PRELIMINARY CHARACTERIZATION OF MICROBIAL AND ALGAL COMMUNITIES IN A ZERO WATER EXCHANGE SUPER INTENSIVE SYSTEM FOR PRODUCTION OF FOOD-SIZE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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Introduction

- Disease outbreaks and nutrient-rich outflow are significant issues to overcome for shrimp aquaculturists world-wide
- Heterotrophic dominated super-intensive growth systems may alleviate these issues
- Maintained biofloc may:
  - Prevent proliferation of pathogenic bacteria
  - Supplement feeding regimes
  - Maintain water quality

Objectives

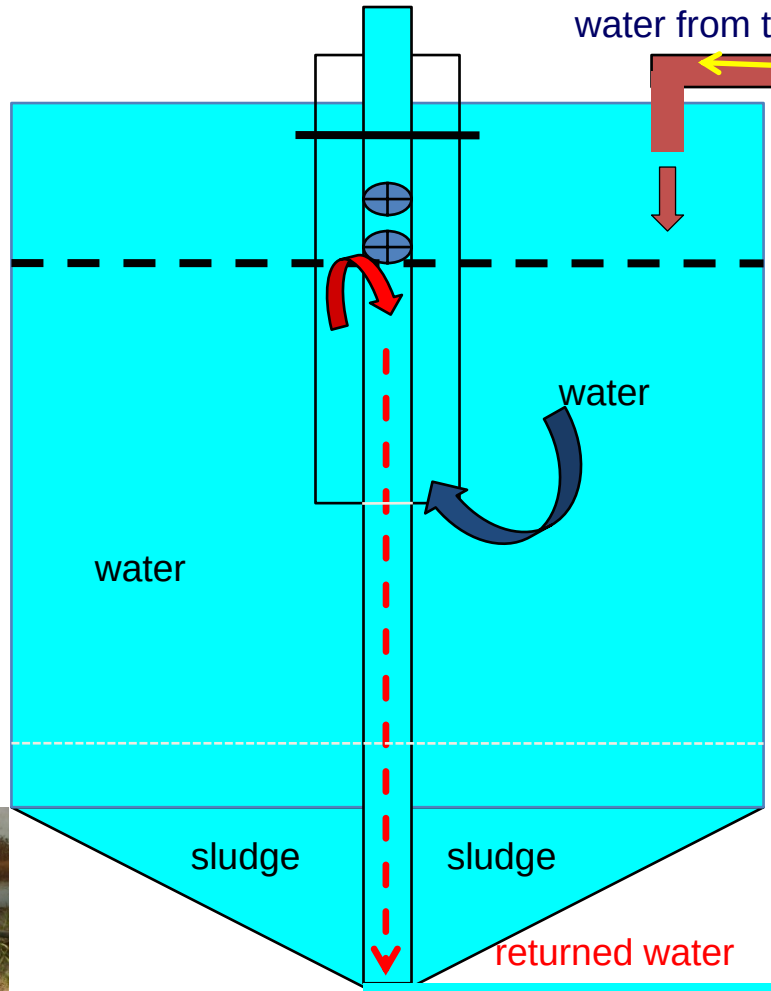
- Evaluate foam fractionation (FF) and settling tanks (ST) as methods to control particulate matter using bacterial and algal communities as indicators
- Determine the efficacy of flow cytometry (FCM) to monitor changes in bacterial and algal communities
  - Algal = auto-fluorescence
  - Bacteria = novel gram staining procedure

Methods

- Juveniles (0.99 ± 0.17 g) of *Litopenaeus vannamei* stocked (450 m^{-3})
- 4 ethylene propylene diene monomer (EPDM) lined raceways (RWs) (40 m^3)
- Water previously used in 62-d nursery trial
- 5.1 cm airlift pumps, 0.9 m long diffusers, and a longitudinal partition throughout the center line
- 5.1 cm PVC pipe with spray nozzles fed by a Venturi injector powered by a 2 hp pump
- Two RWs = small commercial FF
- Two RWs = 8.6 m^3 ST (4.9 m^3 water volume)
- Growth period = 108-d

