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Use of Copper Sulfate and the Disinfectant Peracetic Acid in Aquaculture

Dave Straus

US Department of Agriculture, Agricultural Research Service
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Stuttgart, Arkansas, USA

presented to

2018 Ohio Aquaculture Conference

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Introduction

- Copper sulfate is approved by the EPA as a general use material (algaecide, fungicide, insecticide, water treatment, molluscicide); it is not a restricted-use pesticide.
- It is a safe and economical treatment to control Ich on fish and fungus on fish eggs and is widely used by the catfish industry.
- Our research has focused on getting copper sulfate approved through the U.S. FDA for treating catfish with Ich (*Ichthyophthirius multifiliis*) and catfish eggs for fungus (*Saprolegnia* spp.).

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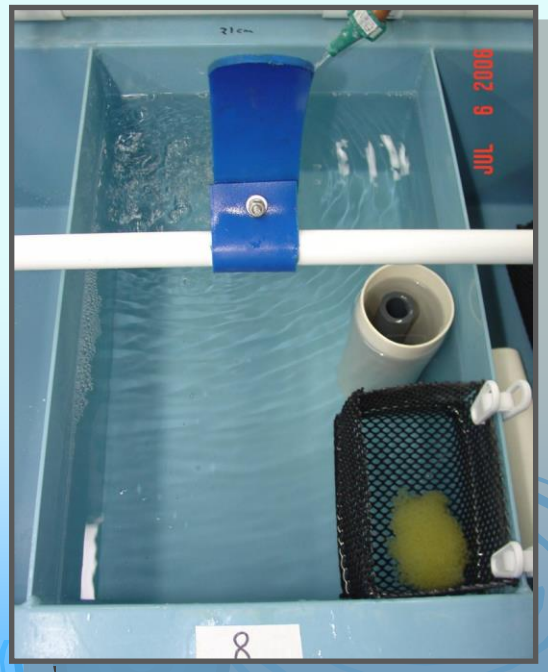
Introduction

- Designed a hatching system to parallel catfish industry practices.
- Evaluated the safety of copper sulfate to eggs (TAS) and hatching fry.
- Treatment limits fungus/improves survival; safe and effective for catfish eggs.
- Additional studies determined 100 ppm did not harm eggs or lower hatch rate.
- Also determined the toxicity to catfish yolk-sac and swim-up fry.
- Most catfish fry producers (farmers and state agencies) now use this treatment.



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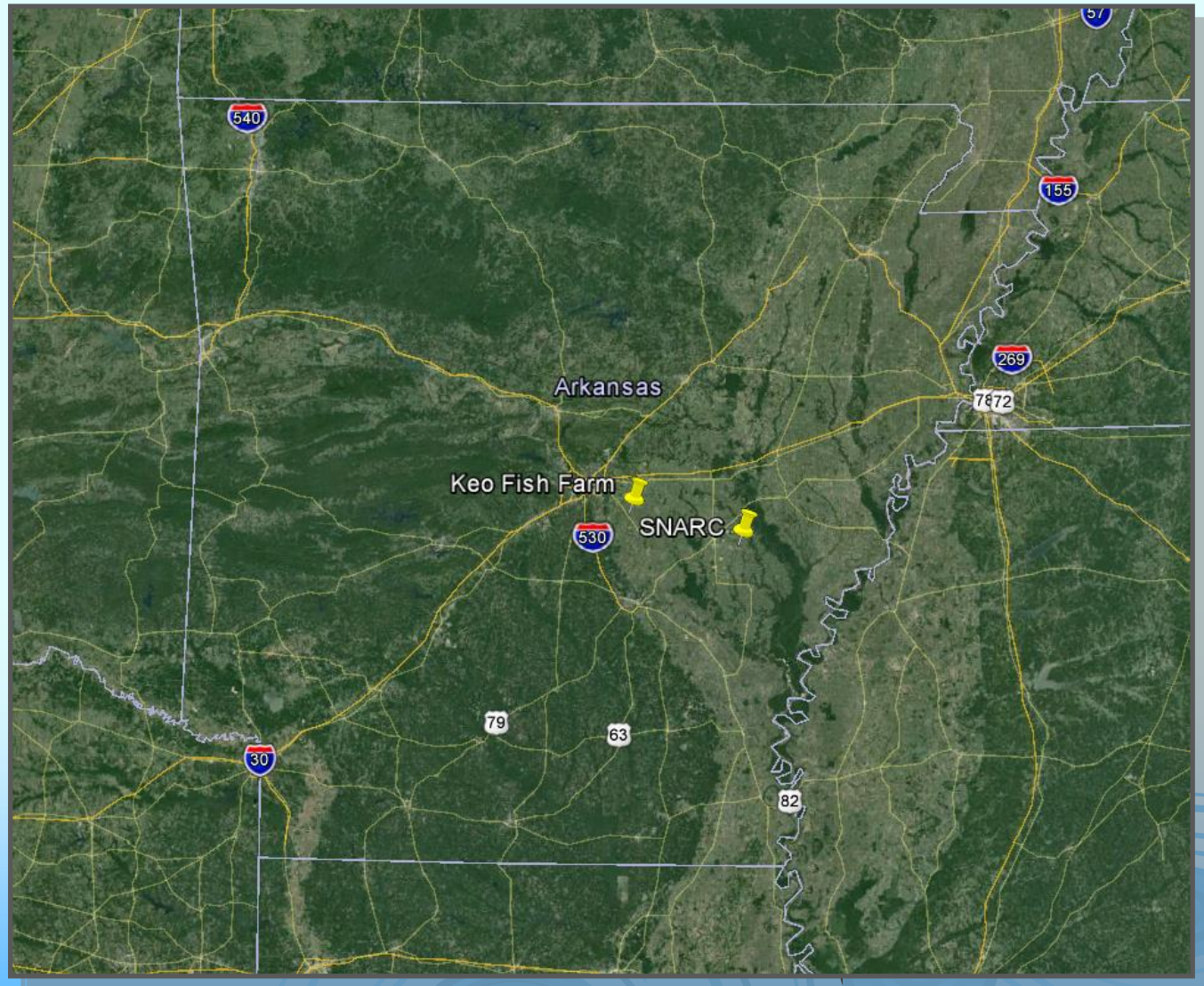
Where *is* SNARC?



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Keo Fish Farm

Spawning building

Hybrid Striped Bass

(female white bass *Morone chrysops* X male striped bass *M. saxatilis)*

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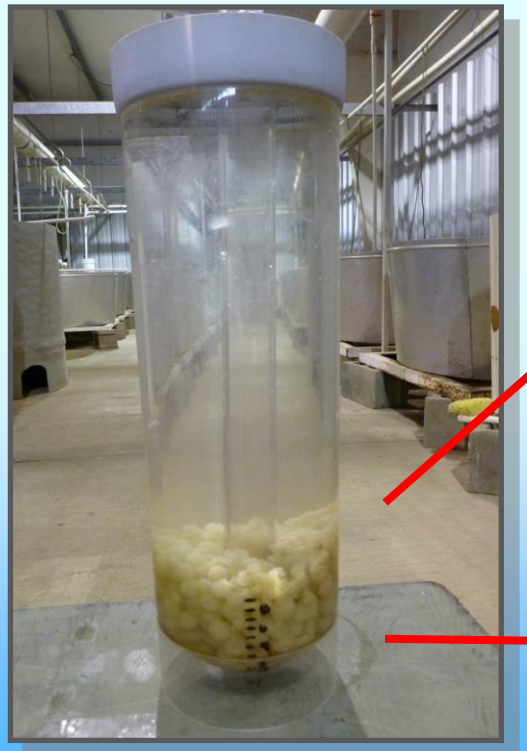
Keo

- Copper sulfate research at Keo:
  - Had severe fungus (*Saprolegnia* spp.) problems when hatching eggs.
  - Fungus can grow on fish eggs in cooler water (spring cold-fronts).
  - When fry are shipped long distances, fungus present in the water can greatly reduce survival.



