EFFECTIVENESS OF AQUAFLOR® AGAINST BKD

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GREGG ANDERSON,
AND DR. PHIL MAMER

OR BETTER FISHING THROUGH CHEMISTRY
PRESENT THE FINDINGS OF TWO PIVOTAL STUDIES CONDUCTED AT EFHL WITH AADAP

DEMONSTRATE THAT BOTH ERYTHROMYCIN AND FLORFENICOL ARE EFFECTIVE AGAINST BKD
GROSS LESION IN SOCKEYE SALMON
GROSS LESIONS IN CHINOOK SALMON: PIVOTAL STUDY FISH
DISEASE IN THE AQUEOUS ENVIRONMENT

- Virulent
- Environment
- Host

Result: Disease
INAD 6013 METAPHYLACTIC TREATMENT OF BACTERIAL KIDNEY DISEASE (BKD)

WILL USE AS A FEED ADDITIVE FOR JUVENILE SALMON AGAINST BKD (100 MG/KG/DAY)

WILL INJECT INTRA-PERITONEALLY ADULTS AGAINST BKD (10–20 MG/KG)

BACTERIOSTATIC OR BACTERIALCIDAL

INHIBITS PROTEIN SYNTHESIS AT THE 50S RIBOSOMAL UNITS
ERYTHROMYCIN

- FEED TARGET DOSE IS 100 MG/KG/DAY FOR 28 DAYS
- INJECTION TARGET DOSE IS 10–20 MG/KG
- TOXICITY IN JUVENILES (TETANY/DEATH)
- TOXICITY IN ADULTS (JAUNDICE/DEATH)
- NEED AN INAD TO USE IN MEDICATED FEED
- NEED VETERINARY EXTRA-LABEL PRESCRIPTION FOR ADULT INJECTIONS
- UNPALATABLE
- KRILL COATING CAN CAUSE MYCOTIC INFECTION
FUNGUS MOUTH
ERYTHROMYCIN TOXICITY

OVER 70,000 FISH LOST TO ERYTHROMYCIN TOXICITY AT RAPID RIVER (2005)
WHAT ELSE COULD WE USE?

- IN TIMES OF HIGH STRESS CLINICAL SIGNS WOULD APPEAR

- INAD (9332) HIGH DOSE OTC (10 g active OTC/100#’s of fish, for 14 DAYS)

- CALLED JIM BOWKER WOULD AADAP BE INTERESTED IN EXPANDING LABEL FOR OTC

- JIM CALLED BACK AND SUGGESTED AQUAFLOR®
NEED FOR ANOTHER ANTIBIOTIC

- CURRENTLY WE MUST PREDICT OUR ERYTHROMYCIN NEEDS IN JANUARY

- UNDER STRESSFUL CONDITIONS, CHINOOK HATCHERIES STILL EXPERIENCE LOSSES TO BKD

- ANTIBIOTIC RESISTANCE

- AVAILABILITY OF INJECTABLE ERYTHROMYCIN IS DUBIOUS
AQUAFLOR (FLORFENICOL)

- FIRST FEED ANTIBIOTIC FOR U.S. AQUACULTURE IN MORE THAN 20 YEARS

- BACTERIOSTATIC or BACTERIOCIDAL
  - CONCENTRATION OR DURATION DEPENDENT

- BROAD SPECTRUM

- HIGHLY PALATABLE

- SAFE (POSSIBLE UV SENSITIVITY IN STHD/RBT)
AQUAFLO\textregistered

- CAN BE USED WITH A VETERINARY FEED DIRECTIVE

- INHIBITS PROTEIN SYNTHESIS BY BINDING TO RIBOSOMAL SUBUNITS OF SUSCEPTIBLE BACTERIA
EGGS WERE COLLECTED, FERTILIZED, AND WATER HARDENED **WITHOUT** IODOPHOR FROM A FEMALES WITH GROSS SIGNS OF BKD (SOUTH FORK OF THE SALMON RIVER SUMMER CHINOOK SALMON)

THESE FEMALES DID NOT RECEIVE AN INJECTION OF ERYTHROMYCIN

EGGS WERE INCUBATED AT McCALL HATCHERY

FRY WERE TRANSPORTED TO EFHL AND HELD UNTIL SIGNS OF BKD WERE NOTICED
COMPARE MORTALITY RATES BETWEEN AQUAFLOR MEDICATED FEED AND CONTROL FEED

BOTH TREATMENTS FED AT 4.0% BODY WEIGHT

AQUAFLOR MEDICATED FEED TARGET DOSE AT 15 MG/KG FOR 10 DAYS

FOUR CONTROL TANKS WITH 206 FISH PER TANK AND FOUR TREATMENT TANKS WITH 206 FISH PER TANK

OBSERVE 14 DAYS POST TREATMENT
DAILY DATA COLLECTION

- Mortality in each tank
- Feeding response
- Dissolved oxygen
- Temperature
- Water chemistry
- All data collected by masked investigator
- Unmasked investigator weighs out feed (knows which tanks are which)
OTHER INFORMATION

