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# Inhibited expression of amylases and cellulases in crabs with hepatopancreatic necrosis disease and their regulation by EcR and HR38

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Hepatopancreatic necrosis disease (HPND) is a serious threat to the stable and sustainable development of the Chinese mitten crab farming industry. Studies have found that the hepatopancreas is the main lesion organ in HPND crabs, capable of causing metabolic abnormalities and non-feeding (starvation state). Here, four amylases (named as *EsAmy1-4*) and four cellulases (endo-beta-1, 4-glucanase, named as *EsGlu1-4*) that relate to carbohydrate metabolism were cloned from *Eriocheir sinensis*. Our studies found that the expressions of *EsAmy1-4* and *EsGlu1-4* were significantly decreased in the hepatopancreas of crabs with HPND. Moreover, the expressions of two important regulatory factors of carbohydrate metabolism, *hormone receptor 38 (EsHR38)* and *ecdysone receptor (EsEcR)*, were remarkably decreased in the hepatopancreas of crabs with HPND. Further studies found that knockdown of *EsHR38* and *EsEcR* could down-regulated the expressions of *EsAmy1-4* and *EsGlu1-4*. In addition, downregulation of *EsEcR* could decrease the expression of *EsHR38*. Hence, HPND causes down-regulation of *EsEcR* expression, inhibits the transcription of *EsHR38*, further inhibits the expression of *EsAmy1-4* and *EsGlu1-4*, and then leads to the abnormal carbohydrate metabolism. The abnormalities of carbohydrate metabolism may result in the abnormal food intake in crabs. This study provides new evidence for further understanding the mechanism of HPND.

## KEYWORDS

hepatopancreatic necrosis disease, amylase, cellulase, hormone receptor 38, ecdysone receptor, *Eriocheir sinensis*

## 1 Introduction

Chinese mitten crab, *Eriocheir sinensis*, is an important economic breeding species with high nutritional value, delicious taste, and rapid growth (Cheng et al., 2008). In the middle and lower reaches of the Yangtze River, especially in Jiangsu of China, Chinese mitten crabs are widely farmed (Dai et al., 2021). However, the expansion of crab farming scale and the increase of farming density has caused the outbreak of various diseases, such as tremor disease (TD) (Huang et al., 2019), edema disease, black gill disease (Wang, 2011), hepatopancreatic necrosis disease (HPND), and so on. HPND, commonly known as “shui biezi”, has received a lot of attention since its large-scale outbreak in Xinghua, Jiangsu Province in 2015 because of the huge economic losses it causes (Yang et al., 2016). There are different opinions on the possible causes of HPND, including the microsporidia fungus (Ding et al., 2016), abnormal fatty acid metabolism, high concentration of alanine (Gao et al., 2018), imbalance of hepatopancreatic flora (Shen et al., 2021), abiotic factors related to cyanobacteria bloom (Chi et al., 2021), environment and nutrition (Huang et al., 2021). Until now, there is no unanimous conclusion about the cause of HPND. Therefore, the cause and mechanism of HPND need to be urgently explored to ensure healthy crab production.

The main target and lesion organ of HPND is the hepatopancreas, which is an important digestive and immune organ of crustaceans with high digestive enzyme activity (Yang et al., 2018; Huang et al., 2021). Hepatopancreatic lesions are characterized by degeneration and atrophy, and the color changes from gold to light yellow and white (Pan et al., 2017). Studies have also found that there was abnormal nutrient metabolism in hepatopancreas and signs of non-eating and starvation in crabs with HPND (Ding et al., 2018; Yang et al., 2020). However, how HPND affects carbohydrate metabolism is not clear. Amylase and cellulase are two common digestive enzymes in carbohydrate metabolism, in which  $\alpha$ -amylase is responsible for the hydrolysis of  $\alpha$ -1,4-glucosidic bonds in starch and related  $\alpha$ -glucans (Janecek et al., 2014), and endo- $\beta$ -1, 4-glucanase is responsible for the hydrolysis of cellulose like  $\beta$ -1,4-glucans (Watanabe and Tokuda, 2001). In the current study, we explored the effects of HPND on the expression of these two kinds of digestive enzymes for explaining the influences of HPND on hepatopancreas carbohydrate metabolism.

Studies have found that the carbohydrate metabolism can be regulated by the 20-hydroxyecdysone (20E), which is an insect steroid hormone that involves in the regulation of molting (Colombani et al., 2005; Kh and Keshan, 2021). 20E is able to induce hunger by slowly reducing food consumption (Liu et al., 2010), and can inhibit the transcription of key glycolytic enzymes (Tian et al., 2010). 20E-regulated gene expression is accomplished by ecdysone receptor (EcR), where EcR is a homolog of the vertebrate FXR/LXR receptor (Riddiford et al., 2000; King-Jones and Thummel, 2005). EcR belongs to the nuclear receptor superfamily and has been proved to be a key regulator of carbohydrate metabolism in mosquitoes (Dong et al., 2018). Therefore, whether the expressions of *amylase* and *cellulase* genes

are regulated by *EsEcR* in *E. sinensis* is studied to reveal the possible roles of EcR in hepatopancreas carbohydrate metabolism.

