High density production of the Pacific White Shrimp, *Litopenaeus vannamei*, in zero-exchange, biofloc-dominated grow-out system: the challenge of *Vibrio*

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Introduction

- Numerous studies documented excellent performance of *Litopenaeus vannamei* in no exchange, high-density, biofloc-dominated systems
- However, recent findings suggest that shrimp in these systems can be affected by bacterial disease outbreaks
- In most cases, these outbreaks caused by pathogenic *Vibrio* species
- Better monitoring, prevention & control methods of pathogenic *Vibrio* are needed to improve the economic viability of these systems

Objectives

- Document the impact of *Vibrio* outbreaks on shrimp after exposure to multiple stressors
- Study the performance of *L. vannamei* juveniles and the changes in selected WQ indicators in high-density, biofloc-dominated, no water exchange RWs operated with a^3 injectors
- Determine if the a^3 injectors can maintain adequate water mixing and DO levels in RWs stocked with juveniles of *L. vannamei* at high-density in biofloc-dominated water operated with no exchange

Materials & Methods

- A 38-d GO study in two 100 m^3 greenhouse-enclosed RWs, stocked with juveniles (6.45 g) at 458 shrimp/m^3
- RWs filled with biofloc-rich water (87.5%) from an earlier 62-d nursery trial + NSW (12.5%)
- Temp., salinity, DO, pH: 2/d; SS: 1/d; TSS: ≥1/wk; NH_4-N, NO_2-N, NO_3-N, VSS, turbidity, RP: 1/wk; Alkalinity: 2/wk adjustments using $NaHCO_3$ to maintain 160 mg/L as $CaCO_3$
- pH adjusted to >7 using $Ca(OH)_2$ from day 33 on

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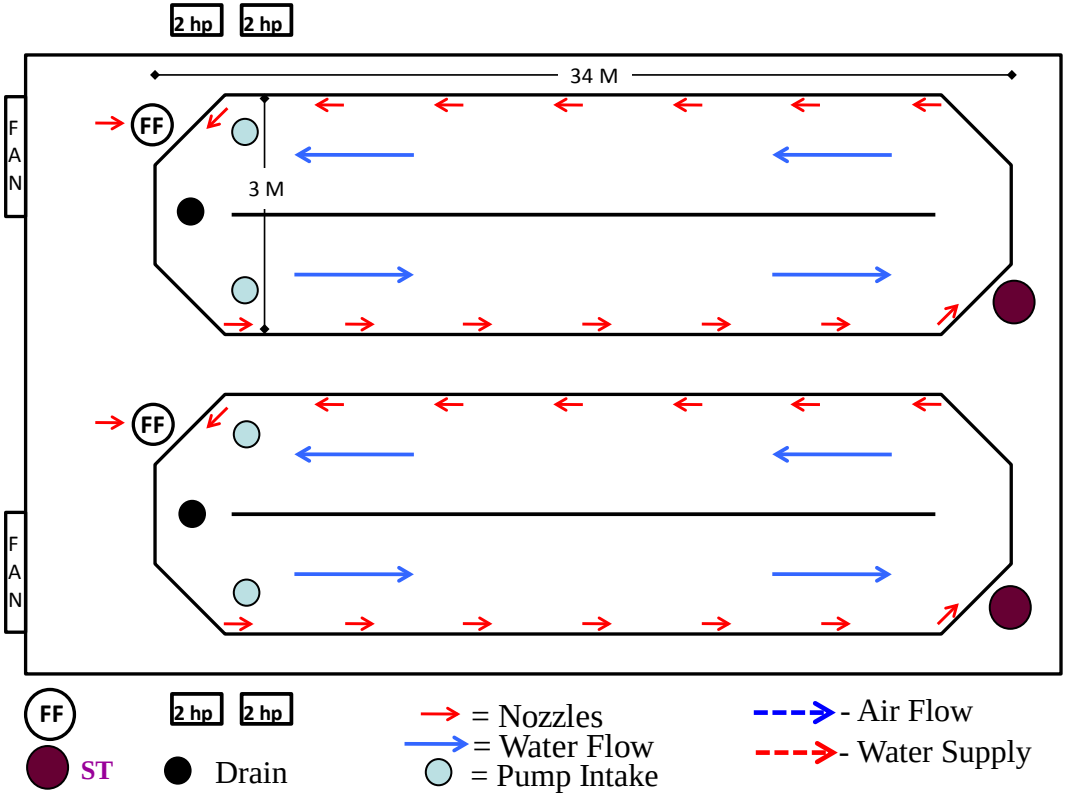
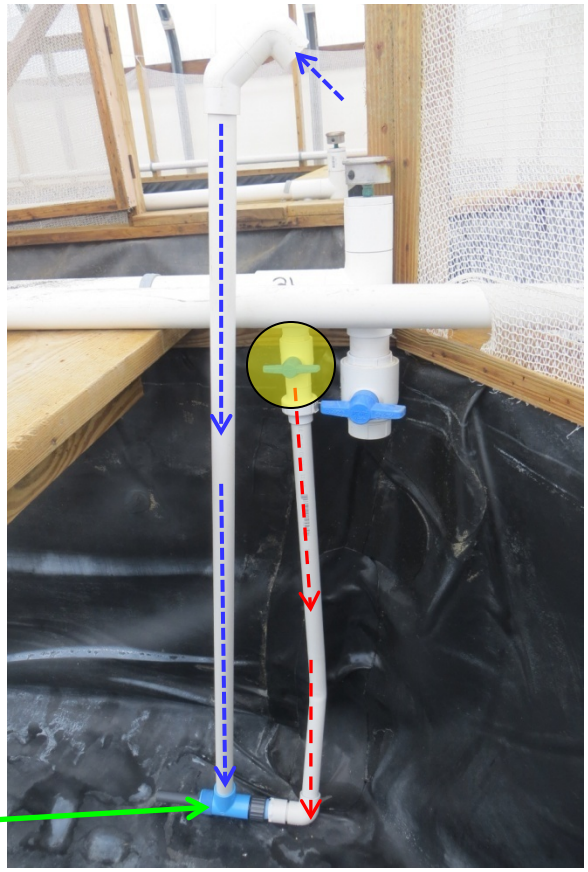
100 m^3 RWs – Greenhouse

Water & Air Flow





- Each RW has 14 a^3 injectors
- One ST & one FF per RW
- Two 2 hp pumps per RW that can be operated independently or simultaneously, depending on loading factors (e.g., biomass, DO concentration)

