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Effects of Algae Paste Substitutes on the Larval Rearing Performance and Microbial Communities in the Culture of Cobia (Rachycentron canadum) and Yellowtail Kingfish (Seriola lalandi)

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EFFECTS OF ALGAE PASTE SUBSTITUTES ON THE LARVAL REARING PERFORMANCE AND MICROBIAL COMMUNITIES IN THE CULTURE OF COBIA (*Rachycentron canadum*) AND YELLOWTAIL KINGFISH (*Seriola lalandi*)

By

Zachary N. Daugherty

A THESIS

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EFFECTS OF ALGAE PASTE SUBSTITUTES ON THE LARVAL REARING PERFORMANCE AND MICROBIAL COMMUNITIES IN THE CULTURE OF COBIA (*Rachycentron canadum*) AND YELLOWTAIL KINGFISH (*Seriola lalandi*)

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Microbial management in the culture of marine pelagic fish is crucial for the refinement of larval rearing protocols and strategies. Standard larval rearing methods employ the addition of live or paste microalgae to culture environments, termed “green water” culture, which promotes the growth of undesired opportunistic bacteria associated with microbial “pioneer” communities. A non-selective approach for the reduction in bacteria by substituting microalgae paste with a non-organic clay additive was evaluated for two marine pelagic fish, cobia (*Rachycentron canadum*) and yellowtail kingfish (YTK; *Seriola lalandi*). Both trials induced turbidity using Old Mine #4 Kentucky ball clay and Reed Rotigrow *Nannochloropsis* algae paste set to similar Secchi disc depths (55 – 65 cm). In addition to paste and clay additives an experimental artificial algae (solgel) was used in the YTK trial. Final growth (mm), survival (%), and total *Vibrio* communities (CFU ml⁻¹) were measured in both trials. Moreover, in the YTK trial, total bacteria levels and submerged (downwards and upwards facing) light intensities were assessed between treatments. Results were evaluated using independent t-tests for the cobia trial and one-way ANOVAs with Tukey HSD post-hoc tests for the YTK trial. No
significant differences were found in growth ($P = 0.40$) and survival ($P = 0.54$) between treatments in the cobia trial. Significant differences were seen in total *Vibrio* counts at day 7 and 10 ($P < 0.05$) between treatments. Although differences in total *Vibrio* were found, the bacterial population in the algae paste tanks did not have a negative impact on the larvae’s growth or survival. The cobia trial results indicate that clay may successfully function as an algae paste substitute without any detrimental effect. The response of YTK trial to clay was very different to that of cobia. Both clay and solgel had a negative impact on YTK larvae’s growth, survival, SBI, FI, and prey consumption ($P < 0.05$). Differences were seen at 5 DPH for total bacteria and total *Vibrio* ($P < 0.05$) between treatments. Submerged upwards facing light intensity in clay was significantly greater than the algae paste and artificial algae treatments ($P < 0.0001$). Immediate disorientation and stress in yolksac YTK at initiation of the clay turbidity treatment was present. It was hypothesized that light scatter on clay particles and back-scatter from tank color may have caused the negative impacts seen on the larvae. This study warrants more research regarding interactions between light intensities, tank color, tank dimensions, and the use of non-organic algae substitutes.
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CHAPTER 1: INTRODUCTION

Professionals of marine aquaculture endeavor to develop and discover repeatable protocols for closing the cycle of various species. One of the greatest impediments to this effort is the survival and production of commercially important finfish species from the egg to fingerling phase. This impediment has led aquaculture researchers to investigate various strategies for reducing larval finfish mortality. Bacteria and microbial ecology in hatcheries, specifically opportunistic and pathogenic bacteria, are believed to strongly influence survival and growth during early developmental stages of many marine species (Salvesen et al., 1999; Vadstein et al., 2004, 1993; Verner-Jeffreys et al., 2004). Newly stocked culture systems provide conditions that promote the growth of such opportunistic microbial communities. Vadstein et al. (1993) describes the potential importance of mature microbial communities over “pioneer” communities, or communities that are commonly associated with newly established culture systems and culture systems that practice periodic prophylactic treatments. These pioneer communities and their associated opportunistic microbial communities have necessitated the development of strategies for microbial control in aquaculture. Numerous strategies exist for microbial control in culture settings (Figure 1) such as disinfection of eggs (Hansen and Olafsen, 1999; Harboe et al., 1994; Vadstein et al., 1993), reduction of organic inputs (Vadstein et al., 1993), use of probiotics (Gatesoupe, 1999; Hansen and Olafsen, 1999; Olafsen, 2001; Vadstein et al., 2004, 1993; Verner-Jeffreys et al., 2004; Zink et al., 2013), use of microbial matured water (Attramadal et al., 2012; Skjermo et al., 1997; Vadstein et al., 1993) and periodic prophylactic baths (Benetti et al., 2008a, 2008b; Bergh et al., 2001;
Vadstein et al., 2004). The process of substituting algae paste for an inorganic clay additive would be categorized as a “non-selective” strategy. This occurs by reducing organic inputs that promote microbial growth (Figure 1; Vadstein et al., 1993) and has been shown to absorb organic matter in a minor capacity (Attramadal et al., 2012).

Within the past 20 years, discoveries in the culture of walleye (*Stizostedion vitreum*), have influenced researchers to investigate the introduction of inorganic clay additives. Preliminary work utilizing a suspended clay slurry to increase water turbidity has been shown to significantly improve walleye larvae survival, growth, and increased swim bladder inflation (SBI) over traditional “clear-water” culture practices (Bristow and Summerfelt, 1994; Bristow et al., 1996; Clayton and Summerfelt, 2010; Rieger and Summerfelt, 1997). This research accredits the high survival and growth to an increased
food acceptance, likely caused by uniform larval dispersion and a high contrast of food particles within the turbid water. Most research on the effects of increased turbidity levels in marine settings revolve around anthropogenic activities, for instance dredging in coastal areas and its interactions on various marine species during egg and larval stages (Isono et al., 1998; Partridge and Michael, 2010). Many coastal regions naturally possess low turbidity levels (Auld and Schubel, 1978; Isono et al., 1998) which gives an unorthodox designation to the strategy of exposing marine larval finfish to non-organic introduced turbidity within larval culture systems. Anecdotal evidence in the commercial culture of Almaco jack (*Seriola rivoliana*) implementing a clay turbidity strategy is showing survival rates to fingerling stage of approximately 10 – 20% (Daugherty, pers. obs.). Recent studies on the effects of using a clay additive for marine finfish species have shown either no differences in growth and survival of Atlantic halibut (*Hippoglossus hippoglossus*) (Björnsdóttir et al., 2011) or variable to increased growth and survival in Atlantic cod (*Gadus morhua*) (Attramadal et al., 2012).

The common alternative to using inorganic clay in marine larval finfish rearing is the input of various microalgae species, termed “greenwater culture”, in culture environments. This strategy utilizes live microalgae or concentrated microalgae paste to increase turbidity within culture settings. Palmer et al. (2007) state that green-water culture is widely used internationally in marine larviculture due to the advantages of direct and indirect nutrition, lowered stress levels, light attenuation and its positive effects on prey selection, increased oxygenation through photosynthesis, introduction of chemical and digestive stimulants (e.g., microflora colonization of digestive tracts), and the antibacterial properties of some live microalgae species. A potential disadvantage of
algae additives is that it provides algal carbon and metabolites which can serve as additional substrate for microbial growth (Attramadal et al., 2012; Cole et al., 1984). Greenwater culture has been utilized for many marine finfish species, including, but not limited to, barramundi (Lates calcarifer), Australian bass (Macquaria novemaculeata), dusky flathead (Patycephalus fuscus), sand whiting (Sillago ciliata) (Palmer et al., 2007), Atlantic halibut (Naas et al., 1992), sea bream (Sparus aurata) (Palmer et al., 2007; Papandroulakis et al., 2002), turbot (Scophthalmus maximus) (Øie et al., 1997), snapper (Pagrus auratus) (Palmer et al., 2007; Partridge and Michael, 2010), Seriola spp. (Benetti et al., 1998; Carton, 2005; Stuart and Drawbridge, 2011; Woolley et al., 2012a), and cobia (Rachycentron canadum) (Benetti et al., 2008a, 2008b; Faulk and Holt, 2005).