The NR4A subfamily is the orphan nuclear receptor and belongs to the larger nuclear receptor (NRs) superfamily of eukaryotic transcription factors (Ranhotra, 2015). The NR4A subfamily includes three members, namely Nur77 (NR4A1), Nurr1 (NR4A2) and Nor1 (NR4A3) (Kurakula et al., 2014), which have been shown to be transcriptional regulators of hepatic glucose metabolism (Pei et al., 2006). In arthropods, hormone receptor 38 (HR38) is the only homolog of mammalian NR4As (Nakagawa and Henrich, 2009). Studies have shown that HR38 plays a key role in carbohydrate metabolism and is evolutionarily conserved (Ruaud et al., 2011). We studied the effects of HR38 on the expression of *amylase* and *cellulase* for clarifying the roles of HR38 in carbohydrate metabolism. In addition, HR38 has been suggested to mediate the 20E/EcR/USP signaling cascade that regulates carbohydrate metabolism in response to 20E (Dong et al., 2018). Hence, we further explored the possible role of EcR in the expression of HR38.

In this study, four amylases (*EsAmy1-4*), four cellulases (endo-beta-1, 4-glucanase, *EsGlu1-4*), one *EcR* gene (*EsEcR*), and one *HR38* gene (*EsHR38*) from *E. sinensis* were identified. Quantitative Real-Time PCR (RT-qPCR) was used to analyze the tissue distribution of *EsAmy1-4* and *EsGlu1-4*. Moreover, the expression levels of *EsAmy1-4*, *EsGlu1-4*, *EsEcR*, and *EsHR38* in the hepatopancreas of crabs with HPND were detected by RT-qPCR. RNA interference (RNAi) was utilized to study the effects of *EsEcR* and *EsHR38* knockdown on the expressions of *EsAmy1-4* and *EsGlu1-4* in the hepatopancreas. Furthermore, we explored whether *EsEcR* has a regulatory effect on the transcription of *EsHR38* by using RNAi. This study will reveal the effects of HPND on the carbohydrate metabolism, and provide new knowledge of HPND.

## 2 Materials and methods

### 2.1 Experimental animals and tissues collection

Crabs with HPND ( $10 \pm 2$  g each) were obtained from crab farming base in Xinghua, Jiangsu Province, China. The hepatopancreas of sick or healthy crabs were collected for subsequent RNA extraction. Healthy crabs ( $12 \pm 2$  g each) were provided by Freshwater Fisheries Research Institute of Jiangsu Province, Nanjing, China, and temporary farming in an aerated water tank filled with freshwater at 25 °C–26 °C for 7 days before processing. Six kinds of tissues, including hemocytes, heart, hepatopancreas, gills, stomach, and intestine, were collected from three random healthy crabs. For hemocytes collection, the hemolymph was extracted from three crabs and placed in an equal volume of precooled anticoagulant solution (glucose, 1.47 g; citric acid, 0.48 g; trisodium citrate, 1.32 g; prepared in ddH<sub>2</sub>O and added to 100 mL, pH 7.3). The mixture was centrifuged at 4 °C, 2000 rpm for 10 min to precipitate the hemocytes. All the tissue samples were stored briefly at  $-80$  °C before RNA extraction.

## 2.2 Total RNA extraction and cDNA synthesis

Total RNA was extracted from each collected sample using the High Purity Total RNA Rapid Extraction Kit (BioTeke, Beijing, China) in accordance with the manufacturer's instructions. The quality of RNA was evaluated by 1% agarose gel electrophoresis. The concentration of RNA was obtained by measuring the absorbance at a wavelength of 260:280 nm ( $OD_{260}/OD_{280} = 1.8-2.0$ ) using Nanodrop 2000 (Thermo Fisher Scientific, USA). About 1  $\mu$ g of total RNA was used to synthesize the first-strand cDNA using TransScript<sup>®</sup> All-In-one First Strand cDNA Synthesis SuperMix (One-step gDNA removal) (Transgen Biotech, Beijing, China) in accordance with the manufacturer's instructions. Extracted RNA and synthesized cDNA were stored at  $-80^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$ , respectively.

## 2.3 Bioinformatics analysis

After searching the transcriptome data of crab hepatopancreas, *EsAmy1-4*, *EsGlu1-4*, *EsHR38* and *EsEcR* were found. The homologs of *EsAmy1-4* and *EsGlu1-4* were acquired by the basic local alignment search tool (BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST/>). The amino acids composition of *EsAmy1*, 2, 4 and *EsGlu1-4* were obtained using an Expert Protein Analysis System (<https://web.expasy.org/translate/>). The protein structures of *EsAmy1*, 2, 4 and *EsGlu1-4* were predicted using a Simple Modular Architecture Research Tool (<http://smart.embl-heidelberg.de/>). Phylogenetic tree was built by the neighbor-joining (NJ) algorithm using MEGA 7.0 software.

