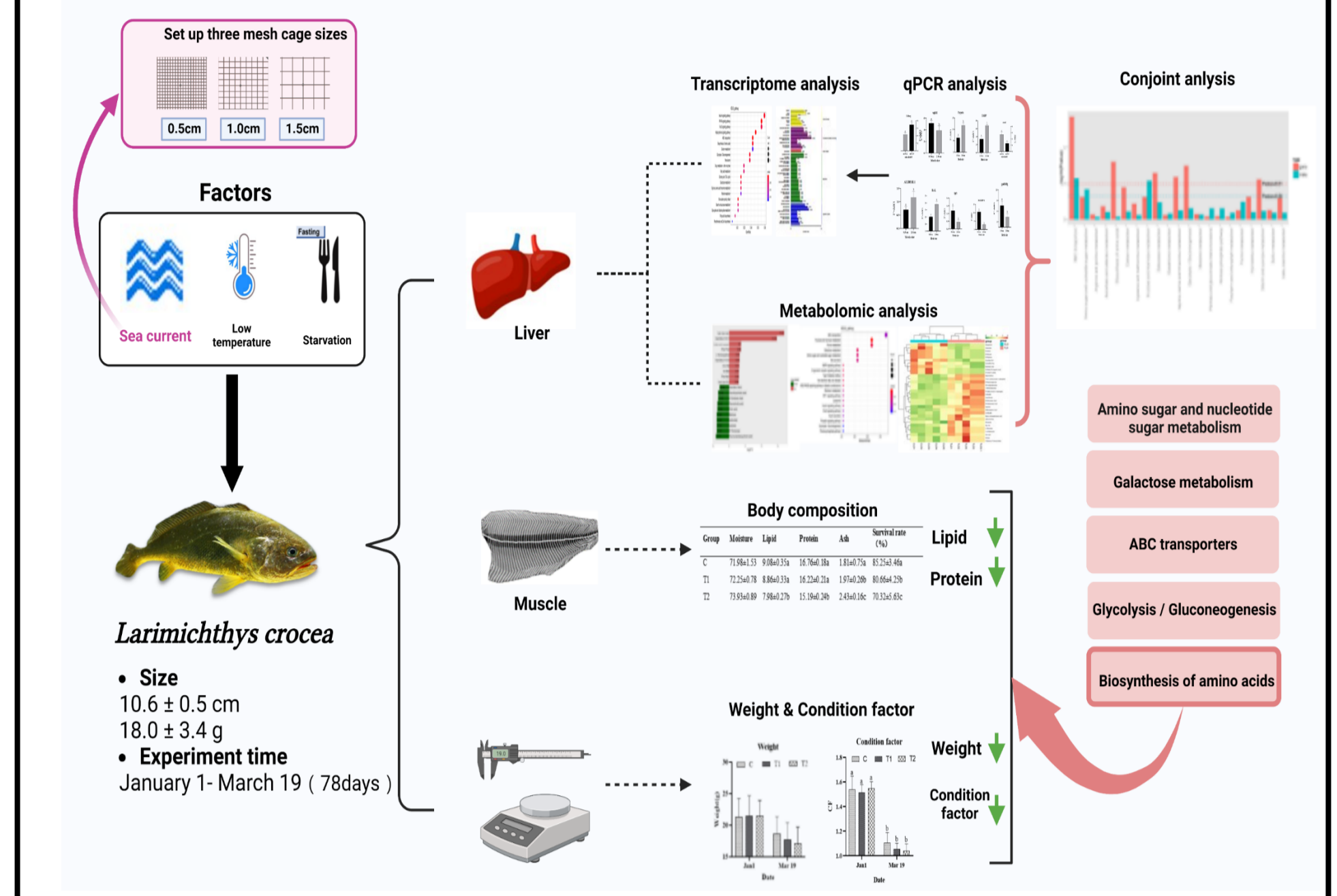


## Introduction

- ❖ **Large yellow croaker:** *Larimichthys crocea*, an economically important marine fish. Cage culture is the primary method.
- ❖ **Overwintering challenges:** Overwintering poses difficulties due to changes in behavior, weight loss, and increased mortality. Factors beyond energy stores affect survival rates at low temperatures.
- ❖ **Environmental influences:** Strong sea currents, including water flow and turbulence, impact large yellow croaker during overwintering.
- ❖ **Aim of this study:** Investigate the behavior and physiological responses of large yellow croaker to these environmental factors.

