# Insights Into The Increased Dietary Levels of Brine Shrimp *Artemia franciscana* Co-Fed With Microparticle Diets in the Rearing of *Litopenaeus vannamei*

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# Introduction

- Most Post larvae and juveniles rely **on Artemia** (Agh and Sorgeloos, 2005) due to their underdeveloped **digestive system**.

- There is still insufficient knowledge about when to start the **Artemia co-feeding** and at what rate it should be given (Khudyi *et al., 2017).*

***Larval stage - how much??????***

- **Aims:**
- Evaluate the performance of *Litopenaeus vannamei* co fed with **increased** dietary levels of Brine Shrimp *Artemia franciscana* with microparticle diets.

**Hatchery stage**
**Nauplii V to PL8**
**PL8 to PL15**

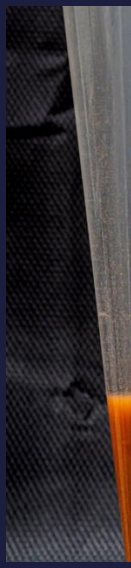
**Trial I (Medium density)**

| | |
|---|---|
| Commercial diet + | Artemia 1kg |
| MP + | Artemia 0kg |
| MP + | Artemia 1kg |
| MP + | Artemia 2kg |
| MP + | Artemia 4kg |
| MP + | Artemia 8kg |

**Hatchery and nursery stage**
**PL1 to PL15**
**PL16 to PL35**

**Trial II (High density)**

| | |
|---|---|
| Commercial diet + | Artemia 2kg |
| MP + | Artemia 1kg |
| MP + | Artemia 2kg |
| MP + | Artemia 4kg |
| MP + | Artemia 6kg |
| MP + | Artemia 8kg |



## Trial design and diets

- Completely Randomized design (CRD) 6 Treatments X 8 reps = 48 tanks
- Mackay Marine MP diet
- Commercial diet
- Artemia dry cysts (90% Hatch quality)

# Trial I

- 48 tanks, 416 Larvae/tank
- RAS: 11.5 L tanks
- Initial weight ~ 0.001 mg
- Statistical analysis: two-way ANOVA, Dunnett's

**Commercial Diet**

Art 1kg

**MP Diet**

Art 0kg

**MP Diet**

Art 1kg

**MP Diet**

Art 2kg

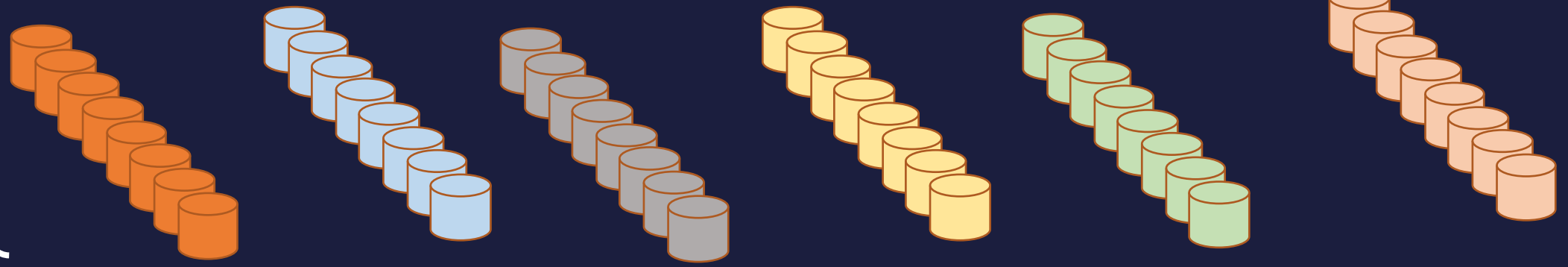
**MP Diet**

Art 4kg

**MP Diet**

Art 8kg

8 reps



# Trial II

- 48 tanks, 650 Larvae/tank
- RAS: 11.5 L tanks
- Initial weight ~ 0.001 mg
- Statistical analysis: two-way ANOVA, Dunnett's

**Commercial diet**

Art 2kg

**MP Diet**

Art 1kg

**MP Diet**

Art 2kg

**MP Diet**

Art 4kg

**MP Diet**

Art 6kg

**MP Diet**

Art 8kg

8 reps

# Algae preparation

# Methods

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**Nauplii V to Zoea II stages:**

***Thalassiosira psudonana*** was diluted in 30 ppt saltwater at 28°C to 30,000 cells/mL

**Zoea III to Mysis III stages:**

The mixture of ***Thalassiosira weissflogii*** at 15,000 cells/mL and ***Thalassiosira Psudonana*** at 15,000 cells/mL was provided

**Culture tanks**

A 12-h photoperiod was provided by artificial lighting controlled by timers.

