Full Life-Cycle Bioassay Approach to Assess Chronic Exposure of *Pseudodiaptomus forbesi* to Ammonia/Ammonium

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Overview

- Integrating ammonia/pH data with abundance and distribution data of *Pseudodiaptomus forbesi* in the San Francisco Estuary
- Chemistry of Ammonia
- Acute and chronic effects of ammonia on *P. forbesi*
- Results and Conclusions

Physicochemicals in Hood and *P. Forbesi* abundance in Cache Slough Region

pH, temperature, and ammonia for Sacramento River at Hood

	Lab pH	Hach pH	Temperature	$NH4^+$-N
4/13/2009	7.63	7.57	14.77	0.53
4/27/2009	7.58	7.41	15.8	0.54
5/11/2009	7.45	7.32	17.2	0.28
5/26/2009	7.4	7.34	19.07	0.65
6/8/2009	7.62	7.45	19.84	0.4
6/22/2009	7.38	7.27	20.99	0.59
7/14/2009	7.57	7.33	20.7	0.24

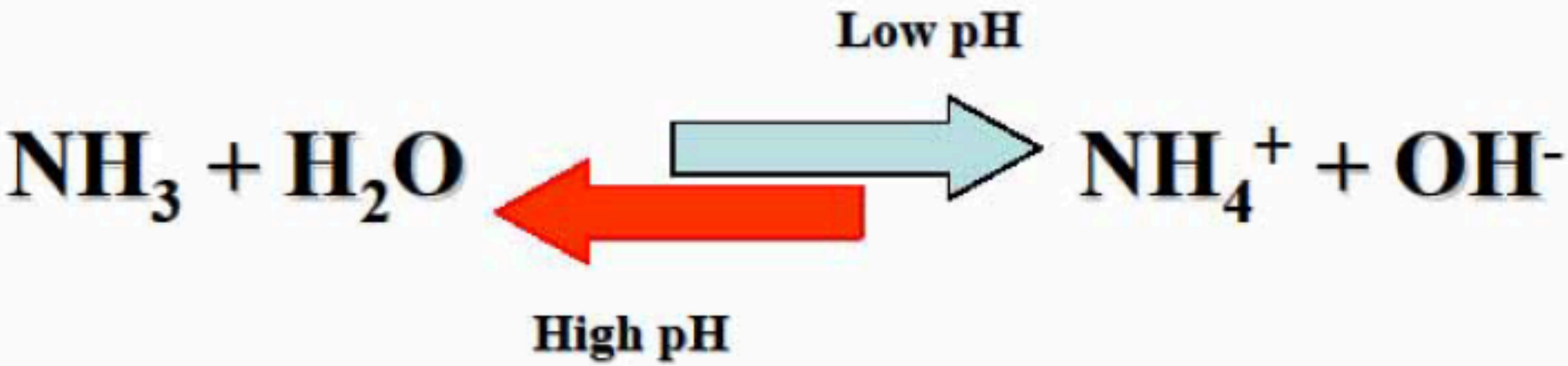
Date	pH	Temperature (°C)
2009		
04/01-04/30/2009	7.35±0.13	15.87 ± 2.47
05/01-05/31/2009	7.38±0.12	18.35 ± 3.75
06/01-06/30/2009	7.34±0.09	21.20 ± 1.79
07/01-07/31/2009	7.22±0.08	21.41 ± 1.22
08/0108/31/2009	7.37±0.10	21.65 ± 1.01
04/01-08/31/2009	Mean=7.33±0.12	Mean=19.71 ± 4.64
2008		
04/01-04/30/2008	N/A	16.46 ± 2.04
05/01-05/31/2008	N/A	20.14 ± 3.31
06/01-06/30/2008	N/A	21.44 ± 1.54
07/01-07/31/2008	N/A	22.86 ± 1.27
08/01-08/31/2008	N/A	23.46 ± 1.10
0401-08/31/2008	N/A	Mean= 20.90 ± 4.89

2008 Zooplankton Counts at Cache Slough region

Site 716	*Pseudodiaptomus forbesi*	*Eurytemora affinis*
4/1/2008	147	26
04/15/208	200	7
04/29/208	157	25
5/28/2008	717	0
6/9/2008	1193	0
6/23/2008	1282	0
7/7/2008	468	0

Site 719	*Pseudodiaptomus forbesi*	*Eurytemora affinis*
4/1/2008	109	6
4/14/2008	66	31
4/29/2008	117	9
5/12/2008	127	3
6/9/2008	636	1
6/23/2008	2561	0
7/7/2008	787	0

