Learning the practical aspects of using clay particles to improve bacterial management during marine larval culture, University of Miami, Experimental Marine Hatchery

Robert Michael

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

Project No. 2012/720

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Office Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042

Postal Box 26, Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042

Tollfree 1300 732 213 Phone 08 8201 7650 Facsimile 08 8201 7659

Website www.seafoodcrc.com ABN 51 126 074 048

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NON-TECHNICAL SUMMARY

PROJECT NO: 2012/720 - Learning the practical aspects of using clay particles to improve bacterial management during marine larval culture

PRINCIPAL INVESTIGATOR: Robert Michael

ADDRESS: Australian Centre for Applied Aquaculture Research, 1 Fleet street Fremantle, Western Australia

(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY

The aim of this travel was to assess the potential for clay to replace green water during marine finfish larval culture and to learn and develop these techniques with the hope of applying this information to the Australian industry.

NON TECHNICAL SUMMARY:

Using clay particles to replace *nannochloropsis* paste during the green water phase of cobia larval culture was recently investigated. The results clearly showed that using this media has the potential to greatly reduce bacterial loads within larval tanks and reduce costs associated with purchasing algal paste concentrates.

ABOUT THE PROJECT/ACTIVITY

BACKGROUND AND NEED

Western Australia's extensive coastline is currently underutilized for marine finfish production, yet a recent Aquaculture Development Council discussion paper pointed out that aquaculture sites along this coastline can collectively host between 25,000 and 100,000 tons of annual finfish production. An industry of this size could directly contribute between $200 and $800 million to the economy, plus multiplier effects. The main species of interest in this state are yellowtail kingfish, mulloway and Yellowfin Tuna. The largest barramundi farm in the country is currently located in Western Australia and ACAAR is contracted to supply their juvenile requirements.

The Marine Fishfarmers Association (MFA) of WA has a long-term objective of supporting the growth of marine finfish aquaculture in this state. The MFA are currently managing a small but very collaborative trial in Geraldton to assess the opportunities and limitations of commercial sea cage farming of yellowtail kingfish in this state. This project is the first step in developing aquaculture in the Midwest area of WA and is linked to the development of the Abrolhos Islands Aquaculture site of 800 ha, as approved in 2004.

This overall MFA CRC project, “2011/74 - Development of Yellowtail Kingfish aquaculture in Western Australia: Removal of barriers to profitable production”comprises three sub-projects aimed at removing some key barriers (parasites) and optimising some key inputs (larval and genetics) to production and improving the commercial viability of the development of a larger scale industry. These projects are:

1. Genetic management strategy for cultured yellowtail kingfish in Western Australia
2. Larviculture of yellowtail kingfish
3. Management and mitigation of impacts of internal parasites within cultured yellowtail kingfish in Western Australia

The project that I have undertaken relates directly to sub-project 2 which involves the testing of innovative methods (including clay particles) for reducing the bacterial loads within yellowtail kingfish larval rearing tanks. High incidences of malformation and low level survival are common during yellowtail kingfish larviculture and it has been hypothesised that this is a result of excessive harmful bacterial loads within the culture tanks. The current rate of malformation and survival continues to impact heavily on the price of juvenile kingfish. There is a need therefore, to reduce the

incidence of such malformations and increase the overall survival rate to reduce the cost of juvenile production and improve the quality and quantity of fish being put to sea.

Financial support from the Seafood CRC has allowed me to travel to the University of Miami's Experimental Marine hatchery (UMEH) to work and train under the supervision of Professor Daniel Benetti and alongside his Masters student, Mr Zack Daugherty, the subject of whose thesis is the use of clay particles in larval rearing of cobia. Over the past 12 months Mr Daugherty has been conducting preliminary trials on the best methods of handling and delivering clay particles to larval rearing tanks.

During my 30 day visit I was able to further develop these techniques with Mr Daugherty prior to conducting a 13 day replicated research trial that assessed the potential bacterial reducing properties of using clay during cobia larval rearing compared with the standard green water method that uses concentrated *nannochloropsis* paste.

PRELIMINARY TESTS

Prior to conducting the main experiment, we needed to develop a method of standardising the 'transparency' levels between tanks that were exposed to nannochloropsis paste and those exposed to the clay. We decided to use Nephelometric Turbidity Units (NTU) to monitor the transparency levels between tanks rather than the routine secchi disk depth (SDD). This was because the trial tanks were too shallow to measure SDD (i.e. the tank bottom could still be seen when adding the same concentration of microalgae used to achieve the SDD of 50-70cm used in commercial rearing tanks).

To do this, we periodically dosed known concentrations of paste (Reed Mariculture *Nannochloropsis* 3600) and clay (20g/L stock solution) to two 400L trial tanks and measured the NTU using a portable turbidity meter and SDD using a turbidity tube. This allowed us to develop standard curves for both paste and clay (Fig. 1 and Fig. 2) which enabled us to determine the different NTU levels required for each product for the desired SDD of 50-70cm which is used in commercial cobia culture. Using this information we were then able to develop a formula to determine how much paste or clay (taking into consideration the flushing effect of new water entering the tanks) to dose each trial tank over the duration of the day to maintain the desired equivalent SDD of 50-70cm (Appendix 1).