One of the primary benefits that increased turbidity is believed to have on larval survival and growth is allowing for improved feeding incidence (FI) or prey selection of larvae due to uniform light attenuation in greenwater culture (Naas et al., 1992) and claywater culture (Bristow and Summerfelt, 1994). A noteworthy benefit of turbidity using live microalgae is its ability to inhibit opportunistic microbial growth such as some Vibrio spp. (Salvesen et al., 2000). However, the recent convenience of using inert Nannochloropsis oculata microalgae paste has the effect of increasing concentrations of opportunistic bacteria (Attramadal et al., 2012). Some chemical constituents of certain clays have shown to possess powerful anti-microbial properties rivaling that of some antibiotics (Williams and Haydel, 2010). Inorganic clays are reported to absorb viruses such as the Infectious hematopoietic necrosis (IHN) that cause substantial mortality in salmonids; however, the infectivity of this virus when absorbed by clay particulates remains (Yoshinaka et al., 2000). Landau et al. (2002) have shown that proteins can also
bind with clay and be effectively removed from water when combined with a foam fractionation system. Studies that substitute live and paste algae with clay additives in marine larvae culture have showed reductions in total and opportunistic bacteria (Attramadal et al., 2012; Björnsdóttir, 2010; Bjornsdottir et al., 2011).
2.1 Background

Many of the reports that exist using suspended clay in larviculture claim to show increased survival and growth. Two questions exist regarding these positive reports: (i) How does clay induced turbidity affect a larvae’s performance in growth and survival?; and, (ii) How does it influence levels of opportunistic bacteria? For the purpose of this study, an experimental design was developed to analyze growth and survival to post-flexion of cobia (*Rachycentron canadum*).

The primary assumption of this study is that the inorganic nature of clay and the possible antimicrobial properties it possesses may inhibit opportunistic bacteria to a greater degree than in greenwater culture. This study analyzed and compared total *Vibrio* concentrations between the treatment groups. Consequently, two hypotheses were tested during this experiment. First, it was determined if significant differences exist in survival and growth between clay versus algae paste treatments. Second, it was determined if significant differences exist in *Vibrio* loads between clay and algae paste treatments. By examining these parameters this study may help indicate if a reduction in opportunistic bacterial loads influence marine larval performance.

2.2 Materials and Methods

2.2.1 Pre-trial Standardization of Algae Paste with Clay

A measure of Secchi depth is the standard practice for measuring turbidity and its effects on light attenuation in marine fish hatcheries. Literature using clay in hatchery settings often measure turbidity in Nephelometric turbidity units (NTU). The
first step allowing for objective comparisons between algae paste and clay was to standardize their light attenuation properties for a known and readily measurable metric. Because these two materials possess different physical properties their NTU differ when analyzed by a nephelometer, even when their light attenuation levels, measured using Secchi disc depth (SDD), seem similar under visual human observation. For example, if two water samples with each of these additives are produced, one with clay and one with algae paste, and each were to have an identical SDD, the algae paste sample would have a much lower NTU reading than the clay sample. This difference in light scatter is likely caused by the physical differences of algae cells versus clay particles. Because the experimental tanks used in this study are too shallow for use of a Secchi disc, measurements of SDD were obtained using a TTG 120 cm Transparency Tube (Water Monitoring Equipment & Supply, P.O. Box 344, Seal Harbor, ME, USA, 04575) in conjunction with NTU measurements using a Hach 2100 Q Portable Turbidimeter (Hach Company, P.O. Box 389, Loveland, Colorado, 80539).

To begin the pre-trial, turbidity measurements were obtained before and after a cumulative incremental dosage of the Reed Rotigrow *Nannochloropsis occulata* algae paste (Reed Mariculture Inc., 971 E Hamilton Ave, Suite D, Campbell, CA 95008 USA) was added to a 250 L tank ranging from 1 – 70 mL across 20 dosages. Old Mine #4 Kentucky ball clay (Paoli Clay Company, Paoli, Wisconsin, 53508) was prepared in a stock solution (SS) at a concentration of 20 g clay L$^{-1}$ of seawater. The clay SS was incrementally added to the second 250 L tank ranging from 1 – 1000 mL across 37 dosages (Figure 2). The number of dosages for each treatment was determined by the measurable bounds of the 120 cm transparency tube (0 – 120 cm depth). SDD and NTU
were recorded with the aforementioned devices after each dosage was administered and allowed to homogenize via a single central air source within pre-trial tanks. Recorded SDD readings were plotted against NTU readings and fit to a power model.

\[ y = c \cdot x^b \]  

(1)

Subsequent power equations were developed for calculating SDD based on NTU readings allowing for a less intrusive and objective measure of SDD during the experimental trial.

*Figure 2.* Pre-trial normalization in 250 L tanks of light attenuation properties measuring Secchi depth (cm) in relation to turbidity (NTU).
2.2.2 Pre-trial Results

Figure 3. Scatterplot of NTU readings plotted against observed SDD readings for algae paste pre-trial.

Figure 4. Scatterplot of NTU readings plotted against observed SDD readings for clay pre-trial.
The Secchi depth ($SDD_{AP}$) in cm for the algae paste treatment is determined by

$$SDD_{AP} \text{ (cm)} = 90.411 \cdot T_{(NTU)}^{-0.782435}$$  \hspace{1cm} (2)

where turbidity ($T$) is measured in NTU ($R^2 = 0.990$) using the power formula derived from Figure 3.

The Secchi depth ($SDD_{CLAY}$) in cm for the clay treatment is determined by

$$SDD_{CLAY} \text{ (cm)} = 454.3107 \cdot T_{(NTU)}^{-0.861168}$$  \hspace{1cm} (3)

where turbidity ($T$) is measured in NTU ($R^2 = 0.995$) using the power formula derived from Figure 4.

2.2.3 Experimental Setup

This study was conducted at the University of Miami Experimental Hatchery (UMEH). Trials were performed using a 12 x 400 L tank experimental nested in a 15 m$^3$ water bath shown in Figure 5. The array was used for testing clay and algae paste additives at concentrations needed for a transformed target SDD range of 55 – 65 cm, which is the standard protocol for cobia larval rearing at UMEH. Each treatment additive was assigned by a systematic approach to insure a maximum interspersion of clay – paste treatments across the experimental units, thus mitigating potential tank effects within the array (Hurlbert, 1984). The experimental system was located outdoors under 90% shade cloth, where day time light intensity ranged from 1000 – 1400 lux (approximately 19 – 26 µmol s$^{-1}$m$^{-2}$ using sunlight conversion from Thimijan and Heins, 1983).

