Use of no water exchange and Zeigler 35% CP HI diet for the production of marketable Pacific White Shrimp, *Litopenaeus vannamei*, in a super-intensive raceway system

A Summary Report prepared for Zeigler Bros. by Tzachi Samocha
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In the last few decades, production of the Pacific White Shrimp, *Litopenaeus vannamei*, has been negatively affected by disease epizootics and environmental concerns over the impact of effluent discharge into receiving streams. Traditional shrimp grow-out methods use outdoor ponds and require substantial exchanges of pond water during the production cycle. The possible introduction of disease with the incoming water and the release of nutrient-rich effluent into receiving streams are matters of great concern. These issues have forced the industry to seek out more sustainable management practices. Several studies show that utilizing limited or zero water exchange practices do not adversely affect shrimp production.

Limited discharge recirculating aquaculture systems (RAS) are an option that can reduce disease introduction and the negative environmental impact created by traditional pond culture. In a 92-day grow-out study conducted in 2007, four 40 m^3 (68.5 m^2) greenhouse-enclosed, ethylene propylene diene monomer (EPDM, Firestone Specialty Products, Indianapolis, IN) lined raceways were filled with water previously used in a 78-d nursery study. The raceways were stocked with juvenile (1.14 g) Pacific White Shrimp at a density of 531/m^3, and operated with no water exchange. To control particulate matter, two raceways were each outfitted with a home-made foam fractionator (FF), and the other two raceways were each equipped with an 8.6 m^3 cylindroconical settling tank (ST) (4.9 m^3 working water volume). Settling tanks were operated on a continuous basis (4 L/min). Due to the large size of the foam fractionators and their ability to quickly reduce particulates, the FFs were only operated for few days at a time. The average weight of the shrimp raised in the RWs operated with a ST was significantly higher than those raised in the RWs with FF (18.45 vs. 17.35 g). It may be possible that the differences in mean final weight between treatments were the result of the intermittent operation of the FFs which produced fluctuations in the concentration and availability of particulate matter as an additional food source. No statistically significant differences were found in total yield, survival, or growth between the two treatments, although an impressive total yield of 9.29 kg/m^3 was found in one of the RWs operated with the ST.]

In 2008 a follow-up study was initiated to determine if using a smaller FF, operated on a more continuous basis than those used in the first study, would perform as well as RW's operated with a ST. However, due to an outbreak of *Vibrio*, the 2008 study was terminated before the outcome could be determined. In 2009, a 108-day grow-out study was conducted with the following objectives: 1. Produce market size *L. vannamei* at a high stocking density with zero water exchange, 2. Monitor shrimp growth, survival, and FCR under zero water exchange, 3. Study the effect of using foam fractionators (FF) and settling tanks (ST) on selected water quality indicators under zero water exchange, and 4. Evaluate the benefit of using continuous DO monitoring equipment in operating a super-intensive shrimp production system.

The 2009 study utilized four 40 m^3 raceways. Each raceway (25.4 x 2.7 m or 68.5 m^2) was lined with EPDM (Firestone Specialty Products, Indianapolis, IN), a liner previously determined to be non-toxic to shrimp. RWs were equipped with a center longitudinal partition positioned over a 5.1 cm PVC pipe with spray nozzles. Raceways also had six banks each with three 5.1 cm airlift pumps. Airlifts were positioned equidistance on both sides of the partition. In addition, each raceway had six 0.91 m long air diffusers (1.9 cm OD, Aero-Tube™, Tekni-plex Aeration, Austin, TX), a centrifugal pump, and a Venturi injector capable of introducing atmospheric air or a mixture of oxygen and air. Raceways were filled with the water used in a preceding 62-d nursery study. Two raceways were each outfitted with a small commercial FF (VL65, Aquatic Eco System, Apopka, FL), while the other two raceways were each equipped with an 8.6 m^3 cylindroconical settling tank (4.5 m^3 working water volume). In this study FFs were operated on a more continuous basis to control particulate matter load. All four raceways were stocked (450 shrimp/m^3) with juvenile Pacific White Shrimp (0.99 ± 0.17 g) from a nursery raceway previously stocked at 5,000/m^3. Shrimp were fed four times a day, seven days a week using a commercial 35% crude protein feed (Hyper-Intensive 35) donated by the feed manufacturer (Zeigler Bros., Gardners, PA). This feed was specially formulated for intensive systems operated with limited discharge. During the first week this feed was mixed with the feed used in the previous nursery trial (Fry #4, 30% CP, Rangen Inc., Buhl, ID) for smooth transition into the new feed. From day 19 on, 2/3 of the ration was fed at four equal portions during the day while the rest was fed throughout the night using belt feeders. Daily rations were adjusted based on an assumed FCR of 1:1.4, growth of 1.4 g/wk and a mortality rate of 0.5%/wk. Use of the ST and the FF was initiated 23 days after the study began. Foam fractionators and settling tanks were operated intermittently, targeting culture water TSS concentrations between 400 and 500 mg/L and settleable solids between 10 and 14 mL/L. Water flow into the settling tanks varied between 2 and 6 L/min. Raceways were maintained with zero water exchange throughout the study. Municipal chlorinated fresh water was added to compensate for water loss due to evaporation and operation of the FFs.

