Management of Indoor Shrimp Culture in Biofloc Based Systems

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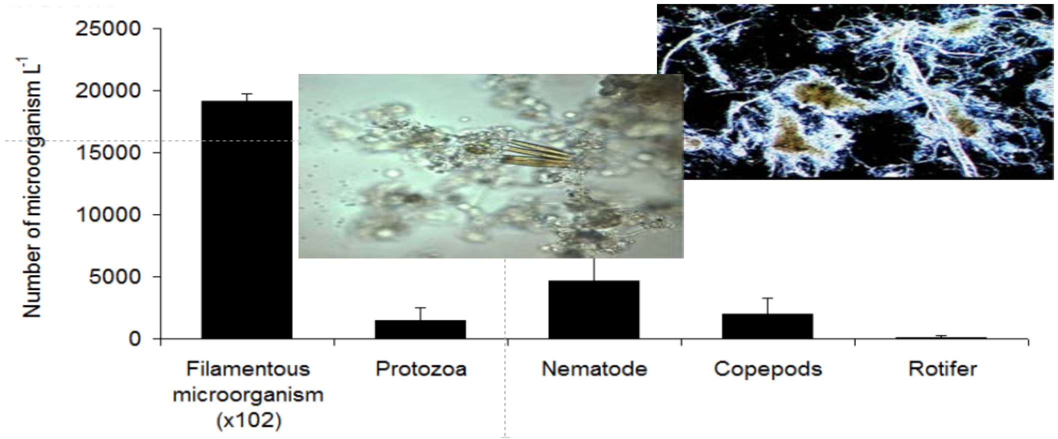
Indoor Shrimp Farming Workshop – September 14-15, 2018, Kentucky State University, Frankfort, Kentucky

Biofloc-dominated Systems

- Previous reports showed the feasibility of producing high shrimp yields in no water exchange in biofloc-dominated systems
- Biofloc is assemblage of living (bacteria, algae, cyanobacteria, fungi, protozoans) & non-living components (uneaten feed, waste products)



Emerenciano et al., 2011 WAS Natal, Brazil

System Management – Major Factors

- Seedstock
- Water Source & Salinity
- Dissolved Oxygen & Water Mixing
- Water Temperature
- Nitrogen Species: TAN, NO_2, NO_3 & Denitrification
- pH, Alkalinity & Particulate Matter
- Growth Monitoring, Feed & Feed Management
- Waste Disposal
- Alarm Systems & Power Backup
- Natural Light & Microalgae
- Biosecurity, Probiotics & Diseases

System Management

Seedstock

- Quality -- Health, PL Age & Average Weight
- Transportation Options
- Acclimation

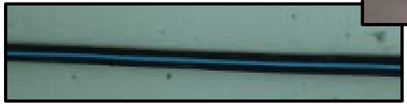
- SPF & SPR -- Fast Growth *vs* Taura Resistant
- PL Size (Length *vs* Wt.) & Feed Management

Water Source & Salinity

- Preparation (Filtration, Disinfection, Probiotics)
- NSW *vs* ASW
  - Ionic Balance
  - Salinity Impact on Nitrogen Species Toxicity

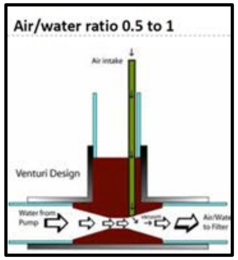
System Management

***Dissolved Oxygen & Mixing** – Available Options*

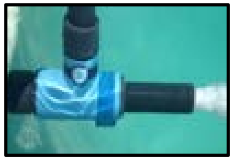


- ***Blower-driven:*** Air Diffusers, Air Stones & ALP - Fouling issues & Expected yields
- ***Pump-driven:***

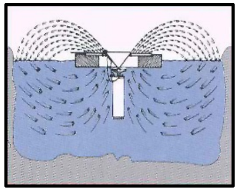
  Venturi Injectors -- Can serve to mix air, pure oxygen & to add chemicals; Can support yields of about 6 kg/m^3 when operated with atmospheric air under no water exchange

  a^3 Injectors: -- Excellent oxygenation & mixing capacity; Support yields of > 9 kg/m^3 in no water exchange when using atmospheric air only

  Fountain Type Aerators – be aware of potential shrimp injuries

System Management

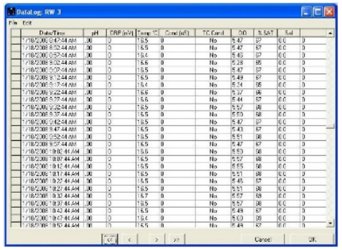
Use of Pure Oxygen:

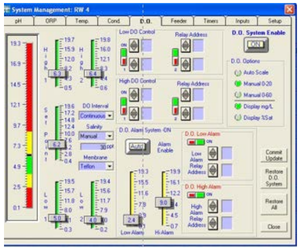
High-density no exchange biofloc systems with shrimp load > 4 kg/m^3 have high oxygen demands; Availability of pure oxygen on-site is highly recommended in case of emergency (power failure, algal bloom crash, overfeeding, excessive dose of organic carbon, etc.)

System Management

Online Oxygen Monitoring System:

Helps maintain optimal DO & minimizes shrimp stress and/or crop losses - Extremely important tool in no-exchange, high-density, biofloc systems - Need for a dependable system that alerts operators of low DO & implements corrective measures Optical DO sensors can withstand exposure to heavy fouling -- The monitoring system software can be programmed to send information to multiple sites via land line, cellphone, or the internet





System Management

Water Temperature:

- Needs & Implications
  - Optimal Range
  - Tank Design (insulation, in ground, cover)
  - Structure Insulations – impact on energy use
- Passive Control -- Greenhouses, Inside/Outside Air Supply, use of Shade Cloth
- Active Control -- Heat Exchangers, Space & Submersible Heaters, Maintenance Requirements

System Management

Nitrogen Species: TAN, NO_2, NO_3 & Denitrification

- Be aware of low salinity on nitrogen species toxicity
- It is common to express inorganic nitrogen compounds by their nitrogen content, e.g., NH_4^+-N (ionized ammonia-nitrogen), NH_3-N (un-ionized ammonia-nitrogen) -- *the sum of the two is oftentimes called total ammonia-nitrogen (TAN) or simply ammonia*, NO_2-N (nitrite-nitrogen), & NO_3-N (nitrate-nitrogen)

Ammonia

- A soluble end-product of protein catabolism excreted in un-ionized form (NH_3) which is toxic to shrimp -- *be aware of* your *test kit!*

