# *Vibrio* dynamics in zero-exchange, biofloc-dominated production systems for the Pacific white shrimp, *Litopenaeus vannamei*

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# Introduction

- As super-intensive biofloc-dominated shrimp production systems have developed, three main limiting factors have emerged: waste (solids) management, ionic changes, and bacterial infections
- *Vibrio* infections are the most common problems in intensive shrimp culture, including biofloc-dominated systems, and these outbreaks can substantially reduce shrimp production
- Simple tools to limit and monitor *Vibrio* infections in shrimp production systems are needed

# Objectives

- To monitor changes in *Vibrio* populations throughout nursery and grow-out trials in two indoor super-intensive biofloc-dominated zero-exchange shrimp production systems
- Establish protocols for *Vibrio* monitoring and potential treatment options in these systems

# Materials & Methods

The presence of *Vibrio* was monitored in two biofloc systems producing *Litopenaeus vannamei* through nursery and grow-out phases in greenhouse-enclosed, EPDM-lined raceways:

Six 40 $m^3$ RWs

Two 100 $m^3$ RWs

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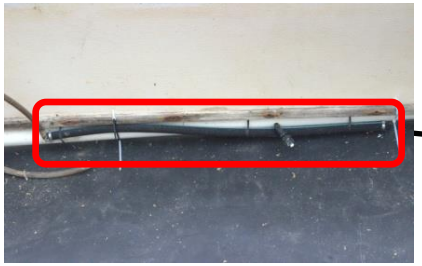
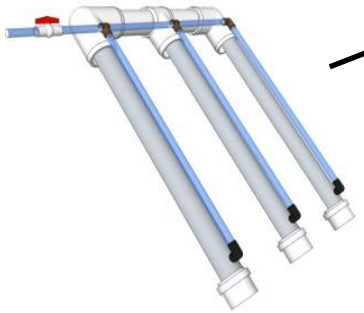
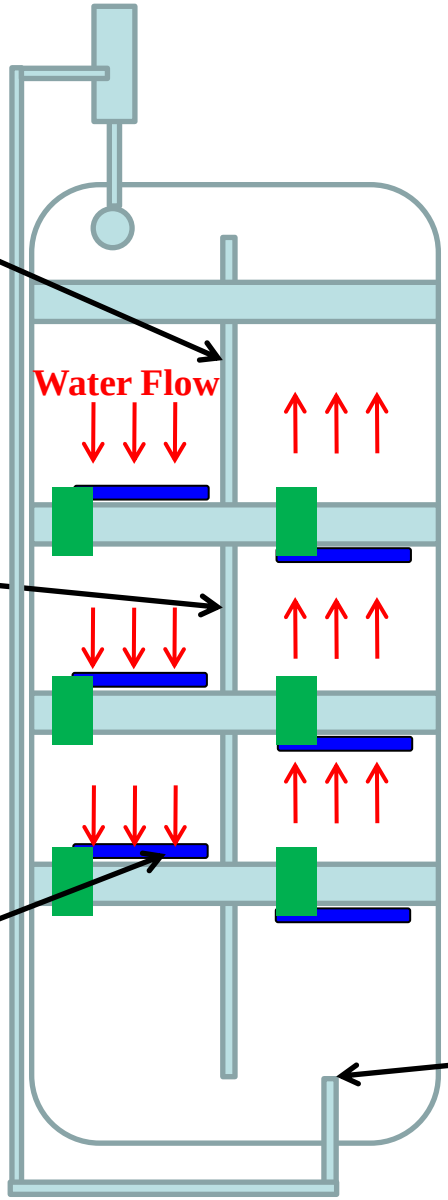
# Materials & Methods

**40 $m^3$ RWs**








Each RW has:

- 18 air-lifts
- 6 air-diffusers
- 1 x 2 hp pump to circulate water through a Venturi and nozzles

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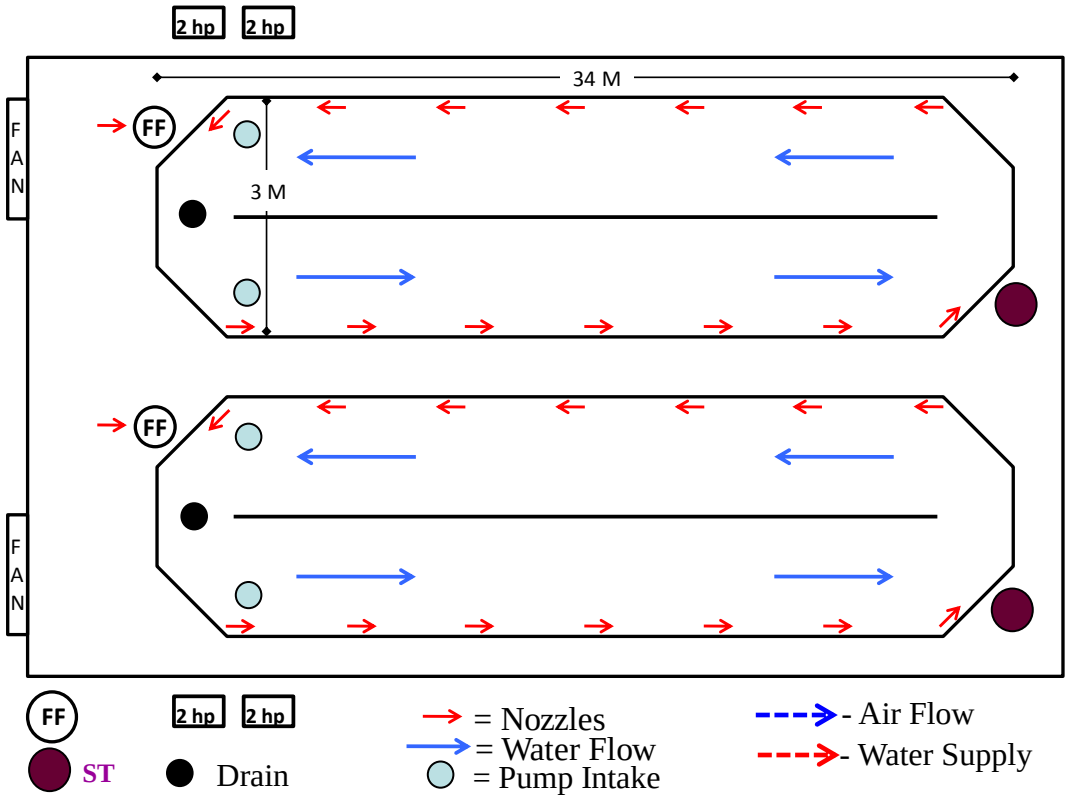
# Materials & Methods

