trend reported by Thurston et al. (1981b). Broderius et al. (1985) found decreased toxicity of NH_3 to smallmouth bass as pH increased from 6.5 to 8.7.

Un-ionized ammonia may be more toxic at pH 6.5 because of direct pH associated stress. Mortality in controls exceeded 10% at pH 6.5 unless test organisms were fed at the beginning and at 24 hours after test initiation. Feeding at pH 6.5 does not alter data interpretation because toxicity in tests at pH 6.5 when organisms were fed was still significantly more toxic than at all the other pH levels.

Feeding Effects

Toxicity of NH_3 is significantly reduced (Spearman Karber Analysis; $p < 0.05$) when test organisms are fed. Without food, mortality was 50% or greater in all test concentrations including the controls at pH 6.5 in medium water (Figure 6). When food was provided, the only substantial mortalities were at the 60% (0.41 mg/L NH_3-N) and 100% (0.66 mg/L NH_3-N) concentrations of ammonium sulfate. Subsequent tests at pH 7, 8, and 9 had 90% or greater survival in the controls even without food; therefore, LC50s were compared between tests run with and without food (Figure 7). In both fed and un-fed tests toxicity decreased as pH increased, however NH_3 was at least twice as toxic in the unfed tests

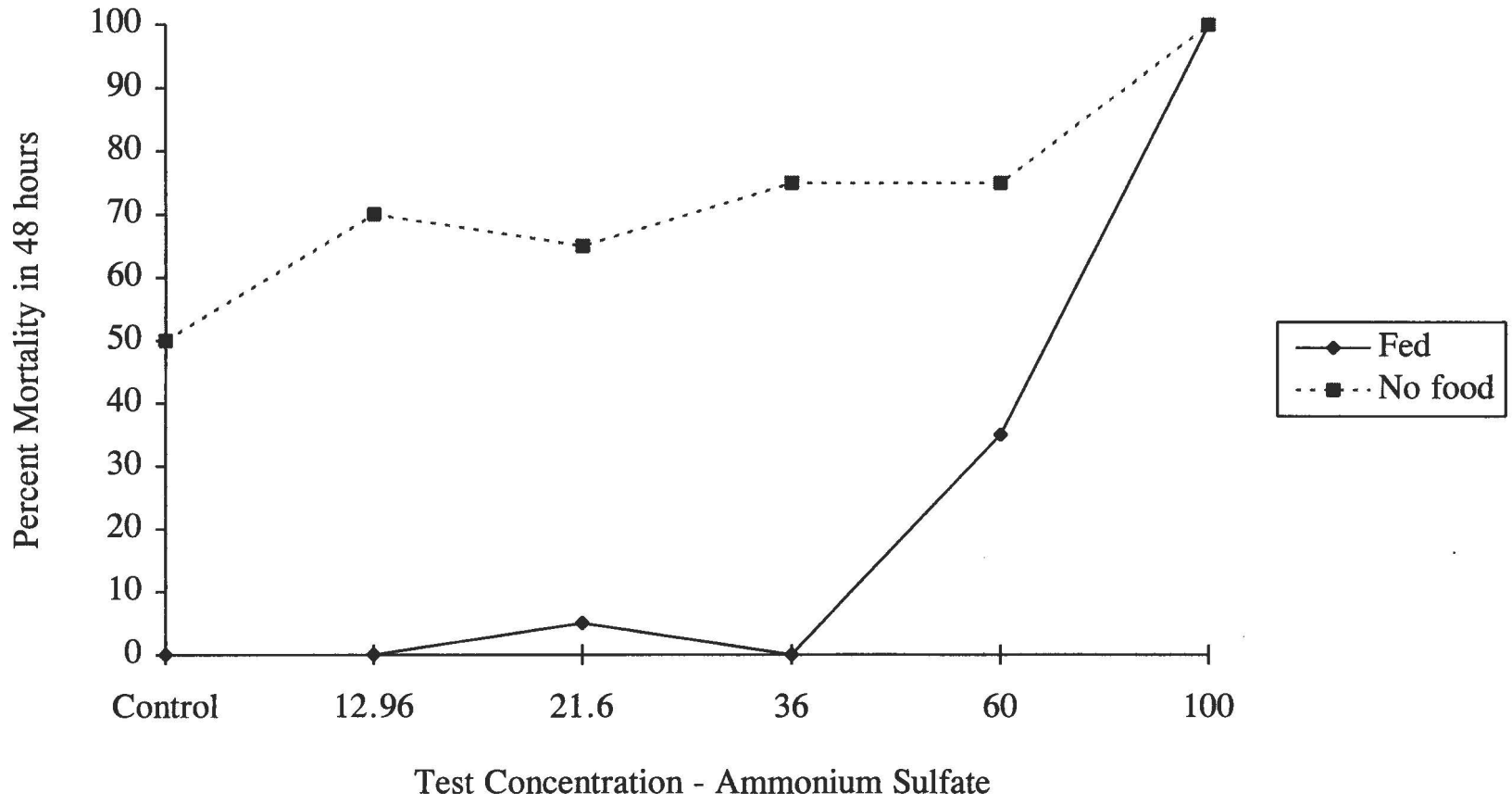


Figure 6. Effects of feeding on acute toxicity of ammonia to *C. dubia* at pH 6.5 in water of medium hardness.

