

# Process Requirements for Disinfection of Recirculating Water Using Ozonation & UV Irradiation

Steven Summerfelt, Mark Sharrer, Scott Tsukuda, &  
Michael Gearheart

*Freshwater Institute, Shepherdstown, WV*

# OUTLINE

- Introduction
- Methods
- Results
  - ✓ Bacteria inactivation in RAS vs O<sub>3</sub> dose
  - ✓ O<sub>3</sub> dose & RAS Water Quality
  - ✓ Process control for full-flow ozonation
- Conclusions

# Introduction

- Obligate and opportunistic fish pathogens can accumulate in RAS
  - ✓ during a disease outbreak when pathogens propagate and shed from their host
  - ✓ when no internal water disinfection process is used

# Introduction

- Ozonation ( $O_3$ ) and ultra violet (UV) irradiation can be used separately or in combination to treat water in RAS before it returns to the fish culture tanks.
  - ✓ Proactively prevent the accumulation of fish pathogens

# O<sub>3</sub> Doses for Disinfection

- Must maintain a residual concentration (C) for a given time (t):

C\*t, mg\*min/L

✓ ISAV	0.3
✓ <i>Aeromonas salmonicida</i>	1.6
✓ <i>Yersinia ruckeri</i>	0.45-0.6
✓ <i>Flavobacterium</i> sp.	2.8
✓ <i>Flexibacter</i> sp.	1.6
✓ <i>Streptococcus</i> sp.	0.015
✓ <i>Vibrio salmonicida</i>	0.45-0.6

# UV Doses for Disinfection

- Dose to inactivate 99.9% of BACTERIA from Wedemeyer (1996) and Liltved (2001):

	<u>mW-sec/cm<sup>2</sup></u>
✓ <i>Aeromonas salmonicida</i>	4
✓ <i>Aeromonas hydrophila</i>	5
✓ <i>Vibrio anguillarum</i>	4
✓ <i>Yersinia ruckeri</i>	3
✓ <i>Pseudomonas fluorescens</i>	5

# UV Doses for Disinfection

- Dose to inactivate 99.9% of VIRUSES from Wedemeyer (1996) and Liltved (2001):

	<u>mW-sec/cm<sup>2</sup></u>
✓ <i>ISA</i>	4-10*
✓ <i>IHN</i>	1-3
✓ <i>IPN</i>	100-200
✓ <i>Channel catfish virus</i>	2
✓ <i>Herpesvirus salmonis</i>	2
✓ <i>White spot syndrome baculovirus</i>	900*