## 100 $m^3$ RWs




Each RW has:

- Two 2 hp pumps
- 14 $a^3$ injectors
- One ST & one FF

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# Materials & Methods

**Nursery**

- RWs were filled with disinfected natural seawater and 10% aged seawater (*KI Nitrifier*™ / 3 wk)
- RWs were operated with no water exchange
- RWs stocked with Fast-Growth/Taura Resistant $PL_{5-10}$ (0.94 mg)- 40 $m^3$: 675 PL/$m^3$; 100 $m^3$: 540 PL/$m^3$ & reared for 62 days
- *KI-Nitrifier*™ & white sugar were used to enhance nitrifying & heterotrophic bacterial activities to control nitrogen species
- Feed was distributed 24/7 using belt feeders (EZ-*Artemia*/Dry - *Zeigler Bros.*)

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# Materials & Methods

## Grow-out

- RWs were filled with a mixture of biofloc-rich water (87.5%) used in the nursery trial, and natural seawater (12.5%)
- RWs stocked with juveniles from the nursery trial-
  - 40 $m^3$: 457/$m^3$ (5.30 g) reared for 48 days
  - 100 $m^3$: 458/$m^3$ (6.45 g) reared for 38 days
- Shrimp were fed either 35% (HI-35) (3 x 40 $m^3$ RWs) or 40% protein (EXP) (3 x 40 $m^3$, 100 $m^3$ RWs) feeds (*Zeigler Bros.*)

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# Materials & Methods

## Water Quality


- Each RW had an optical DO monitoring probe & YSI 5500D monitoring system (*YSI Inc., Yellow Springs, OH*)
- Alkalinity adjusted to 160 mg $L^{-1}$ (as $CaCO_3$) using sodium bicarbonate as required
- pH adjusted to >7 using NaOH during grow-out
- $O_2$ supplemented in 40 $m^3$ RWs grow-out
- Ecopro® (*EcoMicrobials, Miami, FL*) every 1-3 d
- FFs & STs used to control solids, targeting TSS of 200-300 mg $L^{-1}$ & SS of 10-14 mL $L^{-1}$

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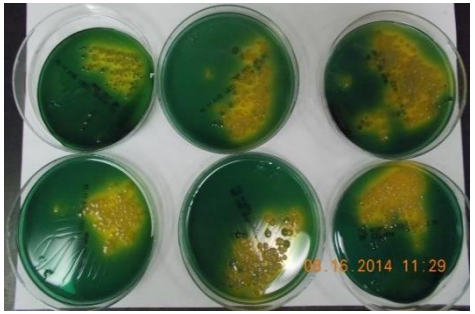
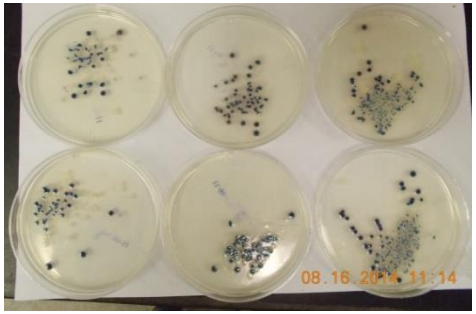
# Materials & Methods

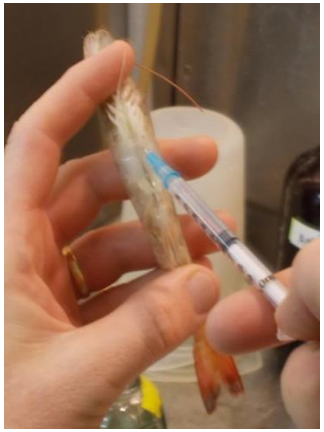
## *Vibrio* & Algae

- *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 grow-out harvest
- Algal pigments in biofloc measured 1/wk







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# Results

## Nursery Water Quality

| | 40 $m^3$ | | 100 $m^3$ | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| Temperature ($^oC$) | 26.6 | 20.8 - 30.6 | 26.6 | 22.2 - 30.2 |
| DO (mg $L^{-1}$) | 6.5 | 4.4 - 8.5 | 6.7 | 4.4 - 8.5 |
| pH | 8.2 | 7.8 - 8.5 | 8.1 | 7.6 - 8.5 |
| Salinity (ppt) | 30.4 | 29.6 - 31.2 | 30.4 | 29.7 - 31.1 |
| TAN (mg $L^{-1}$) | 0.94 | 0 - 4.95 | 0.78 | 0 - 2.72 |
| $NO_2$-N (mg $L^{-1}$) | 2.29 | 0 - 10.93 | 1.84 | 0 - 5.46 |

