# Use Of Molasses As Carbon Source In Limited Discharge Grow-out Systems For *Litopenaeus vannamei*

T. M. Samocha[1], S. Patnaik[1], J. M. Burger[1], R. V. Almeida[1], A. M. Ali[2], Z. Ayub[1], M. Harisanto[1], A. Horowitz[3], and D. L. Brock[4]

[1] TAES, Shrimp Mariculture Research Facility, Corpus Christi, TX
[2] UNM, Earth & Planetary Sciences Department, Albuquerque, NM
[3] UPAH Tec. Shaker Heights, OH
[4] Rangen Inc., Buhl, ID

Texas Agricultural Experiment Station
THE TEXAS A&M UNIVERSITY SYSTEM

# Introduction

- Increased environmental regulations and loss of crops due to viral disease outbreaks have created a demand for more sustainable shrimp production practices
- These methods call for raising the shrimp under limited water exchange with careful monitoring of water quality
- Feed, feed management, and water treatment of the culture systems can affect water quality and consequently the shrimp performance
- Thus, optimization of protein utilization and manipulation of microbial communities can improve water quality and shrimp yields while reducing feed costs

# Introduction

- Findings by several researchers suggest that autotrophic and heterotrophic microbial communities play a major role in limited discharge intensive shrimp culture systems, particularly in maintaining optimum water quality, enhancing natural productivity and nutrient cycling
- Other studies suggest that detritus-rich water from limited discharge systems can enhance shrimp growth
- Beside the nutritional benefit from bacterial flocs, the use of carbon (C) supplementation was suggested as a management tool to control nitrogen species buildup in the culture medium

# Objectives

- Evaluate the effect of carbon supplementation during the nursery and grow-out phases on water quality and the performance of *Litopenaeus vannamei* under limited water discharge conditions

# Materials and Methods

- Nursery study was conducted in four 40-$m^3$ greenhouse-enclosed raceways, while grow-out trial were conducted in twenty-four outdoor 7.9-$m^3$ tanks and in two 2,000-$m^3$ lined ponds at the TAES, Shrimp Mariculture Research Facility, Corpus Christi, Texas
- All three systems were operated with limited discharge
- Poor survival in the ponds due to *Vibrio harveyi* outbreak precluded evaluation of the carbon supplementation on WQ and shrimp performance

# Materials and Methods - Nursery

- A 58-d trial in four RWs stocked at 1,800 $PL_{9\text{-}12}/m^3$
- All RW's were equipped with pressurized sand filters
- Two RWs were provided with foam fractionators and were operated with 1.53% daily water exchange
- The other two RWs had no FF and were operated at 2.62% daily water exchange
- Shrimp were fed mostly 45% CP diet (Rangen Inc., Buhl, Idaho), distributed four times a day seven days a week

# Materials and Methods - Nursery

- DO, temp., pH and salinity - twice daily
- Turbidity, settleable solids, and algal counts - once daily
- $NH_4$-N & $NO_2$-N daily from day 31 on
- $cBOD_5$, reactive phosphorous, TSS, VSS – weekly
- Molasses (24% C) was added (starting 31 days after stocking) only when $NH_4$-N levels were $> 1$ mg/L (assuming 6 g C is needed to convert 1 g of $NH_4$-N to bacterial biomass)

# Materials and Methods – Grow-out

- A 84-day trial in twenty-four 10.5-$m^2$ (7.9-$m^3$) tanks
- Tanks were filled with chlorinated seawater (30 ppt), positioned under a shade and provided aeration from 10 airstones/tank (6-8 L/min/stone)
- Stocking: 81 juveniles/$m^3$ (6.2±0.3 g)
- Use of two commercial diets made by Rangen;
  - "30% Eco", a cost-effective 30% CP diet, and
  - A regular 45% CP diet (fed at iso-N to the 30% diet)
- Feed was distributed four times daily seven days a week

# Tank Study - Experimental Design

| Treatment ID | n | CP (%) | C | Iso-N | Assumed (g/wk) | Assumed FCR | Ration |
|---|---|---|---|---|---|---|---|
| 30% Eco-0% | 4 | 30 | 0% | - | 1 | 1:1.5 | 100% |
| 30% Eco-50% | 4 | 30 | 50% | - | 1 | 1:1.5 | 100% |
| 30% Eco-100% | 4 | 30 | 100% | - | 1 | 1:1.5 | 100% |
| 30% Eco-150% | 4 | 30 | 150% | - | 1 | 1:1.5 | 100% |
| 30% Eco-adj | 4 | 30 | Adj. | - | 1 | 1:1.5 | 100% |
| 45% -100% | 4 | 45 | 100% | + | 1 | 1:1 | 66.6% |

# Diets & Feed Management – Grow-out

- Molasses was provided at 0%, 50%, 100%, and 150% of the estimated carbon needed to convert the $NH_4$-N into bacterial biomass assuming 50% of the nitrogen contributed by the feed is converted into ammonium-N
- Carbon supplementation levels in the adjustable treatment (Raceways & Tanks) were based on the actual $NH_4$-N concentrations in the water assuming the conversion of 1 g of $NH_4$-N into bacterial biomass requires 6 g of carbon

# Calculation & Assumptions – Carbon Supplementation

1. A 100 g ration (30% CP) = 4.8 g N (30 g / 6.25) = 2.4 g $NH_4$-N (4.8 g/2)
2. To convert 2.4 g $NH_4$-N into bacterial biomass, 14.4 g C is needed (2.4 g x 6 g)
3. The 50%, 100% & 150% C supplementation treatments received daily application of: 7.2 g, 14.4 g & 21.6 g C, respectively
4. 1,000 ml molasses = 1,300 g = 312 g C (1,300 x 24%)
5. 14.4 g C requires 46.15 ml of molasses (1,000 x 14.4 / 312)
6. Adjustable treatment received daily C application based on actual $NH_4$-N level in the tanks provided $NH_4$-N > 1 mg/L