Raceway

Settling Tank



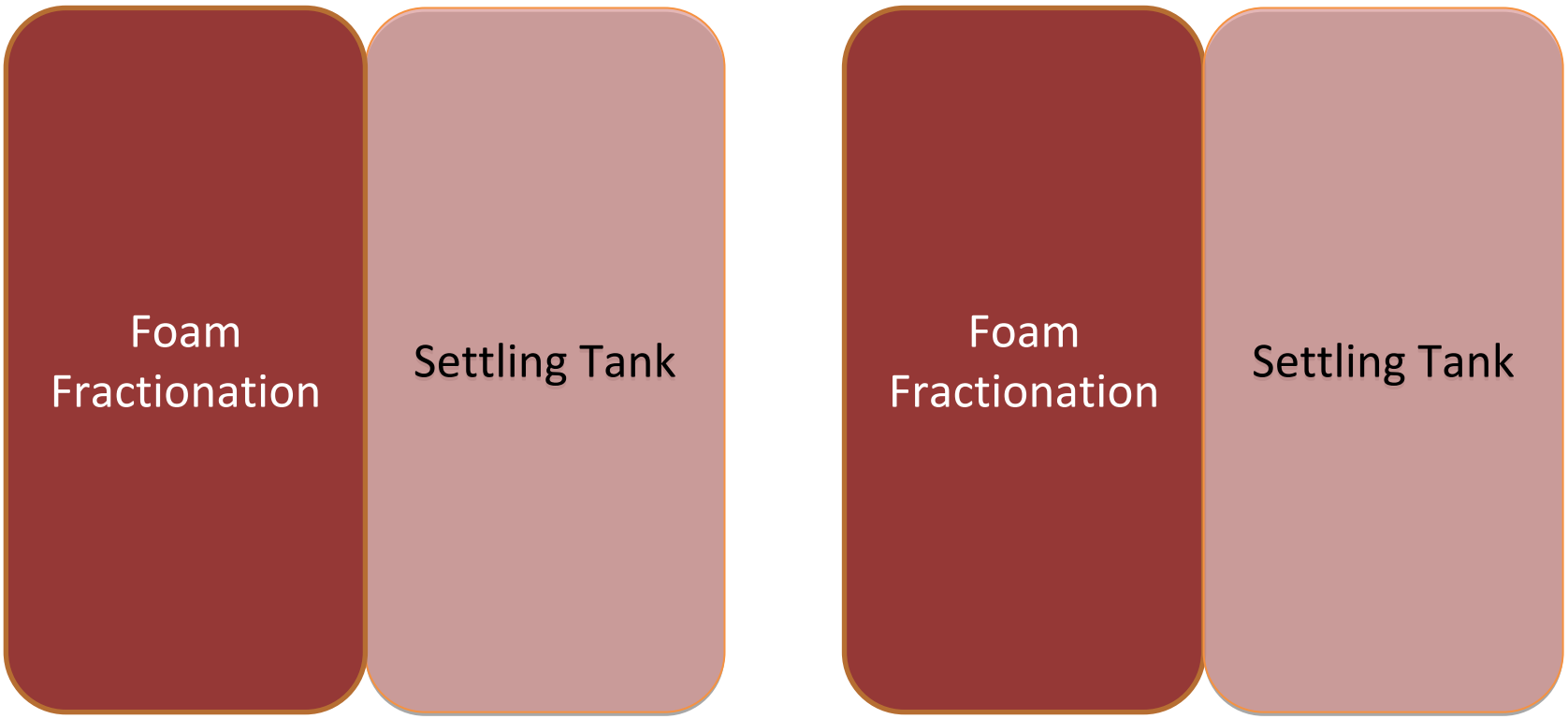
Foam Fractionation

Methods

- Temp., salinity, dissolved oxygen and pH were monitored at least twice daily
- Alkalinity, settleable solids, turbidity, algal counts, TSS, VSS, $cBOD_5$, NO_2-N, NO_3-N, and reactive phosphorus were monitored weekly
- Samples were collected weekly to characterize algal communities and gram negative/positive bacteria
- Each raceway was equipped with YSI 5200 multi-parameter monitoring system to provide continuous DO, and Temp. readings

