



INVESTIGATING ZOOPLANKTON BIOENCAPSULATION AS A PROBIOTIC DELIVERY METHOD FOR CATFISH FRY



Jing Huang*, Charles C. Mischke, Caitlin E. Older, Matt J. Griffin, Heather R. Jordan, Peter J. Allen, Penelope M. Goodman, J. Grant Reifers, and Fernando Y. Yamamoto

Thad Cochran National Warmwater Aquaculture Center, DREC, Mississippi State University

INTRODUCTION

The intestinal microbiota is closely associated with biosynthesis of essential nutrients, digestion, and immune modulation. Dietary supplementation with probiotics has been recognized as a strategy to enhance growth and health performance in aquaculture species. However, conventional probiotic supplementation through manufactured feeds is not optimal for fish at early life stages. This study aims to improve probiotic delivery efficacy to catfish fry by using pond-derived zooplankton as a natural carrier.

Probiotic strain used in this study

Autochthonous probiotic: *Lactococcus lactis* MA5

Host origin: Pond-raised hybrid catfish (*Ictalurus punctatus* × *I. furcatus*)

Documented functions:

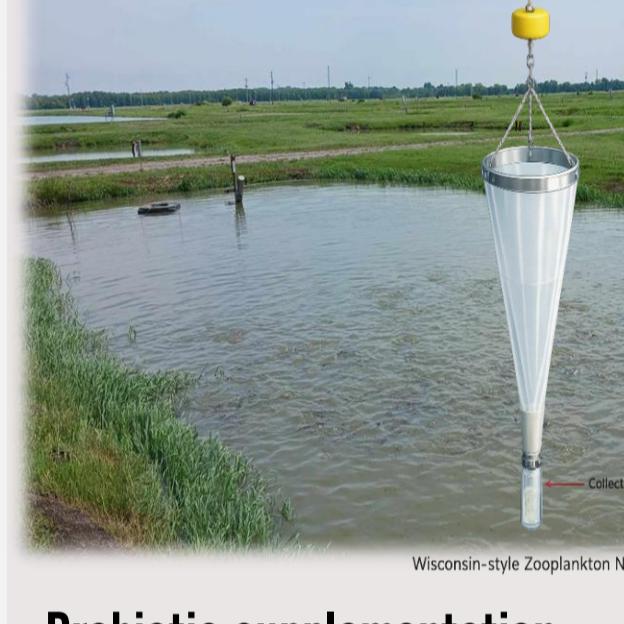
- ✓ Growth promotion
- ✓ Immune modulation
- ✓ Antagonistic activity against *Aeromonas hydrophila*, *Edwardsiella ictaluri*, and *E. piscicida*
- ✓ Support fish recovery from acute hypoxia stress

OBJECTIVES

- Investigate proliferation of zooplankton in an artificial environment supplemented with *L. lactis* MA5.
- Assess the impact of *L. lactis* MA5 on zooplankton microbiota.
- Evaluate probiotic bioencapsulation efficiency in zooplankton preferred by catfish fry.

