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Effect of background color on the growth performance, digestive enzyme activity, antioxidant capacity, and intestinal microbiota of juvenile *Plectropomus leopardus* 

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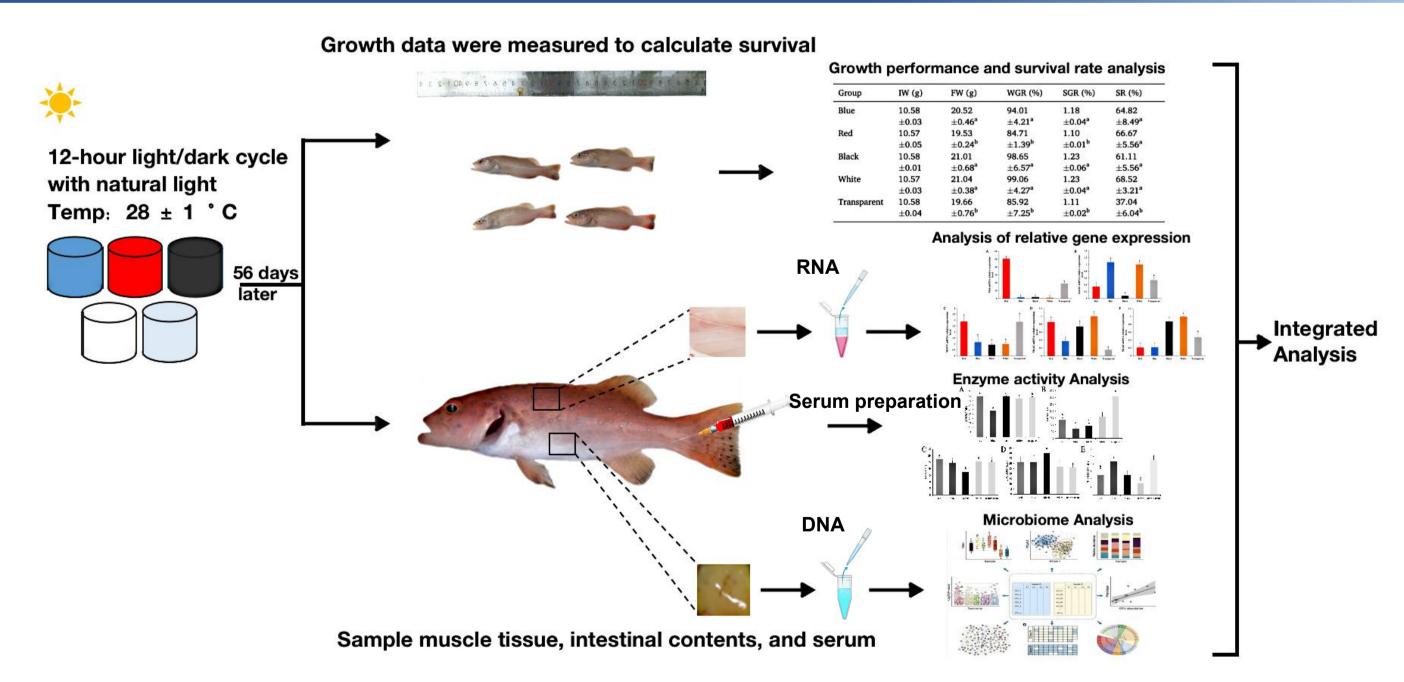
# Highlights

- Plectropomus leopardus showed better growth performance on white background.
- White background color increased digestive enzyme activity and reduced antioxidant capacity of *Plectropomus leopardus* and the white background is beneficial for intestinal microbiota of *Plectropomus leopardus*.