## 2.4 Tissue distribution analysis of *EsAmy1-4* and *EsGlu1-4*

The expression levels of *EsAmy1-4* and *EsGlu1-4* in the hemocytes, heart, hepatopancreas, gills, stomach, and intestine of healthy crabs were analyzed. Specific *EsAmy1-4*-qF/qR and *EsGlu1-4*-qF/qR primers (Table 1) were designed for tissue distribution analysis by performing RT-qPCR using TransStart<sup>®</sup>Top Green qPCR SuperMix kit (TransGen Biotech, China). The reaction system included 5  $\mu$ L of 2 $\times$ TransStart Top Green qPCR SuperMix, 0.4  $\mu$ L of (10 mM) each qF and qR primers, 1  $\mu$ L of cDNA template, and 3.2  $\mu$ L of PCR-grade Water. PCR procedure was performed as follows: 95  $^{\circ}\text{C}$  for 60 s, 1 cycle; 95  $^{\circ}\text{C}$  for 15 s, 60  $^{\circ}\text{C}$  for 30 s, 40 cycles. Melting curve analysis was performed from 60 $^{\circ}\text{C}$  to 95 $^{\circ}\text{C}$ . The  $\beta$ -actin gene in *E. sinensis* was amplified as internal reference using *Es $\beta$ -actin*-qF and *Es $\beta$ -actin*-qR primers (Table 1).

## 2.5 Expression levels of *EsAmy1-4*, *EsGlu1-4*, *EsHR38*, and *EsEcR* in the crabs with or without HPND

The expression levels of *EsAmy1-4*, *EsGlu1-4*, *EsHR38*, and *EsEcR* in the hepatopancreas of crabs with or without HPND

were detected by RT-qPCR. Samples from healthy crabs were set as control. *Es $\beta$ -actin* gene was amplified as an internal reference. Specific RT-qPCR primers, including *EsAmy1-4*-qF/qR, *EsGlu1-4*-qF/qR, *EsHR38*-qF/qR, *EsEcR*-qF/qR, and *Es $\beta$ -actin*-qF/qR were listed in Table 1. All experiments were repeated three times, and the data were calculated using the  $2^{-\Delta\Delta\text{CT}}$  method (Livak and Schmittgen, 2001). Student's *t*-test was conducted for statistical analysis, and  $p < 0.05$  was considered statistically significant.

## 2.6 RNAi of *EsEcR* or *EsHR38* and detection of *EsAmy1-4* and *EsGlu1-4*

Gene-specific *EsHR38*-iF/iR and *EsEcR*-iF/iR primers were designed to amplify the fragment of *EsHR38* and *EsEcR*, respectively. The purified fragments were used as templates to synthesize the *EsHR38*-dsRNA and *EsEcR*-dsRNA by using HiScribe T7 Quick High-yield RNA Synthesis Kit (BioLabs, USA) in accordance with the instruction manual. The dsRNA of *green fluorescent protein* (*GFP*) was synthesized as control by using *GFP*-iF and *GFP*-iR primers (Table 1). The quality and concentration of dsRNA were separately assessed by electrophoresis on 1% agarose gel and the absorbance from Nanodrop 2000 (Thermo Fisher Scientific, USA) at a wave-length of 260:280 nm ( $OD_{260}/OD_{280} = 1.8-2.0$ ). The RNAi experiments were divided into three groups, including the *EsHR38*-dsRNA-injection, *EsEcR*-dsRNA-injection, and *GFP*-dsRNA-injection groups. 20  $\mu$ g of *EsHR38*-dsRNA, *EsEcR*-dsRNA or *GFP*-dsRNA was injected into the healthy crabs. After 24 h, 20  $\mu$ g of *EsHR38*-dsRNA, *EsEcR*-dsRNA or *GFP*-dsRNA was injected into the same crab. At 24 h after the second dsRNA injection, the hepatopancreas from three random crabs in each group were collected for RNA extraction and cDNA synthesis. The expression level of *EsHR38* in the hepatopancreas of *EsHR38*-dsRNA-injected and *GFP*-dsRNA-injected crabs were detected by RT-qPCR. The expression level of *EsEcR* or *EsHR38* in the hepatopancreas of *EsEcR*-dsRNA-injected and *GFP*-dsRNA-injected crabs were detected by RT-qPCR. The expression levels of *EsAmy1-4* and *EsGlu1-4* in the hepatopancreas after knockdown of *EsHR38* or *EsEcR* were detected by RT-qPCR.

## 3 Results

### 3.1 Sequence characters and evolutionary analysis of amylases in crabs

The full-length cDNA of *EsAmy1* was 1909 bp with a 1554 bp open reading frame (ORF) that encodes a protein containing 517 amino acids (Figure S1A). The full-length cDNA of *EsAmy2* was 2541 bp with a 2169 bp ORF that encodes a protein containing 722 amino acids (Figure S1B). The partial cDNA of *EsAmy3* was 1637 bp (Figure S1C). The full-length cDNA of *EsAmy4* was 2560 bp with a 2112 bp ORF that encodes a protein containing 703 amino acids (Figure S1D). Phylogenetic analysis showed that four amylases form two groups. Among which, *EsAmy2-4* and alpha-amylases from arthropods formed one group, whereas *EsAmy1* and alpha-

TABLE 1 Primer sequences used in this study.

