Assessing The Impact Of Enhanced Dietary Levels Of Brine Shrimp *Artemia Franciscana* Co-fed With Microparticle Diet On The Rearing Of *Litopenaeus Vannamei*

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COLLEGE OF AQUACULTURE AND FISHERIES

Motivation - Modernization - Qualification - Internationalization

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 (Low density)

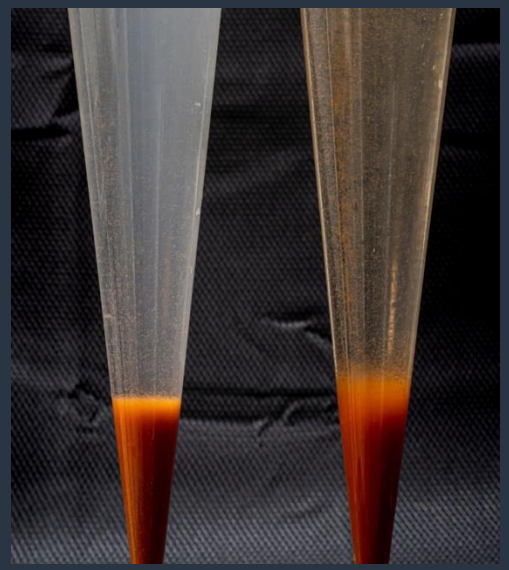
- Commercial diet + Artemia 1kg
- MP + Artemia 0kg
- MP + Artemia 1kg
- MP + Artemia 2kg
- MP + Artemia 4kg
- MP + Artemia 8kg

Hatchery and nursery stage
PL1 to PL15
PL16 to PL35

Trial II (Medium density)

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

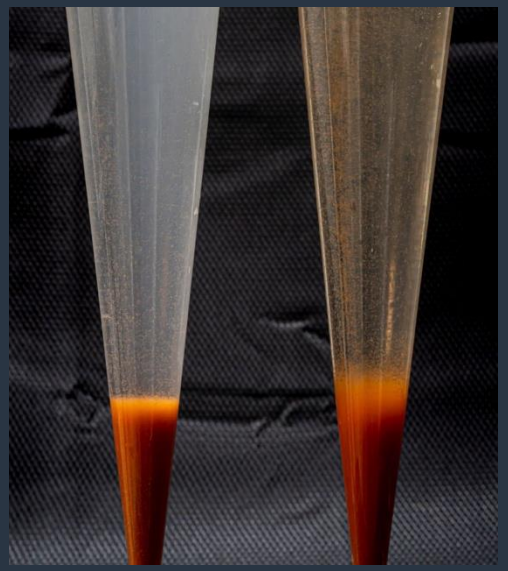
Diets
Isonitrogenous
Isocaloric diets



Trial III

Probiotic @ 1ppm one initial dosage

Nutrient Dense MP	X	Economy (Budget-friendly)
Artemia	X	Artemia
0g	X	0g
3000g	X	3000g
6000g	X	6000g



Trial design and diets: CRD

- **Aims:**
- Evaluate the performance of *L. vannamei* co-fed with increased levels of Brine Shrimp *Artemia franciscana* with a high nutrient-dense microparticle diet and budget-friendly diets.

Algae preparation

Methods

GREAT SALT LAKE BRINE SHRIMP COOPERATIVE, INC

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

GREAT SALT LAKE BRINE SHRIMP COOPERATIVE, INC

Artemia cyst hatching

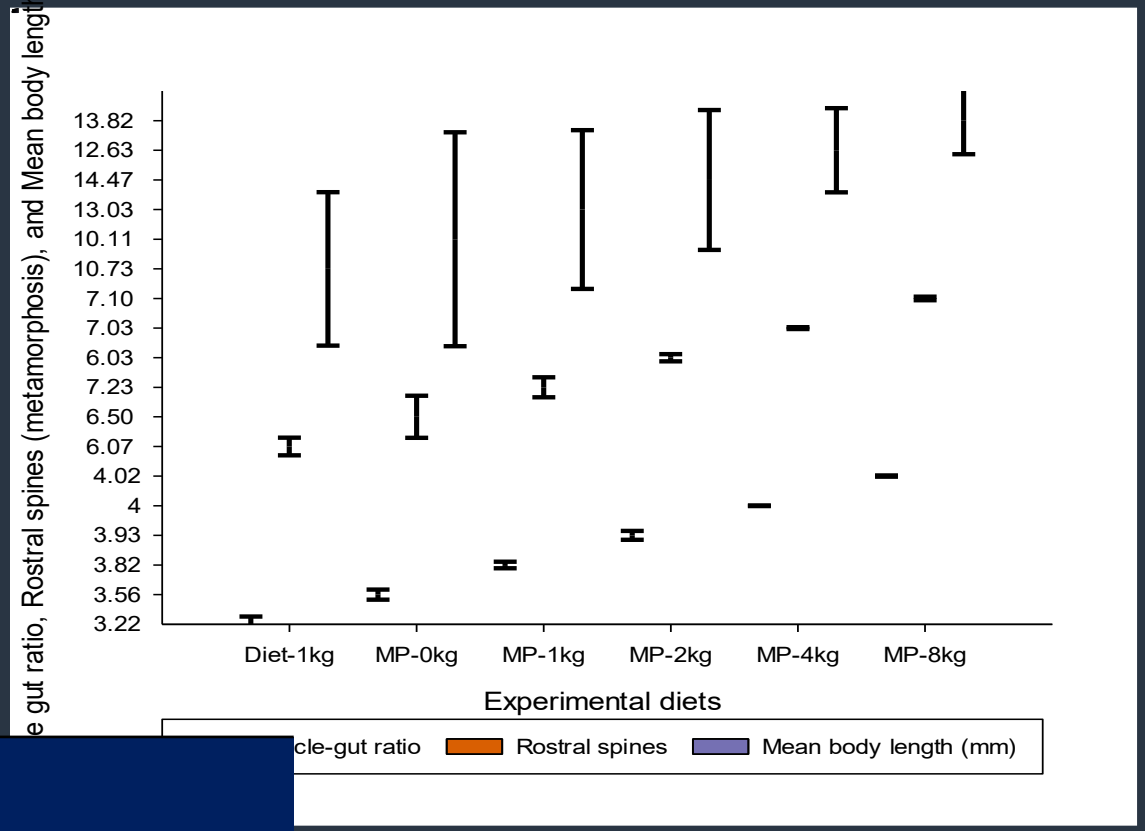
- The requirement of live Artemia nauplii (Instar I) was calculated in every trial
- Dry Artemia cysts at 4% moisture were obtained from Great Salt Lake Brine Shrimp Cooperative, Inc.
- Stocked dry cysts in 28ppt salinity saltwater, hatched for 18h at temp 27°C with a slight aeration