MATERIALS & METHODS

Zooplankton proliferation and population investigation

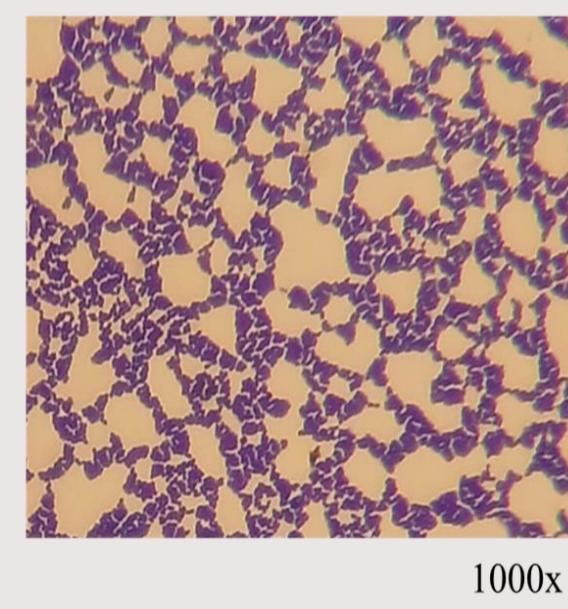


This study was conducted during Sep. 2025 from two 1-acre ponds at the Delta Research Extension Center, Stoneville, MS. The zooplankton were collected using a Wisconsin-style zooplankton net and stocked into 12 indoor tanks (23-L) at equal densities with pond water for seven days.

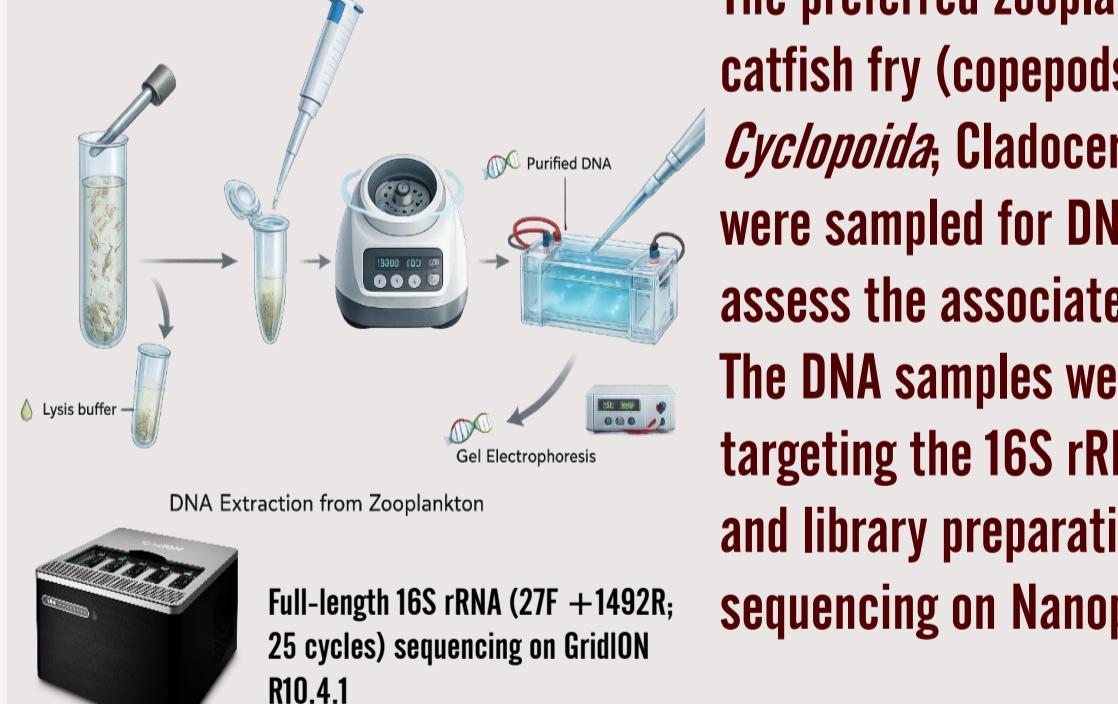
Probiotic supplementation

During the experiment, all tanks were offered ground commercial catfish feed.

Four tanks were supplemented with *L. lactis* MA5 at 3.2×10^4 , and four tanks at 1.6×10^6 CFU/mL. Zooplankton samples were collected and fixed in 5% buffered formaldehyde on day 0 and 7 for quantification and identification.



Microbiota investigation



The preferred zooplankton families for catfish fry (copepods: *Calanoida* and *Cyclopoida*; Cladocerans: *Bosminidae*) were sampled for DNA extraction and to assess the associated microbiome. The DNA samples were amplified targeting the 16S rRNA gene, purified, and library preparation was followed by sequencing on Nanopore platform.