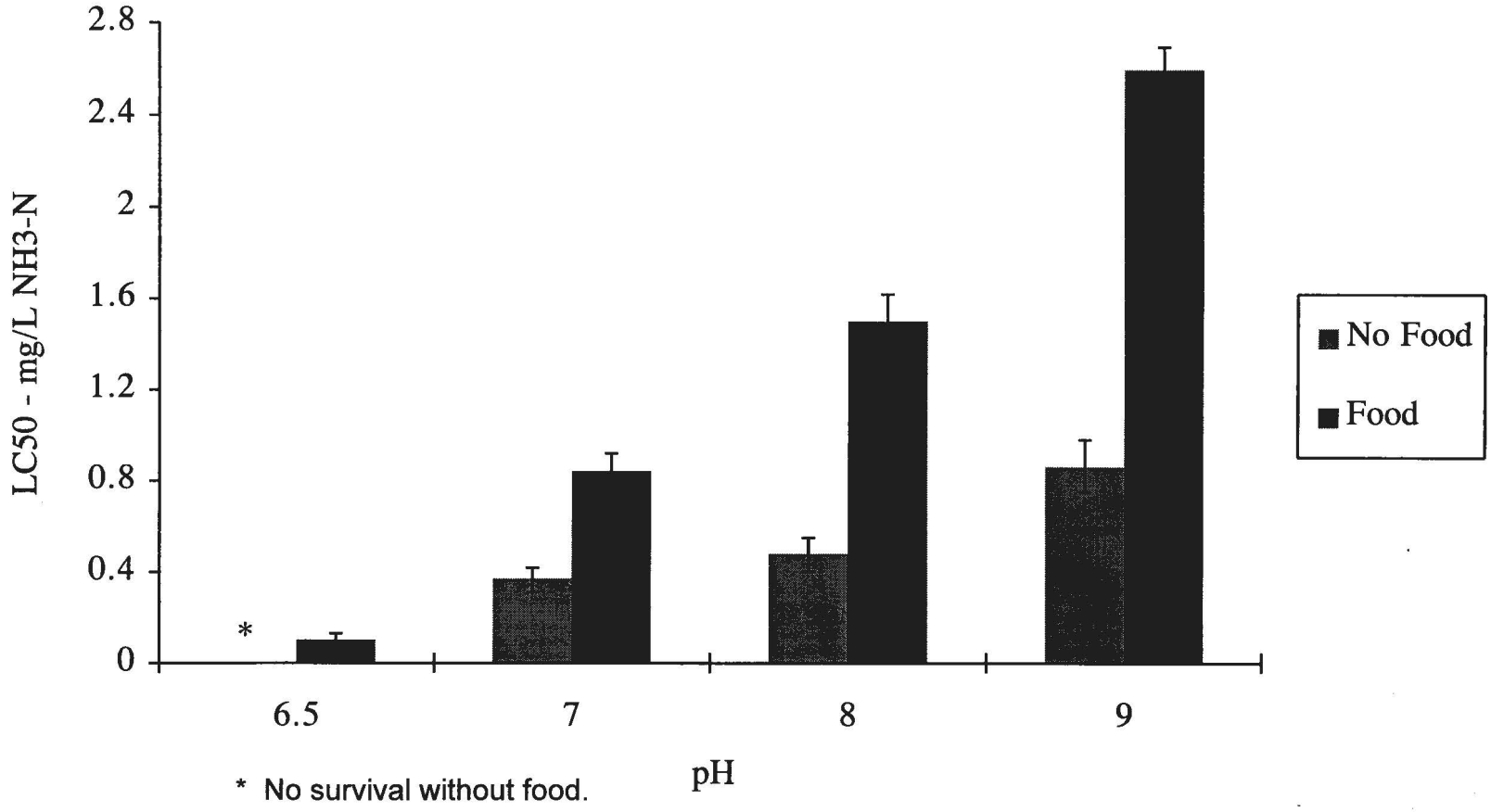


Figure 7. Effects of feeding on acute toxicity (LC50) of un-ionized ammonia (NH_3-N) to *C. dubia* at pH levels of about 6.5, 7, 8, and 9 in medium hardness water. Error bars represent 95% confidence intervals.

(Figure 7).

Comparison of Sensitivity of *C. dubia* to Acute Ammonia Exposure With Other Studies

Compared to other aquatic organisms, *C. dubia* appear to be among the most sensitive to un-ionized ammonia. Whereas my LC50 values ranged from 0.09 to 0.92 mg/L NH_3-N, most other invertebrates had LC50 values of 1.0 mg/L NH_3-N or higher (Table 1 in literature review section).

Rainbow trout, another sensitive organism to ammonia had a 96-hour LC50 range of 0.13 to 0.66 mg/L NH_3-N (Thurston et al. 1981b). In the only other comparable study of *C. dubia* that I found, the 48-hour LC50 was about 0.87 mg/L NH_3-N at pH 8.3 in water with a hardness of 102 mg/L (Cowgill and Milazzo 1991). For my test at pH 7.8 and hardness 92 mg/L $CaCO_3$, the LC50 was 0.48 mg/L NH_3-N. For *D. magna*, Tabata (1962) reported 24-hour LC50 range from 0.18 mg/L NH_3-N at pH 6.0 to 1.4 mg/L NH_3-N at pH 7.0 and 4.9 mg/L at pH 8.0. My most similar test in soft water, resulted in much lower LC50 values of: 0.09 mg/L NH_3-N at pH 6.5, 0.25 mg/L NH_3-N at pH 7.0 and 0.64 mg/L NH_3-N at pH 8.0 which may demonstrate that *D. magna* is more tolerant of un-ionized ammonia than *C. dubia*.

Chronic Toxicity

Low 25% inhibition concentration (IC25) or 50% inhibition concentration (IC50) values indicate greater toxicity and therefore, greater reduction in number of young produced. Concentrations of un-ionized ammonia that resulted in 25% inhibition of reproduction in *C. dubia* ranged from 0.03 to 0.91 mg/L NH_3-N and 50% inhibition of reproduction ranged from 0.07 to 1.01 mg/L NH_3-N (Table 8). Effects of NH_3 on *C. dubia* reproduction were greatest at pH 6.5 in medium water, and least at pH 9.0 in hard water (Table 8).

Hardness Effects

Hardness had an inconsistent affect on chronic toxicity of NH_3 to *C. dubia*. Mean IC25 and IC50 values for NH_3 for each level of pH were not significantly different among the three hardness concentrations. Chronic toxicity of NH_3 at pH levels of about 6.5, 7, 8, and 9 generally appeared to be greater in the softer water, but differences in many of the tests were not significant (Figures 8, 9).

For IC25, changes in hardness were not statistically significant at pH 6.5, 7, and 9 (Figure 8). At pH 8 un-ionized ammonia was significantly more toxic in soft water than in medium water, but results for soft and hard water were not

Table 8. Chronic toxicity of ammonia to *C. dubia* at pH levels of about 6.5, 7, 8, and 9 and hardness levels of about 40, 90, and 180 mg/L $CaCO_3$. Values are expressed as the 25% inhibition concentration (IC25) and 50% inhibition concentration (IC50) for un-ionized ammonia-nitrogen (NH_3-N) and total ammonia-nitrogen (TAN). Data for hardness and pH are mean values and ranges for pH are reported in parentheses following the mean values.