Culture Conditions

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

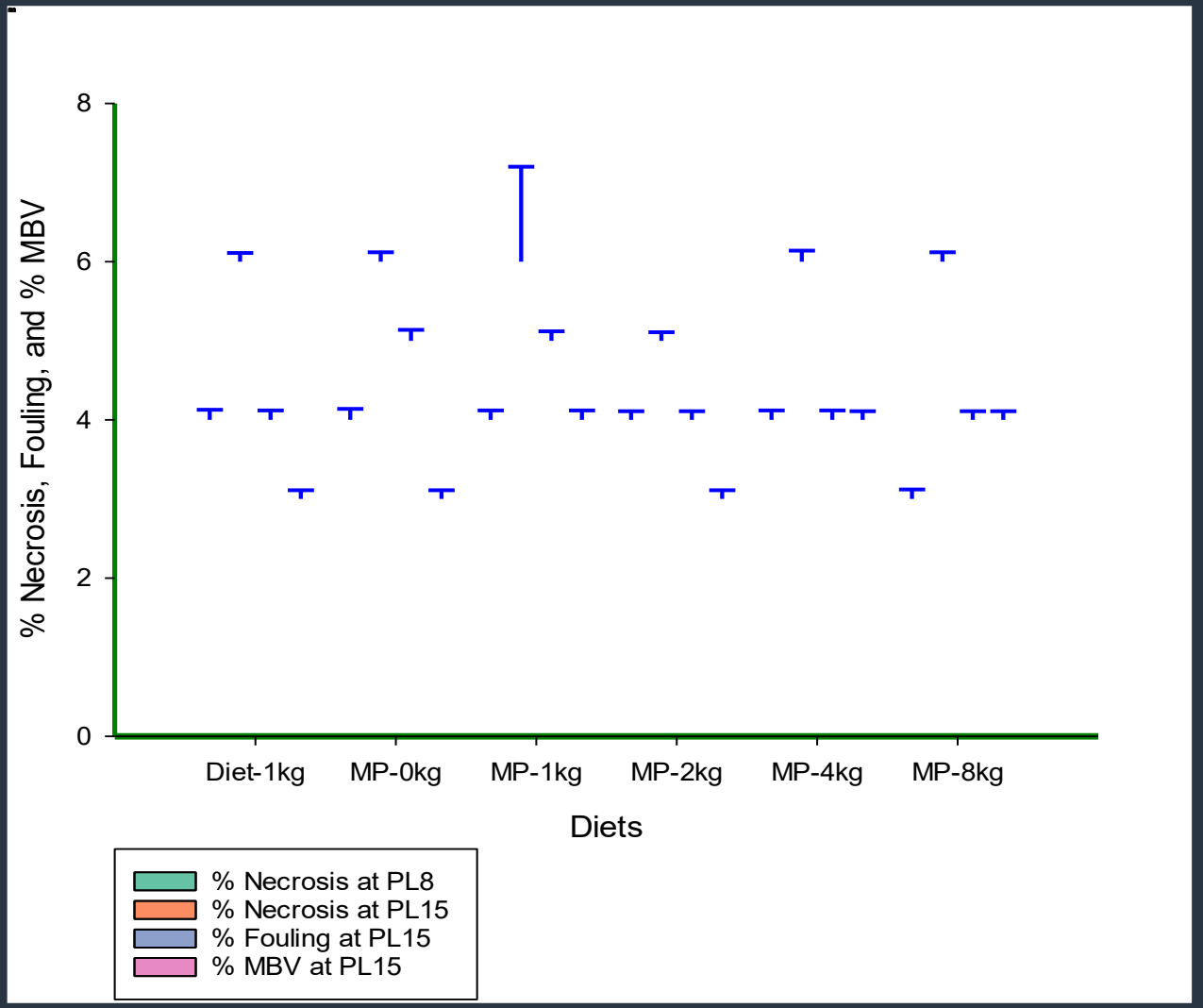
Results: Trial I

Health indices



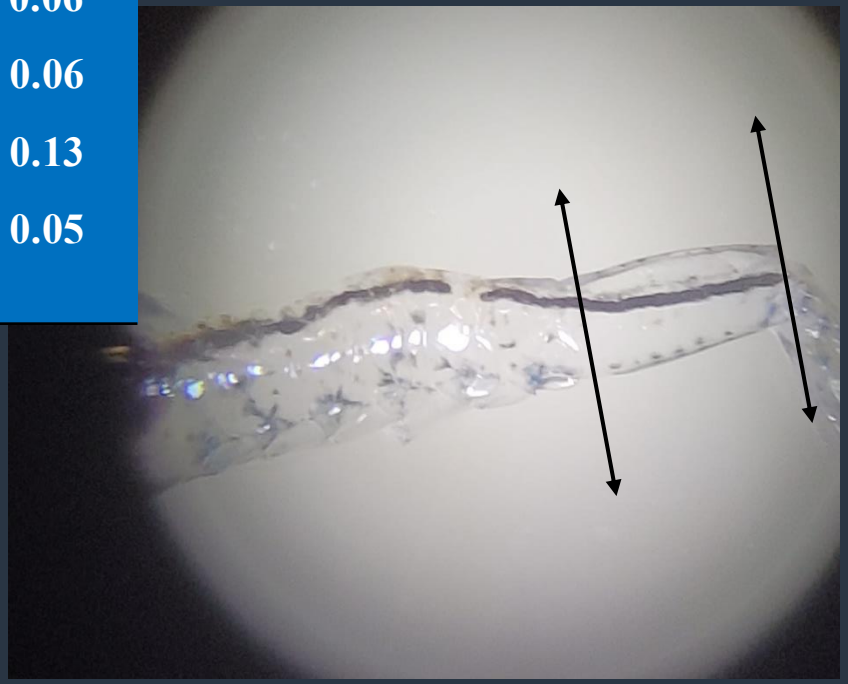
Diet with co-feed Artemia per million	PL 8 Survival rate (%) in the salinity stress test	Mean body length (mm)	Rostral spines	Muscle-gut ratio
Commercial 1kg	91.11 ± 0.20	10.73 ± 2.59	6.07 ± 0.30	3.22^{a} ± 0.26
MP-0kg	91.41 ± 0.25	10.11 ± 3.61	6.50 ± 0.71	3.56^{ab} ± 0.17
MP-1kg	89.90 ± 5.13	13.03 ± 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