STATISTICAL ANALYSIS

The proliferation of zooplankton was analyzed using Kruskal-Wallis (KW) on JMP software, SAS. Alpha (Shannon entropy, Pielou's evenness, observed features, and Chao1) and beta diversity (Bray-Curtis and Jaccard) was analyzed using KW (alpha), PERMANOVA (beta), and PERMDISP (beta) tests on RStudio. Differential abundance of intestine bacteria was tested for using linear discriminant analysis effects size (LEfSe).

ACKNOWLEDGEMENTS

This study was funded by Mississippi Agriculture and Forestry Experiment Station (MAFES) Special Research Initiative (SRI), and by the USDA-ARS project 6066-31320-007-00-D grant through MAFES and the National Institute of Food and Agriculture, USDA, Hatch project MIS-523010. It also used resources provided by SCINet project and the AI Center of Excellence of the USDA-ARS project 0201-88888-003-000D and 0201-88888-002-000D and the Mississippi State University College of Veterinary Medicine.

RESULTS

Table 1. Zooplankton population of incubation with daily supplementation of *L. lactis* MA5 in aquaria for seven days.

	Control	Low	High	PSE	Pro > ChiSq
Total Rotifers (%)	0.25 ± 0.32	0.48 ± 0.65	0.22 ± 0.28	0.42	0.69
Total Copepods (%)	1.16 ± 0.46	1.33 ± 0.33	1.30 ± 0.53	0.44	0.62
Cyclopoida (%)	0.66 ± 0.49	0.81 ± 0.73	0.98 ± 0.68	0.16	0.78
Calanoida (%)	1.37 ± 0.42	1.42 ± 0.30	1.56 ± 0.03	0.22	0.73
Total Cladocerans (%)	0.13 ± 0.22	0.09 ± 0.13	0.09 ± 0.13	0.63	0.88
Bosminidae (%)	0.05 ± 0.00	0.04 ± 0.00	0.07 ± 0.00	0.00	0.55

Copepods, Cladocerans, and ostracods are considered desirable zooplankton for catfish (Mischke et al., 2003). In the present study, zooplankton communities composition in ponds consisted primarily of rotifers (22%), copepods (52.4%), and Cladocerans (24.9%). Based on their dominance and feeding relevance, *Bosminidae*, *Calanoida*, and *Cyclopoida* were selected as potential probiotic carriers.

