Schizochytrium sp. as a promising *Artemia tibetiana* nauplii fortifier for yellow drum (*Nibea albiflora*) larviculture

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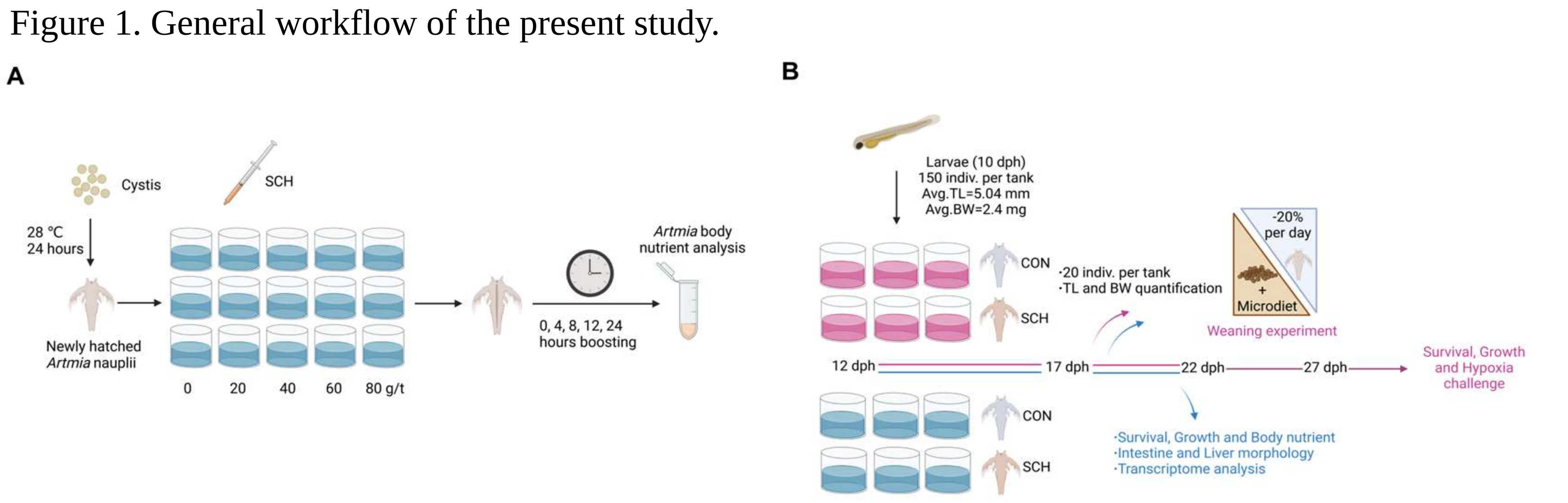
Abstract

Optimal nutrition for *Artemia* nauplii is crucial for marine larvae rearing, as it underpins their growth, development, and overall health. This study aimed to enhance the nutritional profile of *Artemia tibetiana* using *Schizochytrium* sp., assessing its feasibility for yellow drum larvae. Results showed that 50 nauplii/mL seawater and enriching nauplii with 40 g of *Schizochytrium* sp. per ton of seawater for 12 h significantly increased the DHA levels in *A. tibetiana* without affecting survival or size. In a 10-day feeding trial using 12dph yellow drum larvae, results showed significant improvements in growth, liver and intestinal morphology in larvae fed with *Schizochytrium* sp.-enriched *A. tibetiana*. RNA-seq analysis indicated that *Schizochytrium* sp. reduced lipid synthesis gene expression, increasing fatty acid catabolism in intestinal and liver tissues while elevating the expression of immune-related genes. In addition, *Schizochytrium* sp. improved the weaning effect of larvae and showed a higher survival rate under hypoxia stress.

Project designs

Figure 1. General workflow of the present study.