The water quality parameters that were routinely measured within the experimental array included turbidity (Nephelometric turbidity units, NTU), dissolved oxygen (DO, mg L$^{-1}$), pH, salinity (PPT), and temperature (degrees Celsius). Turbidity was measured 4 times daily (0800, 1100, 1400 and 1700) using a Hach 2100 Q Portable
Turbidimeter (Hach Company, P.O. Box 389, Loveland, Colorado, 80539). Dissolved oxygen levels, pH, salinity and temperature were measured twice daily (0800 and 1700) using a YSI Professional Plus Meter (YSI, Inc., 1700/1725 Brannum Lane, Yellow Springs, Ohio, 45387).

Figure 5. Diagram of the 12 x 400 L tank experimental array supplied with water from a 15 m³ reserve tank. Clay and algae paste stock solutions introduced into array via peristaltic pump to 2 x 6-way manifolds.
Figure 6. Alternating treatments within experimental array.

Figure 7. Experimental delivery of additives suspended and agitated via upwelling in 2 x 15 L reservoirs (A) which is then peristatically pumped (B) and mixed with salt water (C) from the header tank (D) and finally passed through 2 x 6-way manifolds (E) to their designated tanks.
Seawater sterilized with UV and filtered to 1 µm, was pumped to a header tank (Figure 7d) to provide a constant head and subsequently even flow to the system. Clay and paste stock solutions were suspended in 1 µm filtered and UV treated seawater then stored in 15 L Aquatic Eco-systems Commercial Brine Shrimp Hatchers (Pentair Aquatic Eco-Systems, Inc., 2395 Apopka Blvd., Apopka, FL 32703) with continuous air injected at the base of each cone to insure a homogenized additive-water mixture (Figure 7a). Each stock solution was pumped into a mixing chamber (Figure 7c) using a 12 rpm Masterflex L/S fixed-speed peristaltic pump outfitted with two-channel Easy-Load II pump heads (Cole-Parmer, 625 East Bunker Court Vernon Hills, IL 60061 USA; Figure 7b) before gravity flowing to each replicate tank via 2 x 6-way manifolds (Figure 7e).

2.2.4 Larval Rearing

Cobia eggs (0 days post hatch, DPH, or one day before hatching) were obtained using methodology described in (Benetti et al., 2008b) and stocked into 2 x 1000 L incubators at densities of 500 eggs L\(^{-1}\). Larvae hatched approximately 12 hours after initial stocking and were siphoned and skimmed to remove unviable eggs and hatching detritus. Larvae remained in incubators during their endogenous feeding stage (2 DPH) and then were enumerated and stocked into each tank at a density of 15 L\(^{-1}\) under 300% water exchange day\(^{-1}\) (Table 1). All standpipes were outfitted with 300 µm mesh and central porous air rings at the base to provide a laminar current.

At 3 DPH, the two treatment additives were introduced into each tank and parameters were recorded for the remainder of the study. The trial was conducted until 13 DPH, or post-flexion stage, which is commonly associated with a significant drop in survival as a consequence of metamorphosis (Benetti et al., 2008b). Prior to first daily
feedings, initial dosages of treatments were added to each designated tank with clay concentrations of 0.013 g L\(^{-1}\) of culture water (5.2 g in 400 L tanks) and algae paste of 0.03 ml L\(^{-1}\) of culture water (12 ml in 400 L tanks). Each initial treatment was mixed into 1500 ml stocked solution using saltwater to facilitate the addition into each experimental unit with 31.2 g clay for six tanks and 72 ml algae paste for six tanks. Treatment additives were added to dosing reservoirs at adjusted concentrations commensurate with changing trial exchange rates for maintenance of desired SDD levels throughout the study (Table 2).

L-type rotifers (*Brachionus plicatilus*) were enriched (Benetti et al., 2008b) and fed at 0800 hrs for first feeding at 5 ml\(^{-1}\) concentrations. Thirty minutes prior to subsequent feedings, residual rotifers were counted and the appropriate number of new rotifers were added to maintain 5 ml\(^{-1}\) prey densities. Remaining live feeds were cold-stored at 10°C and fed at 1100, 1400, and 1700 hrs. Live feed concentrations were increased to 7 ml\(^{-1}\) at 11 DPH to account for increased demand of prey. Only rotifers were fed during the trial to compensate for mortality commonly associated with the period of live feed transition from rotifers (3 – 10 DPH) to *Artemia* (8 – 24 DPH).

2.2.5 Bacteriology

Water samples were obtained in sterile 1.5 ml microcentrifuge tubes from the reservoir tank, header tank, and experimental units on days 5, 7, 10, and 12 to determine changes in total cultivable *Vibrio* communities. Each sample was plated in triplicate on TCBS (thiosulfate citrate bile sucrose, BD Diagnostics) using the membrane filter technique and incubated at 40 °C for 24 hrs following a protocol outlined by Hernández-
López et al. (1995). Total *Vibrio* colony forming units (CFU ml\(^{-1}\)) were enumerated on days following plating.

2.2.6 Larval Sampling

Larval growth was assessed by measuring total length (TL, mm) at the end of trial period using digital microscopy (OptixCam Summit OCS 10.0, The Microscope Store, LLC, 1222 McDowell Ave, Roanoke, VA 24012). Survival was enumerated at end of the trial as a percentage of larvae remaining from the initial stocking estimate.

2.2.7 Data Analysis

Independent t-tests were employed to determine if significant differences exist in growth, survival, and total *Vibrio* concentrations between treatments. All results are reported as means ± SE and are summarized in Table 2. All statistical analyses were achieved using SAS JMP®, Version 10 (SAS Institute Inc., Cary, NC, 1989-2013).
Table 1. Cobia green water alternatives trial protocols. Days represent age of larvae (DPH), trial exchange flow rates were actual water exchanges used per day (%), and initial algae paste-clay represent the algae paste added to 400 L experimental units at beginning of each day. Dosages of additives represent the amounts delivered to each experimental unit daily, rotifer feed schedule of 5 rotifers mL⁻¹ each feeding from DPH 3 – 10 then 7 mL⁻¹ on DPH 11 and 12. Water samples collected for *Vibrio* enumeration on DPH 5, 7, 10, and 12.

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<th>Trial exchange rates (mL min⁻¹)</th>
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<th>Doser Algae Paste (ml)</th>
<th>Initial Clay SS⁺ (ml)</th>
<th>Doser Clay (grams)</th>
<th>Rotifers mL⁻¹</th>
<th>Vibrio Sample</th>
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a Premixed stock solution (SS) of 31.2g clay with 1500 mL salt water.
b Periods where larvae were held in incubators during endogenous feeding phase
c Stocking and endogenous feeding phase continued
d Onset of exogenous feeding and addition of treatment additives to culture tanks
2.3 Results

