EFFECTS OF CULTURE SPACE AND STOCKING DENSITY ON THE BEHAVIORAL RESPONSES AND MOLECULAR MECHANISMS OF GRASS CARP Ctenopharyngodon Idella

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Abstract

While high-density aquaculture maximizes production, the concurrent spatial restriction induces chronic stress and compromises fish welfare. Although previous studies have extensively investigated the impacts of crowding stress on growth performance, immunity, and metabolism in fish, research on how rearing space and stocking density shape fish behavior remains insufficient. In this study, grass carp (Ctenopharyngodon idella) were subjected to a 65-day rearing experiment under five treatments: small-space low-density (SL; 0.375 m³, 5 kg/m³), small-space medium-density (SM; 0.375 m³, 10 kg/m³), small-space high-density (SH; 0.375 m³, 20 kg/m³), large-space low-density (BL; 2.5 m³, 5 kg/m³), and large-space high-density (BH; 2.5 m³, 20 kg/m³). By integrating multidimensional behavioral paradigms with transcriptomic analysis, it was revealed that expanding rearing space significantly mitigates high-density-induced anxiety-like behaviors in grass carp. Furthermore, transcriptomic data indicated that a larger rearing space modulates the organism's stress regulation primarily through the calcium signaling pathway and hormone signaling pathway. This study provided novel insights into the correlation between spatial behavioral phenotypes and the underlying molecular mechanisms in grass carp, offering a robust theoretical foundation for developing welfare-oriented aquaculture practices and optimizing the spatial design of modern farming facilities.

Introduction

Rearing space and stocking density are critical environmental factors affecting fish growth, behavior, and welfare in intensive aquaculture. Spatial restriction may impair normal behavioral expression and induce metabolic stress and oxidative damage. However, the effects of rearing space on behavioral phenotypes, physiological homeostasis, and brain molecular regulation in grass carp remain unclear. This study integrated growth performance, behavioral assays, serum biochemistry, oxidative stress indices, and whole-brain transcriptomics to evaluate the adaptive responses of grass carp under different space–density conditions, providing theoretical support for rearing-space optimization and welfare assessment.

Features of Intensive Aquaculture: High Stocking Density

- Intensive aquaculture offers advantages of high input, high output, and high production efficiency.
- However, high stocking density restricts the activity space of fish and increases crowding stress.

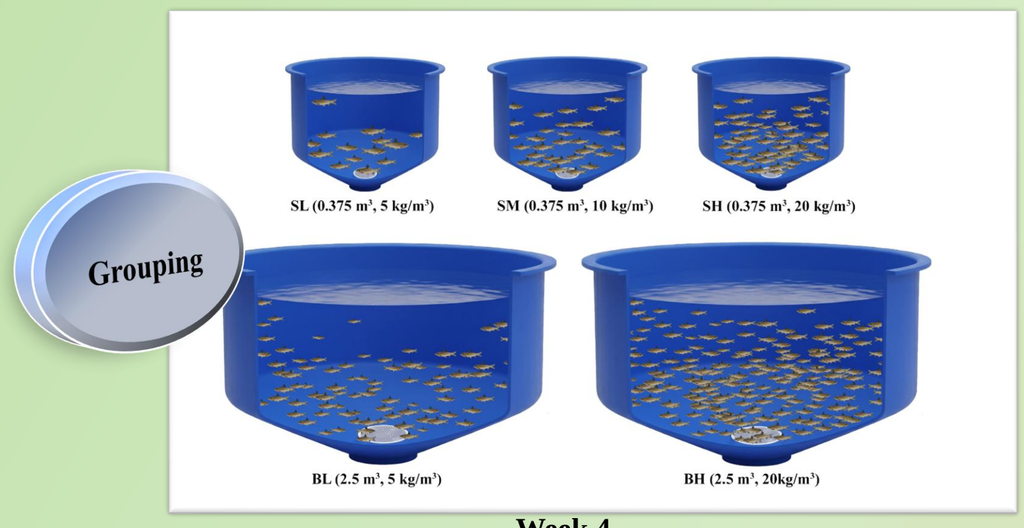


Industrial recirculating aquaculture system (RAS)