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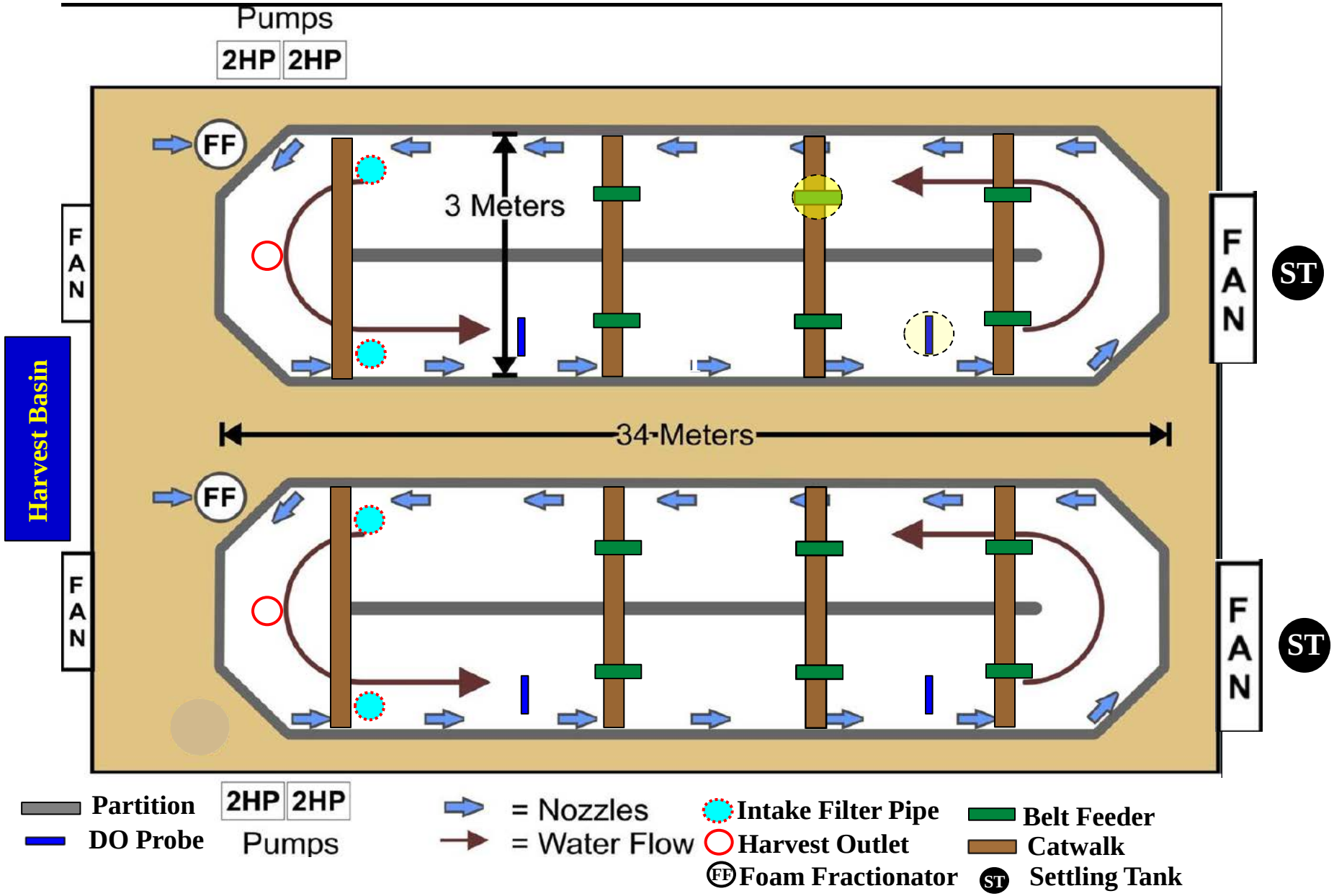
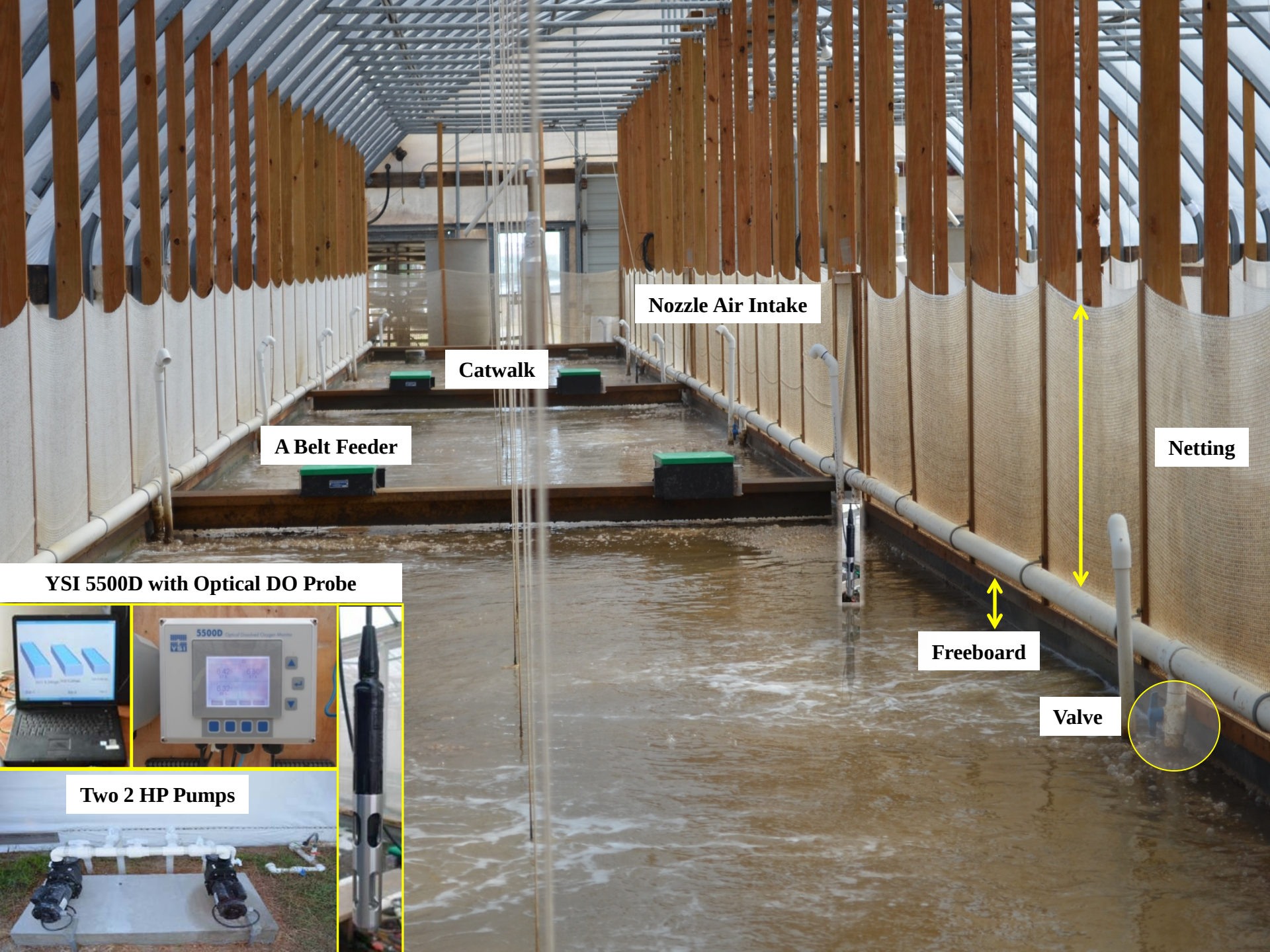
Top View - 100 m^3 RWs at Texas AgriLife