#### Introduction

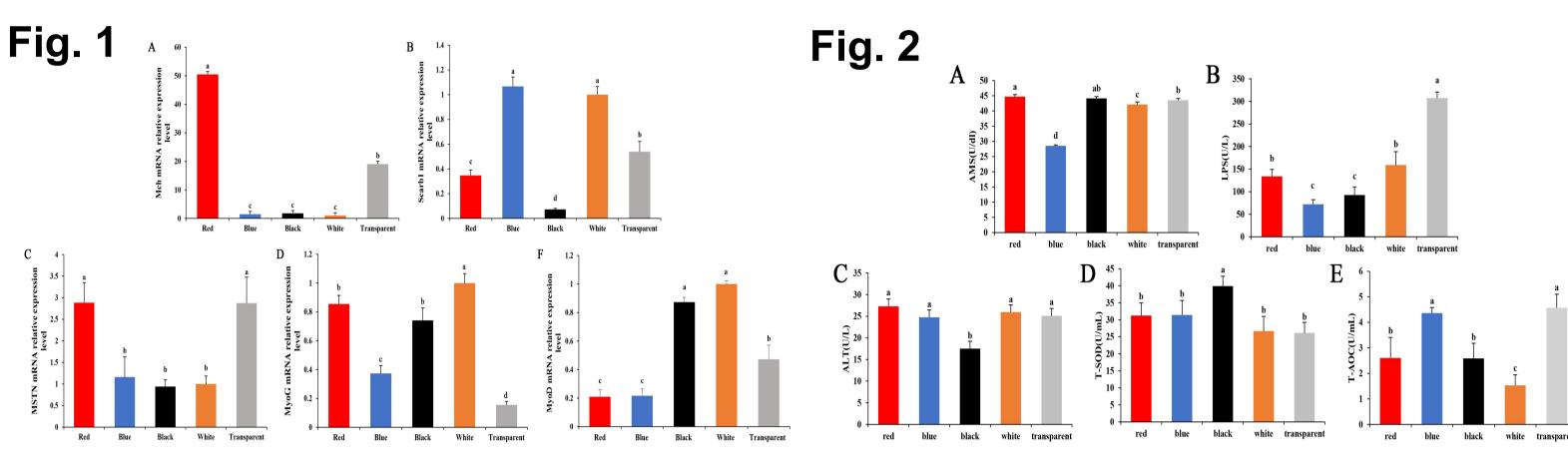
- The important influence of background color on the growth, survival, feeding, movement, metabolism and reproduction of aquatic animals.
- Studies have shown that different background colors have significant effects on the growth and survival of different fish species, for example, a light background helps the growth and survival of Eurasian sea bass, while a white background is most favorable for specific growth rates and feed conversion rates of goldfish and carp.
- Gut microbiota is closely related to animal growth performance, and its composition and structure are affected by stress, diet and environmental factors.
- The aim of this study was to investigate the effects of background color on growth performance, digestive enzyme activity, antioxidant capacity and intestinal microbiota of juvenile *P. leopardus* grouper, so as to optimize its breeding conditions. The white background can promote the growth