pH[a]	Hardness mg/L $CaCO_3$	IC25 mg/L NH_3-N (95% CI)[b]	IC50 mg/L NH_3-N (95% CI)	IC25 mg/L TAN (95% CI)	IC50 mg/L TAN (95% CI)
6.45 (6.37-6.48)	41.3	0.06 (0.02-0.07)	0.08 (0.06-0.09)	39.2 (12.1-50.9)	53.7 (40.7-58.4)
6.51 (6.49-6.55)	88.0	0.03 (0.02-0.06)	0.07 (0.04-0.10)	20.9 (13.6-35.7)	45.9 (31.1-55.7)
6.55 (6.54-6.56)	173	0.03 (0.02-0.07)	0.09 (0.04-0.12)	19.7 (12.1-42.1)	49.0 (21.1-70.0)
7.12 (7.01-7.19)	41.3	0.20 (0.12-0.27)	0.30 (0.20-0.36)	33.5 (18.8-48.5)	54.5 (33.1-72.1)
7.13 (7.05-7.19)	85.3	0.07 (0.05-0.35)	0.34 (0.09-0.43)	20.8 (12.6-56.0)	58.0 (52.7-68.0)
7.13 (7.07-7.18)	164	0.41 (0.12-0.45)	0.50 (0.45-0.52)	65.8 (18.5-72.0)	81.9 (73.7-85.9)
7.96 (7.76-8.01)	42.7	0.14 (0.09-0.23)	0.27 (0.17-0.34)	2.8 (1.7-4.5)	5.4 (3.4-7.1)
7.96 (7.84-8.04)	88.0	0.34 (0.29-0.60)	0.75 (0.60-0.83)	8.4 (7.0-17.8)	23.4 (17.9-26.4)
8.05 (8.00-8.09)	168	0.81 (0.17-0.93)	1.01 (0.84-1.09)	16.5 (2.97-19.4)	21.6 (17.2-23.7)
8.24 (8.13-8.29)	44.0	0.27 (0.09-0.49)	No 50% reduction	3.2 (0.93-6.67)	No 50% reduction
8.59 (8.45-8.68)	82.7	0.35 (0.28-0.50)	0.88 (0.77-1.05)	1.99 (1.53-3.01)	5.93 (5.05-7.38)
8.77 (8.71-8.78)	173.3	0.91 (0.43-1.27)	No 50% reduction	4.2 (1.19-6.08)	No 50% reduction

[a] Dissolved oxygen ranged from 7.5-8.0 mg/L. Temperature ranged from 22.1-23.6 C.

[b] CI = 95% confidence intervals provided in parentheses.

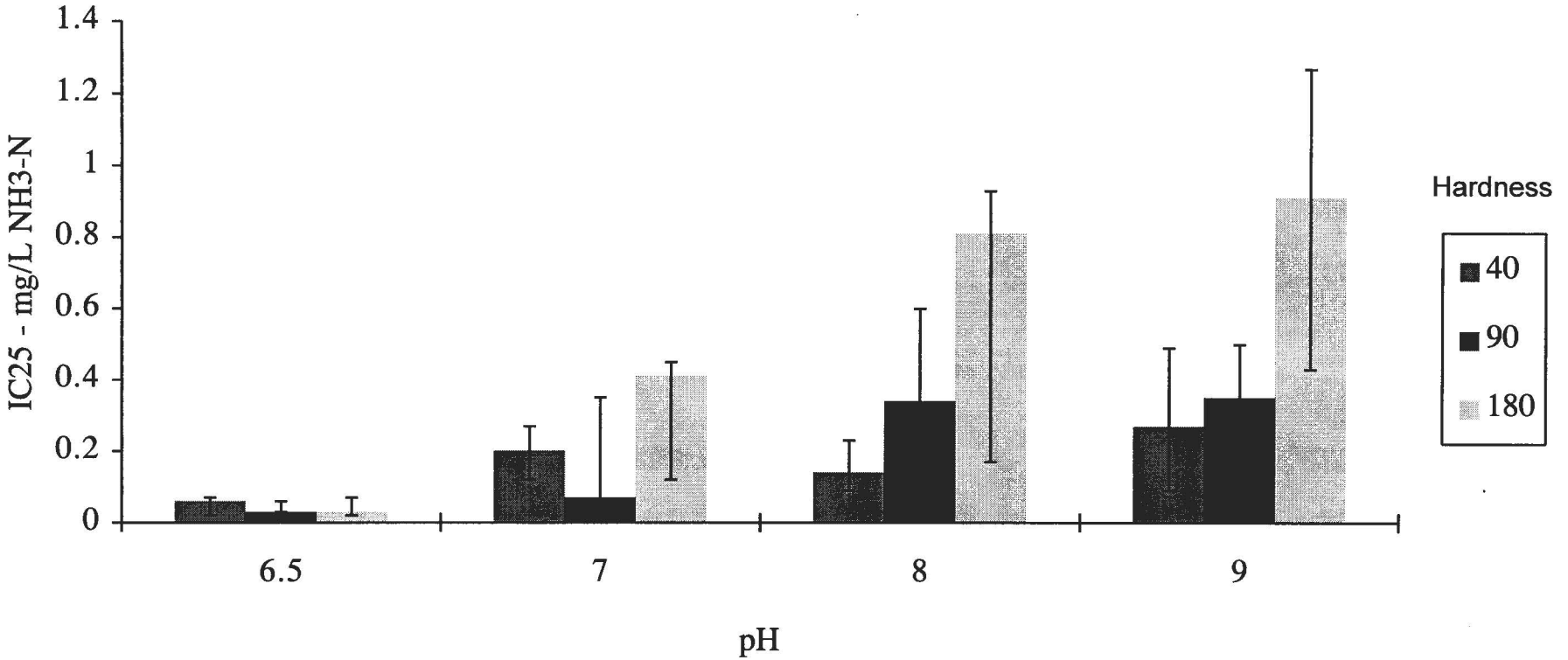


Figure 8. Effects of hardness on chronic toxicity of un-ionized ammonia (NH_3-N) to *C. dubia* at pH levels of about 6.5, 7, 8, and 9. The IC25 is the NH_3-N concentration at which there is a 25% reduction in reproduction as compared to the controls. Error bars represent 95% confidence intervals.