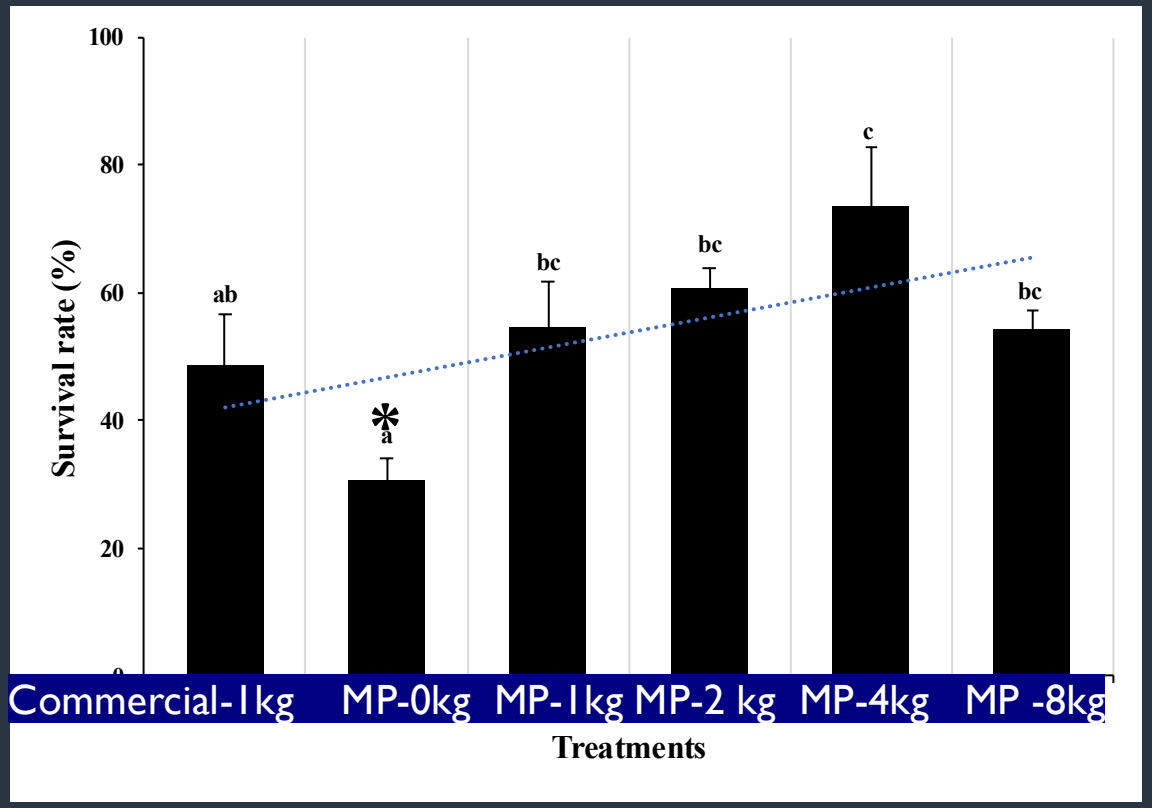
Results: Trial I

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

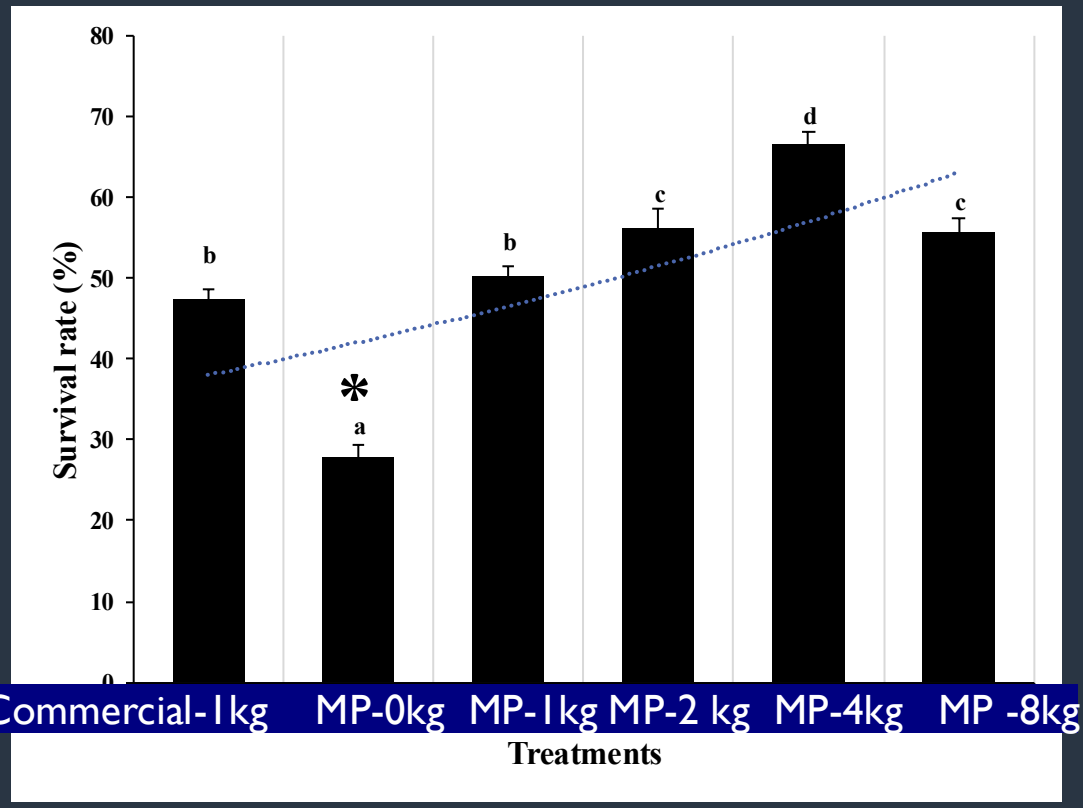


Results: Trial I

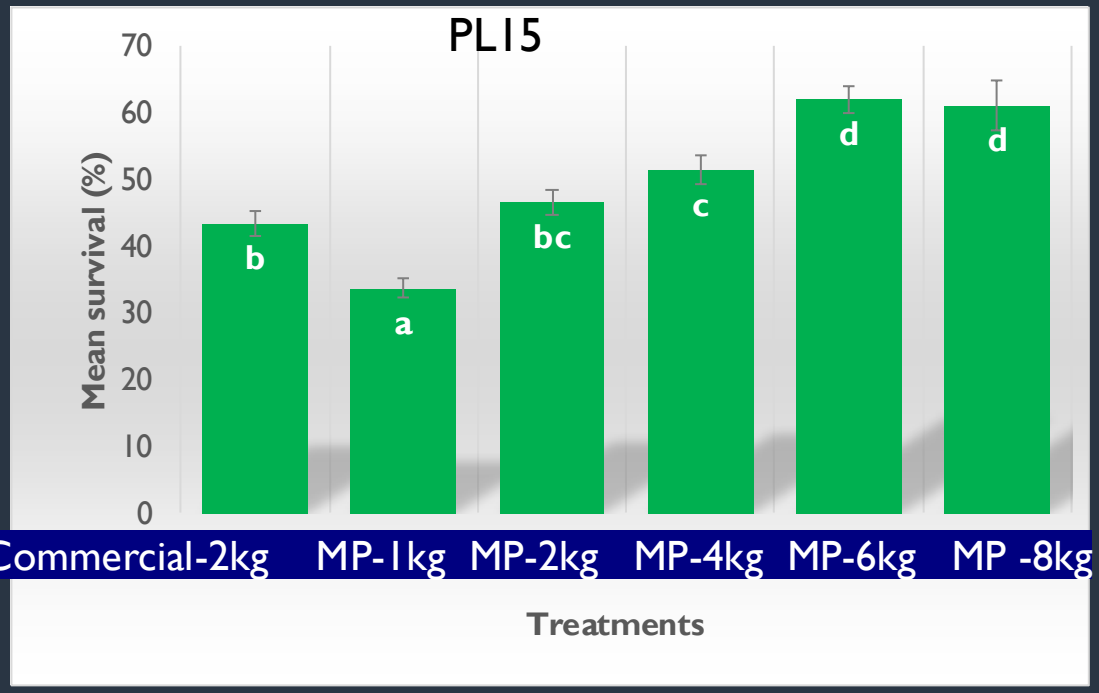
PL8

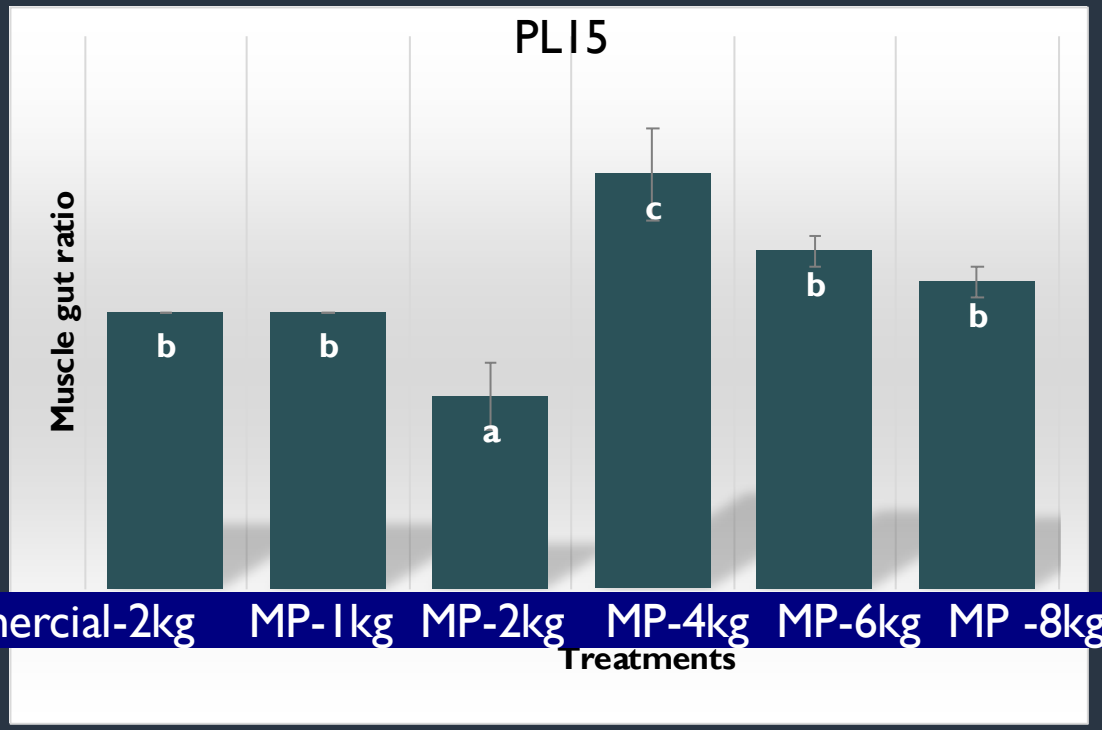


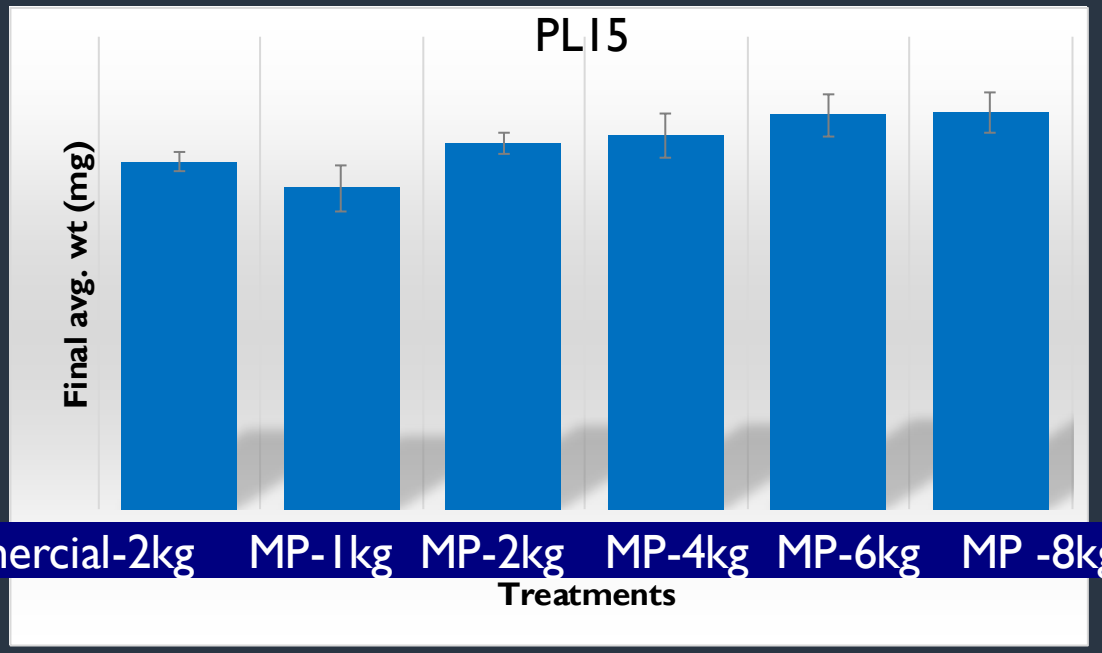
PL15



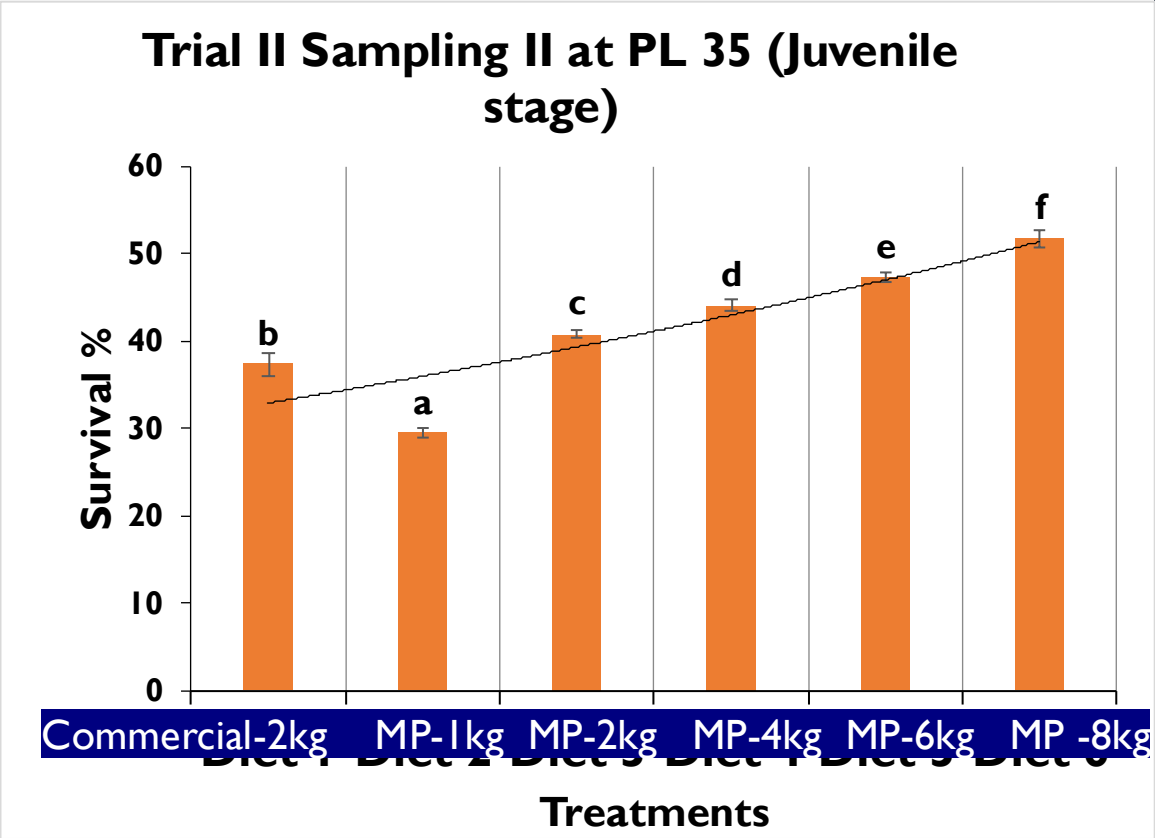
Results: Trial II

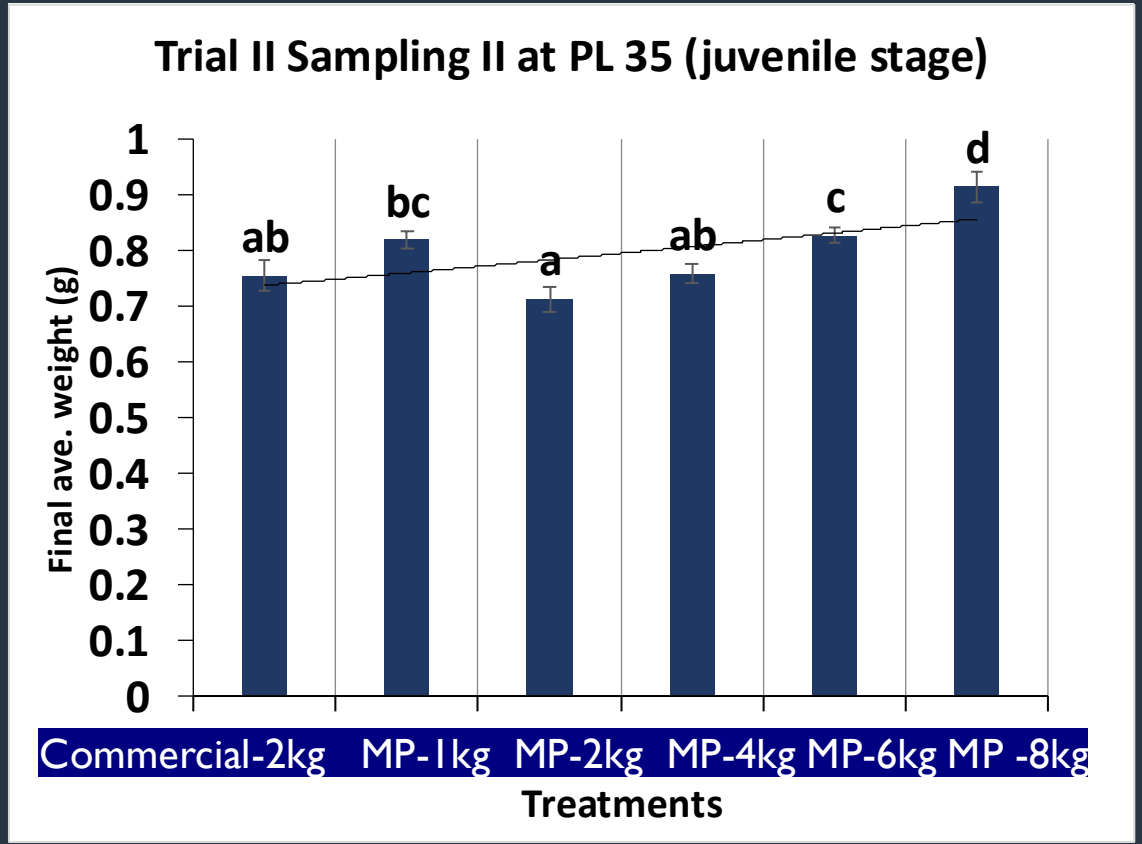






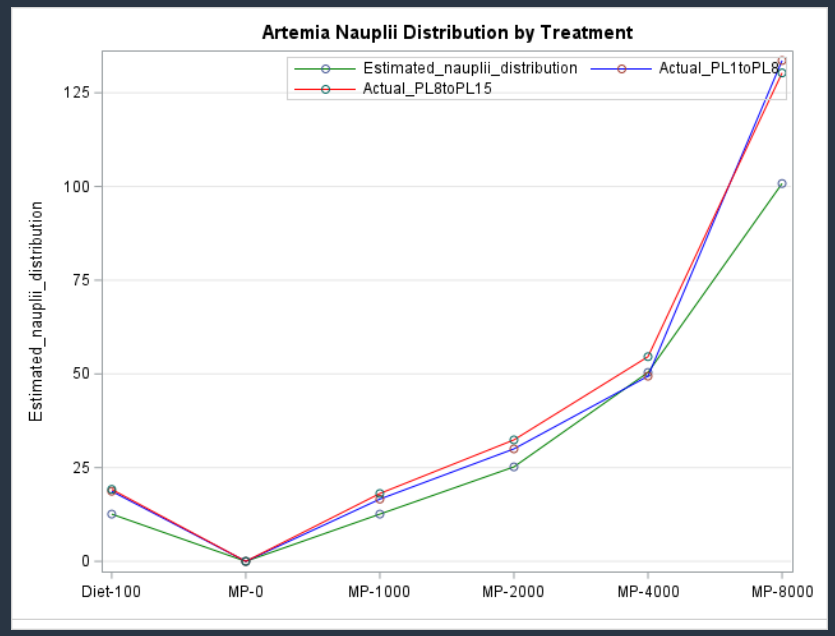
Results: Trial II





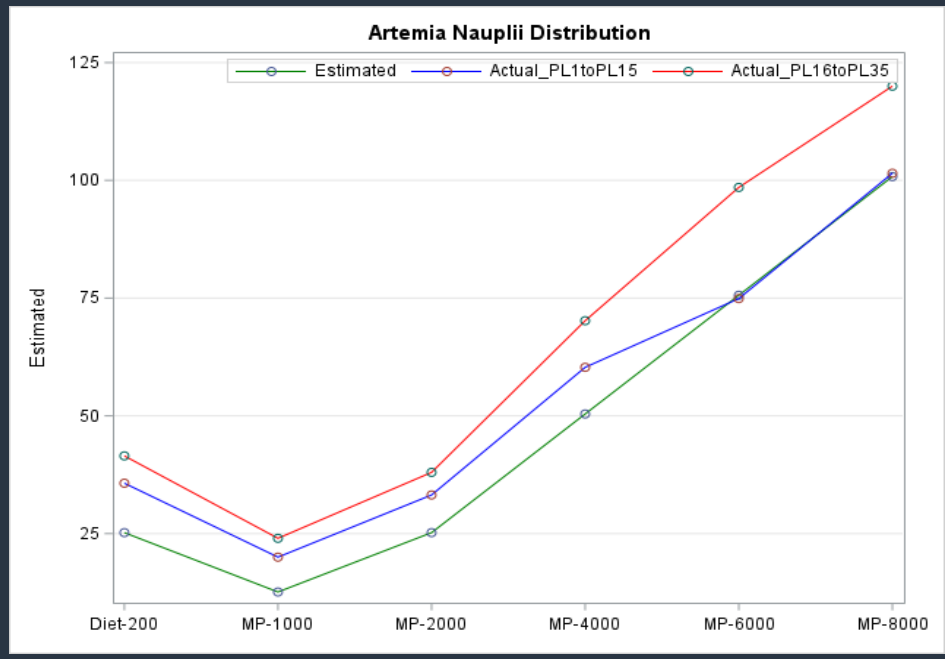