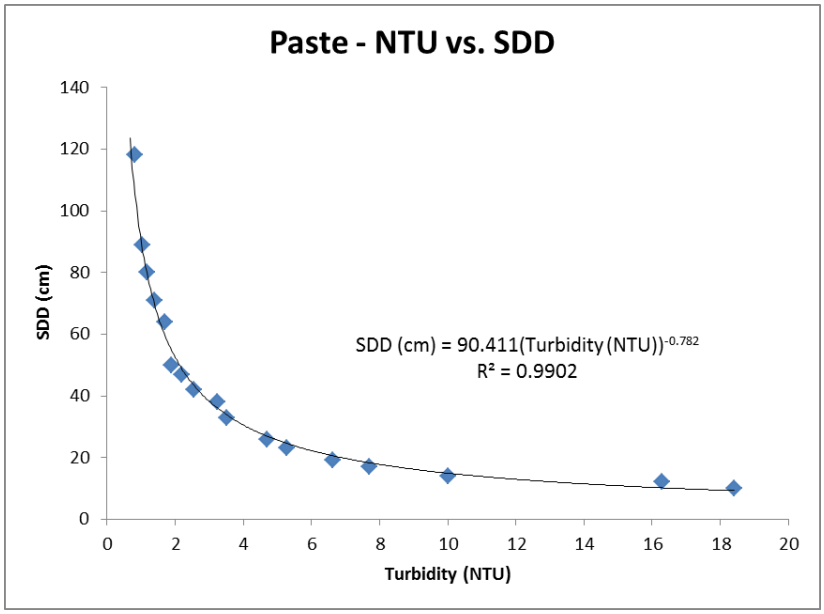

Figure 1: NTU vs. SDD using various concentrations of REED mariculture 3600 *nannochloropsis* paste ranging from 0.012mL/L to 0.274mL/L.

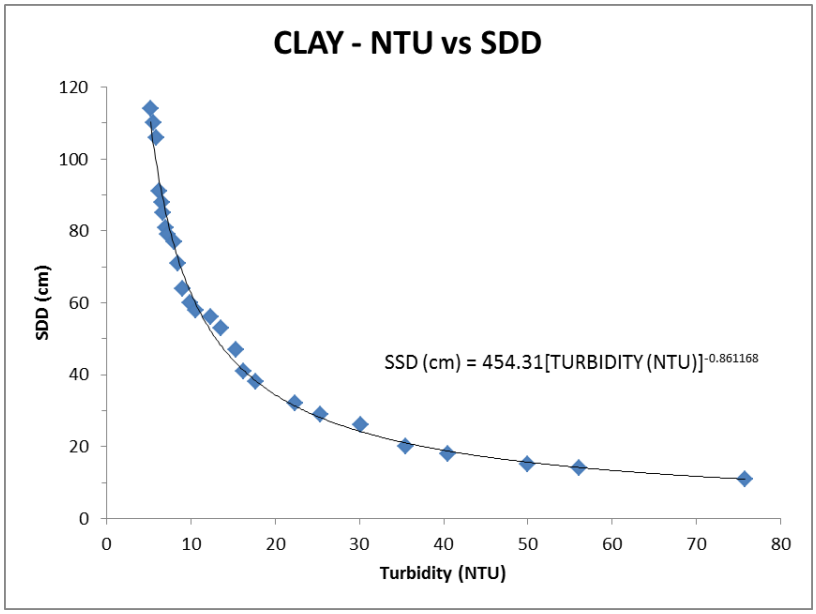

Figure 2: NTU vs. SDD using various concentrations of clay particles ranging from 0.0058g/L to 0.0786g/L.

MAIN EXPERIMENT

Once the aforementioned preliminary trials we begun the main experiment. The aim of this experiment was to assess and compare growth, survival and *Vibrio spp*. loads in larval tanks dosed with either *Nannochloropsis* paste or clay particles.

The trial system consisted of an array of 12 x 400L larval tanks (6 x clay and 6 x paste) housed within a 40,000L water bath which was used to help maintain a stable temperature (Fig 3). Water to the system was continuously delivered to each tank via a 500L header tank which was constantly supplied by a 40,000L reservoir. This design was critical as UMEH regularly experiences pressure drop and periodic water shut down. This method ensured a constant and consistent supply of new water to each of the larval tanks. A known concentration of clay solution or paste was then continuously dosed into the main supply lines and distributed to the allocated tanks ensuring adequate SDD was maintained (Fig 4).