Water temperature, salinity, dissolved oxygen, and pH were monitored twice daily using a YSI 600 Series multiprobe (YSI Inc. Yellow Springs, OH). Alkalinity and settleable solids were monitored every two to three days. Turbidity, total algae count, TSS, VSS, $cBOD_5$, TAN, NO_2, NO_3, and ortho-phosphate were monitored weekly. Alkalinity and pH were controlled by adding sodium bicarbonate, targeting an alkalinity of 160 mg/L $CaCO_3$. Each raceway was equipped with a YSI 5200 multi-parameter monitoring system donated by YSI Inc. (Yellow Springs, OH). Data collected by the monitoring system was uploaded to a computer in the lab which could also be accessed from remote locations for real time monitoring of the DO in the culture system. Daily and weekly water quality data from the two treatments was analyzed by Repeated Measures ANOVA. Shrimp mean final weight, weekly growth rate, survival, FCR, and total yields were analyzed using one-way ANOVA. A significance level of 0.05 was used for all statistical tests.

Oxygen supplementation did not begin until Day 68 of the study. For a period of 40 days (Days 68 through 108) oxygen was used only intermittently, specifically for 30-60 minutes following the day time feeding and only if DO levels dropped below 3 mg/L, at a flow rate of l.0 LPM. During the final week (Day 102 through termination at Day 108), supplemental oxygen was used 24 hr/day at a flow rate of 0.3-0.5 LPM. Total oxygen consumption was 37.0 L/kg of shrimp produced.

A small, but statistically significant difference, was found in salinities between treatments, where the RWs operated with the FFs had lower salinity than the RWs operated with the ST (30.3 vs. 30.8 ppt, see Table 1). Furthermore, although alkalinity in the raceways was readjusted at least twice a week to the level of 160 mg/L $CaCO_3$, the mean alkalinity in RWs operated with the FFs was significantly lower than the RWs operated with the STs (124 vs. 129 mg/L $CaCO_3$). Aside from these small differences, no other differences were found between treatments in the daily water quality indicators monitored in this study. Statistical analyses of the weekly water quality indicators from this study showed significantly higher NO_3 level in the RWs operated with FF (1,027 vs. 855 mg/L, see Table 2). It is also interesting to note that the nitrate on the day of termination in the RWs operated with FF reached a concentration of 2,028 mg/L while the concentration in the RWs operated with the ST was only 1,585 mg/L. This may be attributed to denitrification occurring at the anaerobic, sludge-water interface in the bottom of the settling tanks. These were the only differences found among the weekly water quality indicators monitored in this trial. Total ammonia nitrogen and NO_2 levels in all four raceways remained very low throughout the study, less than 0.5 mg/L during the last few weeks of the study. Bacterial floc and algae were found in the culture medium throughout the study. Settleable solids reached a concentration of 33 mL/L in one of the raceways on Day 43, but for the most part, concentrations ranged between 10 and 30 mL/L. Although TSS concentrations as high as 790 mg/L were recorded on one occasion, efforts were made to keep the concentrations within the 400 to 500 mg/L range. A large portion of the TSS was in the form of VSS. Unlike the *Vibrio* outbreak experienced in the previous year, no signs of bacterial infection were found during this trial. As was the case in the 2007 study, the results from this current trial demonstrate that a shrimp biomass load of about 7.5 kg/m^3 can be maintained with only occasional oxygen supplementation (e.g., providing oxygen at a rate of 1.0 L/min for 30 to 60 minutes after feeding). Although not required by the shrimp, as the biomass load increased above 7.5 kg/m^3, some additional oxygen was supplemented at a rate of 0.1-0.3 L/min during certain nights (pending bad weather and possible power outage) to maintain the researchers' peace of mind. The YSI 5200 units were a valuable tool for the management of super-intensive shrimp culture with zero water exchange. The real-time DO data allowed the use of far less oxygen than in previous trials. The oxygen supplementation regime described above, combined with continuous supplementation of 0.3-0.5 L/min for the final week of growth, allowed us to produce food-size shrimp with yield as high as 9.75 kg/m^3.