## Materials and methods



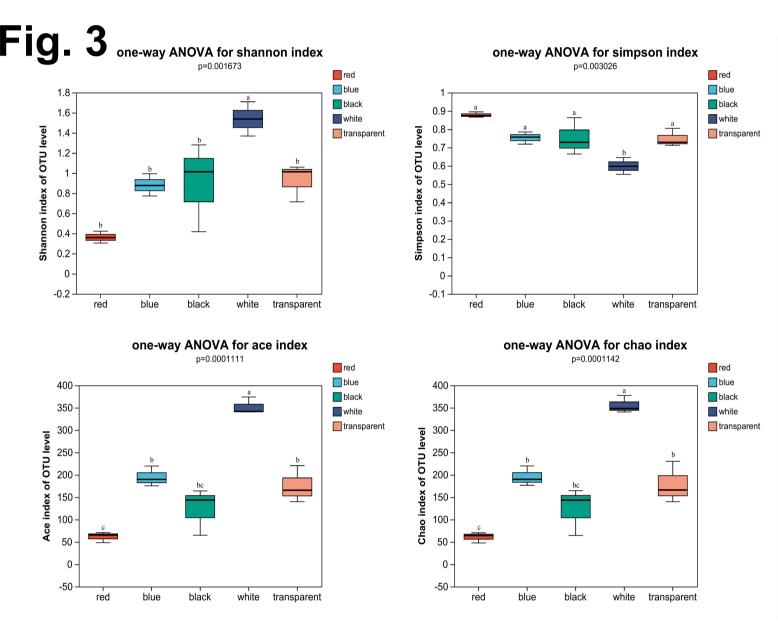
We integrated the growth data of different background color groups and the microbiome of intestinal contents for analysis. They were fed for 56 days in a 12-hour light-dark cycle at a temperature of  $28\pm1^\circ$  C, with commercial feed fed three times a day until full. At the end of the experiment, the survival rate, weight gain rate and specific growth rate were calculated, blood was collected to prepare serum, digestive enzyme activity and antioxidant capacity were measured, RNA was extracted from the back muscles were collected for real-time quantitative PCR analysis and intestinal microbial transcriptome analysis was collected for intestinal microbial contents.

#### Results

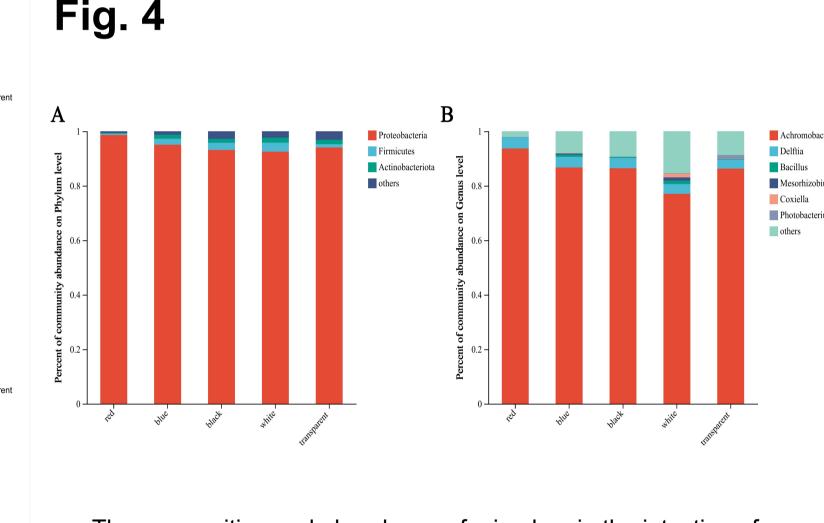


The expression levels of growth-related genes in the muscle tissues of the *Plectropomus leopardus* were changed under different color backgrounds

The activities of α-amylase (AMS) and lipase (LPS) were higher in the white group, while the activities of total superoxide dismutase (T-SOD) and total antioxidant capacity (T-AOC) were lower



The Alpha-diversity indices of the bacterial communities in intestine of *P. leopardus* under different background cultures.



The composition and abundance of microbes in the intestine of *P. leopardus* under different background colors at the phylum and genus level. *Proteobacteria*, *Firmicutes*, and *Actinobacteriota* were the dominant phyla. The six dominant genera were *Achromobacter*, *Delftia*, *Bacillus*, *Mesorhizobium*, *Coxiella*, and *Photobacterium*.