\*loss of infectivity

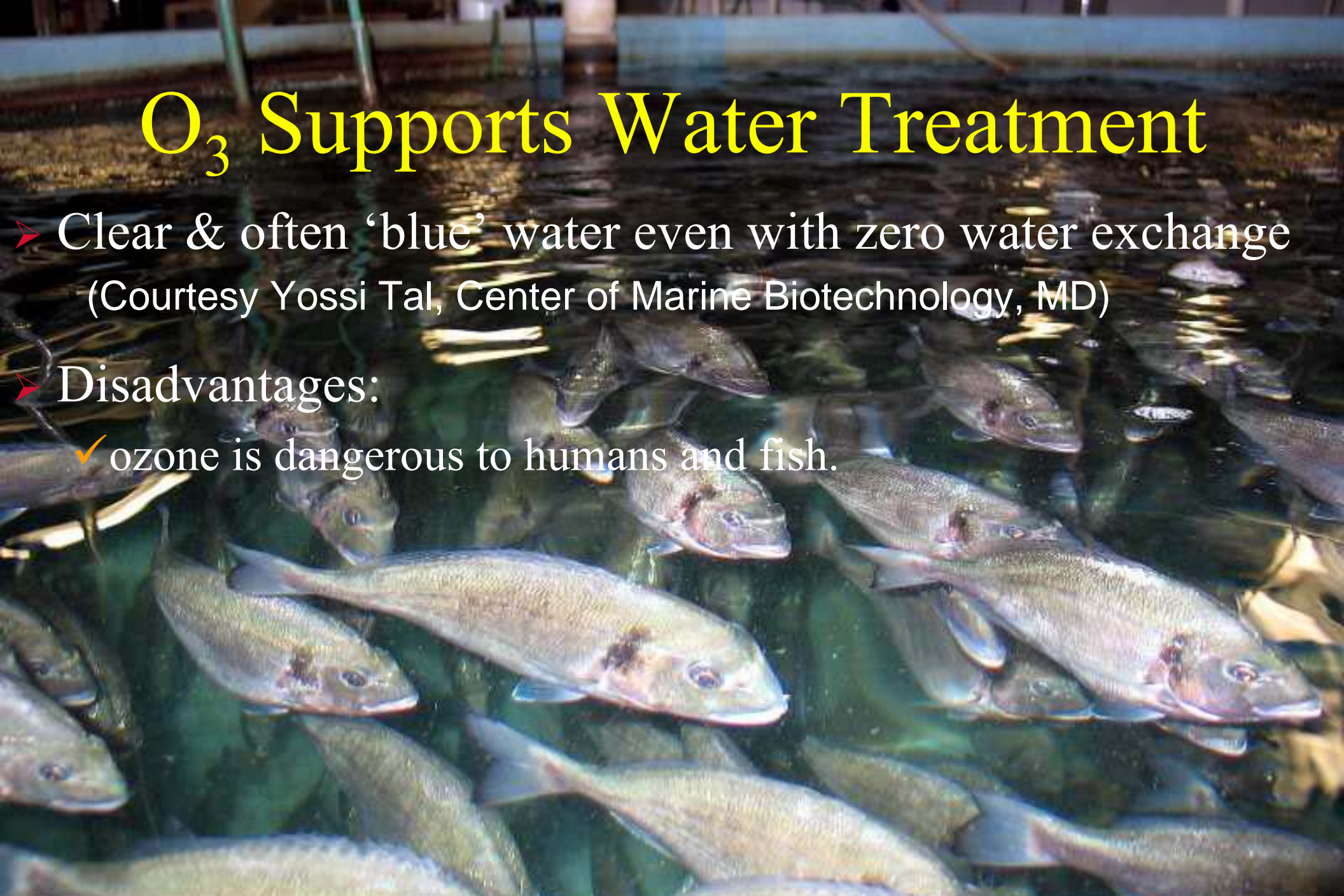
# UV Doses for Disinfection

➤ Wedemeyer (1996):

	<u>mW-sec/cm<sup>2</sup></u>
✓ Dose to inhibit growth of <i>Saprolognia</i>	230
✓ Dose to decrease infectivity of <i>myxobolus cerebralis</i>	28
✓ Recommended dose for recirculated water	50*
✓ Recommended dose for hatchery wastewater	30

# O<sub>3</sub> Supports Water Treatment

- Clear & often 'blue' water even with zero water exchange  
(Courtesy Yossi Tal, Center of Marine Biotechnology, MD)
- Disadvantages:
  - ✓ ozone is dangerous to humans and fish.



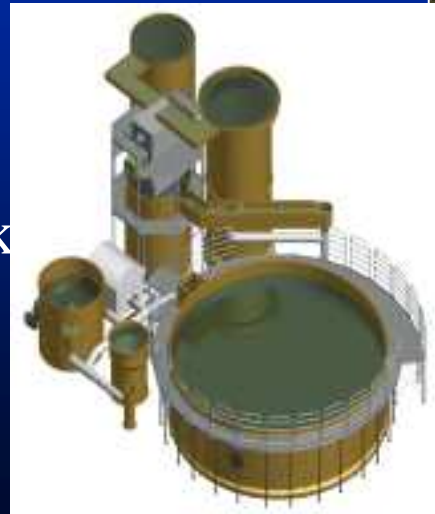
# UV Removes Dissolved O<sub>3</sub>

- 50 mW-s/cm<sup>2</sup> removed 100% of the dissolved O<sub>3</sub> @ inlet O<sub>3</sub> concentration ≤ 0.10 mg/L
  - ✓ 36 mW-s/cm<sup>2</sup> could not remove 100% of the O<sub>3</sub> @ inlet O<sub>3</sub> concentration of ≤ 0.10 mg/L.

# Methods: Full-Flow O<sub>3</sub> + UV Treatment

## ➤ Freshwater Institute's Grow-out System.

- ✓ 4700 L/min recycle flow
- ✓ O<sub>3</sub> added w/ O<sub>2</sub> feed gas in LHO
- ✓ 1.5 min O<sub>3</sub> contact time in LHO sump
- ✓ UV irradiation at 90 MJ/cm<sup>2</sup>
- ✓ O<sub>3</sub> + UV before flow enters culture tank
  - 150 m<sup>3</sup> culture tank
  - 30 min HRT
  - 7.3-8.6 mg/L ΔDO across tank
  - 73-93 kg/day mean feed rate



