High Ammonia Concentration Increases Survival of Channel Catfish Experimentally Infected with *Flavobacterium columnare*

Andrew Mitchell and Bradley Farmer
Harry K. Dupree Stuttgart National Aquaculture Research Center
United States Department of Agriculture, Agricultural Research Service
Stuttgart, Arkansas

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Introduction

- It is generally accepted that elevated ammonia levels in the water serve to:
  - **stress fish** (Noga 1996; Hoole et al. 2001)
  - cause fish to become **more susceptible** to bacterial infections (Ferguson et al. 1992)
  - cause **greater mortality** of infected fish (Walter and Plumb 1980; Chen et al. 1982; Amin et al. 1988)

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Introduction

- **Opposite observation -- preliminary columnaris challenge tests in our ultra-low-flow systems**
  - **When total ammonia nitrogen (TAN) exceeded 10 mg/L -- greater fish survival**
- **Significant increase in survival of Lost River suckers infected with columnaris when unionized ammonia concentrations were increased to about 0.4 mg/L. (Morris et al. 2006)**

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Purpose

- In order to further investigate the effects of ammonia on the survival of fish infected with a bacterial disease, a study was set up to determine if a single high level of ammonia could limit an experimental *F. columnare* infection in channel catfish and reduce mortality.

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Methods

- Channel catfish (~7 g each) stocked at 50 g/L were placed in sixteen—18 L tanks containing 10 L of aerated water
- The tanks received filtered well water at a rate of about 4 water exchanges/day from an ultra-low-flow water delivery system.

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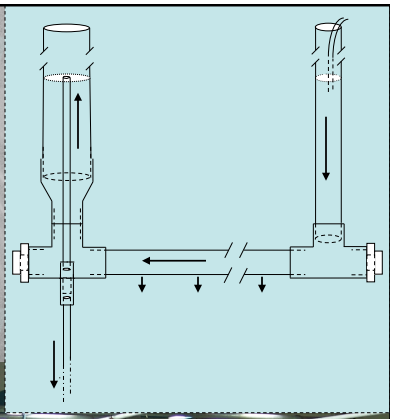
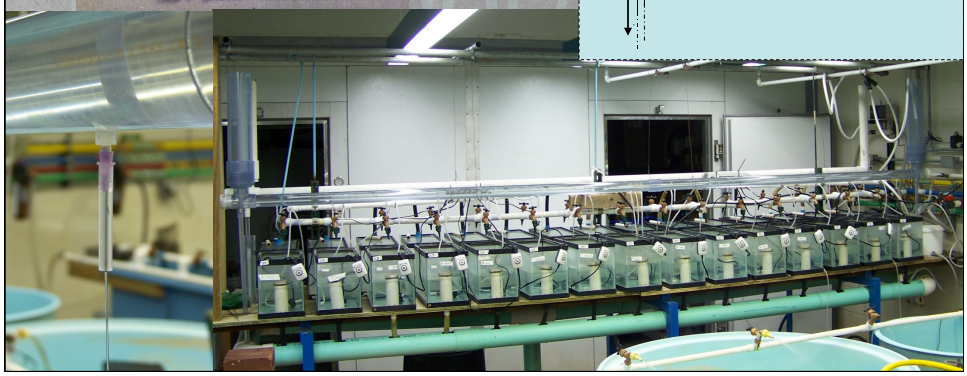
Ultra-low-flow system

Semi-enclosed header trough with needle nozzles that delivers 5 to 130 mL/min (±3% for >7d)

18 gauge needle with Perkin Elmer, Adapter M for ICP





Water quality

- Water temperatures -- 26.3 to 27.3°C
- pH -- 7.3 to 8.2
- Dissolved oxygen -- 4.4 to 8.3 mg/L
- Total alkalinity -- 217-218 mg/L
- Total hardness -- 119-120 mg/L
- Chlorides -- 176 mg/L

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Experimental design

- **Two identical trials**
- **Four treatments**
  - **Treatment 1 - control (no bacteria or ammonia)**
  - **Treatment 2 - ammonia only**
  - **Treatment 3 - bacteria only**
  - **Treatment 4 - both ammonia and bacteria**
- **Four reps/treatment**

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Ammonia exposure

- 15 mg/L TAN exposure -- NH_4Cl stock solution (46 mg/mL)
  - 10 mL of stock solution/10 L of water/tank
- Immersion flush exposure -- chemical added with continuous water flow in each tank; one water exchange in about 6 h.

