ACUTE AND CHRONIC TOXICITY OF AMMONIA ON *PSEUDODIAPTOMUS FORBESI*

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Objective

To determine unionized ammonia and ionized ammonia concentrations and pH levels that cause acute and chronic toxicity in *Pseudodiaptomus forbesi.*

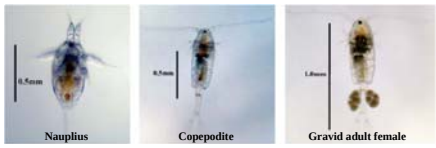


Figure 1. Life Stages of *Pseudodiaptomus forbesi*

Abstract

This study was conducted to determine the acute and chronic effects of ammonia, and the interaction of pH and ammonia to the copepod, *Pseudodiaptomus forbesi*. The 4 days (d) median lethal concentration (LC50) was determined for unionized ammonia (UA) and ionized ammonia (IA) for 1) pH 7.4, 2) pH 7.8 and 3) constant Total Ammonia Nitrogen (TAN) of 3.71 mg/L for pH 7.0, 7.4, 7.8, 8.2 and 8.6. The survival, growth and reproduction will be determined from the chronic exposure study using 0.25, 0.5, 1, 2 and 4 mg/L of TAN at pH 7.8 for 30 d. The results indicate *P. forbesi* was more sensitive to IA toxicity compared to UA at constant pH. However, *P. forbesi* was more sensitive to UA as the pH varied. Results from the chronic exposure study are pending and will be important in determining sublethal ammonia effects on the abundance of *P. forbesi*. These results will help to establish guidelines for water quality maintenance and to better sustain an important food source of pelagic fish in the SFE.

Introduction

The calanoid copepod *Pseudodiaptomus forbesi* is an important food source to larval and juvenile stages of fish such as delta smelt, longfin smelt, and threadfin shad in the San Francisco Estuary. The estuary experiences continuous anthropogenic inputs of ammonia from agricultural runoff and water treatment plants such as the Sacramento Regional Water Treatment Plant (SRWTP). Current treatment protocols at SRWTP indicate that ammonia is not actively removed and may be discharged into the estuary (CVRWQCB, 2010). Data from an ongoing monitoring program in the Delta indicates that ammonia levels progressively decrease downstream of the SRWTP. High levels of ammonia (0.45 mg/L) were detected directly downstream and tapered (0.08 mg/L) further downstream (Foe, 2009). Ammonia equilibrates in water as ionized (IA) and unionized ammonia (UA) based on the pH and temperature of the water. Ammonia can cause toxicity to fish but little is known on the effects of ammonia in *P. forbesi*. Using ambient ammonia and pH levels observed in the estuary, this investigation will determine the effect of pH and ammonia concentrations that will cause toxicity to *P. forbesi*.

Materials and Methods

Acute Toxicity Experiment

Temperature (ºC)	20 ±1
Salinity (ppt)	0.5-0.8
pH	7.4, 7.8
Acceptability of control survival	>80%
Size of test beaker (mL)	600 ml
Volume of test solution (mL)	500 ml
Life stage of copepods	Juvenile
# of copepods	20
# of replicates per concentration	4
Nominal Concentrations (mg/L)	1,2,4,6 and 8
Feeding regime	Once daily, 1 hour before water change
Static- renewal test duration	96 hr, 144 hr

pH Experiment

Temperature (ºC)	20 ±1
Salinity (ppt)	0.5-0.8
pH	7.0, 7.4, 7.8, 8.2, 8.6
Acceptability of control survival	>80%
Size of test beaker (mL)	600 ml
Volume of test solution (mL)	500 ml
Life stage of copepods	Juvenile
# of copepods	20
# of replicates per concentration	4
Measured Total Ammonia Concentration (mg/L)	3.71
Feeding regime	Once daily, 1 hour before water change
Static- renewal test duration	96 hr

Chronic Toxicity Experiment

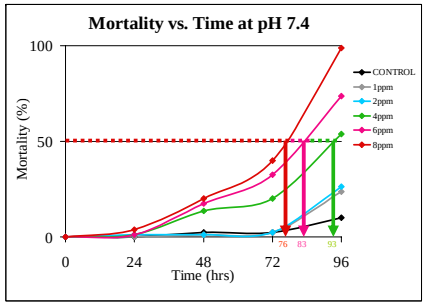
Temperature (ºC)	20 ±1
Salinity (ppt)	2
pH	7.8
Acceptability of control survival	>80%
Size of test beaker (mL)	1000ml
Volume of test solution (mL)	800 ml
Life stage of copepods	Gravid female
# of copepods	3
# of replicates per concentration	4
Nominal Concentrations (mg/L)	0.5, 1,2, and 4
Feeding regime	Once daily, 1 hour before water change
Static- renewal test duration	30 days

