

Article

Comparative Analysis of Enzymatic Activities and Transcriptional Profiles of Various Hepatic Enzymes between Male and Female Yellowfin Tuna (*Thunnus albacares*)

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Abstract: Yellowfin tuna (*Thunnus albacares*) is a valuable pelagic migratory fish with potential for aquaculture. Despite this, there is limited understanding of the biological and physiological characteristics of this species, particularly regarding sex differences in growth performance. The liver, a crucial organ for digestion and metabolism, plays a significant role in regulating fish growth. This study aimed to compare liver enzyme activities and transcriptome profiles between female and male yellowfin tuna to uncover the molecular mechanisms underlying difference between the sexes. The results revealed that female yellowfin tuna exhibited higher amylase and lipid metabolism enzyme activities, while male yellowfin tuna showed higher glucose-6-phosphate dehydrogenase and antioxidant enzyme activities. Additionally, through Illumina sequencing technology, the study generated 37.74 Gb of clean data and identified 36,482 unique genes (UniGenes) in the liver transcriptome. A total of 2542 differentially expressed genes were found, with enriched Gene Ontology terms and pathways related to metabolic processes, particularly lipid metabolism and transport. These findings suggest that female yellowfin tuna have superior digestive enzyme activities and lipid metabolism, while male yellowfin tuna excel in sugar metabolism, ATP production, and antioxidant defense. This study provides valuable insights into sex differences in yellowfin tuna and could aid in advancing full-cycle aquaculture practices for this species.

Keywords: *Thunnus albacares*; liver; metabolism; transcriptome; enzyme activity

Key Contribution: Female and male yellowfin tuna differ in digestion, lipid, and sugar metabolism.



Citation: Liu, D.; Yang, H.; Li, S.; Huang, H.; Li, G.; Chen, H. Comparative Analysis of Enzymatic Activities and Transcriptional Profiles of Various Hepatic Enzymes between Male and Female Yellowfin Tuna (*Thunnus albacares*). *Fishes* **2024**, *9*, 184. <https://doi.org/10.3390/fishes9050184>

Academic Editor: Dariel

Tovar-Ramirez

Received: 18 April 2024

Revised: 10 May 2024

Accepted: 14 May 2024

Published: 17 May 2024



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1. Introduction

Yellowfin tuna (*Thunnus albacares*) is a highly sought-after marine species globally, belonging to the family Mackerelidae and the genus Tuna [1]. This pelagic migratory fish is predominantly found in tropical and subtropical waters of the Pacific, Atlantic, and Indian Oceans [2]. Yellowfin tuna can grow rapidly, reaching lengths of up to 200 cm, with growth rates influenced by sex and body size. Males tend to be larger than females, and growth accelerates after reaching a body length of over 63 cm [3,4]. Due to its distinct flavor and high nutritional value, yellowfin tuna has gained popularity as a premium seafood in the international market [5]. Global landings of yellowfin tuna have averaged

approximately 1.25 million tons annually over the last decade, making it the second largest tuna species worldwide [6]. Studies have indicated a decline in wild spawning of yellowfin tuna since the 1970s, coupled with an increase in fishing mortality among adults and juveniles. The wild populations of yellowfin tuna are currently fully exploited, particularly in the Central and Western Pacific [7]. However, the quality and food safety of yellowfin tuna are susceptible to various environmental and biological factors, including overfishing and climate change [8–10]. Therefore, the establishment of aquaculture-based production systems for yellowfin tuna is crucial. This approach will not only ensure a consistent supply of yellowfin tuna without being limited by seasonal or geographical factors, but also alleviate pressure on wild stocks and promote sustainable resource management.

Currently, yellowfin tuna is farmed mainly through capture-based aquaculture, where wild juveniles or subadults are captured and fattened up in nets for several months before being harvested [11,12]. This practice has been adopted in countries such as Mexico, Panama, and Indonesia [13,14]. In China, the artificial culture of yellowfin tuna is still at an early stage. The Deep-sea Aquaculture Technology and Species Development Innovation Team of the Chinese Academy of Fisheries Sciences has realized the indoor recirculating water and offshore deep-water net-pen culture of yellowfin tuna in Lingshui Lizu autonomous county of Hainan province [15].

To develop the full-cycle aquaculture of yellowfin tuna, it is necessary to understand the biological and physiological characteristics of the species, such as reproduction, nutrition, and metabolism [16]. The liver plays a key role in fish nutrition by receiving and distributing large amounts of dietary nutrients through the portal vein, which is directly connected to the digestive tract [17]. The liver also performs essential metabolic functions such as processing and storing nutrients, synthesizing enzymes and other cofactors, forming and secreting bile, and metabolizing xenobiotic compounds [18]. The liver plays a central role in growth regulation and has, therefore, been extensively studied to reveal the genetic and metabolic mechanisms that lead to differences in growth rates in fishes [19–21], and these findings have contributed to a better understanding of the regulation of growth.

By studying changes in liver enzyme activities, we can provide optimized feeding strategies to improve the growth and health of yellowfin tuna in aquaculture. Transcriptome sequencing technology is a powerful tool to analyze the type, structure, and expression level of all transcription products of a specific tissue or cell under different conditions, which can reveal the molecular regulatory mechanisms of specific biological processes. This technique has been widely used in studies of fish growth and metabolism [22,23].

In this study, we compared the physiological indices of male and female yellowfin tuna and performed comparative transcriptome analysis to identify responsive genes in the liver. Our aim was to gain a comprehensive understanding of sex differences in liver functions. These data will help to add biometric data to yellowfin tuna culture.

2. Materials and Methods

2.1. Fish and Sample Preparation

Wild yellowfin tuna were caught with baited lines in the South China Sea (17°24' N, 110°36' E) in mid-May 2023, and the sex of the fish was determined by gonadal morphology observation. Three females and three males of mature individuals (body length: 100–125 cm, body weight: 13–26.5 kg) were selected for sampling. Liver samples were rapidly frozen in liquid nitrogen and then stored at -80°C until RNA extraction and biochemical analysis. Meanwhile, small pieces of gonadal and liver tissues from each fish were fixed in Bouin's solution for histological analysis.

2.2. Histological Procedures for Liver and Gonad Tissues

Tissue specimens were fixed in Bouin's solution for 24 h and then transferred to 70% ethanol for dehydration. Next, the tissues were further dehydrated in a gradient ethanol series (75–100%), and then cleared with xylene and embedded in molten paraffin. Finally, serial sections were made at 6–8 μm . The sections were stained with hematoxylin and

eosin (H&E) and evaluated histomorphologically using a light microscope (Nikon IQ50, Tokyo, Japan).