Nozzle Air Intake
Catwalk
A Belt Feeder
Netting
Freeboard
Valve
YSI 5500D with Optical DO Probe
5500D
Two 2 HP Pumps

Materials & Methods

- Water circulation, mixing & oxygenation in each RW were maintained by 14 a^3 injectors (*a^3 All Aqua Aeration, Orlando, FL*) & two 2 hp pumps
- Each RW had 2 YSI optical DO probes & 5500D inline monitoring system (*YSI Inc., Yellow Springs, OH*)
- Commercial probiotic - ECOPRO (*EcoMicrobials™, Miami, FL*) - every second day to daily
- No water exchange - FW added to maintain salinity

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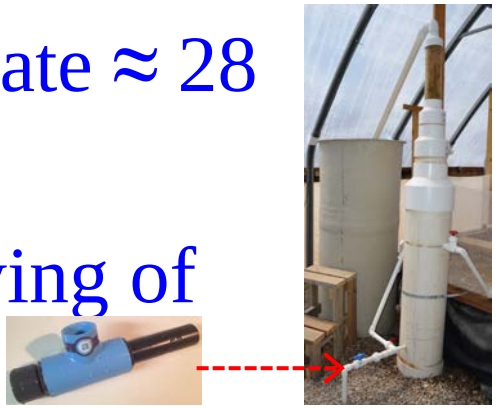
Foam Fractionator

- Operated with one a^3 injector, flow rate ≈ 28 Lpm, fed from the pump's side loop
- Use of fabric for dewatering and drying of the organic particulate matter



Settling Tanks

- Conical tank 2 m^3, flow rate 20 Lpm, fed from the pump's side loop
- Use of fabric for dewatering and drying of the organic particulate matter
- Targeted TSS of 200-300 mg L^{-1} & SS of 10-14 mL L^{-1}

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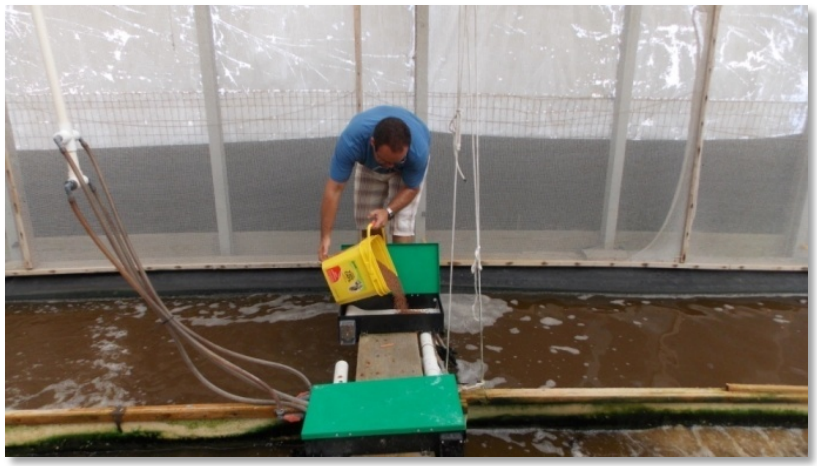
Materials & Methods

- Shrimp were fed 40% CP feed with 9% lipid (*Zeigler Bros., Gardners, PA*)
- Rations were initially determined using an assumed FCR of 1.2-1.3, growth of 1.5 g/wk, and mortality of 0.5%/wk, and were adjusted based on 2/wk growth samples & shrimp mortality
- Feed was distributed continuously 24/7 using belt feeders



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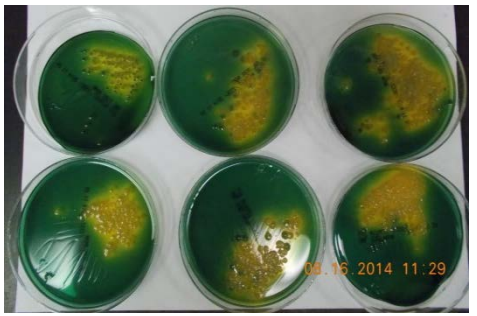
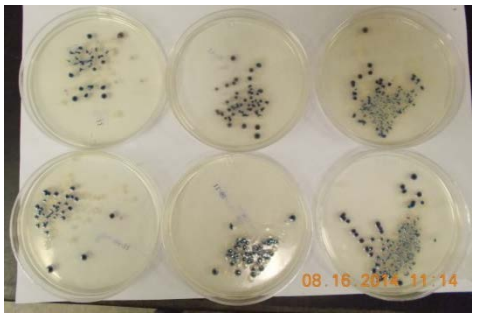
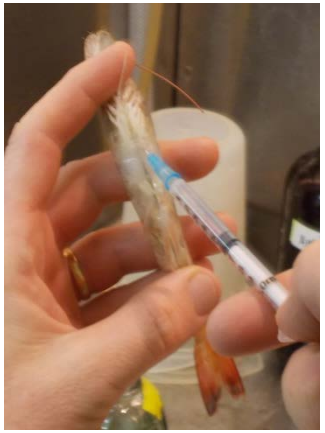
Materials & Methods

Vibrio & Algae

- *Vibrio* in culture medium monitored 2/wk on TCBS and late in grow-out on RambaCHROM
- *Vibrio* in hemolymph of moribund shrimp cultured on TCBS and RambaCHROM at harvest
- Algal pigments in biofloc measured 1/wk







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Summary of water quality parameters* in two 100 m^3 raceways over 38-d period

		Temp. (°C)	Salinity (ppt)	DO (% Sat)	DO (mg L^{-1})	pH
AM	Mean	30.1	30.4	96.7	6.19	7.54
	Min	29.0	29.3	79.2	5.03	6.78
	Max	30.8	30.9	117.9	7.48	7.84
PM	Mean	30.5	30.5	93.7	5.94	7.61
	Min	29.4	29.9	74.4	4.68	6.71
	Max	31.3	30.9	108.2	6.86	7.88