System Management

Ammonia

- The concentration of each of the two forms is pH, temperature, & salinity dependent
- For example: at salinity of 30 ppt, pH 7.0, & temp. of 28^{o}C, less than 1% of the TAN is in the NH_3 (toxic) form compare to 87% in pH 10
- Can be removed by algal (micro & macro) photosynthesis, oxidation to nitrite & nitrate by autotrophic bacteria, & direct conversion to microbial biomass by heterotrophic bacteria

Ammonia Removal:

Heterotrophic Systems

$$NH_4^+ + 1.18\ \underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Alkalinity)}}{HCO_3^-} + 2.06\ O_2 \rightarrow \underset{\text{(Bacteria)}}{C_5H_7O_2N} + 6.06\ H_2O + 3.07\ CO_2$$

- *The increase in microbial biomass production is 40 times greater, consumption of O_2 & CO_2 production are higher than the nitrification process*
- If kept as *fully heterotrophic*, large efforts & resources are needed to maintain these systems
- To operate properly the systems require constant supply of organic carbon & large amount of oxygen

After Ebeling et al. (2006)

Ammonia Removal

- As demonstrated, heterotrophic bacteria incorporate ammonia-N directly into microbial biomass (e.g., no generation of NO_2 or NO_3)
- When using 35% CP feed, only about 1/3 of the dissolve organic carbon requires by the heterotrophic bacteria is available from the feed
- To assimilate all of the available ammonia-N, supplementation of dissolve organic C is needed
- ***To convert 1 g of TAN into heterotrophic bacteria biomass you need 6 g of organic carbon***

Use of Organic C for Ammonia Removal

The *Heterotrophic System*

Examples:

Assuming: 40 m^3 TK with 4 mg/L TAN in the water

Molasses (24%):

1,000 ml molasses = 1,300 g - 312 g C (1,300 x 24%)

TAN in the tank: 160 g (4 x 40,000)

Organic Carbon needed: 960 g (160 x 6)

Molasses needed: 3.08 L (960 / 312) or 4.004 kg (3.08 x 1.3)

White Sugar (43% C):

1 kg white sugar = 430 g C

White sugar needed: 2.23 kg (960 / 430)

Recommended Use of Organic Carbon

- To prevent high levels of ammonia in the culture tank *when nitrifying bacteria were not established*
- To avoid algal-dominated water in the culture tank
- Gradual application to prevent *sharp* DO decreases
- Operator must have the capacity to quickly increase DO in the culture tank

Ammonia Removal:

Autotrophic Nitrification Systems

$$NH_4^+ + 1.83\ O_2 + 1.97\ \underset{\text{(Alkalinity)}}{HCO_3^-} \longrightarrow 0.024\ \underset{\text{(Bacteria)}}{C_5H_7O_2N} + 0.976\ NO_3^- + 2.9\ H_2O + 1.86\ CO_2$$

- Require supply of ammonia, oxygen & inorganic C in the form of alkalinity & adjustments of pH

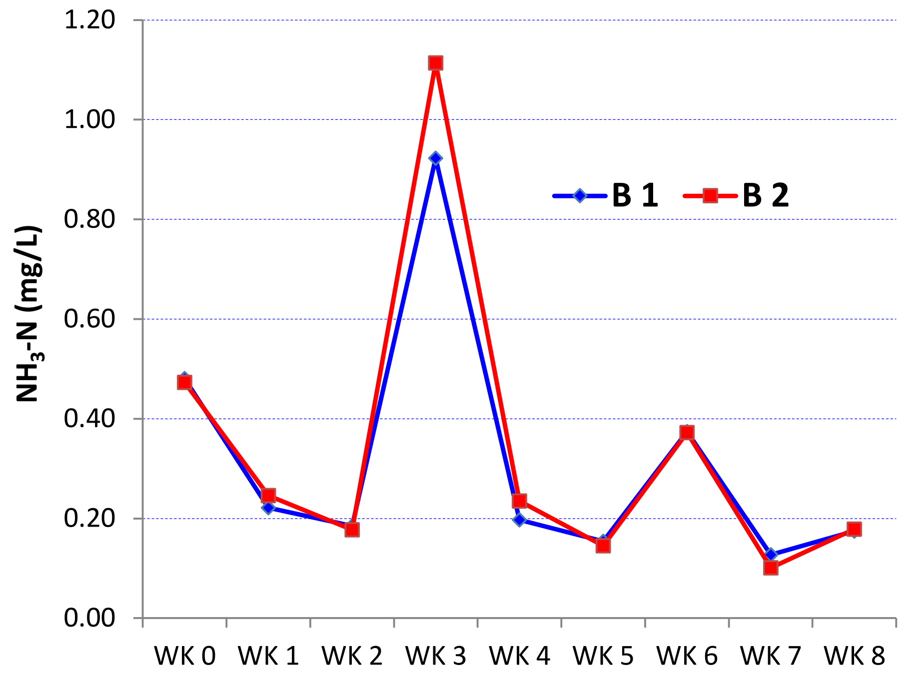
Targeting a *Mixotrophic State* in which:
1/3 of the bacteria are *Heterotrophic* & 2/3 *Autotrophic*

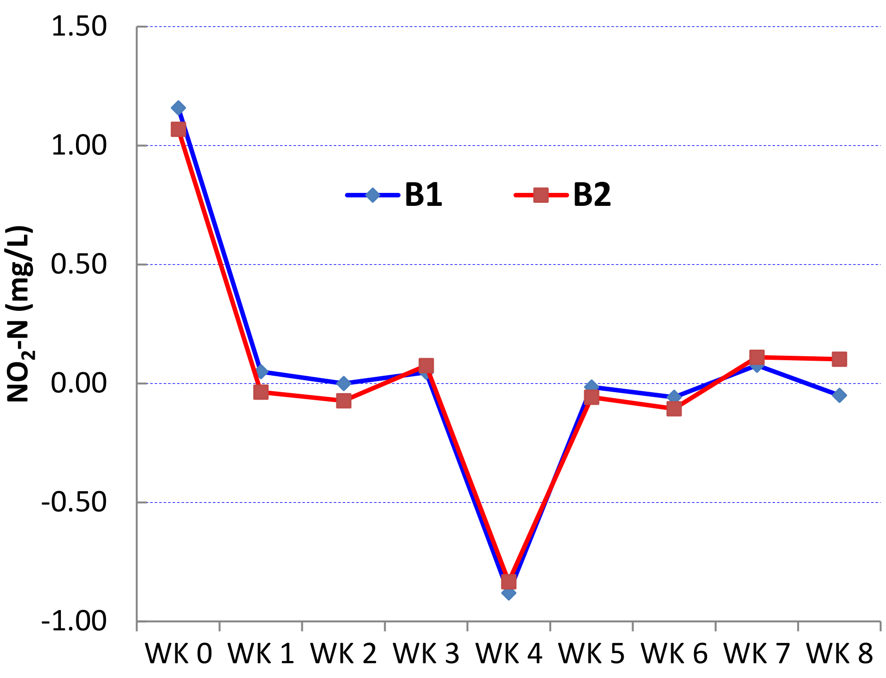
- When a *Mixotrophic State* is reached -- no organic carbon addition is required to control TAN & Nitrite
- Nitrification can be enhanced using commercial nitrifying bacteria (such as *FritzZyme 9*, *KI-Nitrifier*) and/or floc inoculation

