fishes

MDPI

Article

Comparison of Growth Performance, Nutritional Composition, and Muscle Transcriptome between Two Cultured Varieties of the Chinese Mitten Crab (*Eriocheir sinensis*)

Ziyu Wang [1,2], Peiying Huang [1,2], Xiaoyong Li [3,*], Jianmin Pei [3], Wenzhen Liu [3], Jiahao Hou [1,2], Linjie Li [1,2], Hongxiang Fan [1,2], Liugen Zeng [4] and Daxian Zhao [1,2,*]

1 Key Laboratory for Aquatic Germplasm Innovation and Utilization of Jiangxi Province, School of Life Sciences, Nanchang University, Nanchang 330031, China; wangziyu09@email.ncu.edu.cn (Z.W.); 405600220115@email.ncu.edu.cn (P.H.); 405600220034@email.ncu.edu.cn (J.H.); 355600230020@email.ncu.edu.cn (L.L.); 405600230115@email.ncu.edu.cn (H.F.)
2 Laboratory of Aquatic Animal Healthy Breeding, Chongqing Research Institute of Nanchang University, Chongqing 400037, China
3 Department of Animal Husbandry and Aquatic Technology Extension and Application, Jiangxi Agricultural Technology Extension Center, Nanchang 330046, China; 18970055849@163.com (J.P.); liuwenzhen518@163.com (W.L.)
4 Nanchang Academy of Agricultural Sciences, Nanchang 330038, China; bingjianla2008@hotmail.com
* Correspondence: lixiaoyong12@hotmail.com (X.L.); zhaodaxian@ncu.edu.cn (D.Z.)

Abstract: This study evaluated the disparities in growth performance and nutritional composition between two common Chinese mitten crab varieties, "Jianghai 21" and "Changjiang 2", cultured in Jiangxi Province. Over the breeding period, parameters such as body weight, height, carapace length and width, and lengths of the second and third legs exhibited increases. Growth indices of "Jianghai 21" surpassed those of "Changjiang 2" during the intermediate and late breeding phases. While "Changjiang 2" demonstrated significantly higher crude lipid content than "Jianghai 21", the latter exhibited markedly higher levels of total amino acids (TAAs), essential amino acids (EAAs), and delectable amino acids. Although overall nutrient composition did not significantly differ, TAA and EAA contents in the hepatopancreas were notably greater in "Jianghai 21" than in "Changjiang 2". Moreover, a total of 901 differentially expressed genes (DEGs) were identified between the two varieties, with 560 upregulated and 341 downregulated. These DEGs were primarily associated with pathways related to fatty acid degradation, metabolism, and growth regulation. The observed variances in growth performance and nutrient composition between "Jianghai 21" and "Changjiang 2" suggest potential regulation by relevant genes. In summary, the results showed that the growth performance of "Jianghai 21" is greater than that of "Changjiang 2", offering valuable insights for the selection of aquaculture varieties in the region.

Keywords: *Eriocheir sinensis*; cultured varieties; growth performance; nutritional composition; transcriptomics

Key Contribution: This study examined the differences in growth performance and nutritional composition between two varieties of Chinese mitten crabs cultivated in Jiangxi Province; namely, "Jianghai 21" and "Changjiang 2". Through the analysis and comparison of transcriptome data, we aimed to provide a comprehensive understanding of the variations in growth performance and nutritional composition between these two varieties.

check for updates

Citation: Wang, Z.; Huang, P.; Li, X.; Pei, J.; Liu, W.; Hou, J.; Li, L.; Fan, H.; Zeng, L.; Zhao, D. Comparison of Growth Performance, Nutritional Composition, and Muscle Transcriptome between Two Cultured Varieties of the Chinese Mitten Crab (*Eriocheir sinensis*). *Fishes* **2024**, *9*, 132. https://doi.org/10.3390/fishes9040132

Academic Editor: Xugan Wu

Received: 29 February 2024
Revised: 6 April 2024
Accepted: 6 April 2024
Published: 9 April 2024

1. Introduction

Aquaculture serves as a vital protein source to meet the growing demand for this essential component of the human diet and stands as the fastest-growing sector in the global food industry [1,2]. Studies have highlighted the distinct growth performances and

breeding benefits across various aquaculture species [3,4]. Moreover, different strains within the same species can exhibit varied growth performances, even under identical culture conditions [5]. For instance, the Steelhead strain of rainbow trout exhibits a relatively high ability to modulate fatty acid composition compared to other strains, resulting in lower EPA + DHA content [6]. Similarly, evaluation of three *Macrobrachium rosenbergii* strains in Vietnam revealed superior performance in the Hawaii strain among the pure strains [7]. Therefore, selecting suitable breeding species adapted to local environmental conditions is crucial to enhance breeding efficiency.

Within the Chinese aquaculture industry, the Chinese mitten crab (*Eriocheir sinensis*) holds substantial economic significance as a freshwater aquaculture crab, contributing significantly to production and economic value [8,9], with an annual production reaching 815,318 tons in 2022 [10].

Newly cultivated varieties of Chinese mitten crab include "Changjiang 1" [11], "Changjiang 2" [12], "Jianghai 21" [13], "Noah 1" [14], and "Guanghe 1" [15]. "Changjiang 1" demonstrates rapid growth and a high survival rate of $\leq$70% [11]. Conversely, "Changjiang 2" (MOA registration number: GS-01-004-2013) is a river crab variety within the Yangtze River system, exhibiting excellent economic traits and notable attributes such as rapid growth, large size, and stable genetic properties; specifically, "Changjiang 2" exhibits an average individual size increase of approximately 18.5% compared to wild populations. The adult crabs within the farming group display uniform sizes, with the coefficient of variation in body mass for both females and males being less than 10%, which suggests stable genetic characteristics [12,16]. "Jianghai 21" (MOA registration number: GS-02-003-2015), developed in 2015, represents a refined version of the top-quality river crab varieties in the Yangtze River Basin, boasting enhanced aesthetics, robust body shape, and a distinct flavor appealing to discerning consumers. "Jianghai 21" shows traits such as rapid growth, larger size, higher yield, and longer legs. Notably, over 50% of the female crabs exceed a weight of 150 g, and the males surpass 200 g [13,16,17]. "Noah 1" is characterized by rapid growth and high stress resistance [14]. Lastly, "Guanghe 1" is a significant variety cultured in the Liaohe River Basin, with adult crabs boasting an average size and survival rates surpassing those of wild counterparts by 25.98% and 48.59%, respectively [15].

Jiangxi Province plays a pivotal role in Chinese mitten crab cultivation, primarily focusing on "Changjiang 2" and "Jianghai 21". To comprehensively assess and compare the advantages of these two varieties under aquaculture conditions, we conducted a comparative analysis targeting specific growth indices, nutrient composition, and muscle transcriptome. Our study endeavored to establish a robust scientific foundation for the sustainable aquaculture of Chinese mitten crabs in this region.

2. Materials and Methods

2.1. Experimental Protocol and Sample Collection

This study adhered to established guidelines and was conducted at the Jiangshui Hairy Crab Culture Base in Funagata Village, Pengze County, Jiujiang City, Jiangxi Province, with approval from the Animal Conservation and Utilization Committee of Nanchang University. The experimental ponds, ranging from 20,000 to 20,100 m^2 in size, were earthen ponds equipped with ample water supply and maintained water quality conforming to fishery and aquaculture standards (Table 1). The two cultured crab varieties, "Changjiang 2" and "Jianghai 21", were introduced on 28 February 2023, following the selection of robust, agile, uniformly sized, and trauma-free button crab seedlings. The conditions for seedling release are outlined in Table 2.

Table 1. Standard limits for water quality indicators (Class III water) and water quality of *Eriocheir sinensis* "Changjiang 2" and "Jianghai 21" during the culture period.

	WT (°C)	**NH_3-N (mg/L)**	**NO_2-N (mg/L)**	**pH**	**DO (mg/L)**	**S(−II) (mg/L)**	**PO_4^{3-} (mg/L)**
Standard limit	Weekly difference 1~2	≤1.0	≤0.10	6–9	≥5	≤0.20	≤0.05
				E. sinensis "Changjiang 2"			
30th	12.25 ± 0.01	0.07 ± 0.10	0.012 ± 0.005	7.46 ± 0.18	7.24 ± 0.11	0.008 ± 0.004	0.001 ± 0.005
60th	15.57 ± 0.03	0.04 ± 0.01	0.006 ± 0.002	7.21 ± 0.15	7.54 ± 0.07	0.015 ± 0.008	0.003 ± 0.001
90th	23.26 ± 0.01	0.01 ± 0.02	0.005 ± 0.003	6.32 ± 0.13	6.32 ± 0.04	0.007 ± 0.013	0.042 ± 0.006
150th	28.02 ± 0.05	0.02 ± 0.05	0.015 ± 0.010	8.02 ± 0.07	8.02 ± 0.16	0.011 ± 0.004	0.035 ± 0.012
240th	14.66 ± 0.02	0.05 ± 0.04	0.007 ± 0.007	7.02 ± 0.09	7.01 ± 0.26	0.003 ± 0.002	0.004 ± 0.001
				E. sinensis "Jianghai 21"			
30th	12.68 ± 0.05	0.01 ± 0.13	0.004 ± 0.005	8.07 ± 0.06	8.68 ± 0.15	0.008 ± 0.001	0.002 ± 0.004
60th	15.79 ± 0.08	0.05 ± 0.07	0.003 ± 0.006	8.54 ± 0.05	7.97 ± 0.13	0.009 ± 0.003	0.001 ± 0.001
90th	23.69 ± 0.03	0.08 ± 0.02	0.002 ± 0.004	8.64 ± 0.17	8.67 ± 0.24	0.004 ± 0.024	0.004 ± 0.003
150th	28.90 ± 0.02	0.01 ± 0.04	0.003 ± 0.015	8.25 ± 0.01	8.00 ± 0.04	0.002 ± 0.012	0.036 ± 0.018
240th	14.12 ± 0.01	0.10 ± 0.06	0.001 ± 0.009	7.64 ± 0.12	8.12 ± 0.03	0.001 ± 0.002	0.006 ± 0.002

Note: Standard limits for drinking water health standards GB579-2006, GB11607-89, and National Environmental Quality Standards for Surface Water GB-3838-2002 in the third level of water quality. Data are presented as mean ± standard deviation (n = 9).

Table 2. Stocking conditions for comparative pond ecological aquaculture production trials.