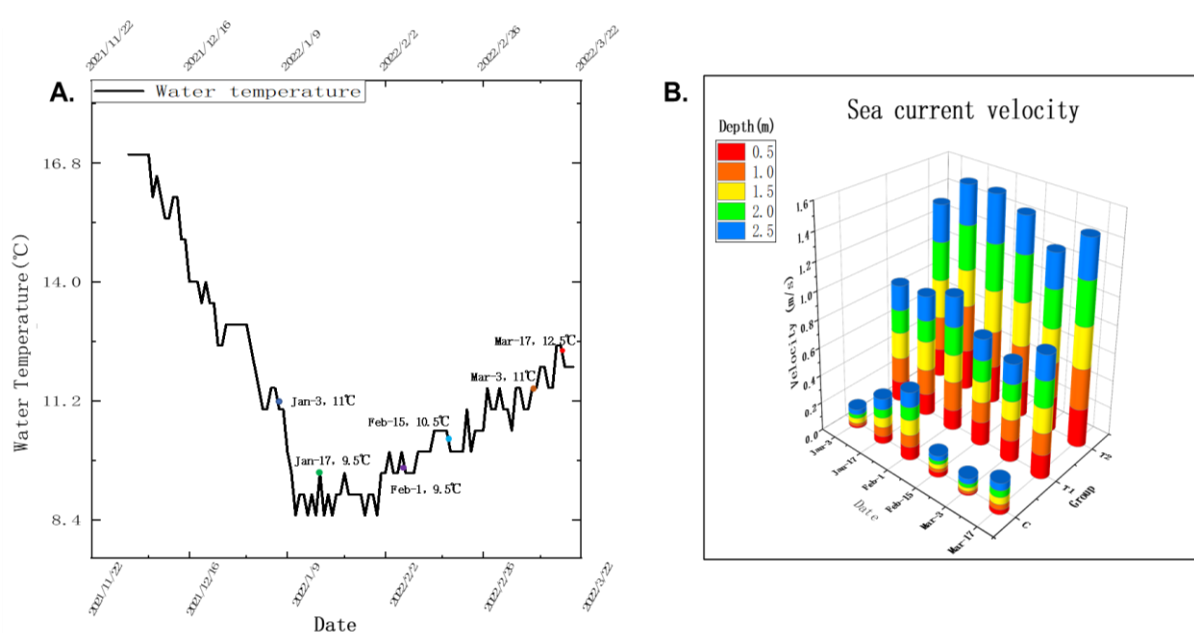
## Methods



## Results

### Temperature and Water Velocity

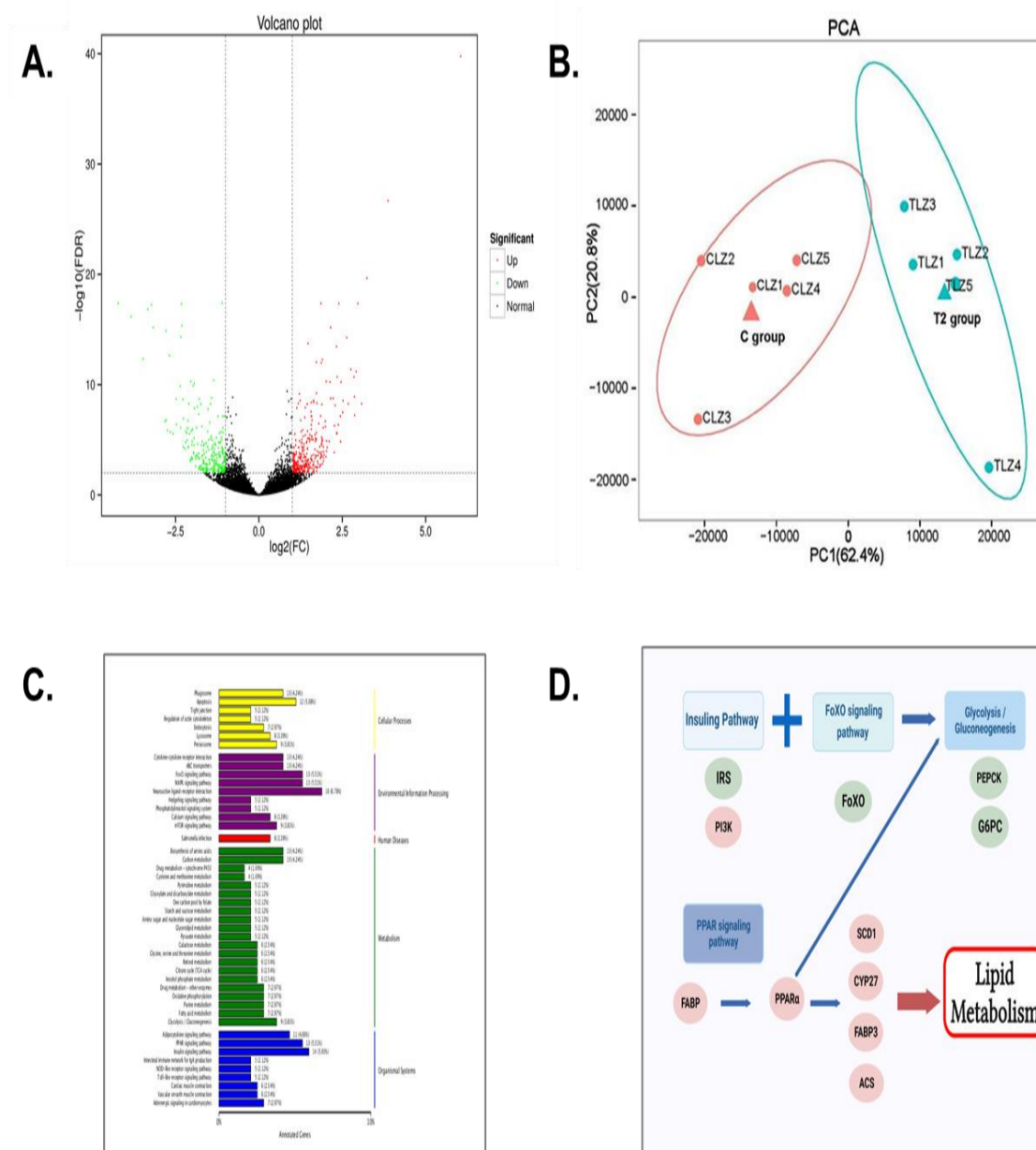
- ❖ Water temperature: 8.5 - 12.5°C (Fig. 1).
- ❖ The average current velocities: C: 0.049 ± 0.007m/s, T1: 0.167 ± 0.011 m/s, and T2: 0.286 ± 0.020 m/s (Fig. 1).