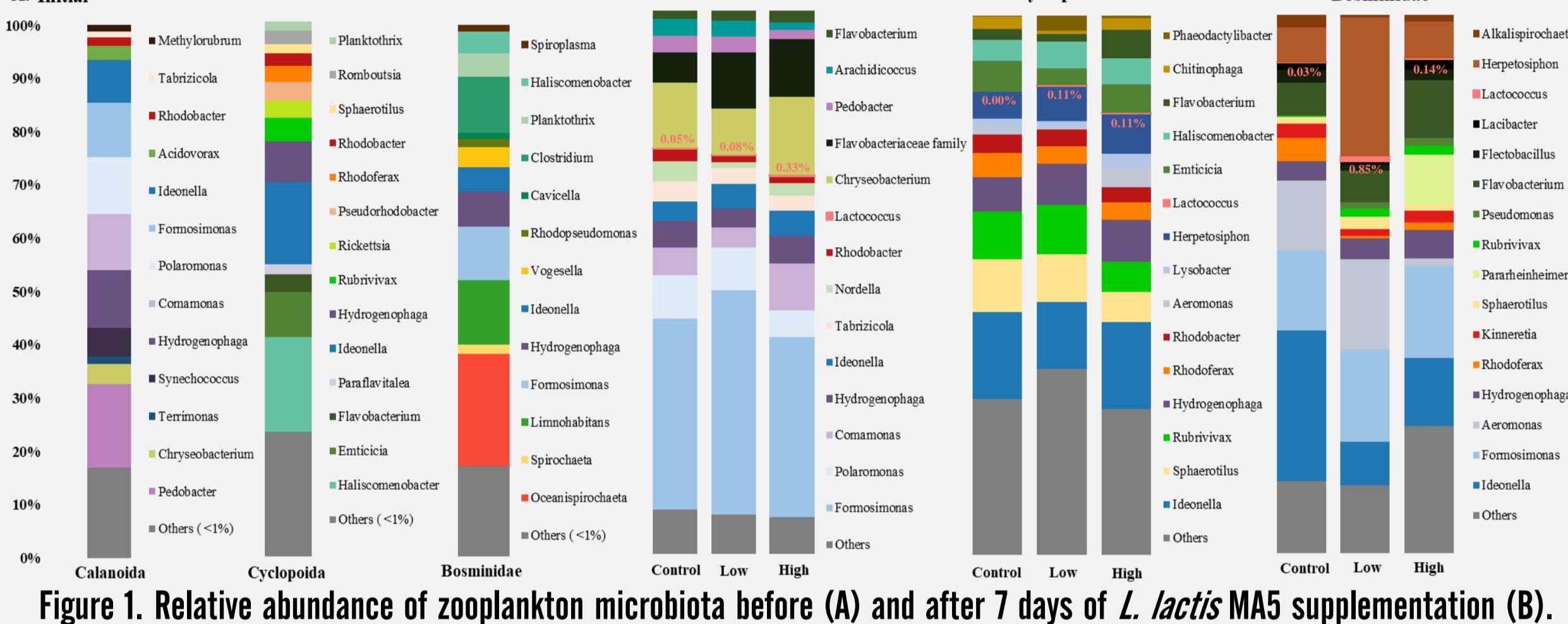


Figure 1. Relative abundance of zooplankton microbiota before (A) and after 7 days of *L. lactis* MA5 supplementation (B). Calanoida (B1) Cyclopoida (B2) Bosminidae (B3). (Low: 3.2×10^4 and High: 1.6×10^6 CFU/mL).