# Methods

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## Artemia cyst hatching

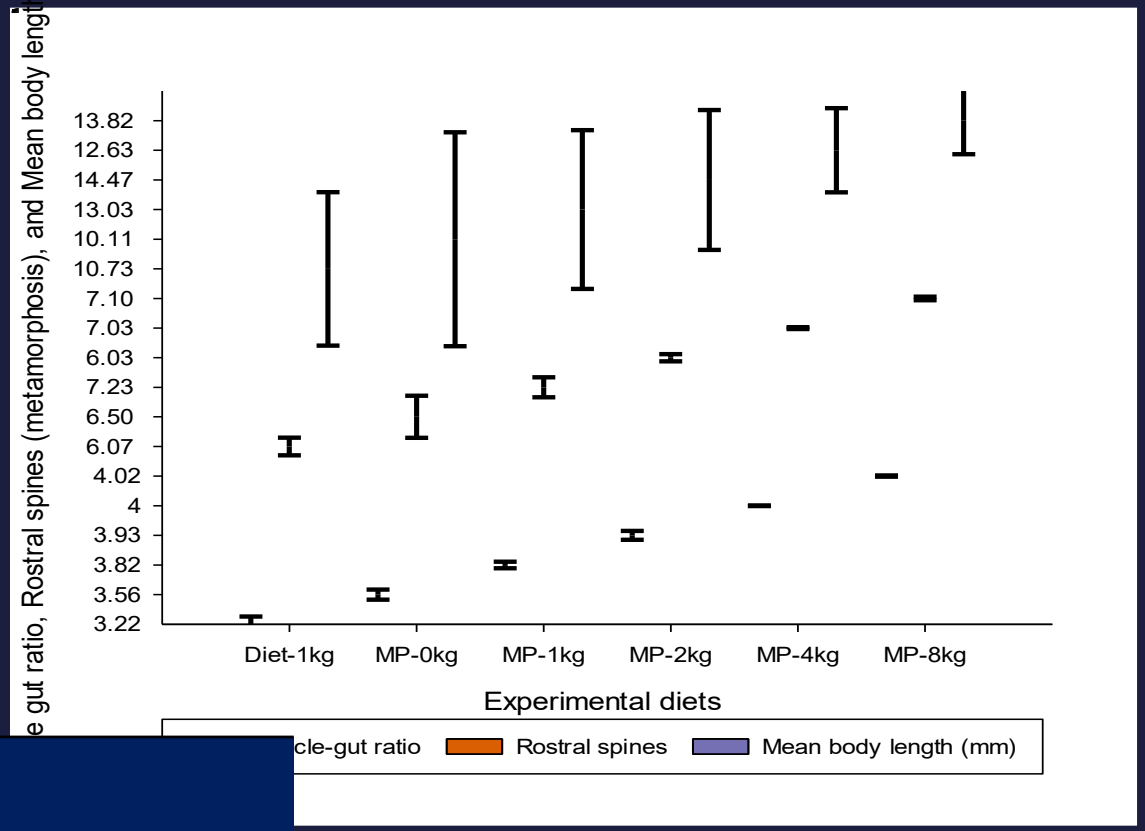
- The requirement of live Artemia nauplii (Instar I) was calculated in every trial
- Dry **Artemia cysts** at 4% moisture were obtained from **Great Salt Lake Brine Shrimp Cooperative, Inc.**
- Stocked dry cysts in **28ppt** salinity saltwater, hatched for **18h** at **temp 27°C** with a slight aeration