*No statistically significant differences between RWs - All WQ indicators were within the acceptable range for *L. vannamei*

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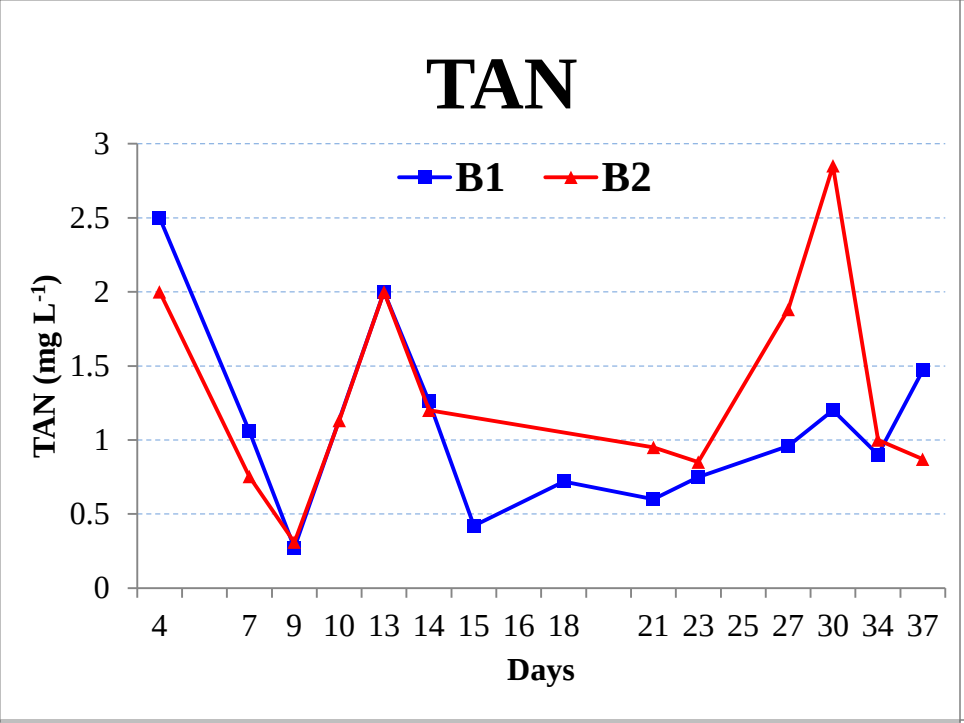
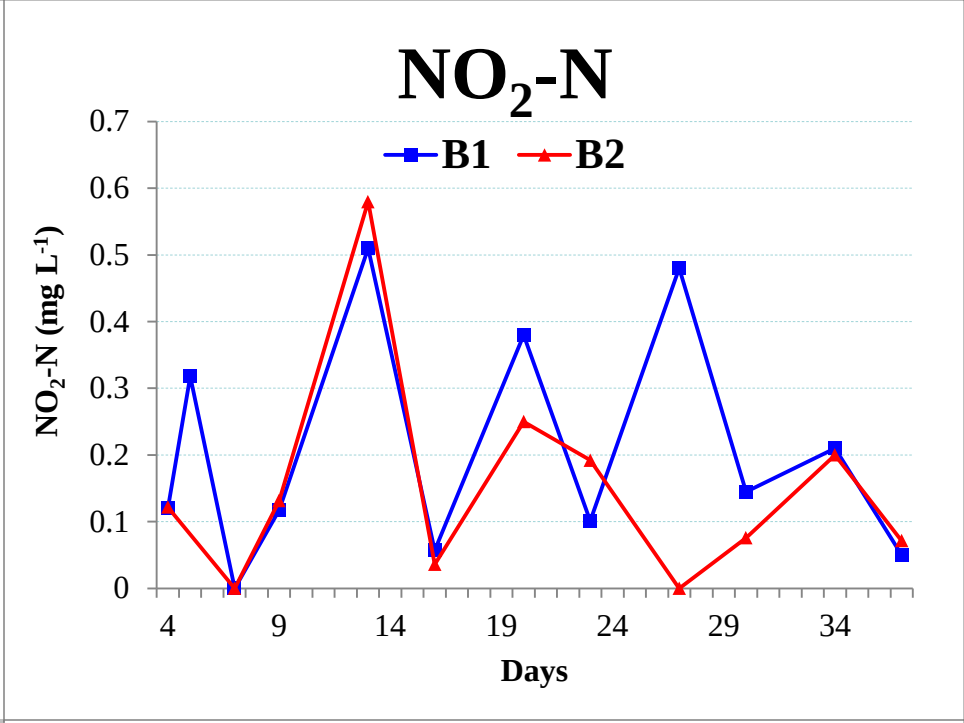
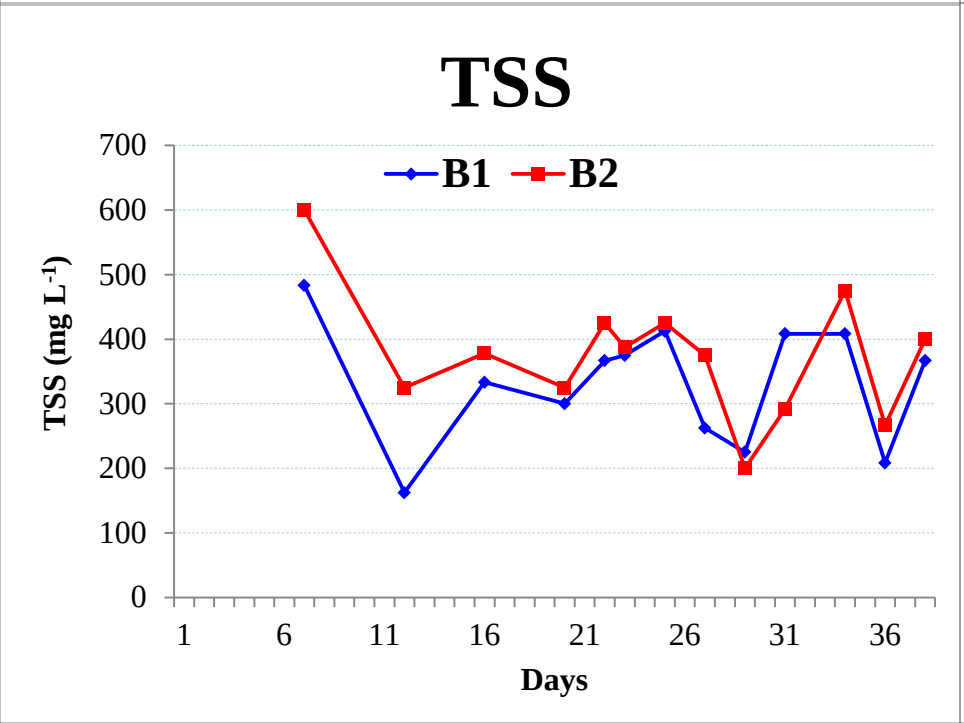
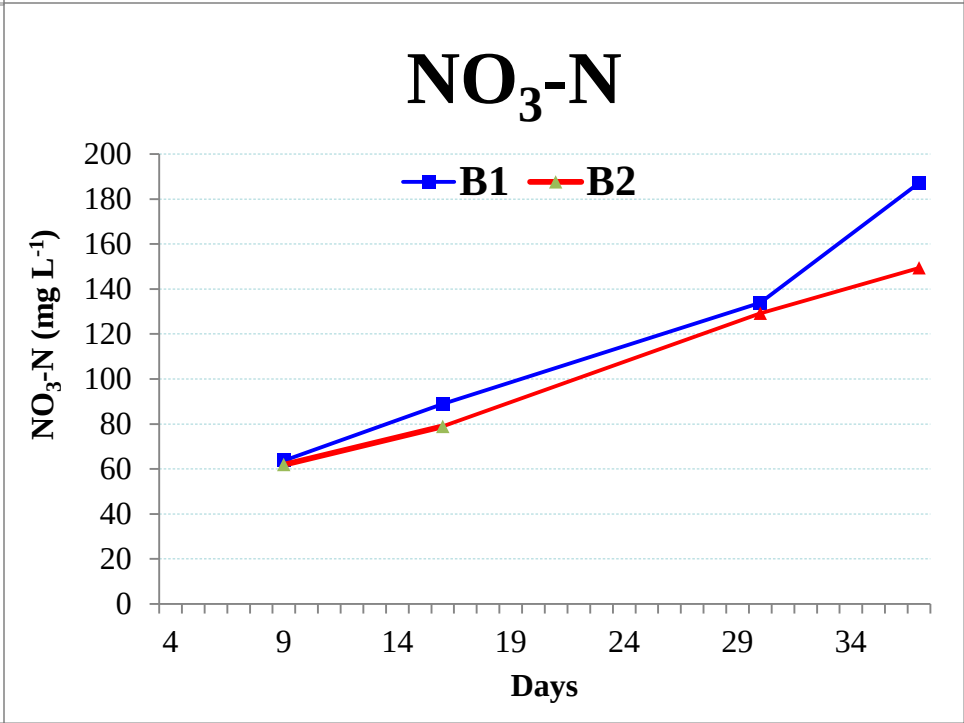
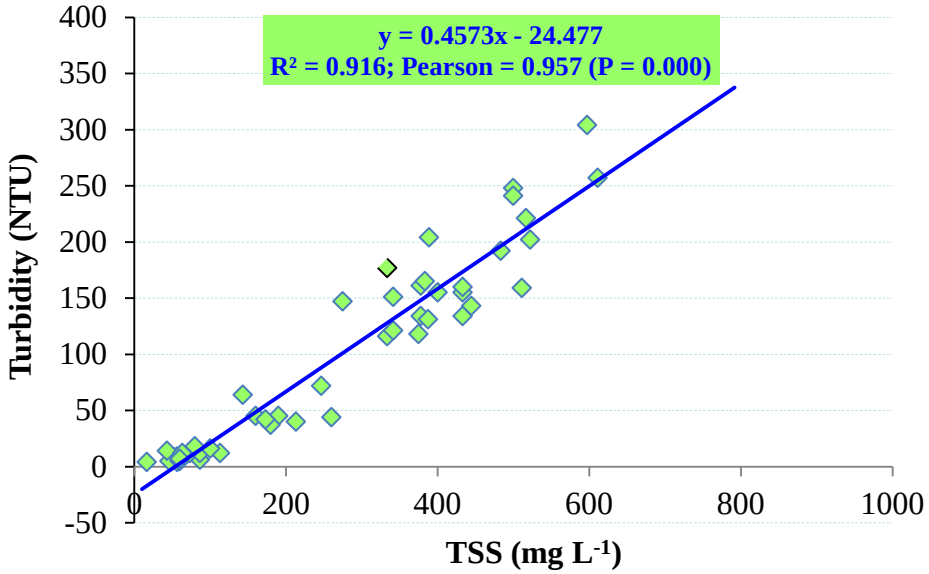