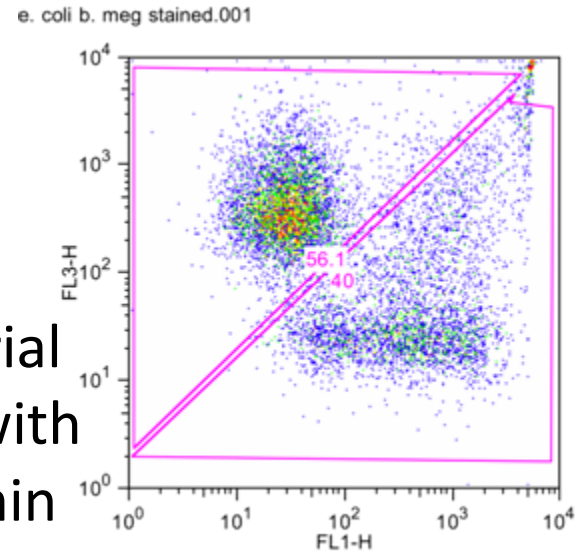
Experimental Design

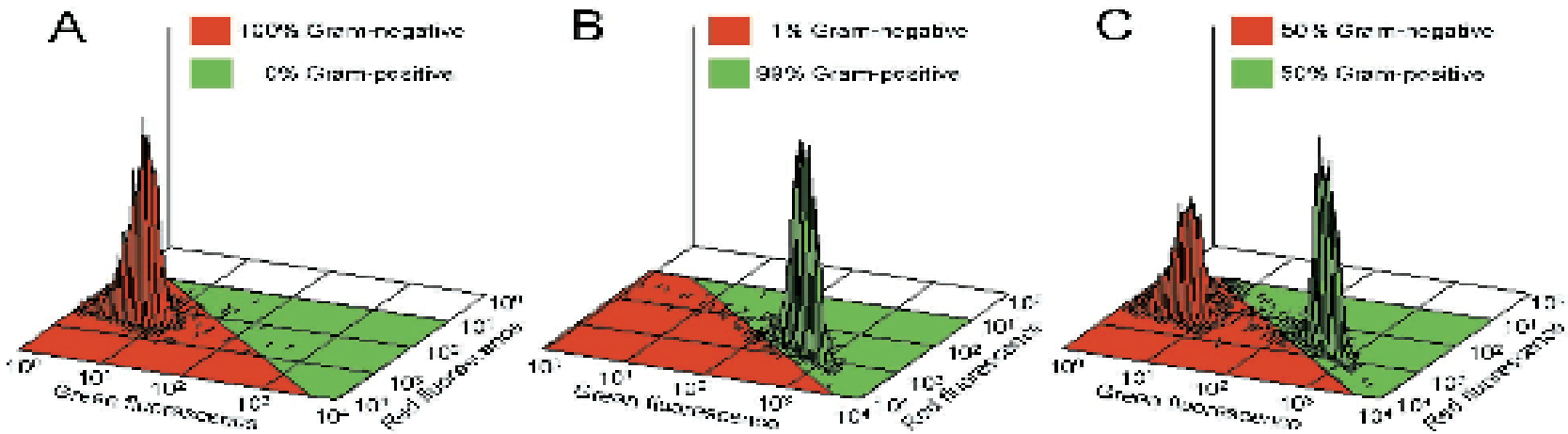
- Particulate control method randomly assigned to 4 raceways



Methods

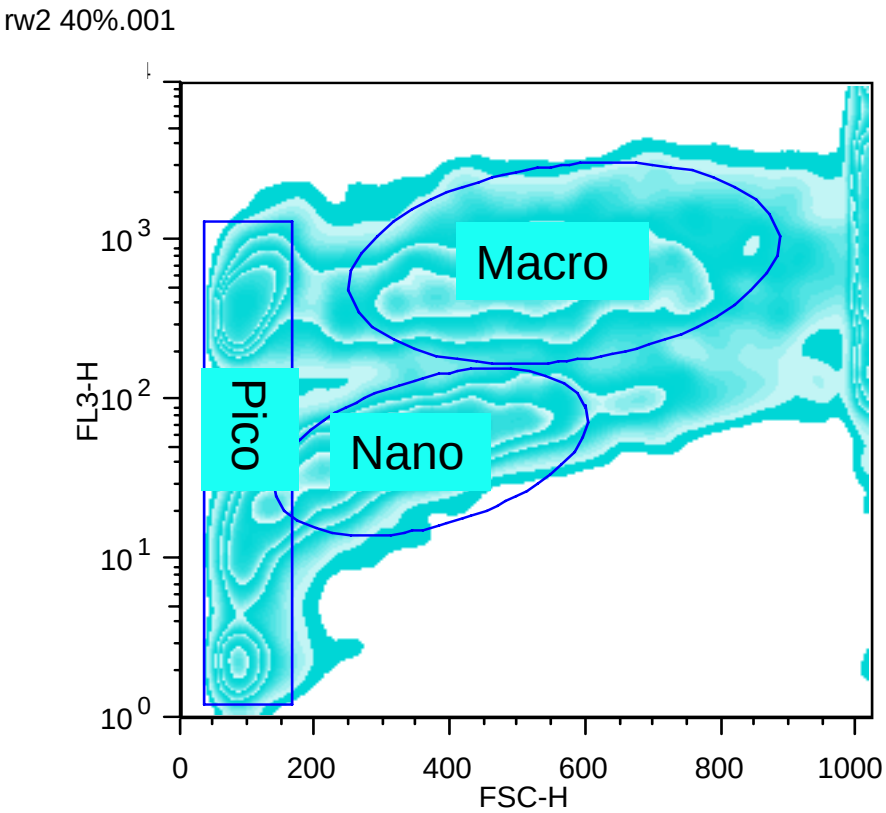
- Bacterial FCM Gram Typing
  - Holm and Jespersen, 2003
    - **Hexidium Iodide** = binds to all bacterial DNA following cell permeablization with EDTA and incubation at 50 C for 15 min
    - **Oregon-green conjugated wheat germ agglutinin** = binds to gram (+) cells when incubated at room temp. with 3 M potassium chloride