Results

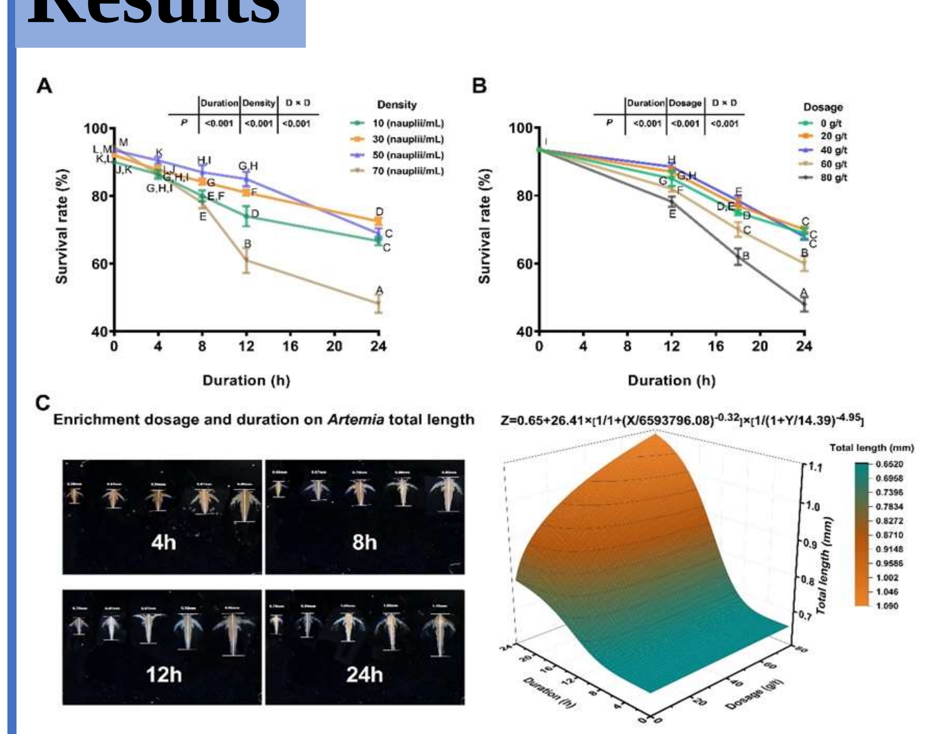


Figure 2. (A) The survival rate of *A. tibetiana* nauplii at different densities and for various durations. (B) The survival rate of *A. tibetiana* nauplii enriched under different dosages and durations. (C) Effect of enrichment dosage and duration on *A. tibetiana* nauplii total length.

The survival rate of *A. tibetiana* nauplii was found to be significantly influenced by both cultivation duration and density, with a noticeable interaction observed between these factors. A significant increase in *A. tibetiana* nauplii mortality was observed with increasing enrichment duration and fortifier dosage ($P < 0.05$).

Table 1. Effects of *Schizochytrium* sp. dosage and duration of enrichment on the fatty acid composition of *Artemia tibetiana* nauplii.