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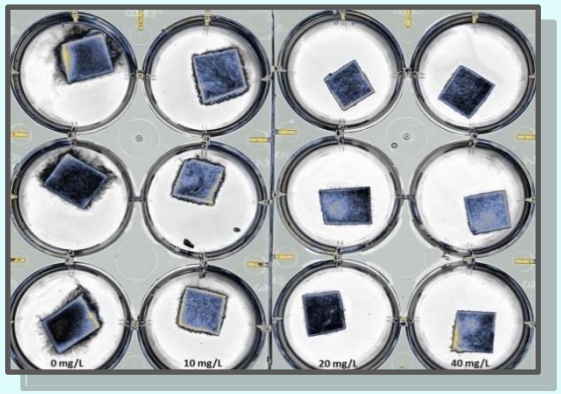
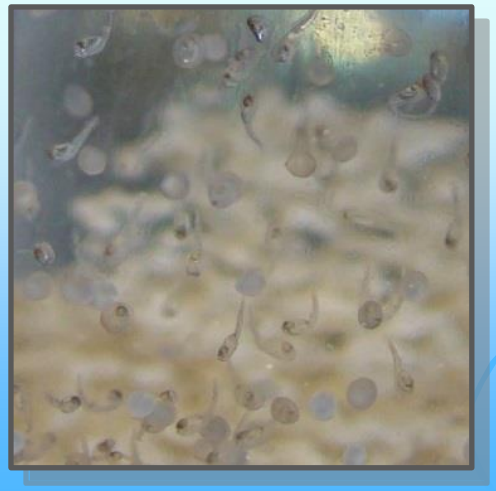
Keo

Making hybrid striped bass

- Female white bass and male striped bass are strip-spawned.
- Eggs are transferred immediately to 6L McDonald jars.
- Tannic acid is added to the jars and they are aerated for 4 min.
- Iodine is added to the jars and they are aerated for 10 min.
- Incubated 48 h until hatch.





Eggs (1000/ml or ~1 mm) and Hatching Larvae

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Largemouth Bass

- Info on Arkansas aquaculture:
  - Birthplace of warmwater aquaculture in the U.S.; started in the 1940s.
  - 2nd in aquaculture-producing states; 20 species of fish/crustaceans.
  - #3 in catfish production - Southeast part of state. (156/1998, 49/2013)
  - 70 - 80% of U.S. baitfish - Lonoke area.
  - 70 - 80% of hybrid striped bass fry produced in the world - Keo.
  - Huge largemouth bass producer - Monroe.
  - Large sport-fish hatchery industry - Lonoke area.

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Regulatory Status

- Copper sulfate ($CuSO_4$) is not approved by the FDA for use on food fish.
    - **But**, regulatory action has been deferred pending ongoing research.
- The only FDA-approved compound(s) for Ich is formalin and for fungus control are formalin and hydrogen peroxide; these are very expensive and have human safety concerns/storage precautions.
- FDA labels we are working on at SNARC:
    - to treat Ich on catfish in ponds
    - to control Saprolegniasis (fungus) on catfish eggs

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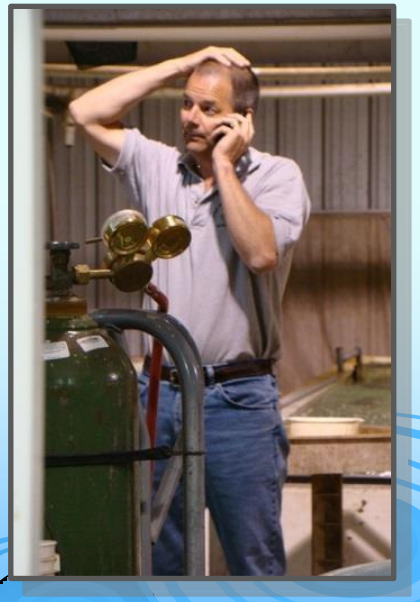
Regulatory Status

- Major technical sections of a New Animal Drug Approval: Product Chemistry, Mammalian Toxicology, Effectiveness, Human Food Safety, Target Animal Safety and Environmental Safety.
- All sections are complete for the Ich label except the Product Chemistry.
- Will follow with Environmental Safety for fungus when we get a Sponsor to complete the Product Chemistry.
- Possible expansion of the label for columnaris?



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Regulatory Status

- Proposed Ich label (drafted with FDA guidance):
  - Treat once daily for 5 – 11 days.
  - Zero withdrawal time.
  - No water discharge for 3 days.

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Regulatory Status

- Copper sulfate is proposed to be an over-the-counter (OTC) therapeutant based upon FDA/CVM Program Policy and Procedures Manual for classification of OTC and Rx drugs:
    - It is a therapeutant with a long marketing history of safe and effective use for the label indications.
    - A diagnosis by a veterinarian is not necessary, as the labeled use conditions are easily recognized by the layman.
    - Studies indicate an adequate margin of safety for the target animal, food products derived from the animal, and for the person administering it when used according to the label directions.
    - Nature of the therapeutant is compatible with OTC use, i.e., the drug has low toxicity and has low misuse and abuse potential incidence of reported adverse reactions when used according to the label directions.
- **So, once we have a label, it can be used for extra-label indications with veterinary oversight.**

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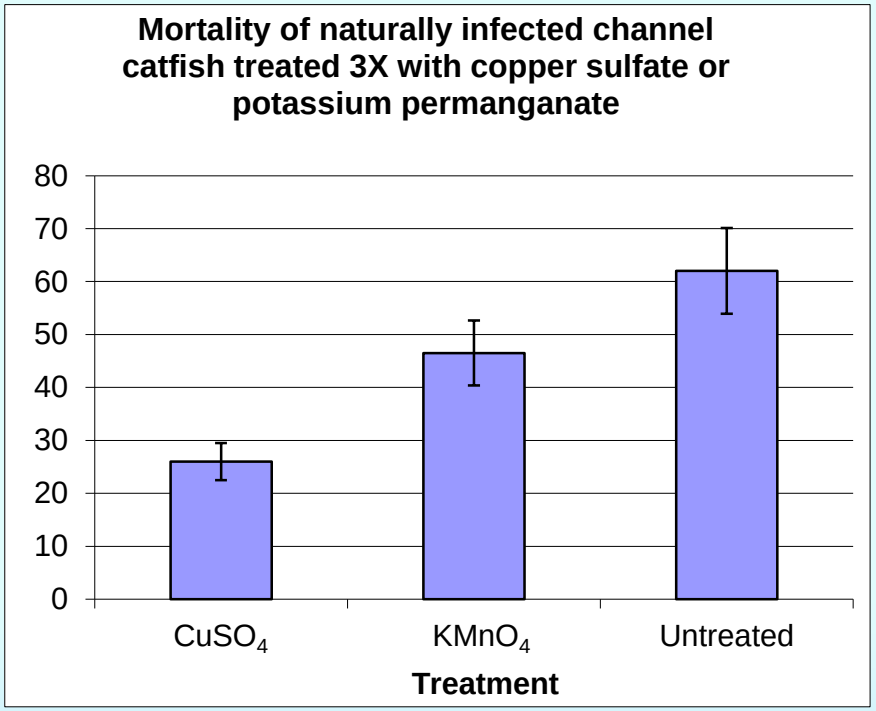
Copper Sulfate vs Potassium Permanganate

- Natural outbreak of columnaris.
- Treatment rates were 2.1 ppm $CuSO_4$ and 3.0 ppm $KMnO_4$.
- Treatments were applied on three consecutive days at 24 h intervals.