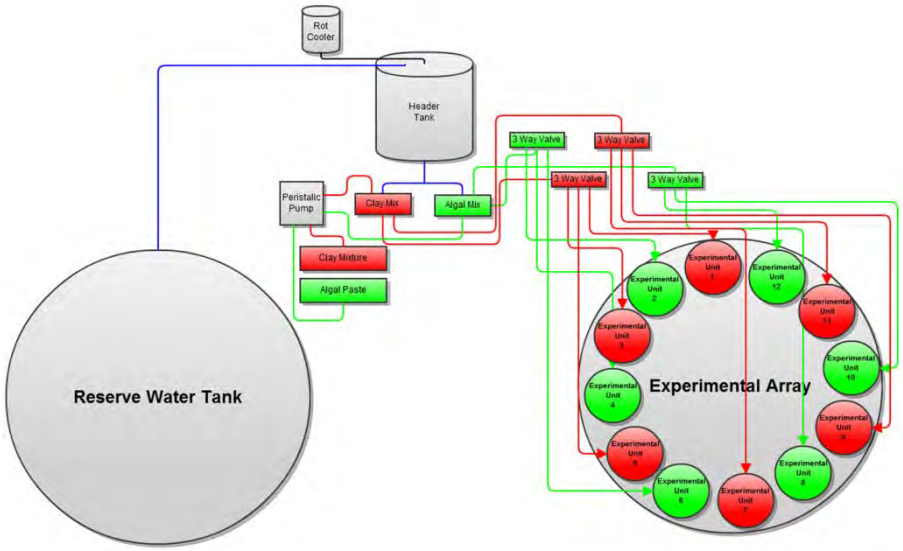

Figure 3: Diagram and photograph displaying the experimental set up

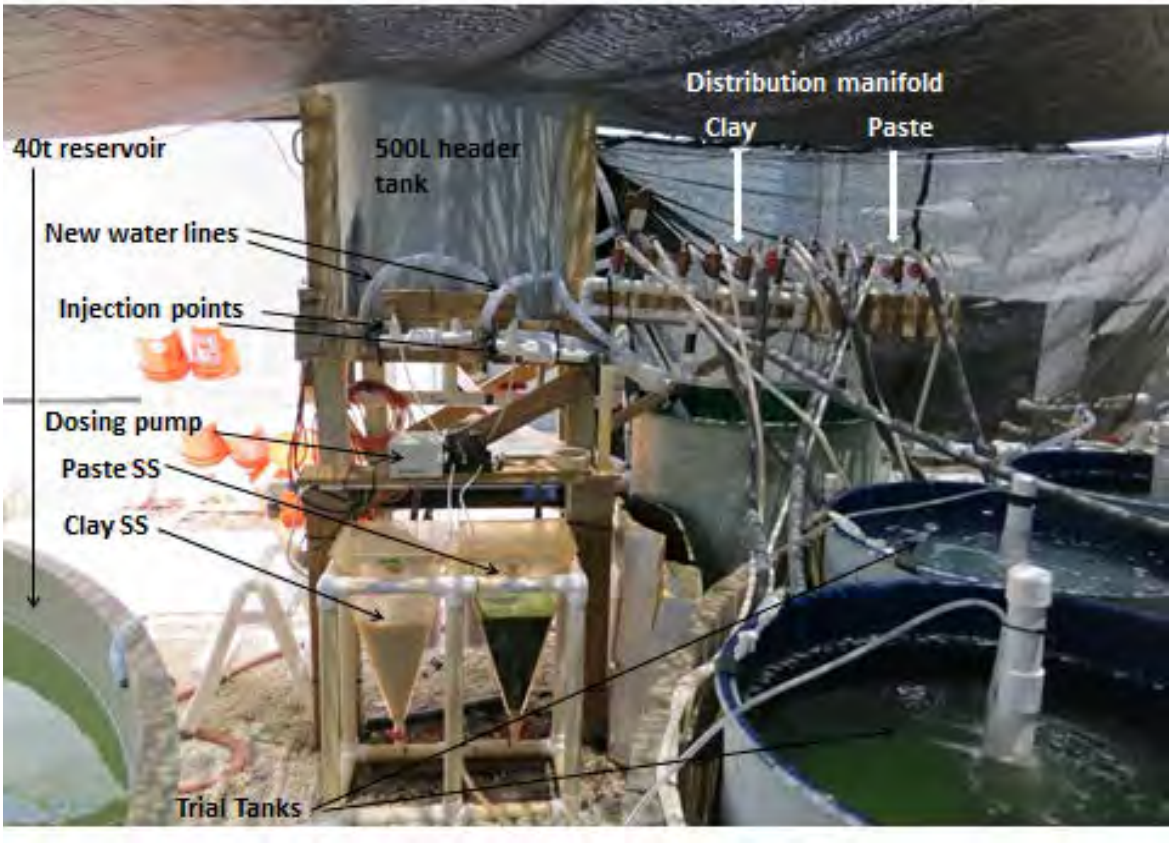

Figure 4: Dosing system

Besides the addition of clay particles, standard UMEH cobia larval rearing protocol was followed. This consisted of the following;

Initial stocking density – 15/L on day 2

Temperature >26°C

D.O. > 6mg/L

First feeding and paste / clay addition – day 3

Flow rate – 300 to 600 % exchange.

Feeding density and frequency – top up to 5 rotifers / mL four times per day (Enriched using standard UMEH protocol).

Temperature, Dissolved Oxygen, pH, Salinity and NTU was measured in each tank twice a day (Appendix 2).

The trial was run until larval flexion was fully complete in both treatments. Given the lower than usual water temperatures experienced at UMEH this occurred at 12 day post hatch (dph). At 13 dph all tanks were pulled down and each individual surviving larvae counted. A representative sample of larvae was also kept from each tank for measurement analysis.

This was achieved by passing samples of culture water through 0.45µm, gridded, cellulose nitrate filter media and plating the filter media on TCBS media and incubating for 24hr at 28°C. Each sample was done in triplicate at 5, 7, 10 and 12 dph.

RESULTS

Survival and growth was assessed at 13 dph and the results across each treatment were averaged (Fig 5). Although a slightly higher survival was seen in the clay tanks compared to the paste tanks (40.7% vs. 35.7%) there is no significant difference between the two.