Dosage (g/t)	Duration (h)	SFAs [1] (%)	MUFAs [2] (%)	n-3 PUFAs [3] (%)	n-6 PUFAs [4] (%)	DHA [5] (%)
0	0	14.78 ± 0.45^{a}	41.78 ± 0.22^{a}	30.65 ± 0.81^{c}	12.15 ± 0.81^{e}	ND
0	4	15.44 ± 0.12^{ab}	42.81 ± 0.07^{abcd}	29.30 ± 0.30^{abc}	11.65 ± 0.14^{cde}	ND
0	8	15.62 ± 0.37^{abc}	43.19 ± 0.40^{abcd}	28.94 ± 0.52^{abc}	11.48 ± 0.16^{abcd}	ND
0	12	16.09 ± 0.60^{bc}	43.59 ± 0.75^{bcd}	28.38 ± 0.57^{ab}	11.32 ± 0.19^{abcd}	ND
0	24	16.52 ± 0.53^{c}	44.15 ± 1.03^{d}	27.79 ± 1.36^{a}	10.86 ± 0.74^{a}	ND
20	4	15.73 ± 0.09^{bc}	42.81 ± 0.32^{abcd}	29.15 ± 0.06^{abc}	11.48 ± 0.26^{abcd}	0.47 ± 0.02^{B}
20	8	15.82 ± 0.37^{bc}	43.01 ± 0.49^{abcd}	29.10 ± 0.64^{abc}	11.44 ± 0.15^{abcd}	0.49 ± 0.02^{B}
20	12	15.92 ± 0.68^{bc}	43.59 ± 1.85^{bcd}	28.50 ± 2.22^{ab}	11.30 ± 0.30^{abcd}	0.51 ± 0.02^{B}
20	24	16.22 ± 0.63^{bc}	43.96 ± 0.71^{cd}	27.93 ± 0.52^{ab}	11.08 ± 0.30^{abc}	0.57 ± 0.03^{BC}
40	4	15.76 ± 0.51^{bc}	42.81 ± 0.43^{abcd}	29.26 ± 0.94^{abc}	11.58 ± 0.45^{bcde}	0.55 ± 0.04^{BC}
40	8	16.10 ± 0.33^{bc}	43.01 ± 0.68^{abcd}	28.92 ± 0.62^{abc}	11.51 ± 0.07^{abcd}	0.65 ± 0.03^{CD}
40	12	16.37 ± 0.27^{bc}	43.53 ± 0.03^{bcd}	28.44 ± 0.14^{ab}	11.21 ± 0.28^{abcd}	0.86 ± 0.09^{E}
40	24	16.43 ± 0.81^{c}	43.81 ± 0.28^{bcd}	28.09 ± 0.10^{ab}	11.03 ± 0.48^{abc}	1.22 ± 0.17^{FG}
60	4	15.92 ± 0.40^{bc}	42.28 ± 0.29^{ab}	29.75 ± 0.39^{bc}	11.77 ± 0.22^{de}	0.71 ± 0.01^{D}
60	8	16.30 ± 0.28^{bc}	42.56 ± 0.22^{abcd}	29.06 ± 0.28^{abc}	11.46 ± 0.19^{abcd}	0.88 ± 0.07^{E}
60	12	16.36 ± 0.52^{bc}	42.98 ± 1.98^{abcd}	28.68 ± 1.72^{ab}	11.37 ± 0.17^{abcd}	1.20 ± 0.22^{F}
60	24	16.55 ± 0.51^{c}	43.73 ± 0.45^{bcd}	28.13 ± 0.80^{ab}	10.94 ± 0.08^{ab}	1.35 ± 0.05^{GH}
80	4	15.94 ± 0.67^{bc}	42.30 ± 1.04^{abc}	29.58 ± 0.33^{abc}	11.59 ± 0.07^{cde}	0.97 ± 0.08^{E}
80	8	16.21 ± 0.71^{bc}	42.79 ± 0.37^{abcd}	28.84 ± 1.11^{ab}	11.50 ± 0.11^{abcd}	1.26 ± 0.06^{FGH}
80	12	16.42 ± 0.32^{c}	43.19 ± 0.58^{abcd}	28.59 ± 0.97^{ab}	11.21 ± 0.16^{abcd}	1.37 ± 0.12^{H}
80	24	16.52 ± 0.21^{c}	43.88 ± 1.01^{bcd}	27.90 ± 1.22^{a}	11.14 ± 0.21^{abcd}	1.51 ± 0.01^{I}
Two-way	Dosage	0.174	0.485	0.945	0.989	< 0.001
ANOVA	Duration	0.003	0.001	< 0.001	< 0.001	< 0.001
P	D × D	0.996	1.000	1.000	0.993	< 0.001

Table 2. Effects of *Schizochytrium* sp. dosage and duration of enrichment on *Artemia tibetiana* nauplii amino acid content.

Dosage (g/t)	Duration (h)	TAAs [1]	EAAs [2]	NEAAs [3]
0	0	40.15 ± 0.81^{ab}	20.15 ± 1.14^{ab}	20.00 ± 0.35
0	4	41.84 ± 1.17^{ab}	20.68 ± 0.28^{b}	21.16 ± 0.98
0	8	41.34 ± 3.09^{ab}	20.23 ± 0.88^{ab}	21.11 ± 2.22
0	12	42.02 ± 1.85^{ab}	20.53 ± 0.54^{ab}	21.49 ± 1.49
0	24	40.48 ± 2.82^{ab}	19.19 ± 1.62^{ab}	21.29 ± 1.33
20	4	41.66 ± 2.27^{ab}	20.61 ± 1.16^{b}	21.05 ± 1.32
20	8	42.07 ± 0.53^{ab}	20.80 ± 0.53^{b}	21.26 ± 0.82
20	12	42.11 ± 1.39^{ab}	20.58 ± 0.78^{ab}	21.53 ± 0.99
20	24	38.44 ± 3.50^{ab}	18.24 ± 1.89^{ab}	20.20 ± 1.68
40	4	41.38 ± 2.08^{ab}	20.56 ± 1.58^{ab}	20.82 ± 1.17
40	8	41.96 ± 1.76^{ab}	20.32 ± 1.45^{ab}	21.64 ± 0.31
40	12	41.69 ± 2.36^{ab}	20.19 ± 1.57^{ab}	21.50 ± 0.80
40	24	37.12 ± 4.82^{ab}	17.99 ± 2.51^{ab}	19.13 ± 3.07
60	4	40.91 ± 5.16^{ab}	19.94 ± 2.88^{ab}	20.96 ± 2.28
60	8	42.67 ± 0.84^{b}	20.82 ± 0.76^{b}	21.85 ± 0.31
60	12	41.84 ±1.21^{ab}	20.11 ± 0.83^{ab}	21.73 ± 0.64
60	24	36.66 ± 4.65^{a}	17.21 ± 2.38^{a}	19.46 ± 2.42
80	4	40.90 ± 2.20^{ab}	20.05 ± 1.35^{ab}	20.85 ± 0.85
80	8	39.56 ± 5.31^{ab}	19.13 ± 3.23^{ab}	20.43 ± 2.08
80	12	41.55 ± 2.12^{ab}	20.17 ± 1.33^{ab}	21.38 ± 0.91
80	24	39.08 ± 3.97^{ab}	18.55 ± 2.69^{ab}	20.53 ± 1.46
Two-way	Dosage	0.874	0.812	0.931
ANOVA	Duration	0.008	0.002	0.074
P	D × D	0.970	0.991	0.951