Chemistry of Ammonia

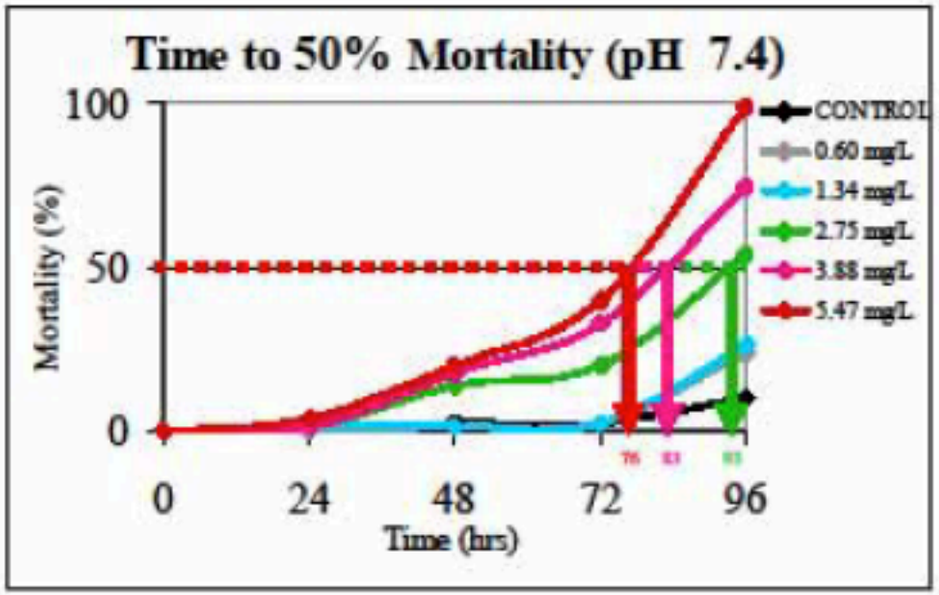


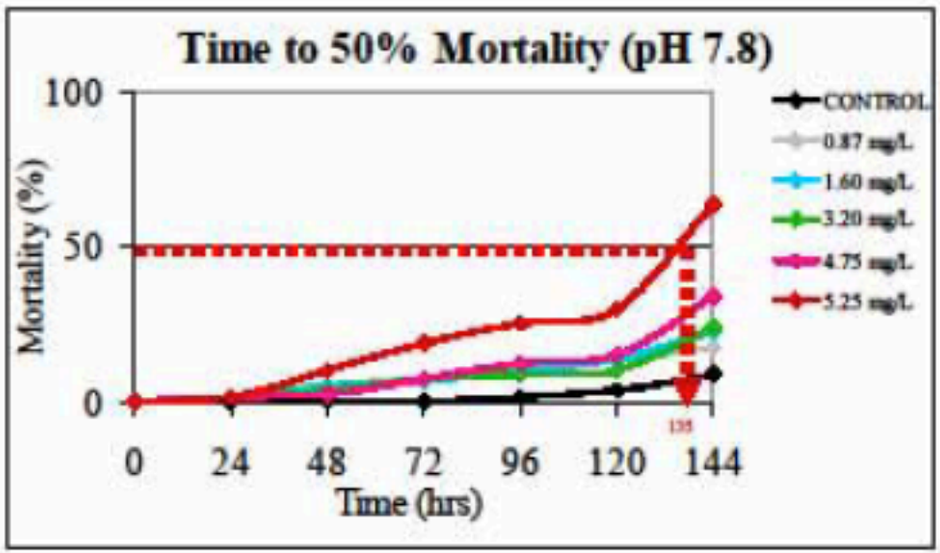
Concentration of unionized (UA) and ionized (IA) ammonia is dependent on temperature, salinity and pH of aqueous systems