TAN
B1
B2
TAN (mg L-1)
3
2.5
2
1.5
1
0.5
0
4 7 9 10 13 14 15 16 18 21 23 25 27 30 34 37
Days


NO2-N
B1
B2
NO2-N (mg L-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
4 9 14 19 24 29 34
Days

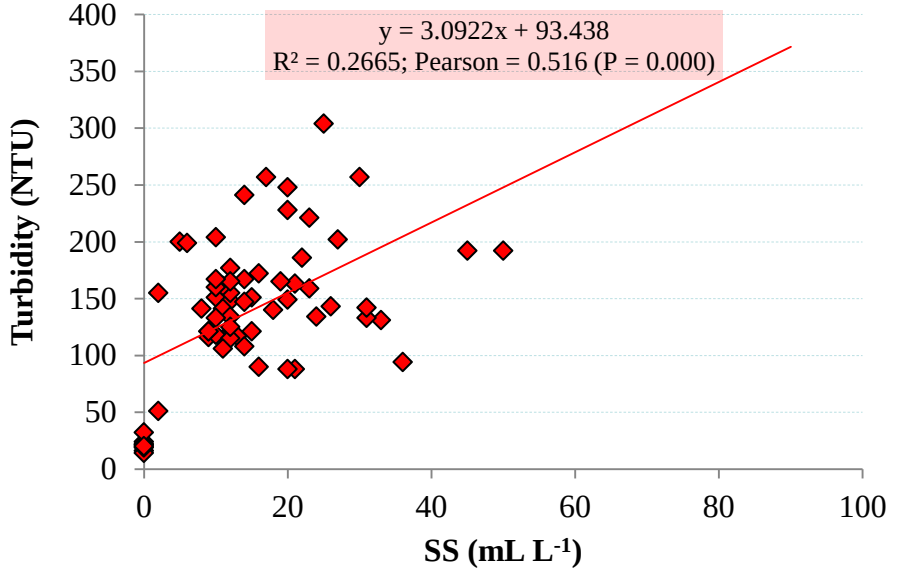

TSS
B1
B2
TSS (mg L-1)
700
600
500
400
300
200
100
0
1 6 11 16 21 26 31 36
Days


NO3-N
B1
B2
NO3-N (mg L-1)
200
180
160
140
120
100
80
60
40
20
0
4 9 14 19 24 29 34
Days