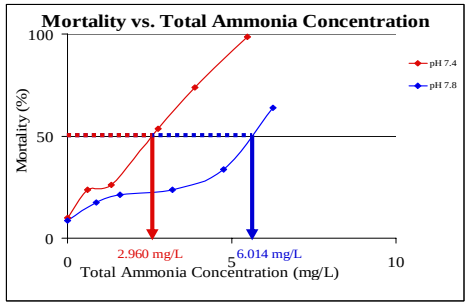
Figure 2. Experimental setup

Ammonia concentrations were analyzed with an ion selective electrode (ISE) and meter. The ISE meter was calibrated using the EPA method 350.3 with stock solutions prepared and certified by Fisher Scientific.

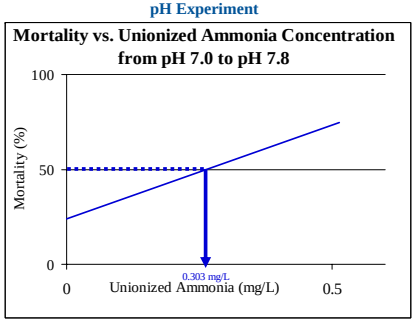
Results

Acute Toxicity Experiment





pH Experiment



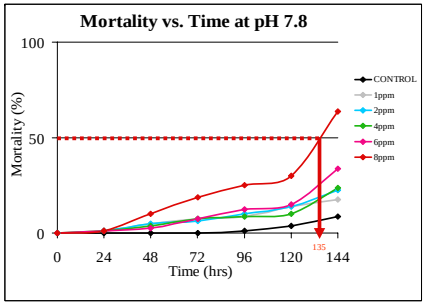
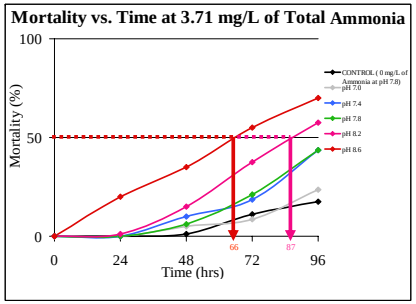


Table 1. Measured Ammonia Concentrations

Nominal Concentration (mg/L)	0.00	1.00	2.00	4.00	6.00	8.00
Measured Concentration at pH 7.4 (mg/L) ±SE	0.00 ±0.00	0.60 ±0.01	1.34 ±0.02	2.75 ±0.02	3.88±0.07	5.47 ±0.03
Measured Concentration at pH 7.8 (mg/L) ±SE	0.00 ±0.00	0.87 ±0.01	1.60 ±0.00	3.20 ±0.00	4.75 ±0.07	5.25 ±0.07

Table 2. LC50 data of Acute Toxicity Experiments (USEPA Probit Analysis Program Version 1.5)

pH	Total Ammonia (mg/L)	Unionized Ammonia (mg/L)	Ionized Ammonia (mg/L)
7.4 (96 hrs)	2.960	0.033	2.927
7.8 (144 hrs)	6.014	0.150	5.864



Summary of Results

- ✧ **Time to 50% lethality was shorter at pH 7.4 compared to pH 7.8**
- ✧ **At pH 7.4, 4-d LC50: UA=0.033 mg/L, IA=2.927 mg/L**
- ✧ **At pH 7.8, 6-d LC50:UA=0.150 mg/L, IA=5.864 mg/L; no LC50 was observed at 4-d exposure**
- ✧ **In the pH experiment, a positive dose response was observed for % mortality with increasing UA at 4-d LC10=0.038 mg/L; 4-d LC50=0.303 mg/L**

Discussion

- ✧ **Ammonia toxicity is dependent on pH levels.**
- ✧ ***P. forbesi* is more sensitive to pH 7.4 compared to pH 7.8 indicating sensitivity to increasing IA concentrations.**
- ✧ **At constant TAN, *P. forbesi* is more sensitive at high pH due to increasing UA concentrations.**
- ✧ **Ongoing experiment will determine the chronic levels of UA and IA and their overall effects on copepod growth and reproduction.**

Acknowledgements

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References

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