Observed mean turbidity was 9.62 ± 0.06 NTU for clay and 1.96 ± 0.02 NTU for algae paste (Table 2). Transformed mean SDD were significantly different \( (P < 0.0001) \) between clay (64.7 ± 0.3 cm) and algae paste treatments (53.5 ± 0.4 cm). No significant differences were seen in DO of 6.47 ± 0.05 and 6.44 ± 0.06 mg L\(^{-1}\) (\( P = 0.70 \)), temperature of 27.2 ± 0.2 and 27.2 ± 0.2 °C (\( P = 0.98 \)), pH of 8.19 ± 0.03 and 8.20 ± 0.03 (\( P = 0.97 \)), and salinity of 30 ± 1 and 30 ± 1 PPT (\( P = 0.97 \)) for clay and algae paste treatments respectively. No significant differences were found (\( P = 0.40 \)) in growth (TL) between clay (8.21 ± 0.09 mm) and algae paste (8.06 ± 0.07 mm) treatments (Figure 8). Additionally, no significant differences were found (\( P = 0.54 \)) in larval survival between clay (40.8 ± 2.7%) and algae paste (35.8 ± 7.2%) treatments (Figure 9). Despite a three-fold reduction in day 5 mean total \( \textit{Vibrio} \) concentrations between algae paste (32 ± 7 CFU ml\(^{-1}\)) and clay (10 ± 9 CFU ml\(^{-1}\)) treatments this difference wasn’t significant (\( P = 0.08 \)). However, significant differences in this parameter were found on day 7 (\( P < 0.01 \)) and 10 (\( P < 0.0001 \)) with the algae paste treatment (215 ± 44 and 598 ± 42 CFU ml\(^{-1}\)) having higher total \( \textit{Vibrio} \) concentrations than the clay treatment (34 ± 4 and 17 ± 5 CFU ml\(^{-1}\)). Despite a 22 fold lower \( \textit{Vibrio} \) count on day 12 in the clay treatment (14 ± 2 CFU ml\(^{-1}\)) relative to the algae paste treatment (320 ± 123 CFU ml\(^{-1}\)), this difference was not significant (\( P = 0.06 \)) due to the high variation around the paste values.
Figure 8. Final growth at 13 DPH (means ± SE) for cobia larvae ($n = 25$) per treatment. Values with different letters are significantly different determined by independent t-tests ($P < 0.05$).

Figure 9. Final survival at 13 DPH (means ± SE) for cobia larvae. Values with different letters are significantly different determined by independent t-tests ($P < 0.05$).
Figure 10. Total Vibrio (means ± SE) on days 5, 7, 10 and 12 between treatments. Values with different letters are significantly different determined by independent t-tests ($P < 0.05$).

Table 2. Transformed Secchi depth (cm), final total length (TL, mm), survival (% of initial stocking) and Vibrio loads (CFU ml$^{-1}$) with means ± SE.

<table>
<thead>
<tr>
<th>DPH</th>
<th>Algae Paste</th>
<th>Clay</th>
<th>Level of Significance</th>
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<tr>
<td>SDD (cm)</td>
<td>-</td>
<td>53.5 ± 0.4$^b$</td>
<td>64.7 ± 0.3$^a$</td>
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<td>Growth (mm)</td>
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<td>8.06 ± 0.07</td>
<td>8.21 ± 0.09</td>
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<tr>
<td>Survival (%)</td>
<td>-</td>
<td>35.8 ± 7.2</td>
<td>40.8 ± 2.7</td>
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<td>Total Vibrio (CFU ml$^{-1}$)</td>
<td>5</td>
<td>32 ± 7</td>
<td>10 ± 9</td>
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<td></td>
<td>7</td>
<td>215 ± 44$^a$</td>
<td>34 ± 4$^b$</td>
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<td>10</td>
<td>598 ± 42$^a$</td>
<td>17 ± 5$^b$</td>
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<td></td>
<td>12</td>
<td>320 ± 123</td>
<td>14 ± 2</td>
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Means in the same row having different superscripts are significantly different determined by independent t-tests ($P < 0.05$).

Significance levels are denoted by: NS (No Significance), * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$)
2.4 Discussion

The methodology associated with transforming turbidity (NTU) into a relational Secchi depth (cm) developed for this study was found to be highly sensitive to the individual tank variations associated with additive dosages and daily exchange rates. Because of this sensitivity there were significant differences in transformed SDD between treatments; however, these differences were not apparent under visual observation. Although cobia foraging efficiency under different concentrations and sources of turbidity have not been studied, Gregory and Northcote (1993) show that juvenile chinook salmon (*Oncorhynchus tshawytscha*) possess the ability to select *Artemia* uniformly at turbidities ranging between 0 – 70 NTU indicating that foraging efficiency may not be significantly effected at such sensitive scales. Therefore, a relaxed approach to SDD was adopted using a targeted range of 55 – 65 cm. which is the approximate depth for standard larval rearing of cobia at UMEH. Thus the statistical differences in SDD were ignored for comparison of treatment effects on larval performance and total *Vibrio* concentrations.

*Vibrio* concentrations were found to be significantly lower in the clay treatment compared to the algae paste treatment. These results are in agreement with the findings of similar studies on clay’s influence on *Vibrio* concentrations in marine larval culture (Attramadal et al., 2012; Bjornsdotir et al., 2011). This study likely displayed much lower bacterial loads than those found by Attramadal et al. (2012) because of the reduced stocking densities of larvae (100 L⁻¹ versus 15 L⁻¹) and the associated reduction in organic inputs. Even though *Vibrio* loads were significantly reduced in clay treatment tanks there appeared to be no relationship with observed larval performance when
compared to algae paste treatment tanks. The effects of Vibriosis in cobia has been documented (Liu et al., 2004; McLean et al., 2008), but negative impacts to cobia larval rearing performance may be influenced more by optimal culture practices and adequate nutrition and less with the overall presence of *Vibrio* spp. within culture systems. The results suggest that either cobia possess resilience to the increased bacterial loads associated with typical greenwater aquaculture methods, or that potentially harmful *Vibrio* thresholds were not reached. Knowledge on the overall microbial composition and its effects on cobia larval performance is likely more important than observing only total opportunistic *Vibrio* concentrations (Attramadal et al., 2012; Björnsdóttir, 2010; Munro et al., 1995; Vadstein et al., 2004, 1993; Verner-Jeffreys et al., 2004). Although no negative effects on survival with different levels of *Vibrio* were observed, clay turbidity may serve to prevent disease outbreaks associated with high levels of pathogenic and opportunistic bacteria.

Survival was similar between treatments as seen in halibut larvae (Björnsdóttir et al., 2011), however algae paste displayed a higher degree of variation. Although no significant differences were found in growth of cobia between additive treatments, clay did possess slightly higher means with less variation across all replicated treatments. The observed stability could provide for more reliable production numbers when expanded to commercial scales. Although prey consumption was not directly measured during this study, a repeating pattern of clay treatment tanks requiring more rotifers to maintain 5 ml\(^{-1}\) prey densities was observed. This requirement for more prey items in clay tanks during feeding periods may be attributed to increased rotifer mortality due to the prey’s consumption of clay particles. In using clay strategies to replace algae, it is important to
flush uneaten prey items from culture tanks to ensure only enriched prey items are being consumed (Stuart et al., 2013). Kirk and Gilbert (1990) have shown that a similar species of rotifer was unaffected by the presence of suspended sediments and were even capable of selecting algae cells when present in turbid environments. This indicates there may be potential benefits to an approach using a paste–clay slurry that would both provide continued enrichment to rotifers and reduced bacterial loads. However, the presence of algae paste may override the buffering capacity for clay to mitigate opportunistic microbial communities.
CHAPTER 3: EXPERIMENT II – YELLOWTAIL KINGFISH (*Seriola lalandi*)

3.1 Background

Yellowtail kingfish (YTK; *Seriola lalandi*) is a valued aquaculture fish (Benetti, 2008; Benetti et al., 2001; Fowler et al., 2003; Tachihara et al., 1997) which has recently undergone studies that seek to optimize larval rearing progress with respect to abiotic manipulation of light intensity, light source, turbidity, and photoperiod (Carton, 2005; Stuart and Drawbridge, 2012, 2011; Woolley et al., 2012b). Carton (2005) demonstrated that feeding incidence is amplified with increased light intensity measured in photosynthetically active radiation units (PAR) of 17 µmol s⁻¹m⁻² (approximately 1258 lux using cool white fluorescent conversion from Thimijan and Heins, 1983) in the presence of greenwater turbidity. It was later determined that increasing the light intensity to 14,850 lux (fluorescent conversion to 208 µmol s⁻¹m⁻²) and even 32,000 lux (metal halide conversion to 448 µmol s⁻¹m⁻²) elicited increased feeding response, SBI, growth, and survival under greenwater conditions (Stuart and Drawbridge, 2011; Woolley et al., 2012b). Furthermore, Stuart and Drawbridge (2011) discuss the possibility of substituting algae paste with other additives for maintaining turbidity while reducing organic inputs.