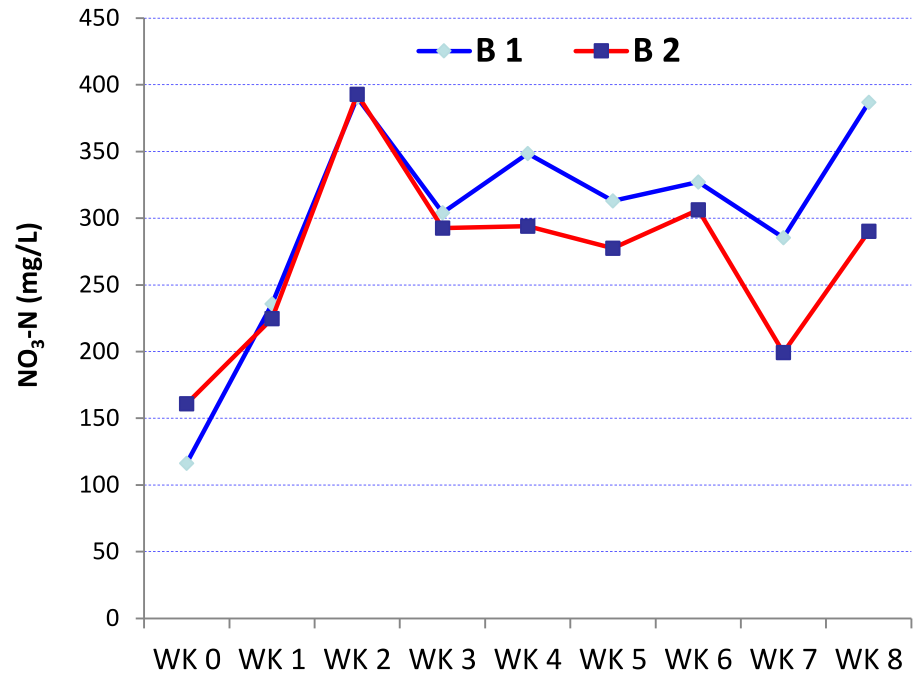
After Ebeling et al. (2006)

Nitrogen Species in *Litopenaeus vannamei* GO trial with 3.6 g juveniles stocked at 500/m^3

RW	Harvest (g)	Growth (g/wk)	Sur. (%)	Yield (kg/m^3)	FCR
1	22.76	2.13	80.2	9.20	1.43
2	22.67	2.12	78.2	8.86	1.53
Av.	22.72	2.12	79.5%	9.03	1.48







System Management

Nitrogen Species Toxicity

- Salinity Impact
  - Increased toxicity of ammonia, nitrite & nitrate with the decrease in salinity
  - Nitrite toxicity can be avoided either by maintaining a heterotrophic or mixotrophic systems
- No nitrate issue in heterotrophic system
- Nitrate build-up in a mixotrophic system requires removal

System Management -- Nitrate Removal

Denitrification

- A four-step anaerobic microbial process that chemically reduces NO_3 to N_2
  - NO_3 is reduced to NO_2, which then is reduced to N_2O with a final step of reduction to N_2
  - To avoid H_2S production it requires adequate source of organic C, redox potential (-50 to +50 mV), DO < 2 mg/L, NO_3 (10-50 mg/L), pH (7.0-8.5), & temp. (25-32^{o}C)
  - In a perfectly balanced system, most of the alkalinity lost during nitrification can be restored

System Management -- Nitrate Removal

Other Options:

Anammox (*anaerobic ammonium oxidation*)

- A process by which chemoautotrophic bacteria combine ammonium & nitrite under anoxic conditions to produce N_2

Photosynthesis

- Predominantly by macroalgae

System Management

pH, Alkalinity & Particulate Matter

pH

- Levels decrease over time
- Requires adjustments to maintain optimal microbial activities

Alkalinity

- Consumed mainly by nitrifying bacteria (use of 7.14 mg $CaCO_3$ for every 1 mg of TAN oxidized to NO_3-N
- Requires adjustment to maintain optimal nitrification activity

System Management - Alkalinity

- Chemicals that can be used to increase alkalinity: sodium & potassium bicarbonate ($NaHCO_3$ & $KHCO_3$), sodium carbonate (Na_2CO_3 - Soda ash), potassium & calcium carbonate (K_2CO_3 & $CaCO_3$ - Agricultural lime)
- Bicarbonates are the most effective, safe, & easy to dissolve, followed by Soda ash
- These chemicals are readily available & have a long shelf life - Na_2CO_3 is generally cheaper & more efficient (less is required to raise alkalinity) than $NaHCO_3$, but is more likely to form a precipitate in the water
- Some liming materials, such as CaO, $Ca(OH)_2$, & $CaMg(OH)_4$ - caustic, difficult to dissolve & can cause large increases in pH

System Management

Particulate Matter

- Biofloc systems are characterized by continued generation of particulate organic matter (POM) to include: feed leftover, feces & microbial biomass
- Monitoring & Control of POM are essential to maintain a healthy biofloc system
    - Implications of too high or low concentrations

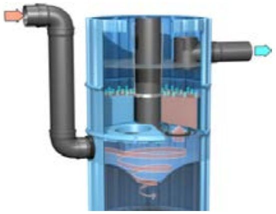
Monitoring Methods:

- Imhoff Cone -- Measures settleable solids (SS, ml/L) – Limitations & Optimal Range
- Gravimetric -- Measures total suspended solids (TSS, mg/L) – Limitations & Optimal Range

System Management

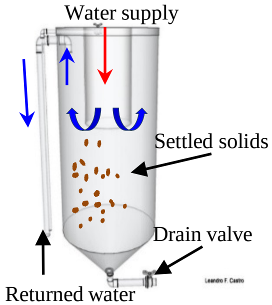
Particulate Matter

Control:



- A variety of equipment is available to manage POM in biofloc system to include: Settling tank; Foam fractionator, Swirl separator; Cyclone filter; Drum filter; Sand filter & Bead filter

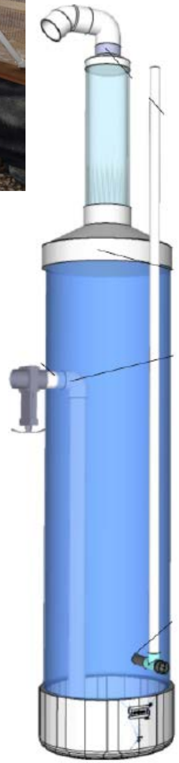
Settling Tanks:



- Remove settleable solids by gravity
  - Can be conical, rectangular, or circular with or w/o baffles to enhance particle settling
  - Effective at removing particles > 60 µm in size
  - Require regular cleaning

System Management - Particulate Matter

Foam Fractionators (Protein Skimmers):



- Effective & inexpensive tools for controlling small (< 30 µm) suspended particles & dissolved organic matter
- A constant supply of small air bubbles captures fine particles & some colloidal material from the tank by adsorption
- The thick foam is collected, de-watered & disposed

System Management

Waste Disposal:

- Particulate control in biofloc tanks results in collection of organic matter with high water content
- One method to reduce the water content includes the use of a shallow tank with false bottom where a filter material is placed over the false bottom for water recovery & to enable drying of the POM
- Potential use of the dried material

System Management

Growth Monitoring, Feed & Feed Management

Growth Monitoring:

- Provides info regarding the shrimp performance
- Collect representative samples: use suitable net (mesh & frame size, cast net), Individual *vs* Group
- Use of observed growth rate, survival, & assumed FCR & to calculate daily ration sizes

  Assumptions: Population @ Time 0: 50,000; Survival @ Sampling Time: 90%; FCR: 1.4; Observed growth: 1.7 g/wk

  Calculated Daily Ration Size:

  15.3 g/day (50K x 90% x 1.4 x 1.7 / 1,000 / 7)

System Management

Feed – Factors to consider

- Palatability
- Better shrimp growth in biofloc than in clear water
- Feed Quality Impact @ high-density cultures
  - Improved growth, yield, FCR & POM control

Feed (HI-35 & SI-35) impact in a 67-d GO trial with juveniles (2.66 g) shrimp @ 500/m^3 in biofloc-dominated system (3 *n*)

	HI-35	SI-35
Final Weight (g)	**22.12 ± 11.35^a**	19.74 ± 8.28^b
Growth (g/wk)	**2.03 ± 0.01^a**	1.76 ± 0.10^b
Total Biomass (kg)	**389.8 ± 1.77^a**	348.5 ± 9.21^b
Yield (kg/m^3)	**9.74 ± 0.04^a**	8.71 ± 0.22^b
Foam fractionator (h)	**812**	1,253
Settling tank (h)	**87**	391
Cost ($/kg)	**1.75**	0.99

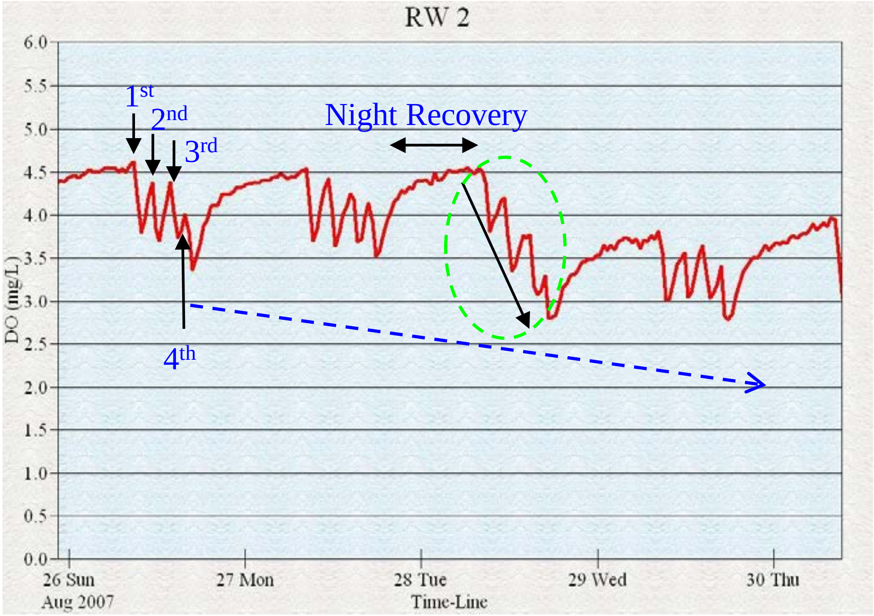
System Management

Feed Management

- Particle Feed Size Selection – Extremely important at the early nursery stage for PL with high CV
- Use of iFCR as feed management tool
- Feed Distribution: Automatic Feeders *vs* Manual
  - Leaching impact on feed quality
  - Impact on growth & FCR
  - Impact on WQ & DO

Four Daily Feedings

- DO Decrease & Recovery from 1st (8:30) to 4th feeding (16:30)
- Cumulative daily DO reduction trends
- Downward DO trends over consecutive days



System Management

Alarm Systems & Power Backup

- Safety Systems -- Theft & Predator Control with Standard Security Measures to prevent entry of unauthorized personnel & predators
- Defensive Responses -- Perimeter fencing, Motion sensors, Security lighting & Cameras, Workers living on-site; Culture tanks in lockable buildings, Electrified wire around the perimeter & Predator traps

System Management

Alarm Systems & Power Backup

- Low DO Sensors
- Power Outage Sensors
- Low/High Air & Water Temperatures Sensors
- Oxygen Flow Sensors
- Low/High Water levels sensors in culture tanks
- Fire detection
- Installation of circuit interrupters (GFCI) on all circuits to protect staff and equipment
- Availability of backup generator with automatic transfer switch

System Management

Natural Light & Microalgae

- Avoid exposure of culture medium to strong direct sunlight -- Use shade cloth when needed
- Better shrimp performance when culture medium has access to natural light to promotes limited microalgal growth
- Presence of diatoms in the culture medium were reported to improve shrimp performance & stress tolerance compare to other microalgal species

System Management

Biosecurity, Probiotics & Diseases

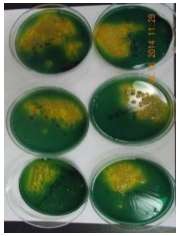
Biosecurity

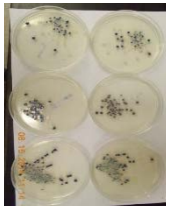
- Purchase PL from commercial hatcheries which provide health certificate
- Limit access of visitors to culture tanks
- Use foot baths and hand sterilizers
- Minimize moving equipment & tools between culture tanks
- Prevent access of disease carriers such as crustaceans, insects, & birds