Programs		
Crab species	"Changjiang 2"	"Jianghai 21"
Seeding time	2023.2.28	2023.2.28
Crab specifications (g)	6.25 ± 1.23	6.86 g ± 1.04
Stocking density (inds/m^2)	2.1	2.1
Types of aquatic plants	Elodea, black algae	Elodea, black algae

2.2. Aquaculture Water Quality Measurements

Pond water quality was analyzed at 30, 60, 90, 150, and 240 d after the initiation of the aquaculture trials summarized herein. Fixed monitoring points (sampling points) were established at the inlet, outlet, and center positions of each test pond. Three samples were withdrawn from each pond, totaling nine samples from the three ponds for each cultured variety. Water samples were collected directly from the preset positions in 250 mL brown glass bottles, sealed, and stored away from light for nitrite nitrogen (NO_2-N, mg/L) analysis. A 1-L collector was used to collect water samples at the preset positions, and concentrated sulfuric acid was added at a ratio of 1:500. The samples were sealed and kept away from light for ammonia nitrogen (NH_3-N, mg/L) analysis. Water temperature (°C), dissolved oxygen (mg/L), and pH were measured using a YSI portable multifunctional water quality analyzer (Professional Plus, YSI Incorporated, OH, USA). Ammonia nitrogen (NH_3-N, mg/L), nitrite (NO_2-N, mg/L), sulfide (S[−II], mg/L), and phosphate (PO_4^{3-}, mg/L) were analyzed and measured using an Octane W-1 Aquatic Water Quality Analyzer and its companion kit.

As indicated in Table 1, all ponds maintained water quality indices within the standard limits throughout the culture period. This suggests that the water quality remained satisfactory throughout the experiment, allowing for the subsequent comparative experiments to be conducted.

2.3. Measurement of Growth Indicators

Thirty Chinese mitten crabs (*E. sinensis*) of the "Changjiang 2" and "Jianghai 21" varieties were randomly selected at 30, 60, 90, 150, and 240 d during the aquaculture experiment, and basic growth data were recorded. Body weight (BW) was measured

using an electronic scale with a precision of 0.01 g, while carapace length (CL), carapace width (CW), body height (BH), second step foot length (SSL), and third step foot length (TSL) were measured using electronic Vernier calipers with a precision of 0.001 cm. The specific measurement method is as follows: CL was measured from the front edge of the cephalothorax (i.e., the front end between the eyes) to the rear edge of the cephalothorax (i.e., the back end of the center line); CW refers to the widest part of the carapace, that is, the lateral distance between the two edges is measured; BH was measured vertically from the highest point of the carapace (i.e., the center of the back) to the lowest point of the abdominal carapace; the length of SFL and TFL refers to the straight-line distance from the base of the leg of the second or third step (i.e., where the leg joins the carapace) to the tip of the leg.

Weight gain rate (WGR), specific growth rate (SGR), growth rate of CL (GR_{CL}), growth rate of CW (GR_{CW}), growth rate of BH (GR_{BH}), growth rate of the second step foot (GR_{SSL}), and growth rate of the third step foot (GR_{TSL}) were calculated using the growth index on day 30 as the initial growth data and growth index on day 240 as the final growth data, employing the following equations:

$$WGR\ (\%) = (W_f - W_i) \times 100/W_i;$$

$$SGR\ (\%/d) = (\ln W_f - \ln W_i) \times 100/T;$$

$$GR_{CL}\ (\%) = (CL_f - CL_i) \times 100/CL_i;$$

$$GR_{CW}\ (\%) = (CWf - CWi) \times 100/CW_i;$$

$$GR_{BH}\ (\%) = (BH_f - BH_i) \times 100/BH_i;$$

$$GR_{SSL}\ (\%) = (SSL_f - SSL_i) \times 100/SSL_i;$$

$$GR_{TSL}\ (\%) = (TSL_f - TSL_i) \times 100/TSL_i;$$

where W_i is the mean initial BW (g); W_f is the mean final BW (g); T is the time interval of breeding; CL_i and CL_f are the means of initial and final CLs, respectively; CW_i and CW_f are the means of initial and final CWs, respectively; BH_f and BH_i are the means of initial and final BHs, respectively; SSL_f and SSL_i are the means of initial and final lengths of the second step foot, respectively; SSL_f and SSL_i are the means of initial and final lengths of the second step foot, respectively; TSL_f and TSL_i are the means of initial and final lengths of the third step foot, respectively.

2.4. Nutrient Component Measurements

At the conclusion of the cultivation trial, 10 *E. sinensis* individuals were randomly selected from each pond, and their muscle and hepatopancreatic tissues were collected and analyzed for nutrient composition, with the nutritional composition measurement of each crab performed with 3 technical replicates. Moisture content was assessed using the GB5009.3-2016 method of dry-weight loss at a constant temperature of 105 °C, measured with an electronic balance with a precision of 0.001 g. Crude lipid content was determined employing the Soxhlet extraction method (GB5009.6-2016) and Soxhlet extractor equipment. Crude protein content was assessed using the Kjeldahl method (GB5009.5-2016) with a Haineng K1160 (Hangzhou Haina Instrument Co., Ltd., Hangzhou, China) fully automatic nitrogen analyzer. Amino acid composition was analyzed using a Hitachi L8900 (Hitachi, Ltd., Tokyo, Japan) automatic amino acid analyzer.

2.5. Transcriptomics Analysis

2.5.1. Sample Collection, RNA Extraction, cDNA Library Construction, and Sequencing

On day 90 after initiating the breeding experiment, three healthy specimens of *E. sinensis* with comparable weights were randomly selected from each of the two cultured varieties, "Changjiang 2" and "Jianghai 21". Muscle samples were extracted from their legs, immediately frozen, and stored in liquid nitrogen until RNA extraction. Total RNA was extracted

using TRIzol reagent (Cat. No. 10296010CN) following the manufacturer's instructions. Subsequently, isolated RNA was treated with RNase I (Omega Bio-tek, Inc., Norcross, GA, USA) to eliminate genomic DNA contamination. The quality and integrity of the mRNA fraction were verified using gel electrophoresis (1.5% agarose gel) and absorption spectroscopy. An Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and Qubit (Qubit, Waltham, MA, USA) were used to measure the RNA integrity and concentration in each sample. Equal amounts of RNA were collected from each group of three crabs for the subsequent phase of analysis.

The RNA utilized for library construction initially comprised total RNA. Subsequently, mRNA containing a poly(A) tail was extracted using Oligo(dT) magnetic beads and fragmented using divalent cations in fragmentation buffer through random interruption. The resulting fragmented mRNA acted as a template to synthesize the first cDNA strand in the M-MuLV reverse transcriptase system using random oligonucleotides as primers. The RNA strand was degraded using RNase H, and a second cDNA strand was synthesized via the DNA polymerase I system using dNTPs. The purified double-stranded cDNA was repaired, A-tailed, and connected to a sequencing adapter. AMPure XP beads were then used for cDNA fragment screening between 250 and 300 bp. A cDNA library was obtained using PCR amplification and AMPure XP bead purification. After successful quality control, the constructed paired-end libraries were sequenced on an Illumina HiSeq 2500 platform (Beijing Novozymes Technology Co, Beijing, China).

2.5.2. Transcriptome Sequencing Data Processing

High-quality clean reads were obtained by discarding reads with junctions, unidentifiable base information, and low-quality reads. Additionally, Q20, Q30, and GC content analyses were performed on clean data (Supplementary Table S1). All subsequent analyses were conducted on high-quality clean data. The reference genome was indexed using HISAT2 v2.0.5, and the paired-end scrubbed reads were aligned against the established *E. sinensis* reference genome (http://www.genedatabase.cn/esi_genome.html, accessed on 1 July 2023) using HISAT2 v2.0.5, and StringTie (1.3.3b) for ab initio assembly of splice transcripts (Supplementary Table S2).

Following transcript assembly, we performed differential expression analysis of the two comparative combinations using DESeq2 software (version 1.20.0) (Supplementary Figure S1). DESeq2 utilizes statistical procedures to identify differential expression in numerical gene expression data (FPKM) using models based on negative binomial distributions. Adjustments were made to the resulting *p*-values to control for false discovery rates using the Benjamini–Hochberg method. Genes with an adjusted *p*-value of $\leq$0.05, identified by DESeq2, were designated as exhibiting differential expression. Functional analysis of differentially expressed genes (DEGs) and transcripts was conducted using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG), providing further insight into the corresponding roles and pathways of the DEG patterns. After normalizing the FPKM values of DEGs, we performed cluster heatmap analysis of the genes related to growth and nutrition among screened DEGs using ChiPlot.

2.5.3. Quantitative RT-PCR (qPCR)

Quantitative qRT-PCR analysis was performed to ensure the accuracy of the transcript results. RNA extraction and cDNA synthesis were conducted as previously described. The QuantStudio™ 6 FLEX (Thermo Fisher Scientific, Waltham, MA, USA) was utilized for qRT-PCR, employing the SYBR ExScrip qRT-PCR kit (Takara, Beijing, China). Primers for qRT-PCR were designed using Premier 5.0 software (Premier Biosoft International, San Francisco, CA, USA) based on the compiled gene sequences (Table 3). Three biological replicates were included for each gene with three technical replicates each. Gene expression levels were normalized using the housekeeping gene β-actin as an internal control. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with CJ samples as calibration controls.

Table 3. Primer sequences used in this study.

Primer Name	Primer Sequence/5′–3′	Product Length/bp	Genbank Accession
BMP2-F	ACGGACCAGAGGACAAAG	181	XM050832375.1
BMP2-R	GAACGGCGAGCATCAA		
EGFL6-F	CCTGTGACCCTGAGACG	263	XM050832091.1
EGFL6-R	GTGGCAGATGGCTTCG		
EGF-β-F	TTTGTCCCTGATGTGAATG	270	NC066537.1
EGF-β-R	CACGAAGGAGCCGAAC		
LRP8-F	TCCGTCACCAGCACCA	164	XM050830481.1
LRP8-R	CCCGACTCCCATCTTTC		
Wnt4-like-F	CGACGATGCCATTGAGG	285	XM050836949.1
Wnt4-like-R	GGCGAGGTGTTCTGATAGTG		
FAT1-F	CGGCACAGCAAGGAT	170	XM050849542.1
FAT1-R	TCTGGGCAGGTGTAGGA		
TBRG1-F	CAGCGGATTGGTGAGG	309	XM050858362.1
TBRG1-R	GCGGGTGATAGCGACA		
RERG-F	GCGTGGCTGGTCGTGTA	297	XM050856896.1
RERG-R	CGAGGTCTGTCCCTTGTG		
GAS1-F	CGGAGGAAGGACACGA	172	NC066514.1
GAS1-R	GCGGCACAGAGGGTAT		
WFIR-F	CACTGCTCCTGCTGTCTGCT	100	XM050881384.1
WFIR-R	TGTTTGCCACACCCGA		
β-actin-F	GCATCCACGAGACCACTTACA	266	NC066571.1
β-actin-R	CTCCTGCTTGCTGATCCACATC		

Note: BMP2, bone morphogenetic protein 2; EGFL6, multiple epidermal growth factor-like domains protein 6; EGF-β, β-epidermal growth factor; LRP8, low-density lipoprotein receptor-related protein 8; Wnt4-like, protein Wnt4-like; FAT1, protocadherin Fat 1; TBRG1, transforming growth factor β-1; RERG, Ras-related and estrogen-regulated growth inhibitor; GAS1, growth arrest-specific protein 1; WFIR, WNT and FGF inhibitory regulator.

