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Temporal changes in the suitability of claywater as a greenwater substitute for rearing larval sablefish (*Anoplopoma fimbria*)

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ABSTRACT

For some species, marine aquaculture facilities increase turbidity to improve larval feeding, growth, and survival, typically by mixing algae with seawater to make "greenwater." Clay is a less expensive and inorganic potential algae substitute that has been shown to reduce bacterial levels relative to algae and promote equal or better growth and survival in some species. Sablefish (*Anoplopoma fimbria*) is a prime candidate species for aquaculture but the rearing of sablefish larvae has not yet been experimentally tested with clay. This study tested whether clay is a viable algae substitute for rearing sablefish, and whether the relative performance of clay versus algae varies as a function of time. In the first week of larval rearing, algae (*Nannochloropsis*) produced more than three times greater survival than clay (Kentucky Ball Clay OM4). However, switching from algae to clay at the beginning of the second week led to 1.5 times greater larval growth compared to tanks where algae was used in both weeks. The performance difference between clay and algae, despite equal turbidity, suggests that clay and algae have potentially harmful and beneficial effects in addition to turbidity effects, and that these effects change through time. However, because the first experiment was a replacement study, it was not possible to know whether algae produced better survival in week one because 1) clay, which might be harmful, was not present, 2) algae, which might be beneficial, was present, or 3) both. A further additive study was conducted to test the second possibility. The experiment found that adding algae to clay in the first week of larval rearing leads to greater growth and survival, suggesting that algae has beneficial effects beyond turbidity. We discuss some possible mechanisms for potential harmful and beneficial effects of algae and clay.

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1. Introduction

Turbid water is needed to successfully rear larvae of many marine species. When reared in non-turbid clear water, larvae of many marine species starve, possibly because they have trouble seeing live feed (Boehlert and Morgan, 1985). Clear water may also cause larval marine fish to swim unproductively against tank side walls (wall-nosing), which wastes energy and may cause deformities (Cobcroft et al., 2012). While high turbidity can be just as detrimental (Boehlert and Morgan, 1985; Carton, 2005; Utne-Palm, 2004), moderate turbidity can improve feeding and prevent wall-nosing (Boehlert and Morgan, 1985; Cobcroft et al., 2012). For marine fish larvae which typically rely heavily on visual cues (Blaxter, 1986; Utne-Palm, 2002), increasing turbidity in culture tanks may more closely mimic natural conditions (Utne-Palm, 2004) and promote normal behavior by enhancing visual contrast, by scattering light, or by reducing perceived predation risk for the larvae (Boehlert and Morgan, 1985; Utne-Palm, 2002).

Most marine fish hatcheries achieve turbidity by adding algae to seawater. The resulting mixture is known as "greenwater" and can improve feeding, behavior, growth, and survival when compared to clear water (Cahu et al., 1998; Cobcroft et al., 2012; Palmer et al., 2007; Papandroulakis et al., 2002; Stuart and Drawbridge, 2011; van der Meeren et al., 2007). However, greenwater has drawbacks. Fish hatcheries can purchase dead algae or grow their own live algae, but purchasing algae is expensive and growing it can be unpredictable and labor intensive. Also, because algae is organic and decays, adding it to rearing tanks can promote bacterial growth and water fouling, particularly if dead algae is used (Attramadal et al., 2012). Nevertheless, greenwater continues to be widely used because there are few well-researched alternatives.

Previous work has tested clay as an inexpensive algae substitute. Attramadal et al. (2012) showed that using clay instead of algae can promote larval Atlantic cod (*Gadus morhua*) survival and growth while reducing organic matter and bacterial abundance. Stuart et al. (2015) found that claywater with continuous feeding led to greater survival

Abbreviations: Low C, low concentration claywater; High C, high concentration claywater; Low C + G, low concentration claywater plus greenwater; G → G, greenwater during weeks 1 and 2; G → C, greenwater during week 1, then claywater during week 2; C → C, claywater during weeks 1 and 2.

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than claywater with batch feeding or greenwater with batch feeding in yellowtail amberjack (*Seriola lalandi*); the latter two treatments did not differ. *Vibrio* colonies were more abundant in the algal paste treatment than either claywater treatment. In other studies, Daugherty (2013) found a negative impact of claywater on yellowtail kingfish (*Seriola* l. *lalandi*) growth and survival, but no effect on cobia (*Rachycentron canadum*). Claywater had fewer *Vibrio* counts than greenwater, though *Vibrio* did not appear to affect growth or survival.

Sablefish has been identified as a prime candidate species for aquaculture, with moist, firm flesh, and high prices off fishing vessels and in the retail market. In the wild, eggs are spawned and develop in deep waters (>200 m), but larvae are found at the surface (Kendall and Matarese, 1987). A significant cost associated with rearing larval sablefish is the price of algae for greenwater, which is used by the nascent North American sablefish aquaculture industry. Continued development of a domestic sablefish industry would be more feasible if rearing methods were refined to reduce costs and increase production quality and quantity. Currently, there are no published studies on the use of clay in sablefish aquaculture.

This study asked whether clay is a viable alternative to algae, and whether the performance of clay differs as a function of time. Here, first-feeding sablefish larvae were reared for two weeks in three treatments: 1) greenwater for two weeks, 2) claywater for two weeks, or 3) greenwater for the first week and claywater for the second week. Comparisons were made for larval body weights among treatments at the end of the first and second weeks, and survival at the end of the second week. Follow-up studies then tested the effects of clay concentrations and algae-clay mixtures on behavior, growth, and survival.