2.3. Measurement of Enzyme Activities

The liver samples were weighed and added to ice-cold 0.85% physiological saline in a sample to saline mass ratio of 1:10. The samples were then homogenized using a tissue cell crusher in an ice bath. After centrifugation at 4 °C and 900× *g* for 10 min, the supernatant was collected as the enzyme source solution. The total protein content of this solution was determined using the BCA method [24]. Enzyme activities related to carbohydrate and lipid metabolism, as well as oxidative stress, were assessed using commercial assay kits following the manufacturer's instructions. The activities were measured by using the absorbance of reaction products or substrates at specific wavelengths with a photometric microplate reader (multiscan MK3, Thermo Fisher Scientific, Chelmsford, MA, USA). The enzymes analyzed and their corresponding assay kits are detailed in Table S1, and include amylase (AMS), lipase (LPS), malondialdehyde (MDA), pyruvate kinase (PK), acyl-CoA oxidase (ACO), malic enzyme (NADP-ME), fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G-6-PD), acetyl-CoA carboxylase (ACC), carnitine-acylcarnitine translocase (CACT), lipoprotein lipase (LPL), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT).

2.4. RNA-Seq and Bioinformatics Analysis

Fish sex and gonadal stages were determined by histological methods. Six liver samples (three of each sex) were used for preparing transcriptome (RNA-Seq) sequencing libraries. The RNA-Seq process was carried out as previously described [25], with the following steps: isolation of total RNA using a TRIzol kit (Invitrogen, Carlsbad, CA, USA); quantification and integrity assessment of RNA using a Nanodrop 2000c spectrophotometer and Agilent 2100 Bioanalyzer system; and construction of cDNA libraries following Illumina RNA sequencing protocol. Sequencing was performed using the Illumina HiSeq™ 2000 platform, which generated paired-end (PE) reads of 125 bp. The sequencing data were submitted to the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA1013240.

The bioinformatics analysis included read quality control, assembly, annotation, and differential expression analysis. The sequences were further processed using the bioinformatics pipeline tool BMKCloud (www.biocloud.net, accessed on 19 July 2023) online platform. First, the quality control of raw sequencing data was performed using an in-house perl script to remove adaptor sequences, reads containing poly-N sequences, and low-quality reads. Clean RNA-Seq data were then assembled using Trinity Assembler with default parameters [26]. Gene expression levels were quantified in fragments per kilobase per million reads (FPKM) using RSEM v1.2.21 [27]. Gene function was annotated using the NR (NCBI non-redundant protein sequences), Pfam (Protein family), KOG/COG/eggNOG (Clusters of Orthologous Groups of proteins), Swiss-Prot (manually annotated and reviewed protein sequence database), KEGG (Kyoto Encyclopedia of Genes and Genomes), and GO (Gene Ontology) databases. All subsequent analyses were conducted with males regarded as the control group.

2.5. Real-Time Quantitative PCR (RT-qPCR) Validation

The liver samples were homogenized and total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA was reverse-transcribed to cDNA using the RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). cDNA was used for RT-qPCR to quantify the expression levels of target genes using the SYBR Premix Ex Taq II (TaKaRa Bio Inc., Shiga, Japan) on a LightCycler 480 system (Roche, Basel, Switzerland). Each sample was analyzed in three biological replicates and three technical replicates. The reference gene β -actin was used as an internal control to standardize the mRNA levels. In addition, the amplification

efficiency and correlation coefficient (R²) were determined by standard curves of 10-fold dilutions (1, 1/10 1/100 1/1000 and 1/10,000) of cDNA template. The primer pairs for the target genes are listed in Table S2. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

2.6. Statistical Analysis

Data are presented as mean \pm standard error of the mean (SEM). One-way ANOVA was performed using SPSS Statistics 24.0 software (SPSS Inc., Chicago, IL, USA) to test the differences among groups. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Histological Observation

All tuna individuals analyzed in this study were sexually mature (Figure S1). Figure 1 illustrates male and female yellowfin tuna liver cells, which exhibited a flattened and compact texture. Although no significant differences were detected in the liver cells between the male and female yellowfin tuna, the male hepatocytes showed a deeper basophilic reaction compared to the female hepatocytes, suggesting higher metabolic activity. Additionally, vacuolization was more pronounced in female hepatocytes, possibly associated with increased lipid metabolism.