**Figure 1.** Temperature (A) and sea current velocity (B) during the experimental period.

### Transcriptome Analysis of Fish Liver

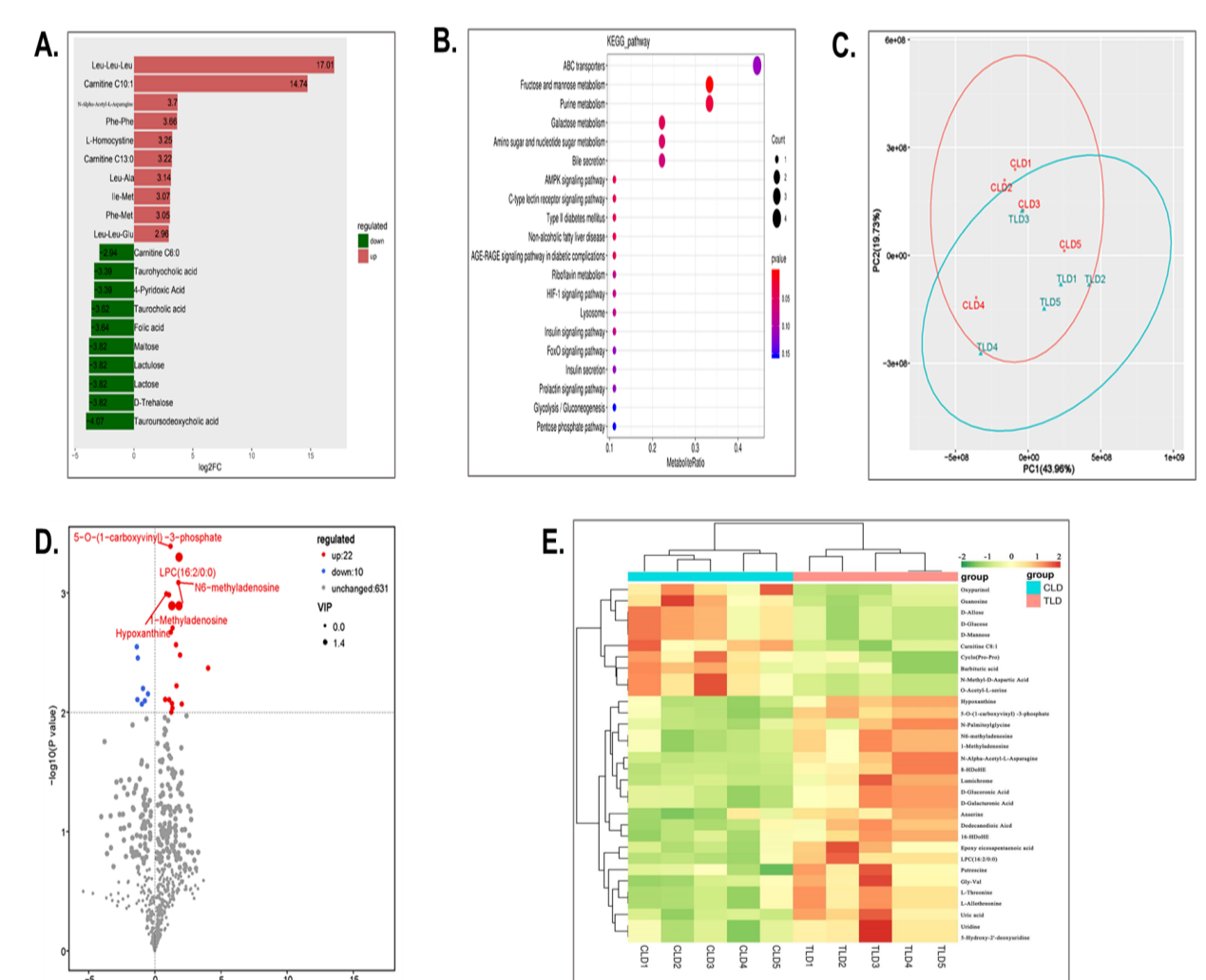
- ❖ A total of 557 genes were differentially regulated, with 300 upregulated and 257 downregulated (Fig 3A).
- ❖ PCA analysis showed sea current had a significant effect on gene expression (Fig 3B).
- ❖ The neuroactive ligand-receptor, insulin signaling pathway, PPAR signaling pathway, FoxO signaling pathway, and MAPK signaling pathway were annotated (Fig 3C).
- ❖ The PPAR signaling pathway is related to regulation of lipid metabolism. Genes FABP, PPAR $\alpha$ , SCD1, CYP27, FABP3, and ACS in the PPAR signaling pathway were increased (Fig 3D).