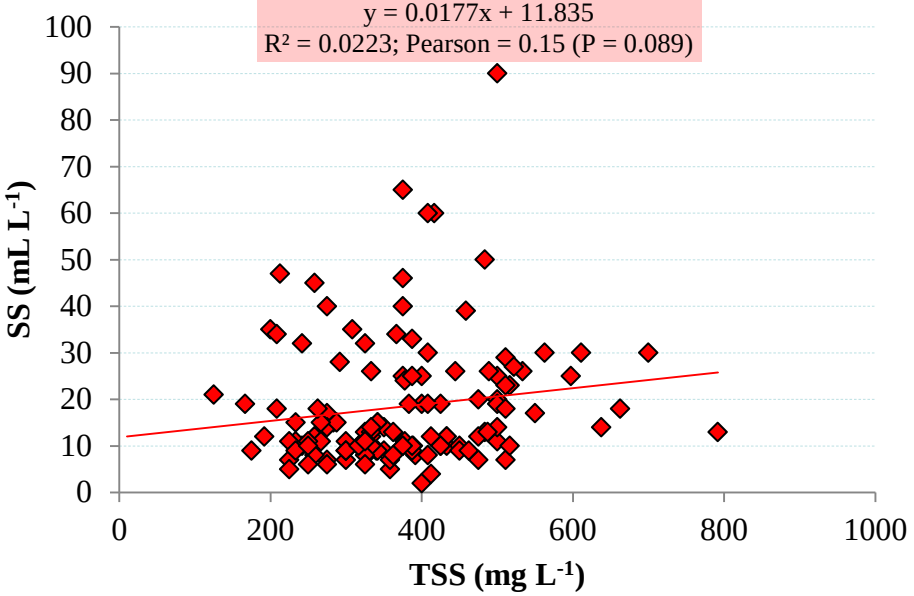
TSS / Turbidity



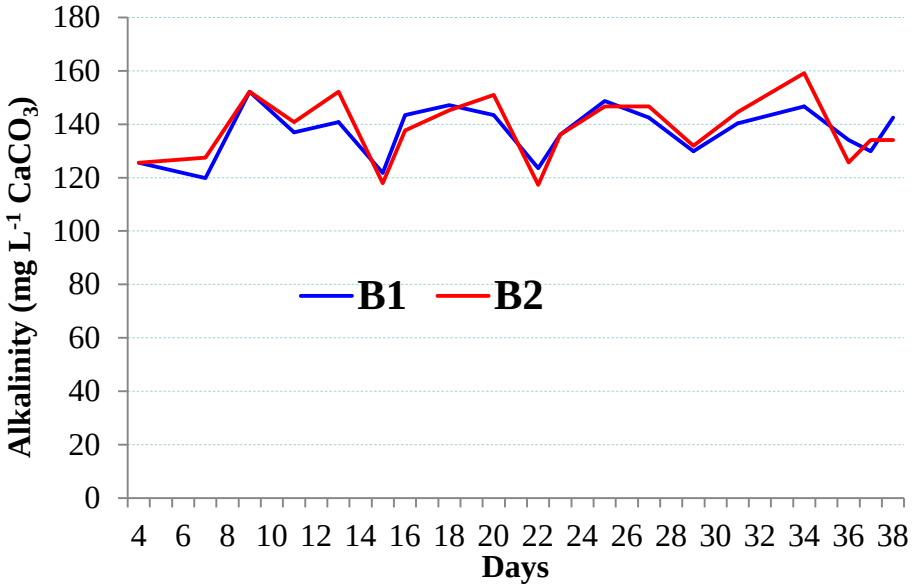
SS / Turbidity



TSS / SS



Alkalinity



Results

- Shrimp stocked in B1 experienced about 20% mortality prior to stocking because of low DO, high water temperature, and high TSS levels when harvested juveniles were stocked in a small tank
- A new wave of shrimp mortality started in B1 7 days after stocking quickly spreading to B2
- Mortality rate increased over time forcing early termination of the study