Table 1. Mean values of daily water quality indicators.

RW ID & Treatments	Temp. (C)		DO (mg/L)		pH		Salinity
	a.m.	p.m.	a.m.	p.m	a.m.	p.m	(ppt)
RW 2 & 3: FF[1]	29.0	29.7	5.2	4.7	6.9	6.8	30.3[a]
RW 1 & 4: ST[2]	28.8	29.6	5.2	4.8	6.9	6.8	30.8[b]

[1] RW's operated with foam fractionators (FF);
[2] RW's operated with settling tanks (ST).

Table 2. Mean values of weekly water quality indicators.

Treatments	TAN	NO_2	NO_3	$cBOD_5$	ALK	TSS	VSS	SS	Turb.
	(mg/L)							(mL/L)	(NTU)
RW 2 & 3: FF[1]	0.15	0.31	1,027[a]	28	124[a]	499	233	15	219
RW 1 & 4: ST[2]	0.15	0.28	855[b]	29	129[b]	429	200	14	213

[1] RW's operated solely with foam fractionators (FF).
[2] RW's operated solely with settling tanks (ST).

The analyses of shrimp performance based on the data obtained at harvest suggest no statistically significant differences in shrimp final mean weight between treatments. Survival rates in all four raceways were high (between 94.5 and 96.8%). Weekly growth varied between 1.35 and 1.39 g/wk. FCR varied between 1.53 and 1.60. Mean final shrimp weight ranged between 21.9 and 22.4 g. Shrimp yield varied between 9.34 and 9.75 kg/m^3. Although a yield as high as 9.75 kg/m^3 was recorded in one of the raceways operated with FF, no statistically significant difference was found between treatments. Water use in the four raceways varied between 98 and 126 L for each kg of shrimp produced (Table 3).

Table 3. Summary of final means for weight, weekly growth, yield, survival, FCR and water usage from the grow-out study with *Litopenaeus vannamei* in greenhouse-enclosed raceways operated with no water exchange.

RW ID	Final Weight (g)	Growth (g/wk)	Yield (kg/m^3)	Survival (%)	FCR	Water Use (L/kg shrimp)
Settling Tank (RW 1)	21.96	1.36	9.34	94.54[a]	1.60	126
Settling Tank (RW 4)	21.81	1.39	9.52	94.51[a]	1.57	107
Foam Fractionator (RW 2)	22.51	1.35	9.51	96.86[b]	1.53	108
Foam Fractionator (RW 3)	22.40	1.39	9.75	96.26[b]	1.57	98

This study showed that market size shrimp can be produced with zero water exchange. The reuse of the same water that served for the nursery study provided for the immediate establishment of a healthy nitrifying microbial community in the grow-out study. This, along with the specially formulated feed provided by Zeigler Brothers Inc. allowed for negligible amounts of ammonia-nitrogen or NO_2 in the culture water throughout the grow-out study.

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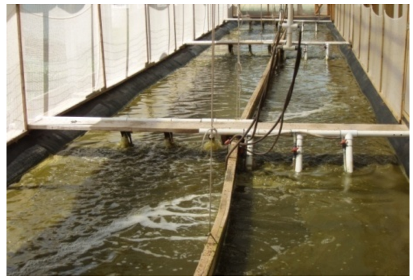

Figure 1: A general view of a raceway full of water

Figure 2: A general view of an empty raceway

Figure 3: View of the center partition, the spray nozzles and one of the air diffusers

Figure 4: A view of the raceway deep end – Note the presence of only one screened outlet to pump water from the raceway.

Figure 5: View of four units for DO monitoring

Figure 6: View of Imhoff cones with bacterial flocs.

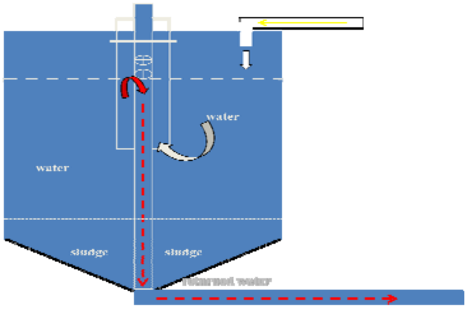

Figure 7: A schematic drawing of the settling tank

Figure 8: A general view of a foam fractionator

Figure 9: Shrimp taken from a raceway one month after the termination of the study the four raceways summarized above