Results: Artemia usage

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)

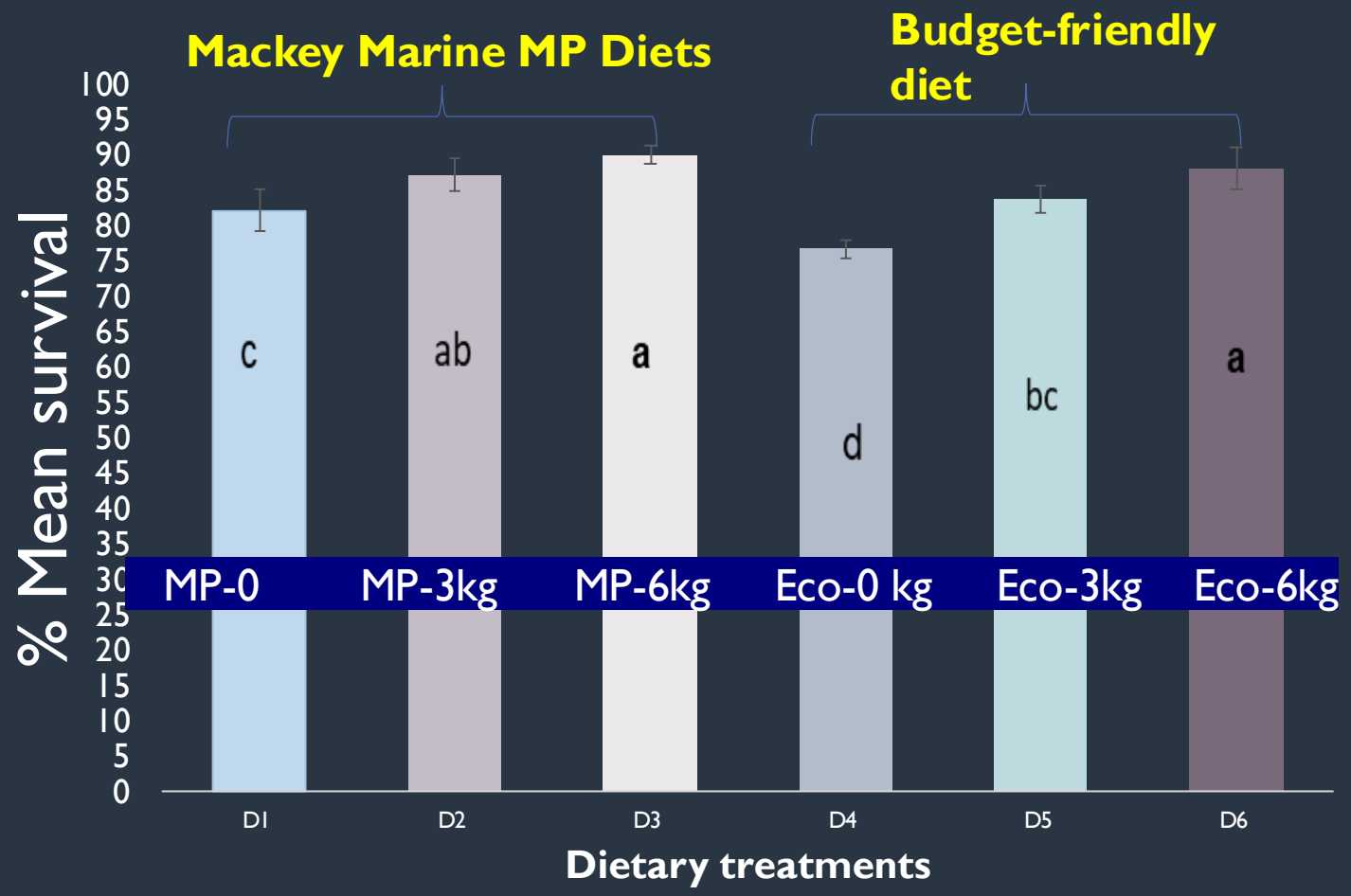


Trial II

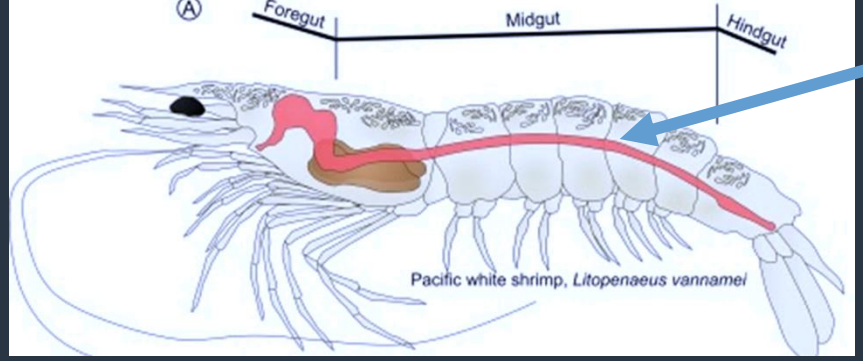
Diet with co-feed Artemia per million	Artemia cyst dosage (g) per Tank/day	Estimated Artemia nauplii distribution/PL/day	Actual Artemia nauplii distribution/PL/day from PL1 to PL15	Actual Artemia nauplii distribution/PL/day from PL16 to PL35
Diet-2000	0.040	25.2	35.7 (10.5)	41.5 (16.3)
MP-1000	0.002	12.6	20.0	24.0
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)



