

Developing columnaris challenge methods –
Ultra low-flow systems, water temperature, fish
density, and bacterial challenge

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Introduction

- Eight studies on columnaris challenge methods
 - Altinok and Grizzle 2001; Bader et al. 2003; Thomas-Jinu and Goodwin 2004; Bader et al. 2006; Gaikowski et al. 2007; Soto et al. 2008; Darwish et al. 2008; Darwish et al. 2009
- Why more study effort???

Introduction

- The eight studies either:
 - Required disrupting the mucus or cutaneous integrity of fish to produce infection.
 - Produced *Flavobacterium columnare* infected fish without a natural disease progression – few signs seen and die-offs occurred in less than 72 h.
 - Produced fish mortality rates less than 15% or greater than 85%.

Problems with disrupting the skin and mucus layer

- Skin damage and mucous removal increases the susceptibility of fish to *F. columnare* bath immersion (Tripathi et al. 2005; Moyer and Hunnicutt 2007).
- We hope to test fish genotypes for their innate immunity to *F. columnare*
- If that immunity is fashioned in the mucus or epidermal layer, disrupting these layers would circumvent a meaningful evaluation of the innate immunity of the fish.
- Desirable to develop a method not requiring cutaneous scraping or injury

Problems continued

- Results from a study using a challenge method that produced infected fish with naturally developing disease signs would be more meaningful.
- We would be able to better demonstrate statistically significant variations between fish genotypes challenged with *F. columnare* if a typical experiment would consistently produce 30 to 70 % mortality on a control standard fish group.

Objective

- To develop an infection method that will not require cutaneous scraping or injury and will consistently produce columnaris disease signs and a 30 to 70 % mortality in a typical experiment.

Ultra-low flow systems

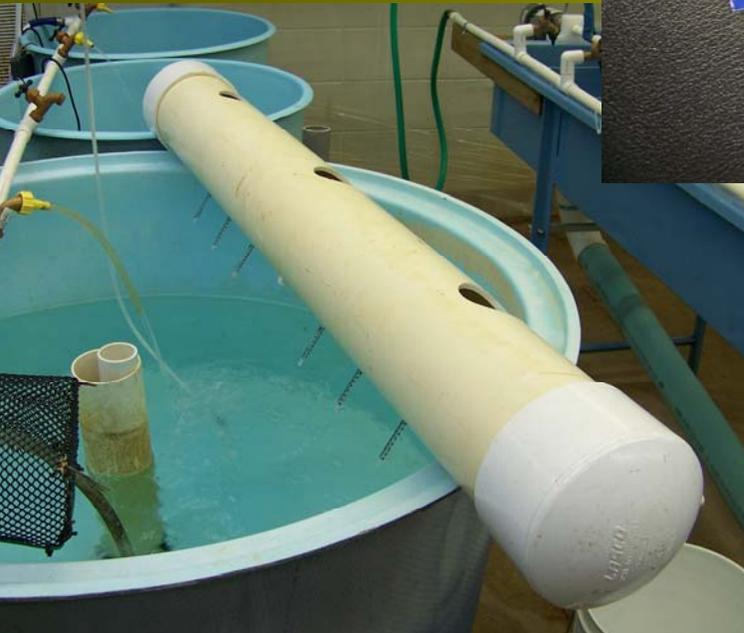
- Low flow
 - Longer time before bacteria are flushed out of the water
 - May allow time for bacteria to establish in the biofilm on aquarium walls, standpipes, heater, and airline potentially producing a continued source of infection – (Welker et al. 2005).
 - Degraded water quality (nitrogenous waste products build up) that could serve to stress fish and further predispose them to infection.

Ultra-low flow systems

- Ultra-low flow systems can provide as little as 1 water exchange per day in small systems (10 L water volumes or less)
- Consistent flows of less than 15 mL/min can be produced
- Ours system does not involve peristaltic pumps or other similar systems and costs less than \$300.00 to build.
- System feeds fifteen 18-L tanks.