# Culture Conditions

- pH
- Temp
- DO
- Total Ammonia Nitrogen (TAN)
- $NO_2$
- Alkalinity

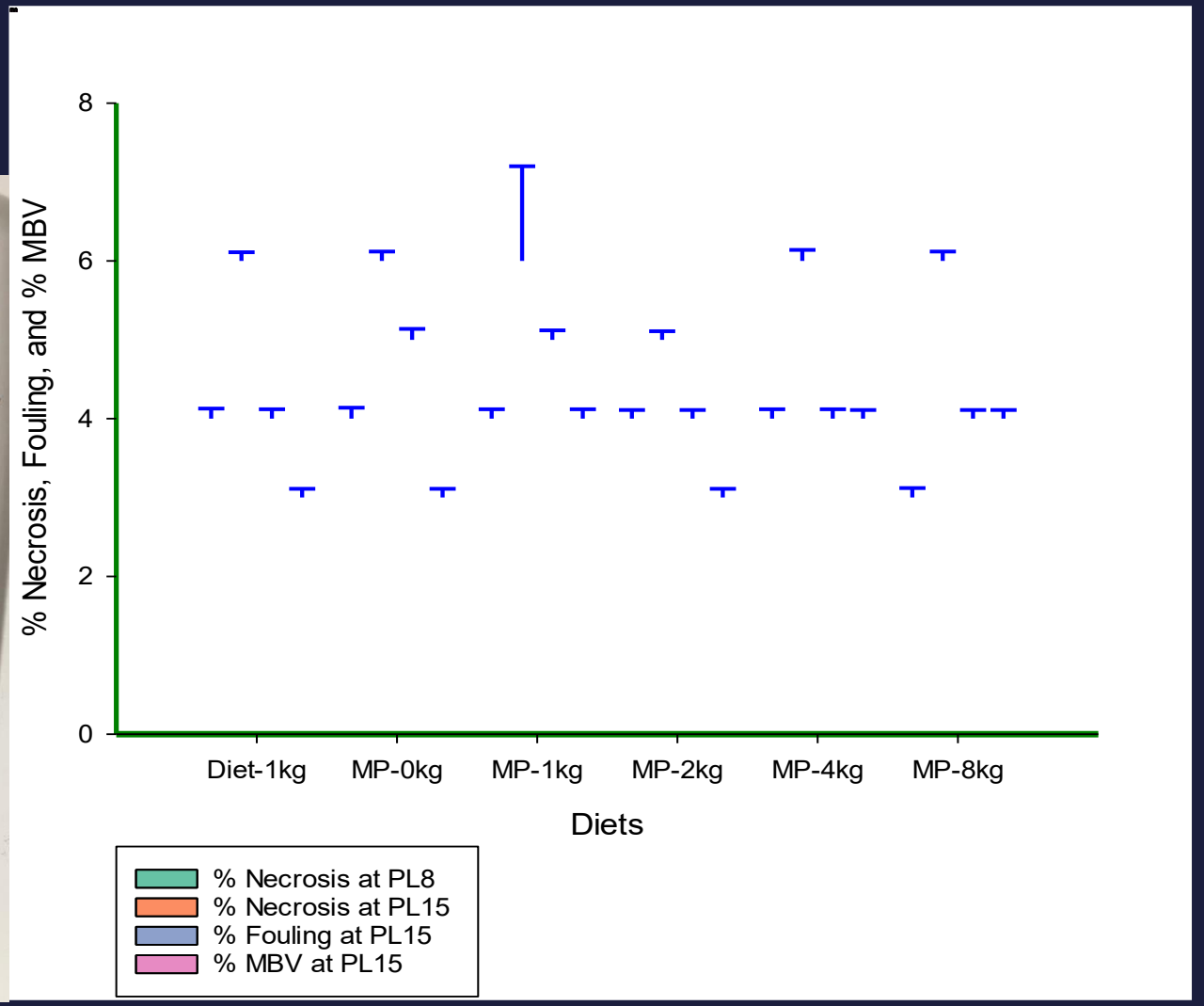
# Results: Trial I

## Health indices



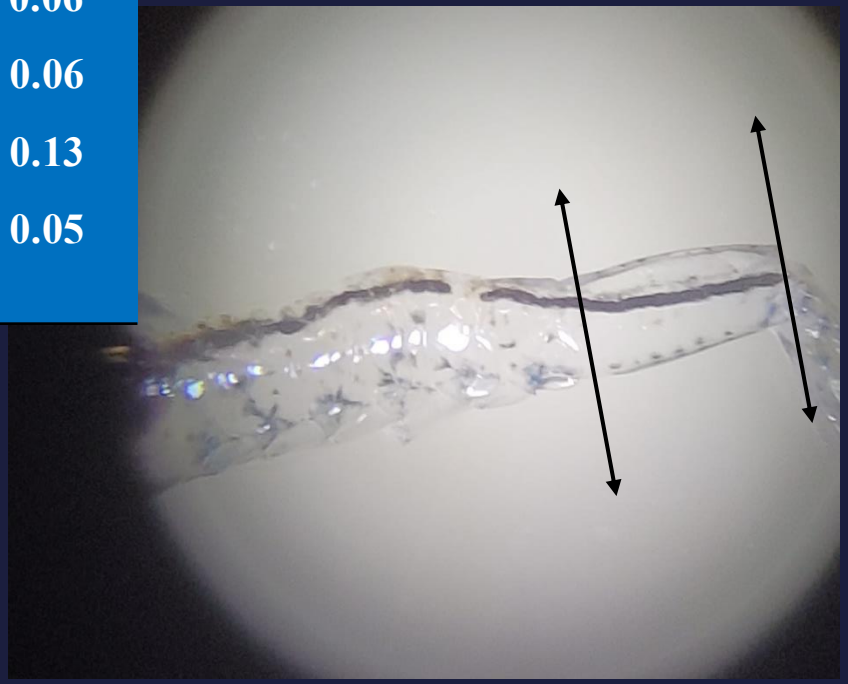

| Diet with co-feed Artemia per million | PL 8 Survival rate (%) in the salinity stress test | Mean body length (mm) | Rostral spines | Muscle-gut ratio |
|---|---|---|---|---|
| Commercial 1kg | 91.11 ± 0.20 | 10.73 ± 2.59 | 6.07 ± 0.30 | $3.22^{a}$ ± 0.26 |
| MP-0kg | 91.41 ± 0.25 | 10.11 ± 3.61 | 6.50 ± 0.71 | $3.56^{ab}$ ± 0.17 |
| MP-1kg | 89.90 ± 5.13 | 13.03 ± 2.68 | 7.23 ± 0.34 | $3.82^{b}$ ± 0.11 |
| MP-2kg | 89.17 ± 5.83 | 14.47 ± 2.36 | 6.03 ± 0.12 | $3.93^{b}$ ± 0.15 |
| MP-4kg | 91.90 ± 0.95 | 12.63 ± 1.42 | 7.03 ± 0.03 | $4.00^{b}$ ± 0.00 |
| MP-8kg | 81.49 ± 4.24 | 13.82 ± 1.13 | 7.10 ± 0.06 | $4.02^{b}$ ± 0.02 |

# Results: Trial I



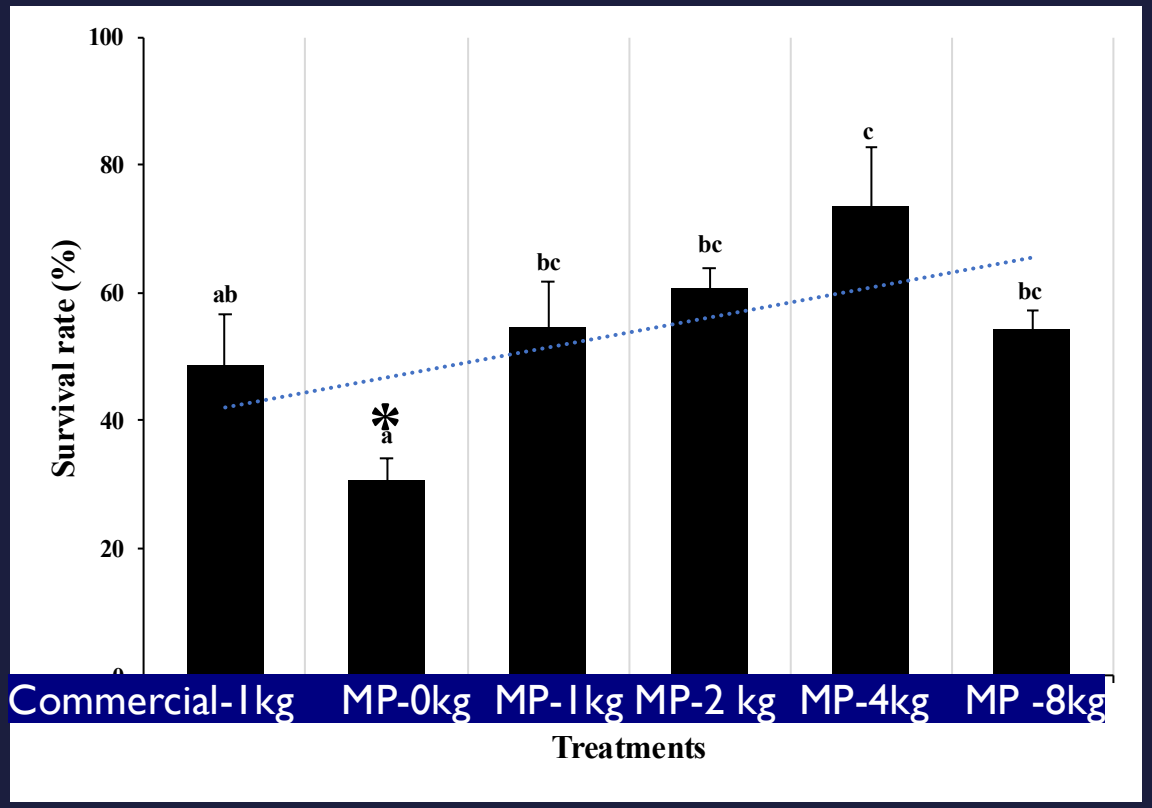
# Results: Trial I

| Diet with co-feed Artemia per million | PL 15 Survival rate (%) in the salinity stress test | Mean body length (mm) | Rostral spines | Muscle-gut ratio |
|---|---|---|---|---|
| Commercial 1kg | 96.46 ± 2.20 | $15.68^{b}$ ± 1.76 | 7.98 ± 0.03 | $3.98^{ab}$ ± 0.03 |
| MP-0kg | 95.68 ± 1.82 | $22.21^{a}$ ± 2.90 | 7.96 ± 0.02 | $3.99^{ab}$ ± 0.01 |
| MP-1kg | 92.51 ± 3.92 | $16.66^{ab}$ ± 2.98 | 7.98 ± 0.03 | $3.81^{a}$ ± 0.06 |
| MP-2kg | 92.64 ± 3.81 | $13.16^{b}$ ± 0.42 | 7.94 ± 0.02 | $4.14^{b}$ ± 0.06 |
| MP-4kg | 98.20 ± 1.11 | $11.92^{b}$ ± 0.42 | 7.70 ± 0.13 | $4.20^{b}$ ± 0.13 |
| MP-8kg | 97.93 ± 1.35 | $15.38^{b}$ ± 1.91 | 7.90 ± 0.08 | $4.06^{b}$ ± 0.05 |