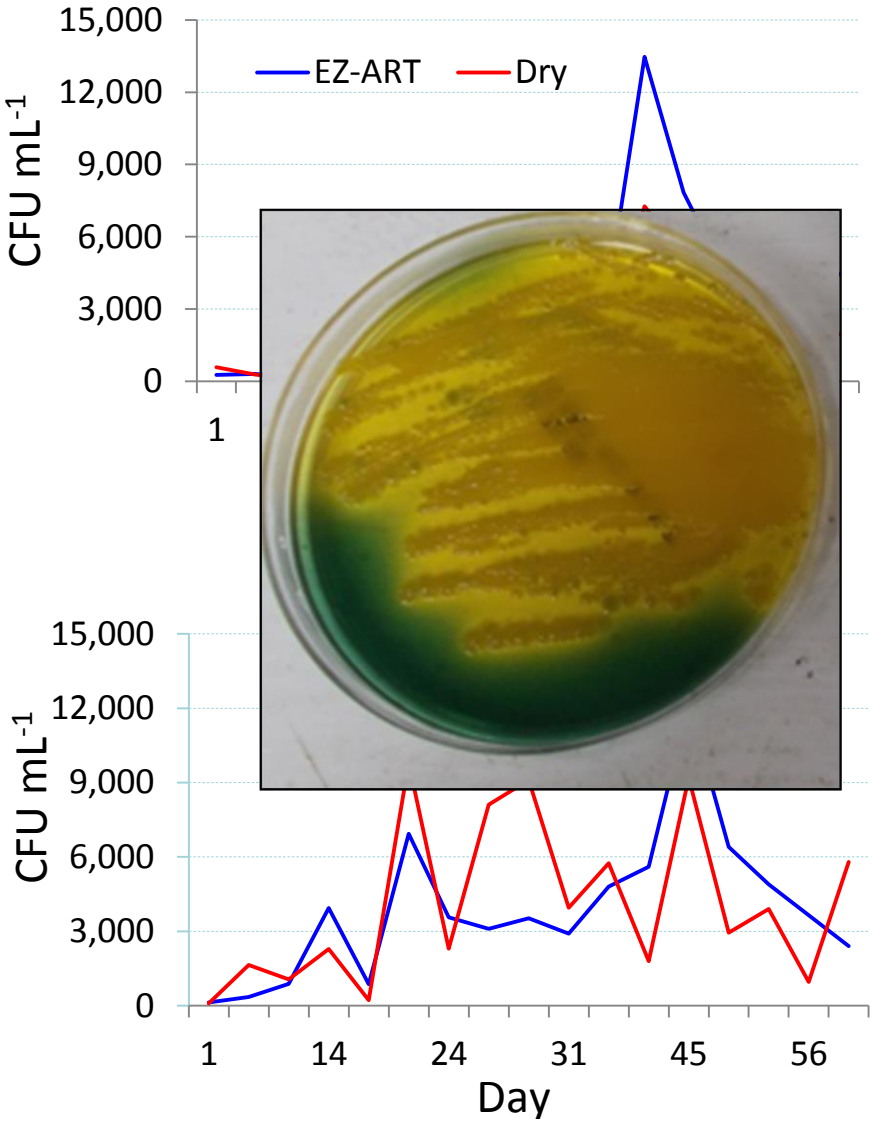
# Results

## Shrimp Performance (Nursery)

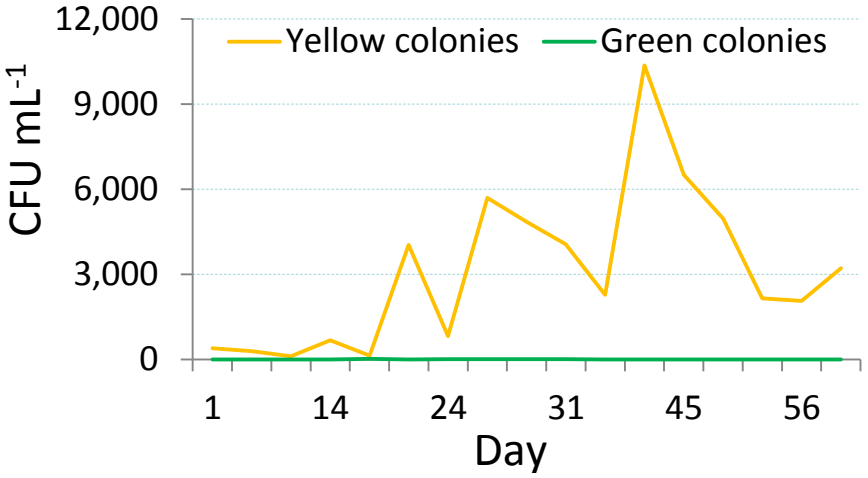
| | 40 $m^3$ | 100 $m^3$ |
|---|---|---|
| Final Weight (g) | 5.57±0.55 | 6.46±0.04 |
| Growth (g/wk) | 0.60±0.06 | 0.73±0.01 |
| Total Biomass (kg) | 126.4±7.28 | 335.6±10.10 |
| Yield (kg/$m^3$) | 3.16±0.18 | 3.36±0.10 |
| FCR | 0.89±0.06 | 0.81±0.00 |
| Survival (%) | 84.86±11.37 | 96.20±2.26 |