# Methods: Full-Flow O<sub>3</sub> + UV Treatment

- O<sub>3</sub> Control Processes:
  - ✓ A proportional-integral-derivative (PID) feed-back control loop automatically adjusted the O<sub>3</sub> generated in the O<sub>2</sub> feed gas to maintain the O<sub>3</sub> residual or ORP at a pre-selected set-point at end of O<sub>3</sub> contact chamber.
    - 20 ppb O<sub>3</sub>
    - 375, 450, & 525 mv ORP



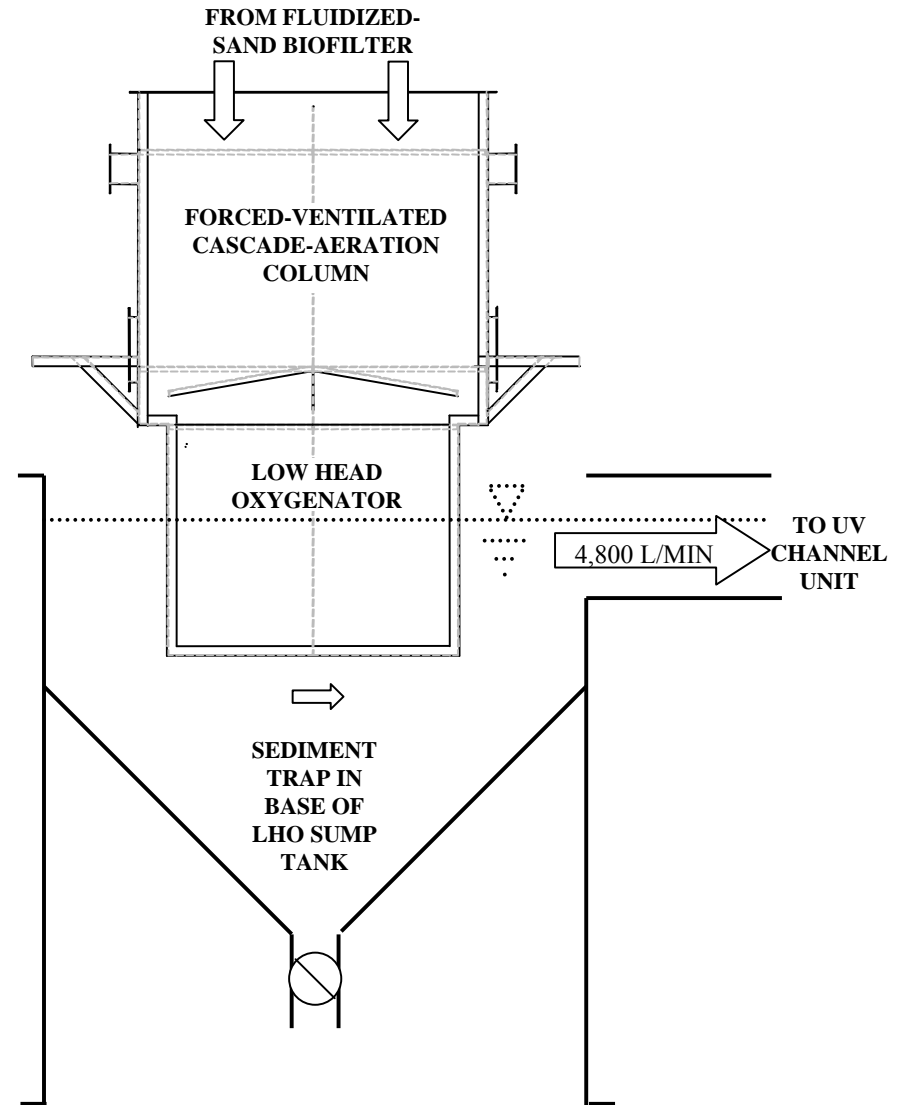
# Methods: Full-Flow O<sub>3</sub> + UV Treatment

- O<sub>3</sub> concentration generated in the O<sub>2</sub> feed gas was **automatically & remotely adjusted** at the PCI-Wedeco model GSO40 ozone generator.