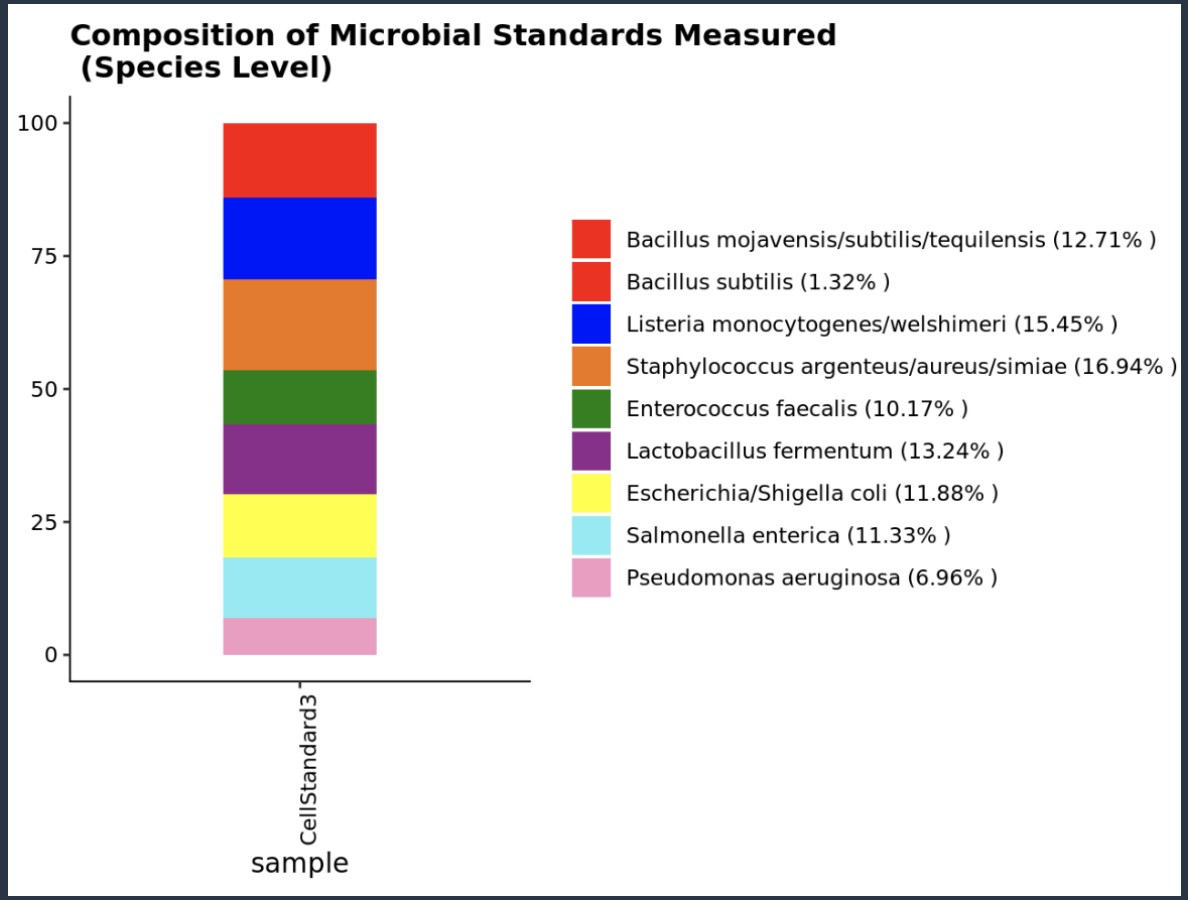
Results Trial III



Gut Microbiome-Juvenile Shrimp

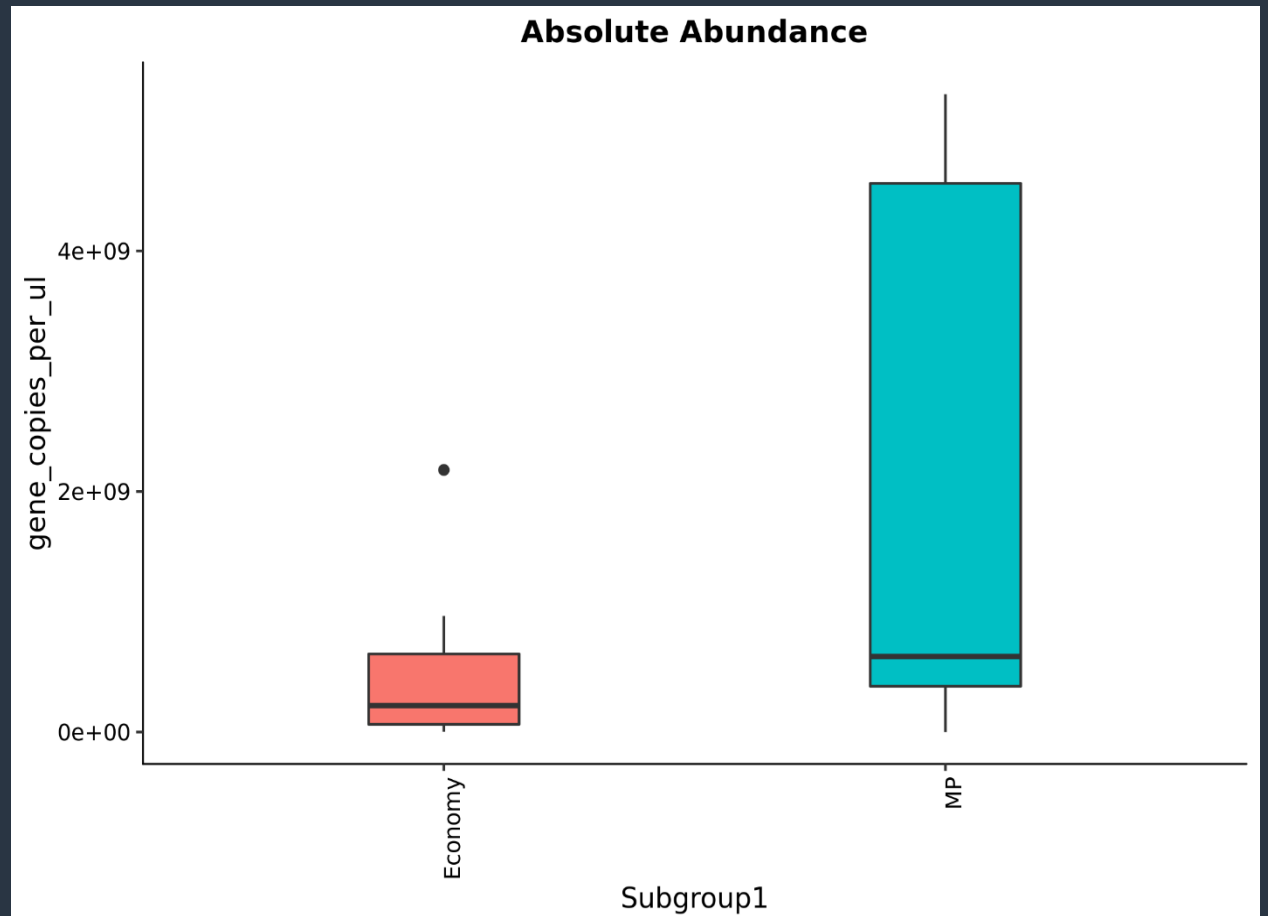


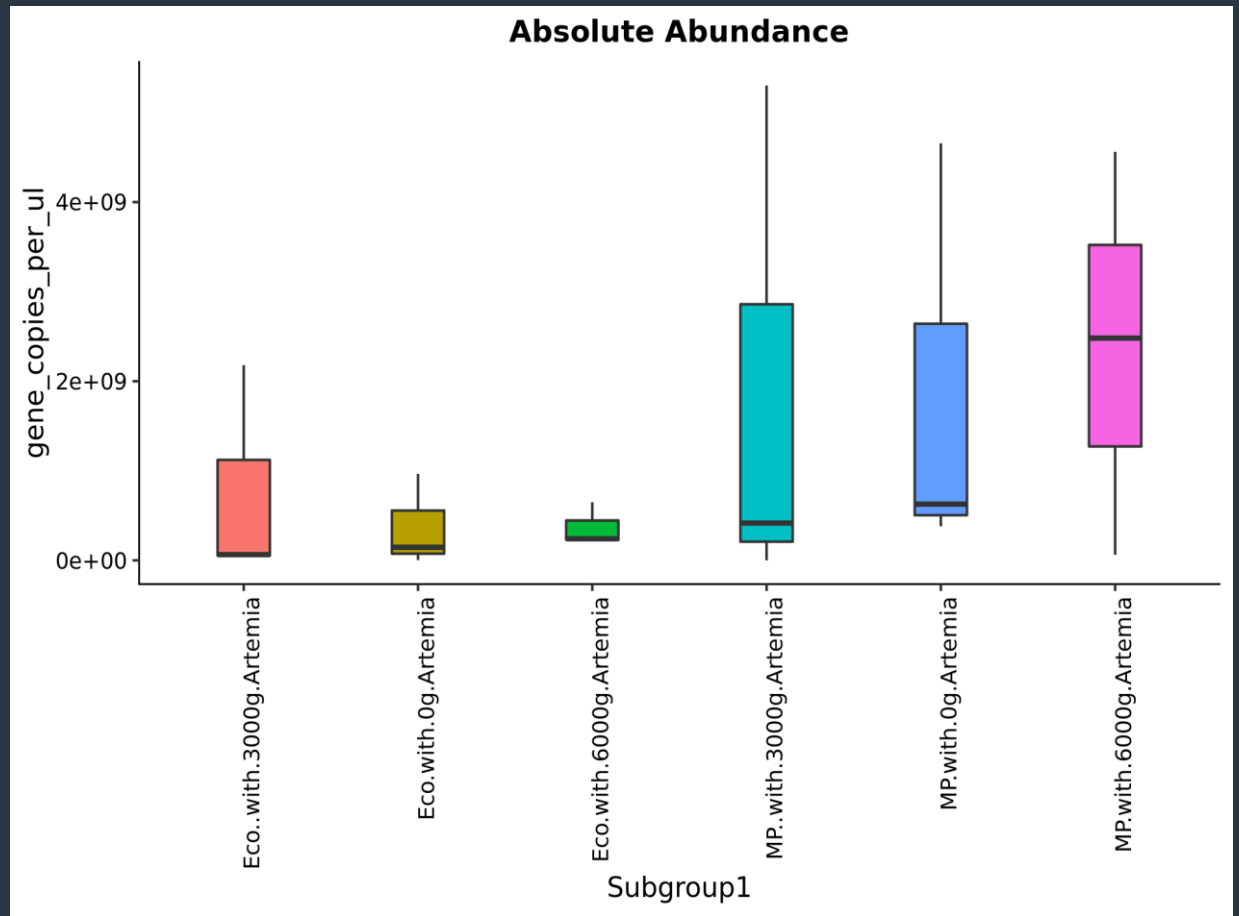
- The microbial colonization of the gastrointestinal tract occurs in the 5th stage of nauplius (Simoes et al. 2002)
- **Positive control.**
- **Negative control.**