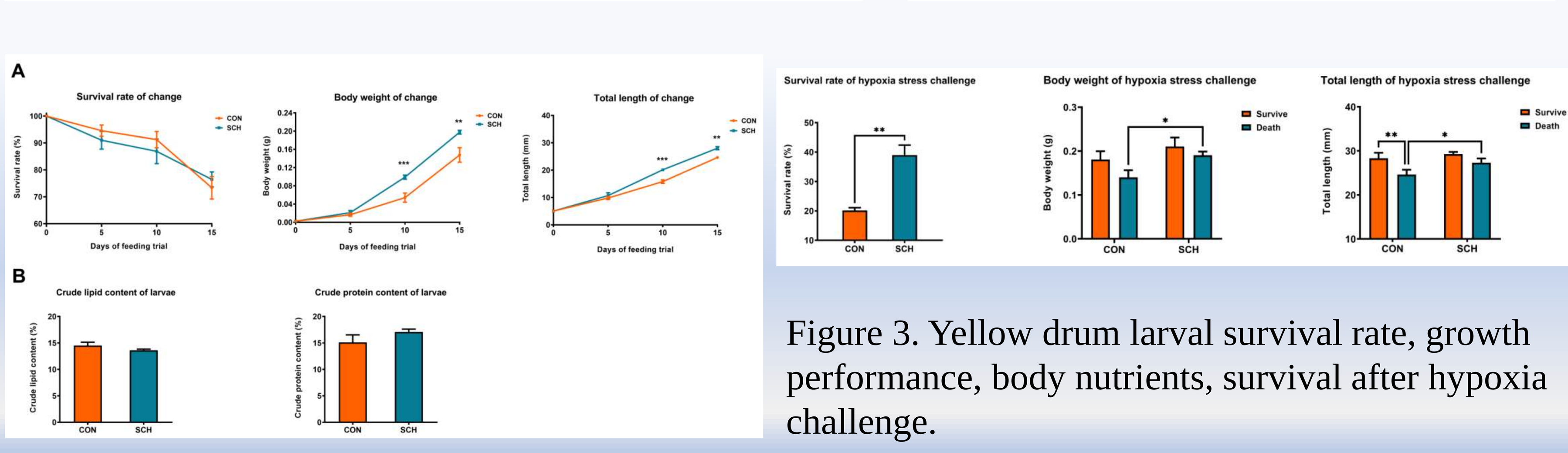


Figure 3. Yellow drum larval survival rate, growth performance, body nutrients, survival after hypoxia challenge.

Table 3. Fatty acid composition (%, identified fatty acids) of 22-dph yellow drum larvae.