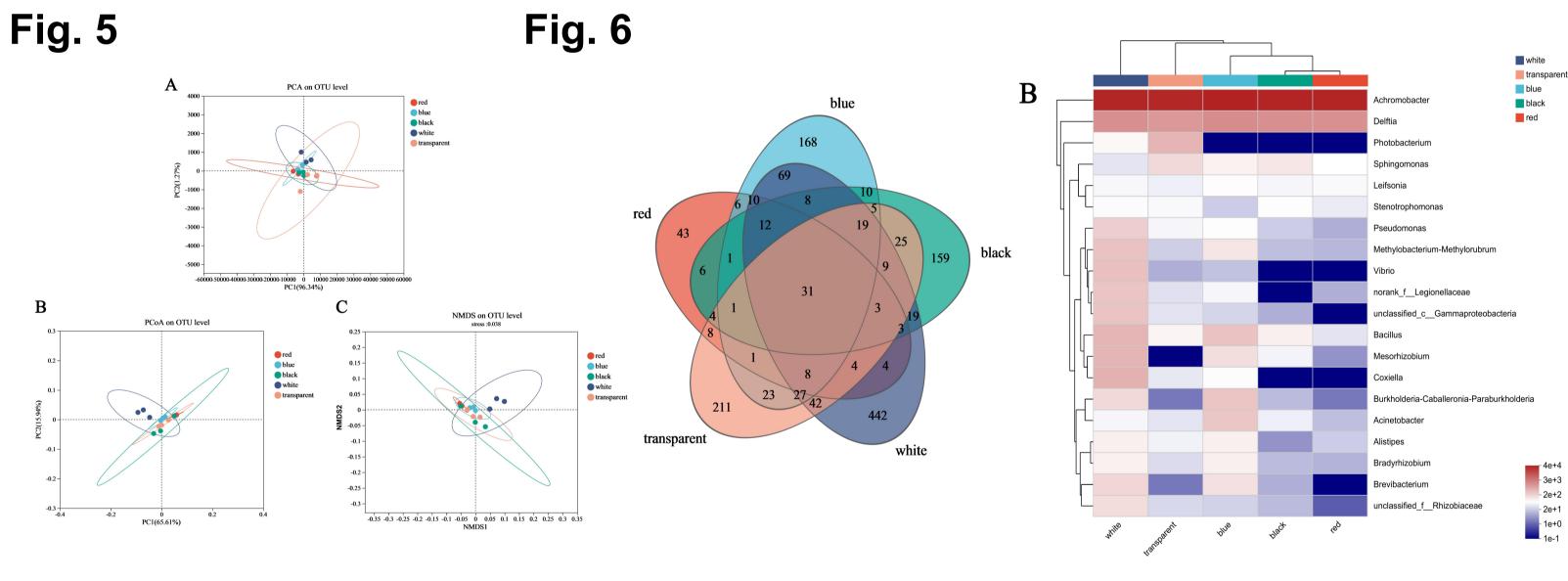
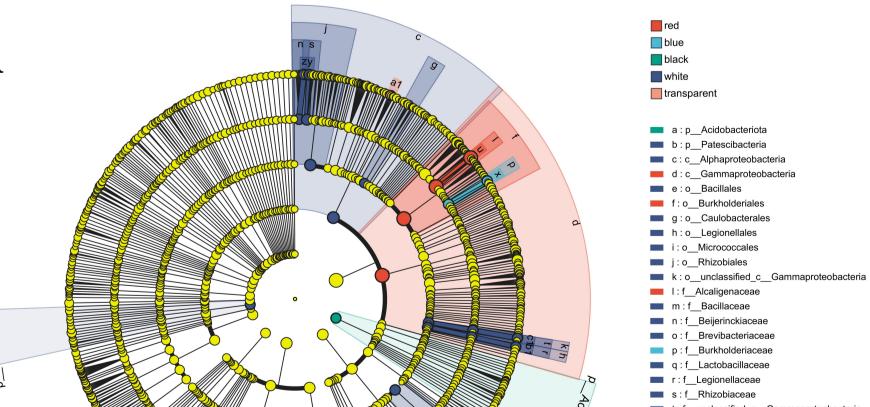


Table. 2

The intestinal microbiota communities of the different background color groups varied along the first axes of both the principal component analysis (PCA) and principal coordinate analysis (PCoA). The structure of the intestinal microbiota of P. leopardus varied among background color treatments. In the NMDS analysis, the stress value was 0.038. This indicates that the data were highly representative.