Ultra low flow systems

- Tried several systems with different nozzles before we settled on the present system



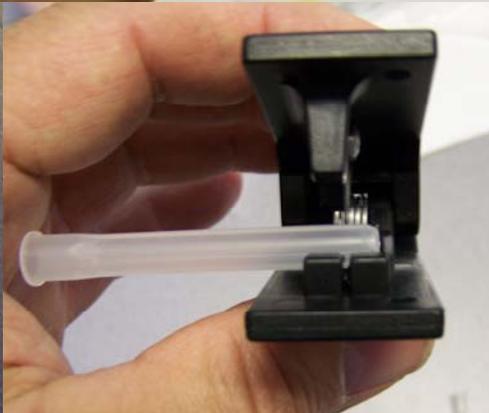
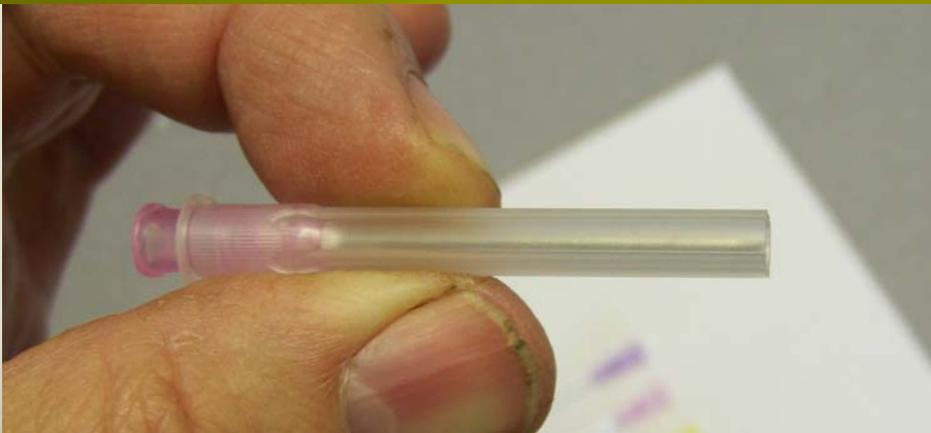
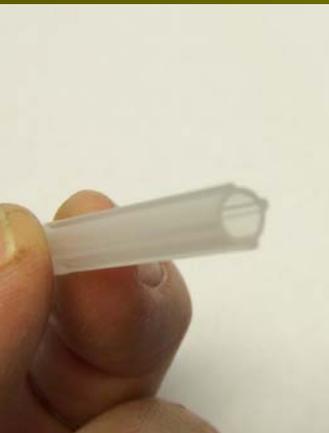
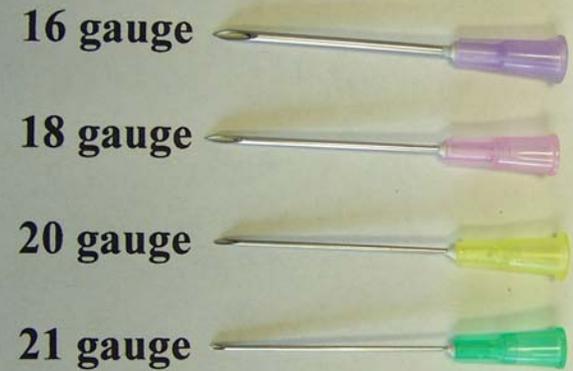
Ultra low flow systems