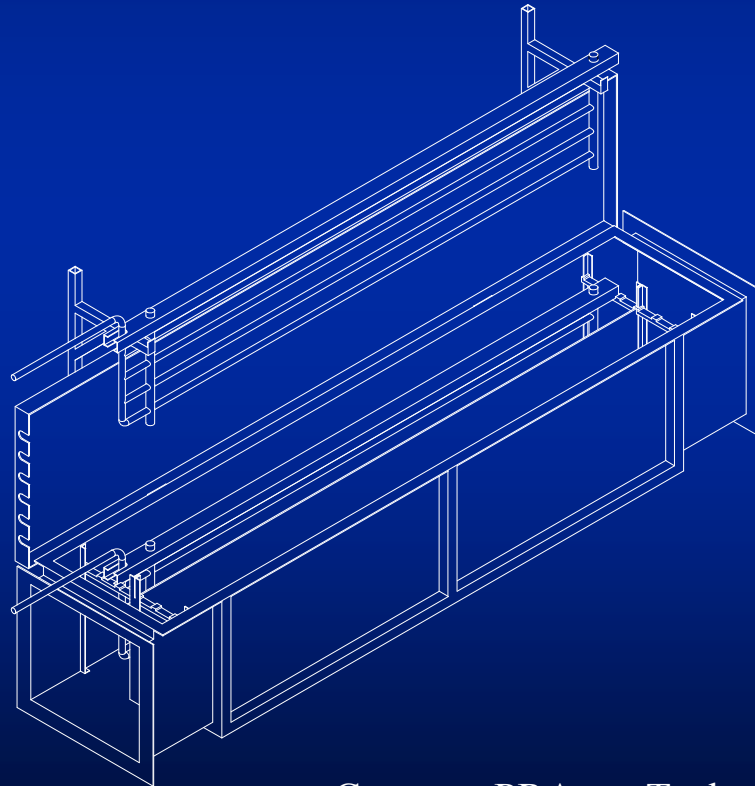
# Methods: Full-Flow O<sub>3</sub> + UV Treatment

- O<sub>3</sub> transfers in LHO
- O<sub>3</sub> contacting in:
  - ✓ LHO
  - ✓ LHO sump
  - ✓ Channel to UV unit
  - ✓ HRT of 1.5 min



# Methods: Full-Flow O<sub>3</sub> + UV Treatment

- UV irradiation channel unit delivered 90 MJ/cm<sup>2</sup>



Courtesy PRAqua Technologies (BC)

# RESULTS: Bacteria Inactivation

# Results: O<sub>3</sub> Followed by UV

## ➤ Total Heterotrophic Plate Counts, cfu/ml

	Before Ozone	After Ozone	After UV	% Removal
<i>No Ozone &amp; No UV</i>	466 ± 147	509 ± 139	<b>530 ± 145</b>	NA
<i>Ozone @ 375 mv &amp; No UV</i>	48 ± 9	22 ± 5	<b>21 ± 3</b>	<b>56.3</b>
<i>Ozone @ 375 mv + UV</i>	124 ± 27	81 ± 18	<b>3 ± 1</b>	<b>97.6</b>
<i>Ozone @ 450 mv + UV</i>	50 ± 12	22 ± 4	<b>0 ± 0</b>	<b>100</b>
<i>Ozone @ 525 mv + UV</i>	386 ± 348	225 ± 209	<b>0.4 ± 0.3</b>	<b>99.9</b>
<i>Ozone @ 20 ppb + UV</i>	47 ± 11	8 ± 2	<b>0 ± 0</b>	<b>100</b>

FULL-FLOW STUDY: Summerfelt et al. (In Prep.)