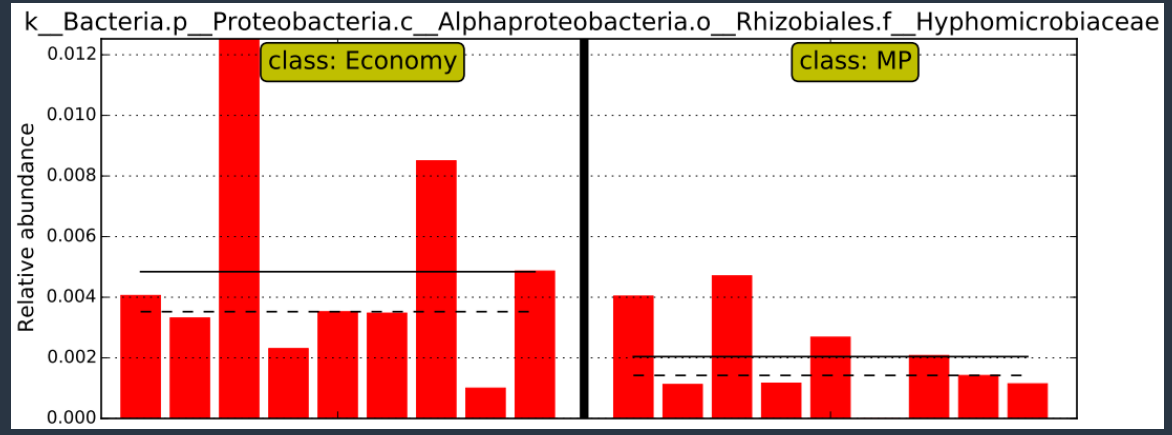
Total DNA = Calculated Total Genome Copies × Assumed Genome Size (4.64 × 106 bp) × Average Molecular Weight of a DNA bp (660 g/mole/bp) ÷ Avogadros Number (6.022 x 1023/mole)

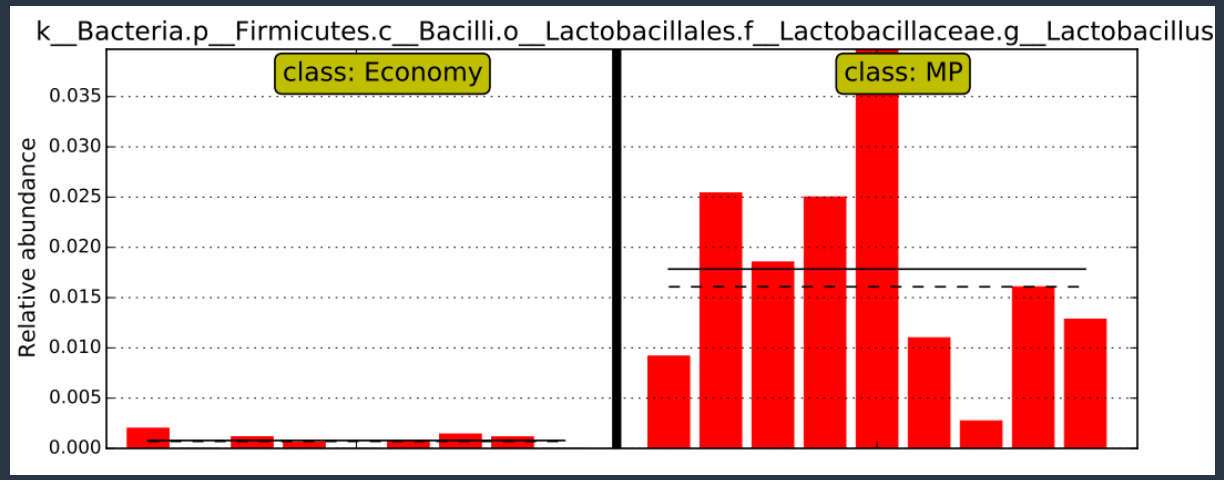
A more species-diverse microbiota in the gut may facilitate resistance to a greater degree of potentially problematic colonizers, as there is consequently a larger set of species-species antagonisms.

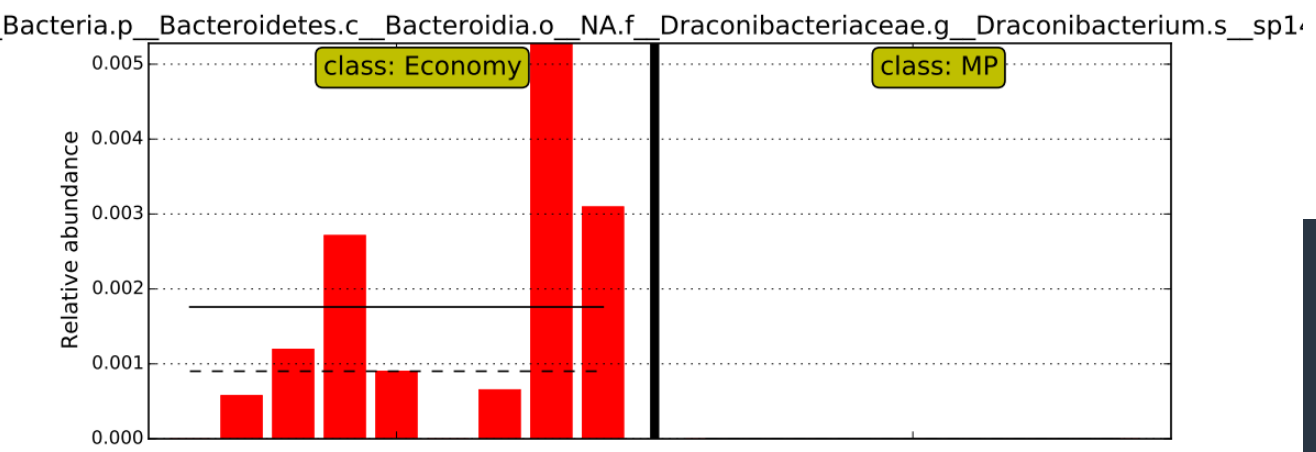


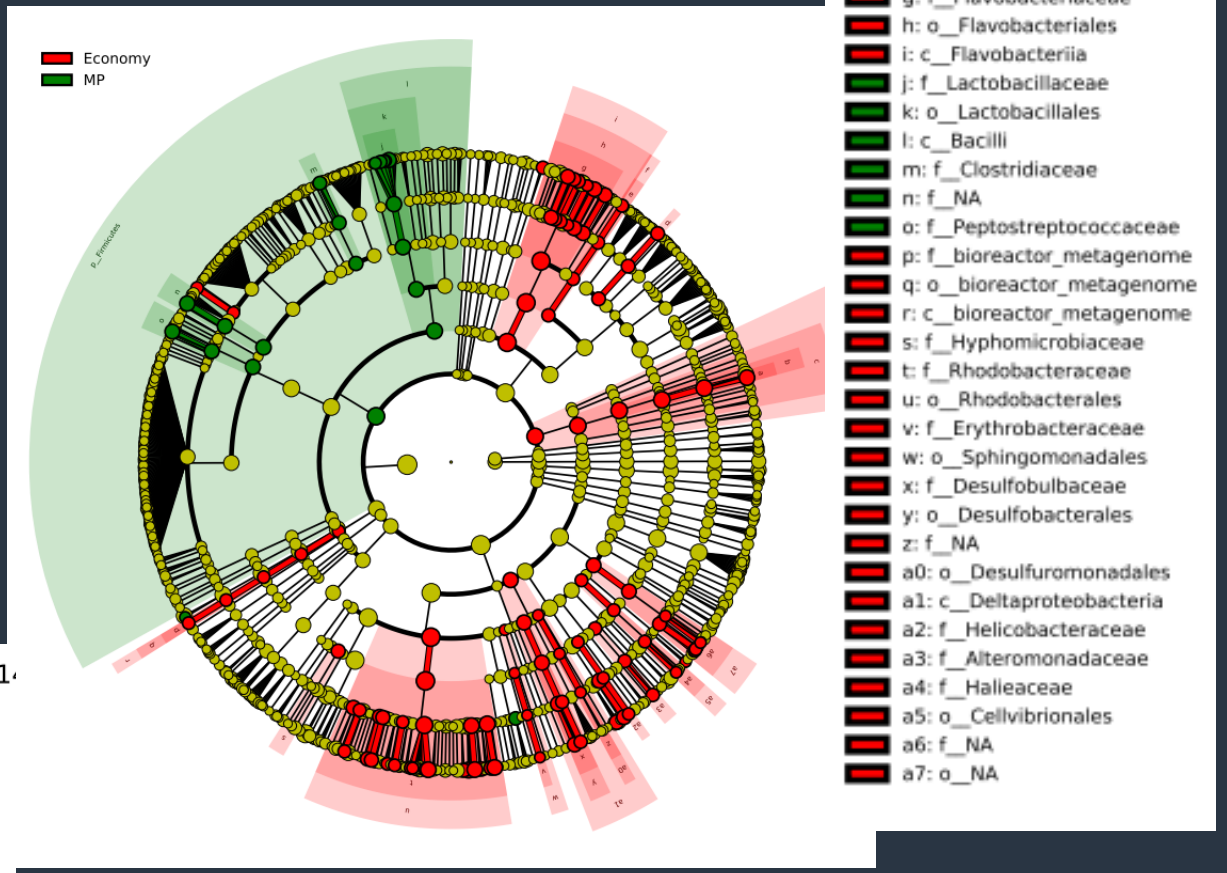


Phylotypes of the gut microbiota responding to diet identified using LEfSe. Color ranges identify phyla within the tree.