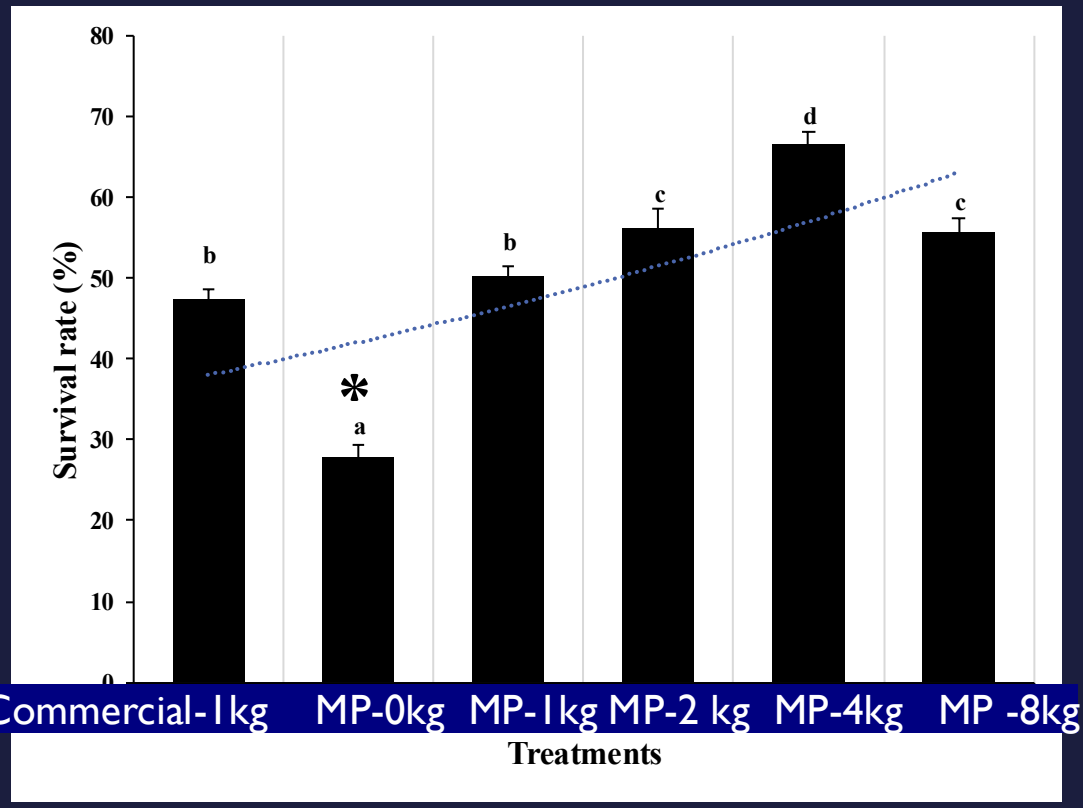


# Results: Trial I

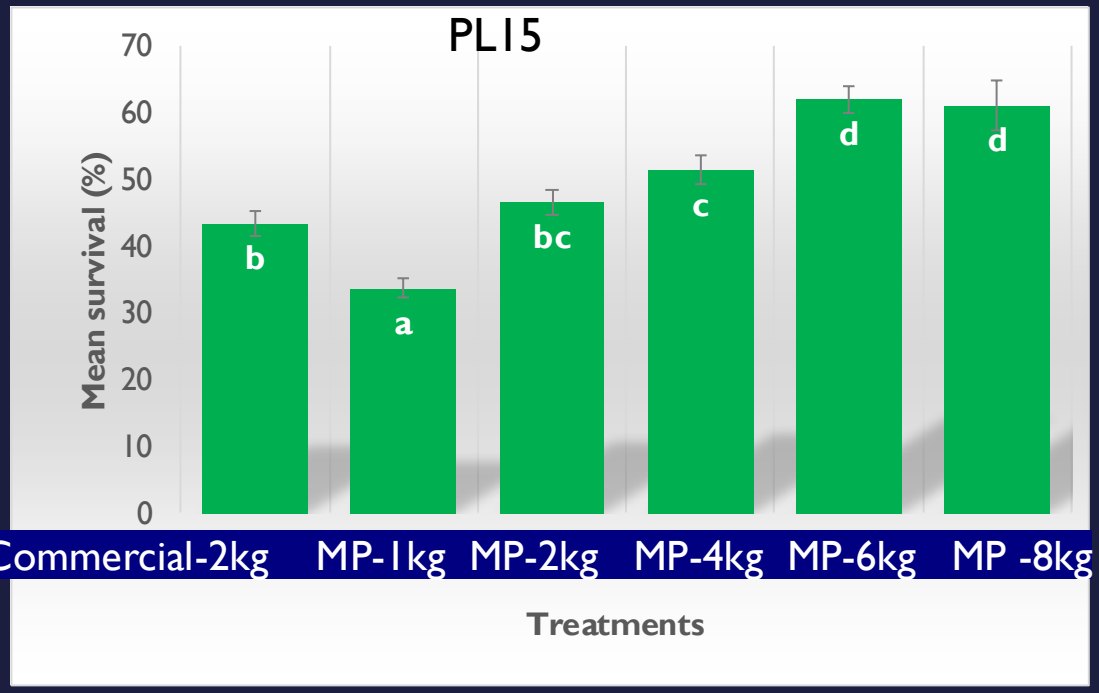
PL8

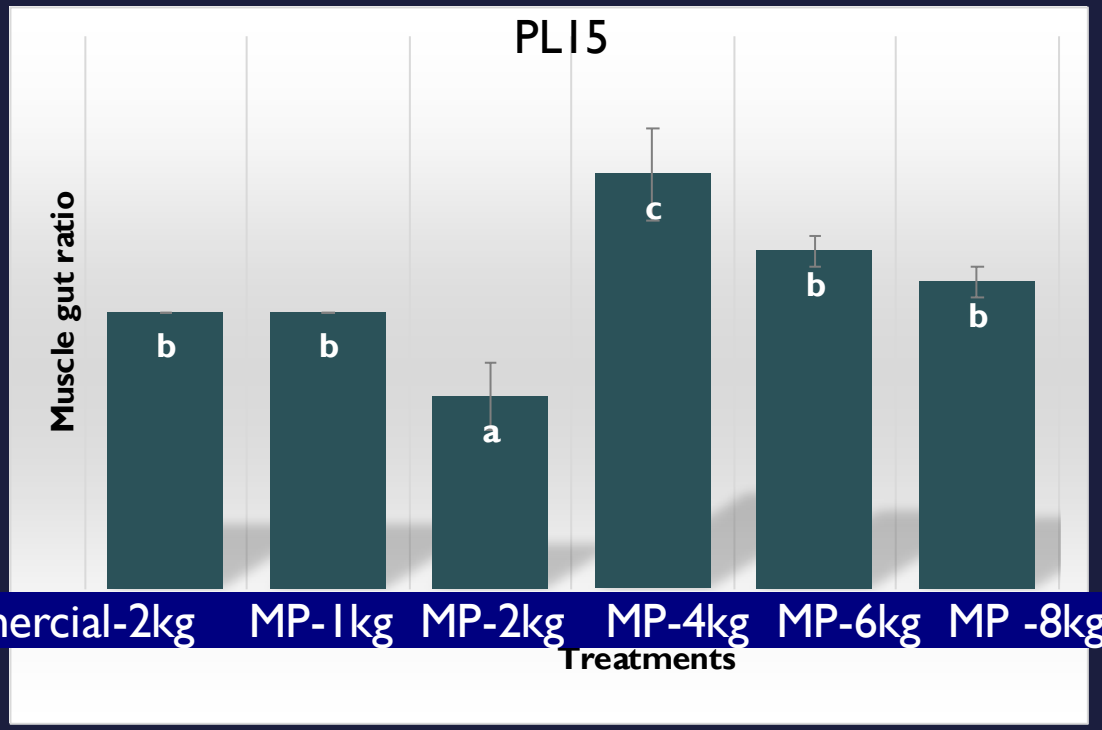


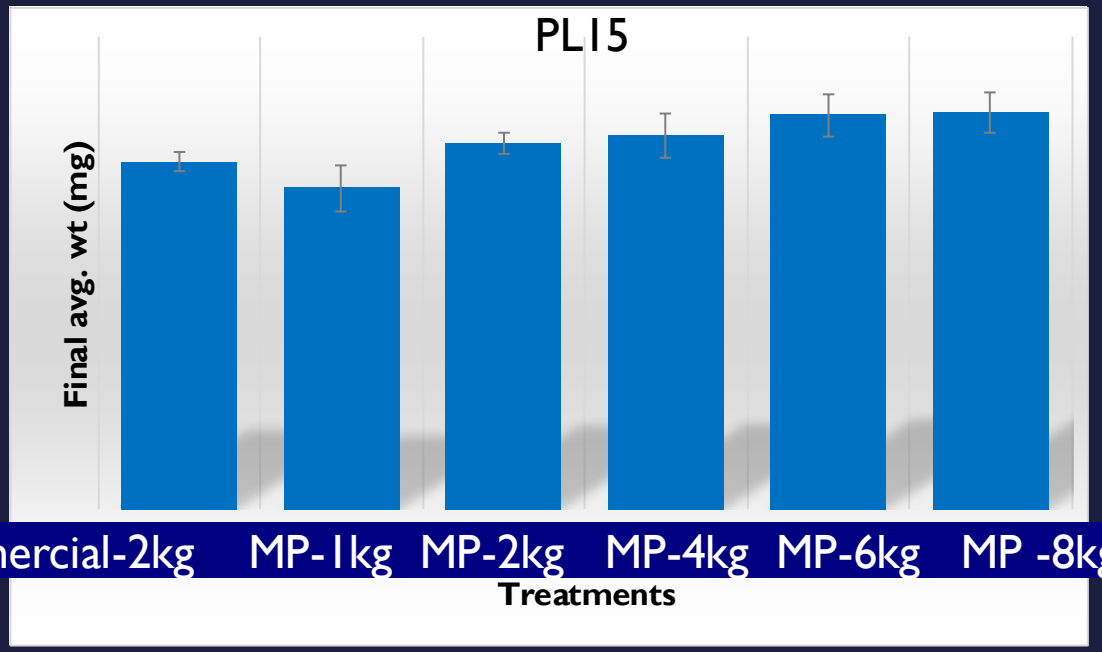

PL15



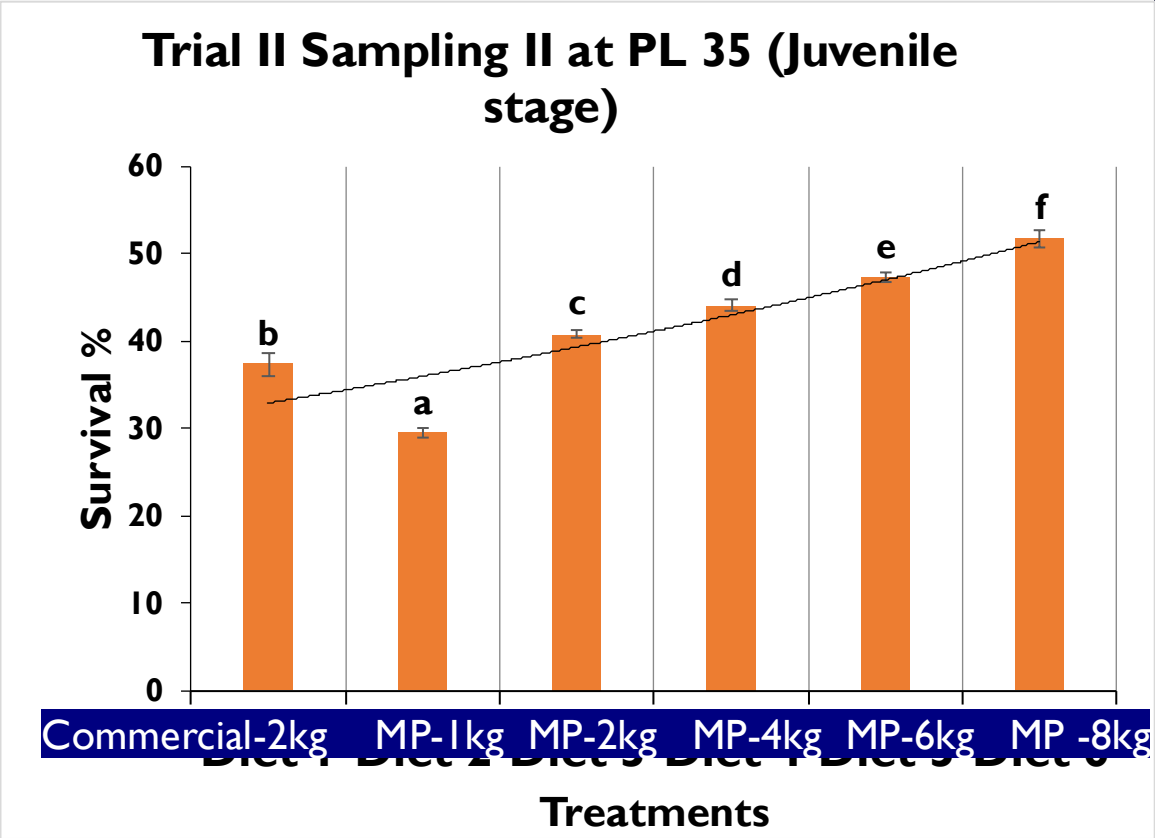
# Results: Trial II

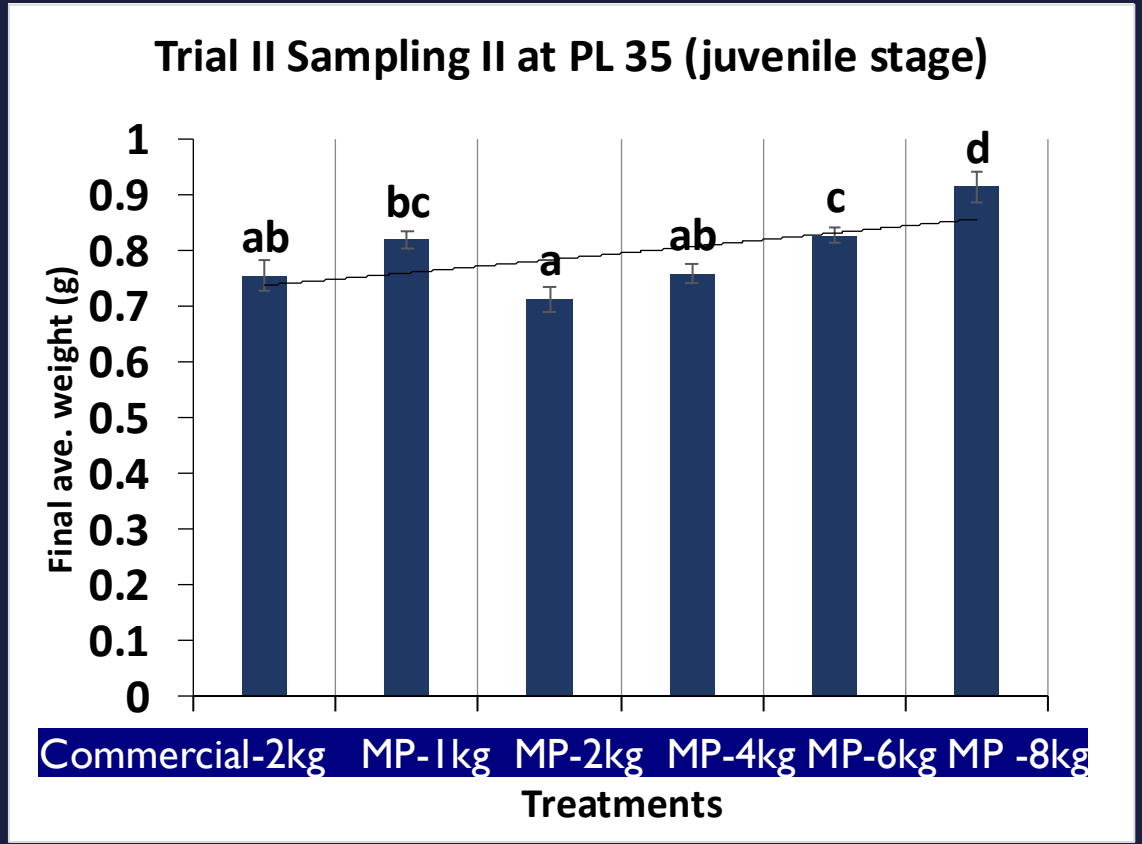


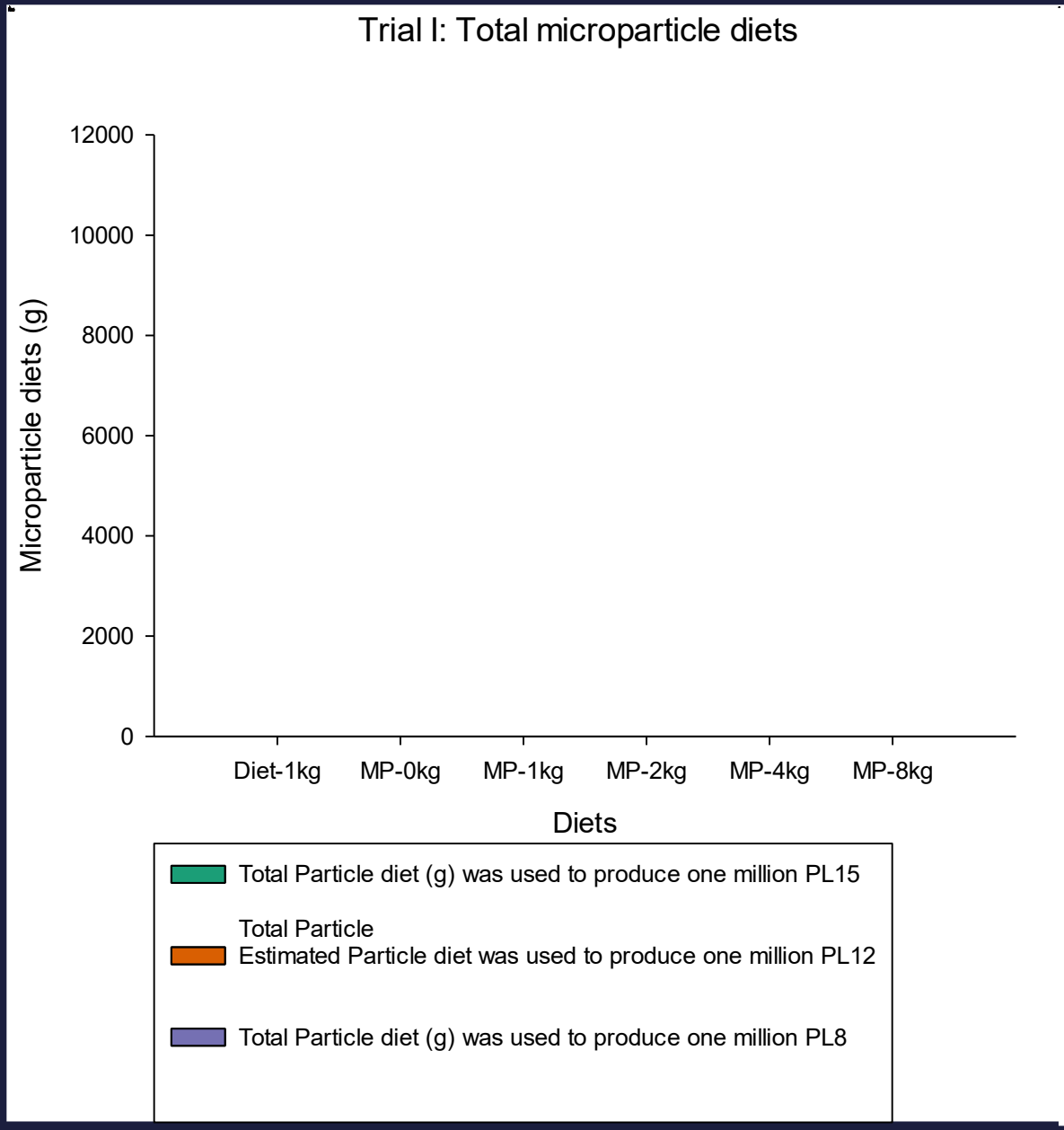



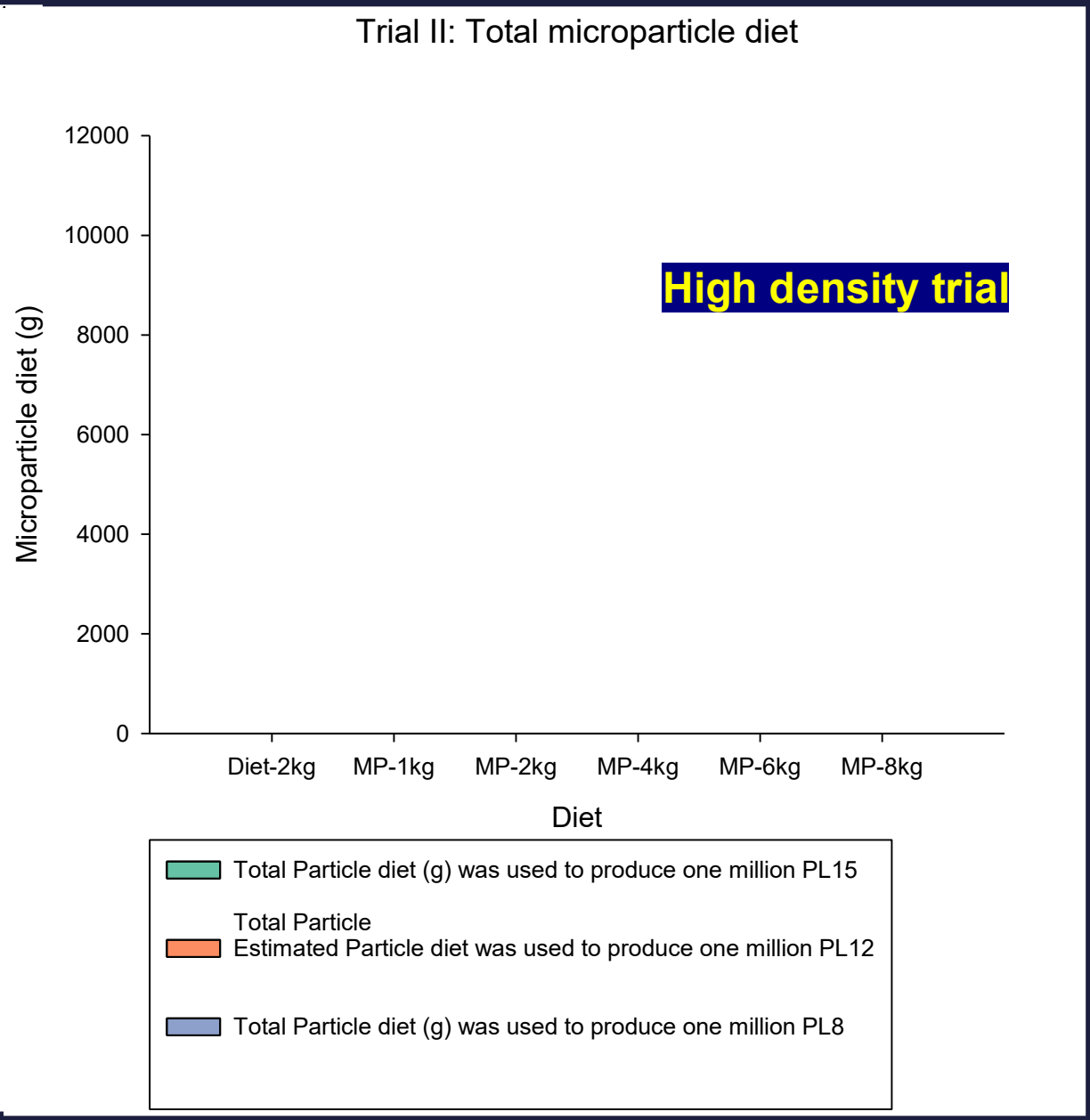
# Results: Trial II



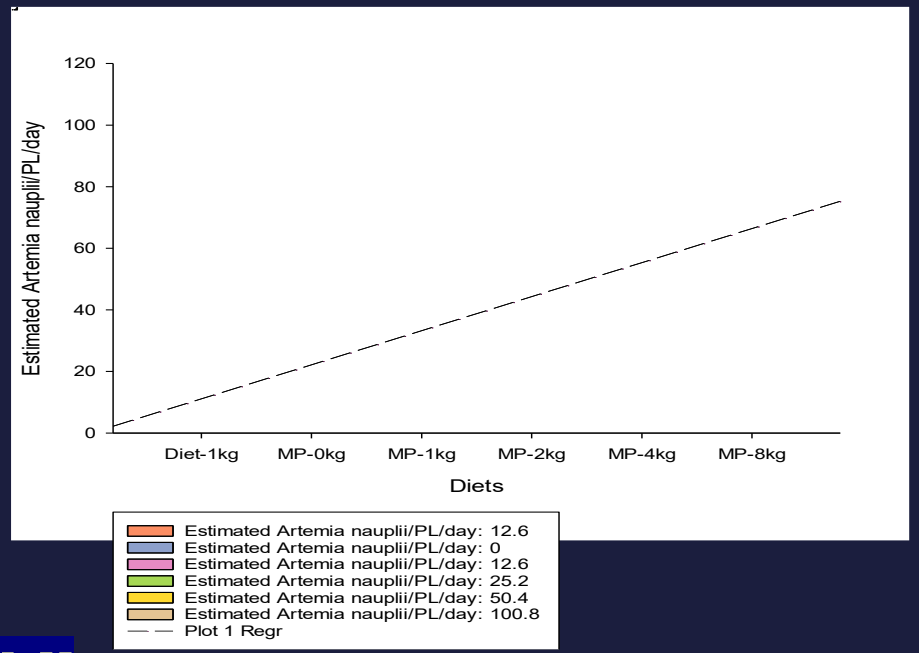