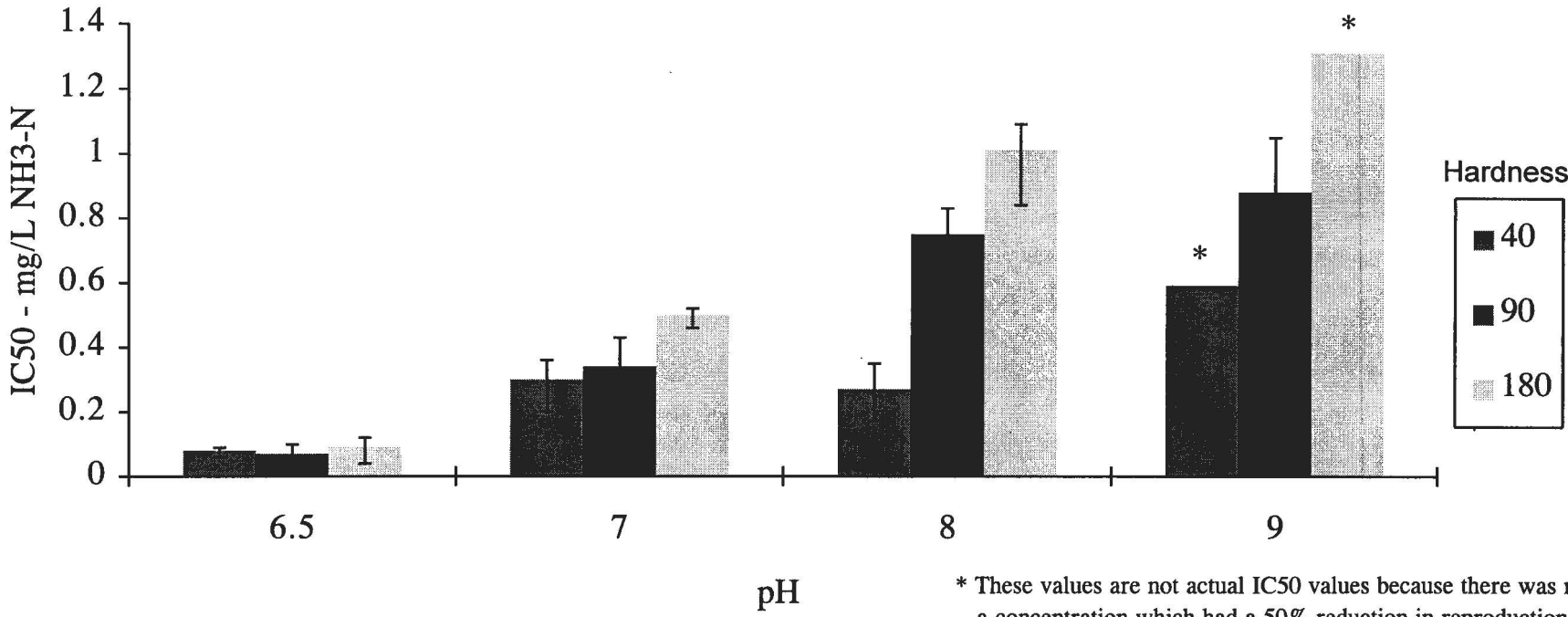


Figure 9. Effects of hardness on chronic toxicity of un-ionized ammonia (NH_3-N) to *C. dubia* at pH levels of about 6.5, 7, 8, and 9. The IC50 is the NH_3-N concentration at which there is a 50% reduction in reproduction as compared to the controls. Error bars represent 95% confidence intervals.

significantly different. For IC50 values, at pH 7, un-ionized ammonia was significantly less toxic in hard water, but soft and medium water were not significantly different from each other (Figure 9). At pH 8, un-ionized ammonia toxicity decreased as hardness increased. No significant differences occurred at pH 6.5 and at pH 9 no comparison was possible. I did not find any information on effects of water hardness on chronic ammonia toxicity in published literature.

Effects of pH

Un-ionized ammonia causes the greatest reduction in reproduction at low pH. Mean chronic toxicity for all hardness levels was significantly greater (lower IC25 and IC50 values) at pH 6.5 than at other pH levels which were not significantly different from eachother (Figures 10). For the IC50 both pH 6.5 and 7 mean values were significantly different from other values, with 6.5 the most toxic level (Figure 11). At each hardness concentration NH_3 was most toxic at pH 6.5 (Figures 12, 13).

For the IC25 at all hardness levels, pH 7, 8, and 9 were not significantly different from each other (Figure 12).

For the IC50 in soft water, NH_3 was significantly more toxic at pH 6.5 than at pH 7, 8, or 9 (Figure 13). In medium water, NH_3 was most toxic at both pH