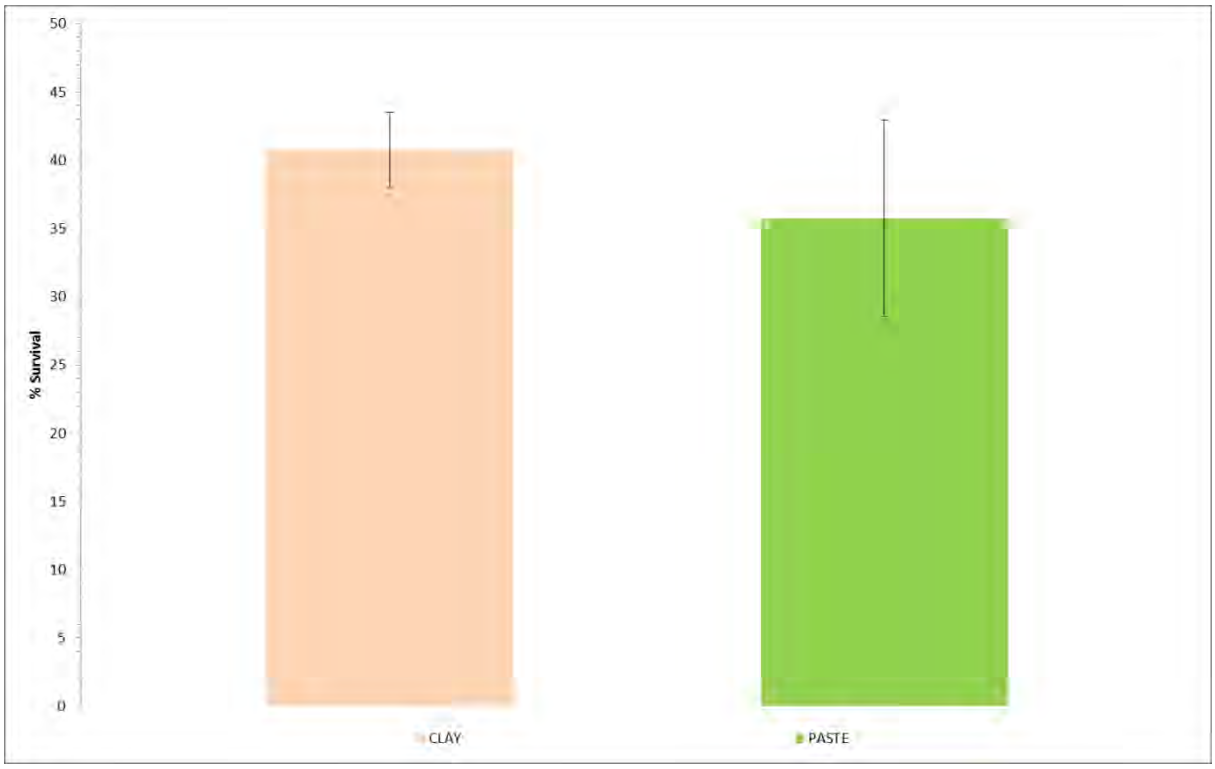

Figure 5: Combined average survival at 13dph in clay treated tanks vs. paste treated tanks

Average length was also assessed between the clay treated tanks and the paste treated tanks (Fig 6), and again, although a slightly longer length was observed in the clay treated tanks (8.21mm vs. 8.06mm) there is no significant difference between the two treatments.

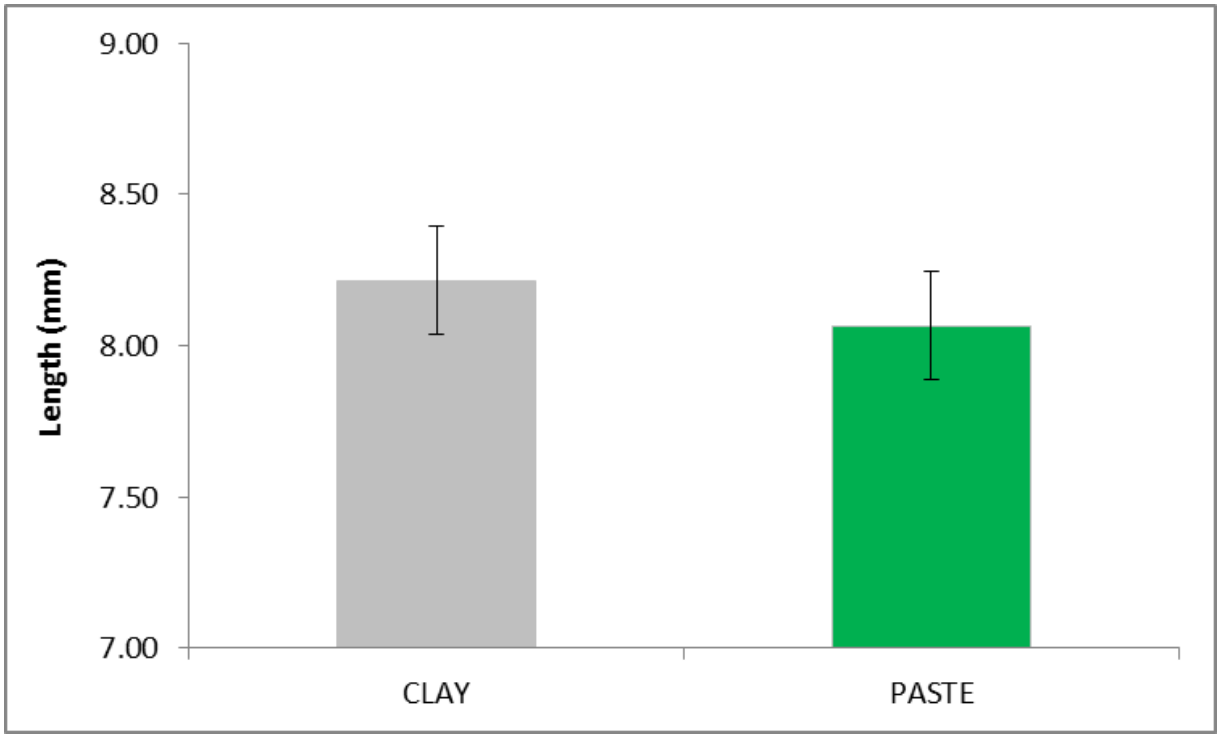

Figure 6: Combined average length (mm) at 13dph in clay treated tanks vs. paste treated tanks

Bacterial plating showed a dramatic reduction in *vibrio* densities in the clay treated tanks compared with the paste treated tanks on each sampling day (Fig 7).