Fatty acid	Larvae fed newly hatched A. tibetiana nauplii and enriched A. tibetiana nauplii	
	CON	SCH
C14:0	2.09 ± 0.38	2.45 ± 0.49
C16:0	13.35 ± 0.61	14.02 ± 0.65
C18:0	4.71 ± 0.28	3.97 ± 1.49
∑SFAs [1]	21.05 ± 0.87	21.31 ± 1.89
C16:1	10.92 ± 1.53	10.20 ± 0.24
C18:1n-9c [2]	32.52 ± 3.00	35.14 ± 1.52
∑MUFAs [3]	44.35 ± 1.48^{a}	46.33 ± 1.67^{b}
C18:2n-6c	8.32 ± 0.37	8.27 ± 0.26
C20:4n-6	2.27 ± 0.18	2.50 ± 0.10
∑n-6 PUFAs [4]	11.10 ± 0.29	11.51 ± 0.17
C18:3n-3	8.18 ± 0.70	8.31 ± 1.50
EPA	11.70 ± 1.33^{a}	13.26 ± 1.72^{b}
DHA	0.54 ± 0.03	0.56 ± 0.04
∑n-3 PUFAs [5]	20.79 ± 2.02	22.60 ± 3.18
∑n-3 LCPUFAs [6]	12.61 ± 1.32	14.29 ± 1.69
DHA+EPA	12.24 ± 1.30	13.82 ± 1.75
Else	3.14 ± 0.06	3.78 ± 0.61

Figure 4. Longitudinal paraffin sections and electron microscopic sections of the intestinal and liver tissue.

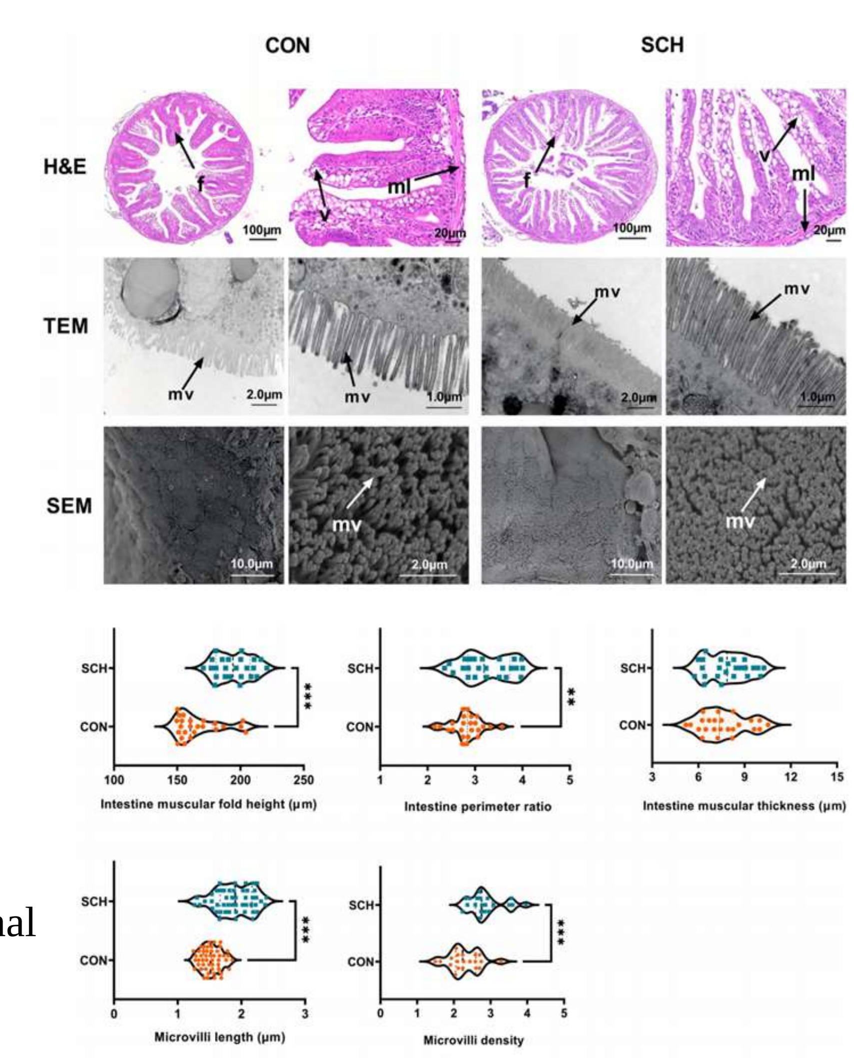


Figure 5. Transcriptome analysis of larval intestinal tissue.