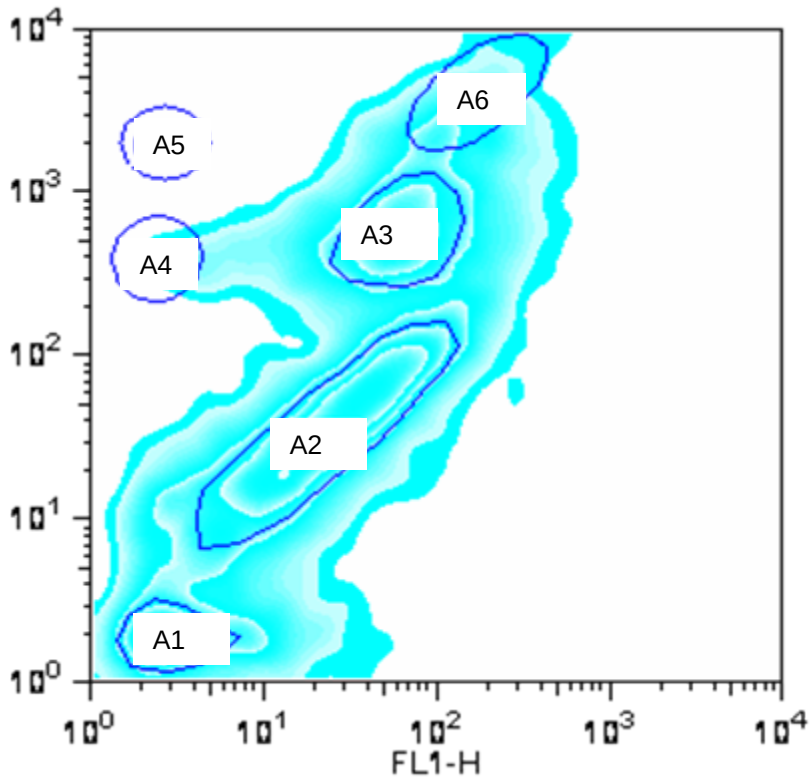




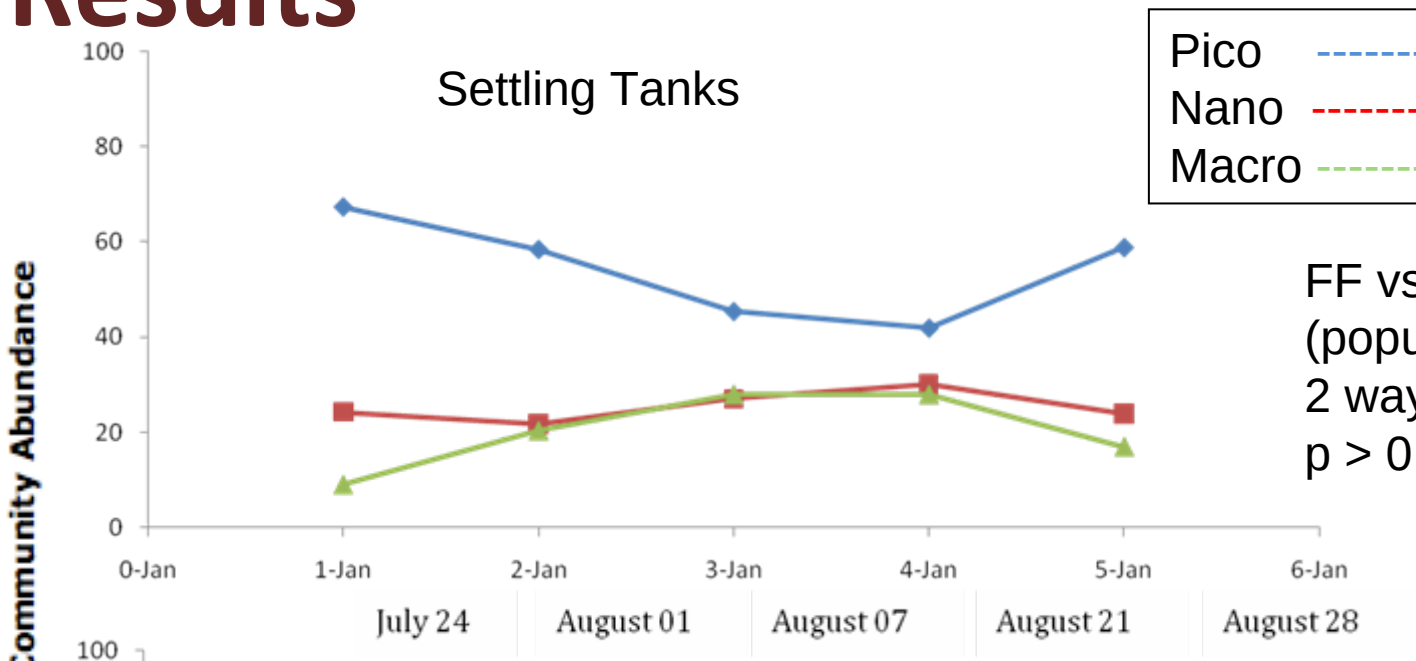
Methods

- Algal Community Characterization
  - Auto-fluorescence and size