- Our system
 - Semi-enclosed header trough

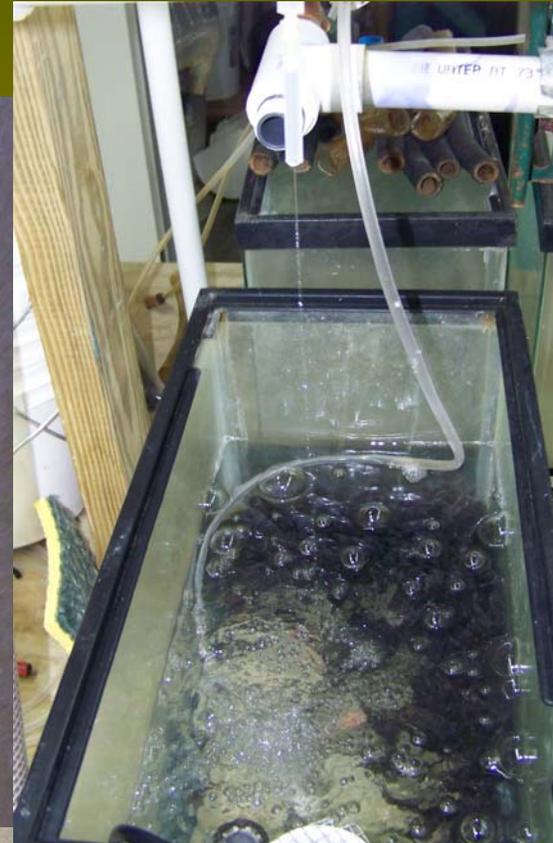
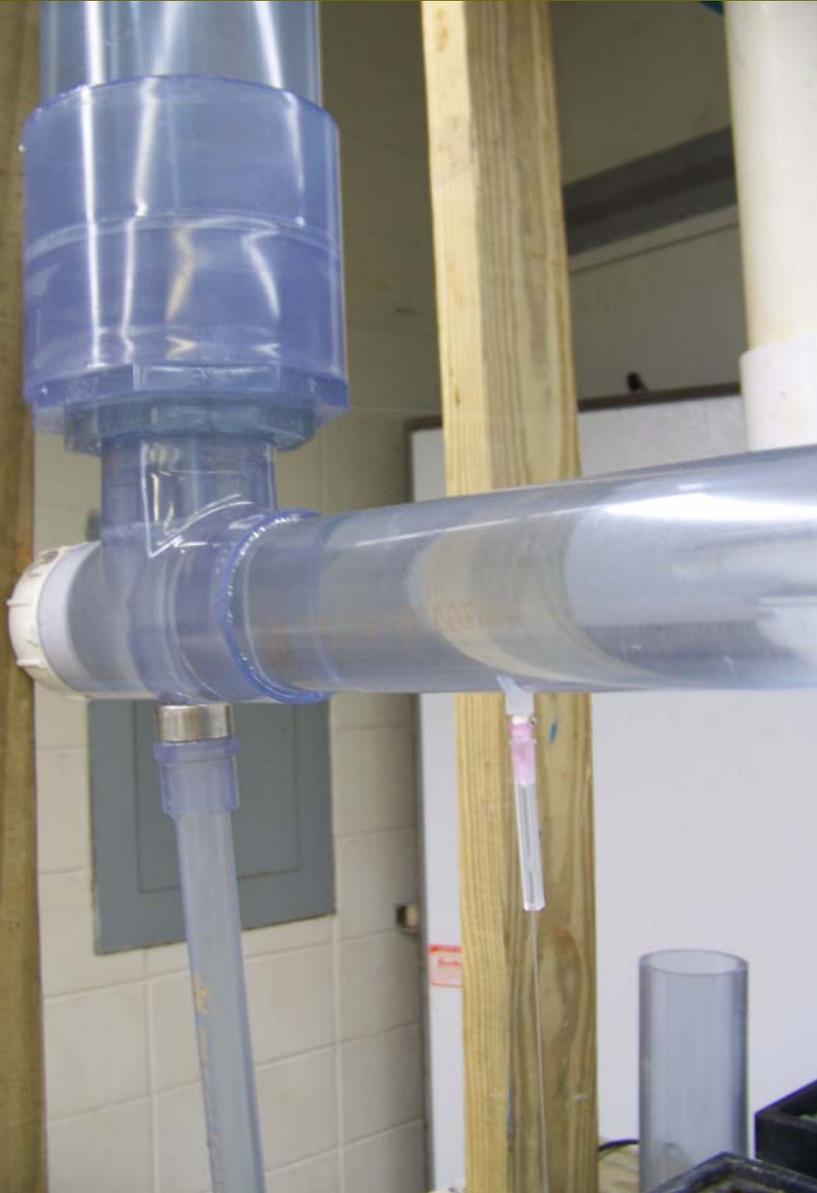


Ultra low flow systems

- Our system
 - Semi-enclosed header trough
 - Needles for nozzles



Ultra low flow systems



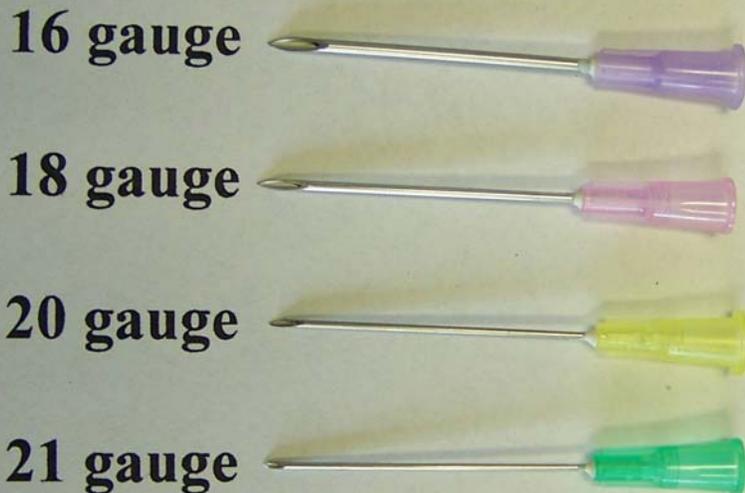
Perkin Elmer, Adapter M, 6.4 mm external screw thread, 4 mm outer diameter nipple

Ultra low flow systems

Potential flows

- Four syringe sizes and three standpipe heights yielded 12 different flows rates.