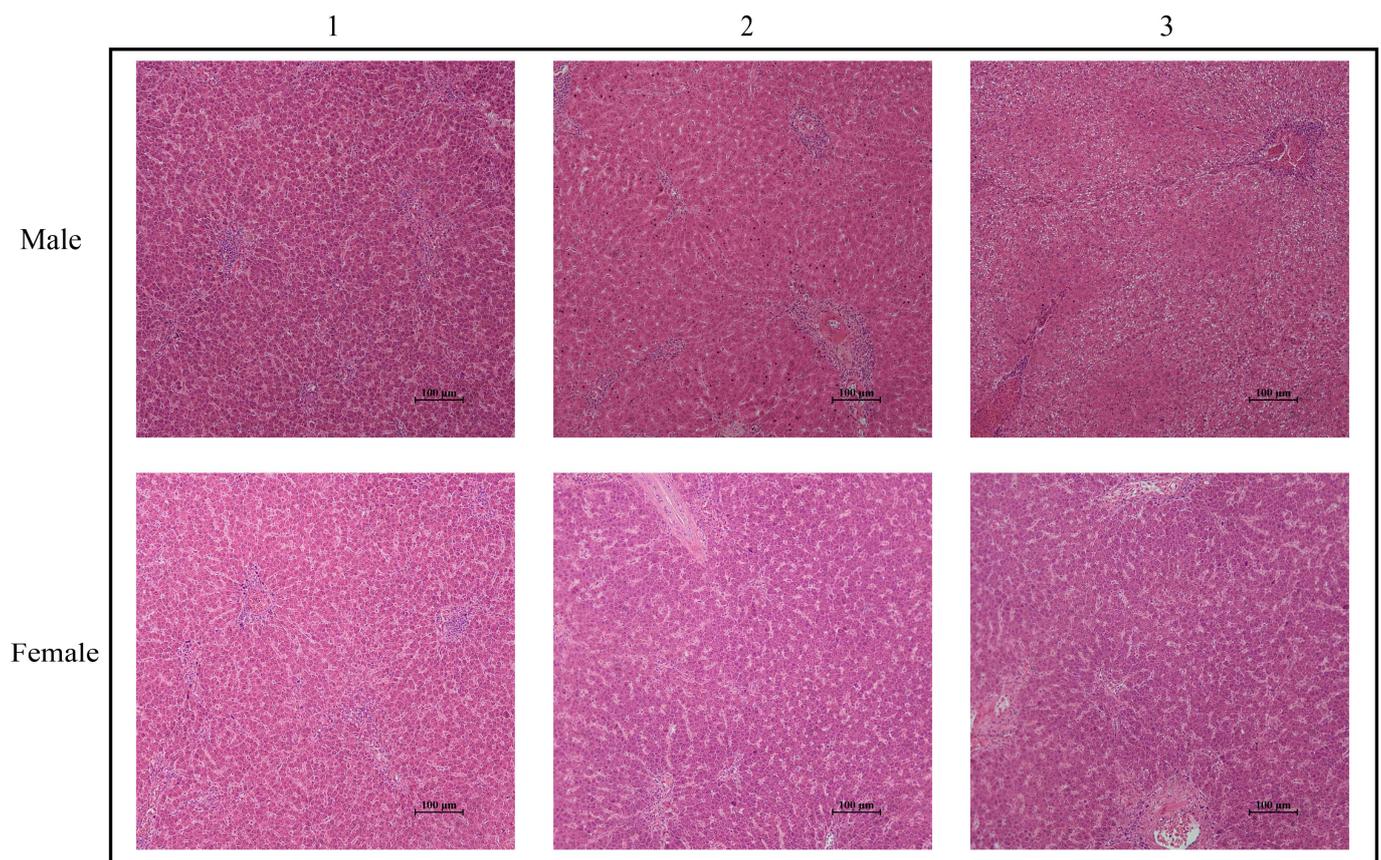


Figure 1. Histological observation of the liver of male and female tuna. Scale bar: 100 μ m.

3.2. Differences in Liver Metabolic Enzymes between Male and Female Yellowfin Tuna

Differences in digestive enzyme activities, energy metabolism, lipid metabolism, and antioxidant enzyme activities in the liver of male and female yellowfin tuna were tested. In terms of digestive enzyme activities (Figure 2A), the AMS activity ($p < 0.01$) and LPS ($p < 0.05$) activity were significantly higher in females than in males. LPL did not differ significantly between the sexes. In terms of energy metabolism (Figure 2B), the hepatic

G6PD activity was significantly higher in males than in females ($p < 0.05$). A similar result was observed for NADP-ME ($p < 0.01$), while no significant difference was found for PK. The activity of hepatic ACO was significantly higher in females than in males ($p < 0.05$). There were no statistically significant differences in the liver for CACT ($p > 0.05$), ACC ($p > 0.05$), or FAS ($p > 0.05$). For antioxidant enzymes (Figure 2D), namely, GSH-PX, males exhibited higher activity ($p < 0.05$).

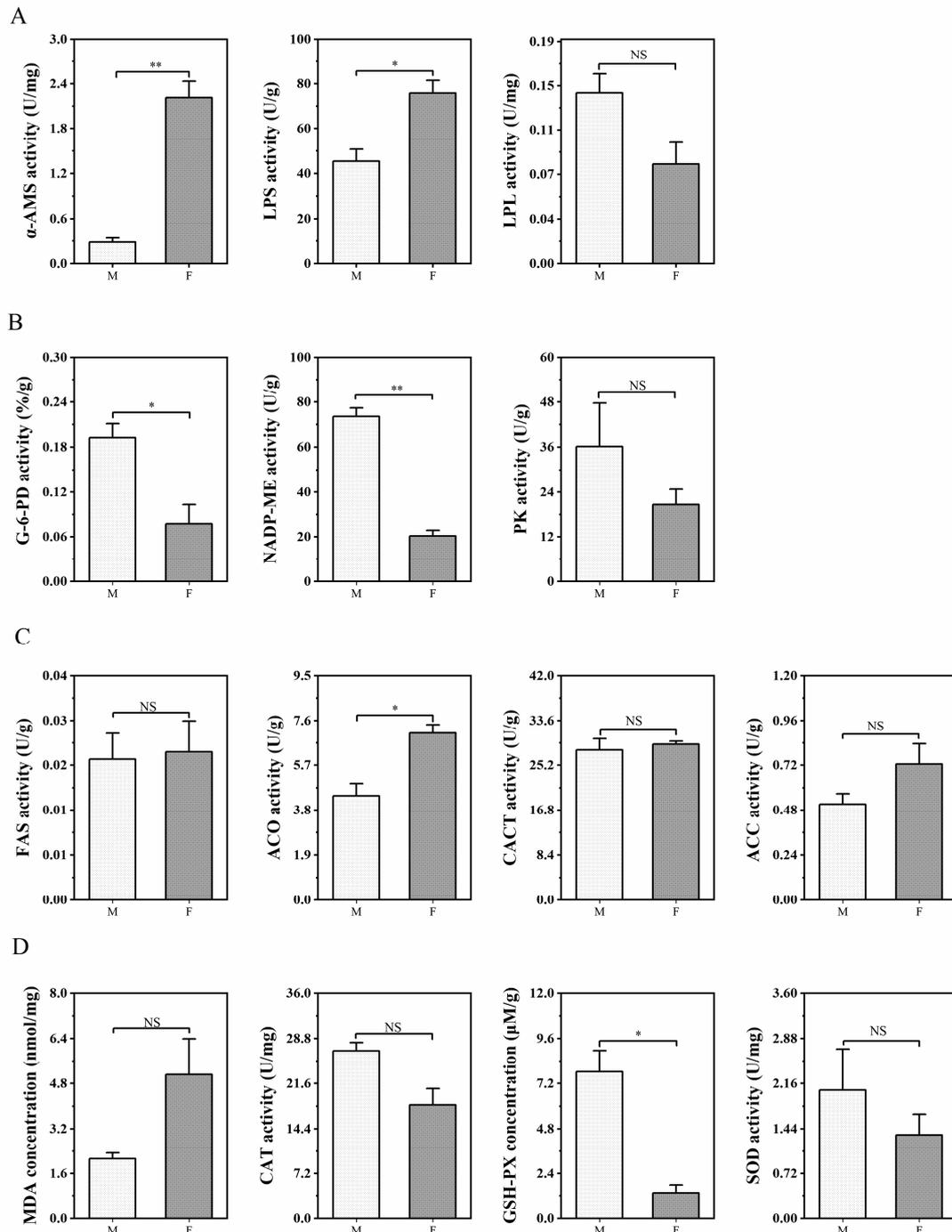


Figure 2. Comparative assessment of liver enzyme activities related to (A) digestion, (B) energy metabolism, (C) lipid metabolism, and (D) antioxidant defense in male and female yellowfin tuna. Error lines indicate SEM ($n = 3$). Asterisks indicate significant differences between the two groups (* $p < 0.05$, ** $p < 0.01$). NS: not significant ($p > 0.05$).

