



Comparative Analysis of Gut Microbiota Structure in Size-Specific Triploid Rainbow Trout (*Oncorhynchus mykiss*) under Low- versus Optimal-Temperature Conditions

Yang Ying^{1,2}, Li Changhong^{1,2}, Yan Qingchun^{1,2}, Wang Linan^{1,2}, Li Zhaonan^{1,2}, Jin Wenjie^{1,2}, Bai Peng^{1,2}, Liang Tianxiu^{1,2}, Huang Shuchen^{1,2}, Chen Yanxia^{1,2*}

1. College of Eco-Environmental Engineering, Qinghai University

2. Key Laboratory of Plateau Cold-water Fish Culture and Eco-environmental Conservation (Co-construction by Ministry and Province), Ministry of Agriculture and Rural

BACKGROUND:

The rainbow trout (*Oncorhynchus mykiss*), native to the cold freshwater habitats of northern North America and the Pacific coast, is now the most widely commercially farmed salmonid species globally.

Water temperature fluctuations markedly affect feeding in rainbow trout (*Oncorhynchus mykiss*). Temperatures below the optimal 12 – 18 °C lower body temperature, depress enzymatic and metabolic activity, and reduce energy demands, thereby curtailing feeding. These thermal shifts also restructure the intestinal microbiota, and stressful temperatures can provoke dysbiosis, increasing pathogen abundance and disease risk.



Figure 1 *Oncorhynchus mykiss*

EXPERIMENT DESING:

Triploid rainbow trout (*Oncorhynchus mykiss*) of three size classes-small, medium, and large-were sampled from Longyangxia Reservoir (Gonghe County, Qinghai Province) in November 2023 (designated SFN, MFN, LFN; n=6 each) and again in February 2024 during minimal water temperatures (designated SFF, MFF, LFF; n=6 each), with mean weights provided. Following anesthesia with 60 µg/mL MS-222, intestinal contents were harvested, rapidly frozen in liquid nitrogen, and stored at -80 °C until analysis.

RESULTS:

Analysis of the dominant bacterial phyla revealed a shift towards Cyanobacteria under low-temperature conditions. While Proteobacteria, Bacteroidota, and Firmicutes were predominant in the SFN, MFN, and LFN groups, respectively, Cyanobacteria dominated the LFF group and, more broadly, all low-temperature groups (SFF, MFF, LFF). Its relative abundance was significantly higher in the MFF vs. MFN and LFF vs. LFN comparisons ($P < 0.01$).

The relative abundance of Mycoplasma exhibited a marked increase in the intestines of all three size classes of triploid rainbow trout under low-temperature conditions (SFF: 14.08%; MFF: 9.12%; LFF: 5.77%), compared to substantially lower levels in their temperature-matched counterparts from the optimal temperature period (SFN: 0.08%; MFN: 1.22%; LFN: 0.66%). This pronounced disparity in Mycoplasma abundance underscores a significant effect of temperature and fish size on the composition of the gut microbiota.

Principal coordinate analysis (PCoA) revealed clear segregation between the gut microbiota structures of the low-temperature and optimal-temperature groups, indicating distinct microbial compositions.