# Materials and Methods – Grow-out

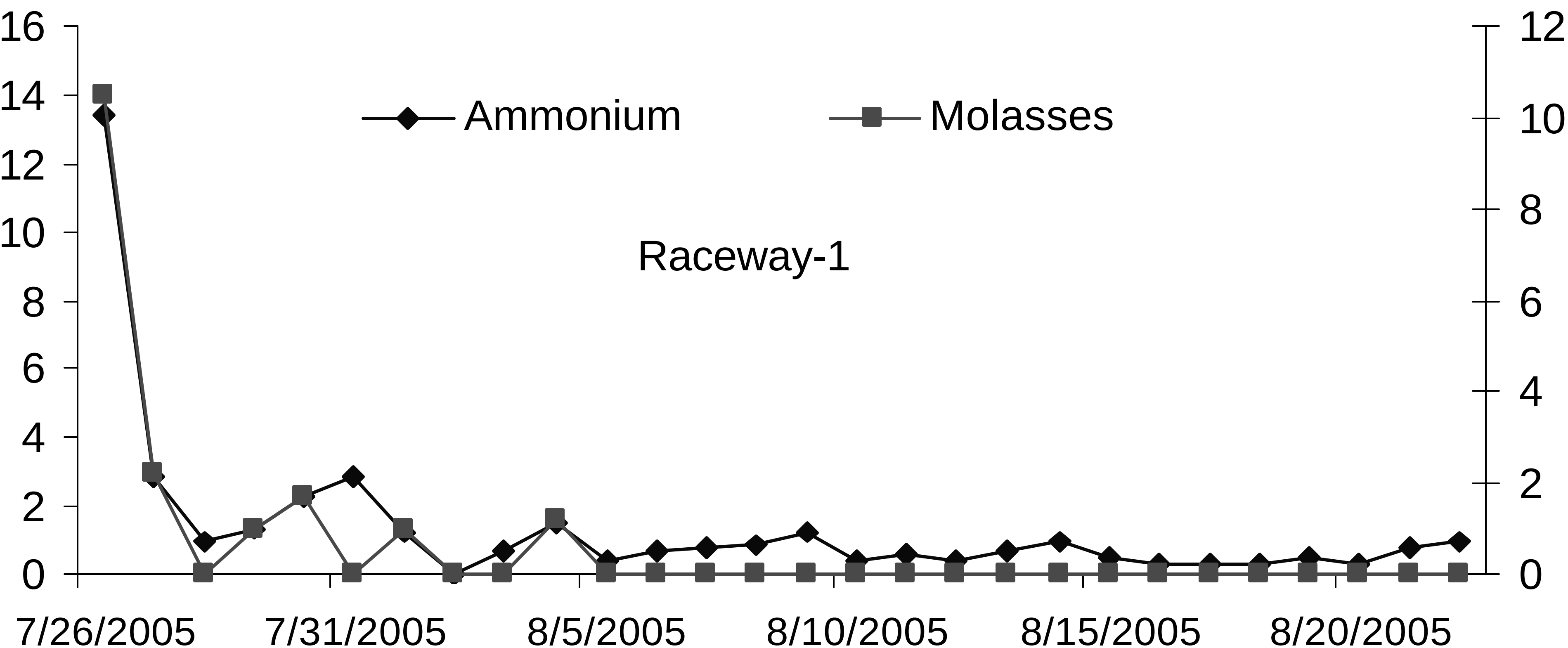
- DO, temp., pH and salinity - twice daily in all tanks
- $NH_4$-N, $NO_2$-N: daily in all tanks from day 26 on
- $cBOD_5$, COD, TSS, VSS, turbidity, reactive phosphorous (RP), settleable solids (SS), & algal counts - weekly in all tanks
- Except for emergency releases due to heavy rains, no water was discharged from the tanks
- Municipal freshwater was added to compensate for evaporation and to maintain salinity
- The study was terminated prematurely due to hurricane Rita

# Materials and Methods

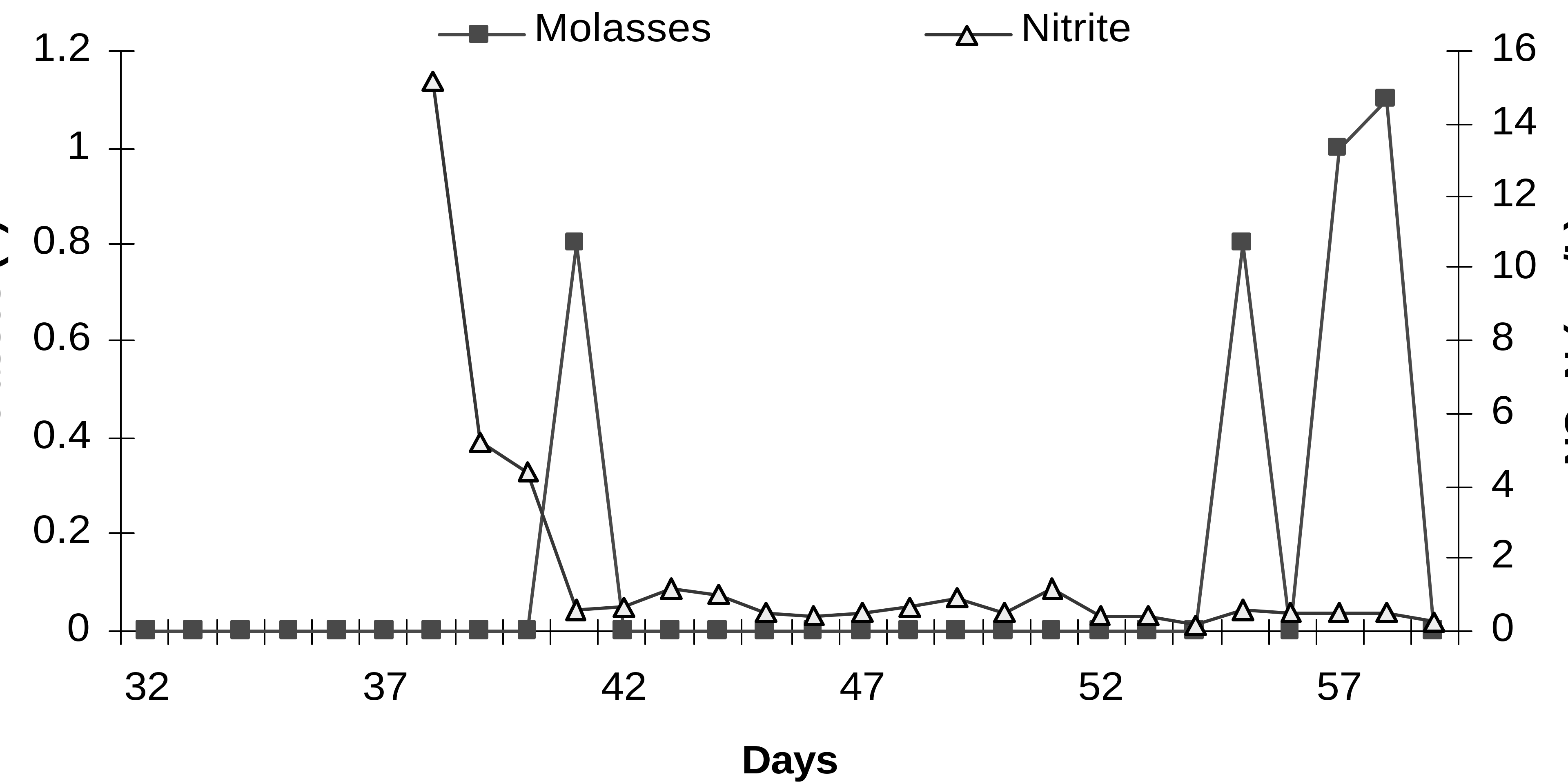
- Data were analyzed using SPSS software
- A significance level of $\alpha=0.05$ was used for all tests
- Repeated Measures ANOVA to identify differences between treatments in daily and weekly WQ
- One-way ANOVA to identify differences between treatments for survival (arcsine transformed), mean final weight and FCR followed by LSD and SNK tests