# Results: Trial I & II comparison for up to PL15

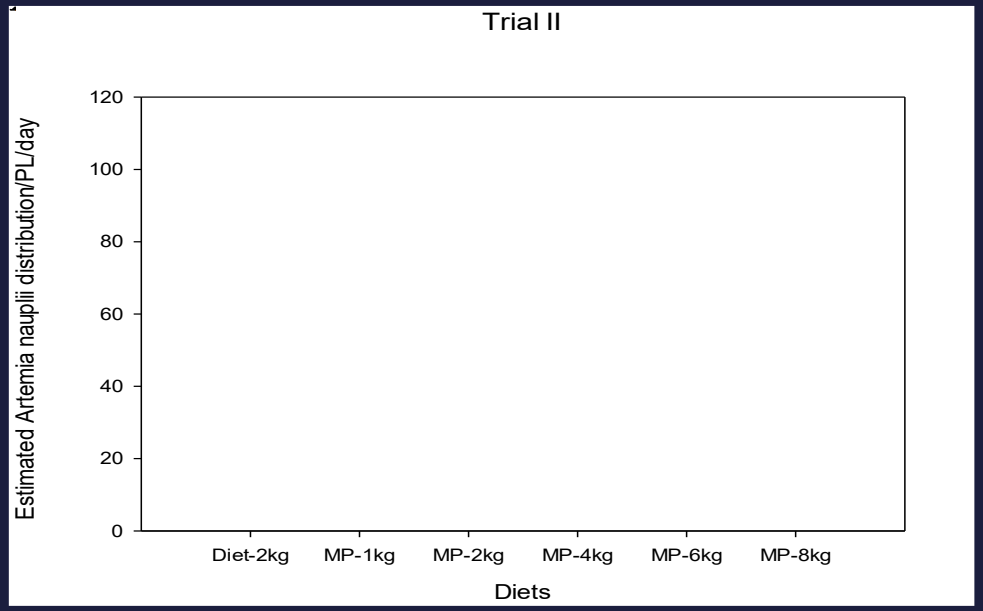


# Results: Trial I

| Treatments | Artemia cyst dosage (g) per Tank/day | Estimated Artemia nauplii distribution/PL/day | Actual Artemia nauplii distribution/PL/day from PL1 to PL8 | Actual Artemia nauplii distribution/PL/day from PL8 to PL15 |
|---|---|---|---|---|
| Diet-1000 | 0.02 | 12.6 | 18.7 (6.1) | 19.2 (6.6) |