Primers name	Sequences (5'-3')
<b>qRT-PCR:</b>	
<i>Es-Amy1</i> -qF	CATGGTGGAGTTCAGGAATGT
<i>Es-Amy1</i> -qR	GCAGAAGGCGATCTGGTTAT
<i>Es-Amy2</i> -qF	ATCAGGACTTGAACACGTATGG
<i>Es-Amy2</i> -qR	AGGTAAGCTTTCACCTCGAAC
<i>Es-Amy3</i> -qF	GCTGAAGAGGGATGGTTTGA
<i>Es-Amy3</i> -qR	ACAGGCAGTCTGTGTGATG
<i>Es-Amy4</i> -qF	GCACTACTGGATGGTCGATATG
<i>Es-Amy4</i> -qR	GTTGCTCAGGTAGGCCTTAAT
<i>Es-Glu1</i> -qF	GAACGCTTTCAGTAGCACAATG
<i>Es-Glu1</i> -qR	ACTCACTGATGAAGACCATGC
<i>Es-Glu2</i> -qF	CGGACGCAGCCAACTATTAT
<i>Es-Glu2</i> -qR	TCGAGGTAGGAGTTATCGTTAGT
<i>Es-Glu3</i> -qF	GACCCAACAACAGCCAGTA
<i>Es-Glu3</i> -qR	ATTGGTCGAGGAAGACGAGA
<i>Es-Glu4</i> -qF	CCTTACCGGTGACCAACAATA
<i>Es-Glu4</i> -qR	GTGTACGTGGCACCATTCT
<i>Es-HR38</i> -qF	CCTATCGGCACTGACAATCAT
<i>Es-HR38</i> -qR	TGGTCTCTAAGGGAGCTGAT
<i>Es-EcR</i> -qF	CTCAGAGAGGCCAAACCTAAAG
<i>Es-EcR</i> -qR	AGGCAGTCGTCGATTCTCTA
<i>Es<math>\beta</math>-Actin</i> -qF	CGAGACCACTTACAACCTCCATC
<i>Es<math>\beta</math>-Actin</i> -qR	AGGGCAGTGATTTCCCTCTG
<b>RNA interference:</b>	
<i>Es-HR38</i> -iF	TAATACGACTCACTATAGGGACAGCCACAGCTACAACAA
<i>Es-HR38</i> -iR	TAATACGACTCACTATAGGGTTGTCCGCAAGACATACATACT
<i>Es-EcR</i> -iF	TAATACGACTCACTATAGGGCCAGGTGAAGAGAGAACAGAAA
<i>Es-EcR</i> -iR	TAATACGACTCACTATAGGGGCTTGGAGAACTCTACGATCAG
<i>GFP</i> -iF	GCGTAATACGACTCACTATAGGTGGTCCCAATTCTCGTGGAAC
<i>GFP</i> -iR	GCGTAATACGACTCACTATAGGCTTGAAGTTGACCTTGATGCC

amylases from arthropods formed another group (Figure 1A). SMART prediction showed that *EsAmy1*, *EsAmy2*, and *EsAmy4* all contain signal peptide, Amy domain, and Amy\_C domain. Besides, *EsAmy2* and *EsAmy4* contained the low complexity regions (Figure 1B).

### 3.2 Identification and bioinformatics analysis of cellulases in crabs

The full-length cDNA of *EsGlu1* was 2014 bp with a 1377 bp ORF that encodes a protein containing 458 amino acids (Figure S2A). The

partial cDNA of *EsGlu2* was 2202 bp (Figure S2B). The full-length cDNA of *EsGlu3* was 1840 bp with a 1677 bp ORF that encodes a protein containing 558 amino acids (Figure S2C). The full-length cDNA of *EsGlu4* was 2269 bp with a 1722 bp ORF that encodes a protein containing 573 amino acids (Figure S2D). As shown in the Figure 2A, there are two branches in the phylogenetic tree. Among which, endo-beta-1, 4-glucanases from termites form one branch, whereas *EsGlu1-4* and endoglucanases from crabs form another branch. The protein structures of four cellulases all contain a Glyco\_hydro\_9 domain, which has hydrolase activity (Figure 2B). In addition, *EsGlu1*, *EsGlu3*, and *EsGlu4* contain a signal peptide, whereas *EsGlu2* lacks a signal peptide because of incomplete sequences.