# Molasses Supplementation & $NH_4$-N - Nursery

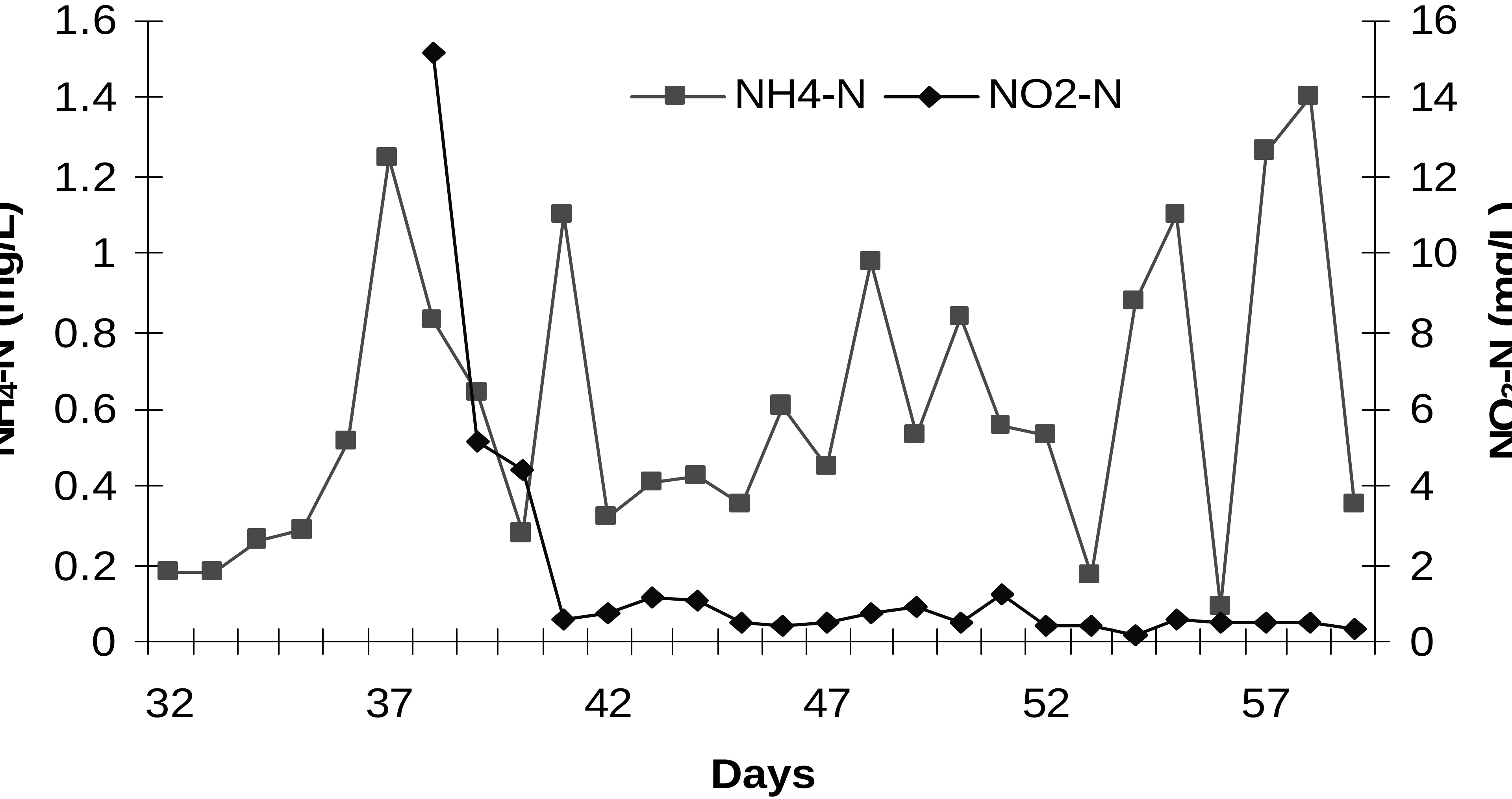
**Molasses can be used to control $NH_4$-N in nursery RWs with limited discharge**



# Relationship Between Molasses & $NO_2$-N - Nursery



# Changes in $NH_4$-N & $NO_2$-N Due To Application Of Molasses



# Results-Nursery

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| FF[3] | 51 | 3.5 | 4.0 | 4.7 | 126 | 72 | 1.5 | 39 | 403 |

[3] 2005 results: RWs operated with FF (71-d, 2.89%/d, 3,600 PL/m$^3$)

# *Litopenaeus vannamei* Performance In A 58-d Nursery Trial In Greenhouse-enclosed Raceways Operated With Different Water Exchange Rates

| Treatment | $Wt_o$ (mg) | $Wt_f$ (g) | Yield ($kg/m^3$) | Survival (%) |
|---|---|---|---|---|
| FF[1] | 20 | 1.32 | 0.36 | 12.6 * |
| FF[1] | 20 | 1.74[a] | 2.96[a] | 92.5[a] |
| WE[2] | 20 | 2.01[b] | 3.14[b] | 85.3[a] |
| WE[2] | 20 | 1.99[b] | 3.73[b] | 99.5[a] |
| FF[3] | 0.6 | 1.91 | 6.79 | 100 |