**Figure 3.** Transcriptome comparison of C group (0.5-cm mesh) and T2 group (2.0-cm mesh).

### Metabolomics Results

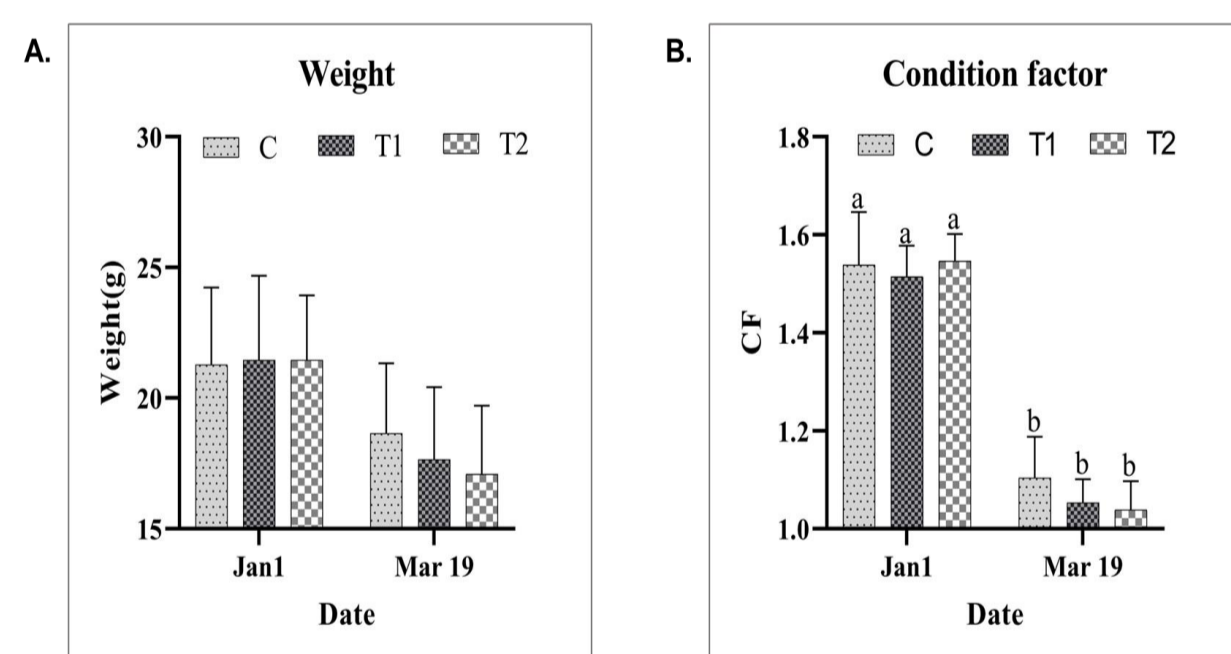
- ❖ The top five significantly increased metabolites: Leu-Leu-Leu, Carnitine C10:1, N-Alpha-L-Asparagine, Phe-Phe, L-Homocystine (Fig 4A).
- ❖ The top three significantly enriched pathways of DMs: ABC transporters, fructose and mannose metabolism, purine metabolism (Fig 4B).
- ❖ The PCA diagram indicated metabolism was influenced by the flow rate (Fig 4C).
- ❖ 663 significantly different metabolites were observed in the two groups (Fig 4D).
- ❖ The main DMs were oxypurinol, guanosine, D-allose, D-glucose, D-mannose, Carnitine C8:1, Cyclo (Pro-Pro), barbituric acid, N-Methyl-D-Aspartic Acid, O-Acetyl-L-serine (Fig 4E).