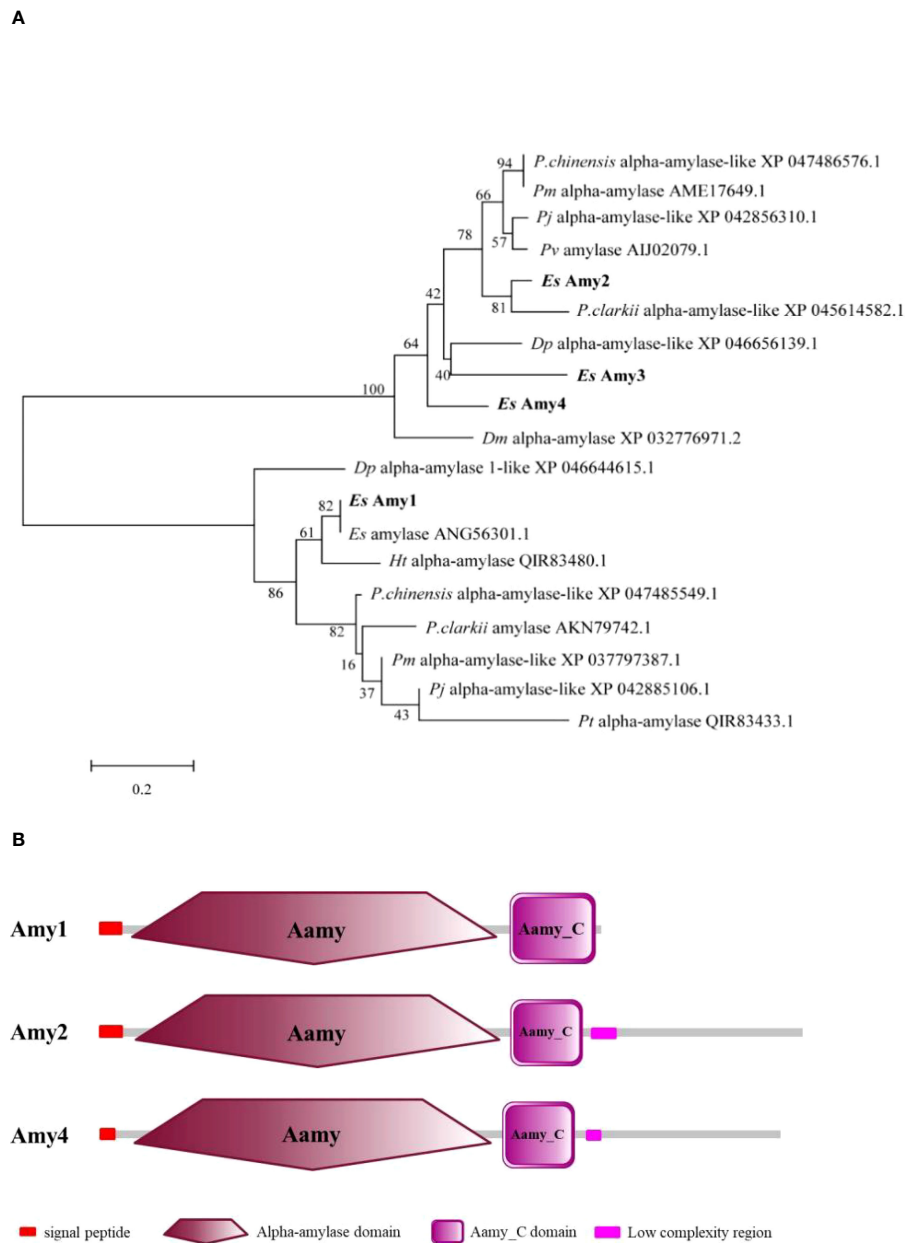


FIGURE 1

Evolutionary analysis and protein domains prediction of amylases. (A) Evolutionary analysis of EsAmy1-4 and their homologs was performed by MEGA 7.0. The EsAmy1-4 are shown in bold. Abbreviation of species, protein name, and GenBank accession number were provided in the evolutionary tree. The full name of each species name is listed as follows: *P. chinensis* (*Penaeus chinensis*), *Pm* (*Penaeus monodon*), *Pj* (*Penaeus japonicas*), *Pv* (*Penaeus vannamei*), *P. clarkii* (*Procambarus clarkia*), *Dm* (*Daphnia magna*), *Dp* (*Daphnia pulicaria*), *Ht* (*Helice tientsinensis*), *Pt* (*Portunus trituberculatus*), and *Es* (*E. sinensis*). (B) Protein structure of EsAmy1, EsAmy2 and EsAmy4. Signal peptide, Alpha-amylase domain (Amy), Amy\_C domain, and Low complexity region are respectively marked in red box, dark red polygon, purple box, and pink box.

### 3.3 The expressions of amylases, cellulases, HR38, and EcR were inhibited in the crabs with HPND

As shown in Figures 3A–H, four amylases (*EsAmy1-4*) and four cellulases (*EsGlu1-4*) were mainly expressed in the hepatopancreas and intestine, and nearly not expressed in the hemocytes, heart, gills, and stomach. The highest expression level in hepatopancreas of *EsAmy1-4* and *EsGlu1-4* suggested that the hepatopancreas is the major organ for

producing amylases and cellulases. RT-qPCR was used to explore the effects of HPND on the expressions of amylases, cellulases, HR38, and EcR. The results showed that the expression levels of four amylases (*EsAmy1-4*) (Figures 4A–D), four cellulases (*EsGlu1-4*) (Figures 4E–H), *EsHR38* (Figure 4I) and *EsEcR* (Figures 4E–J) in the hepatopancreas of crabs with HPND were significantly decreased than that in the normal crabs (as control). These findings indicate that the expressions of amylases, cellulases, HR38, and EcR in the hepatopancreas were inhibited in the crabs with HPND.