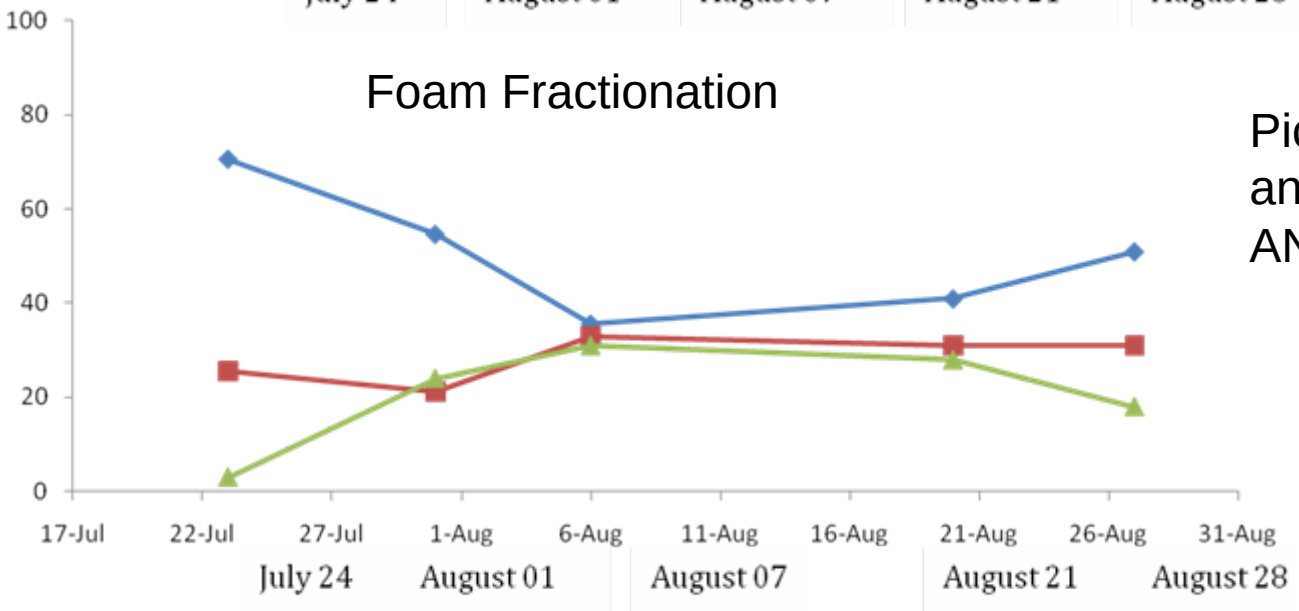




Results



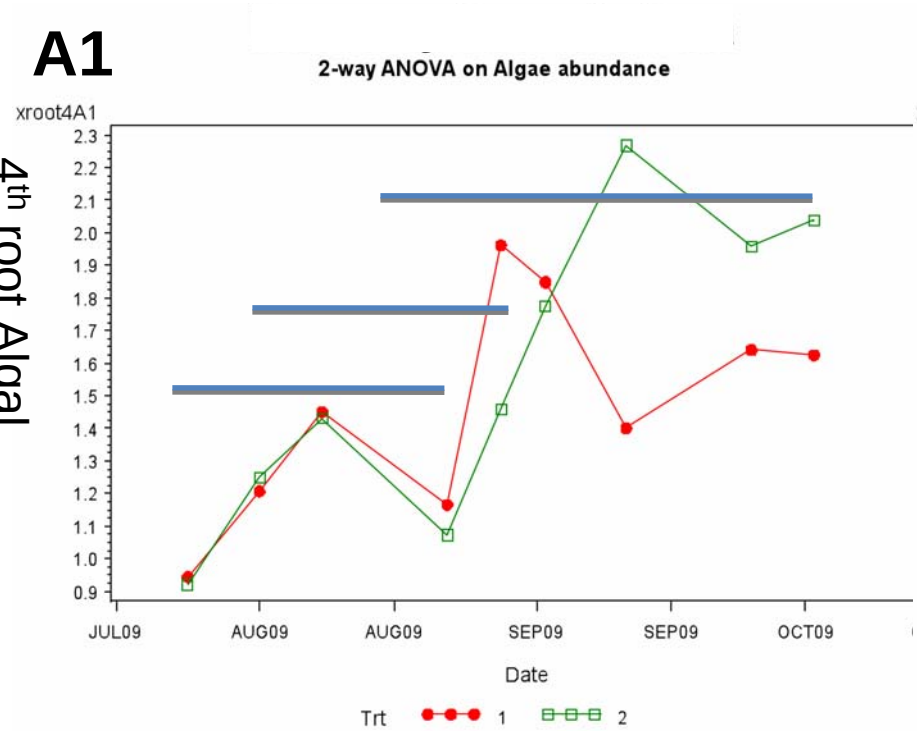
FF vs. ST (populations)
2 way ANOVA
$p > 0.05$