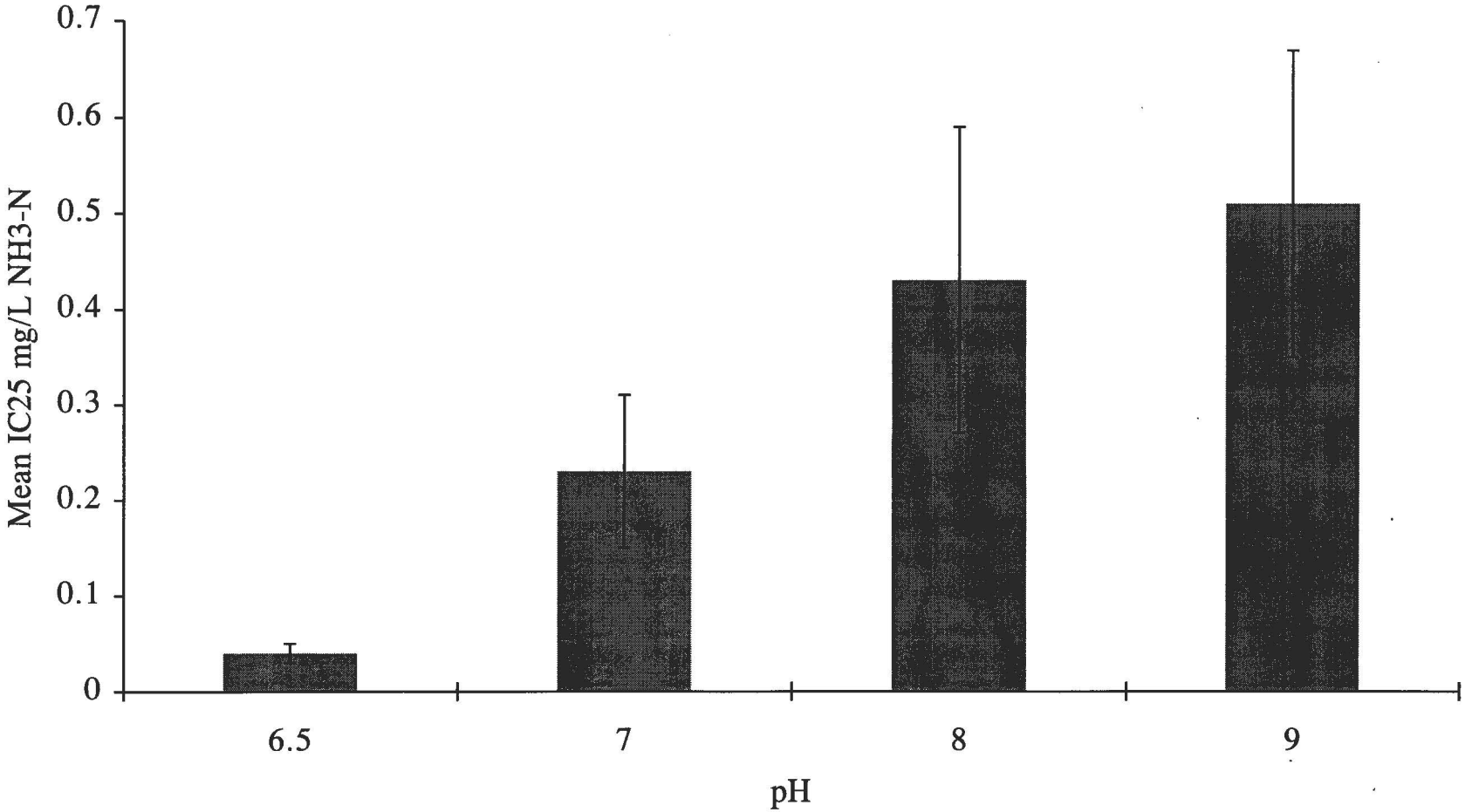


Figure 10. Average effect of pH on chronic toxicity of un-ionized ammonia (NH_3-N) to *C. dubia*. Histogram represents mean 25% inhibition concentration (IC25) values for toxicity tests at hardness levels of about 40, 90, and 180 mg/L $CaCO_3$ at each pH. Error bars represent one standard error.

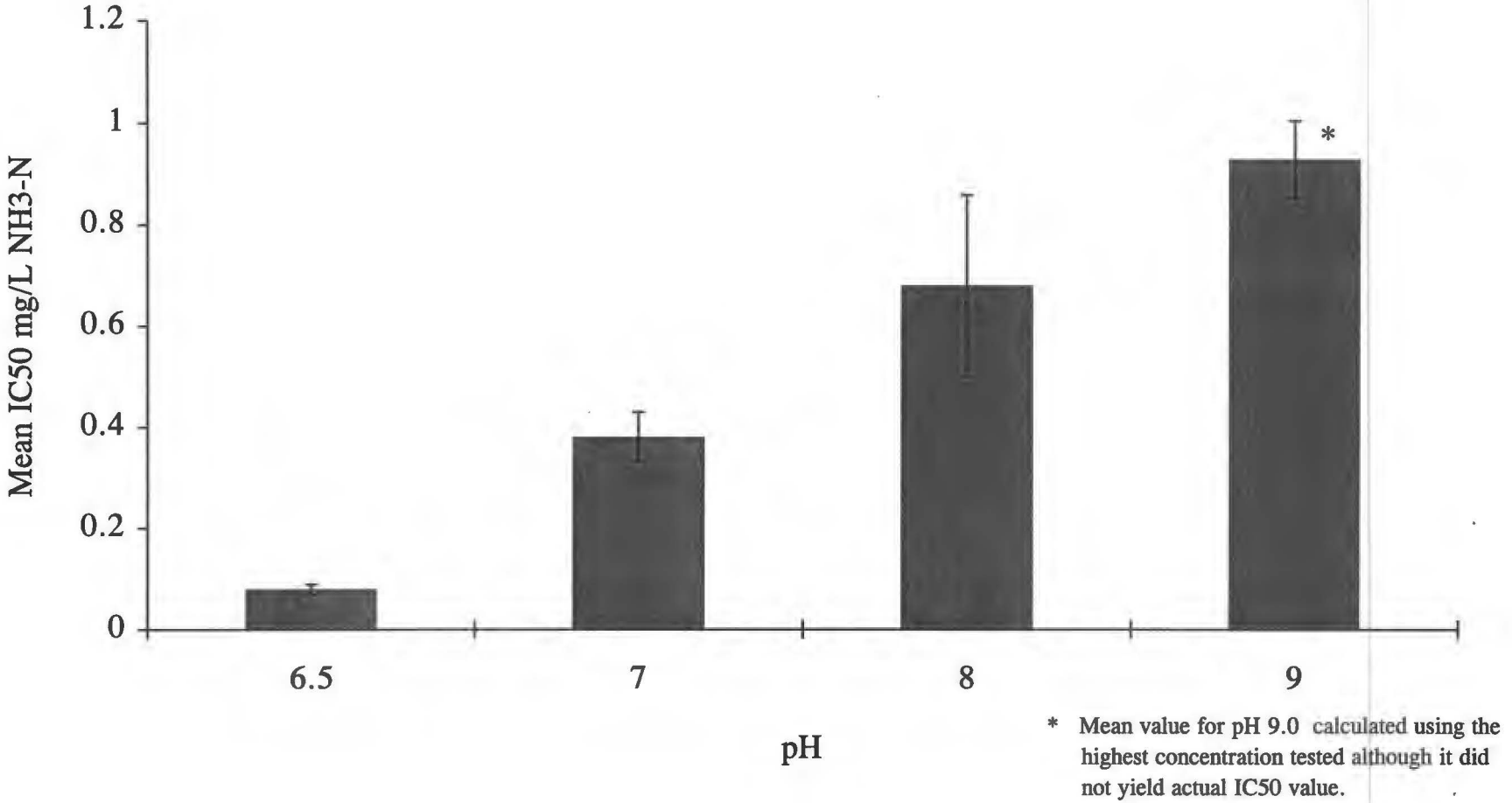


Figure 11. Average effect of pH on chronic toxicity of un-ionized ammonia (NH_3-N) to *C. dubia*. Histogram represents mean 50% inhibition concentration (IC50) values for toxicity tests at hardness levels of about 40, 90 and 180 mg/L $CaCO_3$ at each pH. Error bars represent one standard error.