| MP-0 | 0.00 | 0 | 0.0 | 0.0 |
| MP-1000 | 0.02 | 12.6 | 16.6 (4) | 18.1 (5.5) |
| MP-2000 | 0.03 | 25.2 | 30.0 (4.8) | 32.4 (4.8) |
| MP-4000 | 0.06 | 50.4 | 49.4(-1) | 54.6 (4.6) |
| MP-8000 | 0.12 | 100.8 | 133.6 (32.8) | 130.3 (29.5) |




# Trial II

| Diet with co-feed Artemia per million | Artemia cyst dosage (g) per Tank/day | Estimated Artemia nauplii distribution/PL/day | Actual Artemia nauplii distribution/PL/day from PL1 to PL15 | Actual Artemia nauplii distribution/PL/day from PL16 to PL35 |
|---|---|---|---|---|
| Diet-2000 | 0.040 | 25.2 | 35.7 (10.5) | 41.5 (16.3) |
| MP-1000 | 0.00 | 12.6 | 0.0 | 0.0 |
| MP-2000 | 0.04 | 25.2 | 33.2 (8.1) | 38.0 (12.8) |
| MP-4000 | 0.08 | 50.4 | 60.3 (9.9) | 70.2 (19.8) |
| MP-6000 | 0.12 | 75.6 | 74.9 (-0.7) | 98.5 (22.9) |
| MP-8000 | 0.16 | 100.8 | 101.5 (0.7) | 120.0 (19.2) |

# Conclusions

- Overall, **increased Artemia** co-feeding of 4000g to 6000g significantly improved **survival** during the hatchery stage.

- Additionally, it **further improved** when continually co-feeding during an extended hatchery period or nursery phase.

- Higher inclusion levels of Artemia were shown to be associated with **enhanced** health indices of *L. vannamei* postlarvae.

# Acknowledgments

# Thank you