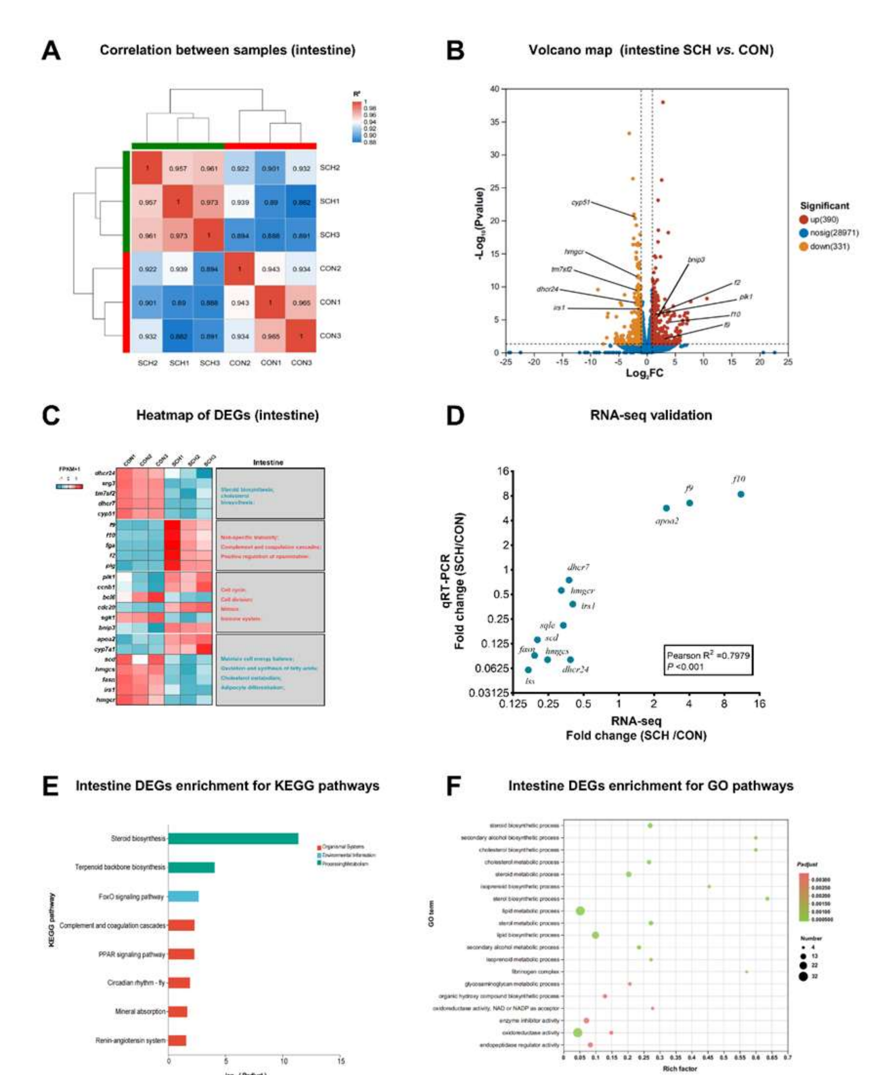


Figure 6. Transcriptome analysis of larval liver tissue.