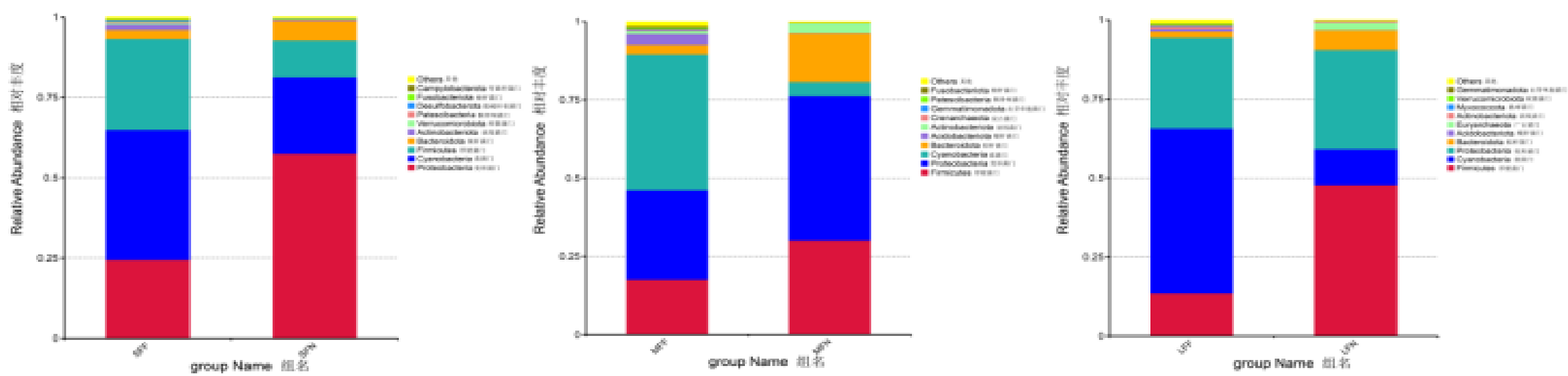


Fig. 2 Structure and relative abundance ratio of intestinal flora of triploid rainbow trout (*Oncorhynchus mykiss*) at the phylum level

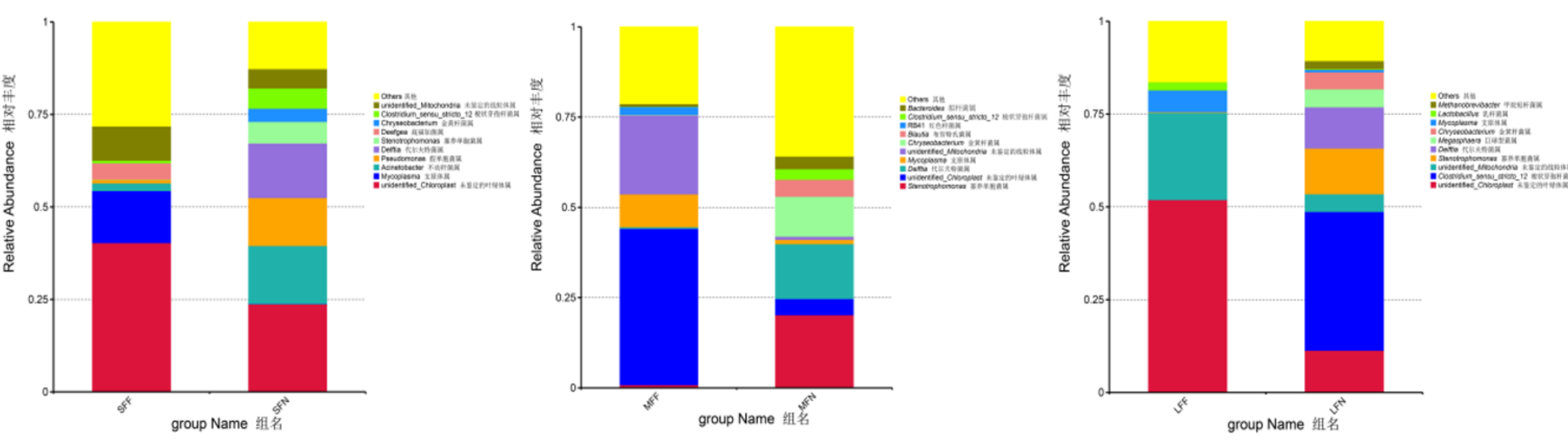


Fig. 3 Structure and relative abundance ratio of gut flora of triploid rainbow trout (*Oncorhynchus mykiss*) at genus level