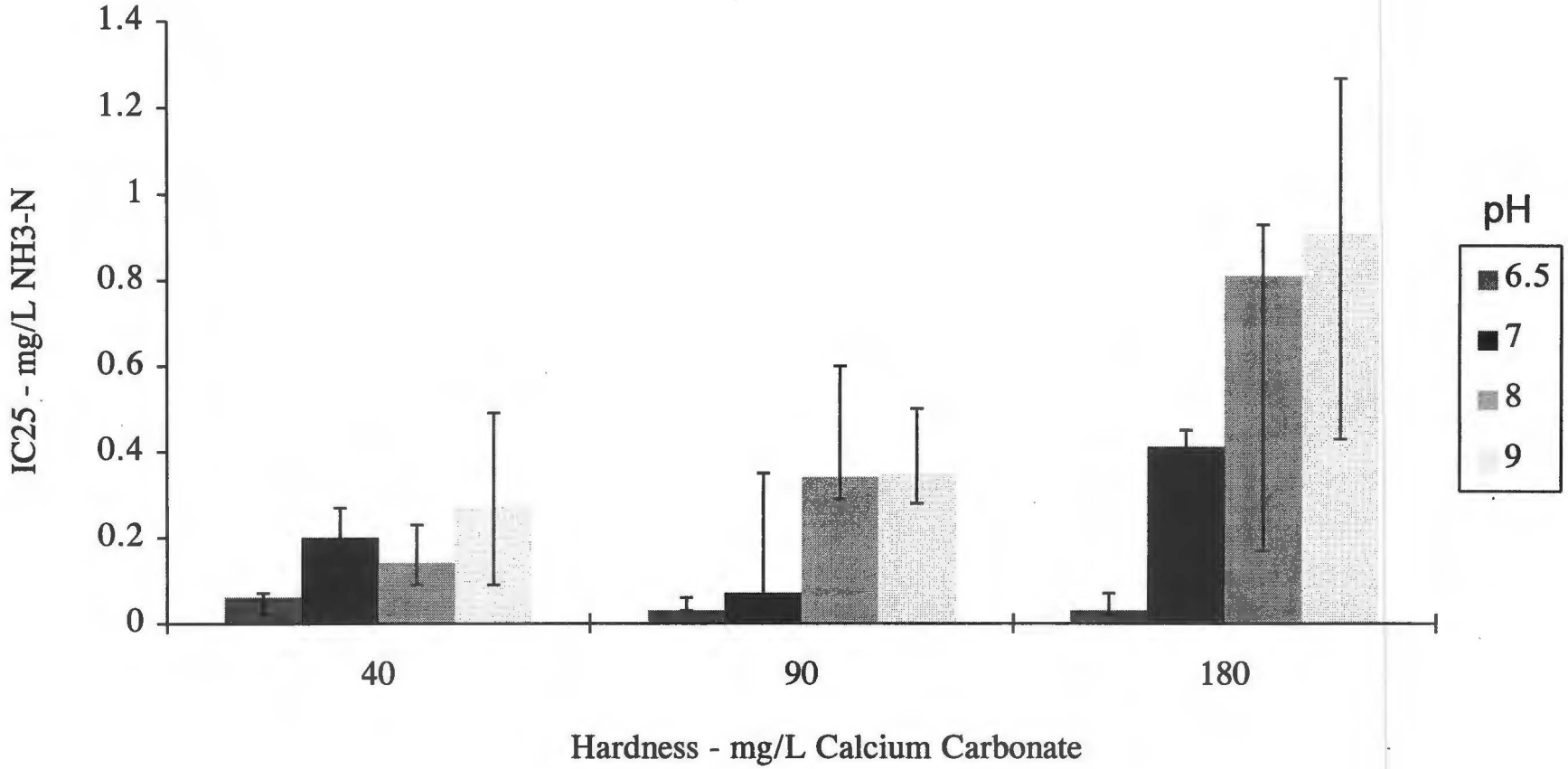
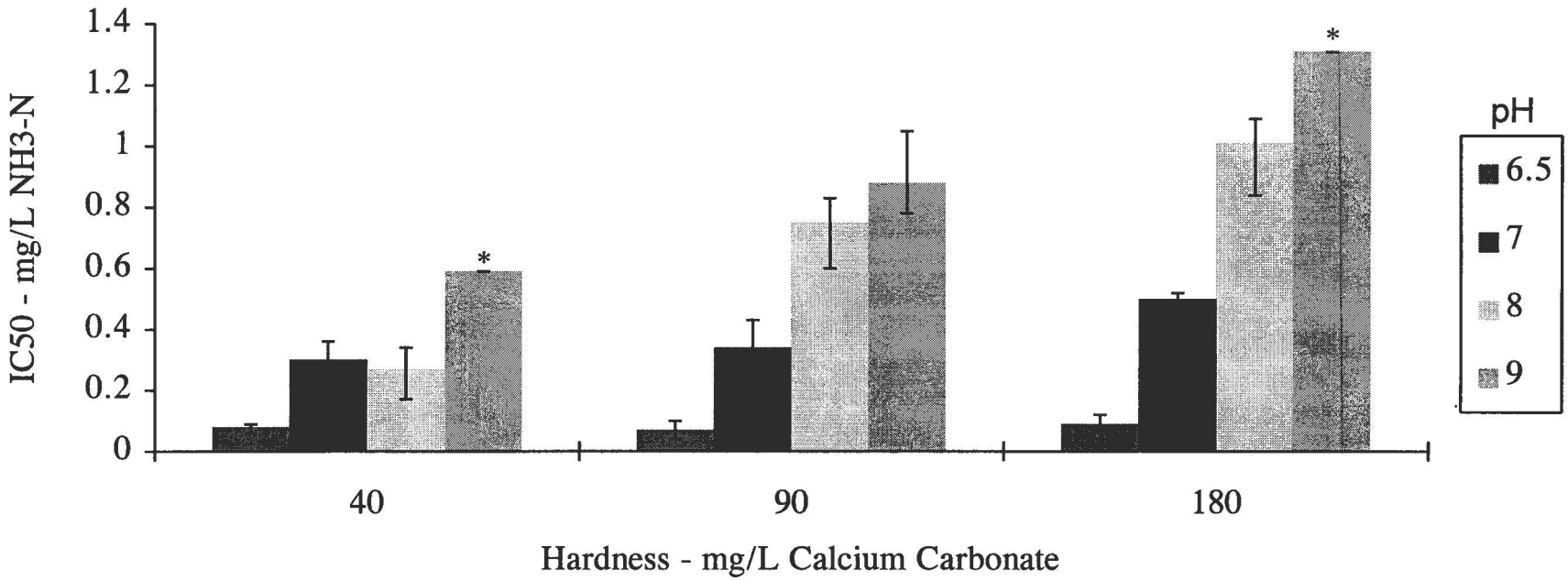


Figure 12. Effects of pH on chronic toxicity of un-ionized ammonia (NH_3-N) to *C. dubia* at hardness levels of about 40, 90, and 180 Mg/L $CaCO_3$. The IC25 is the NH_3-N concentration at which there is a 25% reduction in reproduction as compared to the controls. Error bars represent 95% confidence intervals.