3.3. RNA-Seq of the Liver Transcriptome

RNA from the liver tissues from adult female and male yellowfin tuna was sequenced using synthetic sequencing (SBS) technology on the Illumina HiSeq high-throughput platform. Raw reads from F-1, F-2, F-3, M-1, M-2, and M-3 samples resulted in 37.74 Gb of clean data after quality control. The number of clean reads for each sample ranged from 19,904,230 to 23,628,924. The Q30 base percentages for all samples exceeded 93.57%, with GC content ranging from 43.62% to 49.18% (see Table 1).

Table 1. Statistics for evaluation of sample sequencing data.

ID	Clean Reads	GC Content	% \geq Q30
F-1	21,116,430	48.53%	93.71%
F-2	21,116,430	48.72%	94.01%
F-3	23,628,924	49.18%	94.17%
M-1	20,375,537	46.30%	93.57%
M-2	19,904,230	43.62%	93.98%
M-3	20,183,521	47.51%	93.67%

F represents female yellowfin tuna, M represents male yellowfin tuna, and the suffixed number is the sample number.

The de novo assembly yielded 36,482 UniGenes with an average length of 1128 bp. Among these, 7460 (20.45%) UniGenes had lengths between 1000 bp and 2000 bp, while 6879 (18.86%) UniGenes had lengths exceeding 2000 bp (Table S3). The UniGene sequences underwent annotation by comparing them with various databases including COG, GO, KEGG, KOG, Pfam, Swiss-Prot, TrEMBL, eggNOG, and NR (Table 2). A total of 19,440 UniGenes were annotated using BLAST with an E -value of 1×10^{-5} and HMMER with an E -value of 1×10^{-10} . Across the database comparisons, the percentages of genes annotated were as follows: COG (16.55%), GO (84.69%), KOG (59.35%), Pfam (66.89%), Swiss-Prot (46.18%), TrEMBL (98.53%), eggNOG (85.63%), and NR (97.69%). These results demonstrate the robustness and credibility of the assembly.

Table 2. UniGene annotation statistics.

Anno_Database	Number Annotated	$300 \leq \text{Length} < 1000$	$\text{Length} \geq 1000$	Percentage
COG_Annotation	3217	481	2589	16.55
GO_Annotation	16,464	4328	9749	84.69
KEGG_Annotation	15,549	4046	9279	79.98
KOG_Annotation	11,537	2689	7503	59.35
Pfam_Annotation	13,003	2881	8979	66.89
Swissprot_Annotation	8977	1998	6041	46.18
TrEMBL_Annotation	19,155	5279	10,932	98.53
eggNOG_Annotation	16,647	4429	9752	85.63
nr_Annotation	18,991	5205	10,868	97.69
All_Annotated	19,440	5416	10,994	100.00

3.4. Differential Expression Analysis

A total of 2542 genes were identified in this study as being differentially expressed between male and female groups based on expression levels. Among these genes, 1195 were down-regulated and 1347 were up-regulated in females (Figure 3A). Heatmaps of hierarchical clusters of DEGs were used to visually represent the overall gene expression pattern between the sexes. The male samples were first clustered separately and then grouped with the female branch, indicating significant differences in transcription patterns between the male and female livers (Figure 3B). The differentially expressed genes were annotated, with a total of 1894 genes annotated across various databases including COG, GO, KEGG, KOG, NR, Pfam, Swiss-Prot, and eggNOG (Table 3). Additionally, 10 DEGs were selected and validated using RT-qPCR (Figure 3C), confirming the reliability and accuracy of gene expression levels determined through transcriptomics analysis.

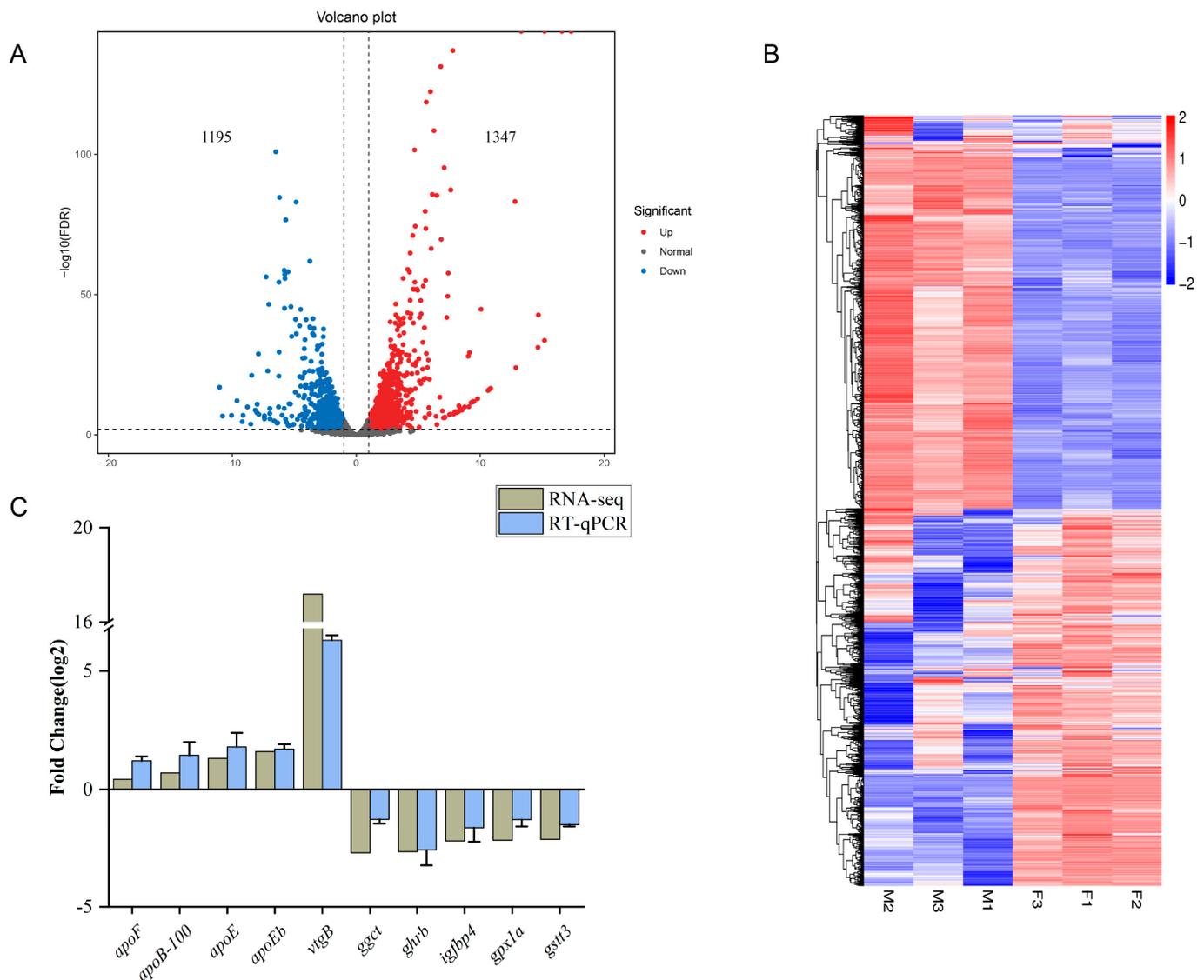


Figure 3. Transcriptome differential gene validation and its clustering analysis. **(A)** Male and female yellowfin tuna liver basal differential genes. **(B)** Clustering diagram of differential gene expression patterns for illustrating the overall pattern of gene expression in different liver samples. **(C)** RT-qPCR differential expression validation. Error lines indicate SEM ($n = 3$).