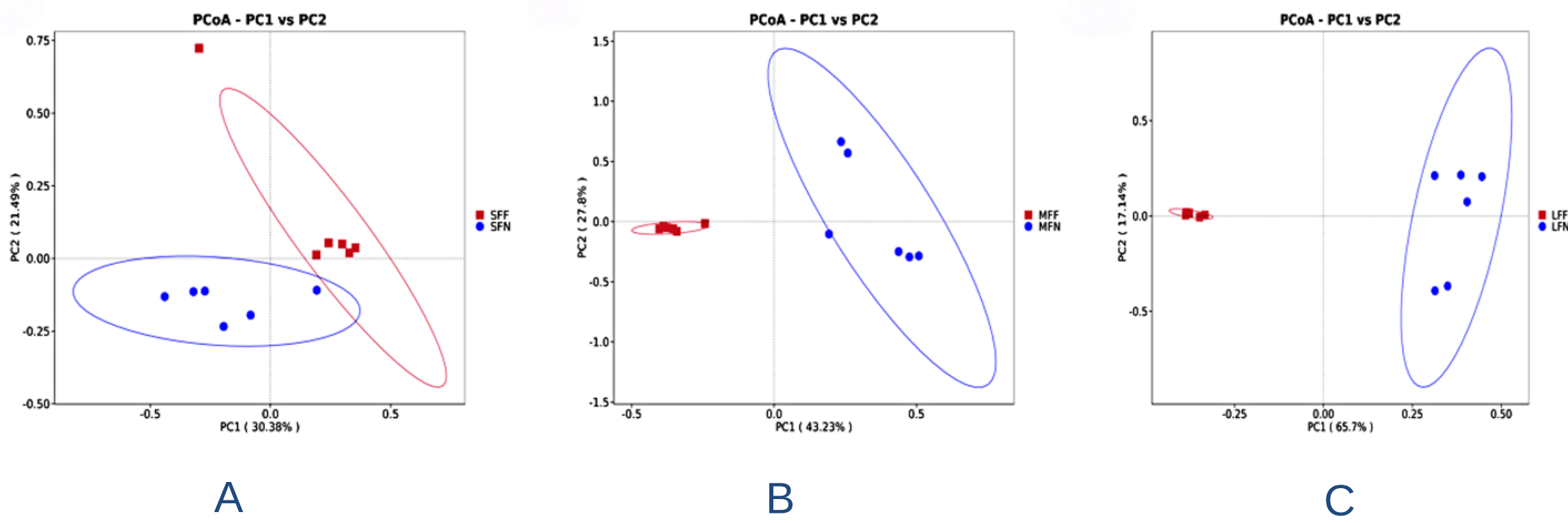


Fig. 4 PCoA analysis of the intestinal flora of different sizes of triploid rainbow trout (*Oncorhynchus mykiss*) at low and moderate temperatures

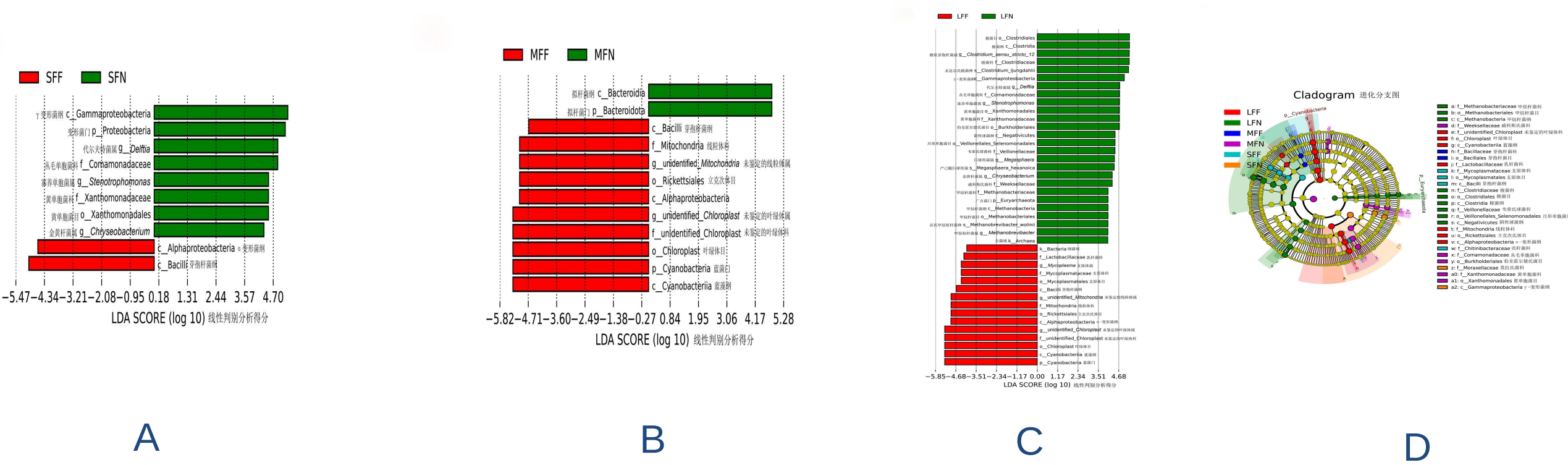


Fig. 5 Differences in LefSe among different sizes of triploid rainbow trout (*Oncorhynchus mykiss*) between low and moderate temperature groups

CONCLUSION:

Our findings demonstrate that low-temperature exposure induces gut microbial dysbiosis in *Oncorhynchus mykiss*, characterized by reduced beneficial taxa and proliferation of pathobionts. Size-dependent microbial restructuring was observed across temperature variants, with distinct taxonomic shifts in intestinal communities. These results provide critical insights for developing thermal adaptation strategies in aquaculture management.