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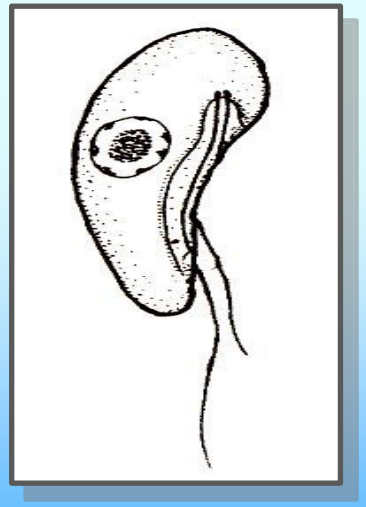
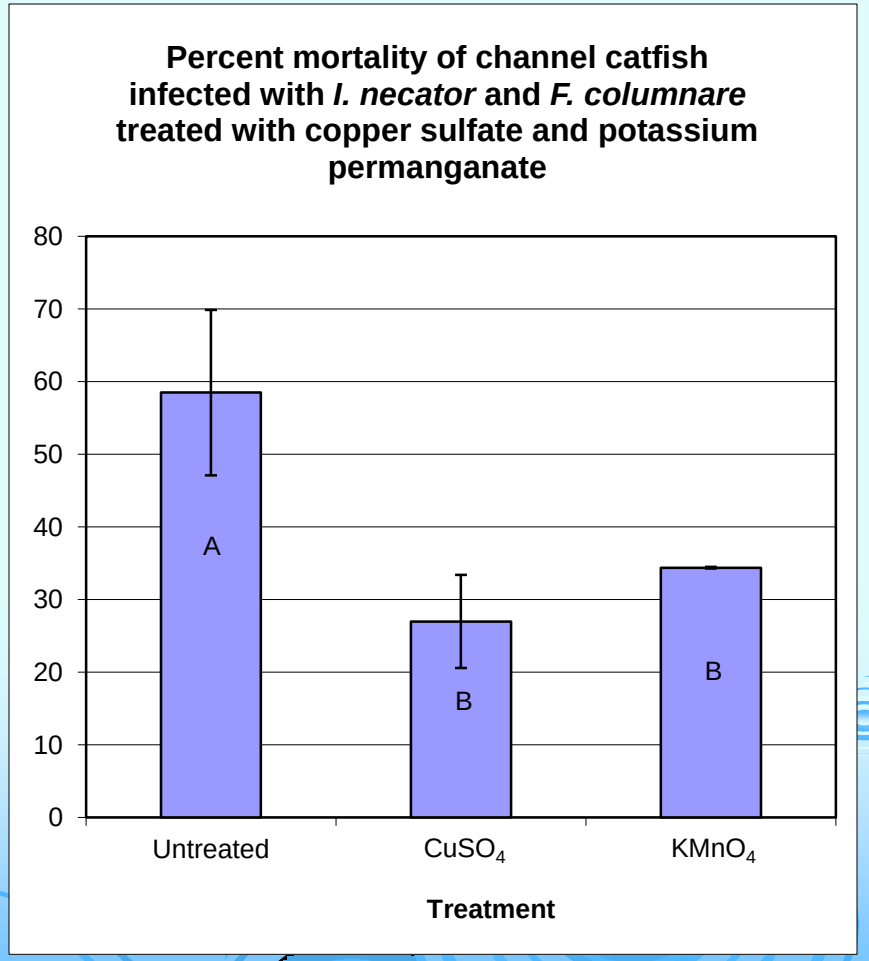
Treating Columnaris and a Parasite

- Natural outbreak of columnaris and costia.
- Treatment rates of $CuSO_4$ or $KMnO_4$ were 2.1 ppm and 3.0 ppm respectively, and were applied once daily on three consecutive days (24 h interval).



Medicated feed
WILL NOT
treat both!



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Low-Flow Challenge System

- Needed a system that could produce diseased fish (columnaris initially).
- Wanted a system to closely approximate industry parameters.
- Didn't want the disease to progress too quickly or kill all the fish.
- Wanted to leave the mucous barrier intact.
- Wanted the results to be repeatable.

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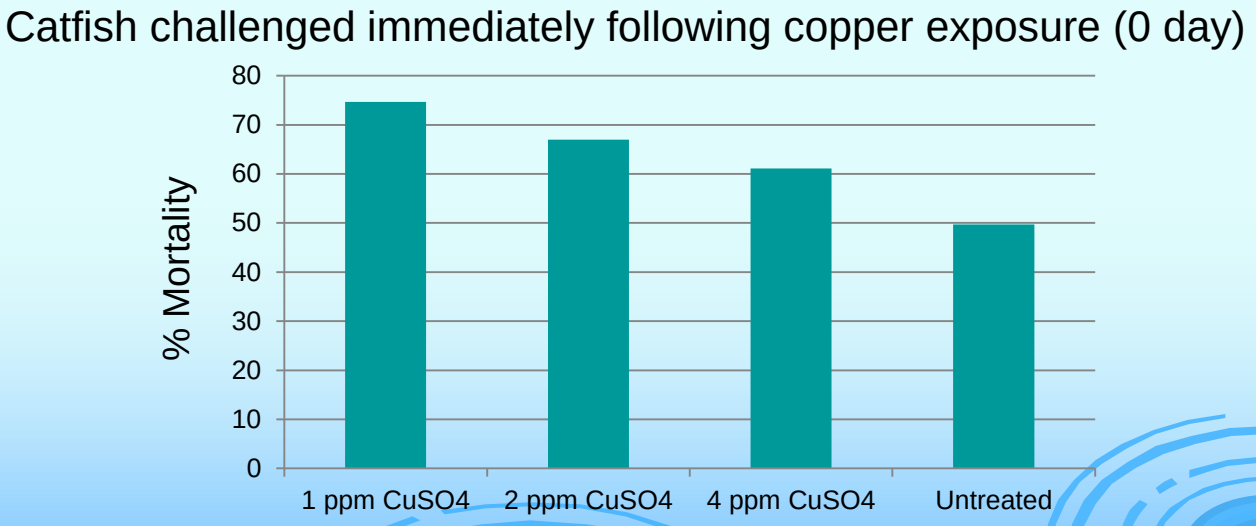
Pre-treating Catfish with Copper

- 24 hour static exposure to either 0, 1, 2, or 4 ppm copper sulfate.
- Our alkalinity is 200, making 2 ppm the recommended treatment.
- Challenged with columnaris at 0, 1, or 7 days after copper exposure.