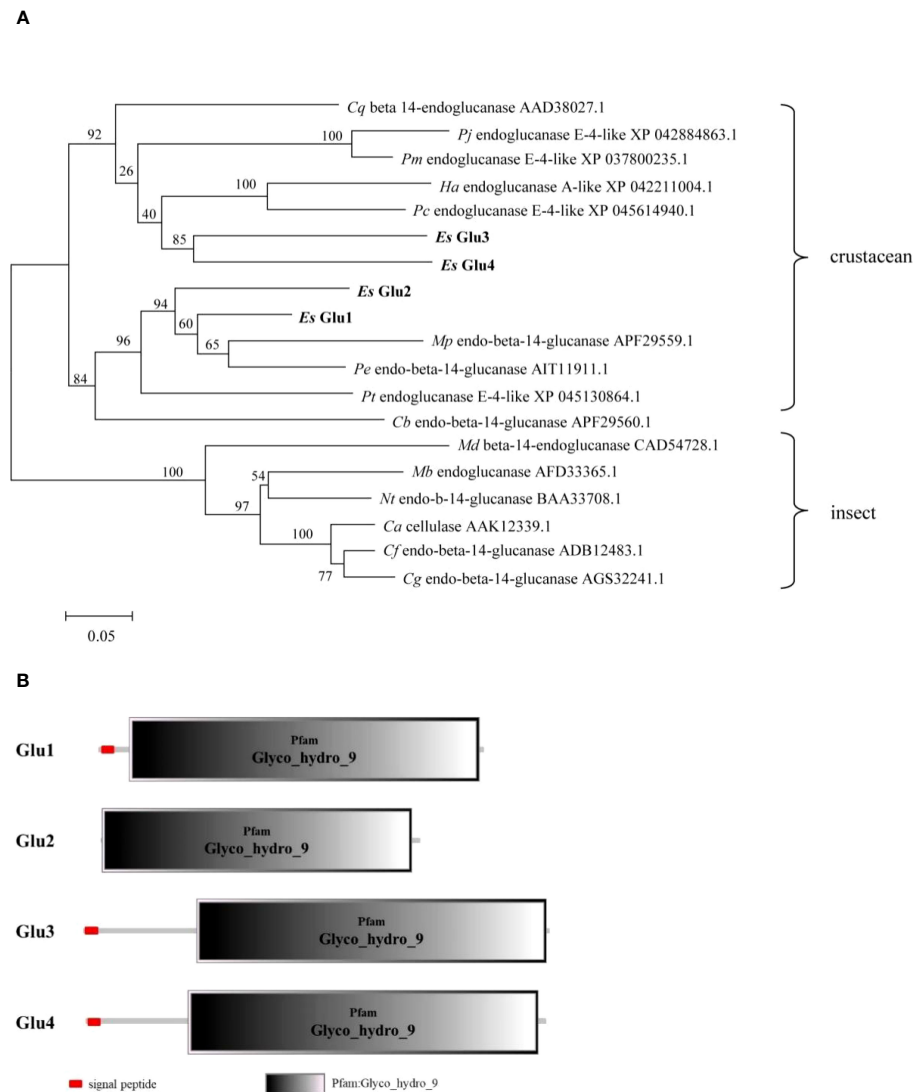


FIGURE 2

Evolutionary analysis and protein domains prediction of cellulase. (A) Evolutionary analysis of *EsGlu1-4* and their homologs was performed by MEGA 7.0. The *EsAmy1-4* are shown in bold. Abbreviation of species, protein name, and GenBank accession number were provided in the evolutionary tree. The full name of each species name is listed as follows: *Cq* (*Cherax quadricarinatus*), *Pj* (*P. japonicas*), *Pm* (*P. monodon*), *Pc* (*P. chinensis*), *Ha* (*Homarus americanus*), *Es* (*E. sinensis*), *Mp* (*Mictyris platycheles*), *Pe* (*Parasesarma erythroclactyla*), *Pt* (*Portunus trituberculatus*), *Cb* (*Coenobita brevimanus*), *Md* (*Mastotermes darwiniensis*), *Mb* (*Macrotermes barneyi*), *Nt* (*Nasutitermes takasagoensis*), *Ca* (*Coptotermes acinaciformis*), *Cf* (*Coptotermes formosanus*), and *Cg* (*Coptotermes gestroi*). (B) The protein structures of *EsGlu1-4*. Signal peptide and Glyco\_hydro\_9 domain are marked in red box and gray box.

### 3.4 RNAi of *EsHR38* or *EsEcR* inhibited the expression of amylases and cellulases

DsRNA-mediated RNAi was utilized to study the relationship between *EsHR38* or *EsEcR* and the production of amylases and cellulases. As shown in Figure 5A, specific *EsHR38*-dsRNA injection could remarkably reduce the transcription of *EsHR38* gene in the hepatopancreas of healthy crabs. Further RT-qPCR detection found that knockdown of *EsHR38* significantly downregulate the expressions of *EsAmy1-4* (Figure 5B) and *EsGlu1-4* (Figure 5C) in the hepatopancreas of healthy crabs. In addition, knockdown of *EsEcR* (Figure 6A) could also evidently decrease the expressions of *EsAmy1-4* (Figure 6B) and *EsGlu1-4* (Figure 6C) in the hepatopancreas of healthy crabs. These results suggest that

*EsHR38* and *EsEcR* play positive regulatory roles in the synthesis of amylases and cellulases in the hepatopancreas.