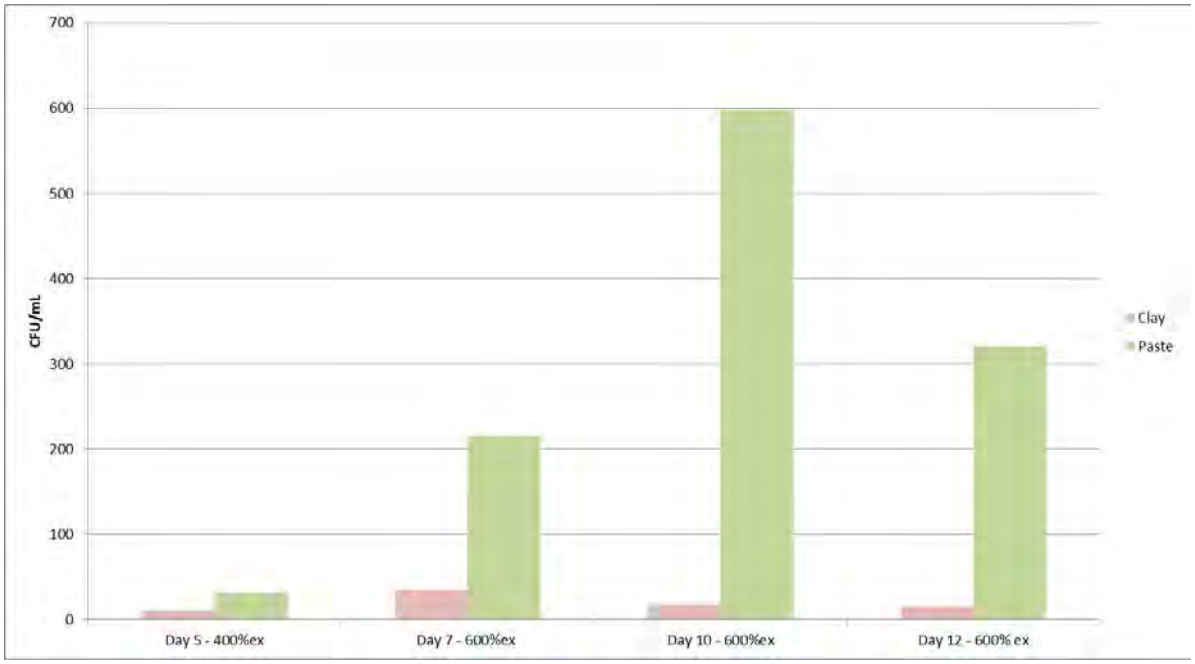

Figure 7: Combined average CFU/mL of *Vibrio* in clay treated tanks vs. paste treated tanks at 5, 7, 10 and 12 dph.

The observed differences in bacterial loads is likely due to the inert nature of the clay particle being unable to provide an organic substrate for bacteria to thrive on. It can be seen from figure 7 that *Vibrio* densities within the paste treated tanks continue to increase as the biological load within the tank also increases. This dramatic increase stops after 10 dph. It is hypothesised that this is due to a shift in the dominant bacterial species within the tank having an effect on the *Vibrio* densities.

It appears that the reduced *Vibrio* densities that are experienced with the addition of clay to the larval rearing tanks do not have any effect on the growth or survival of cobia larvae, at least in the early stages of culture (pre-*Artemia*). However it is worth noting that the use of clay instead of algal paste had significant financial benefits. It was calculated that replacing algal paste with clay will reduce green water costs by 98%.

Although reducing *Vibrio* loads within the cobia tanks appeared to have no effect on survival or growth, the same may not occur with other species that are commonly stocked at much higher rates and are known to be affected by high bacterial loads, i.e. barramundi and yellowtail kingfish.

INDUSTRY IMPACT

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

Data has been obtained demonstrating that clay has significant benefits in marine fish larval rearing for reducing bacterial loads and the high costs associated with using algal paste.

SUMMARY OF CHANGE IN INDUSTRY
(What immediate changes might be expected for business/industry?)

It is unlikely that Australian hatchery operations will adapt these findings until they have been proven for local species.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?
(What will be the impact?)

If these findings are also proven to be relevant to Australian species the methods are likely to be adopted.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

The most significant short term barrier is the lack of confidence that the results with cobia are directly relevant to Yellowtail kingfish and Barramundi. This barrier will be addressed in ACAAR trials proposed with yellowtail kingfish under Sub-project 2 of project number 2011/74 - Development of Yellowtail Kingfish aquaculture in Western Australia: Removal of barriers to profitable production.

IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?

(e.g. 2 businesses will adopt project findings and two more are expected to adopt findings within 12 months)

It is likely that businesses will begin to adopt these changes immediately if ACAAR trials demonstrate similar positive findings using local species.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

(e.g. 50% chance that four businesses will adopt project findings)?

It is highly likely that if no negative impacts of clay are found in our upcoming trials that these changes will occur. This will occur due to financial pressure even if there appears to bacterial advantage.

WHAT BARRIERS ARE THERE TO ADOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

(e.g. to adopt project findings will require group training/sharing equipment/invest additional capital etc.)

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COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

That clay can reduce bacterial loads and costs.

WHO IS/ARE THE TARGET AUDIENCE/S?

Hatchery Managers and technicians in all Australian marine fish hatcheries.

WHAT ARE THE KEY MESSAGES?

Replacing algal paste with clay particles for green water has the potential to reduce bacterial numbers and reduce production costs.

WHAT IS THE CALL TO ACTION?

(What is it you want people to do once you communicate the key message to them –i.e. what change of behaviour or action do you want them to take?)

Stays tuned for results from upcoming trials with commonly cultured Australian marine species and implement the changes if positive results are found.

COMMUNICATION CHANNELS

(How can these messages be communicated and by who?):

All results from the training and research trials conducted at UMEH on cobia will be made available to other CRC participants through a presentation to the yellowtail kingfish larval rearing committee. Given that it is the intention of both UMEH and ACAAR to publish their findings on cobia and yellowtail kingfish, respectively, the data will be widely disseminated if it is worthy of publication.

Channel	*Who by*	*When*
Hatchery Network Participants	ACAAR	After completion of YTK trials

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

WHAT IS YOUR FEEDBACK?

(e.g. *What difficulties were experienced in undertaking this research and how did this affect the project, what improvements and/or considerations can be recommended for future projects in this area* and *what barriers are there to undertaking further research in this area and how could these be overcome?)*

Research trials involving the use of larval marine fish are always difficult and rely on the support of reliable spawning broodstock. There were times at UMEH when we were unable to obtain larvae when required and as a result the main trial was delayed for some time. However the broodstock eventually commenced spawning and we were able to proceed. It is possible that this kind of obstacle may also be experienced at ACAAR when trying to obtain Yellowtail kingfish and it is recommended that alternative populations of broodstock are utilised to overcome this potential barrier.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?