The purpose of this study was to compare a clay additive strategy that would reduce organic inputs with algae paste and an experimental artificial algae additive. Similar to the cobia experiment, this trial evaluated YTK larval performance in the presence of these three additives and enumerated changes in bacteria concentrations throughout the study.
3.2 Materials and Methods

3.2.1 Pre-trial Standardization Additives

An experimental algae replacement was used in addition to the clay and algae paste treatments, requiring the development of a new NTU – SDD curve. This additive, called solgel, is composed of green food grade dye microencapsulated within silica cells ranging from 2 – 5 µm in size. Using the aforementioned devices, measurements were obtained before and after cumulative incremental dosages of the solgel additive were introduced into a 400 L tank with central aeration ranging from 1 – 160 mL across 33 dosages. Similarly, NTU data was plotted against observed SDD and fitted to a power model trendline (see equation 1).

3.2.2 Pre-trial Results

![Scatterplot of NTU readings plotted against observed SDD readings for solgel pre-trial.](image)

\[
SDD \text{ (cm)} = 382.930\text{Turbidity}_{\text{NTU}}^{0.892127} \\
R^2 = 0.985
\]

*Figure 11.* Scatterplot of NTU readings plotted against observed SDD readings for solgel pre-trial.
The Secchi disc depth (SDD$_{SG}$) in cm for the solgel treatment is determined by

$$ SDD_{SG} \text{ (cm)} = 382.930 \cdot T_{(NTU)}^{-0.892127} $$

(4)

where turbidity ($T$) is measured in NTU ($R^2 = 0.985$) using the power formula derived from Figure 11.

### 3.2.3 Experimental Setup

This study was established in Fall 2012 at the Australian Center for Applied Aquaculture Research (ACAAR) in Fremantle, Western Australia as part of ongoing research funded by the Cooperative Research Center (CRC) Australia to optimize yellowtail kingfish larvae protocols. The larval rearing system used for this trial is described in Woolley et al. (2012a). Each treatment was assigned to four replicate 300 L tanks floated in a 5 m$^3$ water bath to stabilize the water temperature of the experimental tanks. Rearing tanks were illuminated by diffused Philips Tango MMF283 outfitted with GE 43828 400W metal halide lights providing approximately 37 – 69 µmol s$^{-1}$m$^{-2}$ (2600 – 4900 lux) at the water surface. Experimental tanks used a marbled granite pattern 3M™ DI-NOC™ Stone, ST-440 Film (3M Corp. Headquarters, 3M Center, St. Paul, MN 55144, USA) for wall coloration (Figure 12) to prevent “walling” behavior (Cobcroft and Battaglene, 2009; Cobcroft et al., 2012) with white conical bottoms at a 35° slope. The experimental array was housed in a room that excluded all natural sources of light and the artificial photoperiod was automated for 12 h light and 12 h dark (0900 – 2100 hrs). Treatment additives were assigned and housed in 3 x 300 L dosing tanks where the additive were pumped evenly to each experimental tank.
3.2.4 Larval Rearing

YTK eggs were acquired from Cleanseas (Cleanseas, PO Box 159, Port Lincoln 5606, South Australia) and incubated in 2 x 400 L incubators at 22 °C. One DPH larvae were stocked randomly into each 12 x 300 L tanks at a density of 60 L⁻¹ with 400% water exchange day⁻¹ (Table 3). All standpipes were outfitted with 300 µm mesh and central air stones at the base to provide a laminar current. Temperature and D.O. were monitored and maintained at 24 °C and > 6 mg L⁻¹ as per Woolley et al. (2012a).

Exogenous feeding was initiated at 3 DPH following an optimized hybrid feeding schedule from previous CRC YTK studies. Treatment additives were introduced into each tank prior to first daily feedings, initial dosages of treatments were added to each designated tank with clay concentrations of 0.013 g L⁻¹ of culture water (3.9 g in 300 L tanks), algae paste of 0.03 ml L⁻¹ of culture water (9 ml in 300 L tanks), and solgel of 0.06 ml L⁻¹ of culture water (19.4 ml in 300 L tanks). Each daily initial treatment additive was mixed into an 800 ml stock solution using saltwater to facilitate the addition into each tank. Treatment additives were added to dosing reservoirs at concentrations commensurate with trial exchange rates for maintenance of desired SDD levels throughout the study (Table 3).
L-type rotifers (*Brachionus plicatilus*) were enriched with Spresso (Inve Technologies, Hoogveld 93, 9200 Dendermonde, Belgium) and fed at 0900 hrs for first feeding. Thirty minutes prior to subsequent feedings residual rotifers were measured to maintain desired concentrations (Table 3). Remaining live feeds were cold-stored at 8 °C and fed at 1200 and 1500 hrs.

### 3.2.5 Bacteriology

Water samples were obtained in sterile 10 ml vials from water source, dosing tank, and experimental units on days 5, 9, and 12 to determine changes in total cultivable *Vibrio* communities. Samples were analyzed for total *Vibrio* and total bacteria (CFU ml⁻¹) by the Department of Agriculture and Food Animal Health Laboratories (3 Baron-Hay Court South Perth, WA 6151, Australia).

### 3.2.6 Sampling and Data Gathering

NTU readings were obtained at 0900, 1200, and 1700 hrs each day for SDD conversion. Feeding incidence (FI) was measured as a percentage of sampled larvae from each tank (*n* = 20) that contained rotifers within their digestive tract on 4, 6, 8, and 10 DPH. Additionally, these samples were used to determine growth (TL, mm) on 4, 6, 8, 10, and 12 DPH and SBI as a percentage of sampled larvae with or without inflated swim bladders by 4 and 6 DPH. Live prey consumption was determined by enumeration of undigested rotifer mastaxs (Downing and Litvak, 2001) in larval guts (*n* = 5) two hours after first feeding on 5, 7, 9, and 11 DPH. Sampled larvae were anaesthetized with 0.2 g L⁻¹ Aqui-S (540 g L⁻¹ isoeugenol, Aqui-S New Zealand Ltd, PO Box 44-269, Lower Hutt, New Zealand). Survival was enumerated at end of trial as a percentage remaining from initial stocking estimate. PAR measurements were obtained on 2, 5, and 8 DPH.
using a LI-COR LI-1400 data logger (LI-COR, Inc., 4647 Superior Street, Lincoln, Nebraska, USA) outfitted with a LI-192 Underwater Quantum Sensor. Submerged PAR readings were recorded upward (upwards facing light) and downward (downwards facing light or reflected light) at 30 cm depth and upward at the surface in each experimental tank. Samples of the algae paste, clay, and solgel were sent to Microanalysis Australia (Microanalysis Australia Pty Ltd, Suite 6, 642 Albany Hwy, Victoria Park, WA 6100, Australia) for particle distribution analysis (PSA) by laser diffraction and sedimentation to assist in describing each materials physical properties. All three samples underwent PSA by laser diffraction using a Mastersizer 2000 (Malvern Instruments Ltd, Enigma Business Park, Grovewood Road, Worcestershire WR14 1XZ, United Kingdom) and the clay’s sedimentation rate was determined using a Sedigraph 5100 (Micromeritics, One Micromeritics Drive, Norcross, GA 30093, United States).