2.6. Statistical Analysis

Using SPSS software (version 26.0; IBM Corporation, Armonk, NY 10504, USA, 2019), we performed data analysis. Data are presented as means ± standard deviation. Percentage data underwent square root inverse-chord transformation to achieve chi-square variance before statistical analysis. The results were assessed for significance using one-way analysis of variance and the post-hoc Duncan's multiple range test. Significance was considered at $p < 0.05$.

3. Results

3.1. Growth Performance

Both studied crab varieties exhibited enhanced growth performance to varying degrees over the culture period. As indicated in the data summarized in Table 4, after 240 d of stocking, *E. sinensis* "Jianghai 21" demonstrated significantly greater mean final BW, WGR, GR_{CL}, GR_{CW}, GR_{BH}, GR_{SSL}, and GR_{TSL} than "Changjiang 2" ($p < 0.001$). Additionally, as depicted in Figure 1, there were no significant differences in BW, CL, CW, or BH between the two varieties during the 30–150 d of stocking ($p > 0.05$). However, after 240 d of stocking, all these indices were significantly higher for "Jianghai 21" than for "Changjiang 2" ($p < 0.001$). "Jianghai 21" displayed significantly higher values than "Changjiang 2" at 240 d of stocking ($p < 0.001$). Moreover, the mean SSL for "Jianghai 21" was significantly higher than that for "Changjiang 2" on days 90 and 150 of stocking, and after 240 d of stocking, the difference became highly significant ($p < 0.001$). Regarding TSL, no significant difference was observed between the two varieties during the 30–150 d ($p > 0.05$), but after 240 d of storage, the mean TSL value for "Jianghai 21" was significantly higher than that for "Changjiang 2" ($p < 0.01$).

Table 4. Growth performance of *Eriocheir sinensis* varieties "Changjiang 2" and "Jianghai 21".

	Changjiang 2	Jianghai 21
Wi (g)	12.42 ± 2.14	11.96 ± 3.65
Wf (g)	148.10 ± 10.56	180.82 ± 11.37 ***
WGR (%)	1081.03 ± 8.37	1513.70 ± 9.26 ***
SGR (%/day)	3.80 ± 0.07	4.08 ± 0.16
GR_{CL} (%)	113.74 ± 0.36	151.88 ± 0.54 ***
GR_{CW} (%)	117.18 ± 0.43	165.63 ± 0.59 ***
GR_{BH} (%)	138.87 ± 0.24	176.28 ± 0.44 ***
GR_{SSL} (%)	112.99 ± 0.56	155.39 ± 0.79 ***
GR_{TSL} (%)	119.89 ± 0.32	151.74 ± 1.36 ***

Note: Data are presented as mean ± standard deviation (n = 30). *** $p < 0.001$.

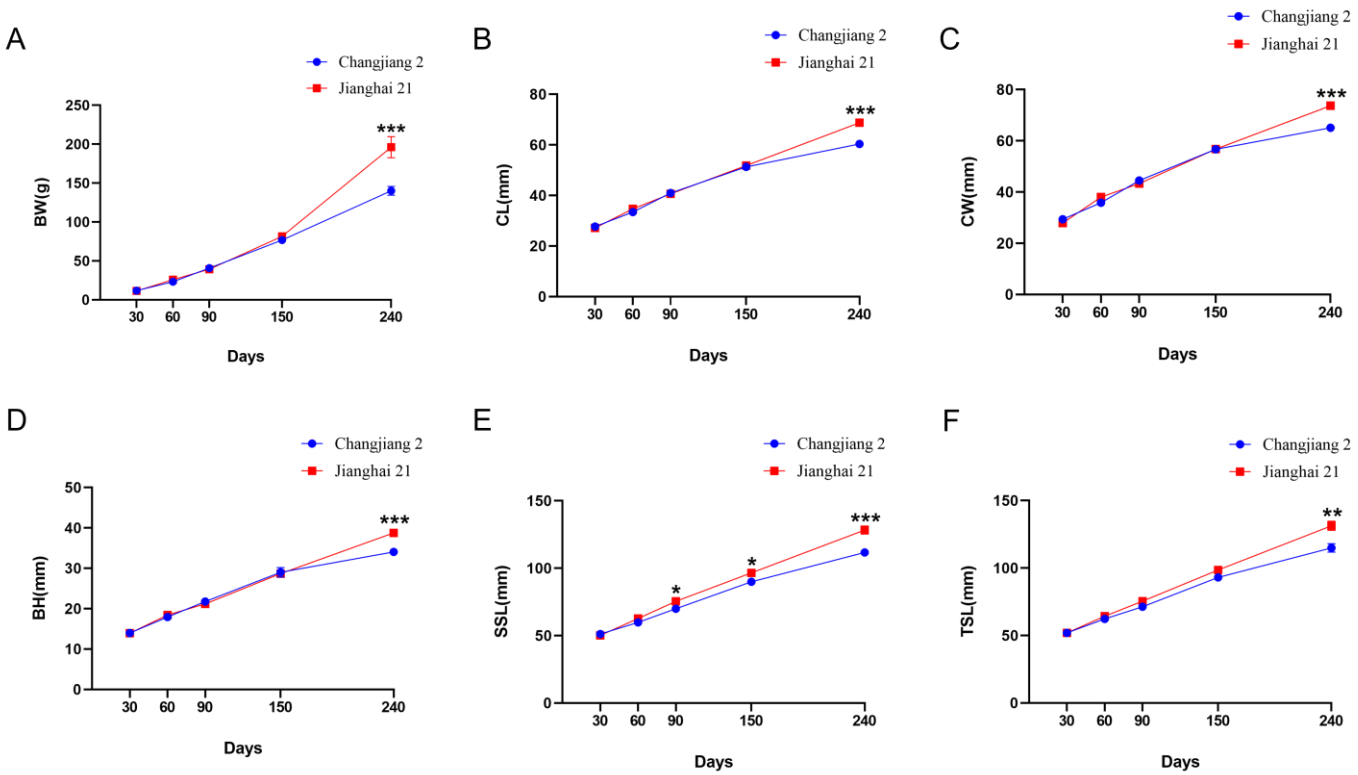

Figure 1. Growth performance of *Eriocheir sinensis* "Changjiang 2" and "Jianghai 21". (**A**) Body weight (BW); (**B**) carapace length (CL); (**C**) carapace width (CW); (**D**) body height (BH); (**E**) second step foot length (SSL); (**F**) third step foot length (TSL). Data are presented as mean ± standard deviation (n = 30). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.2. Proximate Composition

There were no significant differences ($p > 0.05$) in muscle moisture or crude protein contents between "Changjiang 2" and "Jianghai 21". However, the crude lipid content of "Changjiang 2" was significantly greater than that of "Jianghai 21" ($p < 0.001$). Conversely, there were no significant differences ($p > 0.05$) in hepatopancreas moisture, crude protein, or crude lipid contents between the two cultured varieties (Figures 2 and 3).

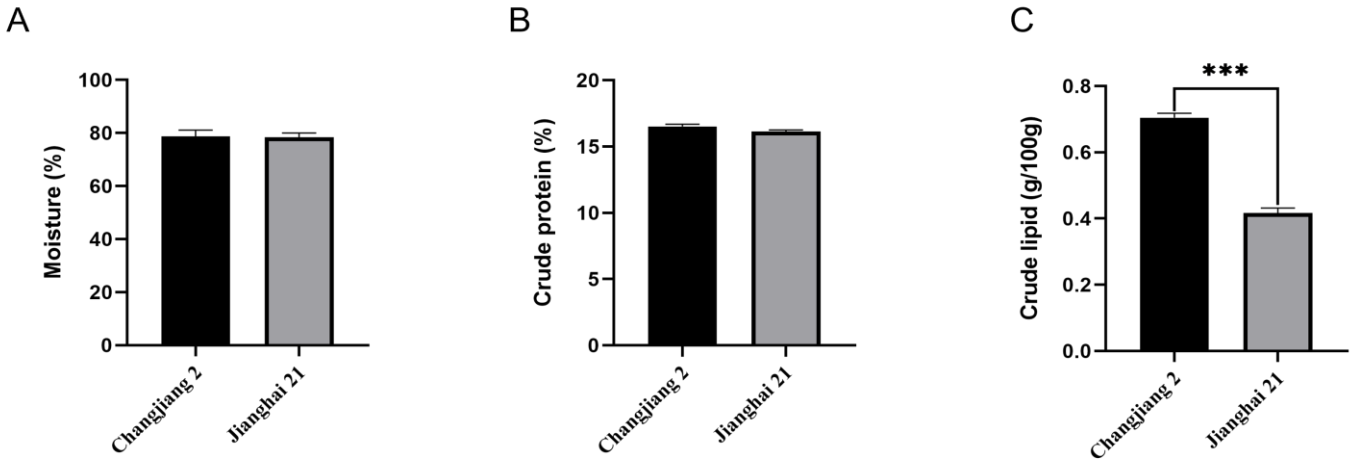

Figure 2. Proximate composition in the muscle of *Eriocheir sinensis* "Changjiang 2" and "Jianghai 21". (**A**) Moisture content; (**B**) crude protein content; (**C**) crude lipid content. Data are presented as mean ± standard deviation (n = 30). *** $p < 0.001$.

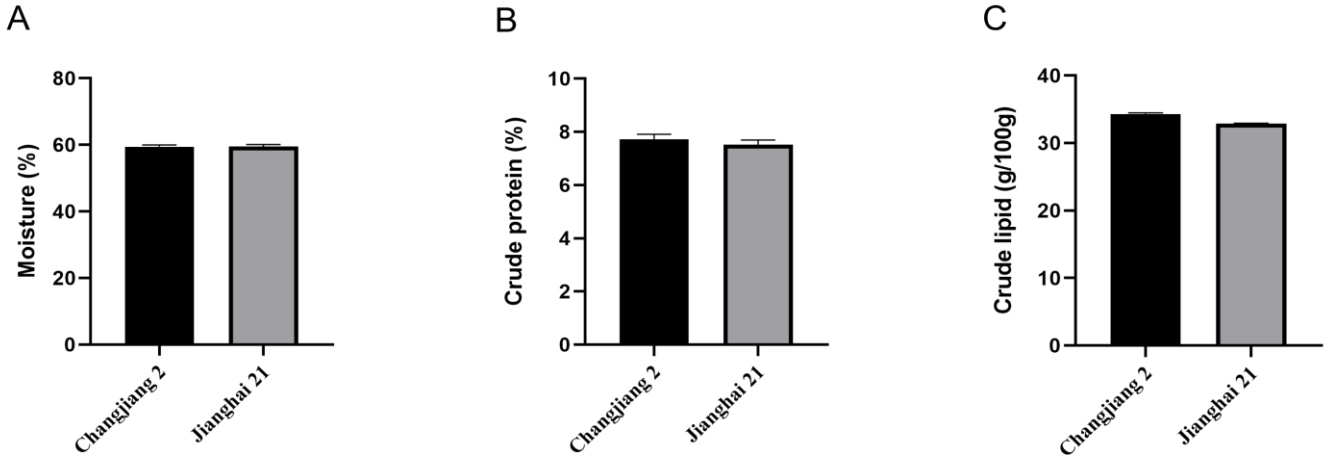

Figure 3. Proximate composition in the hepatopancreas of *Eriocheir sinensis* "Changjiang 2" and "Jianghai 21". (**A**) Moisture content; (**B**) crude protein content; (**C**) crude lipid content. Data are presented as mean ± standard deviation (n = 30).