(e.g. IP protection, licensing, sales, revenues etc)

Nil

ACKNOWLEDGEMENTS

I would like to acknowledge Professor Daniel Benetti, Mr Zack Daugherty and the rest of the staff at UMEH for hosting me at their facility and allowing me to undertake this research.

APPENDIX 1 – trial outline

TRIAL 1 OUTLINE

Parameters taken from current UMEH cobia Protocols.

Initial stocking density	15	per L on in afternoon of day 2
Maintain temp above	26	degrees
Maintain DO above	saturation	

Add paste and live feeds on day 3

We will take 2 x 2ml samples before each feed to determine rot residual levels and top up.

Number of Clay tanks	6	TW TANKS	250L
Number of Paste tanks	6	TRIAL TANKS	400L

Volume of trial tank 400 L

DAY	Protocol exchange rates	Trial exchange flow rates (%)	Trial exchange rates (mL/min)	Initial Paste (mL)	doser paste (mL)	initial clay ss (mL)	doser clay (grams)	Rotifers per mL Feed 1	Feed 2	Feed 3	Feed 4	Bacto sample	comments
0	0	0	0	0	0	0	0	0	0	0	0	NO	incubator
1	0	0	0	0	0	0	0	0	0	0	0	NO	incubator
2	100	300	833	0	0	0	0	0	0	0	0	NO	stock into trial tanks in the afternoon
3	100	300	833	72	72	250	45	5	5	5	5	NO	add paste/clay/ feed first thing am
4	100	300	833	72	72	250	45	5	5	5	5	NO	
5	200	400	1111	72	98	250	61	5	5	5	5	YES	
6	200	400	1111	72	98	250	61	5	5	5	5	NO	
7	200	400	1111	72	98	250	61	5	5	5	5	YES	This is when arts are usually introduced, but we will keep feeding rotifers
8	200	600	1667	72	144	250	90	5	5	5	5	NO	
9	300	600	1667	72	144	250	90	5	5	5	5	NO	
10	300	600	1667	72	144	250	90	5	7	7	7	YES	Larvae beginning flexion - run for two more days - development slower due to cooler water temps
11	300	600	1667	72	144	250	90	7	7	7	7	NO	first signs of dropout
12	300	600	1667	72	144	250	90	10	10	10	10	YES	Last day of feeding
13	300	600	1667	0	0	0	0	10	10	10	10	NO	Pull down trial and asses survival and final length
					1877.76		**1035.6**						
				COST	**$187.78**		**$2.94**						

CLAY STOCK SOLUTION

Needs to be made fresh each morning.