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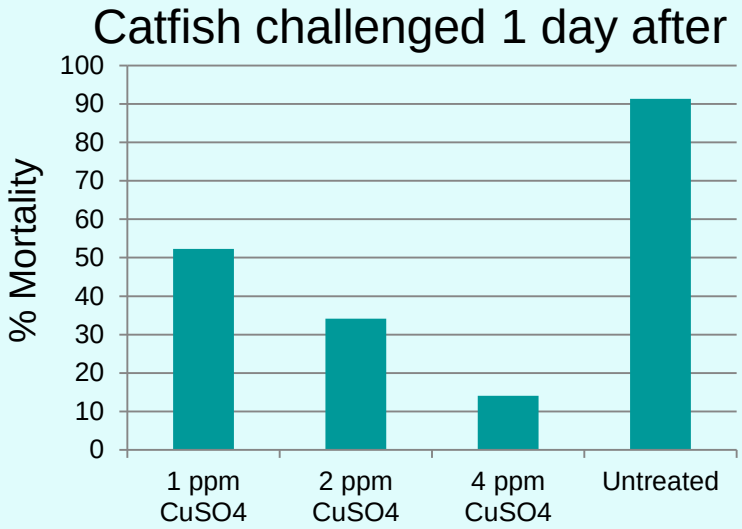
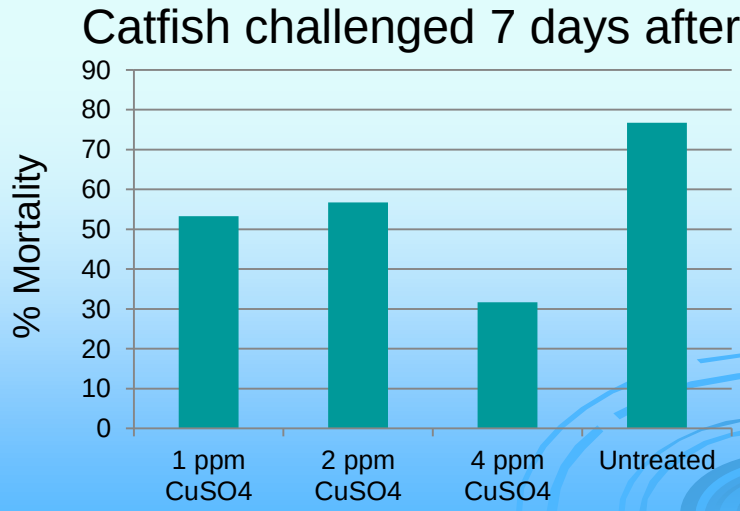
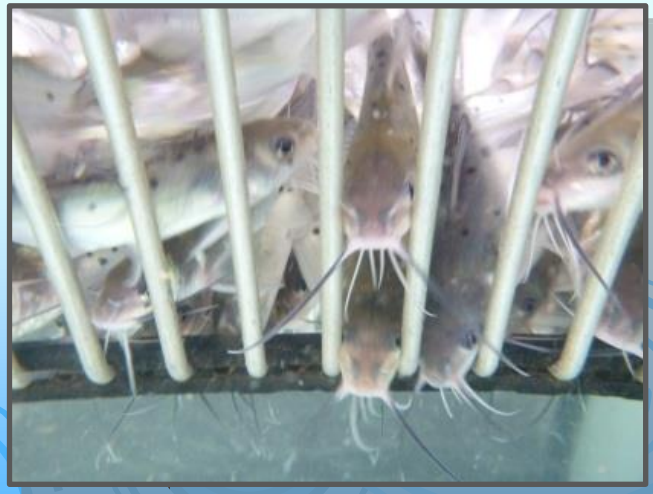
Oh, wait a minute …..







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Elevated Copper Levels in Feed

- Used 2X and 4X the amount of copper sulfate in standard 32% protein catfish feed.
- Fed catfish 3 diets (standard, 2X, or 4X for 30 days).
- Challenged fish at 2 and 4 weeks with columnaris.
- After 2 weeks of copper-supplemented feed, fish fed the 2X and 4X diets had 20% and 27% higher survival than fish fed the base diet.
- At 4 weeks, no difference in survival was found; there was no difference in growth among the 3 diets and no buildup in the fillet.

Bonus: Copper slows feed from molding

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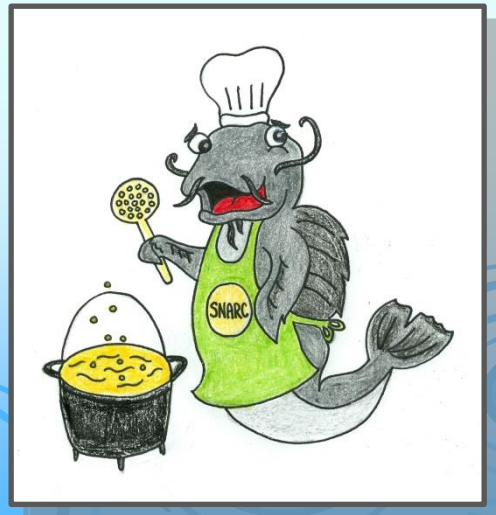
Farmers know their stuff!

“Copper works better than potassium if you have the right water.”

“I harden my fish with copper before I seine them.”

“Why would I try to feed fish that are sick and not eating a medicated feed? Is there something else I can use for immediate help?”

“We used copper all the time for columnaris in the 80’s; what’s changed?”



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Introduction - PAA

- Requested a manuscript on Ich from a researcher in Berlin - Sep 2007.
- Discussed many similar interests (Ich, toxicology, beers, etc.) and finally his new work on peracetic acid (PAA).
  - Treatment of nonspecific mycosis with peracetic acid (Wofasteril E400) during rearing of the small whitefish (*Coregonus albula*). (2005)
- There was very little information on the use of PAA in aquaculture, both in the U.S. and Europe, so we devised a plan to develop research and promote it.
- Along the way we have developed relationships with many other collaborators.

IGB Leibniz-Institute of Freshwater Ecology and Inland Fisheries

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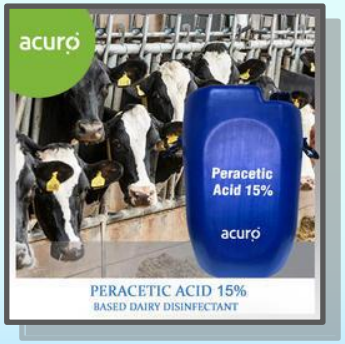
Introduction - PAA

- Peracetic acid (PAA or peroxyacetic acid) is an antimicrobial disinfectant.
- Originally registered by the U.S. Environmental Protection Agency (1985) for indoor use on hard surfaces - mainly hospitals.
- EPA registrations now include: sanitation in food/beverage plants, agricultural facilities, wineries/breweries, greenhouse equipment, animal housing, used to prevent bio-film formation in paper/pulp industries and as a disinfectant for wastewater treatment (2005). Also, commercial laundries and poultry processing (USDA).



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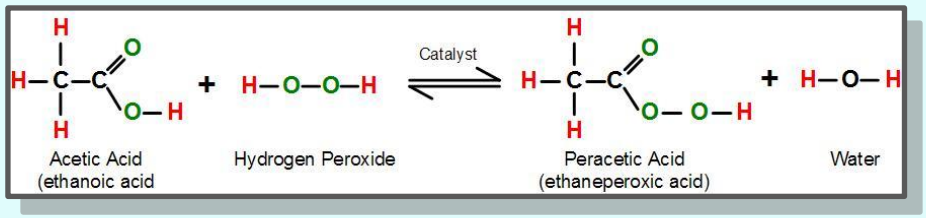
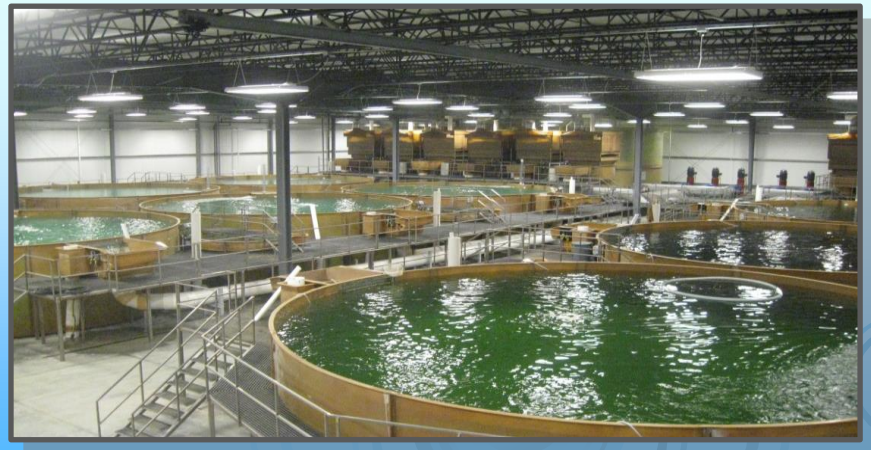
Introduction - PAA