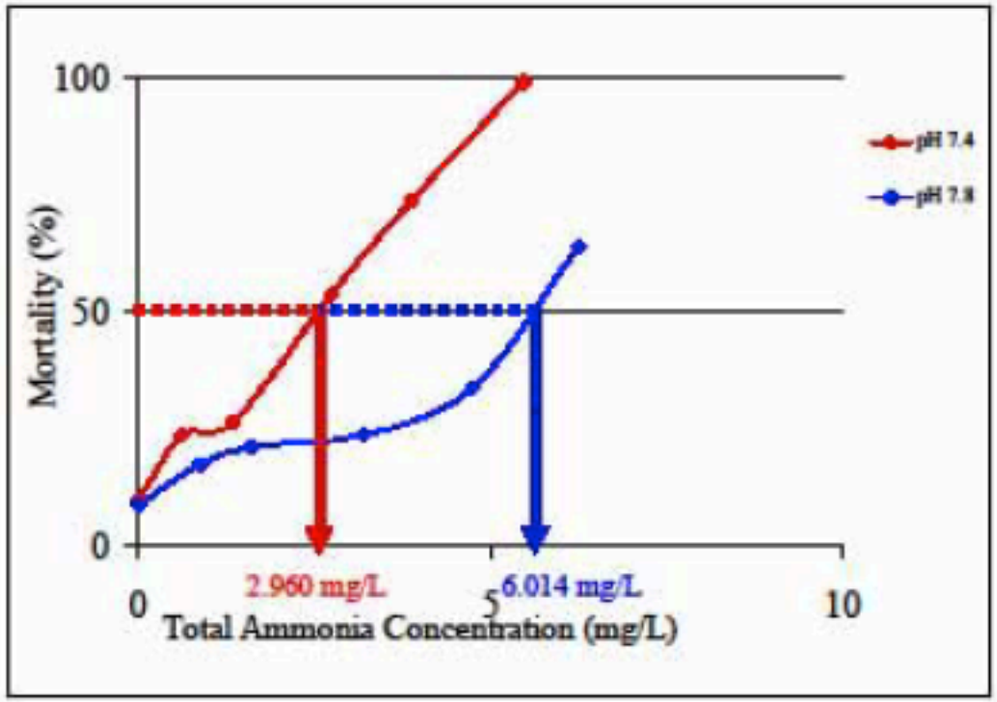
P. forbesi Standard Acute Toxicity Bioassay

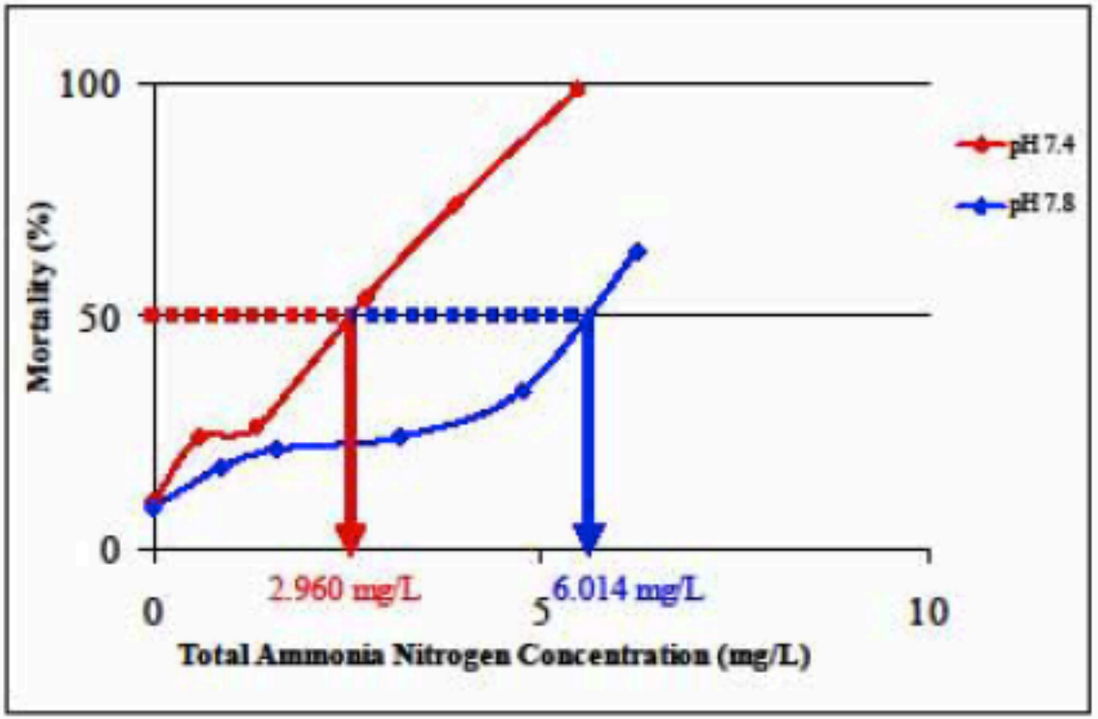
Temperature (°C)	20 ±1
Salinity (ppt)	0.5-0.8
pH	7.4, 7.8
Acceptability of control survival	>80%
Size of test beaker (mL)	600 ml
Volume of test solution (mL)	500 ml
Life stage of copepods	Juvenile
# of copepods	20
# of replicates per concentration	4
Nominal Concentrations (mg/L)	1, 2, 4, 6, and 8
Feeding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