1 Raceway operated with 1.53% daily water exchange

2 Raceway operated with 2.62% daily water exchange

3 2005 results: RW's operated with FF (71-d, 2.89%/d, 3,600 PL/$m^3$)

* Mortality due to mechanical failure

# Results – Grow-out

Before adding the molasses:

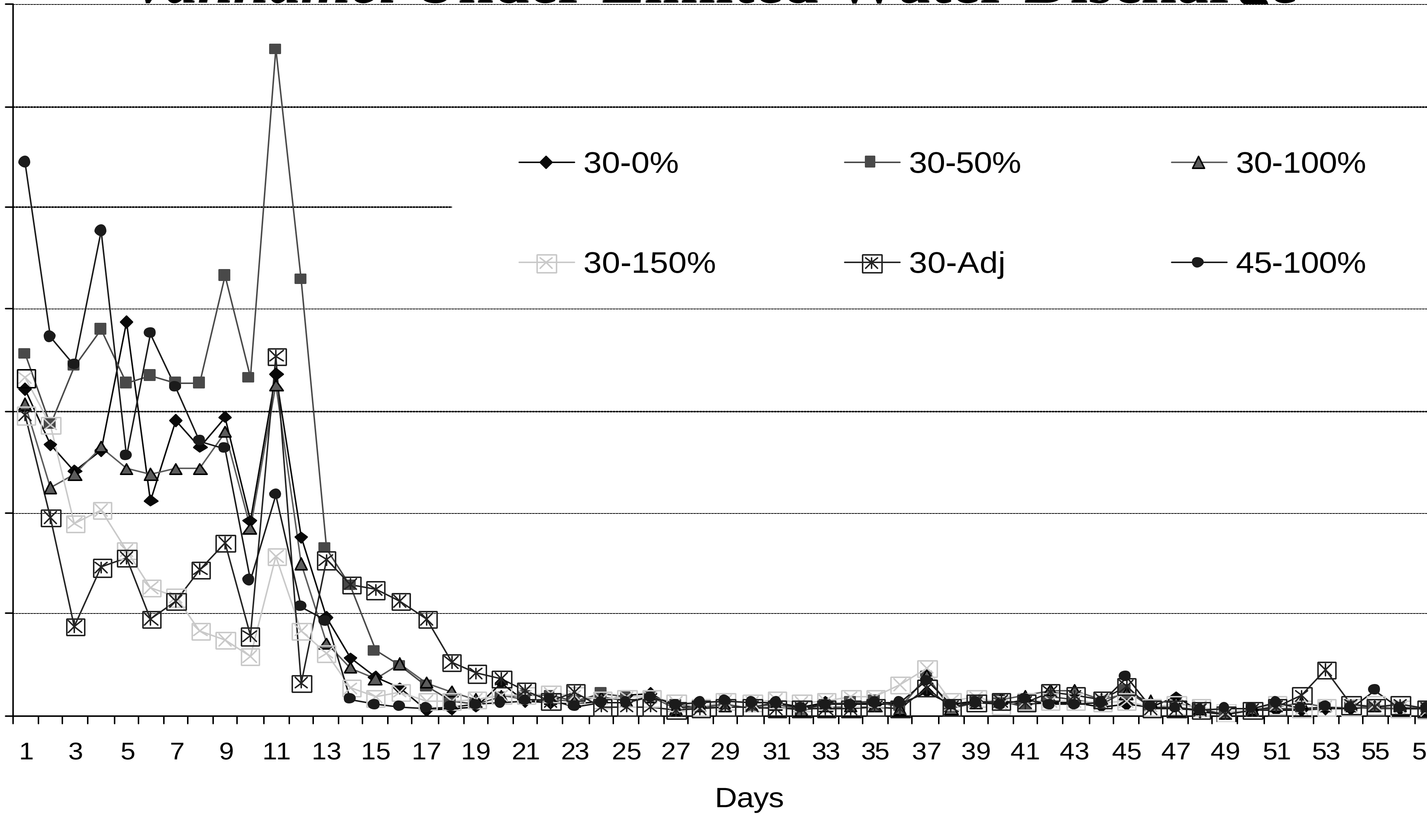
- No statistically significant differences were found between all treatments in nitrite, RP, COD, $cBOD_5$, SS & algal counts
- The $NH_4$-N and turbidity levels in the 45% CP treatment were statistically significantly higher than all other 30% CP diet treatments except for the 30% Eco-150% treatment