**Figure 4.** Metabolites of C group (0.5-cm mesh) and T2 group (2.0-cm mesh).

### Weights and Condition Factor

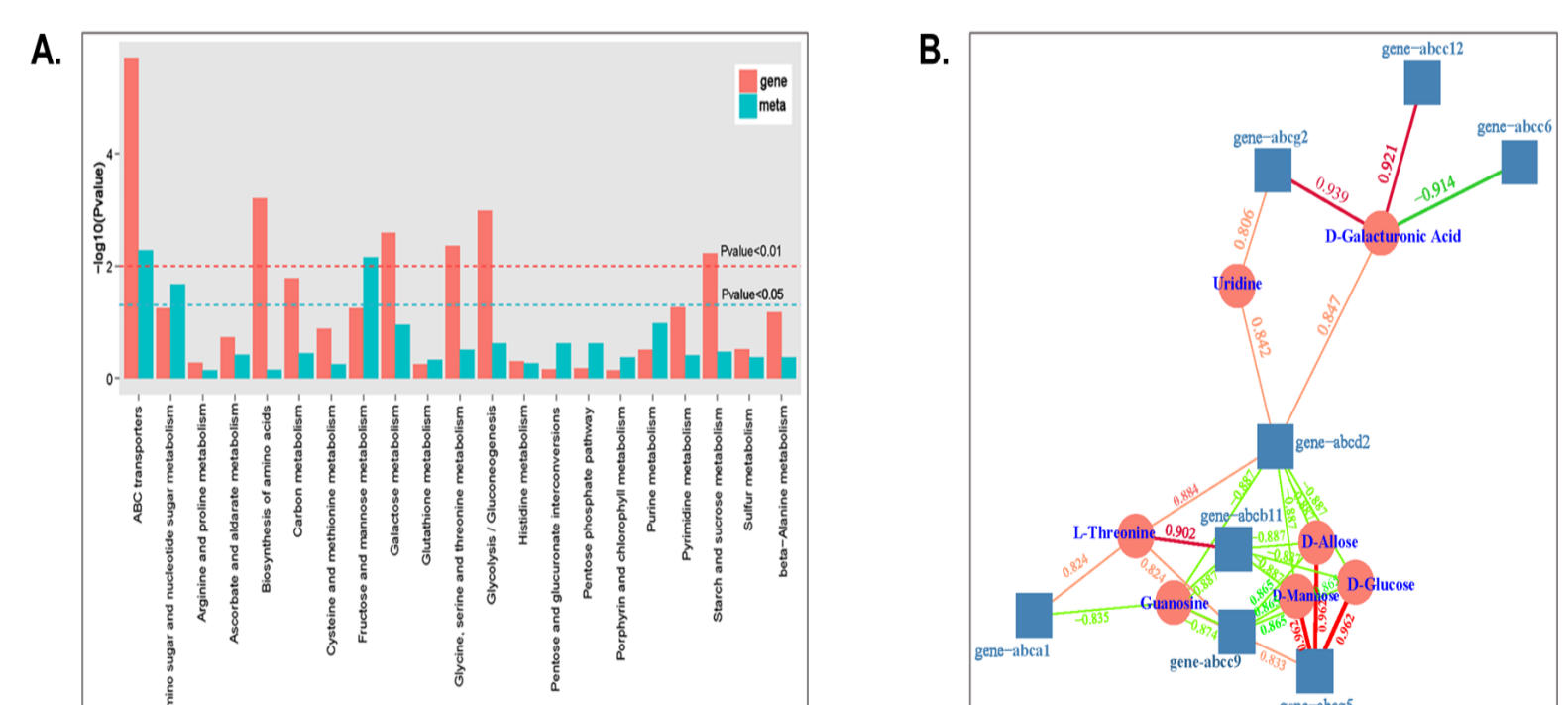
- ❖ The weights and condition factor of the fish decreased significantly in all groups (P < 0.05).



**Figure 2.** Weight (A) and condition factor (B). C: mesh size 0.5 cm, T1: mesh size 1.0 cm, T2: mesh size 2.0 cm.

### Integrated Pathway Enrichment Analysis

- ❖ ABC transporters pathway met the P value < 0.05 criterion for both DEGs and DMs (Fig 5A). Gene *abcg5* was positively related with D-allose, D-mannose, and D-glucose (Fig 5B). Only the ABC transporters pathway met the P value < 0.05 criterion for both DEGs and DMs.
- ❖ In the ABC transporters pathway, genes *ABCA1*, *ABCB11*, *ABCC9*, *ABCC5*, *ABCC12*, *ABCD2*, and *ABCG2* showed significant downregulation, and genes *ABCC6* and *ABCG5* showed significant upregulation
- ❖ Gene *abcg5* was positively related with D-allose, D-mannose, and D-glucose.



**Figure 5.** (A) Column chart of different metabolites and genes co-enrichment. (B) Correlation network diagram of the ABC transporters pathway.

## Conclusion

- ❖ In addition to low temperature and starvation, sea current plays an important role in the overwintering of large yellow croaker. Lipid and protein are heavily consumed for energy. The insulin and PPAR pathways are involved in regulating energy metabolism. The ABC transporter is responsible for the transport of small molecules during metabolism.

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