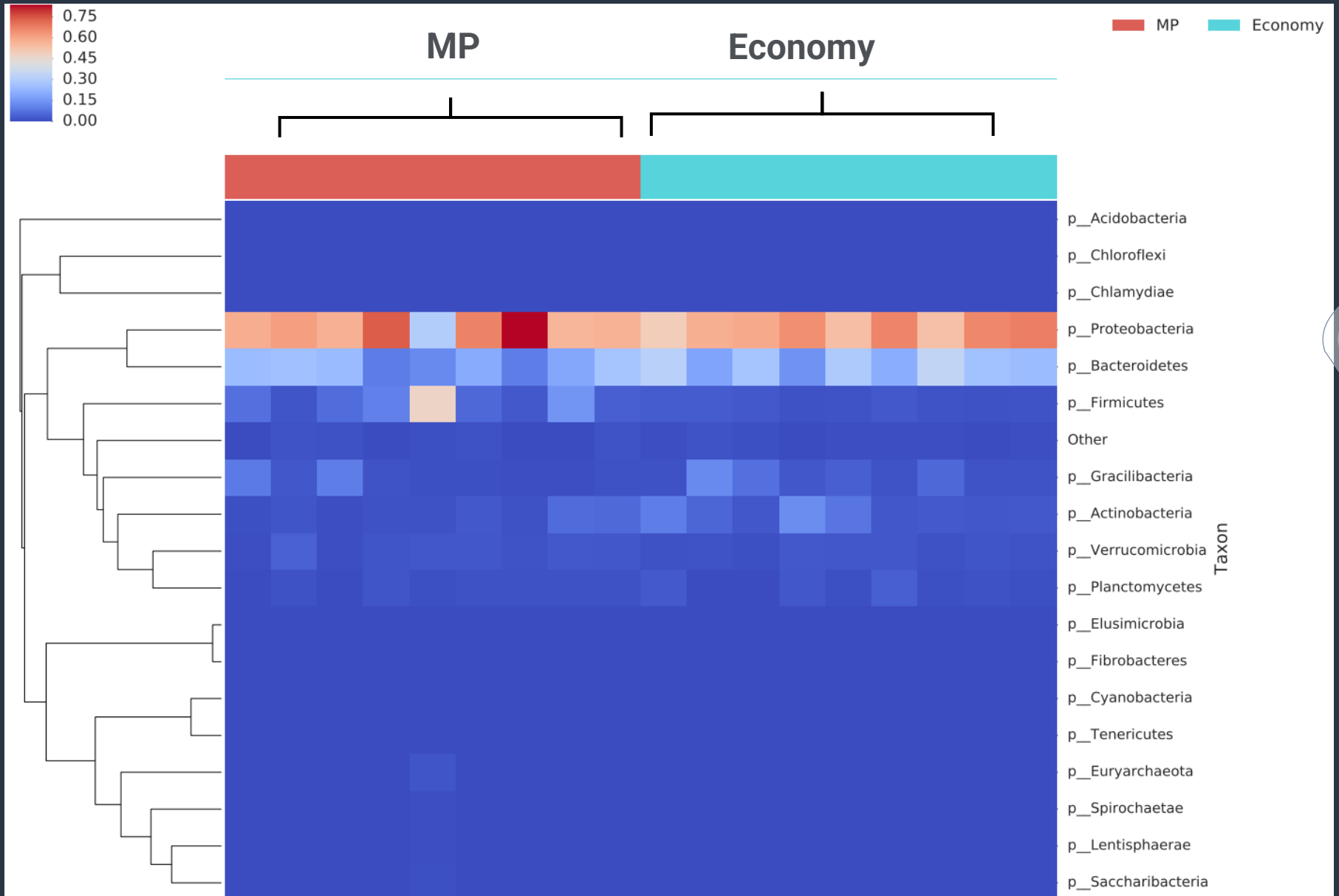




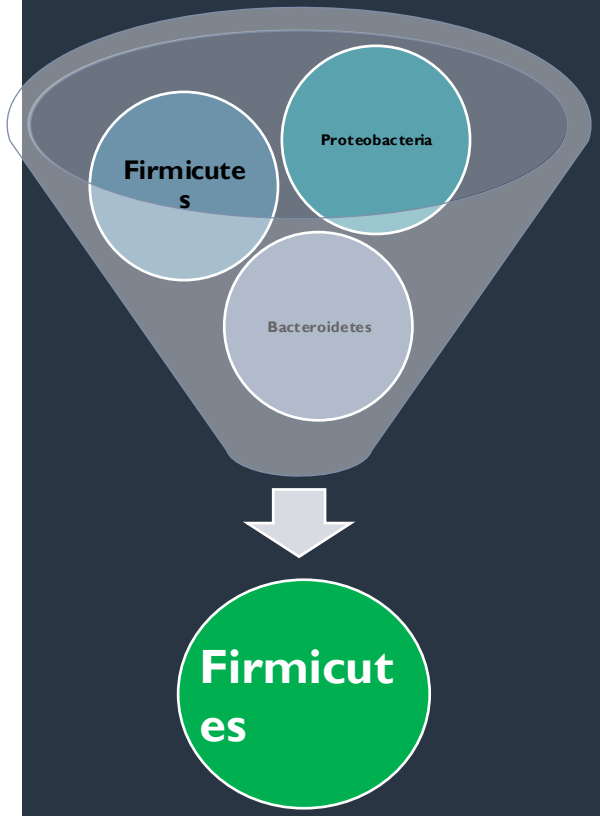


Relative abundance

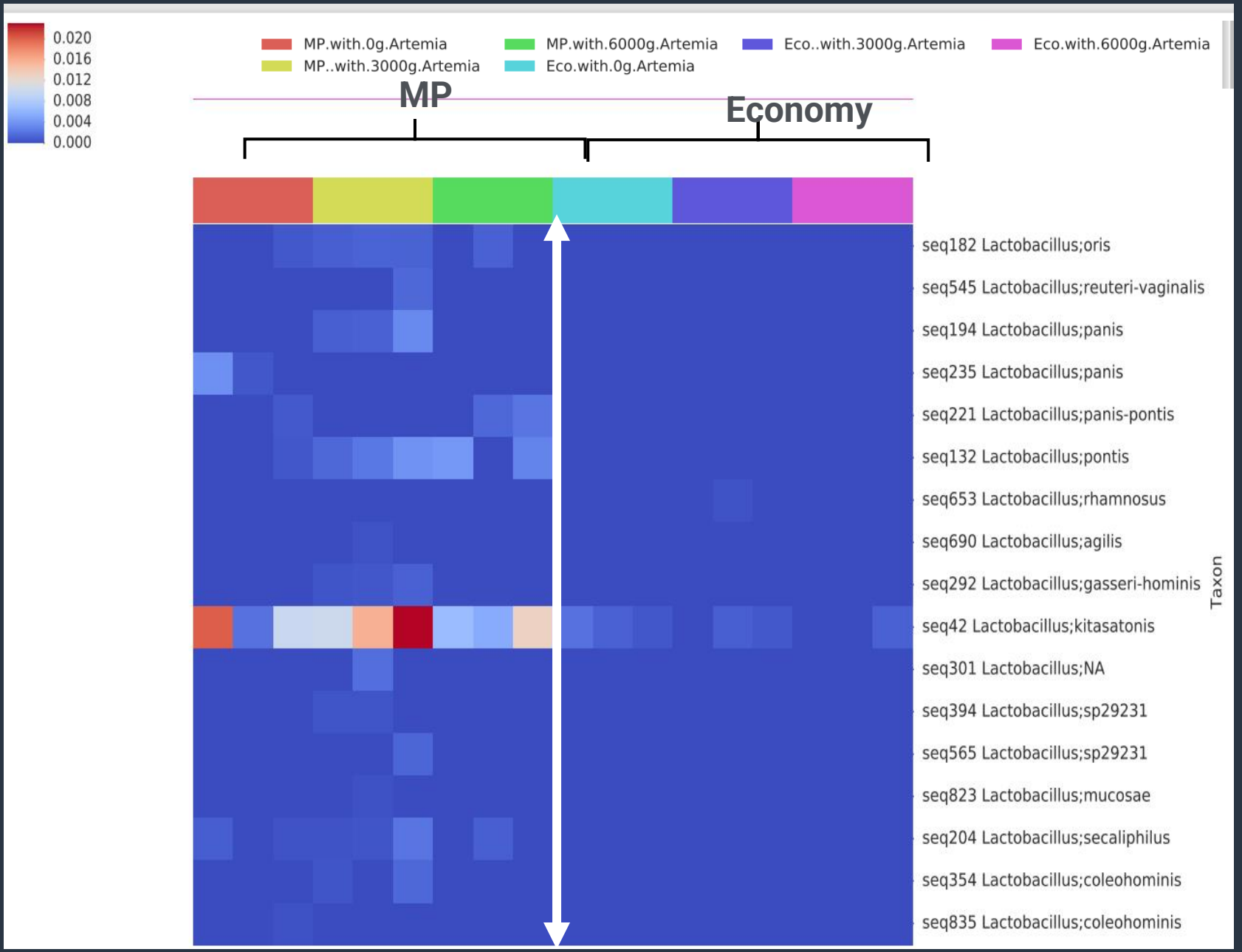
Results



Taxonomy that has significant abundance among different **phylotype groups** were identified by LEfSe (Segata et al., 2011) using default settings.