### 3.5 Knockdown of *EsEcR* inhibited the expression of *EsHR38*

There are studies showing that HR38 and EcR are linked (Baker et al., 2003; Dong et al., 2018). However, the relationship between them is unclear. In this study, we studied the effect of knockdown of *EsEcR* on the expression of *EsHR38* in the hepatopancreas of healthy crabs. The result showed that the expression level of *EsHR38* in the hepatopancreas of *EsEcR*-dsRNA-injected crabs was significantly decreased compared with that of *GFP*-dsRNA-

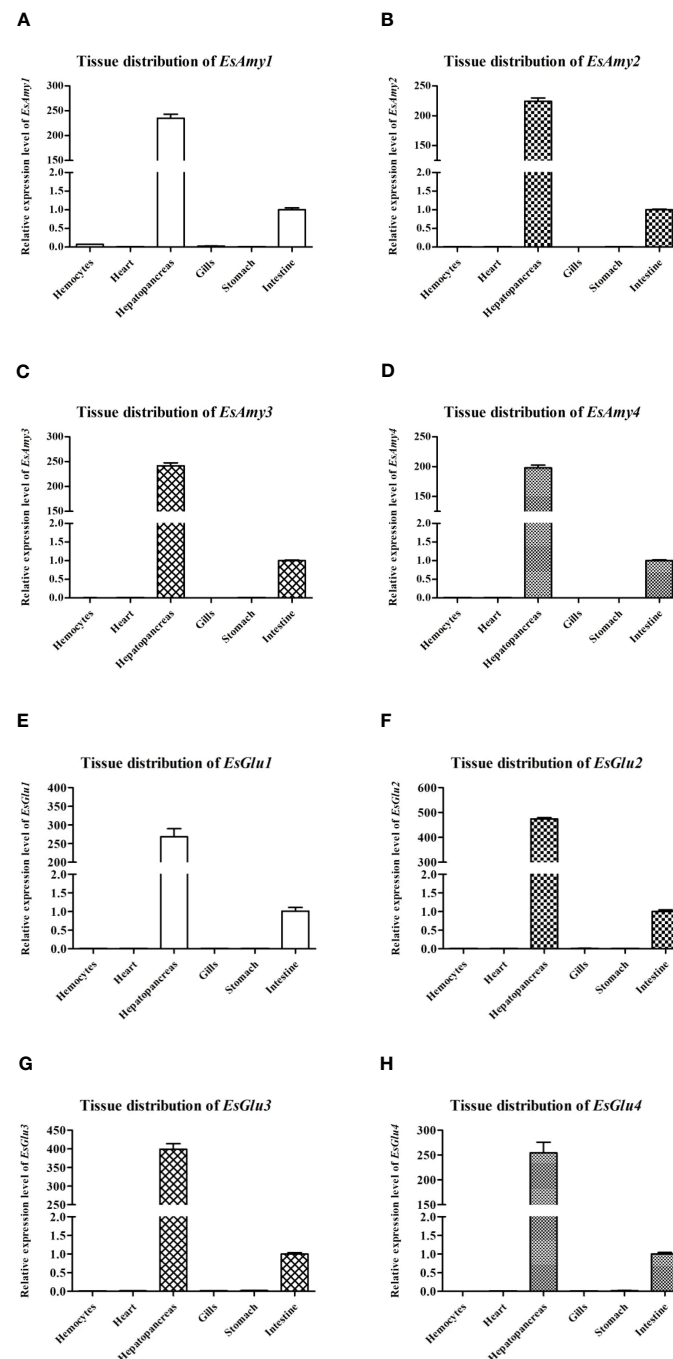


FIGURE 3

The expression levels of (A-D) amylases (*EsAmy1-4*) and (E-H) cellulases (*EsGlu1-4*) in multiple tissues of healthy crabs. The tested tissues included the hemocytes, heart, hepatopancreas, gills, stomach, and intestine. *Esβ-Actin* gene was amplified as an internal reference.

injected crabs (Figure 7), indicating that *EsEcR* positively regulates the expression of *EsHR38* in the hepatopancreas.

## 4 Discussion

In decapods, glucose homeostasis is necessary to maintain normal organ function and response to external stimuli (Asaro

et al., 2018). The hepatopancreas is an important organ for glucose homeostasis and the main site of digestion (Asaro et al., 2018; Wang et al., 2020). The hepatopancreatic F-cells are the synthesis site of digestive enzymes including amylases and cellulases (Vogt, 2019; Vogt, 2021). Amylase and cellulase are present in the hepatopancreas of many decapod species. And, some types of digestive enzymes in the hepatopancreas are more active than those in the gastric juice (Toullec et al., 1992; Linton et al., 2006).