# Results – Grow-out

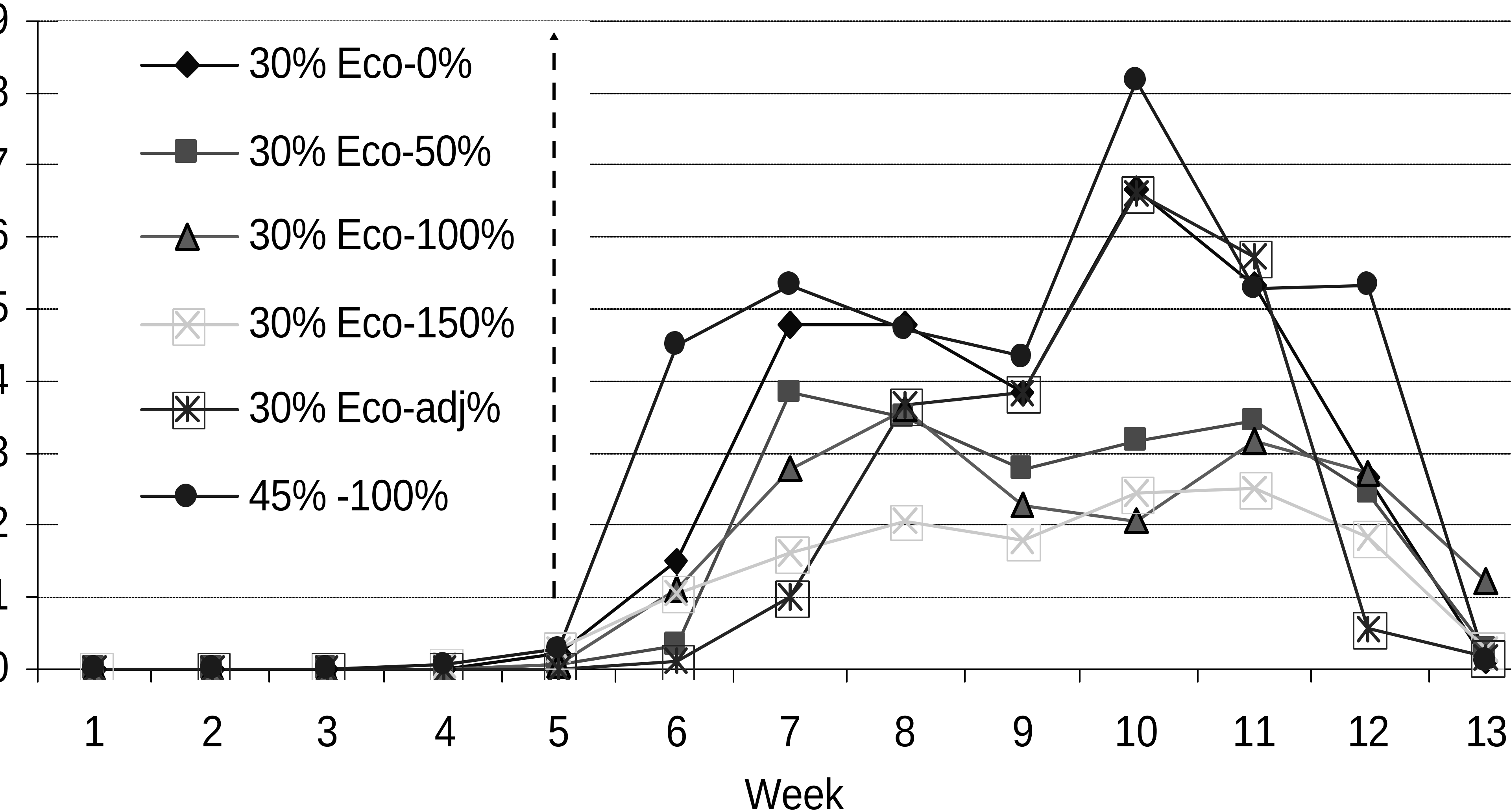
After adding the molasses

- The SNK test showed no statistically significant differences in daily $NH_4$-N levels between treatments
- The RP levels in the 45%-100% were significantly lower than all other 30% treatments
- The $NO_2$-N levels in the 45%-100% were significantly higher than the 30% Eco-150% treatment only
- Turbidity of 30% Eco-adj treatment was significantly lower than all other treatments, in addition, the 45%-100% treatment was significantly higher than 30% Eco-100% treatment
- No significant differences were found between all treatments in COD, $cBOD_5$, TSS, VSS, SS & Algal counts

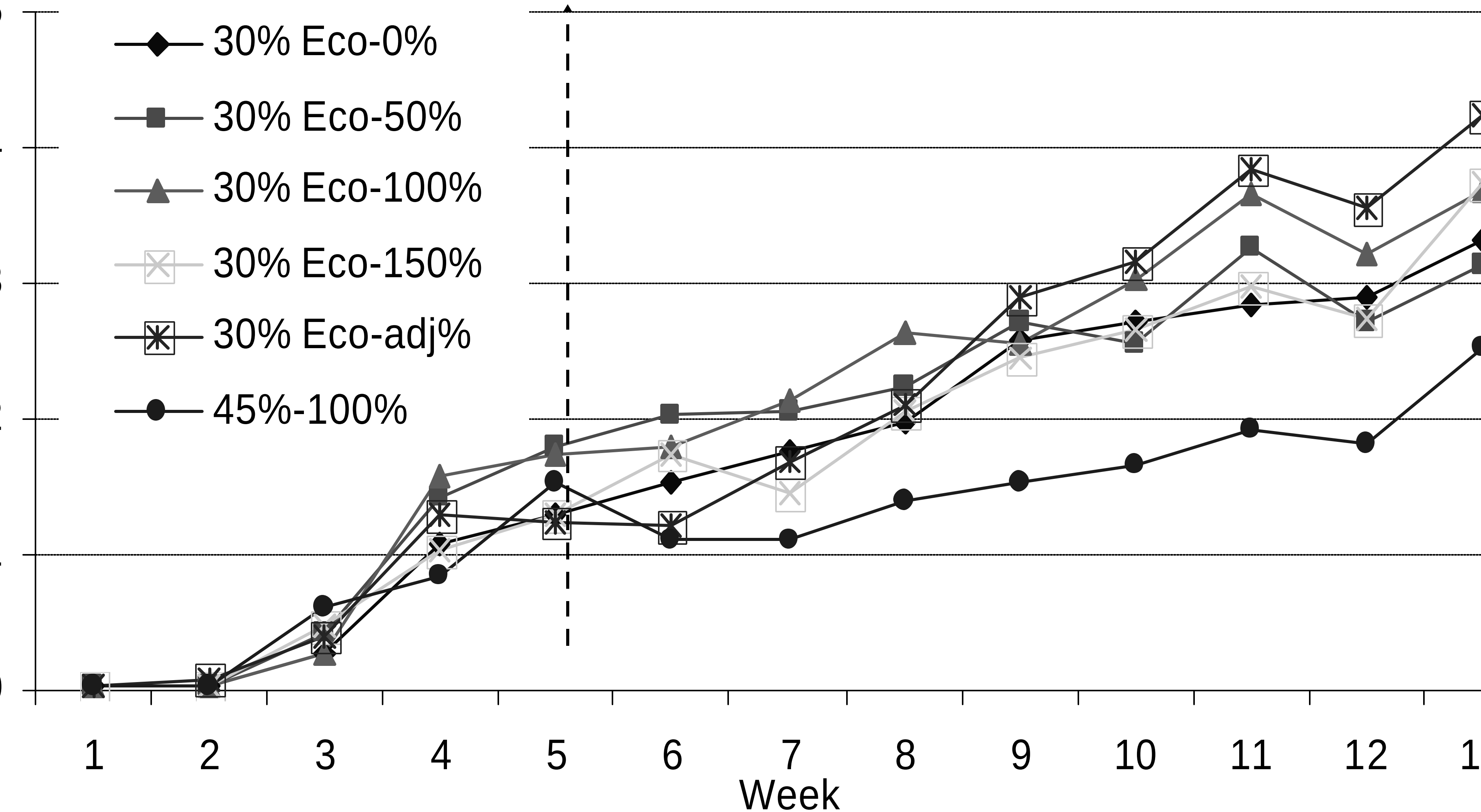
# Changes in $NH_4$-N Levels With & Without Molasses Applications In Tanks Stocked With *Litopenaeus vannamei* Under Limited Water Discharge