Fig. 7

The intestinal microbiota communities of the different background. The Venn diagram revealed 31 OTUs detected across all groups. The heatmap figures for *P. leopardus* show relative abun-dances of the dominant microbiota at the genus level. These findings indicate that the microbiota composition in the intestine of *P. leopardus* changed gradually, and this resulted in changes in the relative abundance of the dominant microbiota.

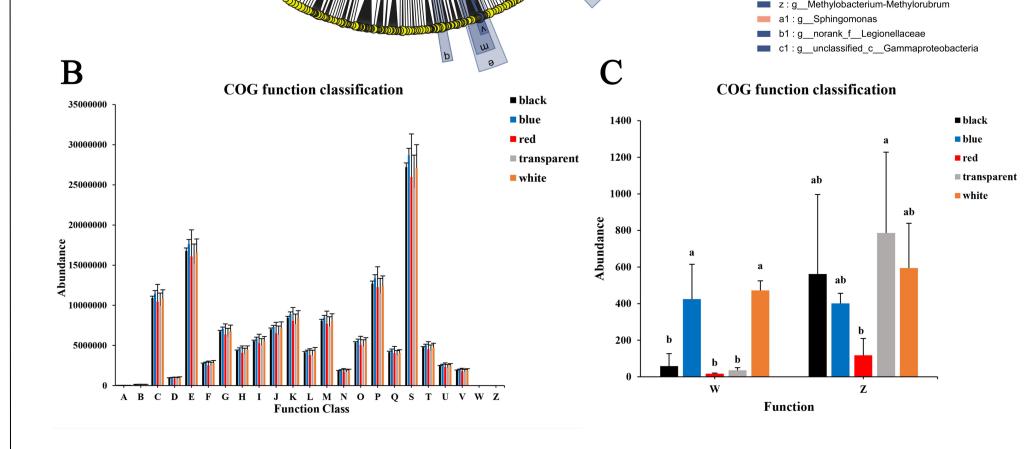


Effect of background color on growth.

Group IW (g) FW (g) WGR (%) SGR (%) SR (%)

Blue  $10.58\pm0.03$   $20.52\pm0.46^a$   $94.01\pm4.21^a$   $1.18\pm0.04^a$   $64.82\pm8.49^a$ Red  $10.57\pm0.05$   $19.53\pm0.24^b$   $84.71\pm1.39^b$   $1.10\pm0.01^b$   $66.67\pm5.56^a$ Black  $10.58\pm0.01$   $21.01\pm0.68^a$   $98.65\pm6.57^a$   $1.23\pm0.06^a$   $61.11\pm5.56^a$ White  $10.57\pm0.03$   $21.04\pm0.38^a$   $99.06\pm4.27^a$   $1.23\pm0.04^a$   $68.52\pm3.21^a$ Transparent  $10.58\pm0.04$   $19.66\pm0.76^b$   $85.92\pm7.25^b$   $1.11\pm0.02^b$   $37.04\pm6.04^b$ 

Note: Different lowercase letters indicate significant differences (P < 0.05). IW: Initial Weight, FW: Final Weight, WGR: Weight gain rate, SGR: Specific growth rate, SR: Survival rate.



background color treatments (LDA score > 2). After the COG enrichment analysis, we found that the different OTUs were mainly enriched in different metabolic processes, including amino acid metabolism, lipid metabolism, and carbohydrate metabolism. The functions of intestinal microbiota were altered by the background color, but significant differences were only observed for two functions: extracellular structures and cytoskeleton.

The intestinal microbiota of *P. leopardu*s varied among

## Conclusions

- Performance of P. leopardus striped Mandarin fish and increase the abundance of beneficial microorganisms in their gut.
- In general, the results of this study can be used to optimize the breeding conditions of leopard-striped Mandarin fish.