System Management

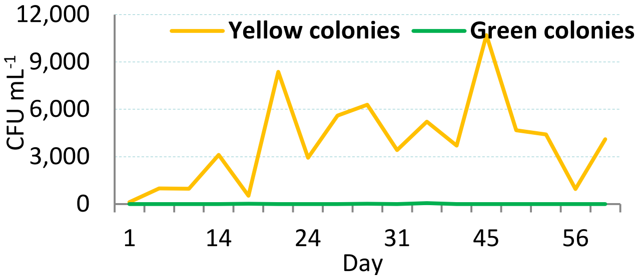
Probiotics

- Use of probiotics with proven performance before stocking & during both the nursery and the grow-out production phases
- Weekly or twice a week monitoring of pathogenic bacteria in the culture medium using TCBS agar plates & Chromagar when needed





- Adjust probiotic application frequency & dosage based on the level of pathogenic bacteria in the culture medium
- Application of several probiotics if needed



System Management

Diseases

- Use PL from a hatchery that provides health certificate with reliable testing methods
- Request the hatchery to provide PL scoring sheet together with the health certificate
- Avoid shrimp exposure to suboptimal water quality conditions such as Low DO, High/Low Temperatures, Low/High pH, High TSS & SS, High TAN, NO_2, NO_3, H_2S, & High Light Intensity

Design and Operation of Super-Intensive Biofloc-Dominated Systems for the Production of Pacific White Shrimp

Litopenaeus vannamei

- The Texas A&M AgriLife Research Experience -

By:
Tzachi M. Samocha, David I. Prangnell, Terrill R. Hanson, Granvil D. Treece, Timothy C. Morris, Leandro F. Castro and Nick Staresinic

Sea Grant
National Sea Grant College Program

Sea Grant
Texas
AT TEXAS A&M UNIVERSITY

The Manual

- Objective: Encourage expansion of sustainable BFD as developed in the Samocha lab
- Funding: NOAA, through National Sea Grant
- Participants:
- Texas A&M AgriLife Research
- Auburn University
- Florida Organic Aquaculture
- Texas Sea Grant Extension Service

The Manual

- Describes design & operation of the biofloc systems developed over 20 years at Texas A&M AgriLife Research Mariculture Lab
- Emphasizes the most recent L. vannamei production trials
- Written in a non-academic style to target a wider group of stakeholders -- especially entrepreneurs interested in building a pilot BFD system

The Manual – some highligths

- 15 Chapters + Excel sheets & short videos
- *Chapter 3: Biofloc* -- *its composition, structure, development, & advantages*
- *Chapter 5: Site Selection & Production System*
- *Chapter 6: System Treatment & Preparation*
- *Chapter 7: Water Quality Management – controlling DO, ammonia, pH, alkalinity, temperature, salinity, suspended solids, turbidity, and waste products in indoor BFD systems*

The Manual – some highligths

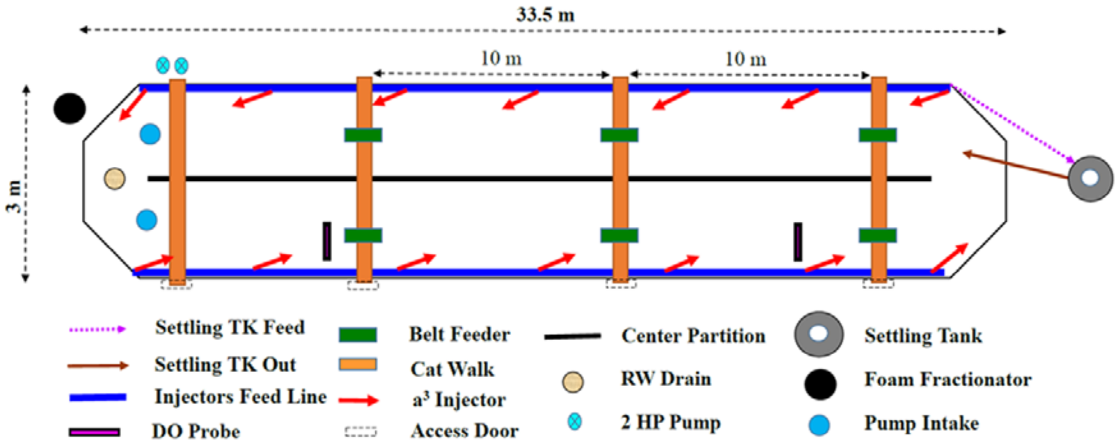
- Chapter 8: Nursery Production
- Chapter 9: Grow-out
- Chapter 12: Disease & Biosecurity
- Chapter 13: Economics of BFD
- Chapter 15: Trouble-shooting Table

Nursery production of the Pacific White Shrimp, *Litopenaeus vannamei*, in 100 m^3 RWs under zero-exchange biofloc-dominated system operated with a[3] injectors

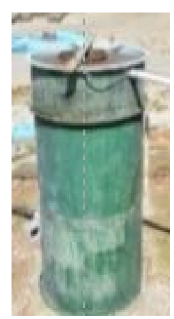
Tzachi Samocha[1], Leandro Castro[1], David Prangnell[1], Tom Zeigler[2], Craig Browdy[2], Tim Markey[2], Darrin Honious[3], and Bob Advent[4]