# Results: O<sub>3</sub> Followed by UV

## ➤ Total Coliform Plate Counts, cfu/100ml

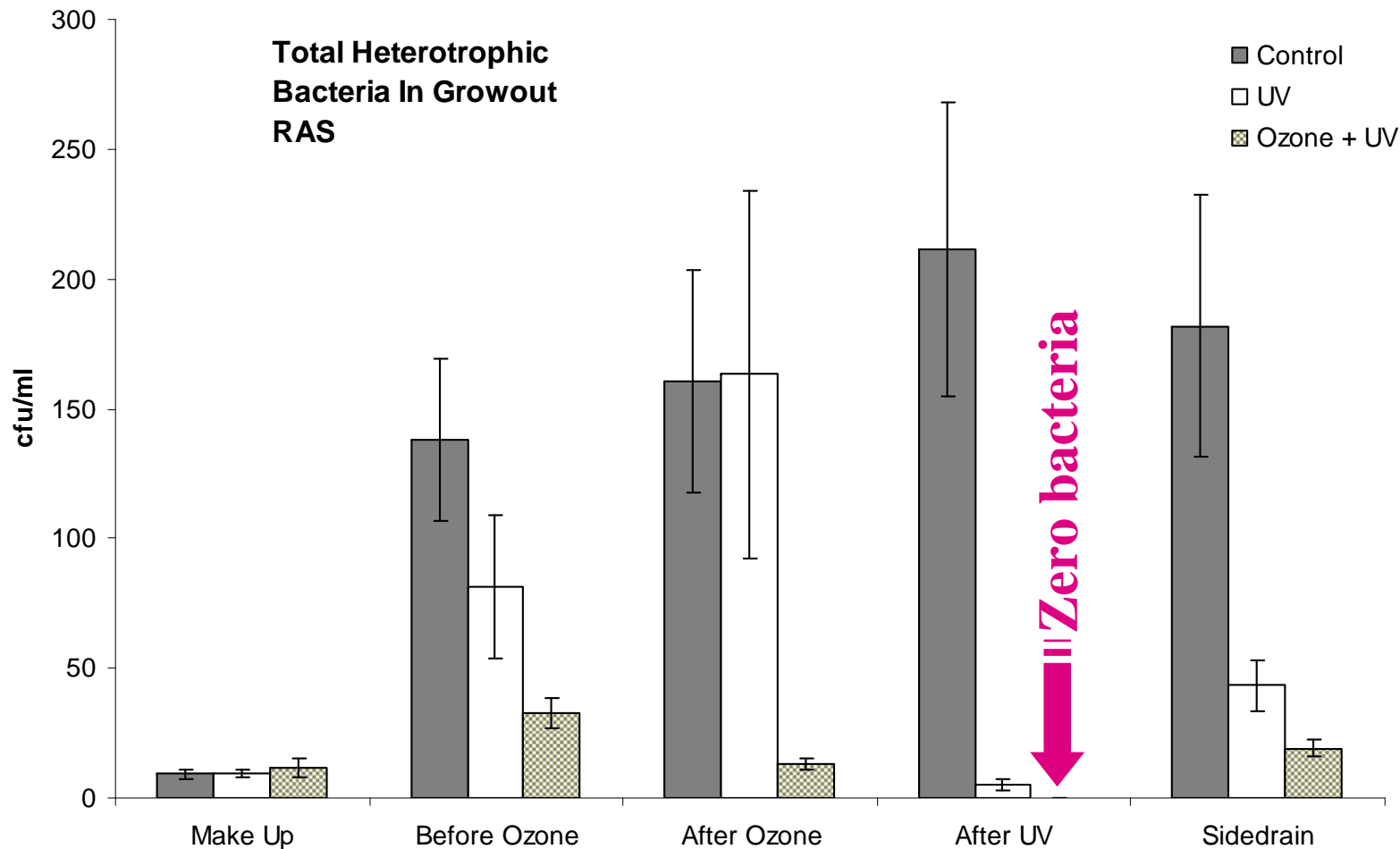
	Before Ozone	After Ozone	After UV	% Removal
<i>No Ozone &amp; No UV</i>	27203 ± 7458	30065 ± 8209	<b>31123 ± 8327</b>	<b>NA</b>
<i>Ozone @ 375 mv &amp; No UV</i>	1293 ± 326	571 ± 229	<b>636 ± 304</b>	<b>55.8</b>
<i>Ozone @ 375 mv + UV</i>	2800 ± 665	2293 ± 763	<b>26 ± 15</b>	<b>99.1</b>
<i>Ozone @ 450 mv + UV</i>	2702 ± 1054	864 ± 236	<b>5 ± 2</b>	<b>99.8</b>
<i>Ozone @ 525 mv + UV</i>	1418 ± 505	439 ± 107	<b>3 ± 2</b>	<b>99.8</b>
<i>Ozone @ 20 ppb + UV</i>	3195 ± 939	498 ± 272	<b>3 ± 1</b>	<b>99.9</b>

FULL-FLOW STUDY: Summerfelt et al. (In Prep.)