Table 3. Statistics on the number of differentially expressed genes annotated with various databases.

DEG Set	Total	COG	GO	KEGG	KOG	NR	Pfam	Swiss-Prot	eggNOG
MvsF	1894	519	1681	1600	1308	1883	1582	1063	1697

3.5. Enriched GO Terms and KEGG Pathways

Based on the GO enrichment analysis, the DEGs were categorized into three main functional classes: cellular components (CCs), molecular functions (MFs), and biological processes (BPs) (Figure 4). The top three GO terms involved in biological processes included serine family amino acid metabolism (GO:0009069), cellular amino acid metabolism (GO:0006520), and lipid transport (GO:0006869). The cellular-component-class DEGs included integral component of membrane (GO:0016021), endoplasmic reticulum membrane (GO:0005789), and proteasome regulatory particle (GO:0005838). The top three molecular function terms were lipid transporter activity (GO:0005319), transmembrane transporter activity (GO:0022857), and amino acid binding (GO:0016597). Additionally, we

noted that a number of lipid-related GO terms were enriched, including fatty acid transport (GO:0015908), lipid localization (GO:0010876), lipid catabolic process (GO:0016042), and triglyceride metabolic process (GO:0006641).

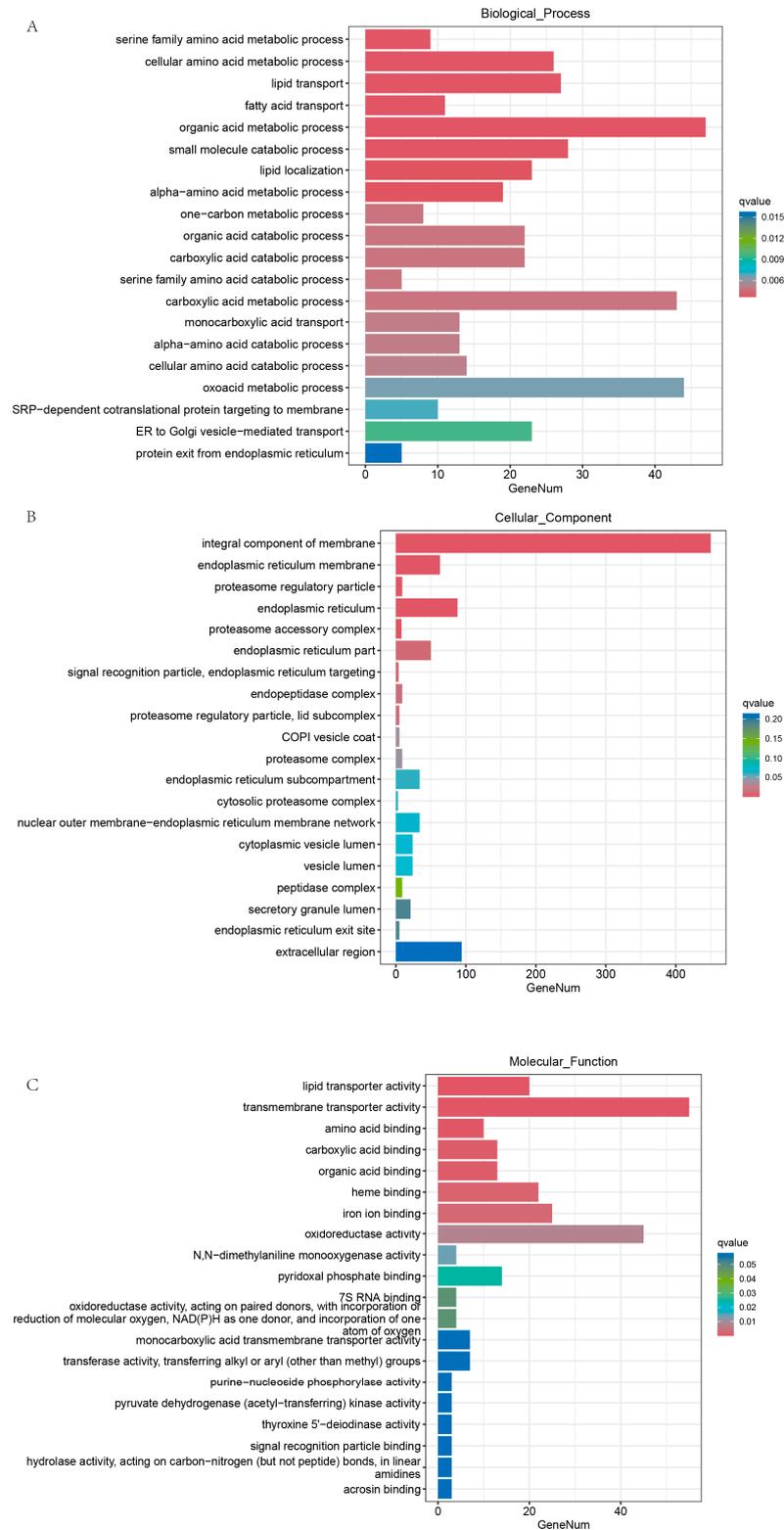


Figure 4. Functional categorization of differentially expressed genes in liver GO enrichment. (A) Biological process, (B) molecular function, and (C) cellular component.

