Pfiesteria “Cell From Hell” or Laboratory Artifact? An Update

Wolfgang K Vogelbein

Dept. of Environmental & Aquatic Animal Health, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia 23062

wolf@vims.edu  (804) 684-7261

Background: The Toxic Pfiesteria Complex

Joann Burkholder et al., 1992-2003

• 2 recognized species: *(P. piscicida & P. shumwayae: Pseudopfiesteria gen. nov.)*

• Secretion of potent exotoxin(s)

• “Ambush predator” dinoflagellates (exotoxin sloughs fish tissues, zoospores feed)

• Cause of fish lesions, fish kills & human disease in mid-Atlantic estuaries

• 10 gal, 3 month “Gold Standard” fish bioassay (Burkholder et al. 92-03)

• Complex life history: (24-29 stages)
**Pfiesteria: A Brief History**

1992

1995
Multiple papers detailing complex 24-stage life cycle and “Ambush Predator Hypothesis”

1996
*Pfiesteria* blooms linked to hog waste
Outbreak of fish lesions in Chesapeake Bay
First alarmist news media reports

---

**Pfiesteria History contd.**

1997
“Below the smooth surface of North Carolina's eastern rivers lurks a deadly one-celled organism that is eating holes in fish and threatening people. The "cell from hell" is as frightening as the Ebola virus, but disinterested state regulators look the other way.”

In the Rivers and Coastal Waters of America an Ancient and Deadly Organism, Reawakened by Man-Made Pollution, May Become the Ultimate Biological Thmet.

RODNEY BARKER
Results of Science & Media Coverage

- Seafood & tourism industries in Chesapeake Bay hurt (> $100 million in economic losses)
- Legislation enacted in Maryland regulating nutrient inputs
- Increased Federal and State funding for research (> $25 million allocated to *Pfiesteria* research)

What have we learned from the research funds invested?

Controversial Issues

Burkholder et al. (1992-2003)

- **Life History** (simple vs. complex; amoebae vs. no amoebae)
- **Adverse Environmental Impacts** (fish lesions, death, human disease)
- **Mechanisms of Pathogenicity** (How does *Pfiesteria* kill fish?)
  - are there toxin-secreting strains?
  - other mechanisms of killing?
  - “Time to fish death”, “% mortality”
- **Structure of *Pfiesteria* "Toxin"**
  - >15 years of effort, structure still unknown
Early Studies: The “Gold Standard” Fish Bioassay

• Concerns & issues
  - cultures not available
  - human exposure
  - water quality (NH₃, DO)
  - assay duration (months)

• Causes of fish mortality
  - *Pfiesteria* toxin
  - ammonia toxicity/DO
  - bacterial/parasitic diseases
  - other?

• Water collected from Pamlico River, NC. (Nov. 1999)
  - Killed tilapia 46 days later
  - ID *P. shumwayae* (SEM, PCR)

Fish Killing Assay

Control Assay

Tilapia Pathology

Identical to *Pfiesteria* toxin exposure
(Noga et al., 1996, Law, 2002)

Control

Exposed
96 Hr Larval Fish Toxicity Bioassay

Wild Mummichog
Embryos
Fish Killing Tank
Clonal *Pfiesteria* Culture

1. Fractionation
2. Membrane Insert
Bioassay Setup

7 day old Larvae

96 Hr Larval Fish Assay: Advantages

- Short assay duration (96 hr)
- High statistical power (large sample size, replication, proper controls) and reproducibility
- Reduced water quality issues (fish not fed)
- Reduced human health concerns
- Dinoflagellate/fish interactions readily observable microscopically (pathogenicity & life history studies)
- Lab-reared, parasite-free fish minimize contamination
Micro-Predatory Feeding on Fish Epithelium (SKIN): The Movie

1. Chemoattraction
2. Swarming
3. Physical Attachment
4. Feeding on Epidermis
5. Detachment (1-3 min)

Electron Microscopy: Fish skin/Zoospore interface

Dinospore
Peduncle
Damaged Epidermis
Pfiesteria shumwayae feeding
Experimental Design: Membrane Insert Study