- PAA is commercially available in an equilibrium mixture of acetic acid, hydrogen peroxide and a stabilizer.
- It has greater reactivity and lipid-penetrating properties than hydrogen peroxide alone, thus penetrating into organisms easier and faster. Not broken down by catalase and peroxidase (like H_2O_2).
- Promising disinfectant for the U.S. aquaculture industry for biosecurity and to control parasites and fungus.





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Introduction - PAA

- In Europe, PAA is approved for use in veterinary medicine and is one of the very few compounds approved for use in aquaculture as a disinfectant.
- It does not leave dangerous residues in the environment when it breaks down like most compounds.
- **So, it is very environmentally friendly!**
- First problem. Where to get it in the U.S.?
- Searched and found very few manufacturers:
  - Solvay
  - PeroxyChem
  - Enviro-Tech
  - MinnTech

- Contacted PeroxyChem to get a sample in 2007.
  - Asked if they would be interested working with us on this research…
  - I don't think the were too familiar with aquaculture…

(Now they are up to speed and we talk often.)

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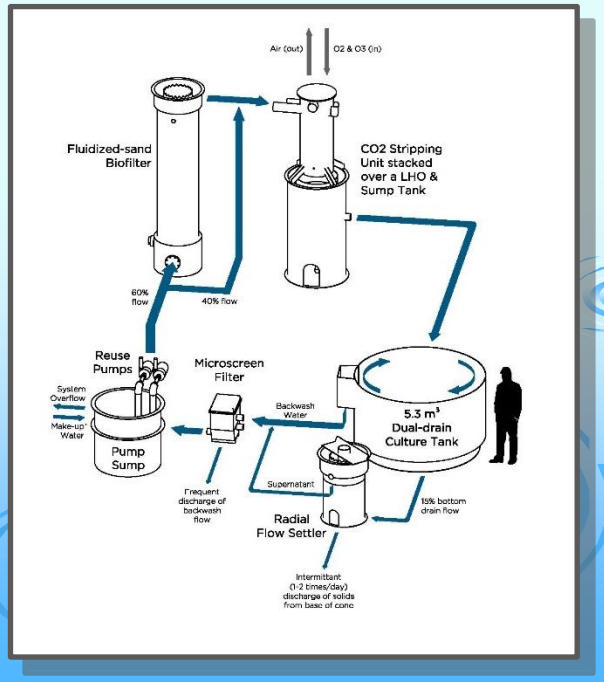
Our collaboration is important because of the expertise of each scientist and the limitations on fish experiments in the EU

- Studies at SNARC:
  - LC50 studies in relevant aquaculture species.
  - Effectiveness studies for aquatic pathogens.

- Studies in Berlin and Denmark:
  - Fungus/bacteria inhibition experiments.
  - Experiments with parasites.
  - RAS (Recirculating Aquaculture Systems).
  - Stress and immune response.
  - Degradation of PAA.



- So let’s see what we’ve done...

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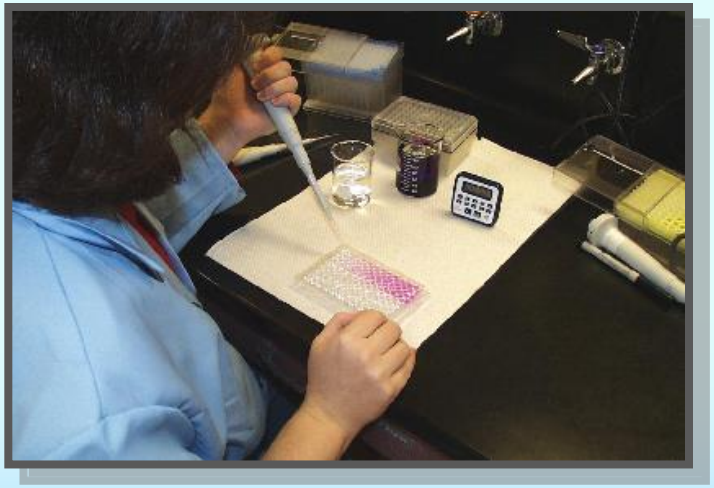
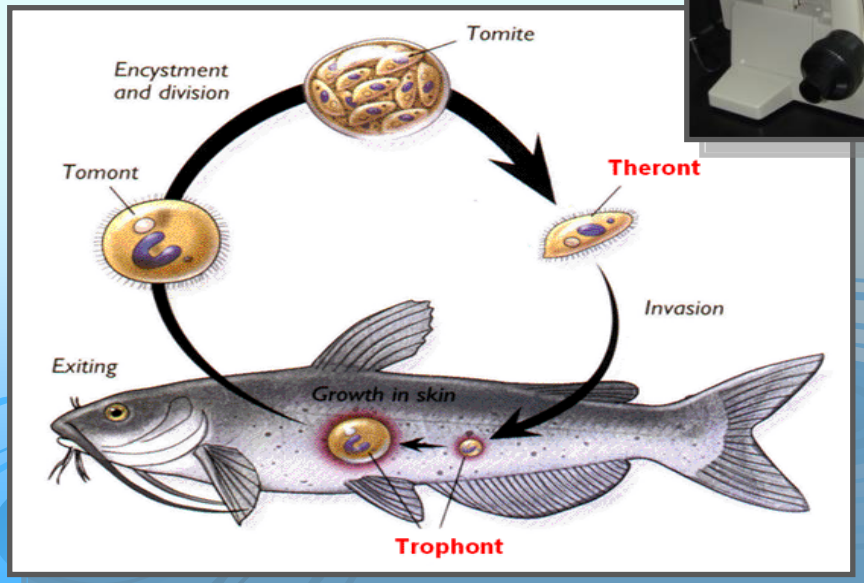
Parasitol Res (2009) 104:1237–1241
DOI 10.1007/s00436-009-1361-9

SHORT COMMUNICATION

Acute toxicity of peracetic acid (PAA) formulations to *Ichthyophthirius multifiliis* theronts

David L. Straus · Thomas Meinelt





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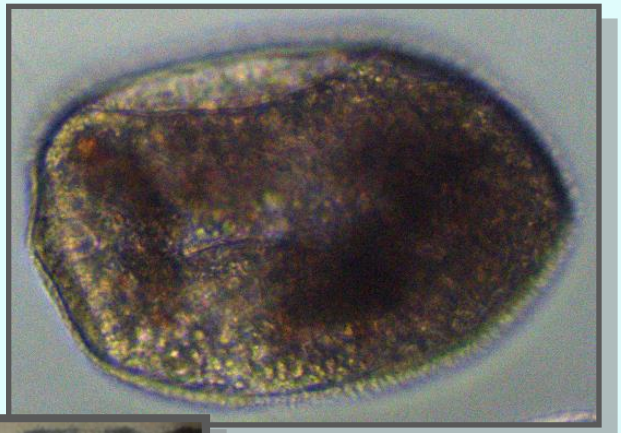
Vol. 86: 51–56, 2009
doi: 10.3354/dao02105