0.5 ppt salinity, Alkalinity (40 mg/L), Conductivity (950 µHMOS), Hardness (140 mg/L), Dissolved oxygen (>8 mg/L)]


Time to 50% Mortality (pH 7.4)
100
50
0
Mortality (%)
0
24
48
72
96
Time (hrs)
CONTROL
0.60 mg/L
1.34 mg/L
2.75 mg/L
3.88 mg/L
5.47 mg/L


Time to 50% Mortality (pH 7.8)
100
50
0
Mortality (%)
0
24
48
72
96
120
144
Time (hrs)
CONTROL
0.87 mg/L
1.60 mg/L
3.20 mg/L
4.75 mg/L
5.25 mg/L


100
50
0
Mortality (%)
0
5
10
2.960 mg/L
6.014 mg/L
Total Ammonia Concentration (mg/L)
pH 7.4
pH 7.8



Ionized Ammonia (mg/L)

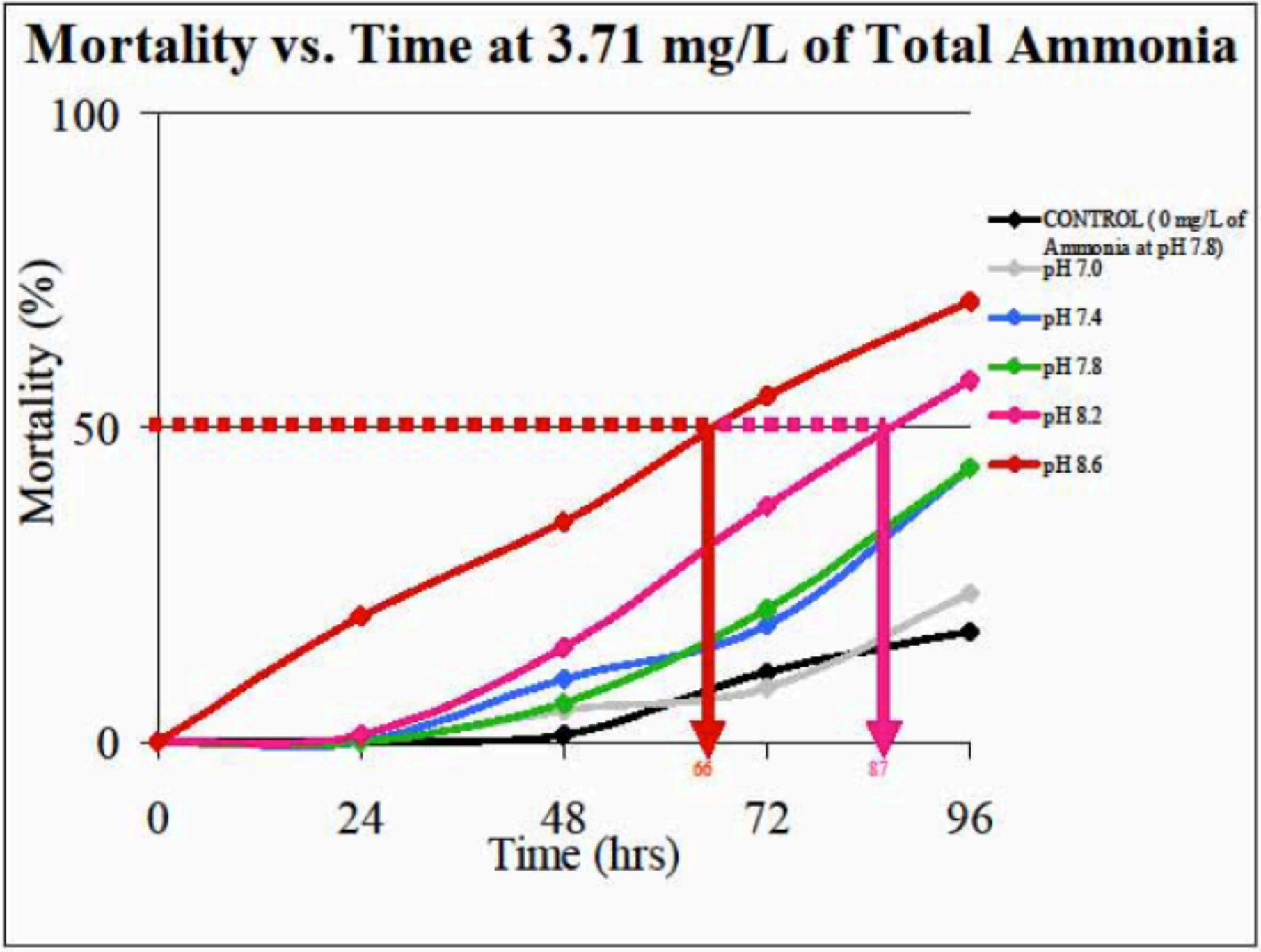
pH	LC 5	LC 10	LC50
7.4 (96h)	1.684	1.903	2.927
7.8 (144h)	3.290	3.738	5.864