- FISH FED BY BELT FEEDERS (FIRST TRIAL)
- DAILY WATER QUALITY PARAMETERS MEASURED BY YSI 556 MPS METER (DISSOLVED OXYGEN, pH, AND TEMPERATURE)
- HACH KIT WAS USED FOR ALKALINITY AND HARDNESS
INFECTION CONFIRMED BY DFAT, ELISA, AND PCR
SAS PROC GLIMMIX (general linear mixed model) $P<0.05$
ACCLIMATION 1d, TREATMENT 10d, POST-TREATMENT 14 d (SECOND STUDY 18 d)
STUDY 1 BIO–OREGON BIOVITA STARTER #2
MEAN WATER TEMPERATURE WAS 13.6°C
AVERAGE D.O. WAS 6.1 mg/L
HARDNESS, ALKALINITY, pH
  ◦ 85 mg/L, 8 mg/L, 7.2
<table>
<thead>
<tr>
<th>TANK NUMBER</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td>TOTAL MORTALITY</td>
<td>47</td>
<td>39</td>
<td>24</td>
<td>45</td>
<td>32</td>
<td>43</td>
<td>27</td>
<td>66</td>
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<tr>
<td>POST TREATMENT</td>
<td>24</td>
<td>18</td>
<td>10</td>
<td>24</td>
<td>12</td>
<td>28</td>
<td>14</td>
<td>44</td>
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</tbody>
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**TREATED TANKS:** MEAN % CUMULATIVE MORTALITY 14.8% (RANGE 11.7–18.9%)
TOTAL MORTALITY: 122 (RANGE 24–39 MORTS/TANK)

**UNTREATED:** MEAN % CUMULATIVE MORTALITY 24.7% (RANGE 20.9–32.0%)
TOTAL MORTALITY: 200 (RANGE 43–66 MORTS/TANK)

\[ P=0.021! \]

GREEN FONT DESIGNATES AQUAFLOR TREATED TANKS
AQUAFLOR VS BKD

- AQUAFLOR TREATED TANKS BENEFITED FROM THE TREATMENT

- MINIMUM INHIBITORY CONCENTRATION NEEDS TO BE ESTABLISHED FOR AQUAFLOR AGAINST *RENIIBACTERIUM SALMONINARUM*

- THIRD PIVOTAL STUDY INVESTIGATION NEXT YEAR?
ERYTHROMYCIN VS AQUAFLOR VS CONTROL

- EGGS WERE COLLECTED, FERTILIZED, AND WATER HARDENED WITHOUT IODOPHOR FROM A FEMALES WITH GROSS SIGNS OF BKD (SOUTH FORK OF THE CLEARWATER RIVER SPRING CHINOOK SALMON)
- THESE FEMALES DID NOT RECEIVE AN INJECTION OF ERYTHROMYCIN
- EGGS WERE INCUBATED AT CLEARWATER HATCHERY
- FRY WERE TRANSPORTED TO EFHL AND HELD UNTIL SIGNS OF BKD WERE NOTICED
FISH WERE RANDOMLY PLACED INTO SEMI-CIRCULAR TANKS

4 REPLICATES PER TREATMENT

AQUAFLOR TANKS RECEIVED 15 MG/KG FOR 10 DAYS (TARGET DOSE)

ERYTHROMYCIN TANKS RECEIVED 100 MG/KG FOR 28 DAYS (TARGET DOSE)

MORTALITY COMPARISON

- DATA ANALYSIS by SAS PROC GLIMMIX
- TUKEY MULTIPLE COMPARISON
RESULTS

- MEAN % CUMULATIVE MORTALITY
  - DAY 24
    - FFC – 12.1% (11.4–12.7%)
    - ERY – 8.7% (4.8–11.0%)
    - CONTR – 20.5% (17.3–22.9%)
  - DAY 42
    - FFC – 17.6% (16.7–19.1%)
    - ERY – 8.8% (4.8–11.0%)
    - CONTR – 28.1% (23.6–31.3%)
RESULTS

- SIGNIFICANT DIFFERENCES
  - ERY – CONTROL – DAY 13
    - $P=0.0253$
  - FFC – CONTROL – DAY 15
    - $P=0.0411$
  - ERY – FFC – DAY 28
    - $P=0.0483$
CONCLUSIONS

- BOTH ANTIBIOTICS EFFECTIVE
  - CONTROLLING MORTALITY DUE TO BKD

- END OF AQUAFLOR®
  - NOT SIGNIFICANTLY DIFFERENT

- END OF ERY
  - DIFFERENCE

- DIFFERENT TREATMENT DURATION
  - 28 d VS 10 d

- DIFFERENT MODE
  - TRANSLOCATION RXN VS PEPTIDE TRANSFERASE RXN

- LIKELY LONGER AQUAFLOR®
  - FLORFENICOL ‘TIME-DEPENDENT’ MICROBIOCIDAL ANTIBIOTIC
ACKNOWLEDGEMENTS

- USFWS AADAP
  - DAN CARTY – DATA ANALYSIS

- FDA OFFICE OF MINOR USE/MINOR SPECIES
STAFF MEETING
IDFG HATCHERY STAFFS

- CLEARWATER HATCHERY
- McCALL HATCHERY
- EAGLE HATCHERY
WESTERN FISH DISEASE WORKSHOP

- HOSTED BY IDFG
  - BOISE, IDAHO
  - 12 JUNE 2012
  - THE GROVE
- CE: NUTRITION, FEEDS AND FEEDING
  - DR. ANN GANNAM
- RACE CREDITS FOR VETERINARIANS
QUESTIONS?

I DON’T WANT TO BE AN EXPERIMENT