Pico vs. Nano and Macro
ANOVA $p < 0.05$

Results

A1



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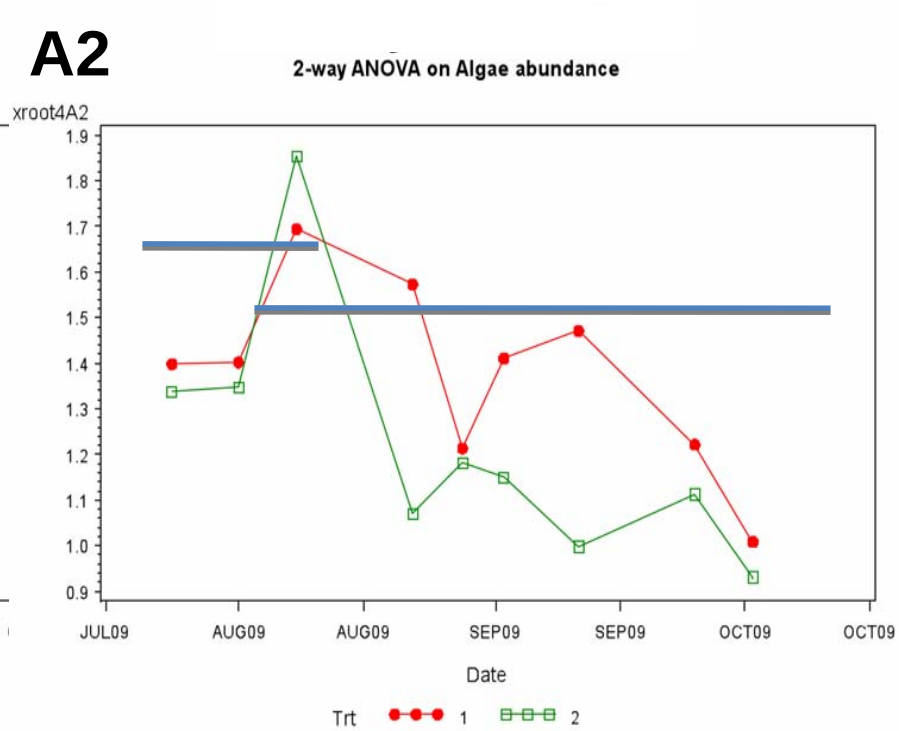
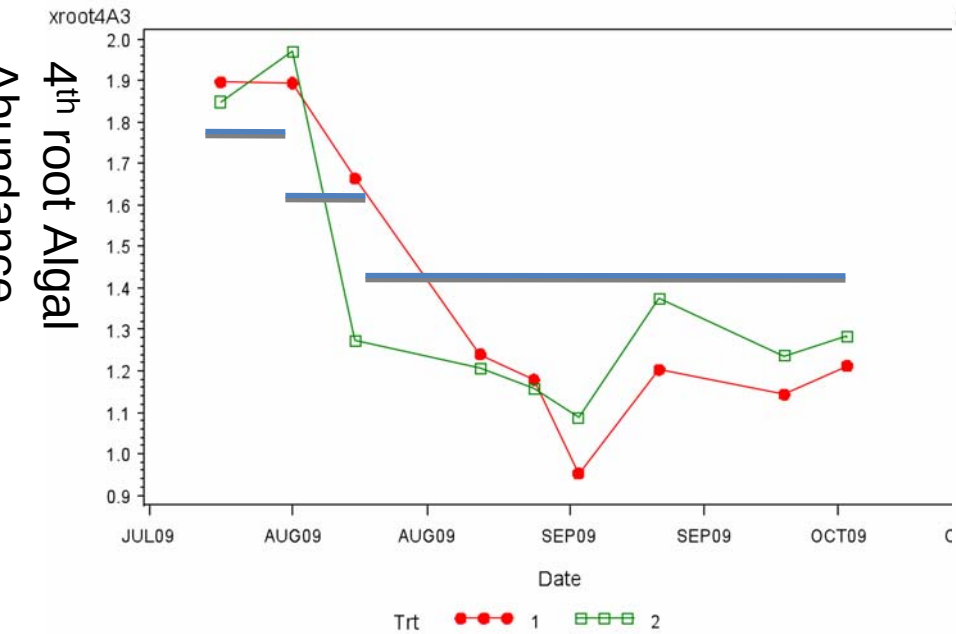