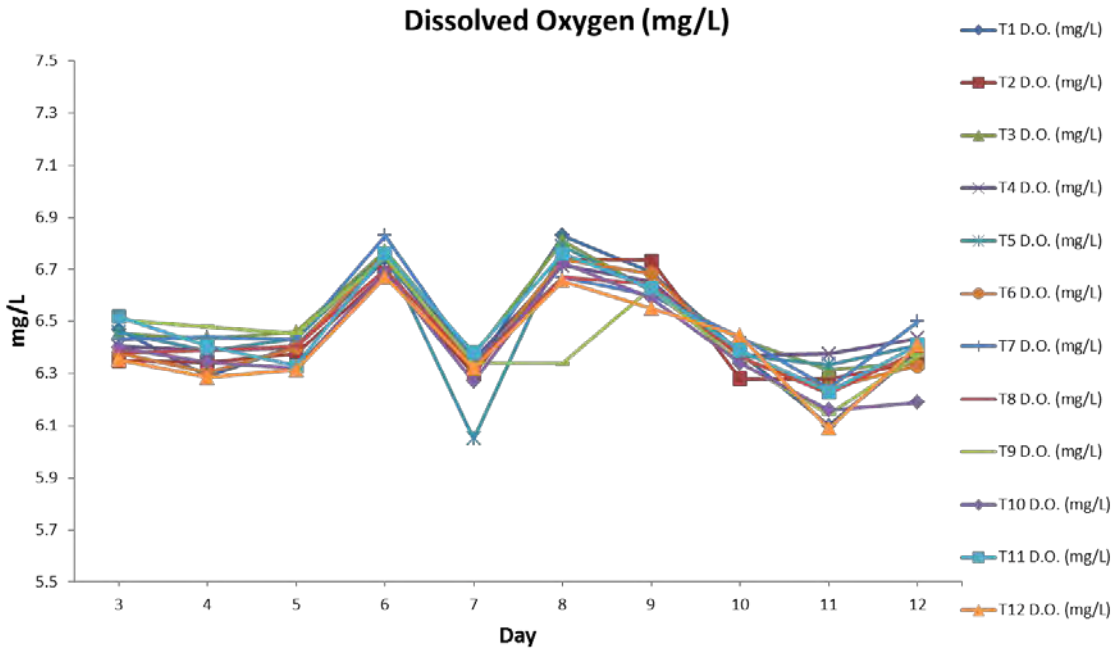
INITIAL TANK RATE

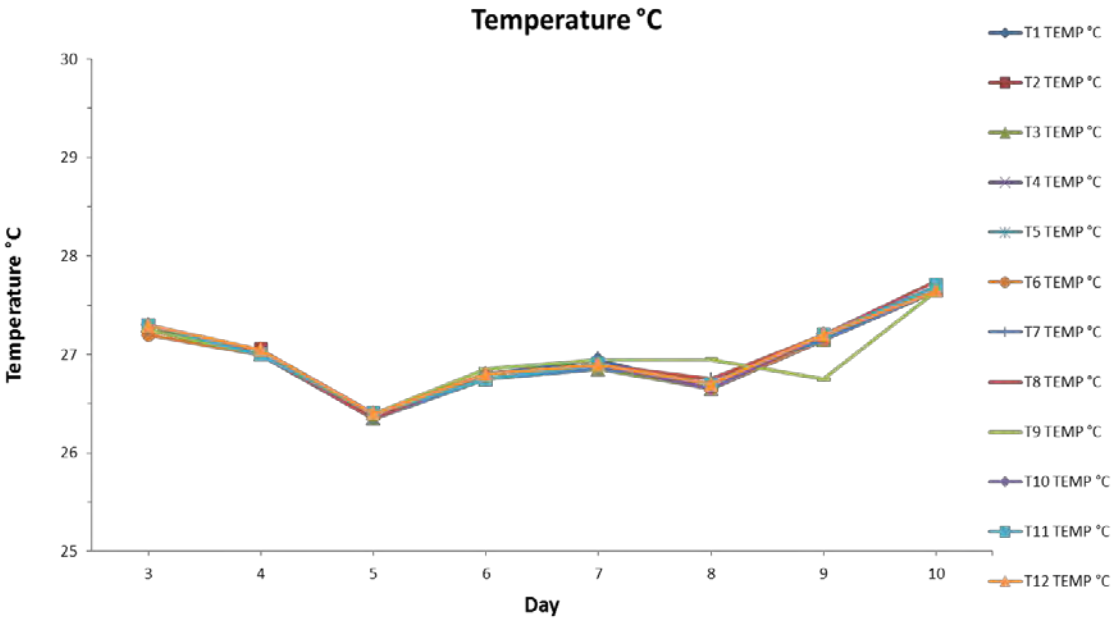
CLAY		**PASTE**	
13	g/m3	30	mL/m3
0.013	g/L	0.03	mL/L
5.2	g needed in each tank	12	ml needed in each tank
31.2	g total	**72**	ml for all 6 tanks
1500	mL is volume that 31.2g needs to be mixed into	1500	mL is volume that 36g needs to be mixed into
250	mlL is the amount that needs to be initially distributed to each tank	250	mlL is the amount that needs to be initially distributed to each tank

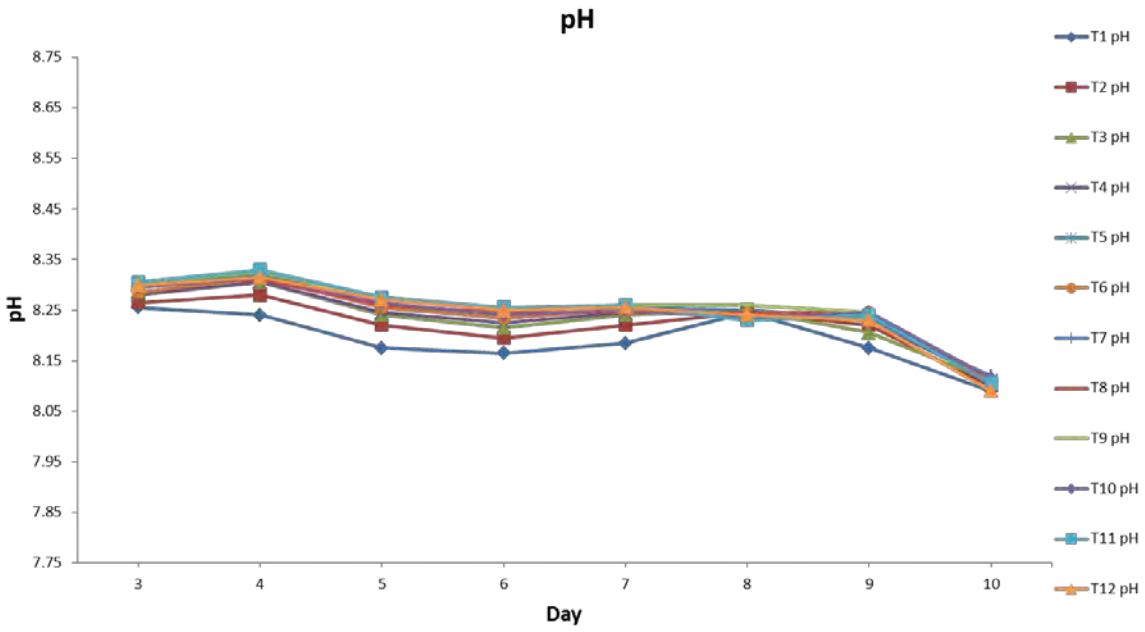
			For 10hrs	
Paste doser	11	ml/min	**6600**	ml
Clay doser	10.4	ml/min	**6240**	ml

Doser Rate - Doser pumping for	**10**	hrs		
45	g/m3/24hr	72	mL/m3/24hr	
0.0019	g/L/hr	0.003	mL/L/hr	
7.5	g per tank	12	ml going to each tank	