The annotation of differentially expressed genes using KEGG identified a total of 205 enriched pathways. These results were further categorized based on pathway types (Figure 5A). The liver transcriptome of yellowfin tuna exhibited involvement in six pathway types, including cellular processes, environmental information processing, genetic information processing, human diseases, and metabolism. Notably, pathways such as protein processing in the endoplasmic reticulum, endocytosis, MAPK signaling pathway, and FoxO signaling pathway showed a high percentage of DEGs. The up- and down-regulation results, along with the numbers of enriched genes, for the top 20 significant pathways are depicted in Figure 5B. Functionally, these pathways and the genes within them primarily relate to metabolism, encompassing lipid metabolism (steroid hormone biosynthesis, glycerophospholipid metabolism, fatty acid degradation, arachidonic acid metabolism, and steroid biosynthesis), carbohydrate metabolism (pentose and glucuronate interconversions, glyoxylate and dicarboxylate metabolism), and amino acid metabolism (glycine, serine, and threonine metabolism; arginine and proline metabolism; tryptophan metabolism; and alanine, aspartate, and glutamate metabolism). These functional categories offer insights into the differences in liver metabolism between male and female yellowfin tuna. Table S4 showcases the gene expression profiles related to these enriched pathways.

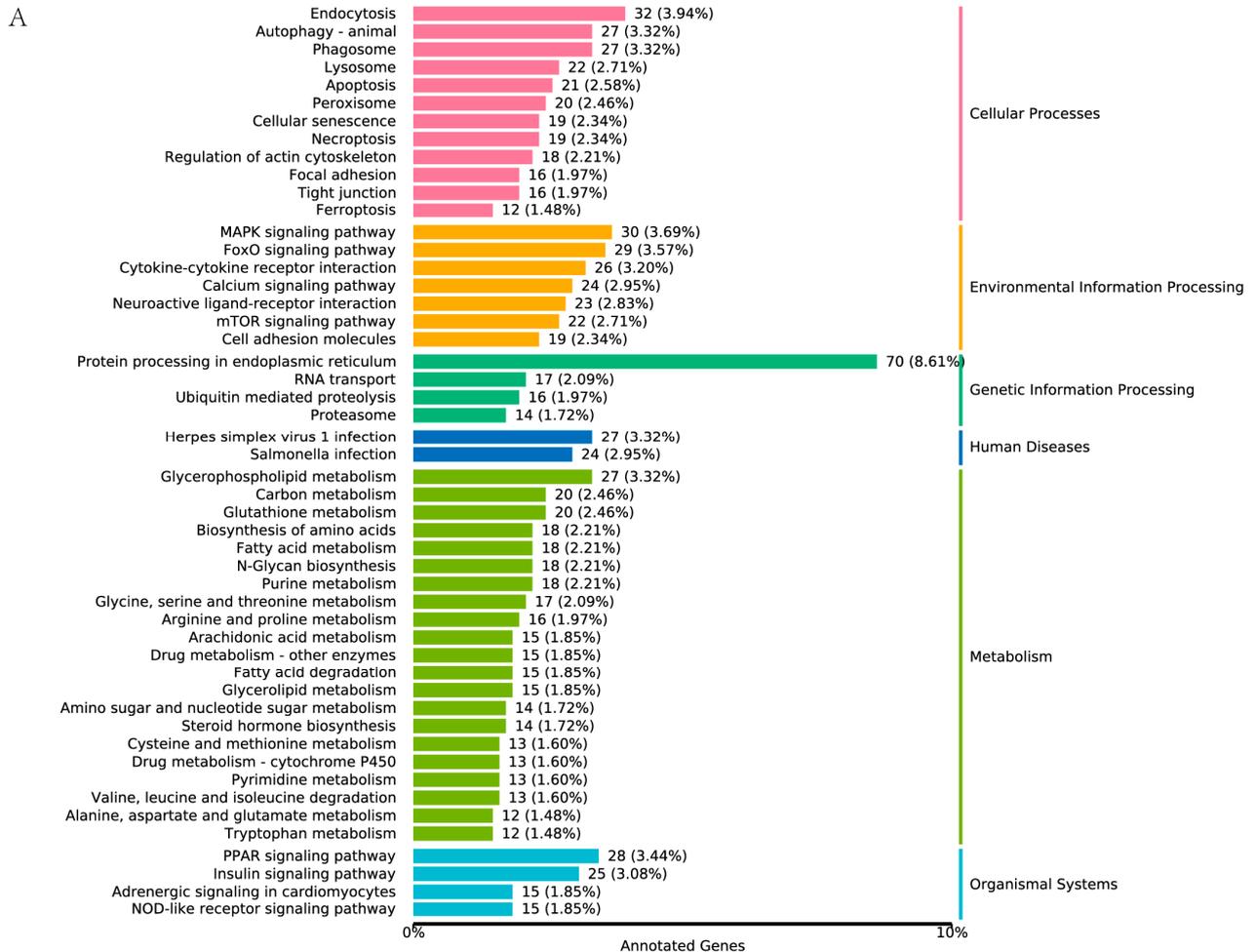


Figure 5. Cont.