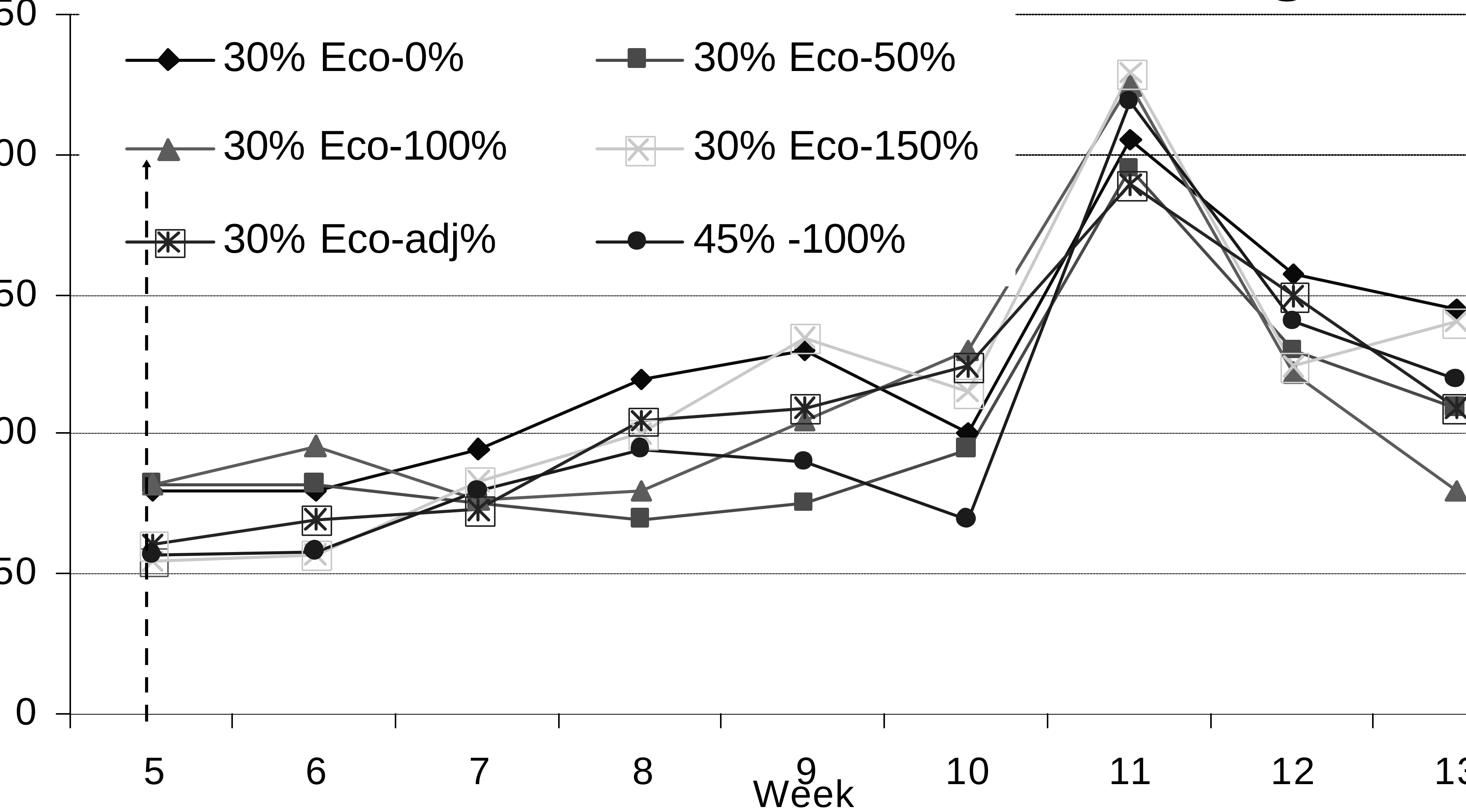
# Changes In $NO_2$-N Levels With & Without Molasses Applications In Tanks Stocked With *Litopenaeus vannamei* Under Limited Water Discharge



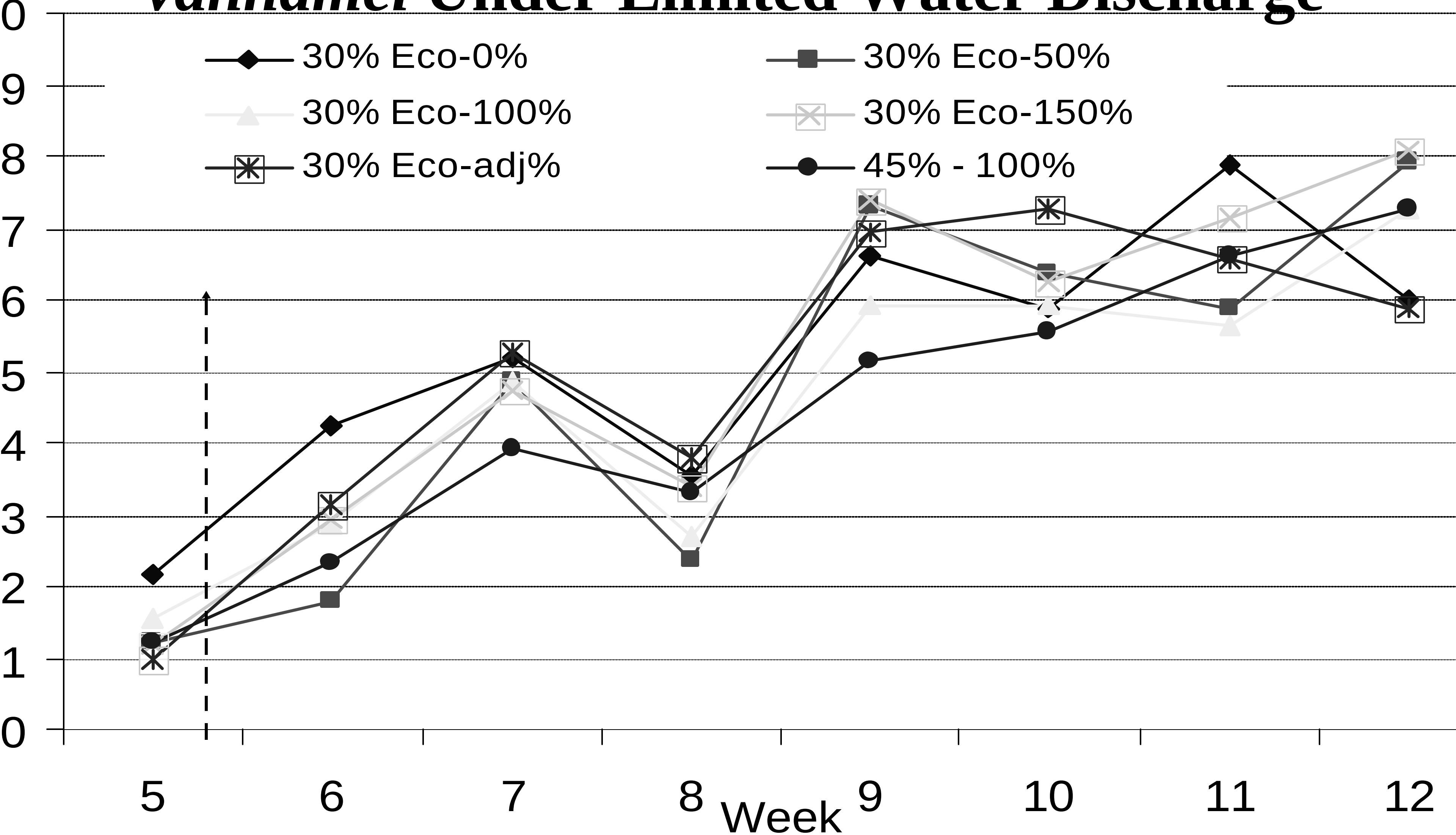
# Changes In RP Levels With & Without Molasses Applications In Tanks Stocked With *Litopenaeus vannamei* Under Limited Water Discharge