APPENDIX 2 – daily water quality data



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8.05
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7.75
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4
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7
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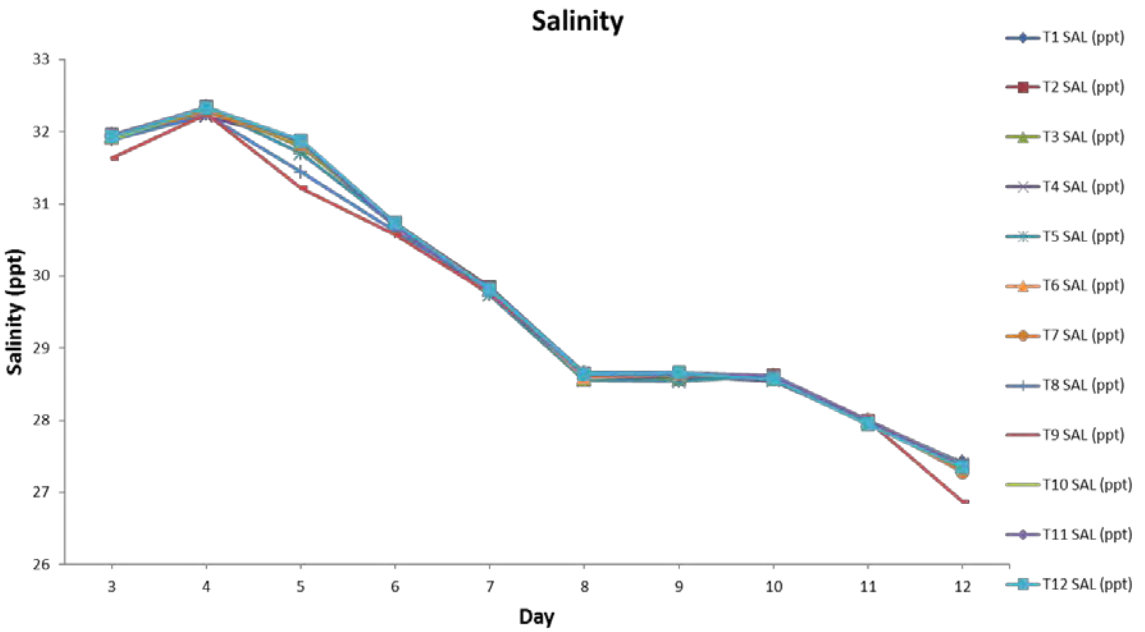
Salinity
33
32
31
30
29
28
27
26
Salinity (ppt)
3
4
5
6
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9
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12
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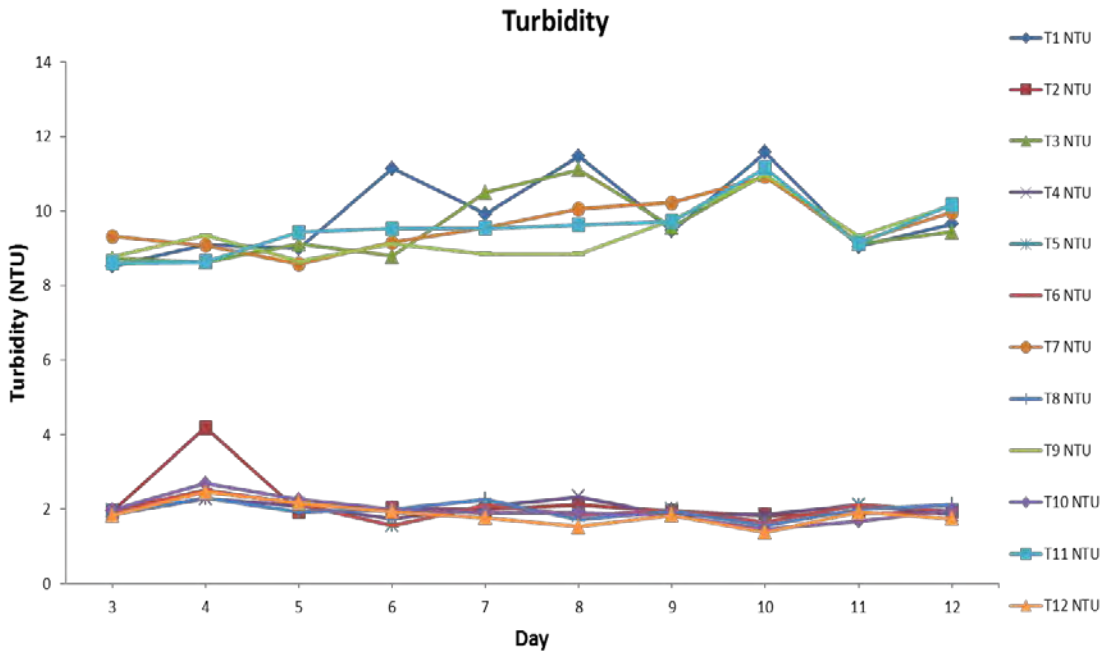
Turbidity
Turbidity (NTU)
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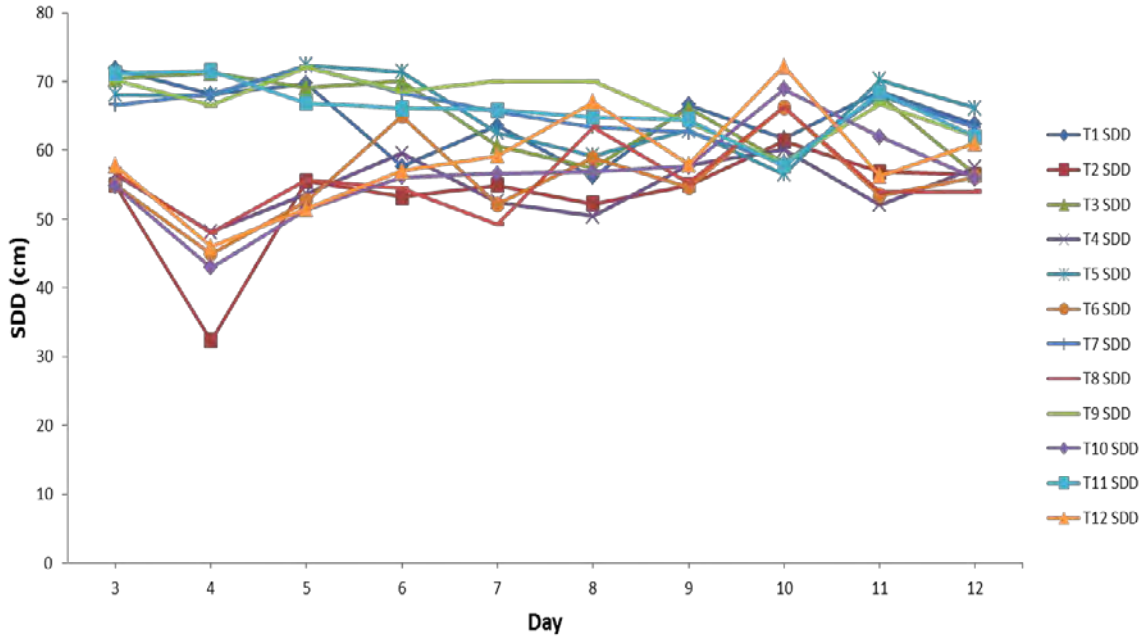
SDD (cm)
Day
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