2. Materials and methods

Larvae originated from crosses of broodstock captured off the Washington coast. For spawning, egg fertilization, and hatching details see Cook et al. (2015). Experiments were carried out in 37 L tanks (40 cm tall, 37 cm diameter) with flat, white bottoms and black walls. A 3.3-cm diameter center standpipe maintained water at a height of 36.5 cm. Plastic tubing (0.635 cm internal diameter) delivered filtered, ambient clear seawater to each tank at a rate of 270 mL/min. A 61-cm tall, 40-cm diameter cylinder made out of Reflectix insulation (Markleville, IN, USA) rested on top of each tank and was topped by a white plastic disk that sealed the tank from external light. A lighting fixture was attached to the underside of the white plastic disk. The lighting fixture contained an LED bulb (Satco S8813, Brentwood, NY, USA) above 14 layers of 1.5-mm stainless window mesh that reduced light intensity to 12 lx at the water surface. Light was on 24 h per day. Larvae were fed three times per day at a density of 20 rotifers per mL of tank water.

Concentrated greenwater was made by mixing *Nannochloropsis* Instant Algae (Reed Mariculture, Campbell, CA, USA, 68 billion cells per mL) and green dye ("Green Shade Color," Esco Foods, San Francisco, CA, USA; see below for ratios). To make concentrated claywater, Kentucky Ball Clay OM4 (Kentucky-Tennessee Clay Company, Roswell, GA, USA) was mixed with water for 2 min (1 min on "low" followed by 1 min on "high") in a commercial blender (Waring MX1000XTX 3.5 HP). These concentrated solutions were kept suspended by aerating them in buckets. Peristaltic pumps (Anko Products, Bradenton, FL, USA) were activated by cycle timers (Cap Controllers, Perris, CA, USA) to deliver the concentrated solutions via manifolds to the experimental tanks.

2.1. Experiment 1—can claywater substitute for greenwater in weeks 1 and 2?

First-feeding larvae were stocked into 37 L experimental tanks. Four broodstock crosses were equally represented among tanks (75 larvae per cross per tank; 300 total larvae in each tank). First-feeding larvae were reared under three different conditions: 1) greenwater for two weeks (n = 6 tanks, G → G), 2) claywater for two weeks (n = 6 tanks, C → C), and 3) greenwater for one week followed by claywater for one week (n = 6 tanks, G → C). Greenwater was maintained at 0.021 mL *Nannochloropsis* instant algae and 0.005 mL of green dye ("green shade color," Esco Foods, San Francisco, CA, USA) per L of tank water. Claywater was maintained at 12 mg of Kentucky ball clay OM4 per L of tank water. These concentrations produced equal Secchi-disk turbidities between treatments (Carolina turbidity tube, Carolina Biological Supply Company, Burlington, NC, USA). In the first week, twelve tanks received greenwater and six tanks received claywater (18 tanks total). On day 8, six of the tanks that had previously received greenwater were switched to claywater. The six tanks that were switched to claywater were selected so that the average body weight at switch-over was equal between the G → G and G → C treatments.

2.1.1. Sampling

Fifteen larvae were weighed together for each tank on day 8. Larvae were removed from each tank, overdosed in MS-222, weighed wet, and then weighed again after drying overnight in a 110 °C oven. That weight was divided by 15 to arrive at "average larval dry weight." On day 15, tanks were drained, survivors were counted, and all survivors were bulk-weighed after drying overnight for each tank ("total dry biomass"). Quantification of survivors required stressful larval handling and was therefore only done at the end of the experiment.

2.1.2. Statistics

Separate analyses were conducted to isolate effects during weeks one and two. To isolate the effect of claywater versus greenwater during week one, C → C and G → C treatments were compared with Wilcoxon tests in terms of survival after week two and total dry biomass after week two. Since these two treatments both used claywater during week two, any difference could be attributed to the use of claywater versus greenwater in week one. At the end of week one, six tanks had contained claywater and 12 tanks had contained greenwater. We conducted a Wilcoxon test to compare average larval dry weights between the six claywater and 12 greenwater tanks.

To isolate the effect of claywater versus greenwater during week two, G → G and G → C treatments were compared with Wilcoxon tests in terms of growth during week two, survival after week two, and total dry biomass after week two. Growth during week two was calculated for each tank as the difference in average larval dry weights between the end of weeks 1 and 2. Since these two treatments both used greenwater in week one, any difference could be attributed to the use of claywater versus greenwater in week two.

2.2. Experiment 2—what is the best clay concentration during week two?

Three different clay concentrations were compared in an eight-day rearing trial using one-week-old larvae. The larvae had initially been stocked at first-feeding from three broodstock crosses into a 2000 L holding tank in greenwater. After eight days, 250 larvae were transferred into each of 18 tanks (37 L) for the experiment. The 18 tanks were evenly divided among low, medium, and high clay concentration treatments: 3, 9, and 15 mg clay per L tank water, respectively (n = 6 tanks per treatment). A nephelometer (HI 93703, Hanna Instruments, Woonsocket, RI, USA) measured turbidity to be 2.4, 11.1, and 13.9 NTU in the low, medium, and high concentration treatments, respectively.

2.2.1. Sampling

For each tank on day four, an observer counted the number of larvae visible from the surface that were touching tank walls (wall-nosing) and the number that were not touching tank walls. Counts were made with a hand tally counter and took about ten seconds per tank. On the eighth day, the tanks were drained and larvae counted. All surviving larvae were weighed with the same methods used in Experiment 1.