B

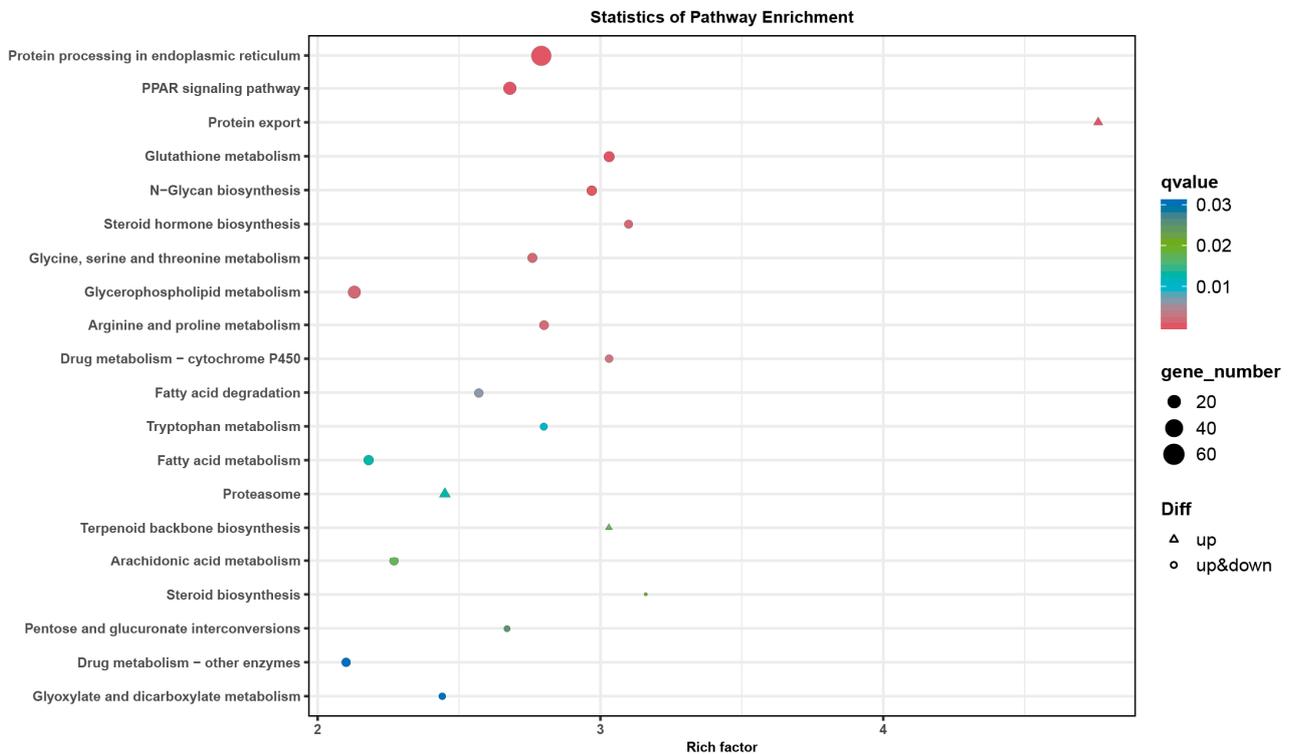


Figure 5. (A) KEGG categorization diagram of differentially expressed genes, with metabolic pathway names on the vertical axis, pathway names on the left, and corresponding categorization categories on the right. Colors in the same column indicate the same category. (B) Scatter plot of KEGG pathway enrichment for differentially expressed genes, with the pathway names on the vertical axis, enrichment factor on the horizontal axis, color representing the q value (p -value corrected by multiple hypothesis testing), and circle size indicating the number of enriched genes in the pathway. In the gene expression pathways context, ‘up’ signifies that all genes in the pathway are up-regulated, while ‘up&down’ indicates a mix of up-regulated and down-regulated genes.

4. Discussion

4.1. Digestive Differences

Fish liver contains a rich enzyme system that plays a crucial role in metabolic processes, particularly in growth and development. Among them, digestive enzymes are the most important class of enzymes in fish liver, which can break down large molecules such as proteins, fats and starches in food, releasing small molecules such as amino acids, fatty acids, and monosaccharides for absorption and utilization [28,29]. We compared the differences in digestive enzyme activity and related gene expression in the liver between female and male yellowfin tuna. The results showed that female yellowfin tuna had higher amylase (α -AMS) and lipase (LPS) activities, and at the same time, the transcriptome results showed that genes relate to glyceride metabolism were significantly up-regulated in females. These genes include *lipc*, *plpp1*, *pipp2d*, and *plpp5*. *lipc* encodes hepatic lipase, one of the three members of the triglyceride lipase family, which can promote the degradation and uptake of vascular lipoproteins and participate in the hydrolysis of triglycerides [30]. *Plpp1*, *pipp2d*, and *plpp5* belong to the PAP2-like superfamily, which are transmembrane phospholipid phosphatases that can convert phospholipids into diacylglycerol, thereby regulating lipid metabolism [31]. These results indicate that female yellowfin tuna have higher digestive capacity, which can improve food utilization efficiency.

4.2. Energy Metabolism Differences

Energy metabolism plays a crucial role in fish, particularly in species with seasonal variations in reproductive activity. The energy allocation pattern shifts throughout the reproductive cycle and can be influenced by hormone levels. For instance, in *Oncorhynchus masou*, enzyme activities like pyruvate kinase, lactate dehydrogenase, and malate dehydrogenase decrease as the spawning season nears [32]. An important indicator of energy metabolism is the activity of G-6-PD, the primary and rate-limiting enzyme of the pentose phosphate pathway. The products of this pathway, NADPH and 5-phosphoribose, are crucial for fatty-acid synthesis and glutathione reduction [33,34]. Our study compared G-6-PD and NADP-ME activities in the liver of male and female yellowfin tuna. The results revealed higher G-6-PD and NADP-ME activities in male yellowfin tuna, suggesting increased NADPH production. Transcriptome analysis also showed the enrichment of energy metabolism pathways in males, such as glycolysis/gluconeogenesis (ko00010) and citrate cycle (ko00020), which are linked to ATP synthesis. These findings indicate that male yellowfin tuna exhibit enhanced glucose metabolism, ATP production, and overall activity in May.

4.3. Lipid Metabolism Differences

Lipid metabolism is a crucial process for fish, especially for those with high reproductive demands, such as yellowfin tuna. The liver is the main site of lipid synthesis, storage, and transport in fish [35]. We investigated the sex differences in lipid metabolism of yellowfin tuna liver by measuring the enzyme activity and gene expression of key enzymes and transporters involved in fatty acid synthesis, degradation, and transport. We found that female yellowfin tuna had a higher lipid metabolism capacity than male yellowfin tuna, which may be needed to meet their reproductive needs.

One of our main findings was that female yellowfin tuna had significantly higher ACO activity than male yellowfin tuna. ACO is the rate-limiting enzyme of fatty acid β oxidation [36]. This finding indicates that female yellowfin tuna can oxidize more fatty acids to produce energy. In addition, we observed a significant increase in the expression of carnitine palmitoyltransferase 1 (*cpt1*) and carnitine palmitoyltransferase 2 (*cpt2*) genes in female yellowfin tuna. These genes encode CPT1 and CPT2, which are key enzymes for transporting fatty acids to mitochondria for β oxidation [37]. These results indicate that female yellowfin tuna have a stronger fatty-acid degradation pathway.

The female yellowfin tuna also had higher expression levels for genes involved in fatty acid synthesis, such as acetyl-CoA carboxylase 1 (*acc1*) and fatty acid synthase (*fas*). ACC1 is a multifunctional enzyme that catalyzes the formation of malonyl-CoA, the precursor of fatty acid synthesis, and also prevents the transfer of acyl groups from acyl-CoA to carnitine [38]. FAS is a homodimeric protein that synthesizes fatty acids from acetyl-CoA and malonyl-CoA [39]. FAS is expressed in most tissues, but mainly in the liver and adipose tissue, and plays a role in lipid metabolism and energy balance [39]. Although we did not detect significant differences in the activities of FAS, CACT, or ACC between male and female yellowfin tuna, the up-regulation of *acc1* and *fas* genes indicates that female yellowfin tuna have higher potential for fatty acid synthesis.

In addition, we also found that female yellowfin tuna had higher expression of genes related to lipid transport and absorption, such as apolipoprotein F (*apoF*), apolipoprotein B-100 (*apoB-100*), apolipoprotein E (*apoE*), and apolipoprotein Eb (*apoEb*). These genes encode apolipoproteins, which are the protein components of lipoproteins that carry lipids in blood and tissues [40]. Apolipoprotein E is particularly important for lipid metabolism, as it can interact with various lipoprotein receptors and mediate the clearance of lipoproteins from the blood circulation [41]. Apolipoprotein E is also involved in oocytes' uptake of yolk [42]. Moreover, we observed a simultaneous up-regulation of vitellogenin B (*vtgb*) and vitellogenin C (*vtgc*) genes in the female yellowfin tuna. These genes encode vitellogenins, which are yolk proteins synthesized in the liver and transported to the ovary [43]. These

results indicate that female yellowfin tuna have a more active lipid transport, possibly to provide lipids for oocyte development and maturation.