3.3. Amino Acids

A total of 16 amino acids, including aspartic acid, threonine, and serine, were analyzed in muscle and hepatopancreas tissues (Tables 5 and 6). In muscle tissue, alanine (Ala), cysteine, valine, phenylalanine, lysine, arginine (Arg), and essential amino acid (EAA) contents were significantly higher in specimens of "Jianghai 21" than in those of "Changjiang 2" ($p < 0.05$). Additionally, total amino acid (TAA) and umami amino acid (DAA) contents were significantly higher in specimens of "Jianghai 21" than in those of "Changjiang 2" ($p < 0.001$) (Table 5). Similarly, cysteine and EAA contents in the hepatopancreas tissue of "Jianghai 21" crabs were significantly higher than those in "Changjiang 2" crabs ($p < 0.05$), and TAA content in the hepatopancreas tissue of "Jianghai 21" specimens was highly significantly higher than that of "Changjiang 2" specimens ($p < 0.001$). However, hepatopancreas DAA content did not show a significant difference between the two cultured varieties of *E. sinensis* ($p > 0.05$) (Table 6).

Table 5. Amino acid composition in the muscle of *Eriocheir sinensis* "Changjiang 2" and "Jianghai 21" (mg/g).

Amino Acid Composition of the Muscle	Changjiang 2	Jianghai 21
Aspartic acid	18.37 ± 0.44	19.37 ± 0.46
Threonine	9.78 ± 0.37	9.87 ± 0.18
Serine	8.90 ± 0.26	9.67 ± 0.16
Glutamic acid	25.10 ± 0.24	27.17 ± 1.00
Glycine	10.90 ± 0.60	12.47 ± 0.95
Alanine	9.66 ± 0.11	10.27 ± 0.09 *
Cysteine	4.54 ± 0.13	5.27 ± 0.12 *
Valine	7.55 ± 0.19	8.23 ± 0.06 *
Methionine	4.66 ± 0.10	4.87 ± 0.20
Isoleucine	6.20 ± 0.17	6.57 ± 0.14
Leucine	12.70 ± 0.13	13.27 ± 0.68
Tyrosine	9.53 ± 0.22	9.87 ± 0.06
Phenylalanine	8.46 ± 0.15	9.17 ± 0.15 *
Histidine	5.74 ± 0.08	6.07 ± 0.12
Lysine	16.80 ± 0.30	18.67 ± 0.39 *
Arginine	15.10 ± 0.46	17.37 ± 0.56 *
TAA	173.00 ± 3.42	188.07 ± 3.95 ***
DAA	81.80 ± 1.33	88.27 ± 2.14 ***
EAA	72.0 ± 1.44	76.67 ± 1.33 *
DAA/TAA	0.47 ± 0.00	0.47 ± 0.00
EAA/TAA	0.41 ± 0.00	0.47 ± 0.00

Note: Data are presented as mean ± standard deviation (n = 30). TAA, total amino acids; DAA, umami amino acids; EAA, essential amino acids. * $p < 0.05$, and *** $p < 0.001$.

Table 6. Amino acid composition in the hepatopancreas of *Eriocheir sinensis* "Changjiang 2" and "Jianghai 21" (mg/g).

Amino Acid Composition of the Hepatopancreas	Changjiang 2	Jianghai 21
Aspartic acid	7.76 ± 0.85	8.00 ± 0.17
Threonine	5.20 ± 0.45	5.66 ± 0.26
Serine	3.83 ± 0.25	4.18 ± 0.12
Glutamic acid	9.23 ± 0.55	10.10 ± 0.29
Glycine	3.85 ± 0.35	4.43 ± 0.16
Alanine	3.67 ± 0.25	4.16 ± 0.20
Cysteine	4.29 ± 0.15	5.10 ± 0.22 *
Valine	4.41 ± 0.35	5.10 ± 0.13
Methionine	2.06 ± 0.05	2.19 ± 0.17
Isoleucine	2.67 ± 0.35	2.79 ± 0.04
Leucine	5.38 ± 0.25	6.09 ± 0.13
Tyrosine	4.97 ± 0.45	4.81 ± 0.15
Phenylalanine	4.22 ± 0.25	4.73 ± 0.18
Histidine	3.08 ± 0.15	3.52 ± 0.15
Lysine	6.65 ± 0.35	7.11 ± 0.10
Arginine	5.06 ± 0.45	5.61 ± 0.18
TAA	76.30 ± 4.45	83.60 ± 0.19 ***
DAA	33.70 ± 2.25	36.30 ± 0.30
EAA	33.70 ± 1.95	37.20 ± 0.16 *
DAA/TAA	0.44 ± 0.05	0.43 ± 0.00
EAA/TAA	0.44 ± 0.05	0.44 ± 0.00

Note: Data are presented as mean ± standard deviation (n = 30). TAA, total amino acids; DAA, umami amino acids; EAA, essential amino acids. * $p < 0.05$, and *** $p < 0.001$.

3.4. DEG Analysis

The objective of the differential expression analysis was to identify DEGs between specimens of the two cultured varieties under study and further explore their functions. The screening criteria for DEGs were padj ≤ 0.05 and fold change ≥ 2. In the JH versus CJ

comparison group, a total of 901 DEGs were identified, with 560 (62.2%) upregulated and 341 (37.8%) downregulated (Figure 4).

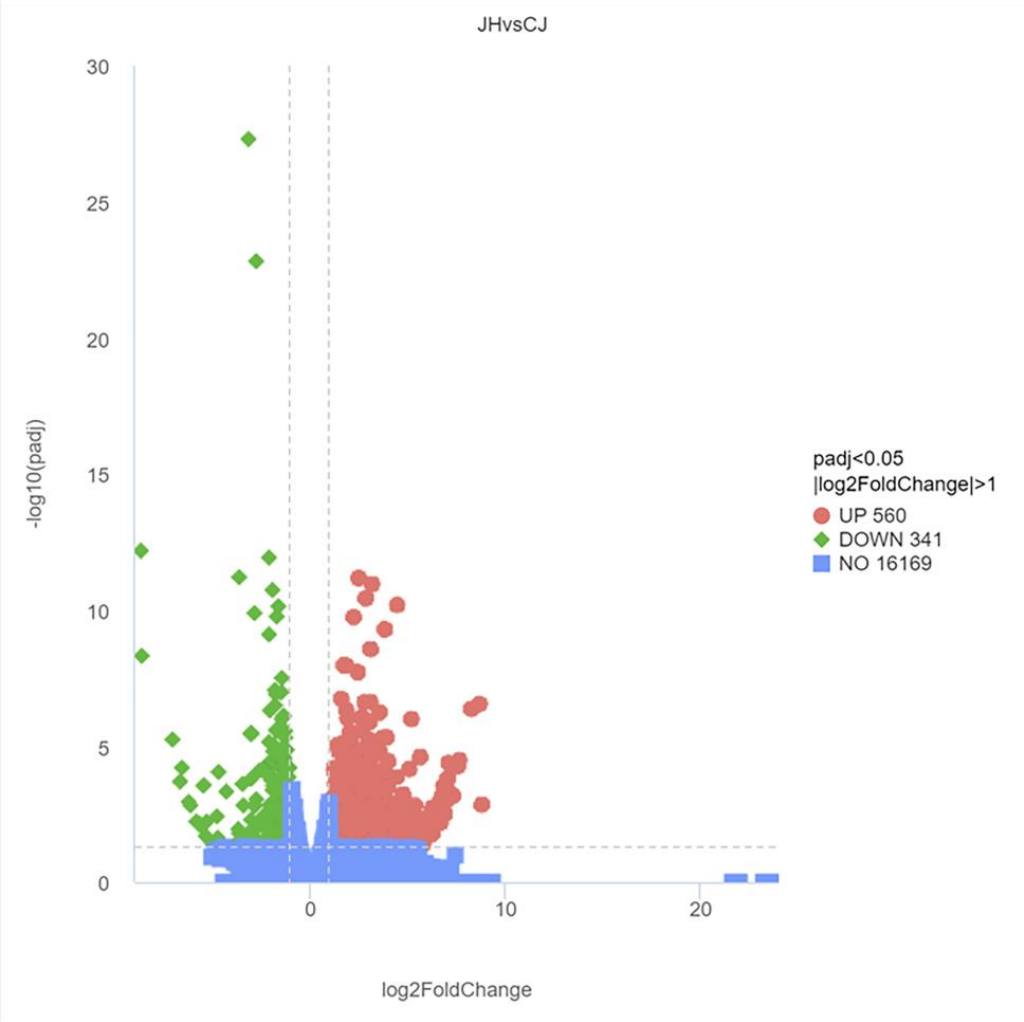

Figure 4. Volcano plot of DEGs between the JH and CJ groups.

3.5. GO and KEGG Analysis of DEGs

We conducted GO analysis to annotate the biological roles of the identified DEGs. The GO annotation results showed that in the JH versus CJ comparison group, 901 DEGs obtained GO annotation information. These DEGs, comprising 560 upregulated and 341 downregulated genes, were classified into 30 subcategories of the three major GO ontologies: biological process (BP), cellular component (CC), and molecular function (MF). In the BP ontology, significant enrichments were observed in redox processes, proton transmembrane transport, and cation transmembrane transport. In the CC ontology, significant enrichments were found in extracellular regions, mitochondria, mitochondrial periplasm, and mitochondrial membrane fractions. In the MF ontology, significant enrichments were identified in oxidoreductase activity, cofactor binding, and structural components of the cuticle (Figure 5).