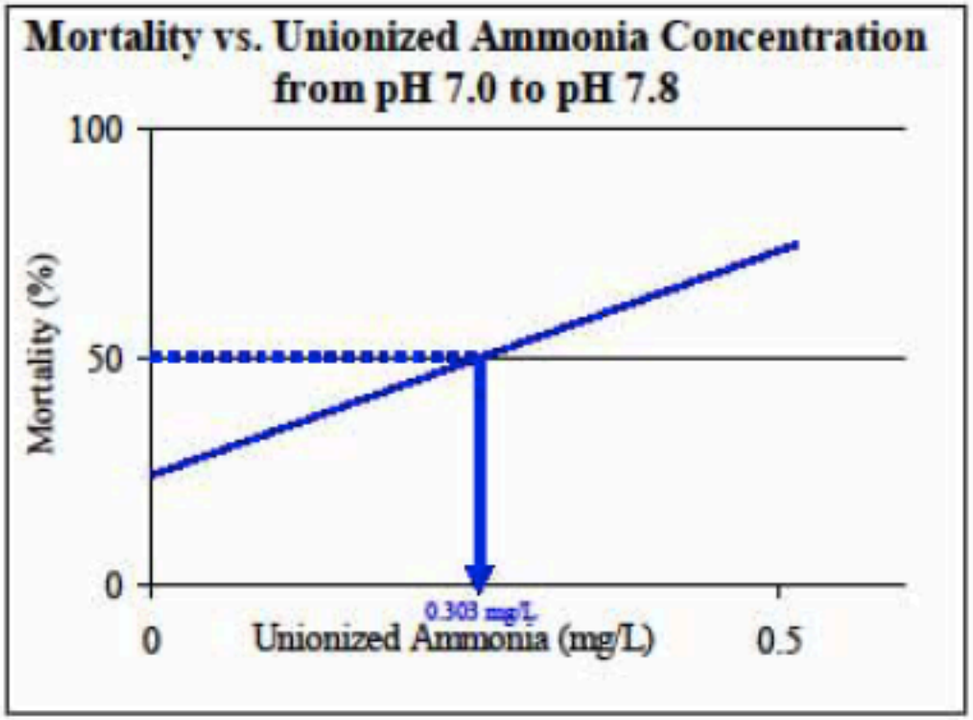
Unionized Ammonia (mg/L)

pH	LC 5	LC 10	LC50
7.4 (96h)	0.019	0.021	0.033
7.8 (144h)	0.084	0.096	0.150

P. forbesi Toxicity Bioassay at 5 pH Levels

Temperature (°C)	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


Mortality vs. Time at 3.71 mg/L of Total Ammonia
100
50
0
Mortality (%)
0
24
48
72
96
Time (hrs)
66
87
CONTROL (0 mg/L of Ammonia at pH 7.8)
pH 7.0
pH 7.4
pH 7.8
pH 8.2
pH 8.6