3.2.7 Data Analysis

One-way analysis of variance (ANOVA) was employed to determine if significant differences existed in SBI, FI, consumption, growth, survival, total Vibrio, total bacteria, and submerged PAR between treatments. Individual differences between treatments was determined using Tukey HSD post-hoc tests. All results are reported as means ± SE and Tukey HSD post-hoc analysis results are summarized in Table 4. All statistical analyses were achieved using the SAS JMP®, Version 10 (SAS Institute Inc., Cary, NC, 1989-2013).
Table 3. YTK green water alternatives trial protocols. Days represent age of larvae (DPH), trial exchange flow rates were actual water exchanges used per day (%), and initial additives represent the amount added to 300 L experimental units at beginning of each day. Dosages of additives represent the amounts delivered to each experimental unit daily, hybrid rotifer feed schedule is following past CRC studies. Water samples collected for *Vibrio* and total bacteria enumeration on DPH 5, 9, and 12.

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<th>DAY</th>
<th>Trial exchange flow rates (% day⁻¹)</th>
<th>Trial exchange flow rates (mL min⁻¹)</th>
<th>Initial Algae Paste (ml)</th>
<th>Doser Algae Paste (ml)</th>
<th>Initial Solgel (ml)</th>
<th>Doser Solgel (ml)</th>
<th>Initial Clay SS⁺ (ml)</th>
<th>Doser Clay (grams)</th>
<th>Rotifers ml⁻¹</th>
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ᵃ Premixed stock solution (SS) of 15.6g clay with 800 mL salt water.
ᵇ Periods where larvae were held in incubators during endogenous feeding phase
ᶜ Stocking and endogenous feeding phase continued
ᵈ Onset of exogenous feeding and addition of treatment additives to culture tank
3.3 Results

Observed mean turbidity was 1.70 ± 0.06 NTU for algae paste, 7.30 ± 0.12 NTU for solgel and 10.31 ± 0.16 for clay. Transformed SDD was significantly different between different treatment additives ($F_{(2,9)} = 21.124, P < 0.001$). SDD was highest in the solgel treatment (65.5 ± 0.6 cm) followed by clay (61.0 ± 0.5 cm) and algae paste treatments (59.4 ± 0.9 cm). Tukey HSD post-hoc analysis revealed that the solgel treatment SDD was significantly higher than algae paste ($P < 0.0001$) and clay treatments ($P < 0.01$), but no other group differences were statistically significant.

Initial 4 DPH FI was significantly different between treatment additives ($F_{(2,9)} = 82.286, P < 0.0001$). Tukey HSD post-hoc analysis revealed that larvae in the algae paste treatment had significantly higher FI (97.5 ± 2.9%) than larvae in solgel (77.5 ± 6.5%, $P < 0.0001$) and clay treatments (57.5 ± 2.9%, $P < 0.001$). This trend in FI between treatments continued on 6 DPH ($F_{(2,9)} = 24.127, P < 0.001$) and 8 DPH ($F_{(2,9)} = 59.912, P < 0.0001$); however, no significant differences were seen at 10 DPH ($F_{(2,9)} = 2.053, P = .18$; Figure 13).

Prey consumption was significantly different among treatment groups at 5 DPH, $F_{(2,9)} = 30.384, P < 0.0001$. Tukey HSD post-hoc analysis revealed that larvae in the algae paste treatment had significantly higher prey consumption levels (44 ± 3) than in clay (15 ± 4, $P < 0.0001$) and solgel treatments (13 ± 2, $P < 0.0001$), but no other group differences were statistically significant. This trend in prey consumption continued at 7 DPH ($F_{(2,9)} = 6.028, P < 0.05$), 9 DPH ($F_{(2,9)} = 38.533, P < 0.0001$) and 11 DPH ($F_{(2,9)} = 10.188, P < 0.001$) with consumption being highest within the algae paste treatment across all sampled periods (Figure 14).
SBI was significantly different between treatment additives at 4 DPH ($F_{(2,9)} = 17.804, P < 0.001$) and 6 DPH ($F_{(2,9)} = 17.804, P < 0.001$). Day 4 Tukey HSD post-hoc analysis revealed SBI was highest in the algae paste treatment ($41.3 \pm 5.9\%$) followed by clay ($18.8 \pm 6.6\%, P < 0.03$) and solgel treatments ($0, P < 0.001$). This trend continued through day 6 with the algae paste treatment having the highest SBI ($47.5 \pm 8.3\%$) followed by clay ($27.5 \pm 5.2\%, P = 0.08$) and solgel treatments ($0, P < 0.001$; Figure 15).

No significant differences were seen in larval growth between treatment groups at 4 DPH ($F_{(2,9)} = 0.886, P = 0.45$). However, significant differences were present at 6 DPH ($F_{(2,9)} = 42.832, P < 0.0001$), 8 DPH ($F_{(2,9)} = 20.950, P < 0.001$), 10 DPH ($F_{(2,9)} = 5.967, P < 0.05$), and 12 DPH ($F_{(2,9)} = 11.041, P < 0.01$). 12 DPH Tukey HSD post-hoc analysis of final growth revealed larvae within the algae paste treatment ($5.80 \pm 0.08$ mm) to have similar growth compared to larvae within the solgel treatment ($5.65 \pm 0.03$ mm, $P = 0.25$), and larval growth in algae paste and solgel to be greater than larval growth within the clay treatment ($5.40 \pm 0.06$ mm, $P < 0.01$ and $P < 0.05$; Figure 16).

Final mean survival was significantly different between treatment groups ($F_{(2,9)} = 25.072, P < 0.001$). Tukey HSD post-hoc analysis revealed that larvae reared in the algae paste treatment had the highest mean survival ($21.6 \pm 2.9\%$) compared to clay ($6.9 \pm 1.0\%, P < 0.01$) and solgel treatments ($2.3 \pm 1.6\%, P < 0.001$), with no significant differences seen between clay and solgel groups ($P = 0.29$; Figure 17).
Figure 13. Feeding incidence (means ± SE) for YTK larvae \(n = 20\) by treatment for days 4, 6, 8 and 10. Values with different letters are significantly different determined by Tukey HSD post-hoc analysis \(P < 0.05\).

Figure 14. Prey consumption (means ± SE) for YTK larvae \(n = 20\) by treatment for days 5, 7, 9 and 11. Values with different letters are significantly different determined by Tukey HSD post-hoc analysis \(P < 0.05\).
Figure 15. Swim bladder inflation (means ± SE) for YTK larvae (n = 20) by treatment on days 4 and 6. Values with different letters are significantly different determined by Tukey HSD post-hoc analysis (P < 0.05).

