Efficacy of florfenicol, copper sulfate and potassium permanganate in controlling a natural infection of *Aeromonas hydrophila* and *Flavobacterium columnare* in sunshine bass

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Introduction

- Sunshine bass (SB) production has been a fast growing segment of the aquaculture industry in the United States over the past decade and has spread to several countries in Europe and Asia.
- SB production has been plagued by many pathogens like *Aeromonas hydrophila* and *Flavobacterium columnare* which also affect other important cultured species (Plumb 1999).
- Currently there are no FDA approved drugs for treating SB diseases.

Objective

- Assess the efficacy of $CuSO_4$, $KMnO_4$ and florfenicol (FFC) against a mixed natural infection of *Aeromonas hydrophila* and *Flavobacterium columnare* in SB.

Method

- SB (30.2 ± 0.5 g) received from a fish farm exhibited clinical signs and many were moribund.
- Microscopic examination of wet mounts from the gill and skin of the fish sampled at the start of the experiment did not reveal notable parasitic infestation.
- The fish condition was assessed by external and internal post mortem examination and bacterial isolation.
- Bacterial isolation was performed on TSA agar supplemented with blood agar and on selective Ordal (Hawke & Thune 1992).

Efficacy Study

- Six hundred fish were randomly allocated to 30 tanks, 20 fish per tank. Five tanks were randomly assigned to each treatment.

The experiment had six treatments:

- FFC-medicated feed at 15 mg/kg body weight (BW) of fish for 10 d.
- Non-treated fish as a control.
- Two waterborne exposures to $CuSO_4$ for 24 h at a rate of 2.1 or 4.2 mg/L (1 and 2 % the total alkalinity, respectively).
- Two waterborne exposures to $KMnO_4$
  a) 2 mg/L above the $KMnO_4$ demand (PPD) for 24 h, or
  b) 10 mg/L, above the PPD for 1 h.

The parameters studied

- The clinical signs were observed.
- Dead and moribund fish were collected daily and the external and internal signs were recorded.
- Bacterial isolation was attempted from the kidney, liver and external lesions.

Statistical Analysis

- The survival percentages within tanks were arcsine transformed and analyzed by ANOVA. The differences among treatment means ($P \leq 0.1$) were determined by Tukey (1953).
- Conducted survival analysis: 1) Kaplan-Meier method to calculate the probability of survival at each day, and 2) logrank test to compare the survival curves (Motulsky 1995).

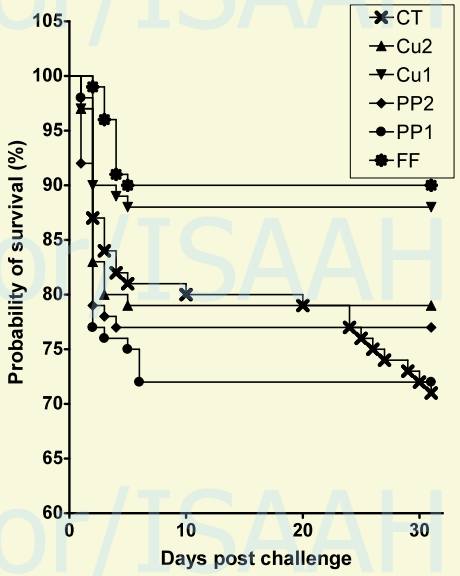
Results

- The final survival of fish fed FFC-medicated feed (90%) was significantly higher ($P<0.1$) than the control fish (71%).
- According to the logrank comparison the survival curves of the FFC-medicated treatment and the $CuSO_4$ treatment at 2.1 mg/L were significantly better than the control fish.

	treatment type					
	CT	Cu1	Cu2	PP1	PP2	FF
Survival % within tanks	71 ± 7[b]	88 ± 3[a,b]	79 ± 6[a,b]	72 ± 4[b]	77 ± 3[a,b]	90 ± 5[a]

Final survival percent of sunshine bass naturally infected with *A. hydrophila* and *F. columnare* and subjected to: 1) waterborne exposure to $CuSO_4$, at 2.1 or at 4.2 mg/L (Cu1 and Cu2, respectively) for 24 h; 2) waterborne exposure to $KMnO_4$ at 2.5 mg/L for 24 h and at 10 mg/L for 1h (PP1 and PP2, respectively); 3) FFC-medicated feeding at 15mg/kg body weight of fish for 10 d (FF); and 4) no treatment to act as control (CT). (P<0.1).

Survival curve of SB naturally infected with *A. hydrophila* and *F. columnare* and subjected to; 1) four waterborne treatments, $CuSO_4$ at 2.1 and at 4.2 mg/L (Cu1 and Cu2, respectively) for 24 h and $KMnO_4$ at 2.5 mg/L for 24 h and at 10 mg/L for 1h (PP1 and PP2, respectively); 2) FFC-medicated feeding at 15mg/kg body weight for 10 d (FF); and 3) non-treated as control (CT).



Bacterial Isolation

- Dead and moribund fish cultured positive (94%) as follows: 43% had *A. hydrophila* only, 47% had mixed *A. hydrophila* and *F. columnare* infection and 4% had *F. columnare* only.
- Both *A. hydrophila* and *F. columnare* were isolated internally from the kidney, indicating a systemic infection, and from external lesions.

Clinical Sign and Pathology

- The fish were lethargic.
- Externally, the skin had depigmentation, loss of scales, erythemia and hemorrhages and the fins were frayed. The eyes had exophthalmia, hemorrhages and corneal opacity. Abdominal distention with swollen and hemorrhagic vents were noted.
- Internally, the fish had ascetic fluid, peritoneal hemorrhages, mottled livers and dark spleens.

Discussion and Conclusion

- This is a pioneer report of controlled experiments investigating the efficacy of FFC, $KMnO_4$ and $CuSO_4$ against a mixed natural infection of *A. hydrophila* and *F. columnare* in SB.
- Florfenicol at 15 mg/kg BW and $CuSO_4$ at 2.1 mg/L significantly increased the survival rate of infected fish.
- Florfenicol was also found efficacious against streptococcosis in SB (Darwish 2007) and against several other pathogens in different fish species (Gaunt et al. 2004).

Conclusion and Discussion (cont.)

- The reduction of mortality in the FFC-medicated fish suggests that FFC concentration reached effective levels in SB tissues (Blood et al. 1979).
- The survival curve of the fish treated with $CuSO_4$ at 2.1 mg/L was significantly better than the control, prolonged survival. Prolonging the survival of fish with secondary infections could prove to be a useful until stress is alleviated.

Conclusion and Discussion (cont.)

- Pre-exposure to $CuSO_4$ protected against *F. columnare* infection in striped bass (MacFarlane et al. 1986).
- Pre-exposure of channel catfish to $CuSO_4$ afforded protection against *Edwardsiella ictaluri* (Griffin and Mitchell 2007).
- Copper provided protection to Zebrafish challenged with *Listeria monocytogenes;* the fish were exposed to copper pre and post challenge (Rougier et al. 1996).

Conclusion and Discussion (cont.)

- The current results need to be further substantiated by field efficacy studies.
- Clinicians need to be cognitive of when a therapeutic intervention will be indicated to reduce mortalities and when it will be more judicial to alleviate stress to enhance recovery from secondary pathogens like *A. hydrophila* and *F. columnare*.

Acknowledgment

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