* These values are not actual IC50 values because there was not a concentration which had a 50% reduction in reproduction. The values shown are the highest concentration tested at the specified pH level.

Figure 13. Effects of pH on chronic toxicity of un-ionized ammonia (NH_3N) to *C. dubia* at hardness levels of about 40, 90, and 180 mg/L $CaCO_3$. The IC50 is the NH_3-N concentration at which there is a 50% reduction in reproduction as compared to the controls. Error bars represent 95% confidence intervals.

6.5 and 7, which were not significantly different from each other. Un-ionized ammonia was least toxic at pH 8 and 9 which were not significantly different from each other. In hard water, un-ionized ammonia toxicity decreased significantly as pH increased.

In the only other study that I found addressing the effect of pH on chronic toxicity of un-ionized ammonia, smallmouth bass were tested. Chronic toxicity of un-ionized ammonia decreased as pH increased. The estimated 32-day no-observed-effect concentrations (geometric means of maximum no-effect and minimum effect concentrations based on decreased growth) were 0.04, 0.15, 0.60, and 0.61 mg/L NH_3-N at pH values of 6.6, 7.3, 7.8, and 8.7 (Broderius et al. 1985).

Comparison of Sensitivity of *C. dubia* to Chronic Ammonia Exposure With Other Studies

My estimates of chronic-effect levels -- IC25 range of 0.03 to 0.91 and IC50 range of 0.07 to 1.01 mg/L NH_3-N -- were somewhat similar to those of two other studies of *C. dubia*. Nimmo et al. (1989) reported a LOEC for reduction in reproduction of 0.88 mg/L *C. dubia* at pH 8 in river water. Cowgill and Milazzo (1991) reported a NOEC of 0.73 mg/L NH_3-N in medium water at pH 8.3.

Chronic NH_3 toxicity also appears to concur for *D. magna*. Gersich and Hopkins (1986) reported a LOEC for reduction in reproduction of 0.87 mg/L NH_3-N at pH 8.0 in hard water. Fish also may have similar sensitivity although chronic tests utilizing fish are not easily compared with invertebrate reproduction data because effects measured for fish are typically growth, embryo survival, and other variables over long periods of time. Lowest-observable-effect-concentrations for fish have ranged from 0.01 to 0.60 mg/L NH_3-N (U.S. EPA 1985).

Comparison of Acute and Chronic Results

Both acute and chronic tests suggested that toxicity generally decreased as hardness increased, but hardness effects were more pronounced in acute tests. Acute and chronic tests demonstrated greater toxicity of NH_3 at lower pH values; toxicity was greatest at pH 6.5 and least at pH 8 or 9. Endpoints for acute and chronic toxicity tests may not be directly comparable because organisms in acute tests were not fed and I found that feeding *C. dubia* increased their tolerance of ammonia. However, ignoring the problem, the LC50 for some tests was similar to or less than the IC50 under the same conditions (Tables 5, 7). In some cases IC50 and LC50 values may be similar if inhibition of reproduction is partially caused by mortality of test organisms which, therefore failed to reproduce.

Possible Mechanisms of Toxicity

Ammonia may be less toxic to *C. dubia* at higher hardness because the higher concentration of ions may decrease the permeability of cell membranes to ammonia (Tomasso et al. 1980), or the extra ions may provide a more optimum osmotic balance and result in a less stressful environment during tests.

Abnormal pH itself may cause stress, thereby increasing toxicity of ammonia (Thurston et al. 1981b). It is also possible that un-ionized ammonia toxicity was not constant over the range of pH levels because at the lowest pH, total ammonia was present in large quantities and NH_4^+ may have been exerting a toxic affect in addition to the un-ionized ammonia (Broderius et al. 1985). The increased concentration of H^+ may also have been increasing the toxicity of un-ionized ammonia (Thurston et al. 1981b).

LITERATURE CITED

Alexander, H.C., P.B. Latvaitis, and D.L. Hopkins. 1986. Site-specific toxicity of un-ionized ammonia in the Tittawasse River at Midland, MI: Overview. Environmental Toxicology and Chemistry 5:427-435.

American Petroleum Institute. 1981. The sources, chemistry, fate and effects of ammonia in aquatic environments. Ecological Analysts, Inc. American Petroleum Institute, Washington D.C.

American Public Health Association (APHA), American Water Works Association and Water Pollution Control Federation. 1989. Standard methods for the examination of water and waste-water. American Public Health Association, Washington DC.

Arthur, J.W., C.W. West, K.A. Allen, and S.E. Hedtke. 1987. Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bulletin of Environmental Contamination and Toxicology 38:324-331.

Bader, J.A., and J.M. Grizzle. 1992. Effects of ammonia on growth and survival of recently hatched channel catfish. Journal of Aquatic Animal Health 4:17-23.

Broderius, S., R. Drummond, J. Fiandt, and C. Russom. 1985. Toxicity of ammonia to early life stages of the small mouth bass at four pH values. Environmental Toxicology and Chemistry 4:87-96.

Burrows, R.E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. Research Report United States Fish and Wildlife Service 66:1-12.

Chen, J., K. Chen, and J. Liao. 1989. Joint action of ammonia and nitrite on *Artemia nauplii*. Aquaculture 77: 329-336.

Colt, J., and G. Tchobanoglous. 1976. Evaluation of short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus*. Aquaculture 8(3):209-224.

Colt, J., and G. Tchobanoglous. 1978. Chronic exposure of channel catfish, *Ictalurus punctatus*, to ammonia: effects on growth and survival.

Aquaculture 15:353-372.

Cowgill, U.M., and D.P. Milazzo. 1991. The response of the three-brood *Ceriodaphnia* test to 15 formulations and pure compounds in common use. Archives of Environmental Contamination Toxicology 21:35-40.

Diamond, J.M., D.G. Mackler, W.J. Rasnake, and D. Gruber. 1993. Derivation of site-specific ammonia criteria for an effluent-dominated headwater stream. Environmental Toxicology and Chemistry 12:649-658.

Effler, S.W., C.M. Brooks, M.T. Auer, and S.M. Doerr. 1990. Free ammonia and toxicity criteria in a polluted urban lake. Research Journal of the Water Pollution Control Federation 62(6):771-779.

Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effects of pH and temperature. Journal of Fisheries Research Board Canada 32:2379-2383.

Flis, J. 1968. Anatomichohistopathological changes induced in carp by ammonia water. Part 1. Effects of toxic concentrations. Acta Hydrobiologica 10:205-214.