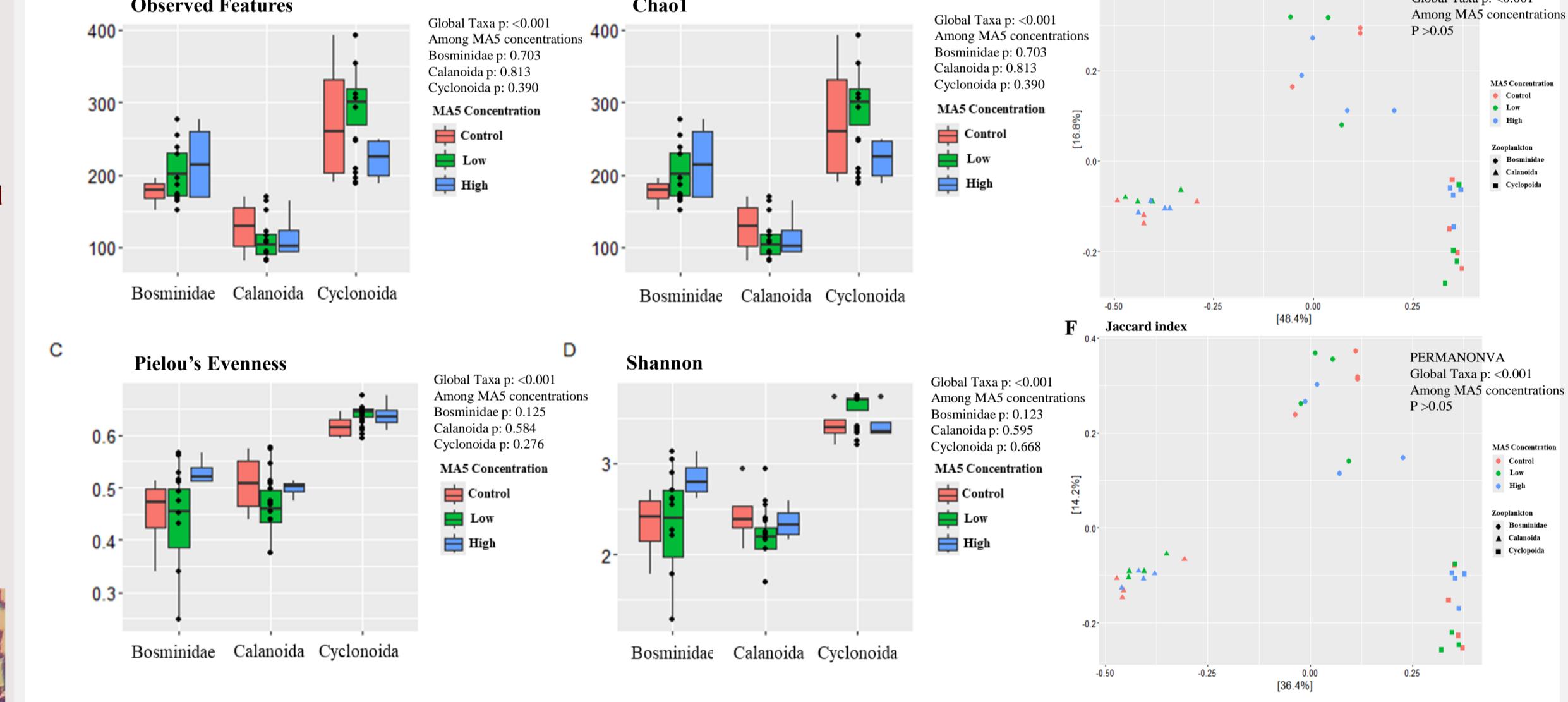
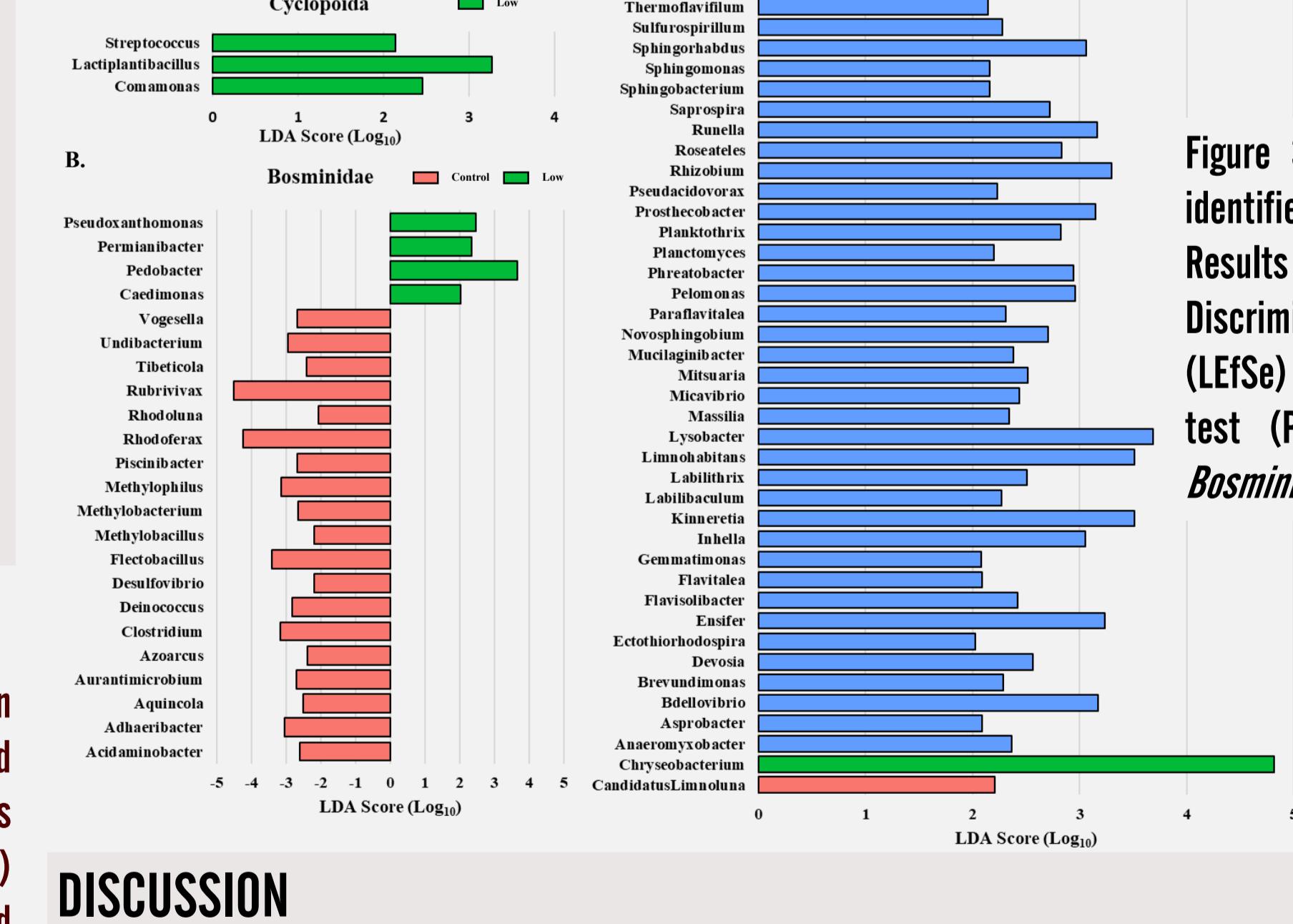