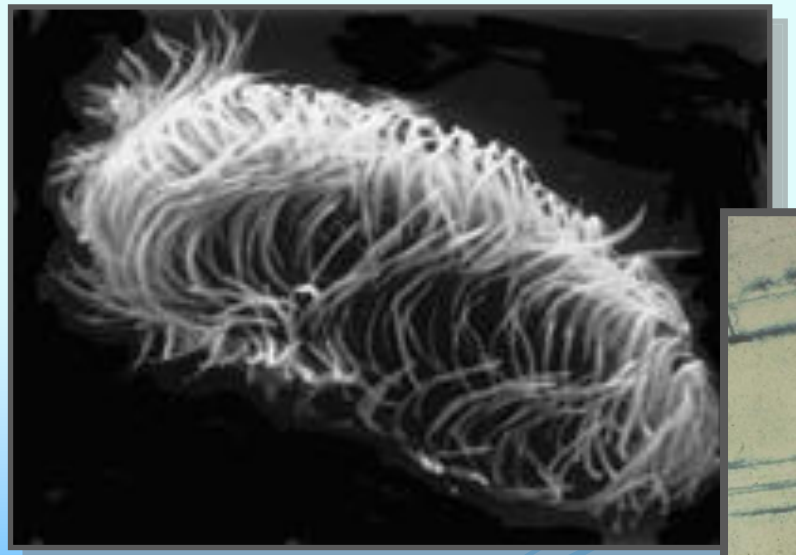
DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

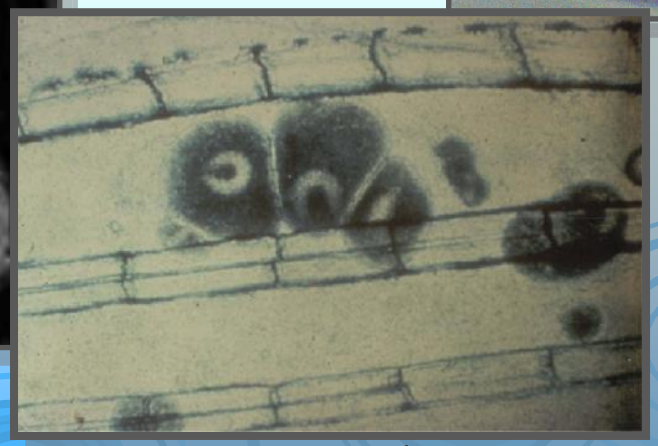
Published September 7

Toxicity of peracetic acid (PAA) to tomonts of *Ichthyophthirius multifiliis*

T. Meinelt[1,*], S. Matzke[2], A. Stüber[1], M. Pietrock[3], A. Wienke[4], A. J. Mitchell[5], D. L. Straus[5]







Tomont

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Journal of Fish Diseases 2012, 35, 505–511 doi:10.1111/j.1365-2761.2012.01383.x

Peracetic acid is effective for controlling fungus on channel catfish eggs

D L Straus[1], T Meinelt[2], B D Farmer[1] and A J Mitchell[1]

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Aquaculture 342-343 (2012) 134–138

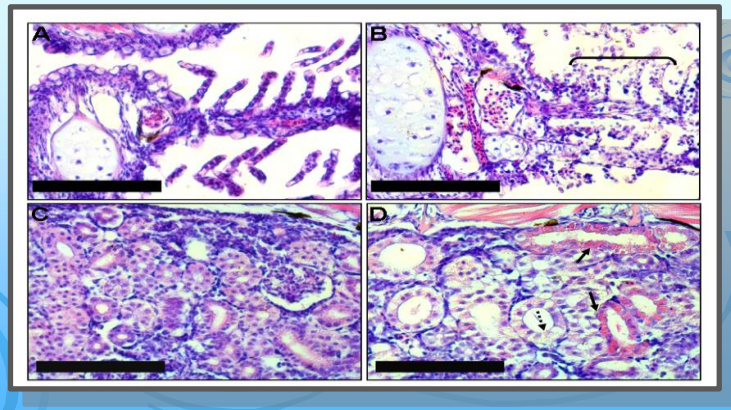
Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Acute toxicity and histopathology of channel catfish fry exposed to peracetic acid

David L. Straus [a,*], Thomas Meinelt [b], Bradley D. Farmer [a], Benjamin H. Beck [a]



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Aquacultural Engineering 53 (2013) 65–71

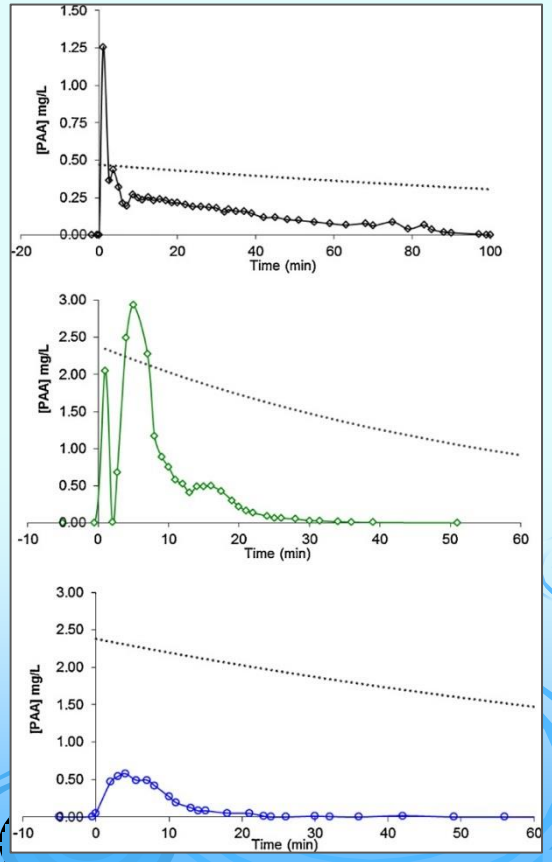
Contents lists available at SciVerse ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

Peracetic acid degradation in freshwater aquaculture systems and possible practical implications

Lars-Flemming Pedersen[a,*], Thomas Meinelt[b], David L. Straus[c]



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Aquacultural Engineering 60 (2014) 35–40

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

Salinity, dissolved organic carbon and water hardness affect peracetic acid (PAA) degradation in aqueous solutions

Dibo Liu[a,b,*], Christian E.W. Steinberg[c], David L. Straus[d], Lars-Flemming Pedersen[e], Thomas Meinelt[b]

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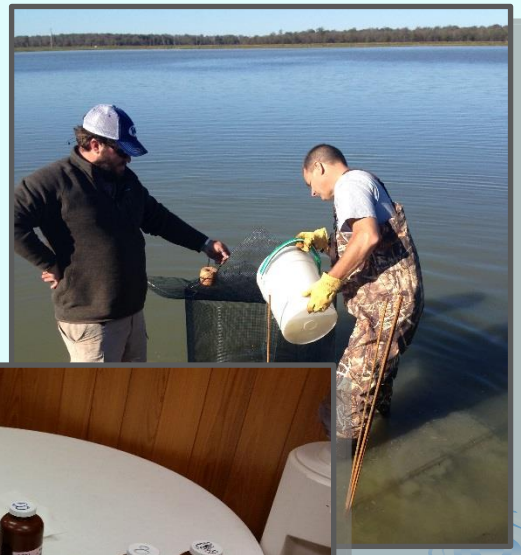
- Jan 2015 - Vipa requested a culture of *Saprolegnia* from a co-worker at SNARC.
- Started setting up a collaboration shortly after.
- Later recruited Vipa to develop a method to quantify the concentration of *Saprolegnia* in ponds and RAS.