4th root Algal Abundance

Results

A3



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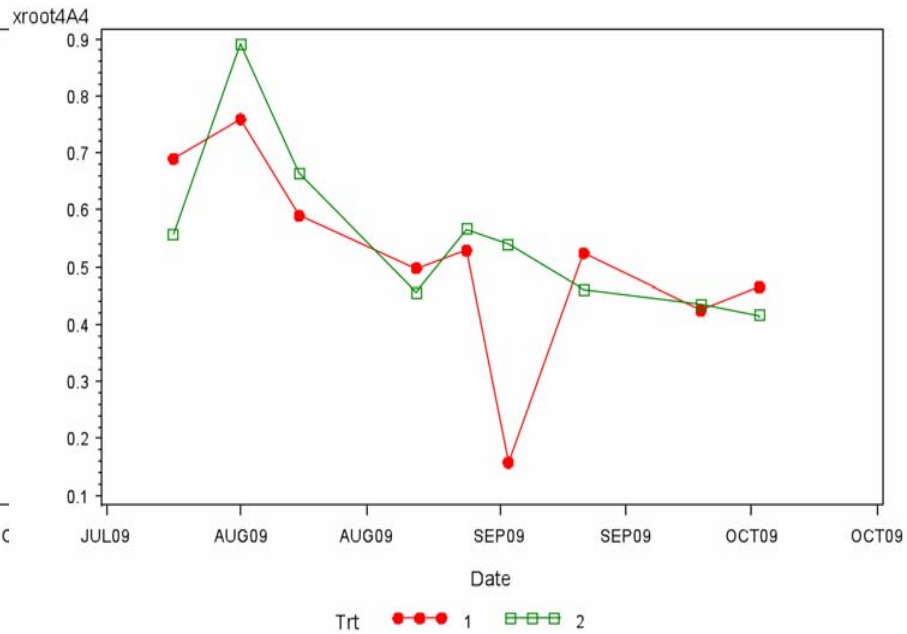
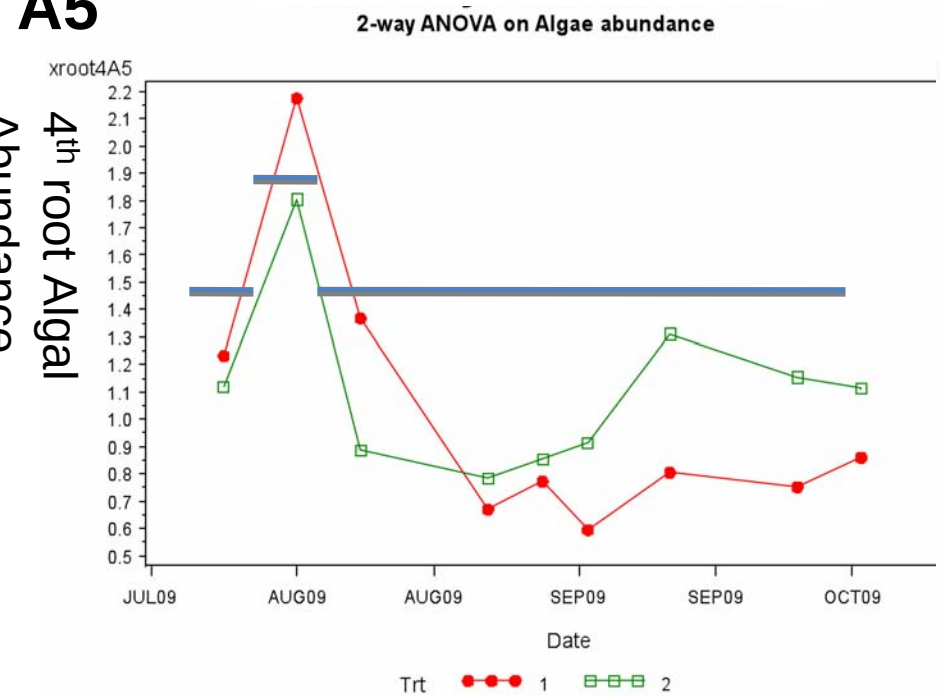