Figure 16. Total length (means ± SE) for YTK larvae (n = 20) by treatment on days 4, 6, 8, 10 and 12. Values with different letters are significantly different determined by Tukey HSD post-hoc analysis (P < 0.05).
There were no significant differences in PAR at the water surface ($F_{(2,9)} = 1.310, P = 0.32$), with pooled average light intensity across the three treatments of 46 ± 3 µmol s$^{-1}$m$^{-2}$ (approximately 3266 ± 313 lux using metal-halide conversion from Thimijan and Heins, 1983; Figure 18a). There was a significant effect of treatment on the upwards facing light intensity ($F_{(2,9)} = 11.995, P < 0.01$) with Tukey HSD post-hoc analysis revealing a significantly lower light intensity for solgel at the mid-water position (15 ± 1 µmol s$^{-1}$m$^{-2}$) than both the clay (26 ± 1 µmol s$^{-1}$m$^{-2}, P < 0.01$) and algae paste (24 ± 3 µmol s$^{-1}$m$^{-2}$) treatments which did not differ significantly from each other ($P = 0.63$; Figure 18b). Attenuation of upwards facing light from surface measurements resulted in light intensity reductions of 54% for algae paste, 63% for solgel and 44% for clay. Significant differences were also observed between all three treatments in the downwards
facing light intensity \( (F_{(2,9)} = 50.748, P < 0.0001) \) with Tukey HSD post-hoc analysis showing clay to have the brightest submerged environment \( (9 \pm 1 \mu\text{mol s}^{-1}\text{m}^{-2}) \) compared to algae paste \( (5 \pm 1 \mu\text{mol s}^{-1}\text{m}^{-2}, P < 0.01) \) and solgel treatments \( (3 \pm 0.2 \mu\text{mol s}^{-1}\text{m}^{-2}; \text{Figure 18}) \).

Laser diffraction resulted in volume weighted means \( (D_{(4, 3)}) \) for solgel at 3.87 \( \mu\text{m} \) \( (40.68\% \text{ at } 0 – 2 \mu\text{m}, 45.07\% \text{ at } 2 – 5 \mu\text{m and } 14.25\% > 5 \mu\text{m}, \text{Figure 19a}), \) algae paste 2.68 \( \mu\text{m} \) \( (50.81\% \text{ at } 0 – 2 \mu\text{m}, 44.62\% \text{ at } 2 – 5 \mu\text{m and } 4.57\% > 5 \mu\text{m}, \text{Figure 19b}), \) and clay 5.96 \( \mu\text{m} \) \( (29.55\% 0 – 2 \mu\text{m}, 36.37\% \text{ at } 2 – 5 \mu\text{m and } 34.08\% > 5 \mu\text{m}; \text{Figure 19c}). \) Sedigraph analysis of the clay showed all particles in sub-clay category \( (0 – 1 \mu\text{m}) \) had a settling rate of \( 3.12 \times 10^{-7} \text{ m·s}^{-1} \), clay category \( (1 – 2 \mu\text{m}) \) of \( 2.81 \times 10^{-6} \text{ m·s}^{-1} \) and silt category \( (2 – 60 \mu\text{m}) \) of \( 1.20 \times 10^{-3} \text{ m·s}^{-1} \).

Significant differences were seen at 5 DPH for total bacteria between treatments \( (F_{(2,9)} = 11.487, P < 0.01) \). Tukey HSD post-hoc analysis revealed that the algae paste \( (1.56 \times 10^{5} \pm 3.19 \times 10^{4} \text{ cfu ml}^{-1}) \) and solgel treatments \( (1.31 \times 10^{5} \pm 4.26 \times 10^{3} \text{ cfu ml}^{-1}) \) had similar total bacteria concentrations \( (P = 0.64) \), and clay had the lowest concentration when compared with algae paste \( (P < 0.01) \) and solgel \( (P < 0.05) \). No significant differences were seen in total bacteria concentrations between treatments at 9 DPH \( (F_{(2,9)} = 3.816, p = 0.06) \) and 12 DPH \( (F_{(2,9)} = 1.340, P = 0.31; \text{Figure 20}) \). Additionally, significant differences were seen at 5 DPH for total Vibrio between treatments \( (F_{(2,9)} = 5.242, P < 0.05) \) with Tukey HSD post-hoc analysis revealing higher concentrations in the algae paste treatment \( (3.42 \times 10^{4} \pm 1.40 \times 10^{4} \text{ cfu ml}^{-1}) \) when compared with the clay treatment \( (1.31 \times 10^{3} \pm 0.40 \times 10^{3} \text{ cfu ml}^{-1}, P < 0.05) \). No significant differences were seen comparing the solgel treatment \( (2.98 \times 10^{3} \pm \)
$1.49 \times 10^3$ cfu ml$^{-1}$) with clay ($P = 0.99$) and algae paste treatments ($P = 0.06$).

Significant differences were seen in total Vibrio concentrations between treatments at 9 DPH ($F_{(2,9)} = 4.407, p < 0.05$); however, Tukey HSD post-hoc analysis revealed no differences in pairwise comparisons between treatment groups. No significant differences were seen at 12 DPH ($F_{(2,9)} = 0.155, P = 0.86$) between treatment groups (Figure 21). Total Vibrio at 5 DPH in the algae paste treatment accounted for approximately 22% of the total bacteria concentration. In contrast, total Vibrio in solgel and clay treatments only accounted for 2% and 4% of their respective totals.
Figure 18. Light intensity measured in PAR (means ± SE) pooled by treatment at the (A) surface, (B) upwards and (C) downwards at 30 cm depth. Values with different letters are significantly different determined by Tukey HSD post-hoc analysis ($P < 0.05$)
Figure 19. Particle size distribution for solgel (A), algae paste (B), and clay (C) using laser diffraction.
Figure 20. Total bacteria concentration (means ± SE) by treatment on days 5, 9 and 12. Values with different letters are significantly different determined by Tukey HSD post-hoc analysis ($P < 0.05$).

Figure 21. Total Vibrio concentrations (means ± SE) by treatment on days 5, 9 and 12. Values with different letters are significantly different determined by Tukey HSD post-hoc analysis ($P < 0.05$).
Table 4. Summary of YTK larval parameters assessed by treatment and days post hatch (DPH) during the course of the trial.

<table>
<thead>
<tr>
<th></th>
<th>DPH</th>
<th>Algae Paste</th>
<th>Solgel</th>
<th>Clay</th>
<th>Level of Significance</th>
</tr>
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<tbody>
<tr>
<td>Feeding Incidence (%)</td>
<td>4</td>
<td>97.5 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.5 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.5 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>95.0 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.8 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.5 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>98.8 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.5 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.2 ± 2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
<td>95.0 ± 2.9</td>
<td>98.8 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Prey Consumption</td>
<td>5</td>
<td>44.1 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.0 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.1 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
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<td></td>
<td>7</td>
<td>54.7 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.6 ± 6.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>26.4 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>*</td>
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<tr>
<td></td>
<td>9</td>
<td>67.8 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.6 ± 3.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.5 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>83.9 ± 11.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.0 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.6 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>**</td>
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<tr>
<td>Growth (mm)</td>
<td>4</td>
<td>4.40 ± 0.05</td>
<td>4.48 ± 0.04</td>
<td>4.45 ± 0.05</td>
<td>NS</td>
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<tr>
<td></td>
<td>6</td>
<td>5.03 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.74 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.56 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>8</td>
<td>5.49 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.10 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.06 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>10</td>
<td>5.61 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.40 ± 0.06&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>5.34 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>12</td>
<td>5.80 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.65 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.40 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>**</td>
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<tr>
<td>SBI (%)</td>
<td>4</td>
<td>41.3 ± 11.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>18.8 ± 13.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>47.5 ± 16.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>27.5 ± 10.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>13</td>
<td>21.6 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.9 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Total Bacteria (CFU ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>5</td>
<td>1.56 x 10&lt;sup&gt;5&lt;/sup&gt; ± 3.19 x 10&lt;sup&gt;4&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.31 x 10&lt;sup&gt;5&lt;/sup&gt; ± 4.26 x 10&lt;sup&gt;3&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.93 x 10&lt;sup&gt;4&lt;/sup&gt; ± 1.20 x 10&lt;sup&gt;4&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>*</td>
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<tr>
<td></td>
<td>9</td>
<td>6.55 x 10&lt;sup&gt;4&lt;/sup&gt; ± 1.56 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.15 x 10&lt;sup&gt;4&lt;/sup&gt; ± 5.95 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.73 x 10&lt;sup&gt;4&lt;/sup&gt; ± 1.43 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>*</td>
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<tr>
<td></td>
<td>12</td>
<td>1.66 x 10&lt;sup&gt;4&lt;/sup&gt; ± 3.41 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.65 x 10&lt;sup&gt;4&lt;/sup&gt; ± 1.18 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.18 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.67 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>NS</td>
</tr>
<tr>
<td>Total Vibrio (CFU ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>5</td>
<td>3.42 x 10&lt;sup&gt;4&lt;/sup&gt; ± 1.40 x 10&lt;sup&gt;4&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.98 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.75 x 10&lt;sup&gt;3&lt;/sup&gt;&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.31 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.20 x 10&lt;sup&gt;4&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>*</td>
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<tr>
<td></td>
<td>9</td>
<td>1.57 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.42 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.57 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.12 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.72 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.08 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>*</td>
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<td></td>
<td>12</td>
<td>4.00 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.75 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.97 x 10&lt;sup&gt;3&lt;/sup&gt; ± 2.42 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.95 x 10&lt;sup&gt;3&lt;/sup&gt; ± 0.75 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>NS</td>
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</table>