Additionally, KEGG annotation of the detected DEGs revealed that most DEGs were associated with 84 specific KEGG metabolic pathways, with a total of 347 DEGs involved. Major pathways that were significantly enriched included valine, leucine, and isoleucine degradation (25 DEGs); oxidative phosphorylation (20 DEGs); carbon metabolism (18 DEGs); fatty acid degradation (14 DEGs); fatty acid metabolism (14 DEGs); motor protein (14 DEGs); and extracellular matrix (ECM)—receptor interaction (five DEGs) pathways (Figure 6).

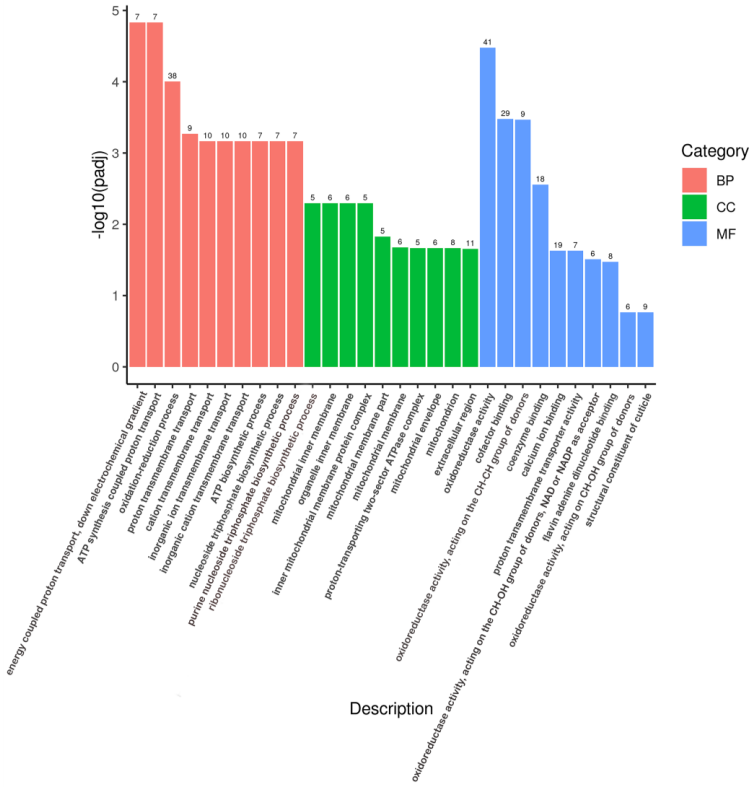

Figure 5. GO annotation of DEGs between the JH group and CJ group.

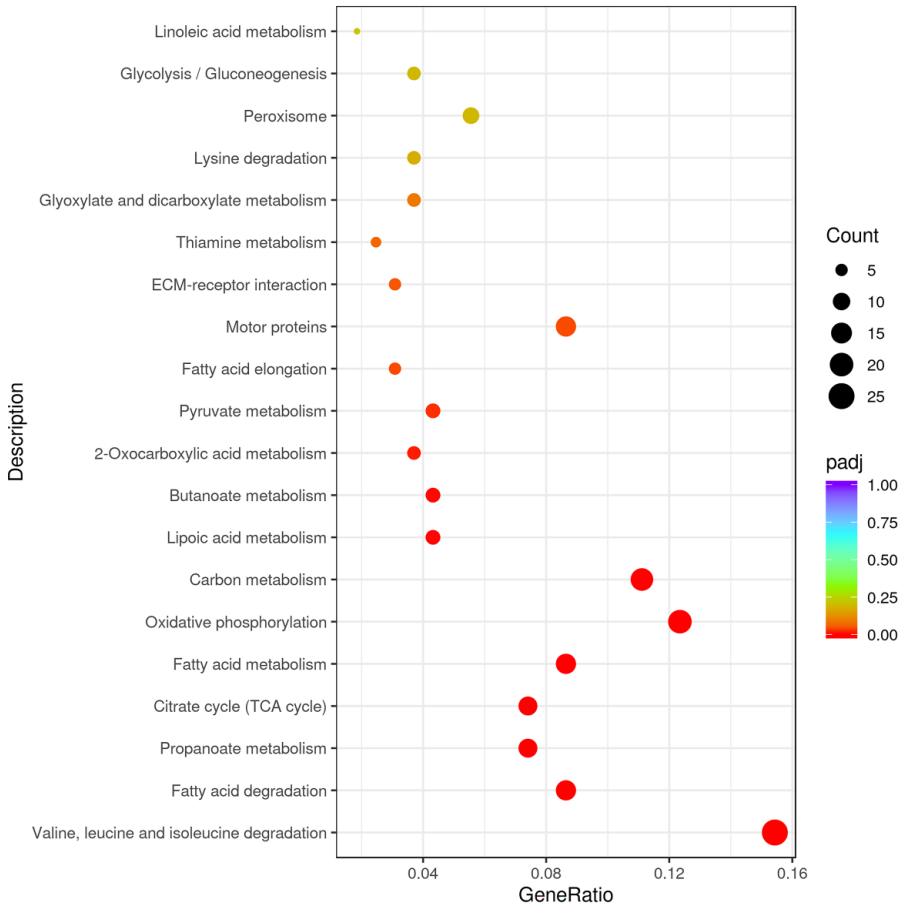

Figure 6. KEGG annotation of DEGs between the JH and CJ groups.

3.6. Clustering Heat Map Analysis of DEGs

DEGs related to growth performance and nutritional components, identified from transcriptome-annotated genes exhibiting significant expression differences, were subjected to clustering heatmap analysis (Figures 7 and 8). As shown in Figure 7, several genes, including Ras-related and estrogen-regulated growth inhibitors (RERGs), growth arrest-specific protein 1 (GAS1), inhibitor of growth protein 1-like, growth arrest and DNA damage, inhibition of growth protein 3, and delta and Notch-like epidermal growth factor, were expressed at higher levels in the CJ group than in the JH group. Conversely, genes such as Activin receptor type-1, unconventional myosin-XV-like, delta and Notch-like epidermal growth factor, unconventional myosin-IE-like, and multiple epidermal growth factor-like domains protein 6 (EGFL6) exhibited higher expression levels in the JH group than in the CJ group.

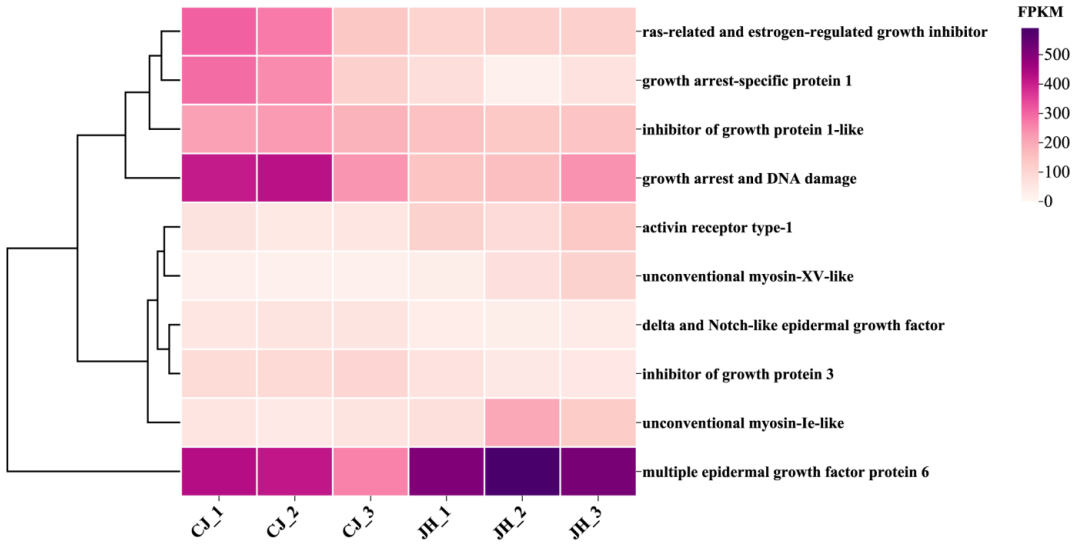

Figure 7. Clustering heat map of growth-related differential genes. FPKM is a measurement unit of gene expression that analyzes differential gene expression between the CJ and JH groups.

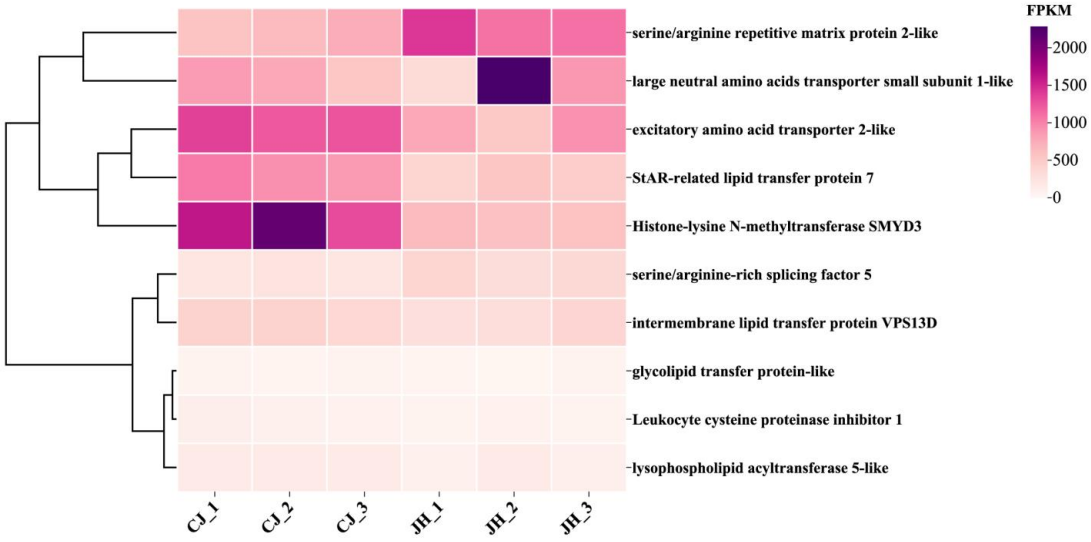

Figure 8. Clustering heat map of nutritional-related differential genes. FPKM is a measurement unit of gene expression that analyzes differential gene expression between the CJ and JH groups.

As shown in Figure 8, genes including large neutral amino acid transporter small subunit 1-like (LAT1), STAR-related lipid transfer protein 7 (STARD7), intermembrane

lipid transfer protein VPS13D (VPS13D), leukocyte cysteine proteinase inhibitor 1 (CST1), excitatory amino acid transporter 2-like (EAAT2), glycolipid transfer protein-like (GLTP), histone-lysine N-methyltransferase SMYD3 (SMYD3), and lysophospholipid acyltransferase 5-like (LPEAT5) showed a higher expression levels in the CJ group than in the JH group. Conversely, serine/Arg repetitive matrix protein 2-like (SRMP2) and serine/Arg-rich splicing factor 5 (SRSF5) exhibited higher expression levels in the JH group compared to the CJ group.