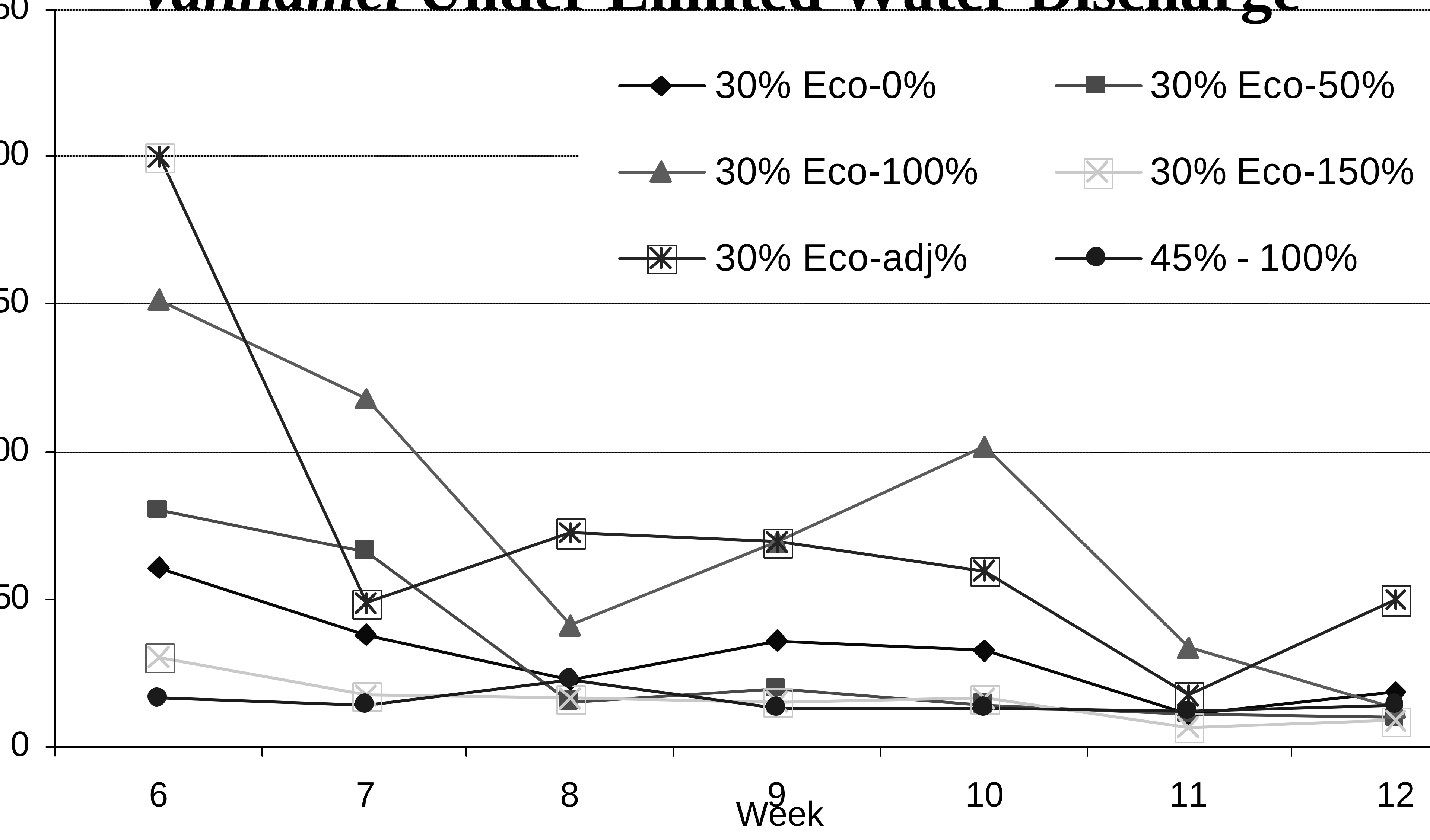
# Changes In TSS Levels With & Without Molasses Applications In Tanks Stocked With *Litopenaeus vannamei* Under Limited Water Discharge