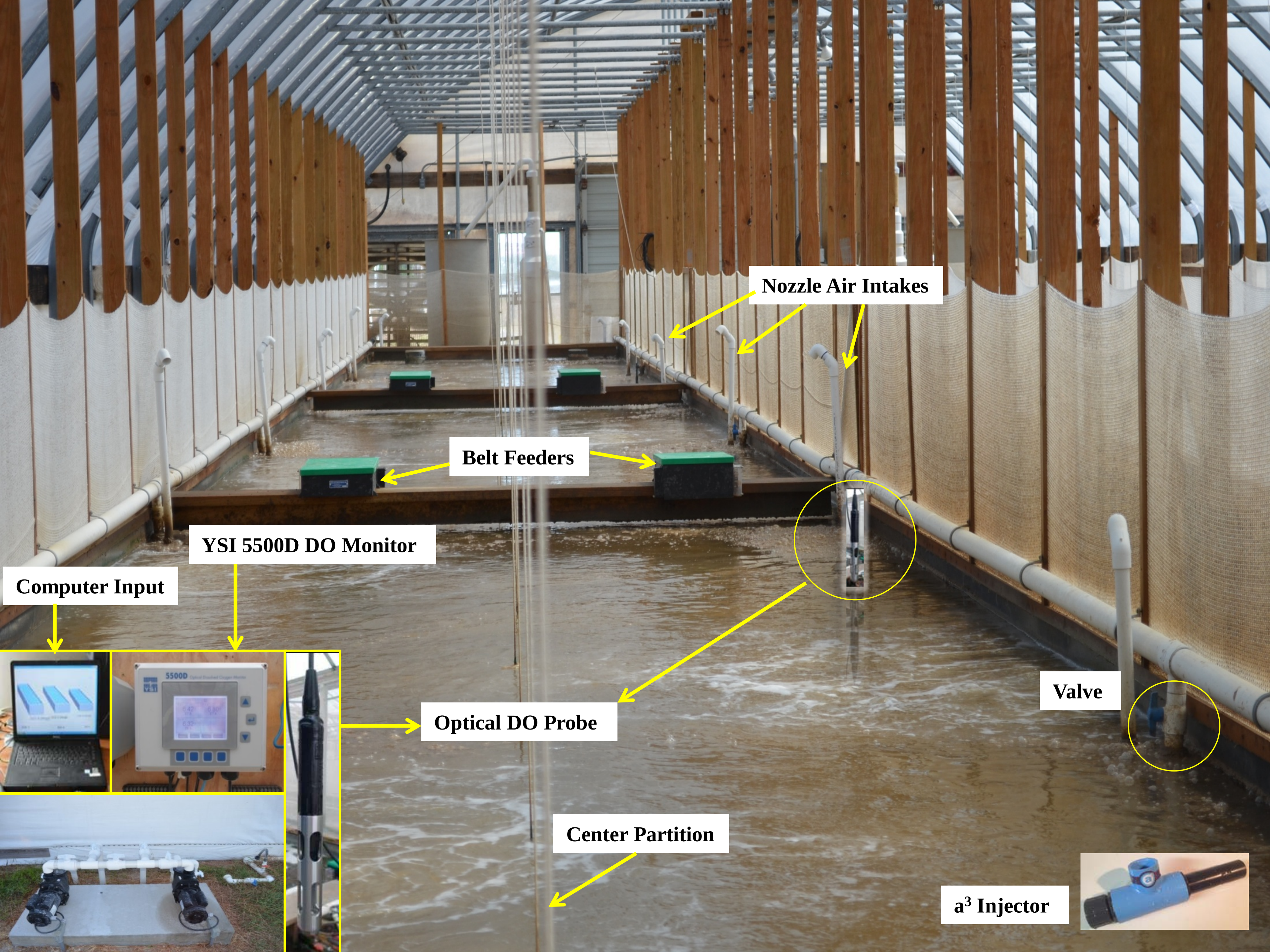
Greenhouse-enclosed 100 m^3 RWs

The Texas A&M AgriLife Research Biofloc System



- Two 100 m^3 RWs
- GH- Shade Cloth & Exhaust Fans
- Online DO Monitoring
- 14- pump-driven a^3 Injectors/RW
- Two- 2 HP Pumps/RW
- One Foam Fractionator/RW
- One Settling Tank/RW
- One Digester





Nozzle Air Intakes
Belt Feeders
YSI 5500D DO Monitor
Computer Input
Optical DO Probe
Valve
Center Partition
a[3] Injector
5500D
YSI


Filter Pipe
Pump Intake
03.16.2014 11:24
Filter Pipe
Filter Pipe
Drain
Center Partition
Pump Intake
06.24.2014 11:28
03.16.2014 11:25

62-d Nursery Trial - 100 m^3 RWs

- RWs filled with 90% disinfected NSW and 10% nitrifying bacteria rich seawater adjusted to 30 ppt salinity
- 540 PL_{5-10}/m^3 (0.94±0.56 mg; **CV: 59.7%!**) - hybrid Fast-growth/Taura-resistant
- Continuous feeding from Day 2
- FW to offset losses to evaporation & solids removal
- Filter pipes fitted with 0.5, 0.8 & 1 mm screens
- Temp., Sal., DO, pH: 2/d; SS: 1/d; TSS: ≥1/wk; TAN, NO_2-N, NO_3-N, VSS, turbidity, RP: 1/wk; Alka.,: adjusted 2/wk using $NaHCO_3$ to maintain 160 mg/L as $CaCO_3$
- Online YSI 5500 DO monitoring with optical probe/RW
- *Vibrio* monitoring 2/wk using TCBS agar for Yellow & green-colony forming

62-d Nursery Trial - 100 m^3 RWs

- *KI-Nitrifier*™ (Keeton Industries, Wellington, CO) & white sugar to boost heterotrophic & nitrifying bacterial activities to control nitrogen species
  - Application: 0.26 mg/L (Day 1, 4, 7, 10 & 32)
- *Ecopro*® (EcoMicrobials, Miami, FL) application: 0.2 mg/L every 3 d + 0.055 mg/L on Day 1, 0.4 mg/L on Day 39 & 0.3 mg/L on Day 42
  - Probiotic contained stabilized spores of *Paenibacillus polymyxa*, *Bacillus megaterium*, *Bacillus licheniformis* (2 strains) & *Bacillus subtilis* (3 strains), at a minimum concentration of 5.5 x 10^8 CFU/g

Foam Fractionator

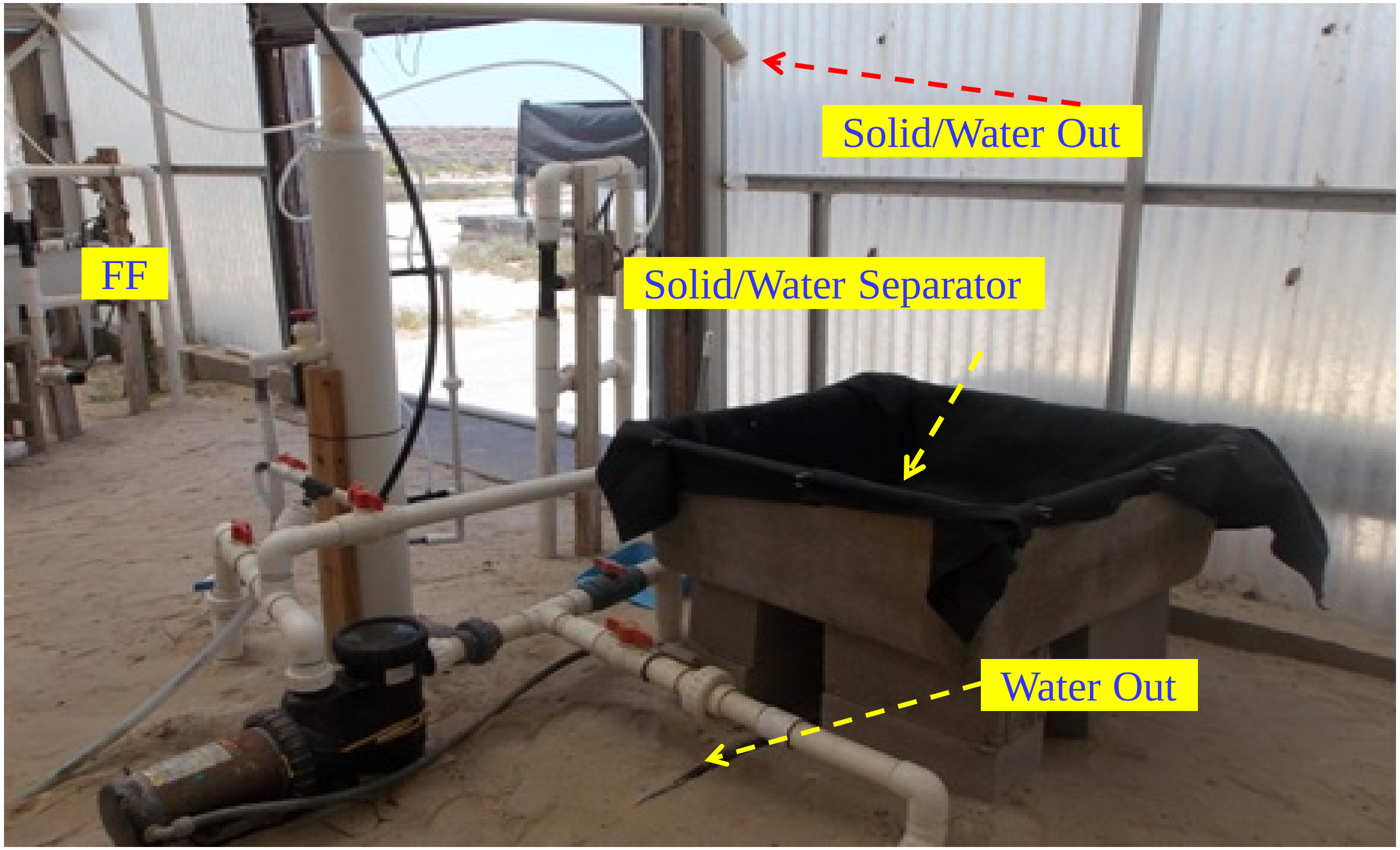
- Operated with one a^3 injector, flow rate ≈ 28 Lpm, fed from the pump's side loop
- Use of fabric for dewatering and drying of the organic particulate matter