Results

A5



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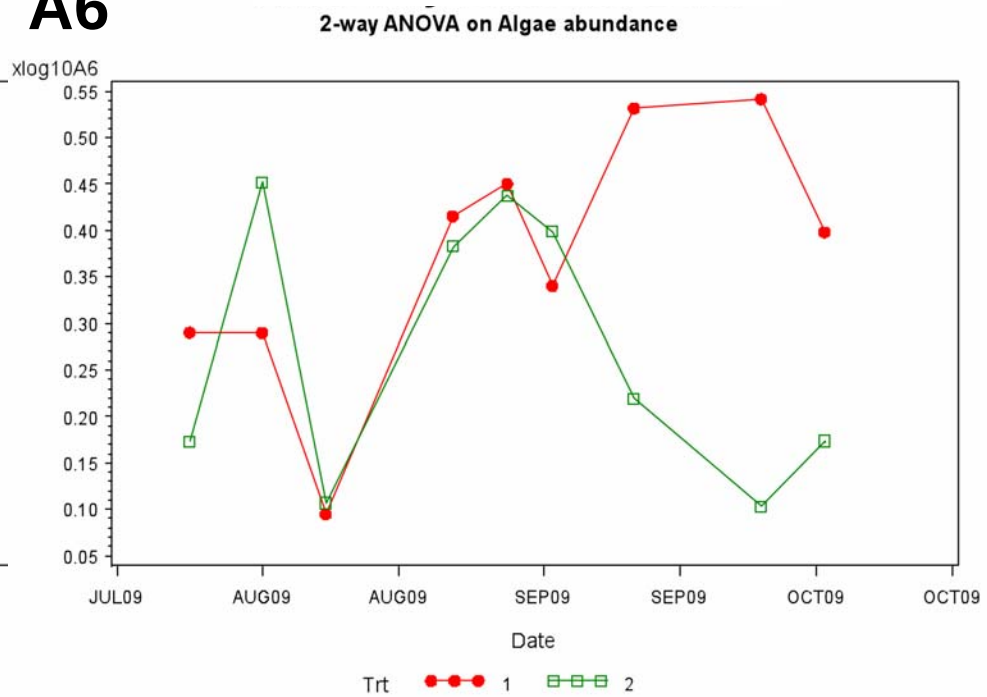
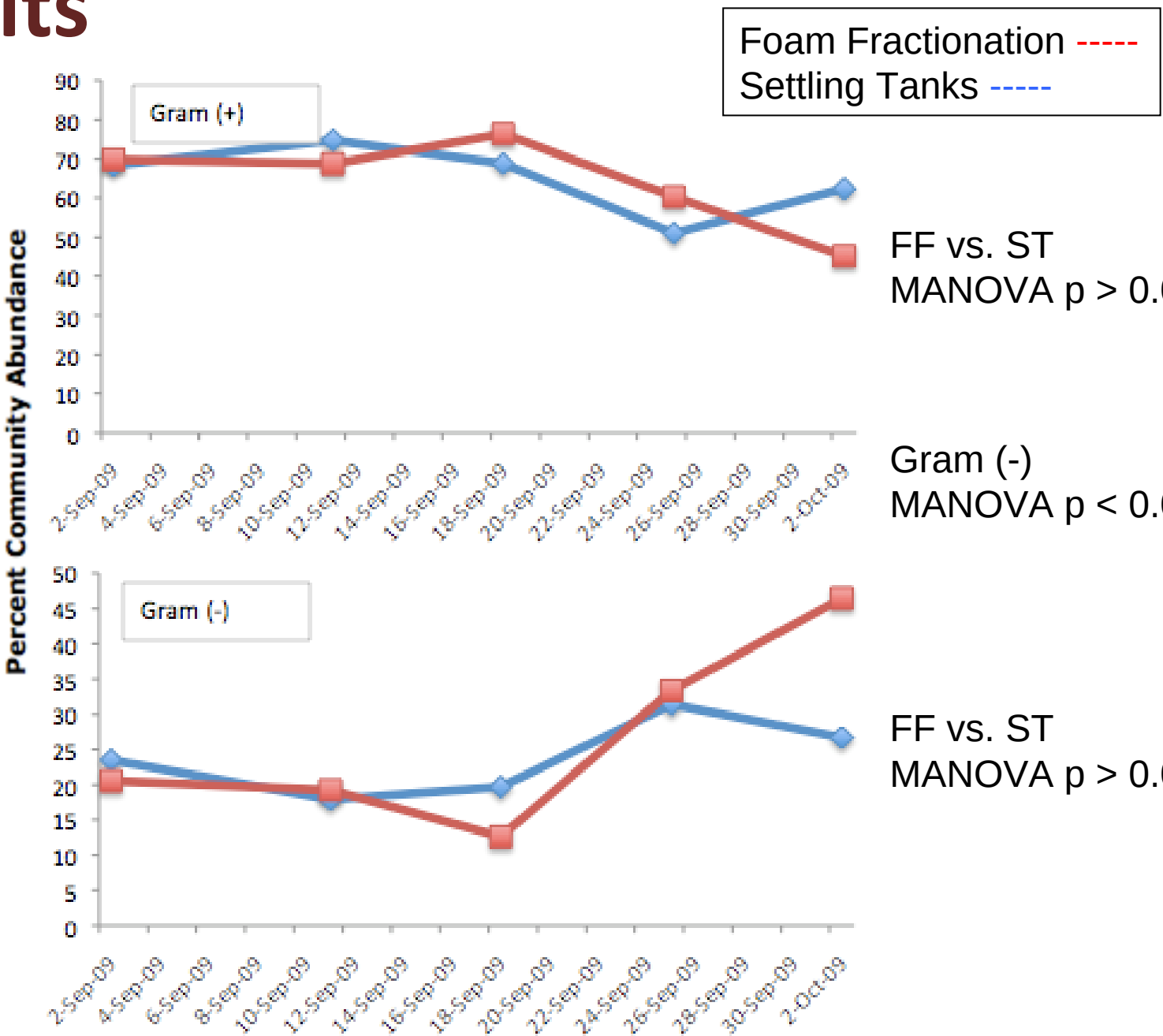


Results



FF vs. ST
MANOVA $p > 0.05$

Gram (-)
MANOVA $p < 0.05$

FF vs. ST
MANOVA $p > 0.05$

Discussion

- Bacteria and algae both beneficial and possibly harful to super-intensive no-exchange shrimp production
- Prevention/monitoring of water quality (i.e., biofloc maintenance) paramount
  - Flow cytometry quick and rapid option for the detection of gram (+) and gram (-) bacteria
    - Gram (-) increase over time
  - Flow cytometry allows for detection of algal population changes (auto-fluorescence detection)
  - Difficult to determine positive/negative impacts of population shifts

Discussion

- Importance of FF vs. ST negligible
  - Each appear to prevent substantial pathogenic gram (-) bacterial proliferation

ID	Yield (kg/m^3)	Av. Wt. (g)	Sur. (%)	FCR	(g/wk)	Freshwater (%/day)	L/kg Shrimp	O_2: last 7 d (L/min)
ST1	9.34	21.96	94.5	1.60	1.36	0.28	126	0.19
ST2	9.52	21.81	94.5	1.57	1.39	0.27	107	0.16
FF1	9.51	22.51	96.9	1.53	1.35	0.24	108	0.36
FF2	9.75	22.40	96.3	1.57	1.39	0.22	98	0.19

Future Direction

- Larger replicate experimentation for strong statistical analysis
- Identification of proliferating gram (-) bacteria/possible pathogenic species and correlation to environmental parameters
  - Principle component analysis
  - Correlate biofloc and water quality parameter adjustments to shrimp yield

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- YSI: DO monitoring systems
- Aquatic Eco System: Foam fractionators
- Firestone Specialty Products: RWs EPDM liner
- Colorite Plastics: Air diffusers
- Zeigler Bros: Feed