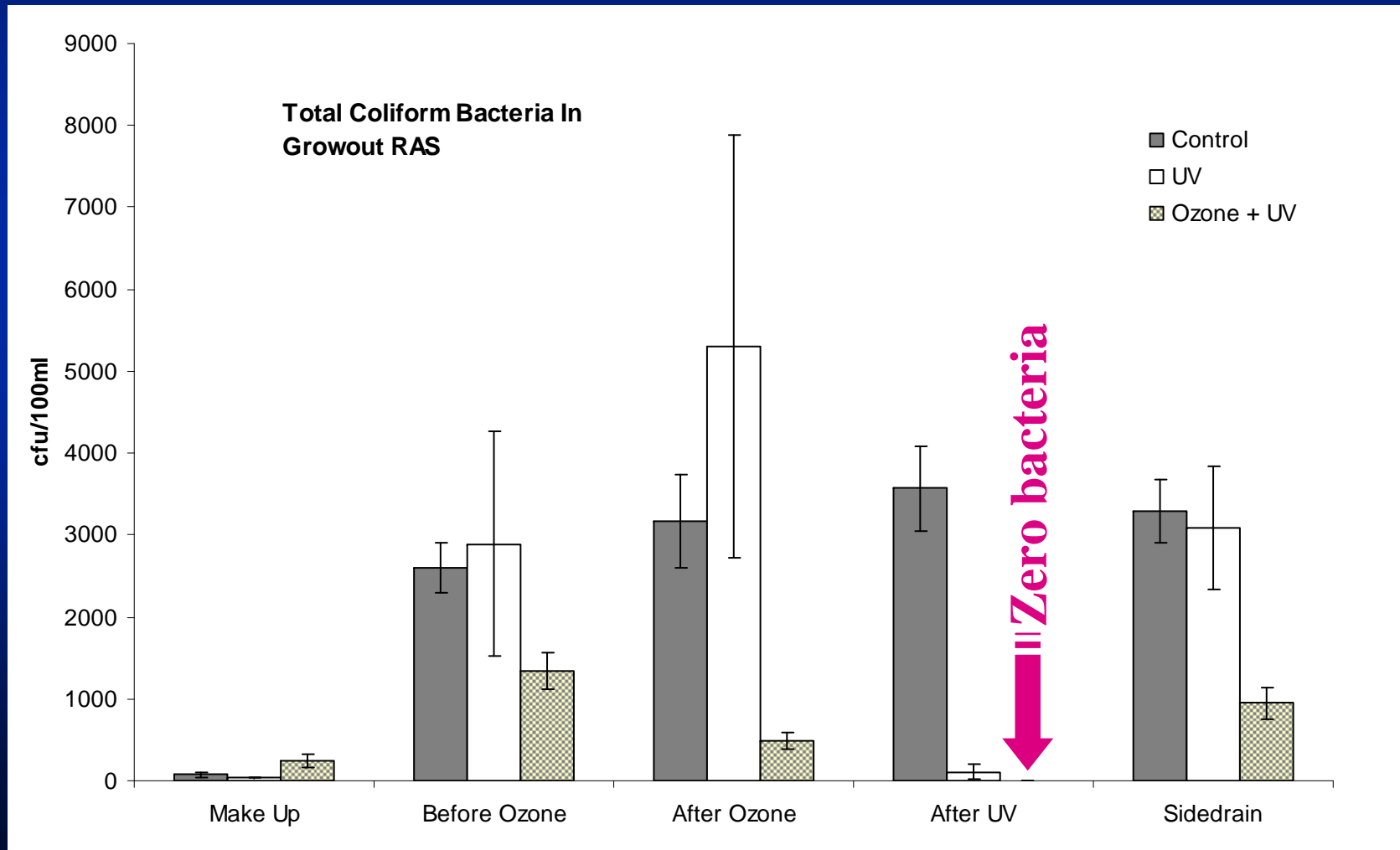
# O<sub>3</sub> Followed by UV Irradiation

- Total Heterotrophic Bacteria Plate Count
  - ✓ < 1 cfu/ml @ ORP of 450 mv & 525 mv & O<sub>3</sub> of 20 ppb
    - 3+ LOG<sub>10</sub> reduction
- Total Coliform Bacteria Plate Count
  - ✓ 3-5 cfu/100ml @ ORP of 450 mv & 525 mv & O<sub>3</sub> of 20 ppb
    - 3 LOG<sub>10</sub> reduction

# Follow-up Study: UV Alone vs. O<sub>3</sub> + UV



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# Results: O<sub>3</sub>/UV & Water Quality

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- Water quality after O<sub>3</sub> & UV treatment  
(flow entering fish tank)