# *Vibrio* colonies in the culture medium (nursery)

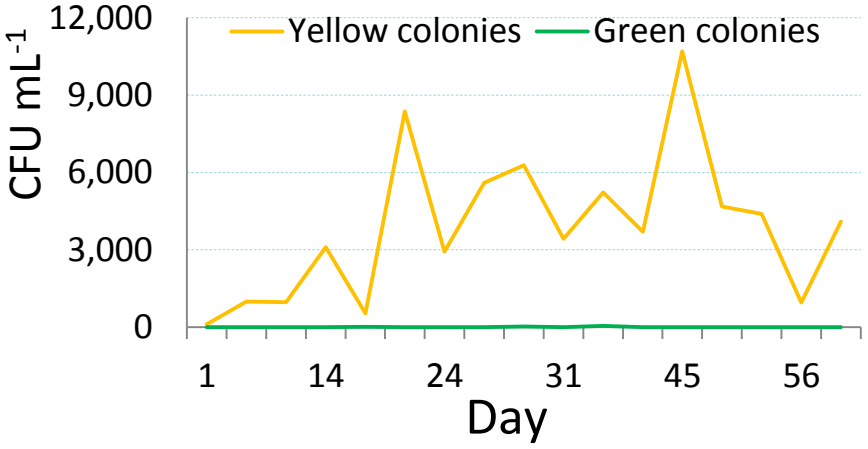
## 40 $m^3$ Total




## 40 $m^3$ Color




## 100 $m^3$ Color



# Results

**Nursery**

- At the end of the nursery phase, survival was high (70.6 - 97.9%) and FCR was low (0.81 - 0.89).
- Green colony forming units (GCFU) *Vibrio* remained below 100 CFU $mL^{-1}$ and <21.5% of the total concentration in 40 $m^3$ RWs and were only observed on 14.7% of plates.
- GCFU *Vibrio* remained below 100 CFU $mL^{-1}$ and <9.1% of the total concentration in 100 $m^3$ RWs and were only observed on 12.1% of plates.
- No evidence of Vibriosis or chitinoclastic bacterial infections in any RW.

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# Results

## Grow-out Water Quality

| | 40 $m^3$ | | 100 $m^3$ | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| Temperature ($^oC$) | 29.9 | 27.8 - 31.8 | 30.3 | 28.8 - 31.6 |
| DO (mg $L^{-1}$) | 5.4 | 3.5 - 6.9 | 6.1 | 4.6 - 7.2 |
| pH | 7.5 | 6.8 - 8.0 | 7.6 | 6.7 - 7.9 |
| Salinity (ppt) | 30.3 | 29.6 - 31.2 | 30.4 | 29.3 - 31.0 |
| TAN (mg $L^{-1}$) | 1.38 | 0.21 - 6.00 | 1.2 | 0.27 - 2.85 |
| $NO_2$-N (mg $L^{-1}$) | 0.24 | 0 - 2.25 | 0.18 | 0 - 0.58 |
| $NO_3$-N (mg $L^{-1}$) | 125 | 46 - 232 | 112 | 62 - 187 |
| $PO_4$ (mg $L^{-1}$) | 29 | 14 - 57 | 32 | 22 - 57 |

# Results

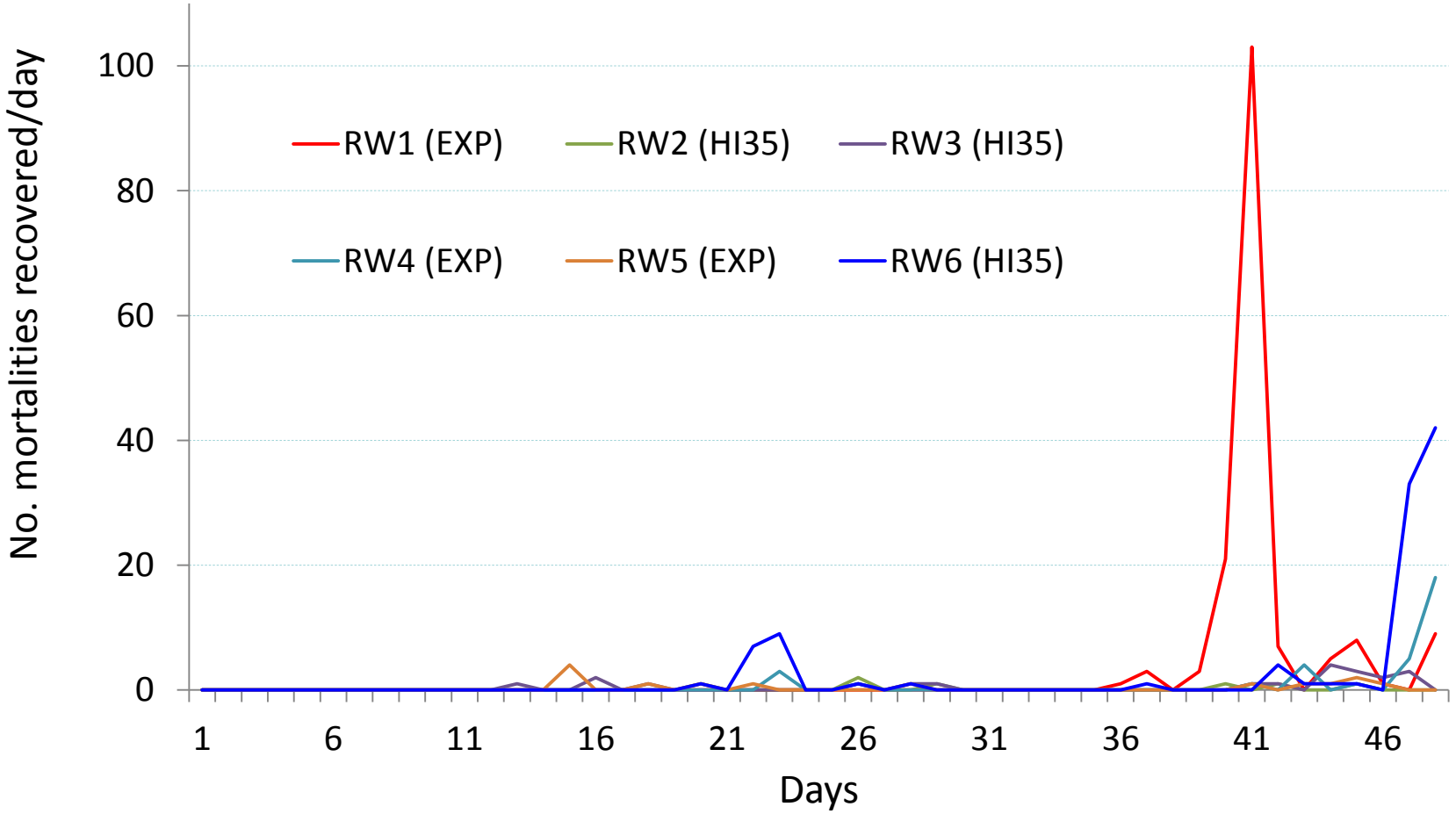
## Shrimp Performance (Grow-out)