Means in the same row having different superscripts are significantly different determined by Tukey HSD post-hoc analysis (P < 0.05). Significance levels from ANOVA are denoted by: NS (No Significance), * (P < 0.05), ** (P < 0.01), and *** (P < 0.001).
3.4 Discussion

Similar to the cobia trial, differences were found in transformed SDD between treatments when statistically assessed, although these differences in attenuation are not visually apparent (Daugherty, pers. obs.). Following similar adopted SDD in the cobia trial, a relaxed approach to SDD of 55 – 65 cm was accepted. This study acquired both surface and submerged light intensity data to better understand how each additive affected light intensities throughout the water column. Similar readings for algae paste and clay treatments were found for upwards facing light intensity at 30 cm depth yet the downwards facing light intensity was significantly higher in clay tanks. This is likely caused by the clay particles reflecting light or a greater degree of reflection of light off the white tank base in this treatment. An important note on observed fish behavior should be mentioned regarding the onset of clay treatment into the system. In the clay treatment tanks an ostensible majority of yolk sack larvae rapidly aggregated at the surface and displayed erratic locomotion and disorientation. This unusual behavior and apparent stress by the larvae likely had a negative impact on the measured parameters used to assess overall larval performance, i.e., FI, consumption, SBI, survival, etc. YTK larvae have been shown to tolerate and thrive in high light intensities in the presence of green water turbidity (Carton, 2005; Hilder, 2013; Stuart and Drawbridge, 2011; Woolley et al., 2012b) which suggests the brighter submerged clay environment may not have caused the immediate disorientation observed in YTK larvae. It is likely that the light scatter caused by clay particles, and back-scatter from tank color, may be responsible for the observed erratic behavior in YTK larvae. Clay turbidity may require different tank colors to achieved desirable larval rearing performance parameters.
Naas et al. (1996) suggests all black tanks would provide for more natural illumination in larval rearing because of the tank’s ability to absorb refracted and reflected light. To determine if the clay – light interaction would be adjusted by tank color, a subsequent trial with only a clay treatment additive (and no larvae) was performed in which downwards facing light intensity was measured at different clay concentrations in a tank of the same dimension as those used in the trial, but with black a base. At a similar clay concentration to that used in the study (10 NTU) downwards facing light intensity was reduced by 90% compared to the white base tanks. Because of the rapid settling of clay particles within silt size category an “albedo” effect may occur in black bottom tanks causing increasing downwards facing light intensity. In the successful utilization of clay at commercial scales for *Seriola rivoliana* using all black tanks larvae do not display disorientation or surface aggregation (Daugherty, pers. obs.). Tank depth could be another important factor to consider given that the tanks used in the YTK trial are much shallower than tanks used for commercial larval rearing. A shallower tank can intensify the effects of light reflected from tank bases. This suggests tank color, tank dimensions, light intensity and turbidity interactions are important factors when substituting algae paste with this clay additive in *Seriola* larval rearing and thus require more research to optimize phototactic larval response at a species level.

Although the substantial reduction in both FI and prey consumption in clay and solgel tanks may have been influenced by light – particle interactions and its effects on visual contrast (Naas et al., 1992), the rapid mortality in the clay treatment suggests that larval mortality preceded periods associated with starvation. Additionally, enumeration of FI and undigested rotifer mastaxs were more difficult to observe in samples from these
two treatments in contrast to algae paste treatments. Rotifers and rotifer mastaxs within these treatments appeared more translucent due to their consumption of these algae paste substitutions. Final growth at 12 DPH was similar between all treatments, yet differences were seen at 6 and 9 DPH and showed an increasing FI in solgel and clay treatments. This suggests that larvae which were able to cope and survive to the end of the trial under algae substitute turbidities, maintained comparable growth to larvae in the algae treatment.

SBI of larvae in solgel treatment was 0 across all samples and periods (4 and 6 DPH). This result can be attributed to the additive’s cohesive properties that resulted in a buildup of surface film. Because *Seriola lalandi* are physostomes this film negatively impacted SBI within this treatment (Woolley et al., 2012b). Although the clay treatment appeared devoid of any surface films in comparison to algae paste and solgel treatments (Daugherty, pers. obs.), larvae in the clay treatment displayed poor SBI. The poor SBI in the clay treatment is likely attributed to the apparent stress larvae were displaying at the onset of additive introduction.

Assessment of total bacteria and total *Vibrio* resulted in similar trends seen in the cobia trial with *Vibrio* accounting for approximately 22% of total bacteria levels and highlighting the “pioneer” phase of the microbial communities within the culture system. This indicates algae paste provides adequate substrate for opportunistic bacteria when compared to solgel and clay (Attramadal et al., 2012). However, the superior larval survival observed in algae paste tanks suggest such early microbial presence of *Vibrio* is of secondary importance to ideal abiotic factors (i.e., light intensity, tank color and turbidity) and its effects on overall larval performance. Total bacteria and total *Vibrio*
concentrations between treatments were similar at day 9 and 12 and lower than day 5 indicating a shift from a “pioneer” to a “mature” or stabilized microbial community (Vadstein et al., 1993).

In contrast to the cobia trial, inorganic substitutes for water turbidity had a negative impact on YTK larval rearing performance. Whereas clay can serve as an alternative to algae paste with no negative impacts in survival and growth in cobia larval rearing, clay used for turbidity in YTK requires optimization of interactive abiotic parameters. Bacteria concentrations followed similar trends between cobia and YTK trials with YTK having much higher concentrations levels. This was likely the effect of higher organic loads caused by increased stocking densities and associated live feeds. In conclusion, the presence of increased bacteria loads under common greenwater larval rearing conditions is of secondary importance to the optimization of various environmental abiotic factors.
REFERENCES


Björnsdóttir, R., 2010. The bacterial community during early production stages of intensively reared halibut (Hippoglossus hippoglossus L.) (Ph.D. Dissertation). University of Iceland, School of Health Sciences, Faculty of Medicine, Reykjavik, Iceland.


Fowler, A.J., Ham, J.M., Jennings, P.R., 2003. Discriminating between cultured and wild yellowtail kingfish (Seriola lalandi) in South Australia. (No. RD03/0159), Aquatic Sciences Publication. South Australian Research and Development Institute, Adelaide, Australia.