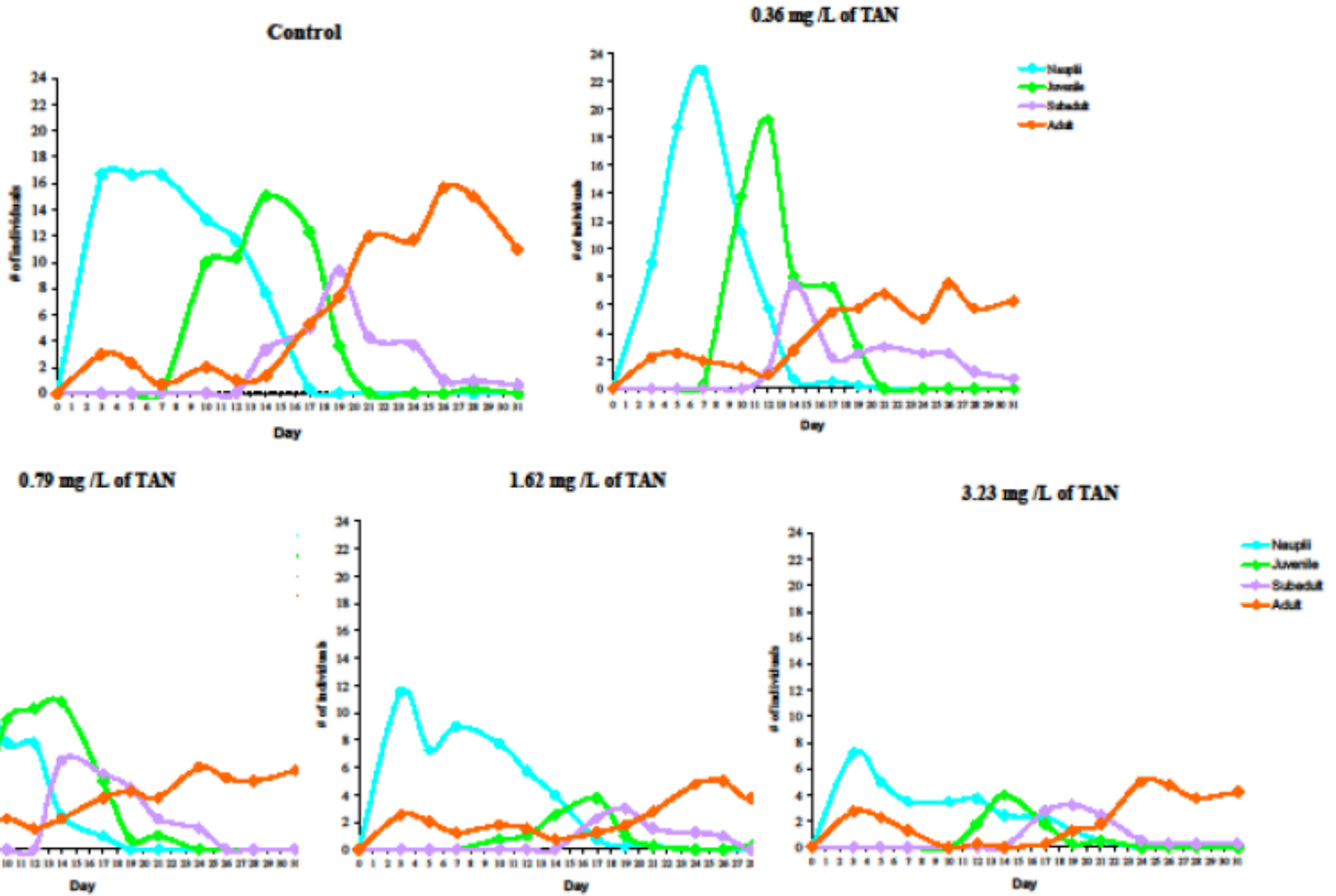


pH	LC 5	LC 10	LC50
7.0- 8.6	0.021	0.038	0.303

30 Days Static-Renewal Bioassay on P. forbesi Growth and Survival

Temperature (°C)	20 ±1
Salinity (ppt)	2
pH	7.8
Acceptability of control survival	>80%
Size of test beaker (mL)	1200ml
Volume of test solution (mL)	1000 ml
Life stage of copepods	Gravid female
# of copepods	3
# of replicates per concentration	4
Nominal Concentrations (mg/L)	0, 0.5, 1, 2, and 4
Feeding regime	Once daily, 1 hour before water change
Static- renewal test duration	30 days