| | 40 $m^3$ | 100 $m^3$ |
|---|---|---|
| Final Weight (g) | 20.64±1.38 | 18.69±0.45 |
| Growth (g/wk) | 2.21±0.18 | 2.25±0.08 |
| Total Biomass (kg) | 292.0±21.0 | 646.7±63.6 |
| Yield ($kg/m^3$) | 7.30±0.53 | 6.47±0.64 |
| FCR | 1.65±0.18 | 1.79±0.30 |
| Survival (%) | 77.72±0.08 | 75.54±5.61 |

# Results

## Shrimp Performance (Grow-out)

### Mortalities recovered per day (40 $m^3$)

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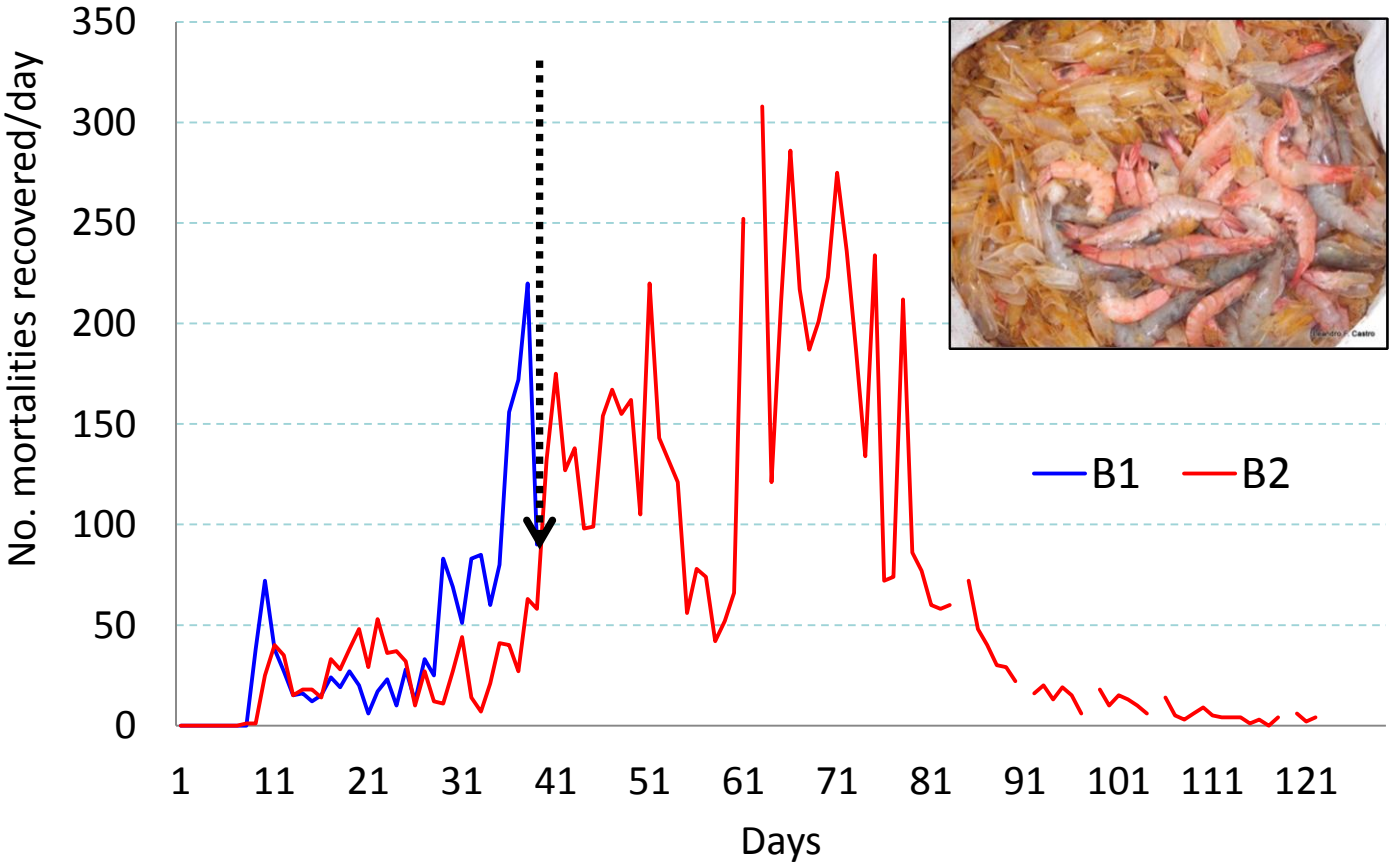
# Results