	<b>TAN (mg/L)</b>	<b>NO<sub>2</sub>-N (mg/L)</b>	<b>TSS (mg/L)</b>	<b>Color (Pt-Co)</b>	<b>UV Trans. (%)</b>
<i>No Ozone &amp; No UV</i>	0.11 ± 0.01	0.06 ± 0.03	4.0 ± 0.9	9.5 ± 2.2	90.2 ± 1.5
<i>Ozone @ 375 mv &amp; No UV</i>	0.10 ± 0.01	0.02 ± 0.01	3.0 ± 1.2	0.3 ± 0.3	95.7 ± 0.3
<i>Ozone @ 375 mv + UV</i>	0.13 ± 0.02	0.01 ± 0.01	2.1 ± 0.4	1.7 ± 0.3	94.9 ± 0.2
<i>Ozone @ 450 mv + UV</i>	0.11 ± 0.01	0.01 ± 0.01	2.5 ± 0.5	0.7 ± 0.3	95.3 ± 0.2
<i>Ozone @ 525 mv + UV</i>	0.14 ± 0.02	0.01 ± 0.01	2.4 ± 0.6	1.0 ± 0.6	95.9 ± 0.3
<i>Ozone @ 20 ppb + UV</i>	0.10 ± 0.02	0.01 ± 0.01	2.2 ± 0.2	1.7 ± 0.3	96.8 ± 1.0

# Results: O<sub>3</sub>/UV & Water Quality

- Water quality after O<sub>3</sub> & UV treatment  
(flow entering fish tank)
  - ✓ Mean NO<sub>2</sub>-N dropped from 0.06 mg/L to 0.01-0.02 mg/L
  - ✓ Mean TSS dropped from 4.0 mg/L to 2.1-2.5 mg/L
  - ✓ Mean True Color dropped from 9.5 Pt-Co to 0.7-1.7 Pt-Co
  - ✓ Mean UV Transmittance rose from 90.2% to 94.9-96.8%

RESULTS: How much  $O_3$   
dose must be added to  
overcome the  $O_3$  demand of  
the RAS water?

# Results: O<sub>3</sub> Dose Required

- Mean O<sub>3</sub> concentration & dose applied per kg feed with only 1.5 min HRT for O<sub>3</sub> contacting

Treatment	ORP (mv)	Dissolved Ozone, Probe (ppb)	Dissolved Ozone, Ampoule (ppb)	Ozone Applied per Feed (g/kg)	Ozone Dose Applied (mg/L)
375 mV + UV	375 ± 0	3 ± 0	0 ± 0	<b>28 ± 4</b>	<b>0.38 ± 0.04</b>
450 mV + UV	450 ± 0	7 ± 2	2 ± 1	<b>29 ± 3</b>	<b>0.39 ± 0.06</b>
525 mV + UV	525 ± 0	12 ± 3	7 ± 2	<b>29 ± 2</b>	<b>0.34 ± 0.04</b>
20 ppb + UV	607 ± 32	20 ± 0	22 ± 3	<b>27 ± 3</b>	<b>0.34 ± 0.05</b>

FULL-FLOW STUDY: Summerfelt et al. (In Prep.)