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TAN determinations

(Hach DR/4000V spectrophotometer using Nessler Method 8038)

- Trial 1 – water samples were taken at 1 min, 1h, 2h, 3 h, 4 h, and 6 h post-exposure.
- Trial 2 – 20 min, 1 h, 2 h, 3 h, 4 h, 6 h, 30 h, and 56 h.
- Unionized ammonia was determined from TAN, pH and temperature for Trial 2.

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F. columnare challenge

- Bacterial suspension was incubated at 28°C for up to 24 h in FCGM (Farmer 2004).
- When suspension reached an absorbance of 0.70 at 550 nm about 60 mL of bacterial suspension was added to each tank immediately after ammonia was added.
- A 10 ml sample was removed from the broth to determine the CFU count.
- **Bacterial density/tank was approximately 1.0E08 bacteria/mL of water**

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In vitro studies - MIC

- *F. columnare* suspension in dilute Mueller Hinton broth was pipetted into a 96 micro well plate
- Ammonium chloride was added using two fold dilutions yielding - 60, 30, 15, 7.5 mg/L TAN.
- Done in triplicate with positive and negative controls.
- Incubated at 28°C for 24 h
- Read visually for the presence or absence of growth.

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In vitro studies - CFU

- Ammonium chloride was added to an F. columnare suspension to give a concentration of 15 mg/L and 30 mg/L TAN.
- These were incubated 6h at room temp.
- Samples were removed, serially diluted and plated on Ordals agar for CFU counts.

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Identification and quantification of *F. columnare* using Roche Lightcycler 480 Real Time PCR system

- Caudal fin (CF) samples were taken at 24 h post-exposure from 3 fish showing no obvious signs of disease from each tank in every treatments
- Genomic DNA was extracted from CF tissue
- Template DNA was used for pathogen detection and quantification (Panagala 2007)

Identification and quantification cont.

- qPCR run in duplicate for each genomic DNA sample with standards included on each plate and a no template control
- Data -- based on a standard curve generated from previously counted bacterial samples.
- The standard curve efficiency was 1.94 and the *r*2 was 0.98

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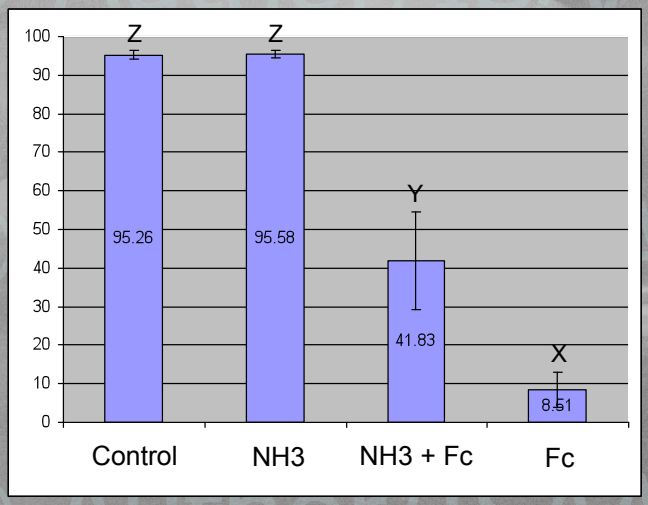
Statistics

- Trial and trial X treatment interaction effects between the two trials were not significantly different therefore survival data from the two trials were combined.
- Arcsine transformed data for percent survival, log transformed QPCR, and CFU data were normally distributed with equal variances
- A GLM ANOVA was performed on the transformed survival, QPCR, and CFU data. Differences among treatment means ($P \leq 0.05$) were separated using the Tukey–Kramer procedure for pair-wise comparisons.