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

Solids & Biofloc Control

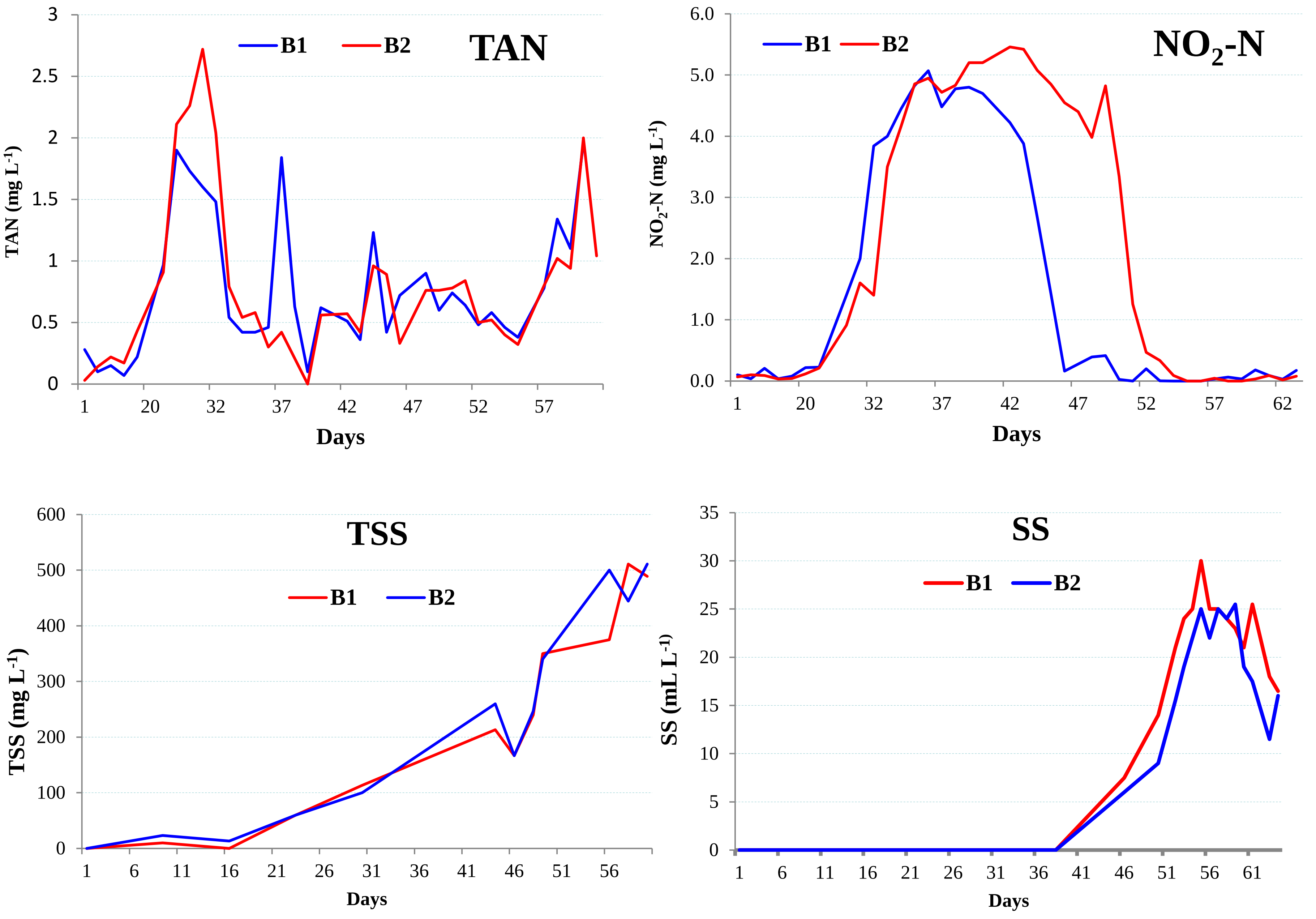
- Maintain TSS levels (250 - 350 mg/L)
- Waste Disposal



62-d Nursery Trial - 100 m^3 RWs

- Shrimp were fed a combination of EZ-*Artemia* & dry feed (Zeigler Raceway Plus <400 µm) for the first 8 d post-stocking and Zeigler Raceway Plus (<400 µm, 400-600 µm, 600-850 µm) + Zeigler Shrimp PL 40-9 with V-pak™ (1 mm, 1.5 mm, 2 mm) for the remainder of the trial
- *Feed size & rates were adjusted based on shrimp growth & size variation - continuous delivery by belt feeders*