3.7. Validation of DEGs by qRT-PCR

Selection of growth- and nutrition-related genes from the JH vs. CJ comparison groups was based on transcriptomic DEGs information, with DEGs meeting the criteria of q value ≤ 0.01 and large multiplicity of differences being chosen (Table 7). Specifically, genes, such as bone morphogenetic protein 2 (BMP2), EGFL6, β-epidermal growth factor (EGF-β), low-density-lipoprotein-receptor-related protein 8 (LRP8), protein Wnt4-like (WNT4-like), protocadherin Fat 1 (FAT1), SRMP2, LAT1, SRSF5, transforming growth factor β-1 (TBRG1), RERG, GAS1, WNT and FGF inhibitory regulator (WFIR), EAAT2, STARD7, SMYD3, VPS13D, GLTP, CST1, and LPEAT5 were included.

Table 7. Screening of growth-related and nutrition-related genes in DEGs.

Gene Title	*p*-Value	log2FC	Regulated
BMP2	0.002	3.099	Up
EGFL6	0.006	4.13	Up
EGF-β	1.03×10^6	3.8	Up
LRP8	0.007	4.86	Up
WNT4-like	0.006	4.54	Up
FAT1	0.004	3.93	Up
SRMP2	2.32×10^7	1.34	Up
LAT1	0.009	1.68	Up
SRSF5	0.002	1.73	Up
TBRG1	3.10×10^6	−1.37	Down
RERG	0.002	−1.08	Down
GAS1	5.03×10^5	−2.001	Down
WFIR	0.007	−2.11	Down
EAAT2	0.002	−1.79	Down
STARD7	1.52×10^5	−1.00	Down
SMYD3	3.13×10^9	−1.49	Down
VPS13D	0.004	−1.31	Down
GLTP	0.008	−1.03	Down
CST1	0.001	−1.05	Down
LPEAT5	0.004	−1.004	Down

The expression levels of these 10 DEGs were determined using qRT-PCR (Table 3). Figure 9 consistently depicts the correlation between RNA-seq and qPCR expression results. With the CJ group used as the control group, the expression trends of these 10 aligned genes in the JH group were consistent with the RNA-seq results, affirming the reliability of our transcriptome data.

To further demonstrate the reliability of the gene expression data obtained from transcriptome sequencing more intuitively, a correlation analysis was conducted between the results of RNA-seq and qPCR experiments. The analysis results indicated a strong correlation between the two datasets ($R^2 = 0.616$, $p < 0.01$, Figure 10), suggesting the reliability of the conducted transcriptome sequencing.

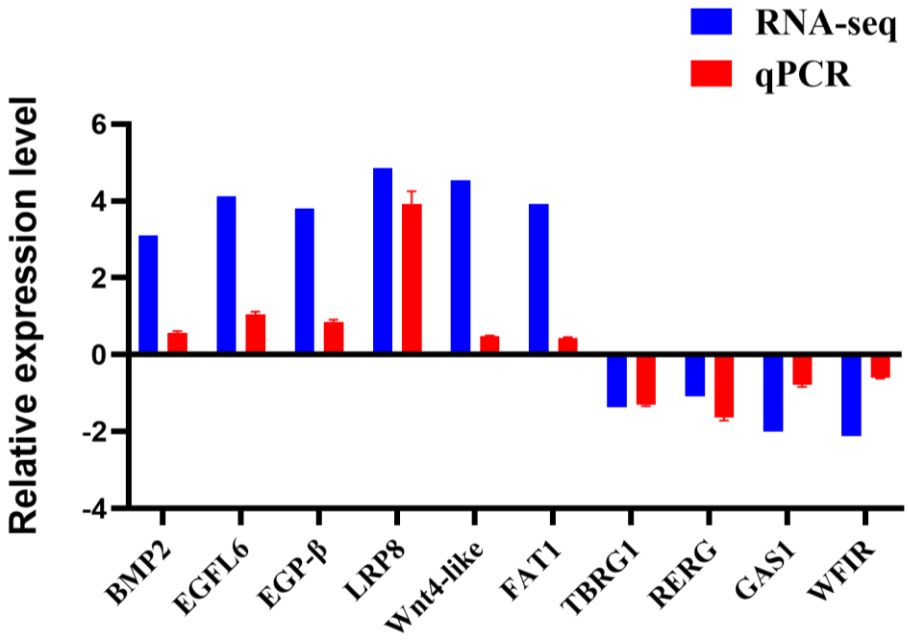

Figure 9. Correlation between the expression profiles of 10 DEGs as determined by RNA-seq and qRT-PCR. The transcript expression levels of the selected genes were normalized to that of the β-actin gene using the $2^{-\Delta\Delta CT}$ method, with CJ samples as calibration controls.

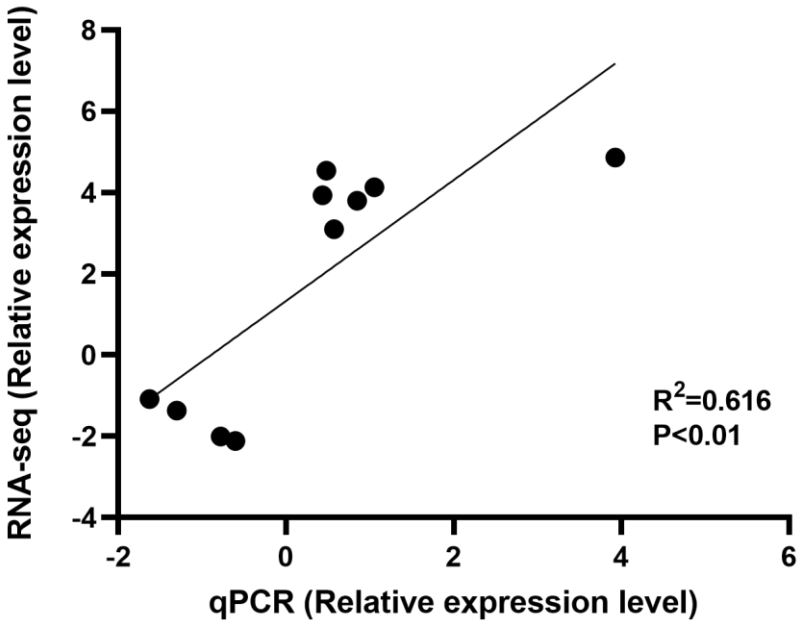

Figure 10. Correlation between RT-qPCR and RNA sequencing of 10 DEGs.

4. Discussion

The growth of Chinese mitten crabs is influenced by multiple factors, including water quality and food availability [18]. Throughout our study, the water quality in all experimental ponds consistently met tertiary fishery and surface water standards (Table 1), with food supply remaining constant across the culture period (Table 2). Larger crabs typically yield higher economic returns, making the comparison of growth performance a critical aspect of crab farming evaluation [19]. Growth performance in Chinese mitten crab varieties is commonly assessed through indicators such as WGR, SGR, carapace dimensions, and appendage length [20,21].

In our investigation, growth performance of the two Chinese mitten crab varieties was assessed using BW, CL, CW, BH, SSL, and TSL. After 240 d of stocking, the final BW, WGR, GR_{CL}, GR_{CW}, GR_{BH}, GR_{SSL}, and GR_{TSL} of *E. sinensis* "Jianghai 21" significantly exceeded those of "Changjiang 2" (Table 7 and Figure 5), indicating superior growth advantages in "Jianghai 21". This aligns with findings from Wang et al., suggesting both varieties may offer high nutritional value, with final crab brood weights ranging from approximately 150 to 200 g [22].

The muscle and hepatopancreas are key edible parts of Chinese mitten crabs, significantly influencing consumer preferences and market prices [23]. Tang et al. conducted a comparative analysis of muscle flavor in wild Chinese mitten crabs sourced from different rivers, revealing that those from the Min River exhibited the highest meat yield (32.94 ± 2.93%) and greatest TAA content (1404.01 ± 2.83 mg/100 g), with sweet amino acids comprising 64.9% [24]. Herein, the muscle crude-lipid content of "Changjiang 2" was notably higher than that of "Jianghai 21" (Figure 2). This aligns with previous findings [25], indicating that "Changjiang 2" displayed lipid levels intermediate between wild and paddy crabs, whereas "Jianghai 21" exhibited lower lipid content than that exhibited by wild crabs, measuring only 0.42 ± 0.01 g/100 g. Additionally, the TAA and EAA levels in "Jianghai 21", particularly in muscle tissue, exceeded those in "Changjiang 2", with a notable elevation in diaminopimelic acid (DAA) in "Jianghai 21" (Tables 5 and 6), suggesting superior flavor potential [26]. Previous research has indicated that glutamic acid, Ala, and Arg play significant roles in imparting the fresh and sweet flavor characteristic of crabs [27,28]. The muscle tissue of "Jianghai 21" exhibited significantly higher levels of Ala and Arg than that in "Changjiang 2", hinting at a potentially enhanced flavor profile in "Jianghai 21".

To explore the differences in growth and nutritional components between the two varieties of the Chinese mitten crab, this study conducted transcriptome analysis of muscle tissues from both varieties. The objective was to uncover the molecular mechanisms driving variations in growth and nutritional aspects between these crab varieties. Previous research has extensively investigated the culture of *E. sinensis* using transcriptomic approaches [29–31]. Herein, we focused on analyzing the muscle transcriptomes of the *E. sinensis* varieties "Changjiang 2" and "Jianghai 21". Overall, 38.5 Gb of pure data were generated (Supplementary Table S1), with comparison efficiencies ranging from 68.31 to 86.8% when aligning sample reads to the reference genome of the Chinese mitten crab (Supplementary Table S2). A total of 901 DEGs were identified between "Jianghai 21" and "Changjiang 2", with 560 DEGs upregulated and 341 DEGs downregulated in "Jianghai 21" (Figure 5). The results of GO enrichment and KEGG annotation of DEGs suggest that growth-related and KEGG annotation of these differential genes revealed enrichment in growth-related pathways such as redox processes, mitochondria, cofactor binding, cuticle structural components, ECM-receptor interactions, and motor proteins (Figures 5 and 6). Among these pathways, the ECM-receptor interactions are essential for a variety of biological processes, including embryonic development, tissue morphogenesis, and wound healing. ECM plays a pivotal role in stimulating cell migration and differentiation [32]. Furthermore, myosin within the motor protein drives the movement of actin filaments and is involved in the spermatogenesis of Chinese mitten crab through the MAPK signaling pathway [33]. The notable upregulation and significant expression of ECM-receptor interaction and motor proteins, in the *E. sinensis* variety "Jianghai 21", in these processes suggests that the regulation of growth-related biological processes may significantly impact the growth performance of *E. sinensis*. Valine, leucine, isoleucine, and fatty acid degradation pathways related to nutritional components were also identified and annotated, especially in crabs, where the fatty acid degradation pathway is associated with various life activities and metabolic processes [34]. Specifically, the process of fatty acid degradation in crabs exerts a regulatory effect on their growth, development, and nutritional status. The essential branched-chain amino acids valine, leucine, and isoleucine play pivotal roles in protein synthesis and energy metabolism in crustaceans [35]. Consistent with the results related to

nutrient components, in the muscle composition of *E. sinensis* variety "Jianghai 21", TAA, EAA, and DAA were significantly higher than in "Changjiang 2", indicating that leucine, isoleucine, and valine may be important regulatory factors. Similarly, the significantly higher muscle fat content in "Changjiang 2" compared to that in "Jianghai 21" may be mainly regulated by the fatty acid degradation pathway. In line with the findings related to nutrient components, the muscle composition of *E. sinensis* variety "Jianghai 21" exhibited significantly higher levels of TAA, EAA, and DAA than those of "Changjiang 2", indicating that leucine, isoleucine, and valine may serve as important regulatory factors. Similarly, the significantly higher muscle fat content in "Changjiang 2" compared to that in "Jianghai 21" may primarily be regulated by the fatty acid degradation pathway.

