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# Copper sulfate treatment decreases hatchery mortality of larval white seabass *Atractoscion nobilis*

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**Abstract**

Culture of white seabass *Atractoscion nobilis* larvae was repeatedly limited by severe mortality during the first 18 days posthatch (dph). Affected larvae examined by phase-contrast and scanning electron microscopy, and by conventional histopathology had numerous bacteria on the epidermal surface, especially on the primordial fin. The bacteria were associated with microscopic epidermal lesions that progressed to fin fraying and ulceration. No other cause of death was found by histopathology. Bacteria cultured from the skin surface included *Vibrio* and *Psuedomonas* spp. White seabass at 12, 15, and 52 dph were given 2 h exposures of 0.1–1.0 mg $l^{-1}$ total copper ion to develop a disease treatment for larval and juvenile fish. Production lots of white seabass were experimentally treated with 0.1 mg $l^{-1}$ total copper ion as a 1 h static bath between 7 and 16 dph. Although all treatments significantly ($P=0.00$) increased survival, a standardized treatment at 10 and 12 dph increased larval survival at 18 dph harvest from under 1% in untreated larvae to 32–66% in treated groups ($P<0.0001$). Copper sulfate treatments significantly reduced bacterial colonization of the epidermis ($P=0.00$) and treated fish had fewer microscopic lesions ($P=0.00$). Fish had no microscopic evidence of toxicity due to treatment at 0.1 mg $l^{-1}$. One h bath treatments of 0.1 mg $l^{-1}$ copper sulfate increase survival of intensively cultured white seabass larvae.


*Keywords:* Marine fish larvae; White seabass; Histopathology; Copper sulfate; Disease therapy; Bacteria

## 1. Introduction

Production of fish larvae often limits the success of marine finfish aquaculture (Katavic, 1986; Dhert et al., 1998). Mass mortality tends to occur during a critical period in early larval development at the time of first feeding (May, 1973; Smigelski, 1975; Skjermo and Vadstein, 1999). Mass larval mortality has been linked to feeding practices (Mobin et al., 2000; Crespo et al., 2001) and to the presence of opportunistic bacteria in live food, particularly *Vibrio* spp. (Wakabayashi et al., 1986; Muroga et al., 1990; Munro et al., 1995; Skjermo and Vadstein, 1999). The mechanisms by which *Vibrio* spp. and other opportunistic bacteria cause mortality in larval marine fish have been described as enteritis (Muroga et al., 1990), gill infection (Austin et al., 1993) and systemic infection (Horne et al., 1977), however bacterial dermatitis caused by the genera *Flavobacterium*, *Pseudomonas*, *Aeromonas* and *Vibrio* spp. are common causes of

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juvenile marine fish mortality in cultured marine fish (Noga, 1996). Minimizing the problem of early larval mortality is essential for successful culture of any marine fish species.

Copper sulfate has been widely used to control finrot and skin lesions of freshwater fish (Gratzek et al., 1992; Leitritz and Lewis, 1980; Davis, 1953). Copper sulfate is also used to control external parasites of fish in marine aquaria (Cardeilhac and Whitaker, 1988; Gratzek and Blasiola, 1992). However, suggested treatment levels and exposure times for large juvenile and adult fish in marine aquaria may not be appropriate for larval fish in marine aquaculture. Also, doses required to remove protozoan or metazoan parasites may be higher than needed to remove or inhibit bacteria. Parker and Geiger (1984) recommend copper sulfate baths of 1 h or longer as an effective treatment for external infections of striped bass caused by *Aeromonas* spp., *Pseudomonas* spp., *Flavobacterium columnare* in freshwater and *Vibrio* spp. in saltwater. Literature on the toxicity of copper sulfate is usually based on freshwater species (Straus and Tucker, 1993). Because of widespread clinical use of copper sulfate, controlled studies on use of copper sulfate in larval and juvenile marine aquaculture species are needed.

White seabass *Atractoscion nobilis* have been cultured experimentally since 1982 with the goal of enhancing a declining natural fishery (Vojkovich and Reed, 1983). High larval mortality repeatedly limited the large scale production of white seabass at a marine hatchery in California during the years 1998–1999. Preliminary studies provided evidence that mortality was associated with bacteria on the surface of the skin. Experiments were carried out with fish removed from production lots at the hatchery to develop safe treatment levels and regimes for use of copper sulfate as a disease therapy for larval and juvenile white seabass.

The primary objective of this study was to determine the effect of low-level copper sulfate baths on the survival of larval white seabass. The secondary objective was to identify potential causes of larval fish mortality.

## 2. Methods

### 2.1. Larval fish culture

White seabass were spawned and reared at the Leon R. Hubbard, Jr. Marine Fish Hatchery at Carlsbad, California, USA. Captive adult white seabass were induced to spawn with temperature and photoperiod manipulation. Fertilized eggs were collected within 18 h of spawning. Buoyant, fertile eggs were enumerated volumetrically in a 4 l graduated cylinder and separated from the non-viable eggs and faecal material at the bottom of the cylinder by decanting. Fertile eggs were routinely disinfected with 100 mg $l^{-1}$ formalin 1 h (Tamaru et al., 1999), and 200–400 ml eggs (100 ml = 36,000 eggs) each were placed in aerated 2000 l incubators (1.2 m diameter × 2 m depth) supplied with sand-filtered seawater maintained at 17–20 °C. Initial flow rates per incubator were 0.5 l $min^{-1}$ per 100 ml eggs for initial incubation until hatching (48 h) and increased to 2–3 l $min^{-1}$ at 8–10 days posthatch (dph). First instar *Artemia* nauplii were added to each incubator starting at 4 dph at a rate of 56–68 nauplii per fertile egg, divided equally among 4 daily feedings. Second instar nauplii enriched with Super Selco were fed at the same rate from 7–18 dph. All nauplii were rinsed in fresh water prior to feeding. Daily photoperiod was maintained at 12 h with overhead artificial lighting. At 18 dph surviving larvae were harvested by draining incubators to a volume of 100 l. The number of live fish in each incubator was determined by counting 5 replicate 0.82 l subsamples (each subsample containing 200–800 fish) prior to transfer into larger rearing containers. Survival from 0–18 days was expressed as the number of harvested fish divided by the number of fertile eggs stocked. Fish from a single spawning event were reared from 19 to 80 dph in a circular, 8000 l pool supplied with sand-filtered 18 °C (range 17.6 to 20.5 °C) seawater at 50–100 l $min^{-1}$. Initial stocking density was 1.5 fish $l^{-1}$. Fish were fed enriched *Artemia* nauplii until 30 dph, frozen adult *Artemia* from 30 to 40 dph and both frozen *Artemia* and commercial pelleted diet from 40 to 80 dph.

### 2.2. Measurement of copper ion concentration

Loss of free copper ion due to complexing or chelation was a concern for this study. The proportion of total dissolved copper present as free copper ion ($Cu^{++}$) was determined in seawater over a 1 h period. Two 1-l bottles were filled with 300 ml each of filtered seawater at 35 parts per thousand (ppt) salinity. Two more bottles received 300 ml distilled water. Copper sulfate standard solution (Hach Chemical) was added to each bottle for a total dissolved copper concentration of 0.1 mg $l^{-1}$. Bottles were placed in an 18 °C shaking incubator for 1 h. At 0 and 60 min, duplicate 25 ml samples were collected from each bottle into acid-washed cuvettes (25 mm pathlength). Total dissolved copper (free and complexed) and free dissolved copper concentrations were