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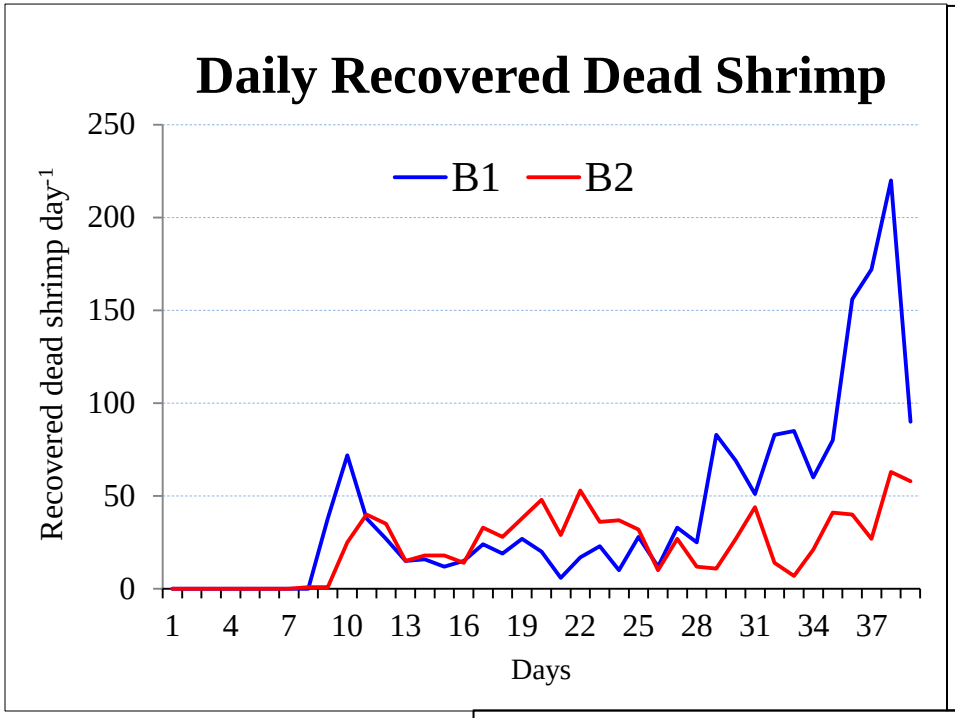
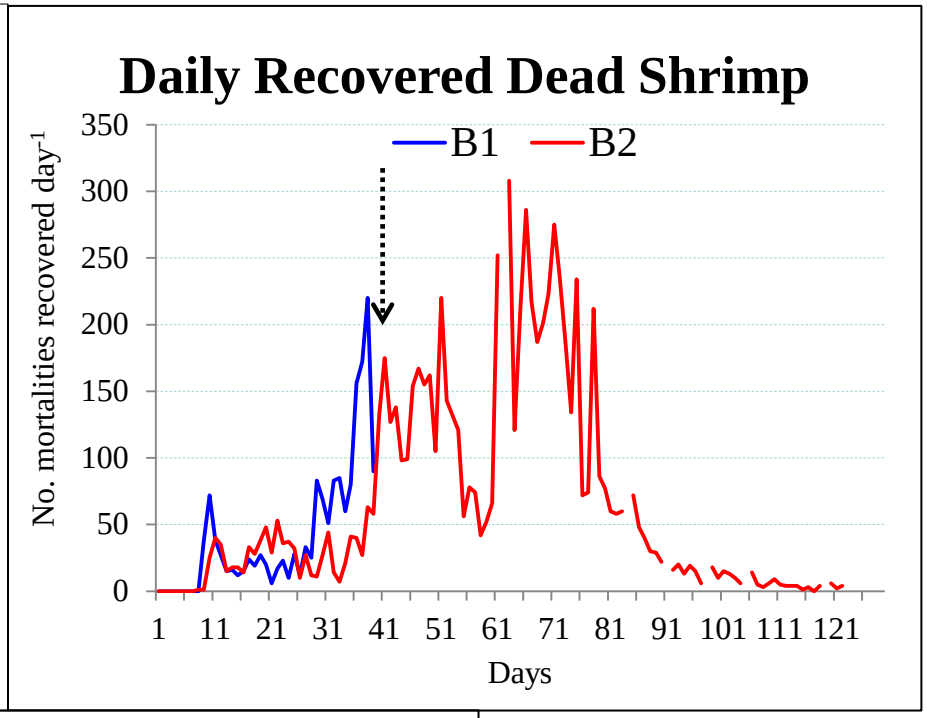
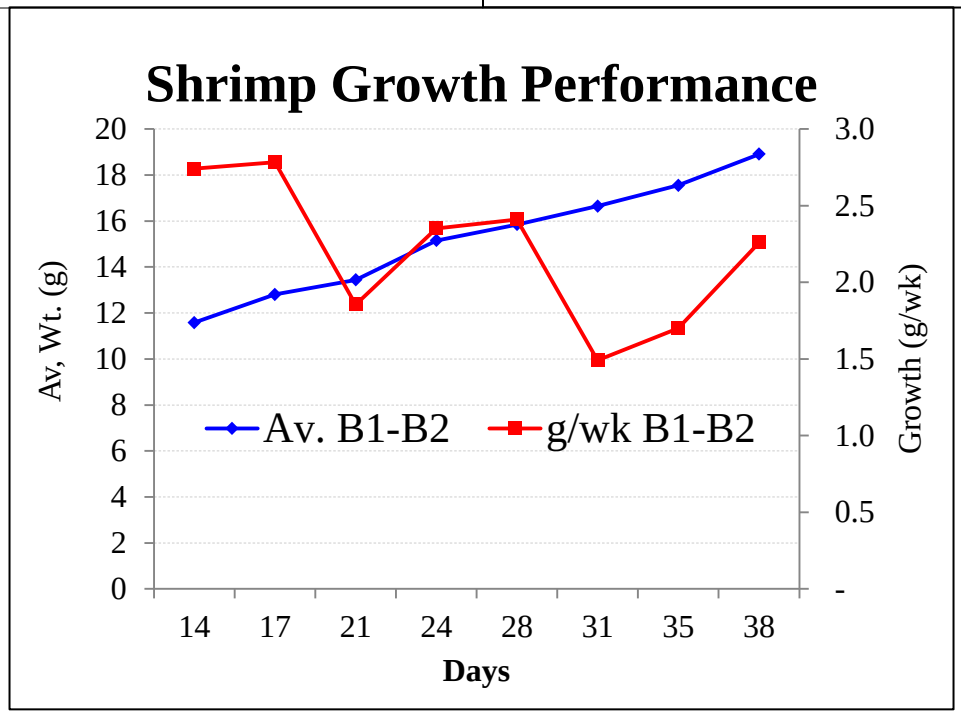
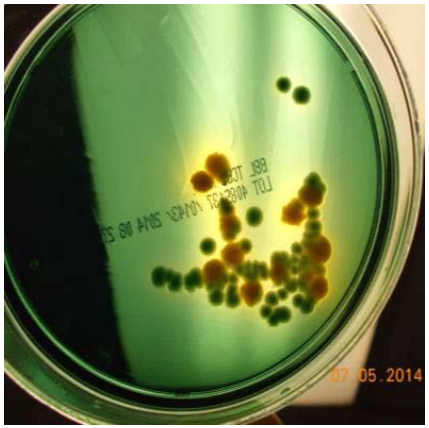

Daily Recovered Dead Shrimp
Recovered dead shrimp day-1
250
200
150
100
50
0
B1
B2
1 4 7 10 13 16 19 22 25 28 31 34 37
Days


Daily Recovered Dead Shrimp
No. mortalities recovered day-1
350
300
250
200
150
100
50
0
B1
B2
1 11 21 31 41 51 61 71 81 91 101 111 121
Days


Shrimp Growth Performance
Av, Wt. (g)
20
18
16
14
12
10
8
6
4
2
0
3.0
2.5
2.0
1.5
1.0
0.5
-
Growth (g/wk)
Av. B1-B2
g/wk B1-B2
14 17 21 24 28 31 35 38
Days

Results

- Green-colony forming *Vibrio* concentrations increased in both RWs from Day 4 on reaching 14,300 CFU mL^{-1}
- The total *Vibrio* concentration increased as the trial progressed, particularly in the final week, reaching up to 27,750 CFU mL^{-1}