In this study, 20 DEGs related to growth performance and nutritional composition were identified. Clustered heat map analysis was conducted to identify potential factors influencing the growth and nutrition of the two cultured varieties of Chinese mitten crabs by identifying different key genes.

From the clustering heatmap results of growth-related DEGs (Figure 7), it was observed that genes negatively regulated growth and development, such as that of RERG, GAS1, and inhibitor of GAS1, exhibiting significantly higher expression levels in *E. sinensis* "Changjiang 2" compared to that in "Jianghai 21". Among these, RERG inhibited growth by suppressing the Ras-mediated signaling pathway [36], while GAS1 and growth protein 1-like inhibitor played key roles in growth inhibition, acting as negative regulators of processes such as AMPK/mTOR/p70S6K, as well as inhibiting cell proliferation [37].

Conversely, genes promoting growth and development, including activin receptor type-1, unconventional myosin-Ie-like, unconventional myosin-XV-like, and EGFL6. EFFL6 exhibited higher expression levels in "Jianghai 21" compared to that in "Changjiang 2". Specifically, activin receptor type-1 participates in the mediation of various biological processes in Chinese mitten crab, and in the production of new blood cells by activating JNK [38]; unconventional myosin-Ie-like and unconventional myosin-XV-like play important roles in fundamental cellular processes [39]. EGFL6 is associated with cell differentiation, proliferation, and apoptosis [40], and decreasing the gene transcription level of EGFL6 in grass carp affects its gluconeogenesis [41] (Figure 8). Large neutral amino acids transporter small subunit 1-like are involved in amino acid synthesis and transport processes, positively contributing to regulating protein synthesis and ensuring an adequate supply of amino acids to the cells [42,43]. Conversely, the excitatory amino acid transporter 2-like, primarily responsible for amino acid transport [43], is enriched to a higher degree in "Changjiang 2" than in "Jianghai 21" [44,45]. According to the aforementioned experiments, we deduced that the significantly higher expression of these lipid-transport-related proteins in the muscle tissue of "Changjiang 2" may be closely related to its markedly higher fat content. These results suggest that the differences in growth performance between the two strains of *E. sinensis* may be regulated by the aforementioned genes.

Judging from the clustered heatmap results of DEGs related to nutritional composition (Figure 8), SRMP2, SRSF5, and LAT1 are involved in amino acid synthesis and transport, positively contributing to regulating protein synthesis and ensuring an adequate supply of amino acids to the cells [42,43]. These genes are enriched to a higher degree in *E. sinensis* "Jianghai 21".

Conversely, the EAAT2, primarily responsible for amino acid transport [43], is enriched to a higher degree in "Changjiang 2". This corresponds with the experimental results where the TAA, EAA, and DAA contents were significantly higher in "Jianghai 21" than in "Changjiang 2", suggesting the potential roles of these genes in amino acid synthesis and transport in *E. sinensis*.

Furthermore, STARD7 and VPS13D are proteins related to lipid transport [44,45], which are significantly upregulated in *E. sinensis* "Changjiang 2" than in "Jianghai 21". According to the aforementioned experiments, we deduced that the significantly higher expression of these lipid transport-related proteins in the muscle tissue of "Changjiang 2" may be closely related to its markedly higher fat content.

To ensure the reliability of our transcriptomic results, we conducted qRT-PCR analysis on 10 randomly selected DEGs. These genes were chosen based on significant differences identified through qRT-PCR screening. Our RNA-seq findings showed the elevated expression levels of BMP2, EGFL6, EGF-β, LRP8, WNT4-like, and FAT1 genes in the muscle tissues of "Jianghai 21" when compared with those of "Changjiang 2". Conversely, the expression of TBRG1, RERG, GAS1, and WFIR genes was lower in the muscle tissues of "Jianghai 21" than those of "Changjiang 2". These results corroborated the trends observed in the RNA-seq data, confirming the reliability of our transcriptome sequencing.

5. Conclusions

We delineated the disparities in growth performance and nutritional composition between two varieties of Chinese mitten crabs, namely, "Jianghai 21" and "Changjiang 2", cultured in Jiangxi Province. Our breeding test outcomes indicate that "Jianghai 21" outperforms "Changjiang 2" in terms of growth performance. Transcriptome analysis unveiled that in the pond ecological aquaculture system, DEGs in "Jianghai 21" and "Changjiang 2" were predominantly enriched in pathways related to fatty acid degradation, metabolism, and growth regulation. Judging from the results of this study findings, "Jianghai 21" emerged as the superior choice when compared to "Changjiang 2" in terms of growth performance. This conclusion lays a theoretical groundwork for selecting cultured varieties within the Chinese mitten crab industry in Jiangxi Province.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fishes9040132/s1. Table S1: Summary of sample sequencing data quality. Table S2: Statistics on the comparison of samples with the reference genome. Figure S1: RNA-Seq results: (A) Pearson correlation coefficient analysis between all biological replicated RNA-seq samples in *E. sinensis*; (B) principal component plot of biological RNA-seq samples in *E. sinensis*.

Author Contributions: Formal analysis, Z.W.; data collection, P.H., J.H. and L.L.; experimental discussion, J.P. and W.L.; validation, P.H.; resources, L.Z. and H.F.; writing—original draft preparation, Z.W.; writing—review and editing, D.Z.; conceptualization, D.Z.; supervision, D.Z.; funding acquisition, D.Z. and X.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Jiangxi Modern Seed Industry (Fisheries) Development Project (Gannong Gui Ji Zi [2022] No. 45), the National Natural Science Foundation of China (grant number 32160870), the Natural Science Foundation of Jiangxi Province (grant number 20232BAB205071), the Chongqing Natural Science Foundation Project (CSTB2022NSCQ-MSX1407), the Jiangxi Agriculture Research System (JXARS-03 and JXARS-10), and the National Key R&D Program of China (2023YFD2401802).

Institutional Review Board Statement: The animal study protocol was approved by the Animal Care and Use Committee of Nanchang University (identification code: NCUACC-2020-563; date of approval: 26 May 2020).

Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of this study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Gjedrem, T.; Akvaforsk, Å. *Selection and Breeding Programs in Aquaculture*; Springer: Berlin/Heidelberg, Germany, 2005; Volume 2005.
2. Yue, G.H.; Tay, Y.X.; Wong, J.; Shen, Y.; Xia, J. Aquaculture species diversification in China. *Aquac. Fish.* **2023**, *9*, 206–217. [CrossRef]
3. Jiang, K.; Chen, C.; Jiang, G.; Chi, Y.; Xu, C.; Kong, L.; Yu, H.; Liu, S.; Li, Q. Genetic improvement of oysters: Current status, challenges, and prospects. *Rev. Aquac.* **2023**, *16*, 796–817. [CrossRef]
4. Duong, T.-Y.; Nguyen, N.-T.T.; Nguyen, T.T.; Huynh, L.T.T.; Bui, T.M.; Pham, L.T.; Dunham, R. Growth and survival of crossbreeds between wild and domestic strains of Mekong Delta bighead catfish (*Clarias macrocephalus* Günther, 1864) cultured in tanks. *Aquaculture* **2022**, *547*, 737507. [CrossRef]