# Previous Research: O<sub>3</sub> Dosing Rate

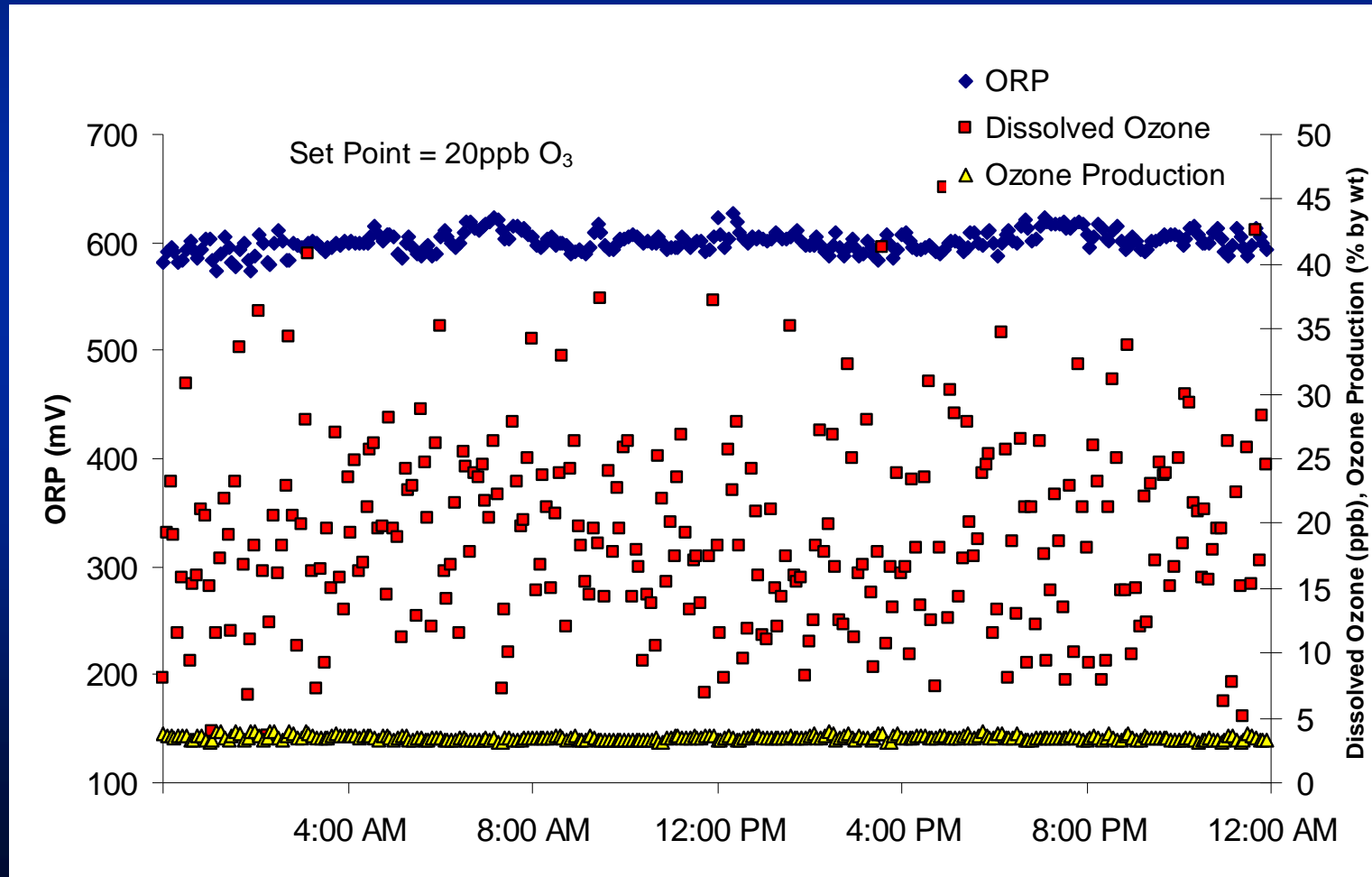
## O<sub>3</sub> Prevents BGD Outbreaks

- Bullock et al. (1997); Summerfelt et al. (1997)
  - ✓ 25 g O<sub>3</sub> per kg feed input
    - improved water quality and microscreen filter performance
    - reduced mortalities associated with Bacterial Gill Disease (BGD)
    - eliminated chemical treatments required to control BGD
    - did not reduce bacteria counts by even 1 log<sub>10</sub>
  - ✓ 36-39 g O<sub>3</sub> per kg feed input
    - same type and magnitude of benefits of lower ozone dose
    - much more likely to kill fish

# Results: Process Control for Full- Flow Ozonation

# Results: Process Control for Full-Flow O<sub>3</sub>

## ➤ ORP & dissolved O<sub>3</sub> probe measurements



# Results: Process Control for Full-Flow O<sub>3</sub>

- ORP probe vs dissolved O<sub>3</sub> probe
  - ✓ ORP was easier to calibrate & maintain
  - ✓ ORP & dissolved O<sub>3</sub> similar to tune for PID control
  - ✓ ORP was just as effective to monitor and automatically control O<sub>3</sub> dose
  - ✓ Dissolved O<sub>3</sub> probe was quick to respond to changes
  - ✓ ORP was slow to respond to sudden drop in dissolved O<sub>3</sub>

# Conclusions

- ORP can be used to reliably monitor and automatically control O<sub>3</sub> dose
- ORP of 450, 525, & 600 mv followed by UV of 90 mJ/cm<sup>2</sup> inactivates total heterotrophic bacteria plate counts
  - ✓ < 1 cfu/ml
  - ✓ 3+ LOG<sub>10</sub> reduction

# Conclusions

- $O_3 + UV$  was more efficient at inactivating bacteria than
  - ✓ UV irradiation w/o  $O_3$
  - ✓  $O_3$  w/o UV irradiation

# Conclusions

- Dose of  $O_3$  that must be added to produce UV disinfection:
  - ✓ 27-29 g  $O_3$  per kilogram feed
  - ✓ 0.34-0.39 mg/L ozone addition
- $O_3$  improves water quality in intensive RAS's:
  - ✓ TSS
  - ✓  $NO_2$ -N
  - ✓ color
  - ✓ % UVT

# Acknowledgements

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- Opinions, conclusions, and recommendations are of the authors and do not necessarily reflect the view of the USDA.
- All experimental protocols involving live animals were in compliance with Animal Welfare Act (9CFR) and have been approved by the Freshwater Institute Animal Care and Use Committee.