Fromm, P., and J. Gillette. 1968. Effects of ambient ammonia on blood and nitrogen excretion of rainbow trout. Comp. Biochemistry and Physiology. 26:887-893.

Gersich, F.M., and D.L. Hopkins. 1986. Site-specific acute and chronic toxicity of ammonia to *Daphnia magna* (*straus*). Environmental Toxicology and Chemistry 5:443-447.

Gulley, D.D. and West, Inc. 1994. Toxstat 3.4. Western Ecosystems Technology,Cheyenne, WY.

Lloyd, R., and L.D. Orr. 1969. The diuretic response by rainbow trout to sub-lethal concentrations of ammonia. Water Research 3:335-344.

Maetz, J. and F. Garcia-Romeu. 1964. The mechanisms of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus*. II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. Journal of General Physiology 47:1209-1227.

Mayes, M.A., H.C. Alexander, and D.L. Hopkins. 1986. Acute and chronic toxicity of ammonia to freshwater fish a site specific study. Environmental Toxicology and Chemistry 5:437-442.

Monson, P.D., M.K. Schubauer-Berigan, and G.T. Ankley. 1993. Interactive effects of hardness and pH on toxicity of ammonia to *Hyalella azteca*. Abstract 1993, Annual Conference of the Society of Environmental Toxicology and Chemistry, Houston, TX.

Mount, D.R., and D.I. Mount. 1992, A simple method for static and static-renewal aquatic toxicity tests. Environmental Toxicology and Chemistry 11:609-614.

Niederlehner, B.R., and J. Cairns, Jr. 1990. Effects of ammonia on periphytic communities. Environmental Pollution 66:207-221.

Nimmo, D.W.R., D. Link, L.P. Parrish, G.J. Rodriguez, W. Wuerthele, and P.H. Davies. 1989. Comparison of on-site and laboratory toxicity tests: derivation of site-specific criteria for un-ionized ammonia in a Colorado transitional stream. Environmental Toxicology and Chemistry 8:1177-1189.

Norberg, T.J. and D.I. Mount. 1984. A seven day life-cycle cladoceran toxicity test. Environmental Toxicology and Chemistry 3:425-434.

Robinette, H.R., J.M. Heinen, D.L. Straus, J.W. Pote, and P.N. Deliman. 1988. Ammonia and pH toxicity and water quality management for freshwater shrimp. Technical completion report. Project number G1431-04. Water Resources Research Instititute, Mississippi State University, Mississippi. Pages iii-19.

Ruffier, P.J., W.C. Boyle, and J. Kleinschmidt. 1981. Short-term acute bioassays to evaluate ammonia toxicity and effluent standards. Journal of the Water Pollution Control Federation 53(3):367-377.

Schenker, S., D.W. McCandless, E. Brophy, and M.S. Lewis. 1967. Studies on the intracerebral toxicity of ammonia. Journal of Clinical Investigation 46:838-848.

Smart, G.R. 1976. The effect of ammonia exposure on gill structure of the rainbow trout. Journal of Fish Biology 8:471-475.

Smart, G.R. 1978. Investigations of the toxic mechanisms of ammonia to fish-gas exchange in rainbow trout exposed to acutely lethal concentrations. Journal of Fish Biology 12:93-104.

Szumski, D.S., D.A. Barton, H.D. Putnam, and R.C. Polta. 1982. Evaluation of EPA un-ionized ammonia toxicity criteria. Journal of the Water Pollution Control Federation 54(3):281-291.

Tabata, K. 1962. Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbonic acid. (English translation). Vull. Tokai. Fish. Lab. 34:67-73.

Thurston, R.V., R.C. Russo, and K. Emerson. 1979. Aqueous ammonia equilibrium tabulation of percent un-ionized ammonia. U.S. Environmental Protection Agency, EPA 600/3-79-091, Duluth, Minnesota.

Thurston, R.V., G.R. Phillips, and R.C. Russo. 1981a. Increased toxicity of ammonia to rainbow trout resulting from reduced concentration of dissolved oxygen. Canadian Journal of Fisheries and Aquatic Science 38:983-988.

Thurston, R.V., G.A. Vinogradov, and R.C. Russo. 1981b. Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. Environmental Science and Technology 15:37-40.

Thurston, R.V., G.R. Phillips and R.C. Russo. 1983. Acute toxicity of ammonia to fathead minnows. Transactions of the American Fisheries Society 112:705-711.

Thurston, R.V. and R.C. Russo. 1983. Acute toxicity of ammonia to rainbow trout. Transactions of the American Fisheries Society 112:696-704.

Thurston, R.V., R.C. Russo, E.L. Meyn, and R.K. Zajdel. 1986. Chronic toxicity of ammonia to fathead minnows. Transactions of the American Fisheries Society 115:196-207.

Tomasso, J.R., C.A. Goudie, B.A. Simco, and K.B. Davis. 1980. Effects of environmental pH and calcium on ammonia toxicity in channel catfish. Transactions of the American Fisheries Society 109:229-234.

U.S. Environmental Protection Agency. 1985. Ambient water quality criteria for ammonia. Office of Water Quality Regulations and Standards, U.S. Environmental Protection Agency, Washington, D.C. EPA-440/5-85-001

U.S. Environmental Protection Agency. 1989. Short term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. U.S. Environmental Protection Agency, Cincinnati, OH. EPA-600/4-89/001.

U.S. Environmental Protection Agency. 1991. Methods for measuring acute toxicity of effluents and receiving waters to freshwater and marine organisms. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. EPA-600/4-90/027.

U.S. Environmental Protection Agency. 1993. A linear interpolation method for sublethal toxicity: the inhibition concentration (ICp) approach. Version 2.0. Environmental Research Laboratory Duluth, MN.

Wajsbrot, N., A. Gasith, M.D. Krom, and D.M. Popper. 1991. Acute toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* under reduced oxygen levels. Aquaculture 92:277-288.

Willingham, W.T., J.E. Colt, J.A. Fava, B.A. Hillaby, C.L. Ho, M. Katz, R.C. Russo, D.L. Swanson, and R.V. Thurston. 1979. Ammonia. Pages 6-18 in: A review of the EPA Red Book: Quality criteria for water. Water Quality Section, American Fisheries Society, Bethesda, MD.

Williams, K.A., D.W.J. Green, and D. Pascoe. 1986. Studies on the acute toxicity of pollutants to freshwater macroinvertebrates 3. Ammonia. Archiv Fuer Hydrobiologie 106(1):61-70.