## Shrimp Performance (Grow-out)

## Mortalities recovered per day (100 $m^3$)

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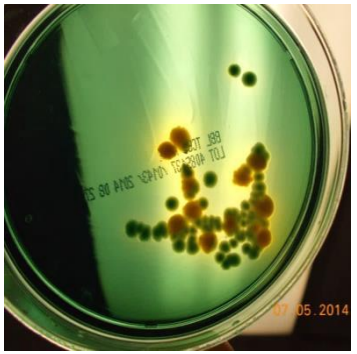
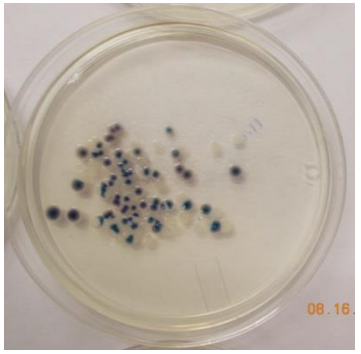
# Results

## Grow-out

- GCFU *Vibrio* were much more abundant than in the nursery phase
- Vibriosis-related mortality observed after 1 week in 100 $m^3$ but not observed until later in the trial in 40 $m^3$ RWs
- RambaCHROM plating & preliminary API: *V. parahaemolyticus*, *V. vulnificus* & *V. alginolyticus* in culture water (1:3.1:6.4), & moribund shrimp (>1:50:50)
- 16S rRNA sequencing: *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. harveyi* & *V. mytili* in moribund shrimp





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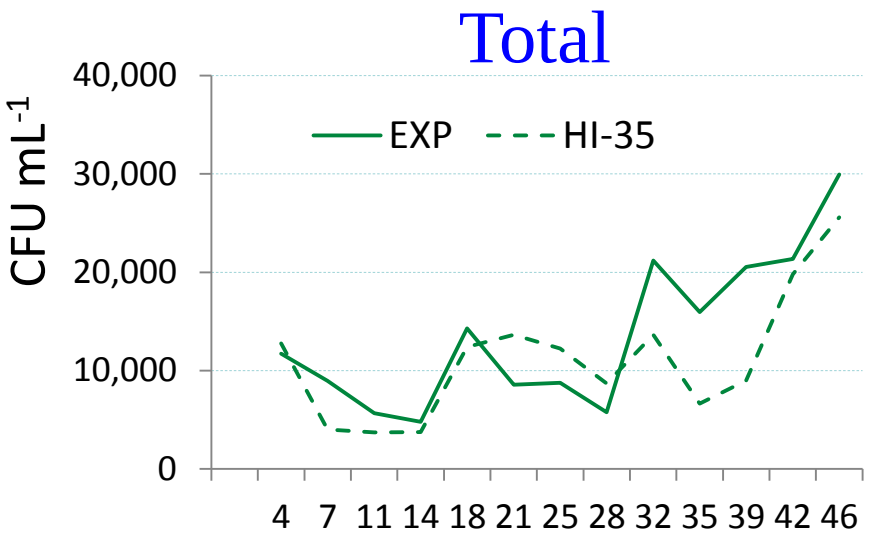
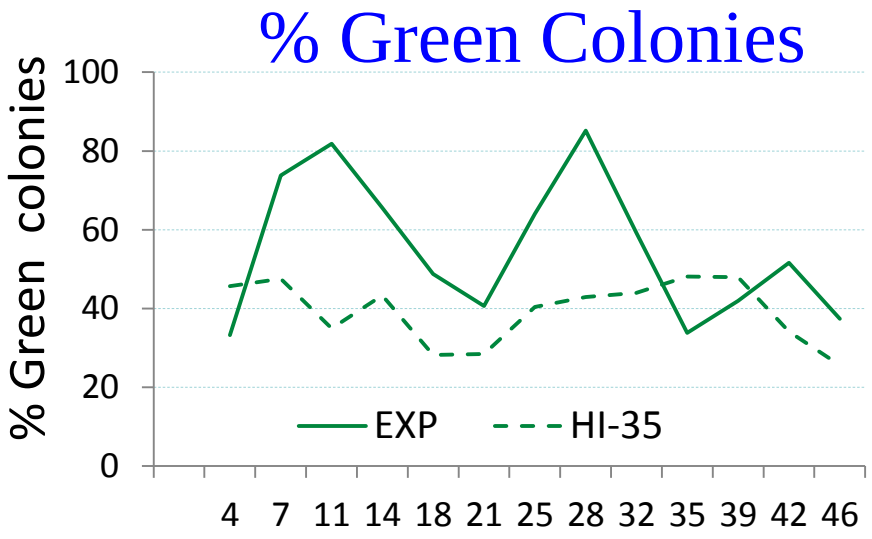
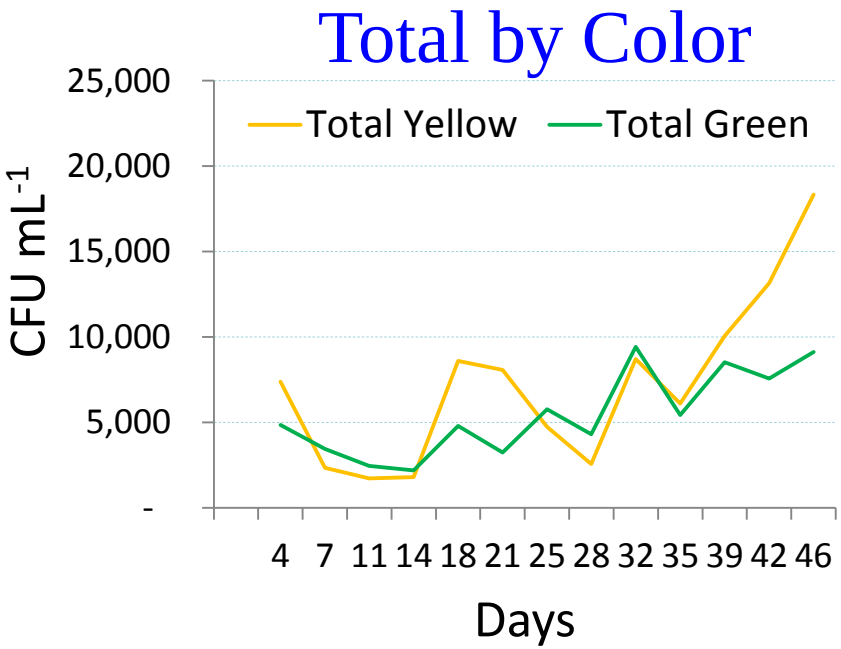
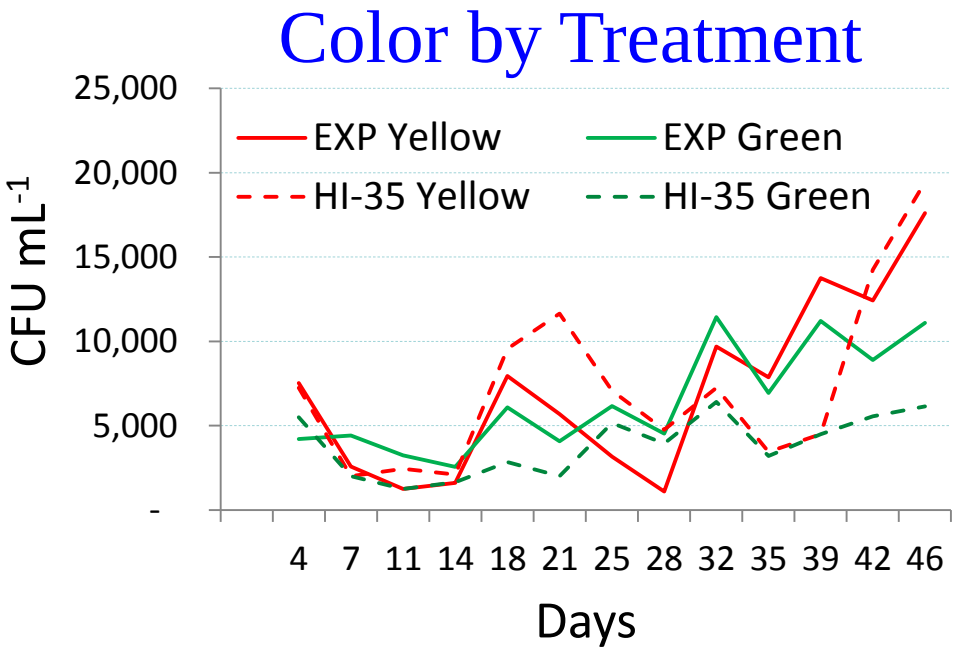
# *Vibrio* colonies in the 40 $m^3$ culture medium (grow-out)