BGSU® Bowling Green State University

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- Feb/Mar 2016 - talked with Chris Good at the Freshwater Institute (Shepherdstown, WV) about using PAA instead of hydrogen peroxide in an upcoming experiment.
- Feb 2016 - After a presentation at Aquaculture America started finding out people were using PAA to disinfect marine fish eggs (halibut, sablefish, baitfish).
  - These people were using Perosan by Zep Products; so I contacted Zep.
  - Zep told me they didn't produce it and gave me another contact.
- Mar 2016 - Contacted PeroxyChem again and resumed discussions.
  - Their regulatory manager gave me credit for my "persistence"!

PeroxyChem

zep Superior Solutions

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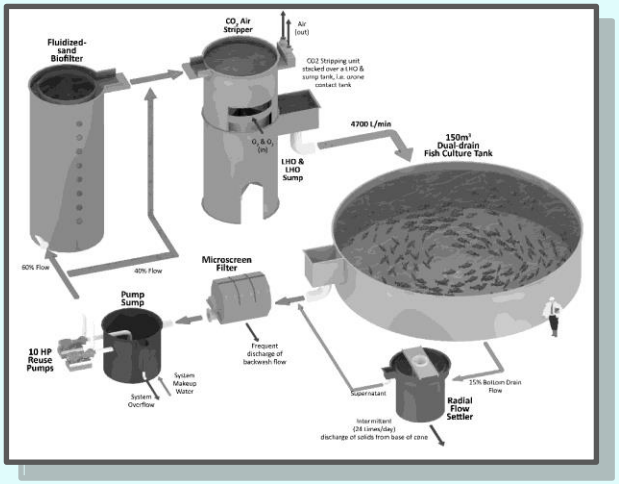
ASSESSING PERACETIC ACID AS A MEANS TO CONTROL POST-VACCINATION SAPROLEGNIASIS IN ATLANTIC SALMON *SALMO SALAR* PARR IN RECIRCULATION AQUACULTURE SYSTEMS

Christopher Good*, John Davidson, David Straus, Susan Harper, David Marancik, Timothy Welch, Christine Lepine, William Wolters, Brian Peterson, Lars-Flemming Pedersen, Vipaporn Phuntumart, and Steven Summerfelt



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- 2016 - Communicated with PeroxyChem about our increasing research efforts with PAA.
- Dec 2016 - PeroxyChem submitted a label request to EPA for:
  - VigorOx SP-15 Antimicrobial Agent is for sanitizing surfaces of harvesting equipment used in the aquaculture industry.
  - VigorOx SP-15 Antimicrobial Agent is for cleaning and disinfecting fish culture tanks and raceways when water is drained and fish are not present.

PeroxyChem

PeroxyChem

VigorOxII Peracetic Acid

Peroxychem's VigorOxII, made of a combination of peracetic acid, hydrogen peroxide, acetic acid, and water, this formula is effective enough to meet Fecal Coliform and E. Coli based permits, however it requires less contact time than chlorine and does not require de-chlorination.

LEARN MORE

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Jun 2017

Obtained the first EPA-registration of PAA as a disinfectant for use in aquaculture!!

ACTIVE INGREDIENTS:

Peroxyacetic Acid	15%
Hydrogen Peroxide	10%
OTHER INGREDIENTS:	75%
TOTAL:	100%

PeroxyChem

Manufactured by:
PeroxyChem, LLC
2005 Market St Ste 3200
Philadelphia PA 19103-7014

ACCEPTED

Jun 26, 2017

Under the Federal Insecticide, Fungicide and Rodenticide Act as amended, for the pesticide registered under EPA Reg. No. 65402-3

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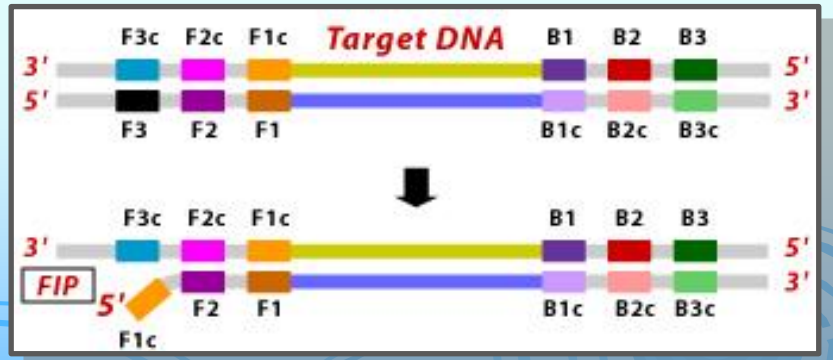
- With Vipa as PI, recently recommended to receive Ohio Sea Grant funding for the project entitled “Development of an efficient approach to quantify and control a fish disease caused by *Saprolegnia* spp.”

  1) To develop molecular approaches to detect, identify and quantify *Saprolegnia spp.* from water samples.

     - Adopt the recently developed molecular technology used to amplify DNA, Loop Mediated Isothermal Amplification (LAMP) to detect and quantify *Saprolegnia* spp.

  2) To investigate the efficacy of PAA in reducing growth of *Saprolegnia* spp. on Atlantic salmon cultured in RASs.

Sea Grant
Ohio Sea Grant College Program



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Introduction - PAA Toxicity

- Since safety to the target animal is key to farmers, and there was hardly any public data available on toxicity to fish:
  - We set out to determine toxicity (LC50) values for 12 species of fish important to aquaculture.
  - More importantly to fish farmers, we determined the No Observable Effects level. (The highest concentration that did not kill the fish.)

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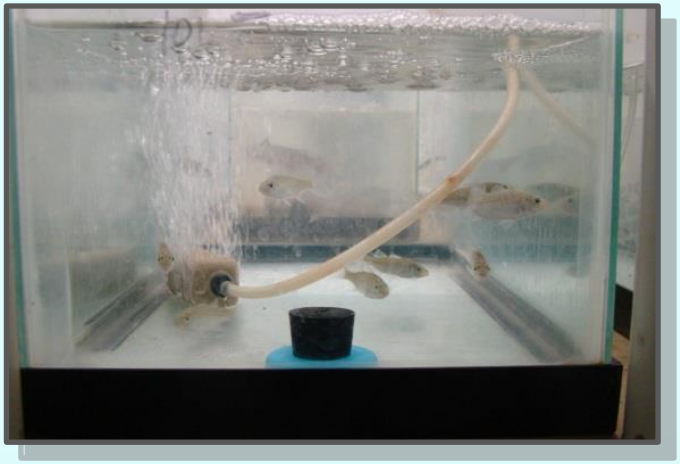
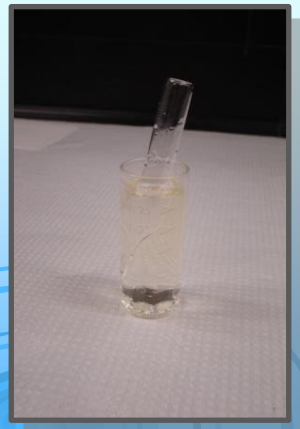
Materials & Methods

- Fish were between 2 – $3^{1}/_{2}$ inches.
- 24 h acute toxicity tests (static) for each species.
- Water chemistry of our well water was: pH = 7.5, total alkalinity = 200 ppm, total hardness = 125 ppm.