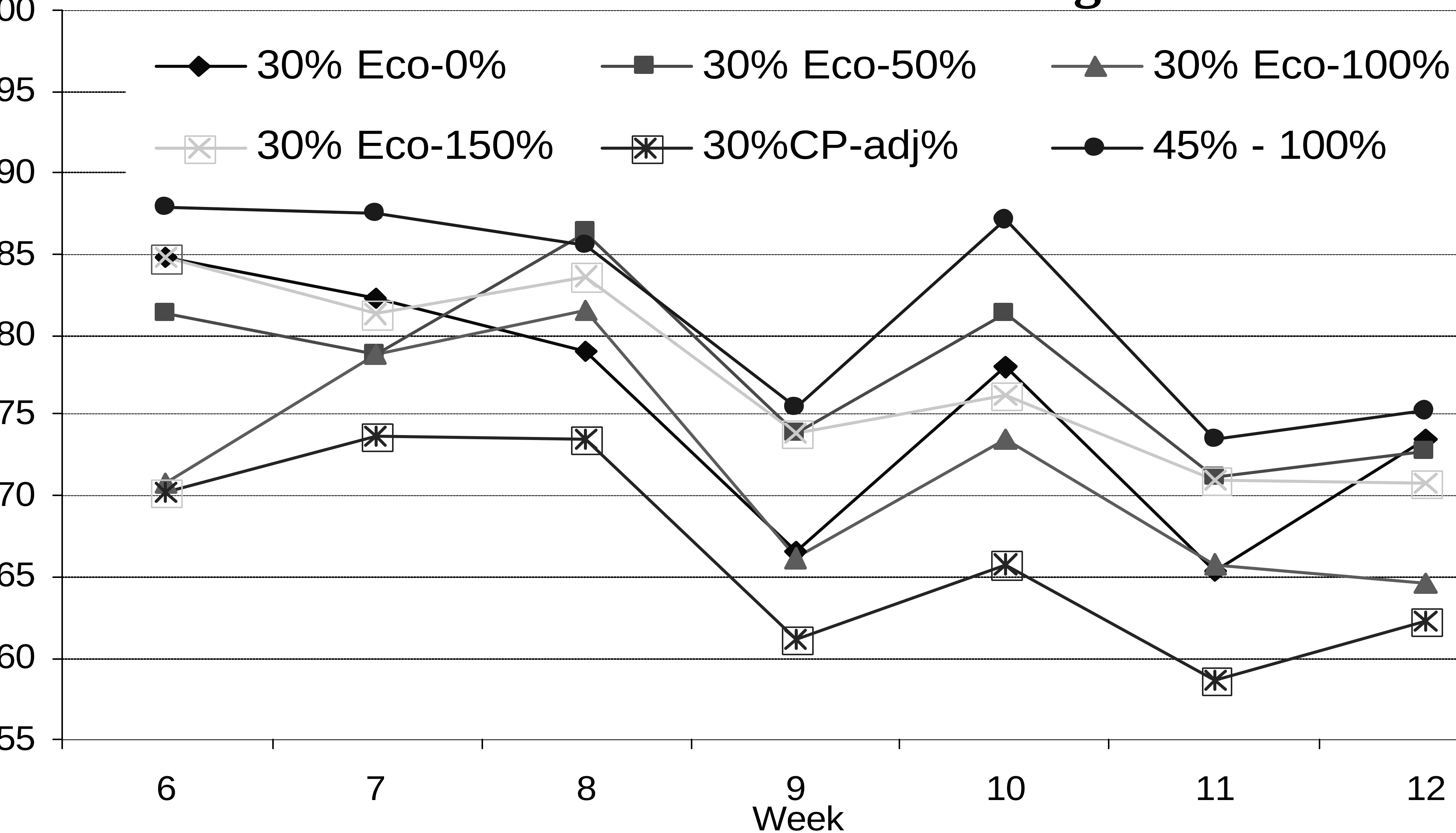
# Changes In SS Levels With & Without Molasses Applications In Tanks Stocked With *Litopenaeus vannamei* Under Limited Water Discharge



# The Effect Of Different Levels Of Molasses On Algal Counts In Tanks Stocked With *Litopenaeus vannamei* Under Limited Water Discharge



## Changes In Turbidity Levels With & Without Molasses Applications In Tanks Stocked With *Litopenaeus vannamei* Under Limited Water Discharge



# Results – Grow-out

- No significant differences in shrimp survival between treatments
- The SNK test showed significantly higher mean weekly growth & final weights of the shrimp fed the 30% CP diet than the shrimp fed the 45% CP
- No statistically significant differences were found between the 30% CP diet treatments in shrimp mean weekly growth, final weights, yields, FCR and survival

# The Effect Of Molasses Applications And Diets (35% & 45% CP) On *Litopenaeus vannamei* In Tanks System Operated With Limited Water Discharge

| Treatment CP–Ration | N | Av. $Wt_f$ (g) | Yield ($kg/m^3$) | Sur. (%) | Growth (g/wk) | FCR | N. Water (%/day) |
|---|---|---|---|---|---|---|---|
| 30% CP-0% | 4 | 16.4[a] | 0.79[a] | 99.0[a] | 0.89[a] | 1.68[a] | 0.39 |
| 30% CP-50 % | 4 | 17.8[a] | 0.70[a] | 86.5[a] | 1.02[a] | 2.04[a] | |
| 30% CP-100 % | 4 | 16.7[a] | 0.76[a] | 95.7[a] | 0.90[a] | 1.79[a] | |
| 30% CP-150 % | 4 | 16.5[a] | 0.76[a] | 95.0[a] | 0.91[a] | 1.79[a] | |
| 30% CP-adj | 4 | 16.8[a] | 0.72[a] | 89.5[a] | 0.94[a] | 1.96[a] | |
| 45% CP-100% | 4 | 12.9[b] | 0.54[a] | 97.5[a] | 0.63[b] | 1.66[a] | |

# Conclusion – Grow-out

- Under the conditions of this study, the 30% CP diet outperformed the 45% CP diet with significantly higher shrimp mean final weight and weekly growth
- The use of molasses did not result in significant differences among treatments in the WQ indicators
- No statistically significant improvement in shrimp growth was noticed due to the use of molasses
- Since previous study showed good growth (1.9 g/wk) and FCR (1:1) when shrimp were fed the 45% CP diet, it is possible that in the presence of the high protein feed molasses had adverse effect on shrimp growth under the conditions of this study

# Conclusions

- Use of carbon in the form of molasses in the nursery phase was suitable tool to prevent increase in $NH_4$-N and $NO_2$-N in the culture water
- The shrimp grow-out tank-system could not be converted successfully into a complete heterotrophic system even with carbon supplementation

# Acknowledgements


- CSREES, USDA Marine Shrimp Farming Program and The Texas Agricultural Experiment Station for funding
- Rangen Inc., Buhl, ID for donating the feeds used in this study
- The employees of TAES Shrimp Mariculture Research Facility, Corpus Christi, TX
- Harlingen Shrimp Farms, Los Fresnos, TX & OceanBoy Farms, Clewiston, FL for the nauplii donations
- West Way, Temple, TX for donating the molasses