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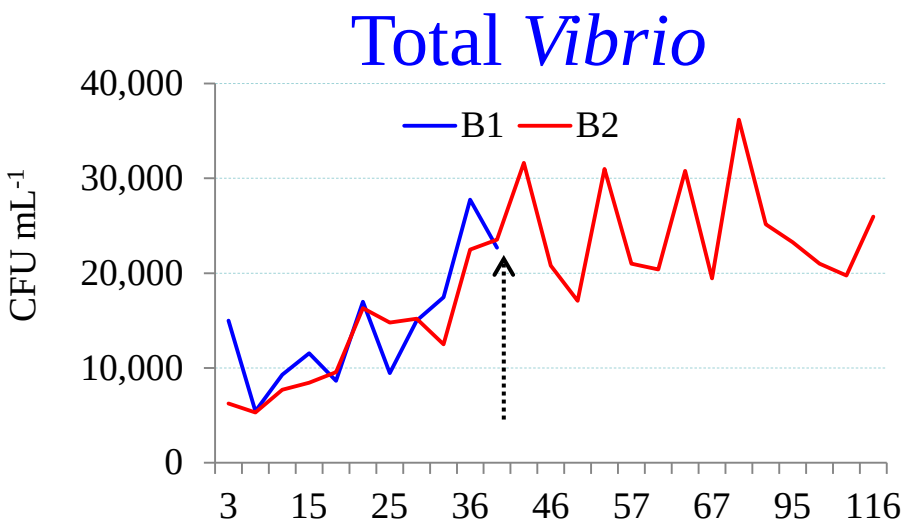
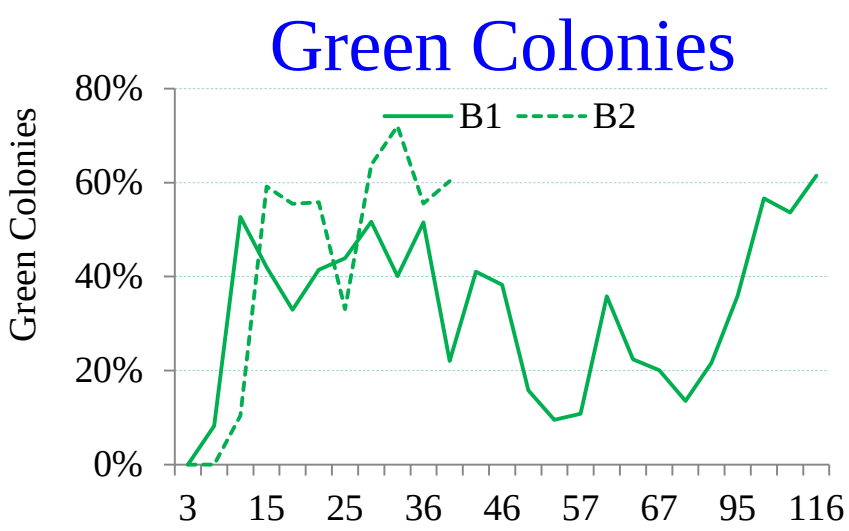
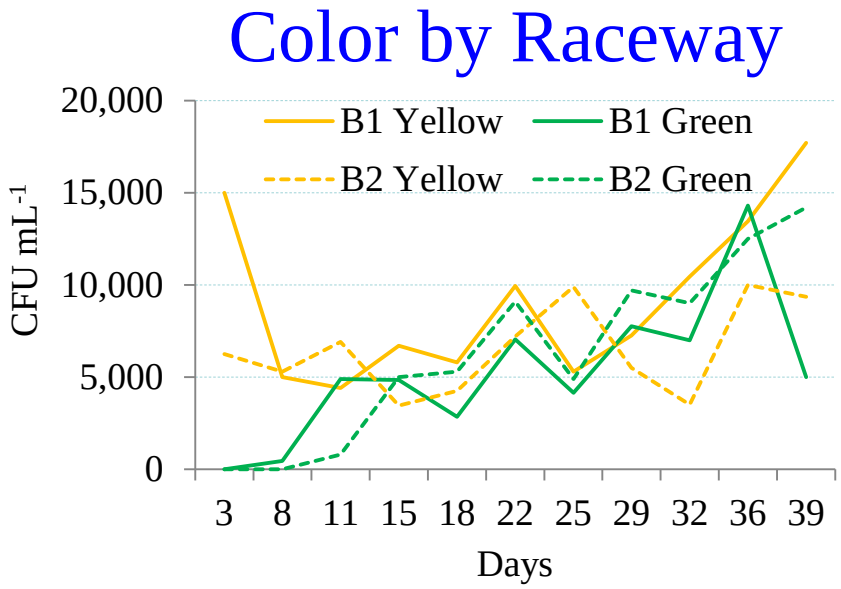
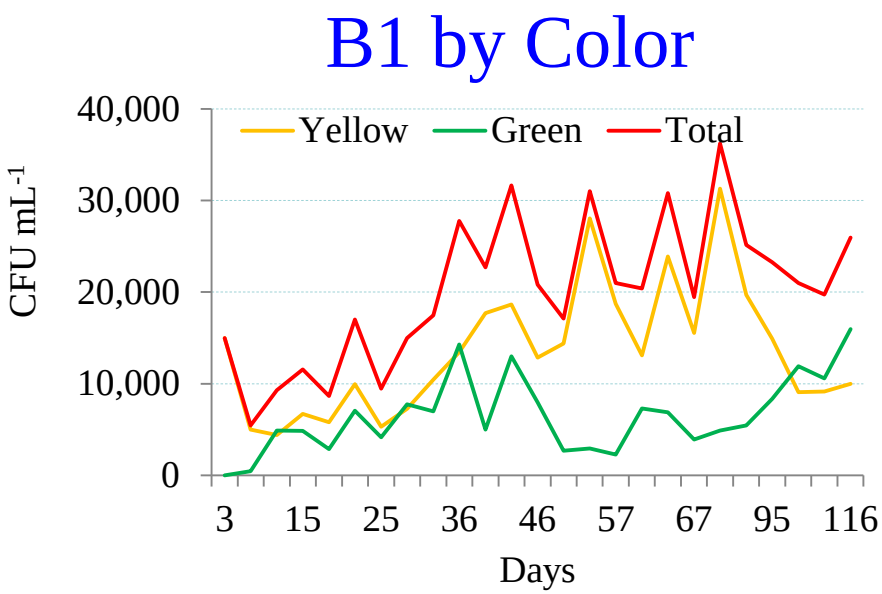
Vibrio colonies in the 100 m^3 culture medium









Litopenaeus vannamei performance in a 38-day grow-out trial in two 100 m^3 RWs

	Raceway 1	Raceway 2
Survival (%)	79.50	71.6
Final weight (g)	18.37	19.01
Growth Rate (g/wk)	2.20	2.31
Yield (kg/m^3)	6.02	6.92
PER	1.25	1.59
FCR	2.07	1.61

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Conclusion

- The commercial probiotic may have suppressed *Vibrio* concentrations but did not prevent mortalities entirely
- Stressors such as low DO, high temp. & TSS should be avoided, especially during transfer, to reduce the risk of triggering pathogenic *Vibrio* outbreak
- Monitoring *Vibrio* is a useful tool for predicting disease outbreaks
- The study demonstrates the detrimental effect of pathogenic *Vibrio* on *L. vannamei* production in super-intensive, biofloc-rich water, and the need for more studies on prevention & control of *Vibrio* in these systems

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Acknowledgements

- *The National Sea Grant, Texas A&M AgriLife Research* for funding
- *Zeigler Bros.* for the feed & funding
- *YSI* for the DO monitoring systems
- *Keeton Industries* for the nitrifying bacteria
- *Aquatic Eco-Systems* for the foam fractionators
- *Colorite Plastics* for the air diffusers
- *Firestone Specialty Products* for the EPDM liner
- *Florida Organic Aquaculture* for funding
- *a^3 All-Aqua Aeration* for providing the injectors