4.4. Antioxidant Differences

The antioxidant capacity of fish is closely related to their health status. Antioxidant capacity is the ability of fish to scavenge reactive oxygen species (ROS) and protect cells from oxidative damage. ROS are generated as by-products of normal metabolism or under environmental stress, such as temperature changes, hypoxia, or pollution. ROS can cause lipid peroxidation, which is a common oxidative stress reaction that causes damage to cell membranes and lipophilic substances. Lipid peroxidation can be measured by MDA content, which is an important indicator of lipid peroxidation [44]. We compared the differences in antioxidant enzyme activity and related gene expression in the liver between female and male yellowfin tuna. While there was no notable disparity in MDA content in the livers of female and male yellowfin tuna, there was a discernible trend towards an increase, potentially linked to females' comparatively lower antioxidant enzyme activities. Antioxidant enzymes are a class of enzymes that can scavenge reactive oxygen species and protect cells from oxidative damage, including GSH-Px, CAT, and SOD.

We found that male yellowfin tuna liver had higher GSH-Px activity than female yellowfin tuna liver, while CAT and SOD activities also showed an upward trend. GSH-Px can remove harmful substances such as lipid hydroperoxides [45], while CAT and SOD can decompose ROS such as hydrogen peroxide and superoxide anion, respectively [46].

At the same time, the transcriptome also detected the expression level of genes related to antioxidants in the liver and found that genes related to glutathione metabolism were significantly up-regulated in males, including *gpx1a*, *gstt3*, and *ggct*. These genes encode GSH-Px, glutathione S-transferase, and γ -glutamyl cycle transferase, respectively, which are involved in the regulation of glutathione metabolism. Other studies have also found sex-related differences in antioxidant defense. For example, in tilapia (*Oreochromis mossambicus*), males had higher SOD and glutathione S-transferase activities than females [47]; in brown trout (*Salmo trutta*), females had higher SOD and CAT activities than males [48]. These differences may be related to the different reproductive strategies, energy metabolism, and environmental adaptability of different fishes [49]. In yellowfin tuna, we suggest that the lower antioxidant level of females may be due to their more efficient lipid metabolism, because lipid metabolism produces more peroxides, and excessive saturated fatty acids or other lipids (such as phospholipids) increase the susceptibility of cell membranes to oxidations [50].

4.5. Expression Differences of Growth-Hormone-Axis-Related Genes

One of the factors regulating fish growth is the growth hormone axis, which includes the pituitary secretion of growth hormone (GH) and liver production of GH-dependent insulin-like growth factor I (IGF-I). GH binds to receptors (GHRs) on various target tissues, activating intracellular signaling pathways that affect growth, metabolism, and immunity [51,52]. IGF-I can stimulate cell proliferation, differentiation, and metabolism, thereby promoting body development and growth. IGF-I also can bind to its binding proteins (IGFBPs), thereby regulating the bioavailability and biological effects of IGF-I. We found that the transcription levels of two isoforms of GHR, *ghra* and *ghrb*, in male liver were higher than those in female liver. This result indicates that male fish have a stronger ability to respond to GH stimulation and activate downstream signaling pathways. We also observed that the expression level of the IGFBP4 gene in male liver was higher than that in female liver. IGFBP4 is a regulatory factor of IGF-I activity, as it can inhibit the binding of IGF-I to its receptor and shorten its half-life. The increase in IGFBP4 expression in male fish may be a feedback mechanism to prevent the excessive transmission of the IGF-I signal and maintain homeostasis [53,54]. Differences in growth axis function may account for the more rapid growth of males. However, growth is a complex biological process with continuous

changes, so more research is needed to elucidate the molecular differences between male and female yellowfin tuna in growth axis function.

5. Conclusions

This study compared the liver enzyme activities and transcriptome profiles of female and male yellowfin tuna to investigate sex-related differences at a molecular level. The findings highlighted differences in digestion, energy metabolism, lipid metabolism, antioxidant defense, and growth axis function between the two sexes. Female yellowfin tuna exhibited superior digestive and lipid metabolism capabilities, likely to support reproductive needs and enhance food efficiency. Conversely, male yellowfin tuna showed higher sugar metabolism, ATP production, and antioxidant defense, possibly due to their more active lifestyle and need to combat oxidative stress. Moreover, males displayed a stronger response to growth hormone and insulin-like growth factor I, indicating differences in growth axis function contributing to the faster growth of males. Transcriptomic analysis revealed significant liver differences between the female and male yellowfin tuna, reflecting distinct physiological states and metabolic demands. Overall, these insights contribute valuable biometric data for yellowfin tuna aquaculture and lay a foundation for the further exploration of sex-related differences at a molecular level.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fishes9050184/s1>: Table S1: Product numbers of commercial assay kits; Table S2: Primers used for detection of DEGs; Table S3: Assembly result statistics; Table S4: Gene expression of partially enriched pathways; Figure S1: Histological identification of male and female yellowfin tuna gonads.

Author Contributions: Data curation, D.L. and H.Y.; Formal analysis, H.Y. and D.L.; Funding acquisition, H.C.; Investigation, H.Y., D.L. and S.L.; Methodology, H.Y. and H.H.; Project administration, G.L. and H.C.; Resources, H.C.; Supervision, H.C.; Writing—original draft, D.L.; Writing—review and editing, H.C. All authors have read and agreed to the published version of the manuscript.

Funding: This article was funded by grants from the Natural Science Foundation of China (32273131), the Science and Technology Plan of Guangdong province (2023B0202010016), the Youth Science and Technology Innovation Talent of Guangdong TeZhi plan talent (2023TQ07A888), the Science and Technology Plan of Zhanjiang City (2022A01214), and the Science and Technology Plan of Yangjiang City (2022011 and SDZX2023027).

Institutional Review Board Statement: Animal handling techniques strictly adhered to the Guidelines for the Care and Use of Laboratory Animals. The Animal Research and Ethics Committee of Guangdong Ocean University authorized this protocol (NIH Pub. No. 85-23, revised 1996).

Data Availability Statement: Sequencing data were submitted to the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA1013240. Data are contained within the article and Supplementary Materials.

Acknowledgments: We wish to thank the anonymous reviewers and the editor of the journal for their valuable comments.

Conflicts of Interest: Dongge Liu was employed by the company Guangdong Havvii Agriculture Group Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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