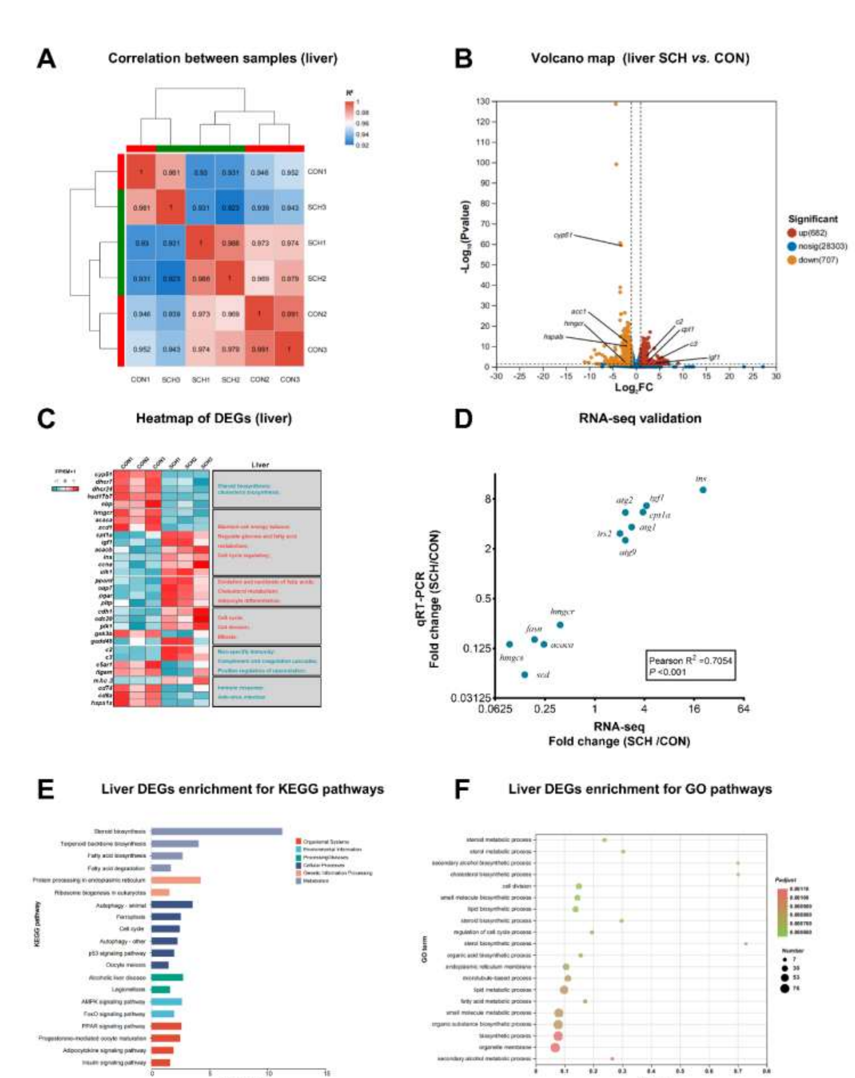
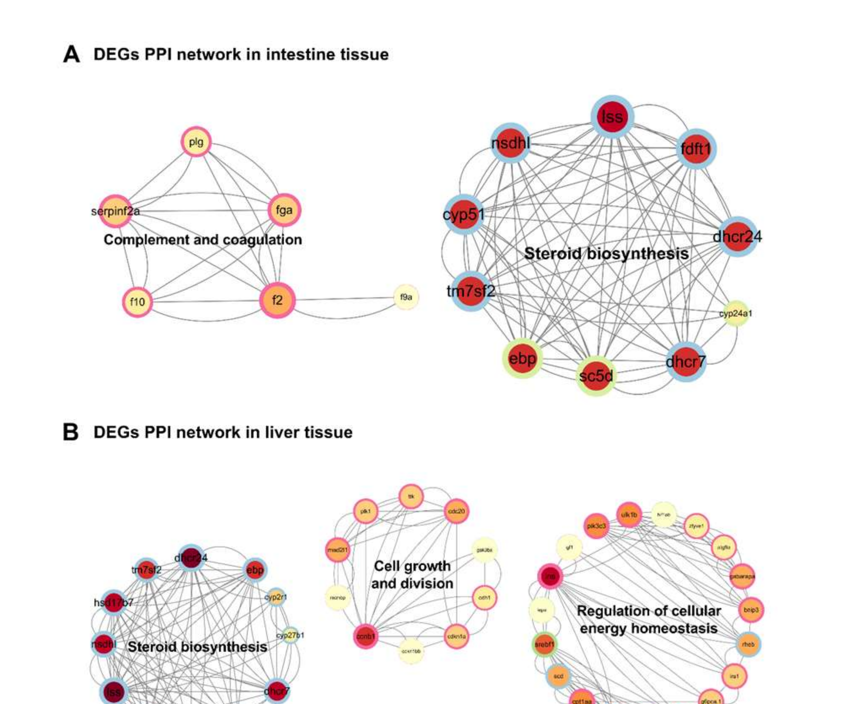


Figure 7. Potential association network for partial DEGs predicted by the String website and Cytoscape software with high confidence.



Conclusions

(1) The optimal duration for enriching *A. tibetiana* nauplii with *Schizochytrium* sp. fortifier was 12 h at a dosage of 40 g/t, and a density of 50 nauplii/mL.

(2) Feeding larvae with *Schizochytrium*-enriched *A. tibetiana* significantly promoted growth performance and enhanced hypoxia tolerance.

(3) The enrichment of *Schizochytrium* sp. in *A. tibetiana* nauplii effectively modulates the expression of genes associated with lipid metabolism and intestinal immunity. This intervention notably enhances the intestinal structure of the larvae and diminishes lipid accumulation in the liver.