P. forbesi populations in control and 4 concentrations of Total Ammonia Nitrogen

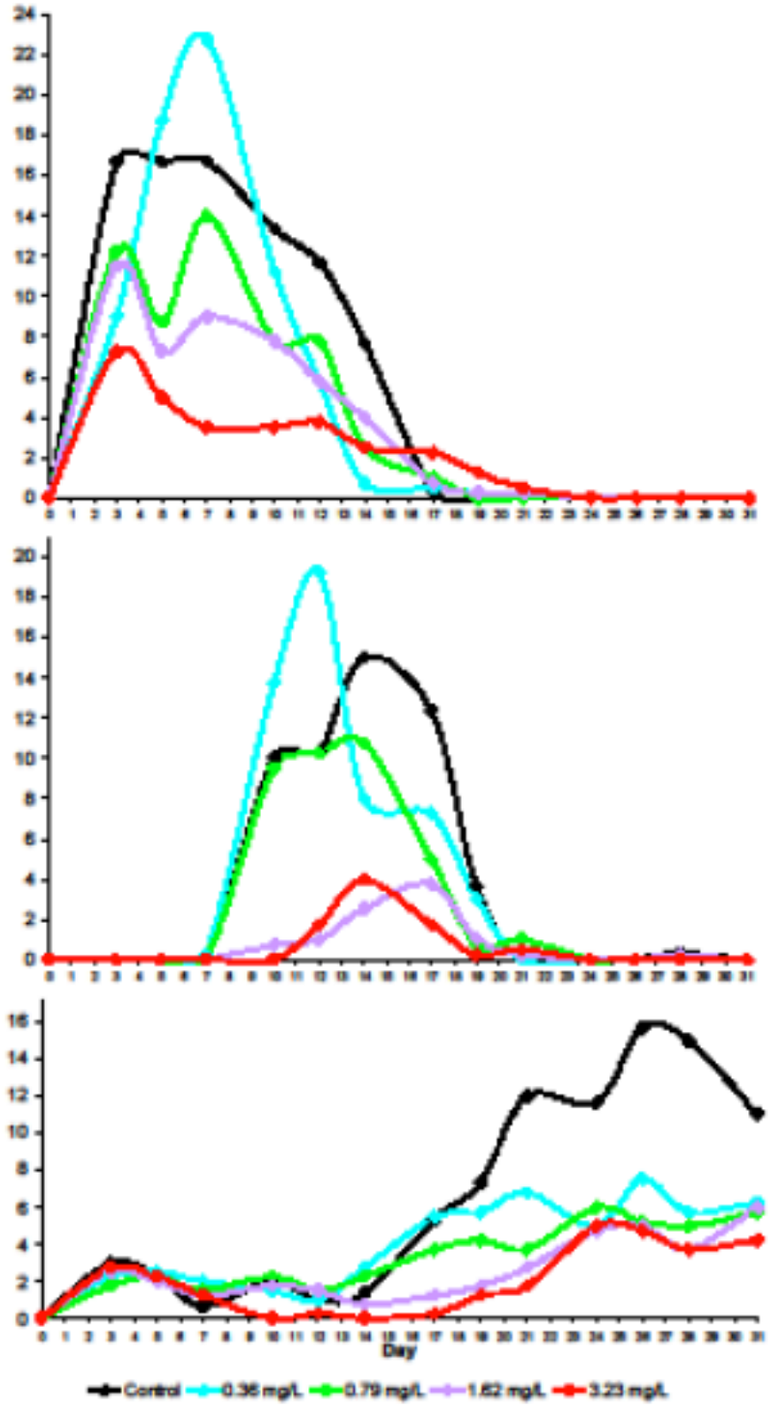


Mean number of Nauplii, Juvenile, and Adult *P. forbesi* exposed to control and 4 concentrations of Total ammonia