Stand pipe heights were:



6 inches (15 cm)

12 inches (30 cm)

18 inches (46 cm)

Flow Rate (ml/min)	Range of Flow (ml/min)	Needle Size (gauge)	Stand-pipe Height (cm)
6	6.1 - 6.5	21	15 cm
9	8.7 - 9.0	21	30 cm
10	10.2 - 10.8	20	15 cm
12	12.0 - 12.4	21	46 cm
15	15.2 - 15.8	20	30 cm
20	19.8 - 20.6	20	46 cm
30	29 - 31	18	15 cm
40	40 - 41	18	30 cm
50	50 - 52	18	46 cm
76	74 - 78	16	15 cm
104	100 - 107	16	30 cm
127	122 - 134	16	46 cm

Ultra low flow systems

Potential flows

- Flow table is useful – Built new system and wanted a 30 mL flow/min
- Used 15 cm stand pipe and 18 gauge needle
- Got 27-28 mL/min
- Replaced standpipe with one 19 cm
- Got 29.5 to 30.5 mL/min
- We can get a lower flow than 6 mL/min
- A 25 gauge needle with 30 cm stand pipe produced about 0.8 mL/min – we thought this was too low to be useful

Ultra low flow systems - heaters

Finnex, 50 W Heater, HPA-50

$27 \pm 0.5^\circ\text{C}$ --
good bacterial
growth



High fish stocking densities

Important for the efficient and rapid spread of pathogens

Crowding stress and associated degradation of water quality (higher total ammonia levels and lower dissolved oxygen) increase chance for disease outbreak



High fish stocking densities

50 g of fish/L of water (have tried 12.5 and 25 g/L)



Bacterial challenges

- *F. columnare* isolate, dose (CFU/ml), and exposure duration (controlled with low flow system) all contribute vital roles in the challenge method.
- Fish density crowding, ammonia, feed, quality of fish, and size also affect the method as well.

Bacterial challenges

- Isolate to Isolate virulence variation has been confirmed by a number of researchers, and the challenge methods will probably vary as well according to what isolate used.
- Varying virulence according to fish species has also been described.
- Previous studies showed $\approx 5 \times 10^8$ CFU/ml to be an effective challenge dose with our isolate to infect abraded catfish consistently (Darwish 08, 09).

CFU's Matter

- Our initial dose $\approx 5 \times 10^8$ = 100% mortality
- Successful attempts at $1, 2, 3,$ and 4×10^7 CFU/ml - mortality increased correspondingly (24%, 46%, 75%, & 94%).
- At the higher doses, mortality progressed too rapidly: most fish dead by 48 hr.
- 2×10^7 CFU/ml (3 reps) gave a consistent mid-range mortality (42 to 51%) at a flow giving a 11 hr water turnover rate and at a stocking density of 50 g fish/L of water.

Promising results

- Now on challenge day there is no need to call in sick.
- This method is less labor intensive, just add the bacteria and walk away.
- Amount of bacteria using this method is much less. 1L will challenge \approx 20 tanks.
- Achievable method for big studies; testing innate immunity of fish genotypes (50 +) to *F. columnare*.
- We were able to achieved 30-70% mortality.
- Got it figured out, right

Oh Crap

- On the next trial (4 reps @ 2×10^7 CFU/ml, 11 hr turnover, 50 g/L), results were variable
 - 2 reps complete die-off in 24 hr
 - 2 reps with 45-55%; right on schedule.
- On the edge: Density, low flow, and bacterial load in water may have “pushed” water quality parameters past levels tolerated by fish in some tanks.

Oh Crap – cont.

- Also had trouble re-isolating *F. columnare* from fish after day 2, possibly related to water quality.
 - Killed *F. columnare*?
 - Selected for an ammonia tolerant species?
 - Other?
- We then tried cutting fish density in half, but lower percent mortality resulted.
- What now?

Future attempts

- Double the flow rate (5.5 hr turnover), and dose titrate again looking for that 30-70% happy zone without water quality being as much of a factor.
- Questions and/or Suggestions

Acknowledgements

- Matt Barnett – for help with all aspects of our current work
- Ahmed Darwish – for laying groundwork for our study efforts with his previous work on challenge models