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Results

LC50 values, Lowest Observed Effect Concentration, and **No Observed Effect Concentration**.

Latin Name	Common Name	LC50 Value (mg/L)	LOEC	NOEC
Pimephales promelas	Black Fathead Minnow	2.76	2.3	1.9
Notemigonus crysoleucas	Golden Shiner	4.10	3.3	2.8
Oncorhynchus mykiss	Rainbow Trout	4.17	3.3	2.8
Ctenopharyngodon idella	Grass Carp	4.25	4.0	3.3
Carassius auratus	Goldfish	4.55	4.8	4.0
Lepomis macrochirus	Blue Gill	4.75	3.3	2.8
M. chrysops x M. saxatilis	Hybrid Striped Bass	5.31	4.8	4.0
Ictalurus punctatus	Channel Catfish	5.64	4.8	4.0
Micropterus salmoides	Large Mouth Bass	5.88	4.8	4.0
Pomoxis nigromaculatus	Black Nose Crappie	5.94	5.8	4.8
Sander vitreus	Walleye	5.91	4.8	4.0
Oreochromis aureus	Blue Tilapia	9.31	6.9	5.8
Ictalurus punctatus	Channel Catfish [a]	4.79	4.0	3.3
Ictalurus punctatus	Channel Catfish [b]	5.78	5.8	4.8

[a] 1/2 Well Water & 1/2 DI Water.
[b] Well Water with 10 mg/L Huminfeed.

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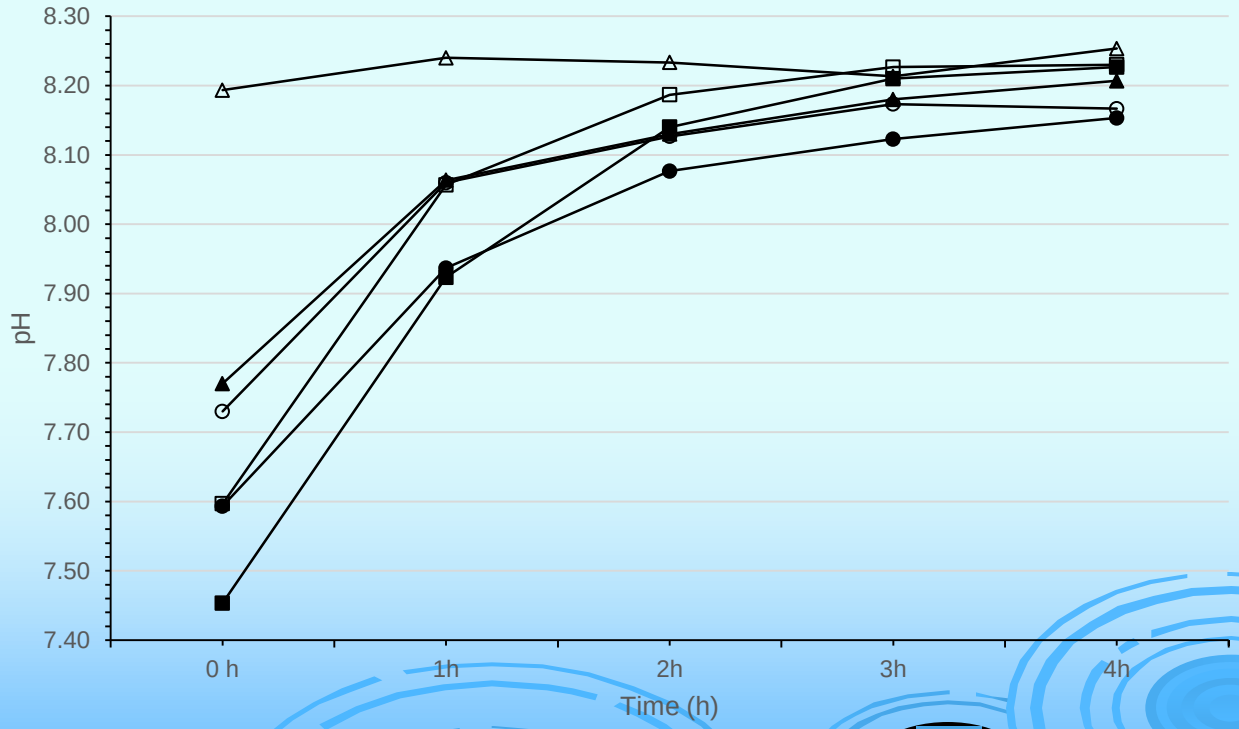
Results

- A profile of the rate of pH recovery over 4 h during the **sunshine bass** bioassay after addition of an aliquot of PAA in static aquaria.
- Nominal concentrations were: Control (open triangle), 4.0 (closed triangle), 4.8 (open circle), 5.8 (closed circle), 6.9 (open square) and 8.3 (closed square) mg/L PAA.



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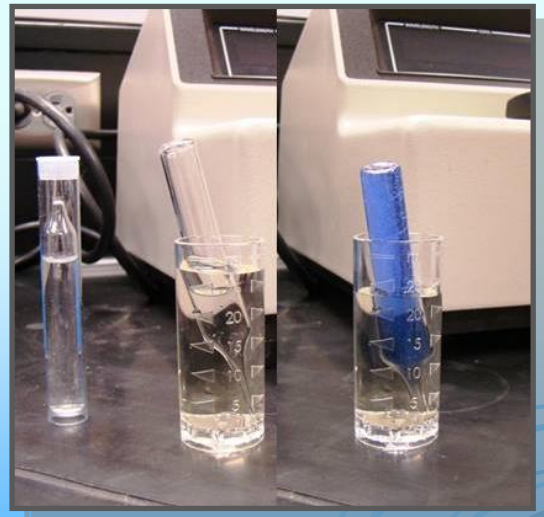
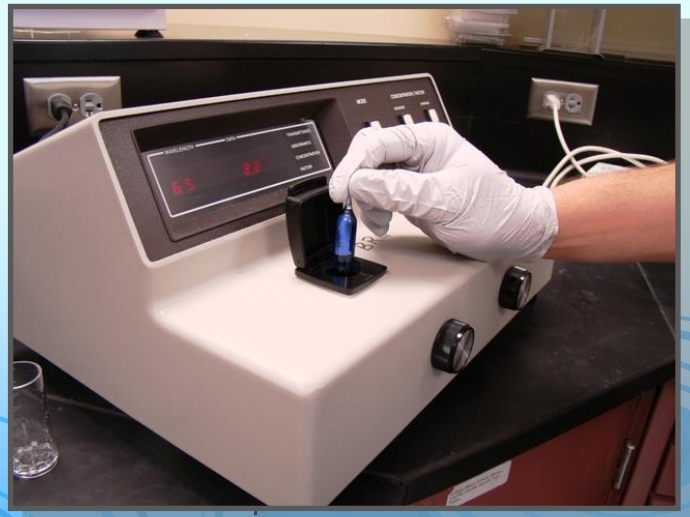
Discussion

- This is some of the first publically generated data to demonstrate the toxicity of PAA to fish.
- Previous data was proprietary.
- Black fathead minnows and blue tilapia were most and least sensitive, respectively.
- This research will be to understand the toxicity variance among species and ultimately determine safe and effective treatment rates.





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Toxicity of Peracetic Acid to Fish: Variation among Species and Impact of Water Chemistry

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