5. Ou, M.; Zhao, J.; Luo, Q.; Hong, X.; Zhu, X.; Liu, H.; Chen, K. Characteristics of hybrids derived from *Channa argus* ♀× *Channa maculata* ♂. *Aquaculture* **2018**, *492*, 349–356. [CrossRef]
6. Gladyshev, M.I.; Makhrov, A.A.; Sushchik, N.N.; Makhutova, O.N.; Rudchenko, A.E.; Balashov, D.A.; Vinogradov, E.V.; Artamonova, V.S. Differences in composition and fatty acid contents of different rainbow trout (*Oncorhynchus mykiss*) strains in similar and contrasting rearing conditions. *Aquaculture* **2022**, *556*, 738265. [CrossRef]
7. Thanh, N.M.; Ponzoni, R.W.; Nguyen, N.H.; Vu, N.T.; Barnes, A.; Mather, P.B. Evaluation of growth performance in a diallel cross of three strains of giant freshwater prawn (*Macrobrachium rosenbergii*) in Vietnam. *Aquaculture* **2009**, *287*, 75–83. [CrossRef]
8. Wang, X.; Zheng, R.-X.; Zhang, D.-M.; Lei, X.-Y.; Wang, S.; Wan, J.-W.; Liu, H.-J.; Chen, Y.-K.; Zhao, Y.-L.; Wang, G.-Q.; et al. Effects of different stocking densities on growth performance, nutritional quality and economic benefit of juvenile female Chinese mitten crab (*Eriocheir sinensis*) in rice-crab culture systems. *Aquaculture* **2022**, *553*, 738111. [CrossRef]
9. Guo, H.; Hua, H.; Wang, J.; Qiang, W.; Xiang, X.; Liu, W.; Jiang, G. The Role of Cholesterol during the Ovarian Maturation and Lipid Metabolism of Female Chinese mitten crab (*Eriocheir sinensis*). *Aquac. Nutr.* **2024**, *2024*, 9933600. [CrossRef]
10. Wang, D.; Fan, W.; Song, D.; Gao, H. *Chinese Fishery Statistical Yearbook*; Fisheries Agency of China Agriculture Ministry: Beijing, China, 2023.
11. Deng, Y.F.; Xia, A.J.; Pan, J.L.; Li, Y.H.; Bai, R.F.; Zhu, Q.S. Breeding of the new variety of *Eriocheir sinensis* named "Changjiang 1". *J. Aquac.* **2013**, *34*, 43–47.
12. Fu, L.L.; Zhu, P. Healthy and efficient breeding technology of Chinese mitten crab "Changjiang 2". *J. Aquac.* **2020**, *41*, 66–67+70.
13. Wang, C.H.; Zhang, G.Y. Chinese mitten crab "Jianghai21". *China Fish.* **2017**, *01*, 69–75.
14. Xu, P.; Dong, Z.J.; Zhuang, H.G.; Zhu, W.B.; Guo, F.Y. Chinese mitten crab "Noah 1". *China Fish.* **2017**, 70–73.
15. Tian, S.J.; Li, X.D.; Jiang, Y.S.; Liu, X.; Yang, H.W.; Yuan, Z.Z.; Zheng, Y. Microsatellite-enriched library construction and polymorphic microsatellite markerisolation in Chinese mitten crab *Eriocheir sinensis* strain "Guanghe 1". *J. Dalian Ocean. Univ.* **2013**, *28*, 230–235.
16. Wang, J.; Xu, P.; Zhou, G.; Li, X.; Lu, Q.; Liu, X.; Zhou, J.; Wang, C. Genetic improvement and breeding practices for Chinese mitten crab, *Eriocheir sinensis*. *J. World Aquac. Soc.* **2018**, *49*, 292–301. [CrossRef]
17. Cui, W.; Ning, B. Development and application of crab culture in the development of Chinese mitten crab industry of Shanghai. *Aquac. Res.* **2019**, *50*, 367–375. [CrossRef]
18. Han, W.; Sun, Y.; Liu, J.; Zhang, Y.; Lu, Z.; Cheng, Y. Effect of different feeding modes on the growth, biochemical composition, and living environment of the juvenile Chinese mitten crab *Eriocheir sinensis*. *Aquaculture* **2021**, *541*, 736687. [CrossRef]
19. He, J.; Wu, X.; Li, J.; Huang, Q.; Huang, Z.; Cheng, Y. Comparison of the culture performance and profitability of wild-caught and captive pond-reared Chinese mitten crab (*Eriocheir sinensis*) juveniles reared in grow-out ponds: Implications for seed selection and genetic selection programs. *Aquaculture* **2014**, *434*, 48–56. [CrossRef]
20. Zhang, B.Y.; Fang, W.H.; Zhu, R.; Wang, N.; Yao, Q.; Liu, H.J.; Wan, J.W.; Chen, Y.K.; Wang, Q.J.; Zhang, D.M. Comparative Study on Growth Index and Nutritional Quality of Female Chinese mitten crab *Eriocheir sinensis* Selected at Different Growth Periods in Rice-Crab Culture Systems. *Aquac. Nutr.* **2023**, *2023*, 4805919. [CrossRef] [PubMed]
21. Wan, J.-J.; Pan, J.-L.; Shen, M.-F.; Xue, H.; Sun, M.-L.; Zhang, M.-Q.; Zhu, X.-H.; Ma, X.-K. Changes in the growth performance, antioxidant enzymes and stress resistance caused by dietary administration of synbiotic (fructooligosaccharide and probiotics) in juvenile Chinese mitten crab, *Eriocheir sinensis*. *Aquac. Int.* **2022**, *30*, 467–481. [CrossRef]
22. Wang, Q.; Wu, X.; Long, X.; Zhu, W.; Ma, T.; Cheng, Y. Nutritional quality of different grades of adult male chinese mitten crab, *Eriocheir sinensis*. *J. Food Sci. Technol.* **2018**, *55*, 944–955. [CrossRef]
23. Czerniejewski, P.; Bienkiewicz, G.; Tokarczyk, G. Nutritional Quality and Fatty Acids Composition of Invasive Chinese Mitten Crab from Odra Estuary (*Baltic Basin*). *Foods* **2023**, *12*, 3088. [CrossRef] [PubMed]
24. Tang, M.; Qu, Z.; Shi, W.; Wang, X.; Wu, X. A comparative study on the flavour of wild Chinese mitten crab (*Eriocheir sinensis*) along the eastern coast of China. *J. Food Compos. Anal.* **2024**, *127*, 105949. [CrossRef]
25. Wu, H.; Ge, M.; Chen, H.; Jiang, S.; Lin, L.; Lu, J. Comparison between the nutritional qualities of wild-caught and rice-field male Chinese mitten crabs (*Eriocheir sinensis*). *LWT* **2020**, *117*, 108663. [CrossRef]
26. Yi, X.; Gao, J.; Li, L.; Du, J.; Nie, Z.; Zhang, X.; Xu, G. Effects of fattening diets on the nutritional quality and flavor of the adult female Chinese mitten crab (*Eriocheir sinensis*). *Aquac. Rep.* **2022**, *25*, 101223. [CrossRef]
27. Wang, S.; Wang, Y.; Wu, X.; Zhang, X.; Zhao, J.; Yang, J.; Cheng, Y. Gonadal development and biochemical composition of Chinese mitten crabs (*Eriocheir sinensis*) from four sources. *J. Food Sci.* **2021**, *86*, 1066–1080. [CrossRef] [PubMed]
28. Wang, S.; He, Y.; Wang, Y.; Tao, N.; Wu, X.; Wang, X.; Qiu, W.; Ma, M. Comparison of flavour qualities of three sourced *Eriocheir sinensis*. *Food Chem.* **2016**, *200*, 24–31. [CrossRef] [PubMed]
29. Wang, T.; Yang, C.; Zhang, T.; Liang, H.; Ma, Y.; Wu, Z.; Sun, W. Immune defense, detoxification, and metabolic changes in juvenile *Eriocheir sinensis* exposed to acute ammonia. *Aquat. Toxicol.* **2021**, *240*, 105989. [CrossRef]
30. Tian, Z.; Jiao, C. Molt-dependent transcriptome analysis of claw muscles in Chinese mitten crab *Eriocheir sinensis*. *Genes Genom.* **2019**, *41*, 515–528. [CrossRef]
31. Shen, M.; Wang, Y.; Zhu, F.; Wei, M.; Xu, D.; Zhang, C.; Du, S.; Jiang, J.; Zhou, J.; Zhang, Z.; et al. Integrative transcriptomic and metabolomic analysis reveals the effects of a sudden drop in salinity on osmoregulation, metabolism, anti-oxidation, and immunity in *Eriocheir sinensis* megalopa and juvenile stages. *Aquac. Rep.* **2023**, *31*, 101656. [CrossRef]
32. Mescher, A.L. The cellular basis of limb regeneration in urodeles. *Int. J. Dev. Biol.* **2004**, *40*, 785–795.

33. Qi, H.-Y.; Li, Z.-F.; Wang, L.-M.; Zhao, Z.; Wang, J.-M.; Tan, F.-Q.; Yang, W.-X. Myosin VI stabilizes intercellular junctions in the testis through the LHR and MAPK signalling pathway during spermatogenesis in *Eriocheir sinensis*. *Int. J. Biol. Macromol.* **2023**, *248*, 125842. [CrossRef] [PubMed]
34. Dvoretsky, A.G.; Bichkaeva, F.A.; Baranova, N.F.; Dvoretsky, V.G. Fatty acid composition of the Barents Sea red king crab (*Paralithodes camtschaticus*) leg meat. *J. Food Compos. Anal.* **2021**, *98*, 103826. [CrossRef]
35. Zhang, Y.; Sully, P.; Spitzer, L.; Dutilleul, P.; Simpson, B.K. Comprehensive biorefinery of invasive European green crab (*Carcinus maenas*) into multiple high-value biomolecules. *J. Clean. Prod.* **2022**, *374*, 134052. [CrossRef]
36. Bernal Astrain, G.; Nikolova, M.; Smith, M.J. Functional diversity in the RAS subfamily of small GTPases. *Biochem. Soc. Trans.* **2022**, *50*, 921–933. [CrossRef] [PubMed]
37. Li, Q.; Qin, Y.; Wei, P.; Lian, P.; Li, Y.; Xu, Y.; Li, X.; Li, D.; Cai, S. Gas1 Inhibits Metastatic and Metabolic Phenotypes in Colorectal Carcinoma. *Mol. Cancer Res.* **2016**, *14*, 830–840. [CrossRef] [PubMed]
38. Jia, Z.; Wang, M.; Wang, X.; Wang, L.; Song, L. The receptor for activated C kinase 1 (RACK1) functions in hematopoiesis through JNK activation in Chinese mitten crab *Eriocheir sinensis*. *Fish Shellfish. Immunol.* **2016**, *57*, 252–261. [CrossRef]
39. Fili, N.; Toseland, C.P. Unconventional Myosins: How Regulation Meets Function. *Int. J. Mol. Sci.* **2020**, *21*, 67. [CrossRef]
40. Shen, J.; Sun, Y.; Liu, X.; Zhu, Y.; Bao, B.; Gao, T.; Chai, Y.; Xu, J.; Zheng, X. EGFL6 regulates angiogenesis and osteogenesis in distraction osteogenesis via Wnt/β-catenin signaling. *Stem Cell Res. Ther.* **2021**, *12*, 415. [CrossRef]
41. Xie, N.; Tian, J.; Meng, X.; Dong, L.; Jiang, M.; Wen, H.; Lu, X. DNA methylation profiling and transcriptome sequencing reveal the molecular mechanism of the high-carbohydrate diet on muscle growth of grass carp (*Ctenopharyngodon idella*). *Aquac. Rep.* **2023**, *30*, 101545. [CrossRef]
42. Veldsman, W.P.; Ma, K.Y.; Hui, J.H.L.; Chan, T.F.; Baeza, J.A.; Qin, J.; Chu, K.H. Comparative genomics of the coconut crab and other decapod crustaceans: Exploring the molecular basis of terrestrial adaptation. *BMC Genom.* **2021**, *22*, 313. [CrossRef]
43. Wan, Y.; Wang, Y.; Shi, Z.; Rentsch, D.; Ward, J.L.; Hassall, K.; Sparks, C.A.; Huttly, A.K.; Buchner, P.; Powers, S.; et al. Wheat amino acid transporters highly expressed in grain cells regulate amino acid accumulation in grain. *PLoS ONE* **2021**, *16*, e0246763. [CrossRef] [PubMed]
44. Galano, M.; Venugopal, S.; Papadopoulos, V. Role of STAR and SCP2/SCPx in the Transport of Cholesterol and Other Lipids. *Int. J. Mol. Sci.* **2022**, *23*, 2115. [CrossRef] [PubMed]
45. Sultan, T.; Scorrano, G.; Panciroli, M.; Christoforou, M.; Raza Alvi, J.; Di Ludovico, A.; Qureshi, S.; Efthymiou, S.; Salpietro, V.; Houlden, H. Clinical and molecular heterogeneity of VPS13D-related neurodevelopmental and movement disorders. *Gene* **2024**, *899*, 148119. [CrossRef] [PubMed]