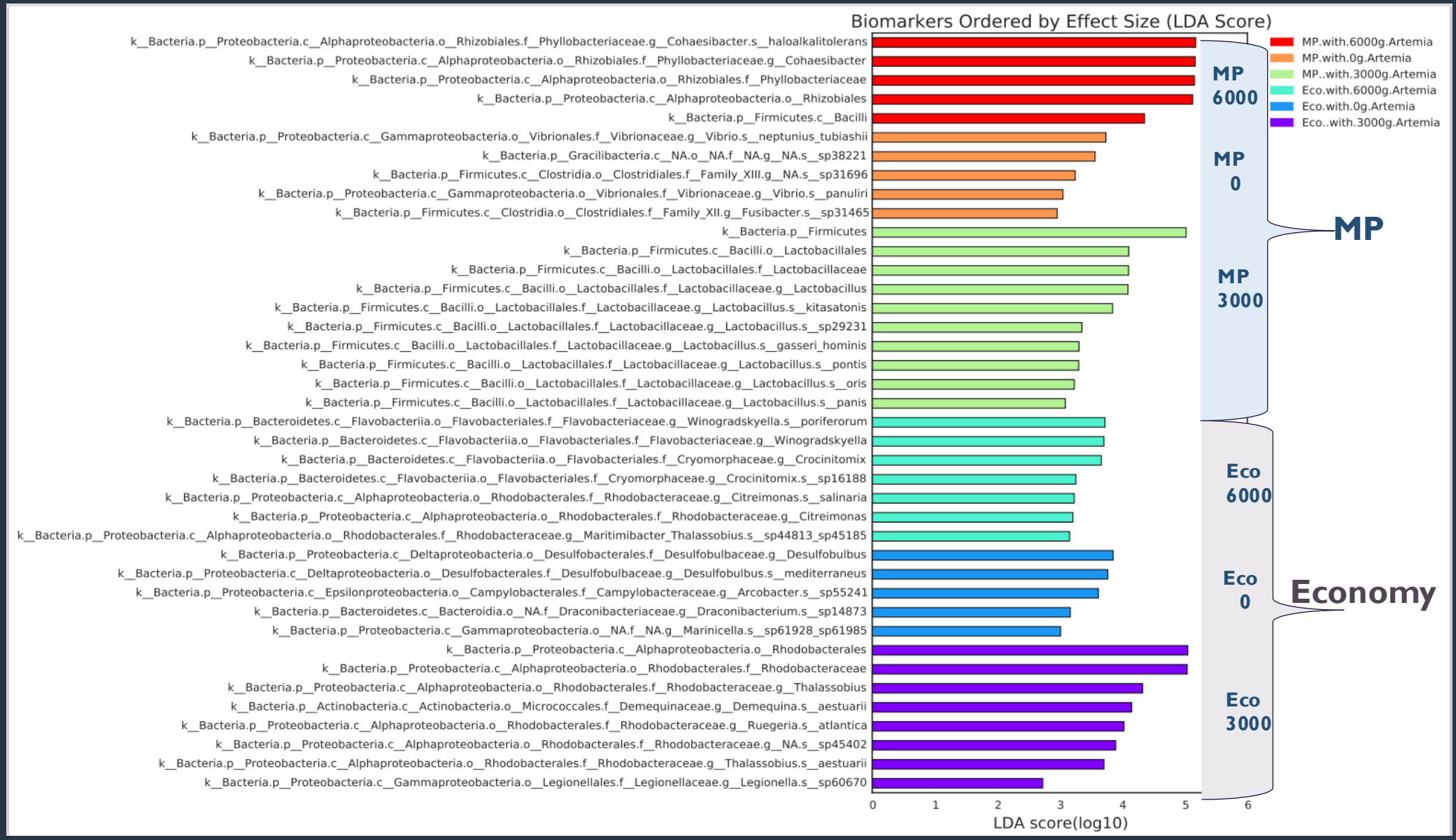
Results



Relative sequence abundance of lactobacillus associated with the MP group.

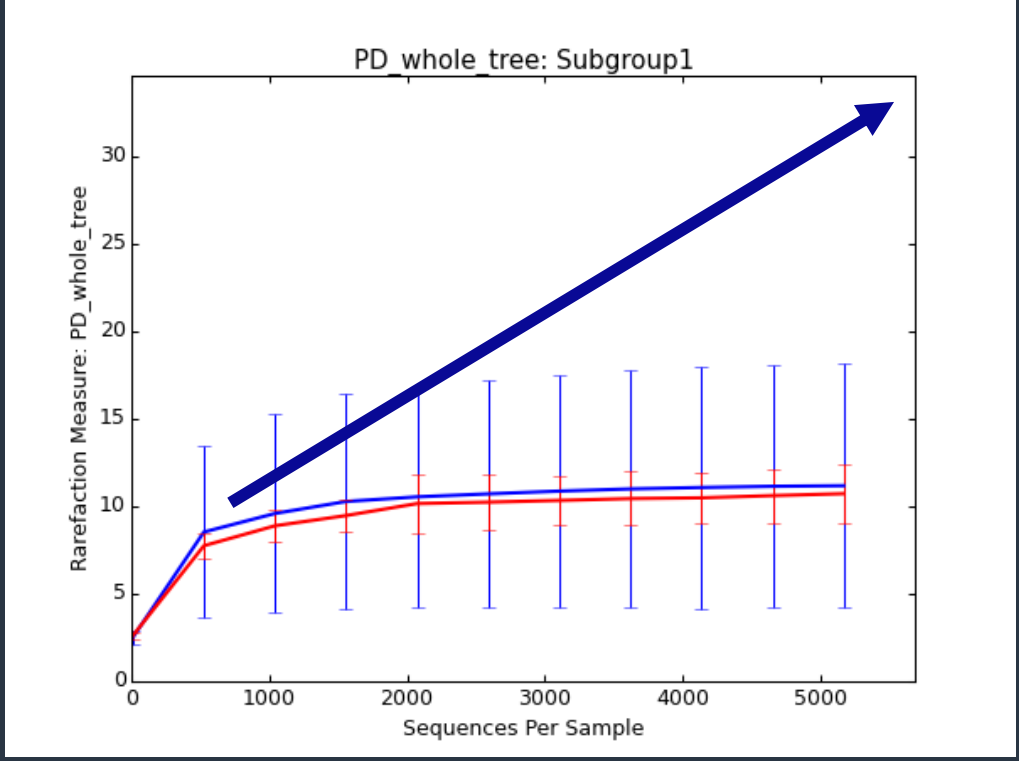
Probiotic potential of novel **Lactobacillus** strains as antagonists for *Vibrio parahaemolyticus*

Linear Discriminate Analysis (LDA) Effect Size (LEFSe) highlighted several taxa of the treatments.



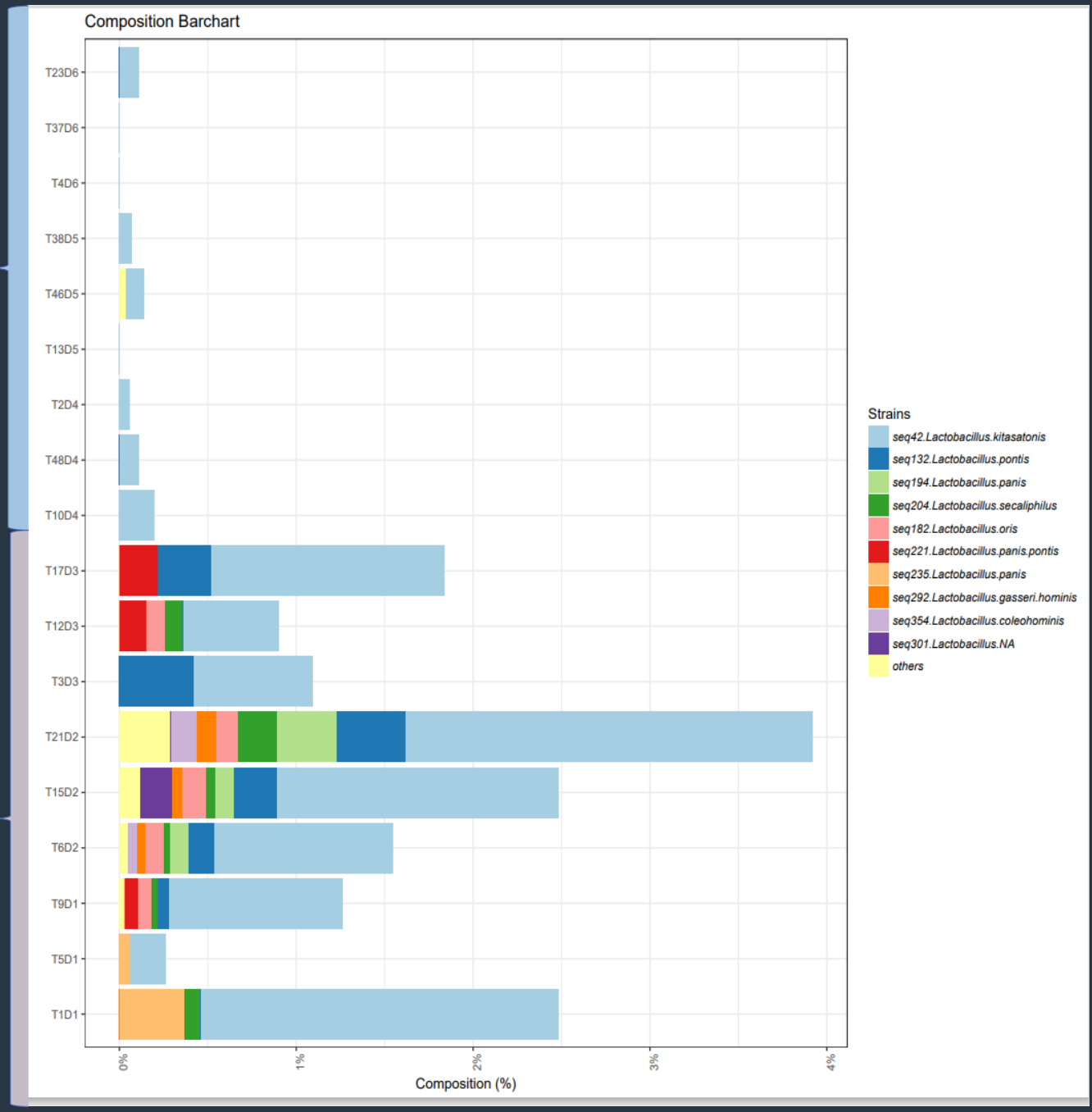
At species level

Rarefaction measures of Phylogenic diversity of whole tree



Economy diets

MP diets



Conclusions

- **Improved** gut health with a higher bacterial abundance of **Firmicutes,** especially **Lactobacillus** colonization, observed with Mackay Marine MP diets comparatively Budget-friendly diet.

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

- *Rhodobacteracea* improved in the high levels of artemia in budget-friendly diet

- Mackay diet showed a significant role as a **prebiotic substrate** in improving ***Lactobacillus*** colonization in the gut of juvenile *L. vannamei* (PL25) with a minimal probiotic dosage (1ppm).

Conclusions

- **High Artemia + Budget-friendly** improved **survival and** ***Rhodobacteracea***

- **High Artemia + Mackay marine MP** diet improved gut health with a higher bacterial abundance of **Firmicutes,** especially **Bacillus and Lactobacillus** colonization.

Acknowledgments

Thank you