A: Fish
B: Fish & P. shum
C: Fish
D: Fish & P. shum
E: P. shum
F: Fish & P. shum
G: Fish & P. pisc

Membrane Insert: low protein binding polycarbonate, 3.0 µm pore size

Membrane Insert Experiment: Fish Mortality And Water Quality

Cumulative Mortality (%)

Time (hours)

Reactive Ammonia (mg/L)

Dissolved Oxygen (mg/L)
Experimental Design: Fractionations

Fish Killing 38 L Aquarium
“Raw” Water Sample

Fractionation of Raw Water (Filtration/Centrifugation)

Water Quality
- DO
- Temp
- pH
- Sal
- NH₃
- NO₂

1. Dinoflagellate Fraction
2. Bacterial fraction
3. Cell-free supernate
4. “Raw” water
5. NH₃ control
6. Clean water control

Larval Fish Assay

Fractionation Study: Fish Mortality

Cumulative Mortality (%)

Time (hours)

- Raw
- Dinoflagellate
- Supernate
- Bacteria
- High Ammonia
- Control
Summary: An Alternate Mechanism of Pathogenicity


- *P. pseudoshumwayae* & *P. piscicida* kill larval, juvenile & adult fish without secreting toxin(s)
- They kill fish by micro-predatory attack; zoospores feed on superficial epithelial tissues
- Do true “toxic” strains of *Pfiesteria* exist?
  - (Moeller et al., 2001)
    - Lipophilic fraction: phthalate ester contaminant
    - Hydrophilic fraction: killed *Artemia*, fish but no skin lesions
  - (Moeller et al., 2002)
    - Glycoside half sugar half something else unidentified
  - (Moeller et al., 2007. *Environ Sci Technol* 41:1166-72)
    - pPITx ligated copper compound, free radical toxicity
  - No convincing evidence for toxicity or complete structure to date
Life Cycle of *Aphanomyces invadans*

Studies on Etiology of Menhaden Ulcers

### Bath Challenges

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (Cells/mL)</th>
<th>Exposure (h)</th>
<th>Morts</th>
<th>Lesions/total (21 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net-stress (high)</td>
<td>700</td>
<td>2</td>
<td>17/17</td>
<td>19/19</td>
</tr>
<tr>
<td>Net-stress (low)</td>
<td>70</td>
<td>2</td>
<td>17/18</td>
<td>20/20</td>
</tr>
<tr>
<td>Trauma</td>
<td>700</td>
<td>1</td>
<td>16/16</td>
<td>14/16</td>
</tr>
<tr>
<td>No-stress</td>
<td>110</td>
<td>5.5</td>
<td>4/17</td>
<td>6/19</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>5.5</td>
<td>1/13</td>
<td>0/14</td>
</tr>
</tbody>
</table>

WIC strain only

---

14 D post-challenge
Aphanomyces invadens: a highly virulent 1º pathogen of menhaden

Results: Menhaden Challenges

- *Aphanomyces invadens*: 1º pathogen in menhaden
- Zoospores infectious via injections & aqueous exp
- Trauma provides a portal of entry
- Koch’s Postulates fulfilled
- *A. invadens* is the causative agent of ulcerative mycosis
- *Pfiesteria* plays no role
- Massive NC fish kills attrib by other to hypoxia events

Other Critical Findings

- *P. pseudoshumwayae* & *P. piscicida* exhibit simple life cycles typical of other heterotrophic dinoflagellates (Litaker et al., 2002, Parrow & Burkholder, 2003)
- Adverse human health effects not supported by 5 year CDC Human Cohort Study
  - (Morris et al., 2006)
  - Unpublished Virginia & North Carolina data
- *Pfiesteria* spp. occur in Chesapeake Bay (<10 cells/ml; Reece unpublished)