Nauplii

Juvenile

Adult

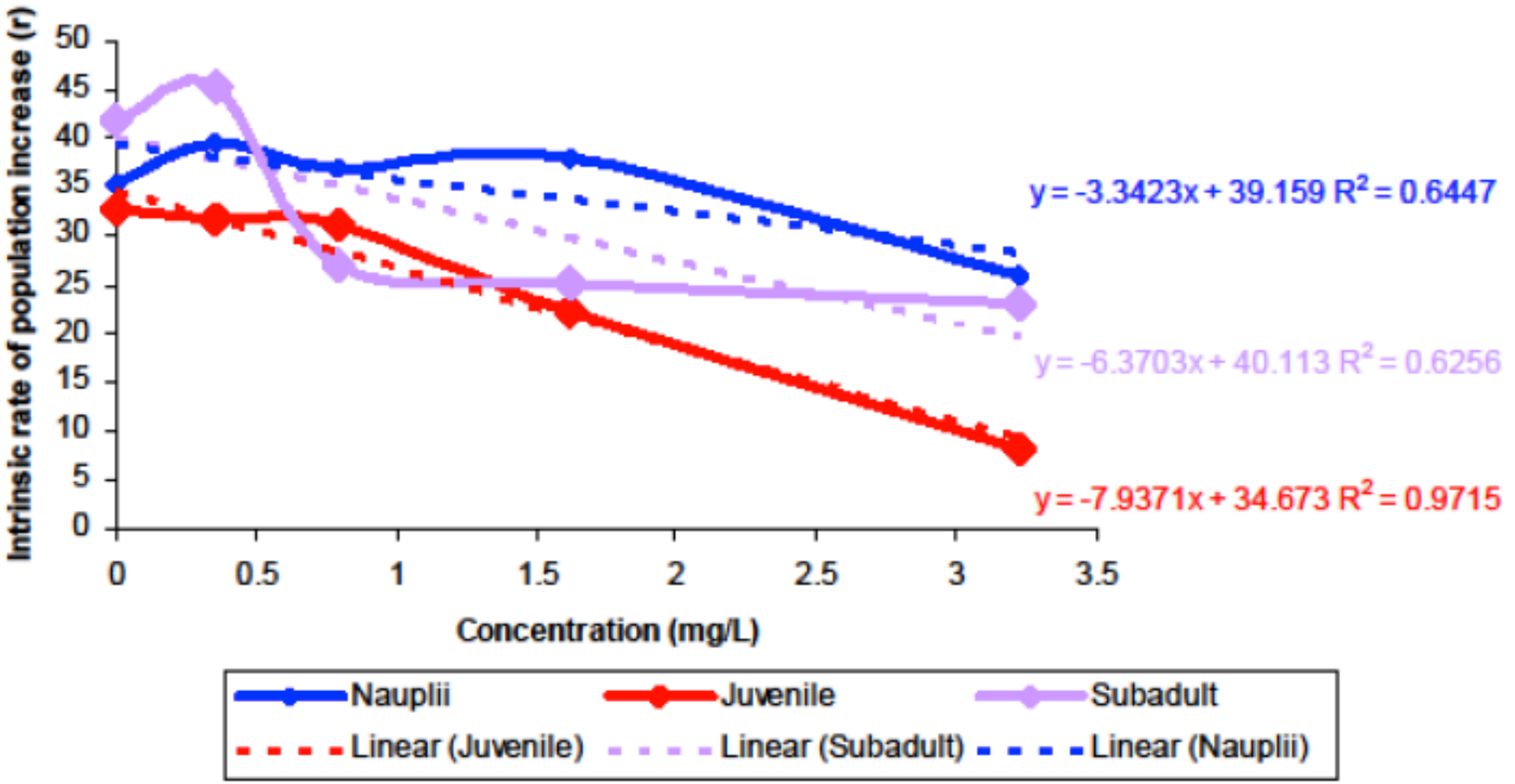


Survival of *P. forbesi* nauplii to adult at the end 30 days of exposure total ammonia

Concentration	Max nauplii	Max adult	% of max adults from max nauplii
Control	16.70	15.7	94.0
0.36 mg/L	22.75	6.75	29.7
0.79 mg/L	12.25	6.00	46.9
1.62 mg/L	11.50	4.75	41.3
3.23 mg/L	5.00	7.25	69.0

- 94% of nauplii survive to adults in control treatment
- Lowest % of nauplii survival to adult in 0.36 mg/L

Intrinsic rate of natural increase (r) of *P. forbesi* Exposed to Total Ammonia Nitrogen



- Life Table equation ($\Sigma l_x m_x e^{-rx} = 1$) is a measure of a population's capacity to increase in an unlimited environment (cf. Ricklefs, 1973), in this study, l_x= number of adult surviving, m_x= number of nauplii or juvenile, r= intrinsic rate of population increase.
- Slope is more negative for juvenile showing faster decrease in population as concentration of total ammonia increases.

Summary of Results

- Time to 50% lethality faster at pH 7.4 than at pH 7.8
  - At pH 7.4 (96h), LC50: UA=0.033 mg/L, IA=2.927 mg/L
  - At pH 7.8 (144h), LC50:UA=0.150 mg/L, IA=5.864 mg/L
- Time to 50% lethality faster at higher pH than lower pH.
  - UA 96h LC10=0.038 mg/L and 96h LC50=0.303 mg/L
- Rate of population increase (r) is negative as concentration of total ammonia nitrogen increases
- Adult *P. forbesi* reproduction are affected by total ammonia nitrogen at concentration $\geq$ 0.79 mg/L
- Nauplii and juvenile *P. forbesi* survivals are affected by total ammonia nitrogen at concentration $\geq$ 0.36 mg/L

Conclusions and Discussion

- Ammonia has acute and chronic effects on *P. forbesi*
- Ammonia toxicity is modulated by pH, UA/IA ratio in current study
- Nauplii and juvenile are more sensitive to TAN than adult *P. forbesi*
- Adult P. forbesi produced less nauplii when exposed to concentrations of TAN $\geq$0.79 mg/L indicating the toxic stress of TAN on gravid female and possibility resulted in decreased brood size (#egg/sac)
- In summary, *P. forbesi* population are affected by total ammonia nitrogen at concentration $\geq$ 0.36 mg/L