Results and discussion

Survival of channel catfish following four treatments



Ammonia by itself did not kill fish.

Ammonia either killed some *F. columnare* or otherwise interfered with the infection process.

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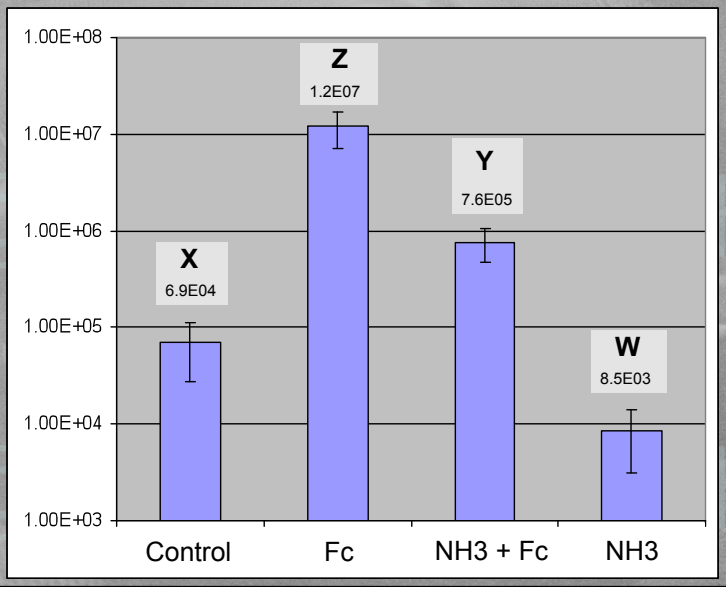
Quantification of *F. col.* from caudal fin of channel catfish in Trial 2



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In Vitro studies – MIC

TAN Treatment	F. col. growth
control	+
7.5 mg/L	+
15 mg/L	+
30 mg/L	+ (slight)
60 mg/L	-

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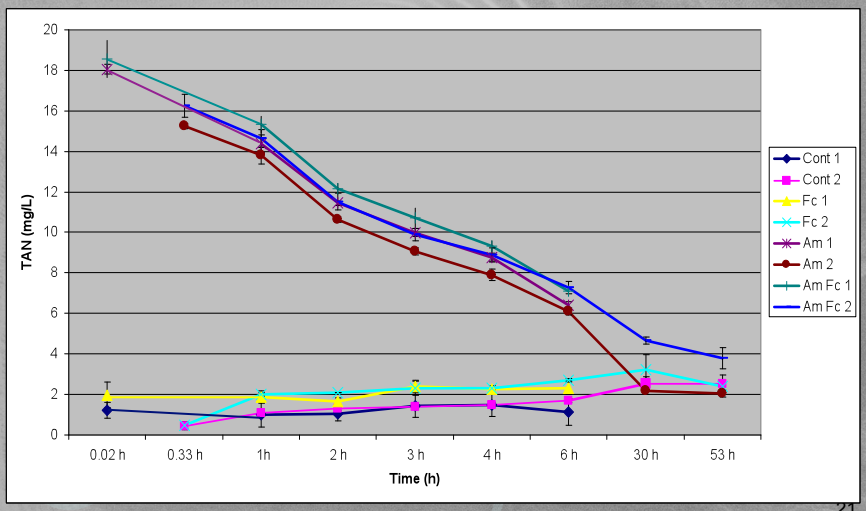
In Vitro studies

Number of colony forming units (CFU) following a 6-h exposure to TAN treatments

Different letters indicate significant differences

TAN Treatment	# of CFU	Reduction from control
0 mg/L	1.95E10 Z	--
15 mg/L	5.18E09 Y	74%
30 mg/L	3.40E09 Y	84%

Total ammonia nitrogen determined at various times post-exposure (Trials 1&2)