Grass carp

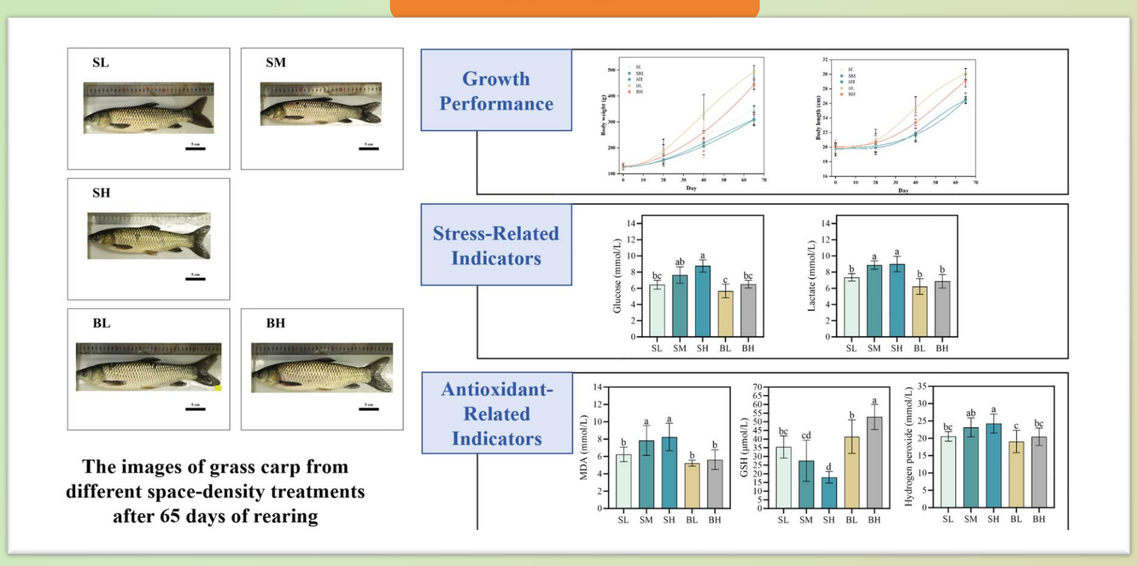
Methods



Body weight and body length measurement, serum biochemical analysis and transcriptome analysis

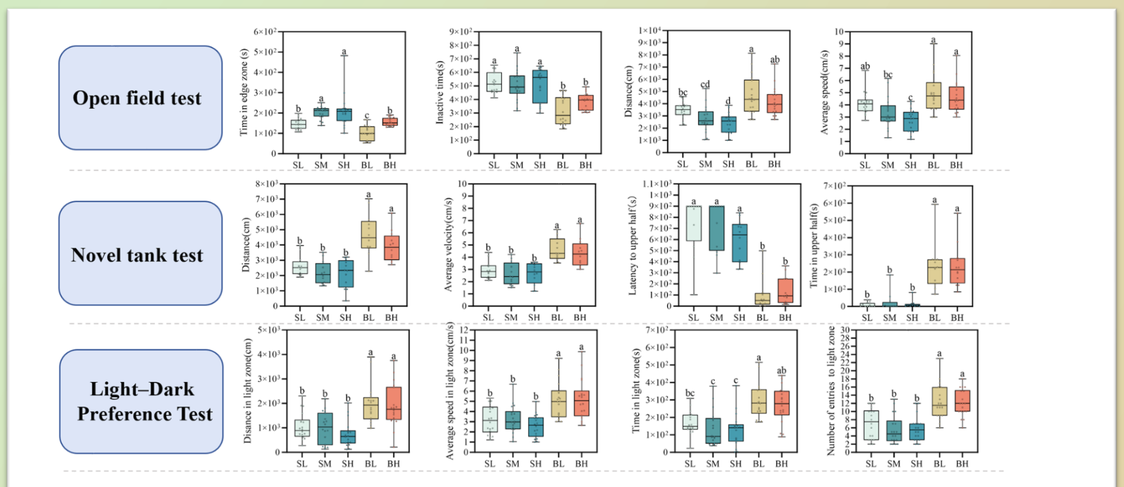
Grass carp were acclimated for 14 days in a recirculating aquaculture system and then assigned to five space–density treatments: small-space low-density group (SL, 0.375 m³, 5 kg/m³), small-space medium-density group (SM, 0.375 m³, 10 kg/m³), small-space high-density group (SH, 0.375 m³, 20 kg/m³), large-space low-density group (BL, 2.5 m³, 5 kg/m³), and large-space high-density group (BH, 2.5 m³, 20 kg/m³). Each treatment included three replicates, and the rearing trial lasted 65 days. Water quality was monitored every two days. Behavioral assays, including the open field, novel tank diving, and light–dark preference tests, were conducted after four weeks. Serum and whole-brain tissues were collected at day 65 for biochemical, oxidative stress, and transcriptomic analyses. GO and KEGG enrichment analyses were performed for differentially expressed genes. Statistical analysis was conducted using one-way ANOVA followed by Duncan's test. Data are shown as mean ± SD, and $P < 0.05$ was considered significant.