Figure 2. Diversity analysis of the zooplankton microbiota after 7 days of *L. lactis* MA5 supplementation. (A) Alpha diversity index: Observed features (B) Alpha diversity index: Chao1 (C) Alpha diversity index: Pielou's Evenness (D) Alpha diversity index: Shannon (E) Bray-Curtis dissimilarity (F) PCoA plot analysis Jaccard index. Zooplankton received probiotic *L. lactis* MA5 with two doses (Low: 3.2×10^4 and High: 1.6×10^6 CFU/mL) in fiber glass tanks supplied with pond water (water volume: 23-L). *Bosminidae*, *Calanoida* and *Cyclonoida* were harvested for the analysis after the trial.



DISCUSSION

Proliferation results indicated that the presence of *L. lactis* MA5 did not enhance zooplankton population growth. Additionally, seven days of co-incubation with MA5 did not significantly affect the relative abundance of *L. lactis* associated with zooplankton. Notably, *Calanoida*, *Cyclopoida*, and *Bosminidae* were not natural carriers of *Lactococcus*, which is consistent with previous findings. The relative abundance of *L. lactis* was low across all taxa ($\max = 0.85\%$); although not significant, it was higher in with high MA5 dose groups. However, bioaccumulation effects may still occur when catfish fry consume MA5-enriched zooplankton, potentially leading to increased probiotic abundance in the fish intestinal microbiota. Further studies are needed to improve the efficacy of this delivery strategy.

CONCLUSIONS

- Introduction of *Lactococcus lactis* MA5 did not enhance zooplankton proliferation under artificial environment.
- Different zooplankton taxa exhibited distinct microbiota compositions.
- No significantly differences in either alpha or beta diversity were observed when offering zooplankton *L. lactis* MA5.
- Mild increases in *Lactococcus* spp. relative abundance were observed after seven days of *L. lactis* MA5 supplementation of zooplankton.

REFERENCE

Charles C. Mischke, David J. Wise, Ryan L. Lane, 2003. Zooplankton Size and Taxonomic Selectivity of Channel Catfish Fry. N. Am. J. Aquac. 65.



MISSISSIPPI STATE UNIVERSITY™
MS AGRICULTURAL AND FORESTRY EXPERIMENT STATION