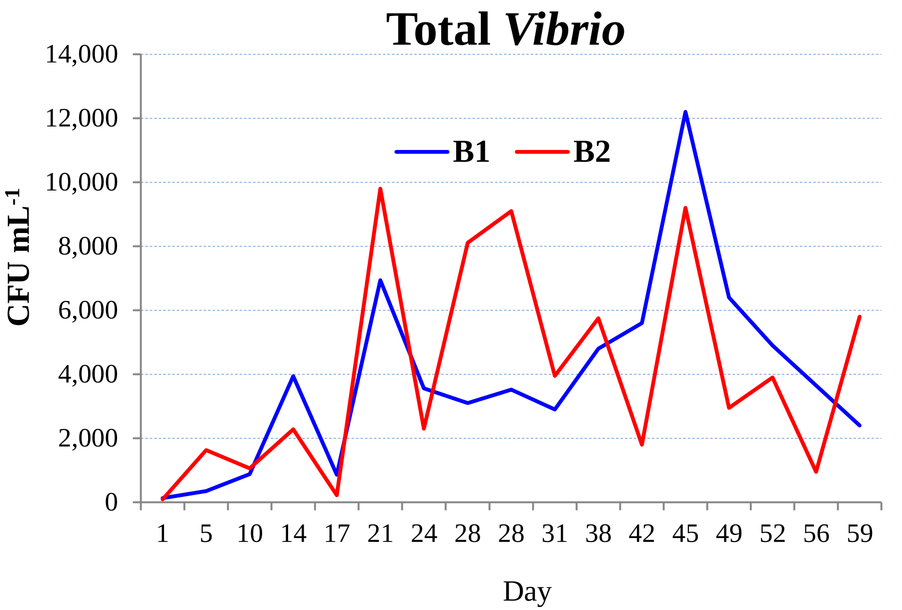
		Temp. (^oC)	Sal. (ppt)	DO (mg L^{-1})	pH
AM	Mean	26.4	30.4	6.8	8.1
	Min	22.2	29.7	4.6	7.6
	Max	29.7	31.1	8.5	8.5
PM	Mean	26.8	30.4	6.6	8.1
	Min	22.9	28.6	4.4	7.6
	Max	30.2	31.1	7.9	8.5

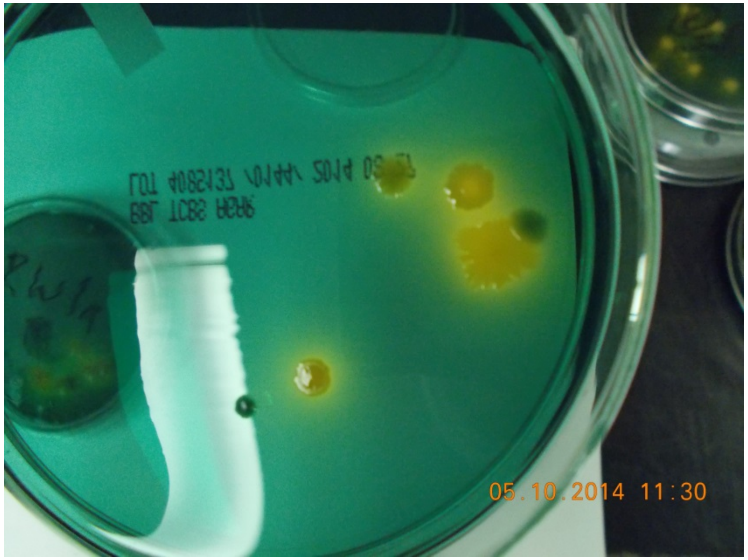


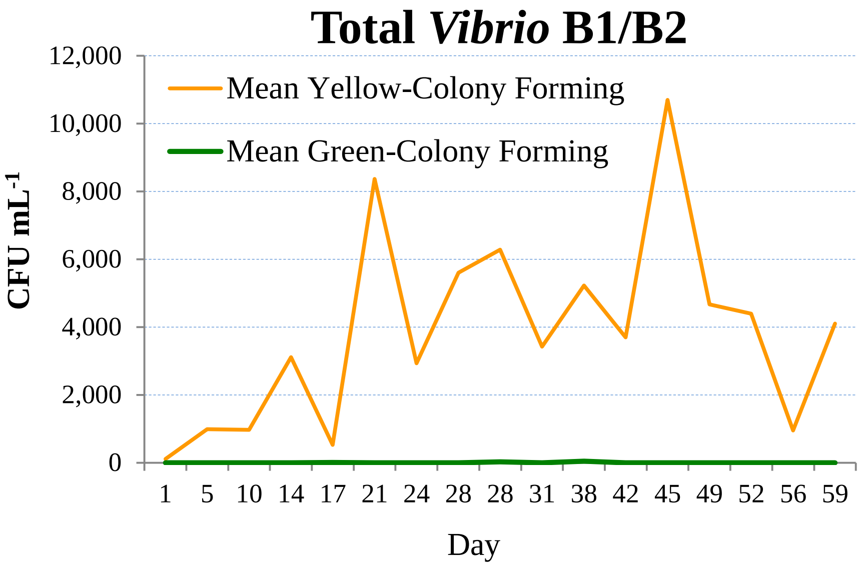
TAN
B1
B2
TAN (mg L-1)
Days
NO2-N
NO2-N (mg L-1)
TSS
TSS (mg L-1)
SS
SS (mL L-1)

62-d Nursery Trial – 100 m^3 RWs

Green colony-forming *Vibrio* concentrations remained below 50 CFU/mL and less than 2% of the yellow colony-forming concentrations throughout the trial







Summary of nursery production in two 100 m^3 raceways with *Litopenaeus vannamei* stocked at 540 PL_{5-10}/m^3

RW	Yield (kg/m^3)	Av. Wt. (g)	Max (g)	Min (g)	CV (%)	Sur. (%)	FCR	Sugar (kg/RW)
B1	3.43	6.49	11.9	0.6	35.6	97.8	0.81	33.4
B2	3.28	6.43	10.5	0.5	31.0	94.6	0.81	33.1

- Low temp. for the 1st three wks resulted a in long trial
- PL high size variation required frequent monitoring of individual weight to determine feed particle size
- The high variation may have prevented full expression of the shrimp growth potential
- High size variation continued throughout the harvest

Conclusion

- Preparing nitrifying bacteria rich water ahead of stocking prevented PL exposure to high TAN & Nitrite
- The use of probiotic may have contributed to the low FCR. A follow-up controlled study is needed urgently
- Use of TCBS agar plates served as a good tool to monitor non- and pathogenic *Vibrio* in culture medium
- Although the a^3 injectors were used with very small PL, shrimp were not damaged
- One 2 hp pump was sufficient to maintain high DO (4.4-8.5 mg L^{-1}) at biomass load of 3.43 kg shrimp m^{-3} with no need for oxygen supplementation
- a^3 injectors provided adequate mixing of the biofloc