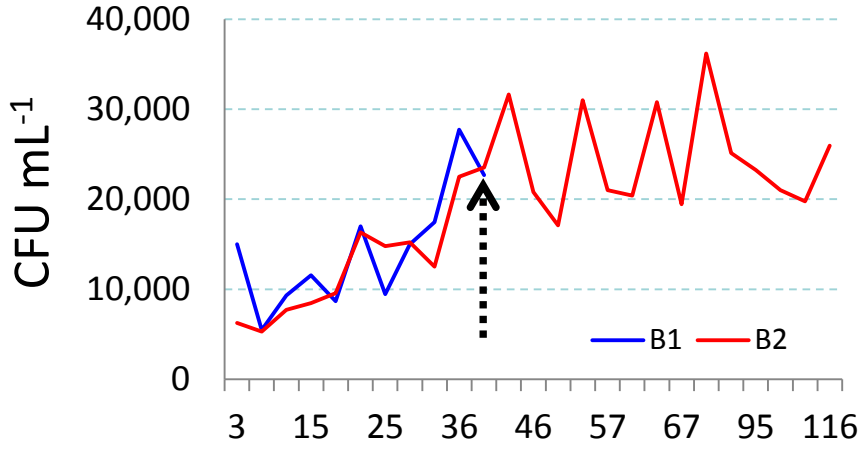



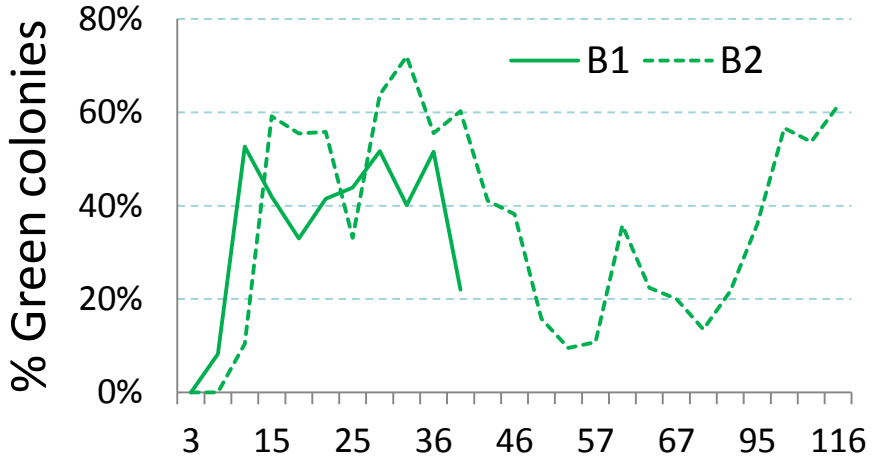



# *Vibrio* colonies in the 100 $m^3$ culture medium (grow-out)

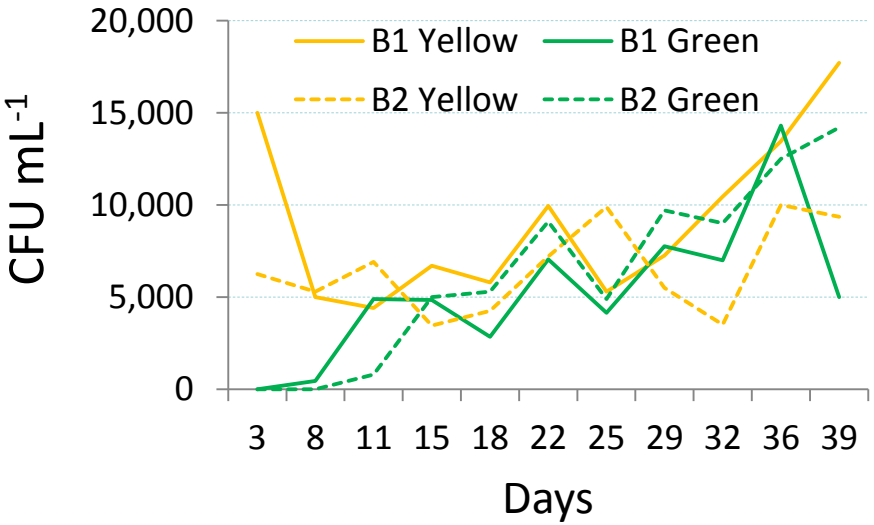
## Total *Vibrio*



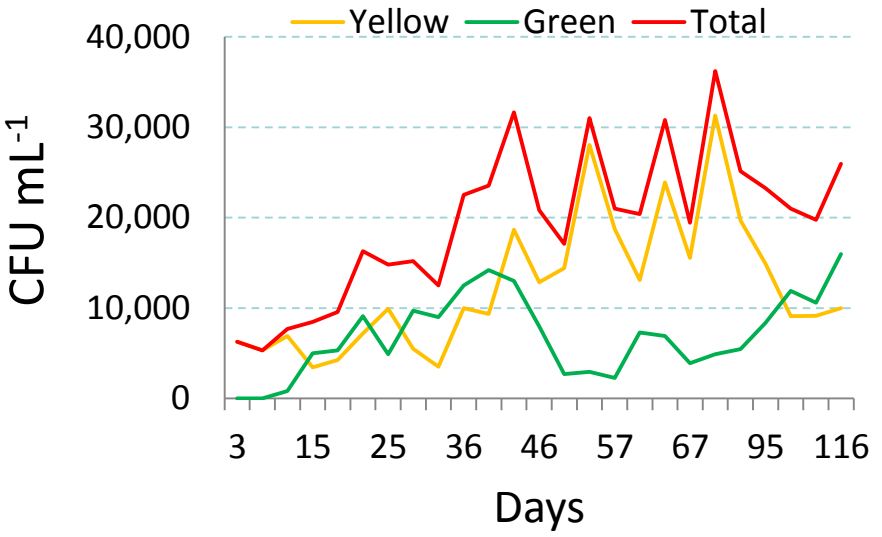

## % Green Colonies




## Color by Raceway




## B2 by Color



# Summary of *Vibrio* counts on TCBS (grow-out)

| | 40 $m^3$ | | 100 $m^3$ | |
|---|---|---|---|---|
| *Vibrio* col. (CFU $mL^{-1}$) | Mean | Range | Mean | Range |
| Total (x 1,000) | 9.63 | 2.20-23.30 | 18.05 | 5.30-31.65 |
| Yellow (x 1,000) | 5.13 | 0.70-20.40 | 12.19 | 3.45-28.05 |
| Green (x 1,000) | 4.50 | 0.30-15.90 | 5.86 | 0.00-14.30 |
| % Green | 48.06 | 3-87 | 38.74 | 0-72 |

Over equivalent time period (38 d of 100 $m^3$ & 1$^{st}$ 38 d of 40 $m^3$ trial)

# Results

## Biofloc content

## Mean algal content in biofloc


| Pigment (ng/µL) | From | 40 $m^3$ | 100 $m^3$ |
|---|---|---|---|
| C2 | Diatoms | 6.65 | 8.08 |
| Diadinoxanthin | | 1.22 | 0.28 |
| Fucoxanthin | | 6.75 | 4.62 |
| Canthaxanthin | Blue-green algae | 0.00 | 0.97 |
| Myxoxanthophyll | | 0.00 | 0.98 |
| Zeaxanthin | | 0.00 | 1.16 |
| Lutein | Green algae / feed | 4.68 | 0.57 |
| Viola | | 4.68 | 0.57 |
| Chlorophyll b | | 3.28 | 0.64 |
| Chlorophyll a | Total algae | 33.02 | 15.20 |
| β-carotene | | 1.68 | 0.60 |

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# Conclusion

- This study demonstrates the detrimental effect of *Vibrio* infections on *L. vannamei* production in intensive biofloc systems and the need for further investigation of *Vibrio* control in these systems
- External stressors such as nursery harvest under unfavorable conditions (e.g., low DO, high temp., high TSS) allow expression of pathogenic *Vibrio*
- Monitoring *Vibrio* can be a useful tool for predicting disease outbreaks

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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
- *a³ All-Aqua Aeration* for providing the injectors