Results



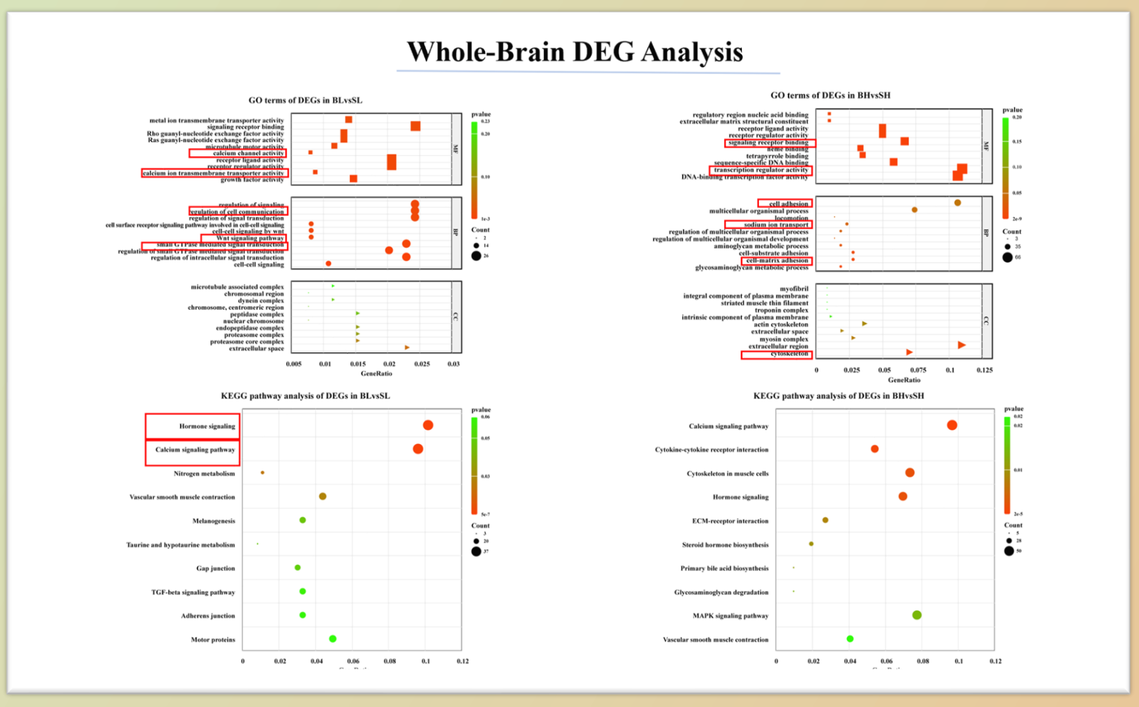
The images of grass carp from different space-density treatments after 65 days of rearing

Compared with the small-space groups, grass carp in the large-space groups showed more rapid increases in body weight and body length. Relative to the SH group, the BH group had significantly lower serum glucose and lactate concentrations. Moreover, significantly decreased MDA and H_2O_2 levels, together with elevated GSH levels, were observed in the BH group compared with the SH group.



Behavioral Test Results

In the open-field test, grass carp in the large-space treatment groups showed a significantly shorter duration of freezing behavior. Compared with the SH group, the BH group exhibited increased total distance moved and average velocity. In the novel tank test, fish in the large-space treatment groups showed a shorter latency to enter the upper zone and a significantly longer duration in the upper layer. In the Light–Dark Preference Test, grass carp in the large-space treatment groups displayed significantly increased distance moved, average velocity, and number of entries into the light zone.



In the GO enrichment analysis, DEGs in the BL vs SL comparison were mainly enriched in calcium channel activity, calcium ion transmembrane transporter activity, the Wnt signaling pathway, small GTPase-mediated signal transduction, and regulation of cell communication. In the BH vs SH comparison, DEGs were mainly enriched in cell adhesion, cell–matrix adhesion, cytoskeleton, sodium ion transport, receptor binding, and transcriptional regulation. In the KEGG enrichment analysis, both the BL vs SL and BH vs SH comparisons were enriched in calcium signaling and hormone signaling pathways.

Conclusion

- Large rearing space improved the growth performance and antioxidant capacity of grass carp, and alleviated stress responses induced by crowding stress.
- Large rearing space enhanced basal locomotor activity, reduced anxiety-like responses, and improved exploratory behavior and adaptability to novel environments in grass carp.
- Under the same density conditions, the large-space treatment groups were commonly enriched in calcium signaling and hormone signaling pathways, suggesting that calcium-dependent signal transduction and endocrine regulation may constitute important molecular mechanisms by which large rearing space improves physiological adaptation and behavioral performance in grass carp.