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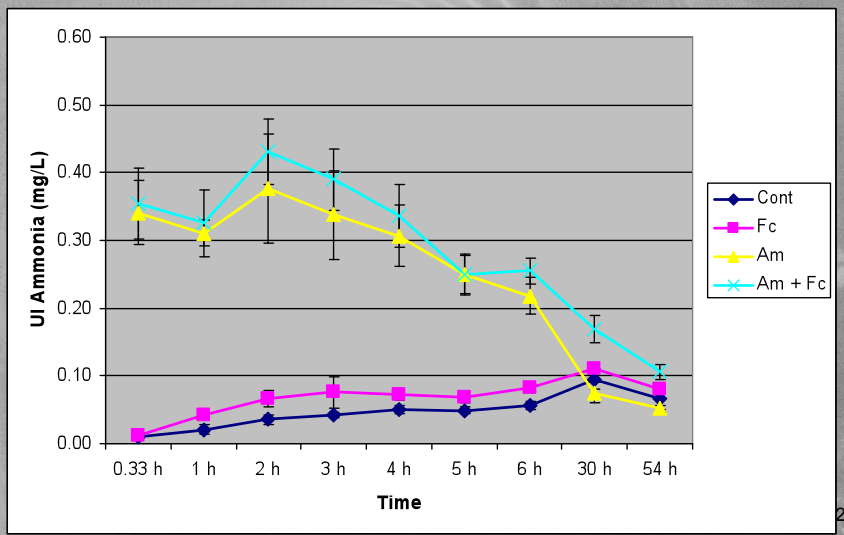
Unionized ammonia (UI) determined from TAN, pH and temperature (Trial 2)



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Summary

- The TAN (~15 mg/L) and UI (~0.4 mg/L) concentrations produced in this study did not overtly affect the health of the catfish
- The ammonia concentration used in these trials was effective in limiting the onset of a columnaris infection in channel catfish.

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Summary

- Channel catfish challenged with *F. columnare* and treated with ammonia had significantly higher survivals than those receiving only a bacterial challenge
- Ammonia significantly reduces the *F. columnare* numbers found in fish tissue
- Apparently healthy fish (control group) had mean *F. columnare* counts of $6.9X10^4$

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Further study needed

- To determine if the ammonia exposure interfered with the attachment of the bacteria to fish tissues
- ??To determine the usefulness of NH_4Cl or another ammonia compound as a management method for columnaris.
  - Optimal rates, safety margins, and contraindications need to be determined.

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Acknowledgements

Matt Barnett for his help throughout the course of the two trials

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TAN toxicity

- Preliminary toxicity tests (20 fish/rep; 3 reps) – immersion flush treatment (4 exchanges/d)

TAN concentration	UI concentration	% fish survival
24 mg/L	0.49 mg/L	100%
49 mg/L	1.25 mg/L	88%
61 mg/L	2.41 mg/L	20%

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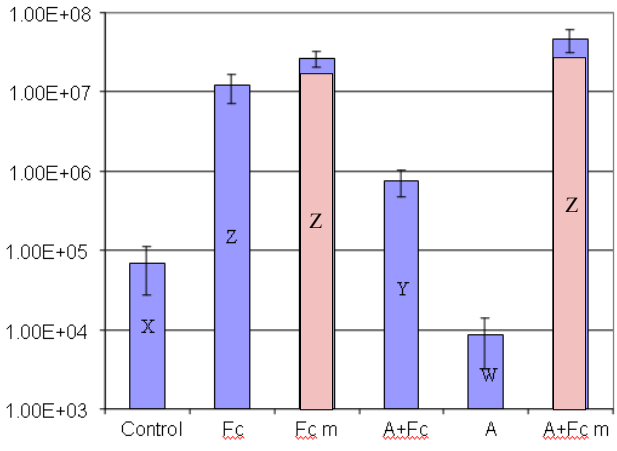
Quantification of bacteria from caudal fin of channel catfish in Trial 2



A = ammonia

Fc = bacterial challenge

m = moribund fish

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