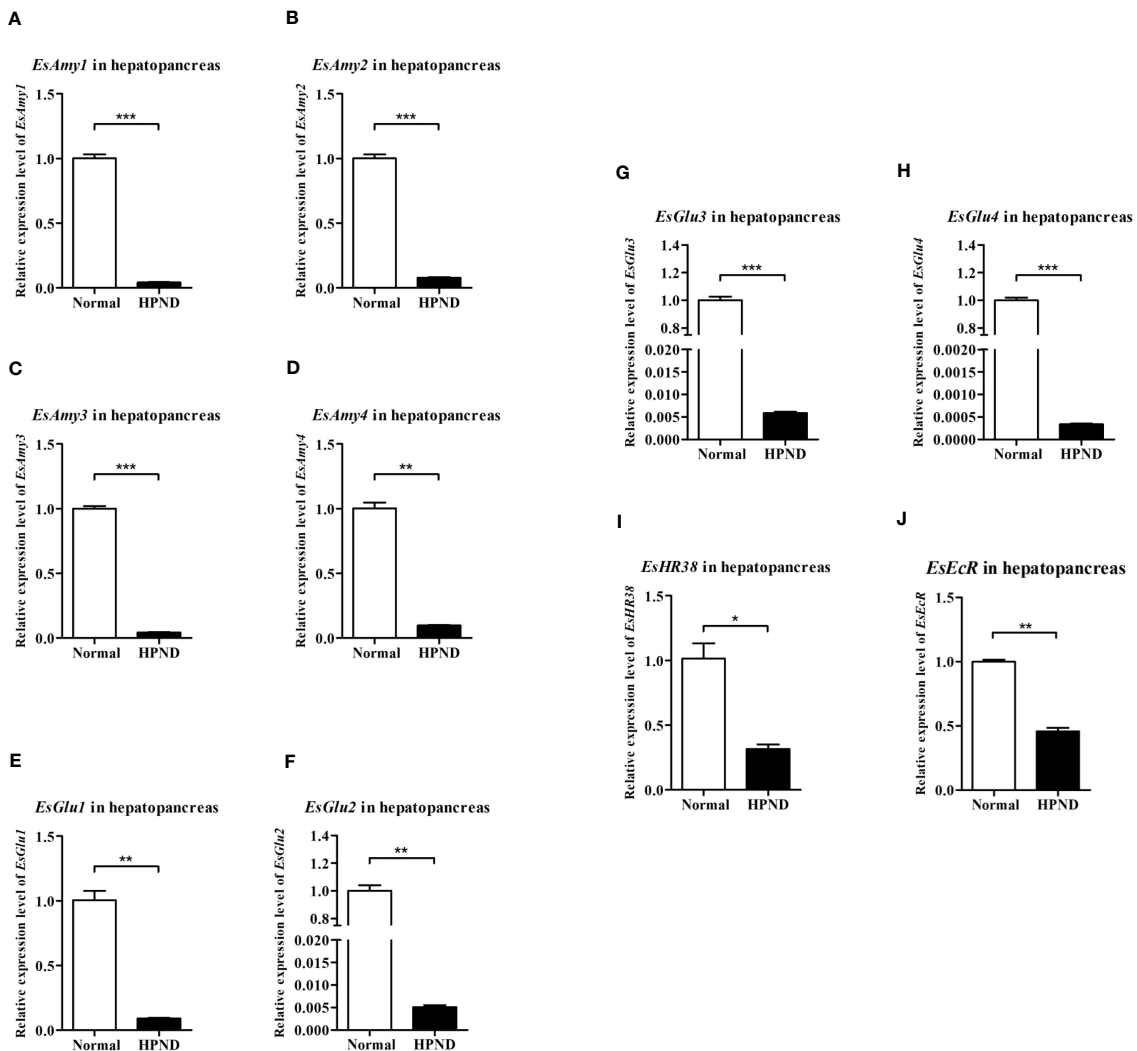


FIGURE 4

The expression levels of (A–D) amylases (*EsAmy1–4*), (E–H) cellulases (*EsGlu1–4*), (I) *EsHR38*, and (J) *EsEcR* in the hepatopancreas of crabs with or without HPND. Sample from healthy crabs (Normal group) was set as control. Asterisks indicate significant differences compared with the values of the control (\* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ ). The data represents the mean  $\pm$  SD of three biological repeats.

In this study, four amylases (named as *EsAmy1–4*) and four cellulases (named as *EsGlu1–4*) were identified from *E. sinensis*. Tissue distribution analysis showed that *EsAmy1–4* and *EsGlu1–4* were highly expressed in the hepatopancreas. HPND threatens the healthy farming of crabs, and the main diseased organ of crabs with HPND is the hepatopancreas (Huang et al., 2021). Our studies found that the expression levels of *EsAmy1–4* and *EsGlu1–4* in the hepatopancreas of crabs with HPND were significantly decreased than that of healthy crabs, indicating that the occurrence of HPND inhibits the synthesis of amylases and cellulases. Since amylase and cellulase are closely related to carbohydrate metabolism, HPND may lead to imbalance of carbohydrate metabolism by inhibiting the synthesis of amylases and cellulases.

HR38 is a homologue of NR4As, whereas NR4As is a subfamily of orphan nuclear receptors, which contain the conserved zinc finger DNA binding domain (DBD) and C-terminal ligand binding domain (LBD) (Ruaud et al., 2011). Moreover, the family of NR4As plays a key

role in carbohydrate metabolism, especially in the glucose transport and glucose utilization (Fu et al., 2007; Zhang et al., 2020). In mice, NR4A receptor has been identified as a downstream mediator of cyclic adenosine monophosphate (cAMP) in gluconeogenic control (Pei et al., 2006), in which NR4A1(Nur77) can affect the glucose metabolism in skeletal muscle and hepatopancreas (Chao et al., 2007; Chao et al., 2009). In *Drosophila*, NR4A homologue Dhr38 plays a key role in carbohydrate metabolism, such as glycogen synthesis (Nakagawa and Henrich, 2009; Ruaud et al., 2011). The research found that the expression of Dhr38 was up-regulated in the fed larvae and down-regulated in starvation (Ruaud et al., 2011). In our study, the expression level of *EsHR38* was significantly down-regulated in the crabs with HPND. In mosquitoes, HR38 was shown to affect the expression of key carbohydrate metabolizing enzyme genes (Dong et al., 2018). Similarly, our study also found that *EsHR38* could positively regulate the expressions of amylases and cellulases in *E. sinensis*. These findings suggest that HR38 may influence the



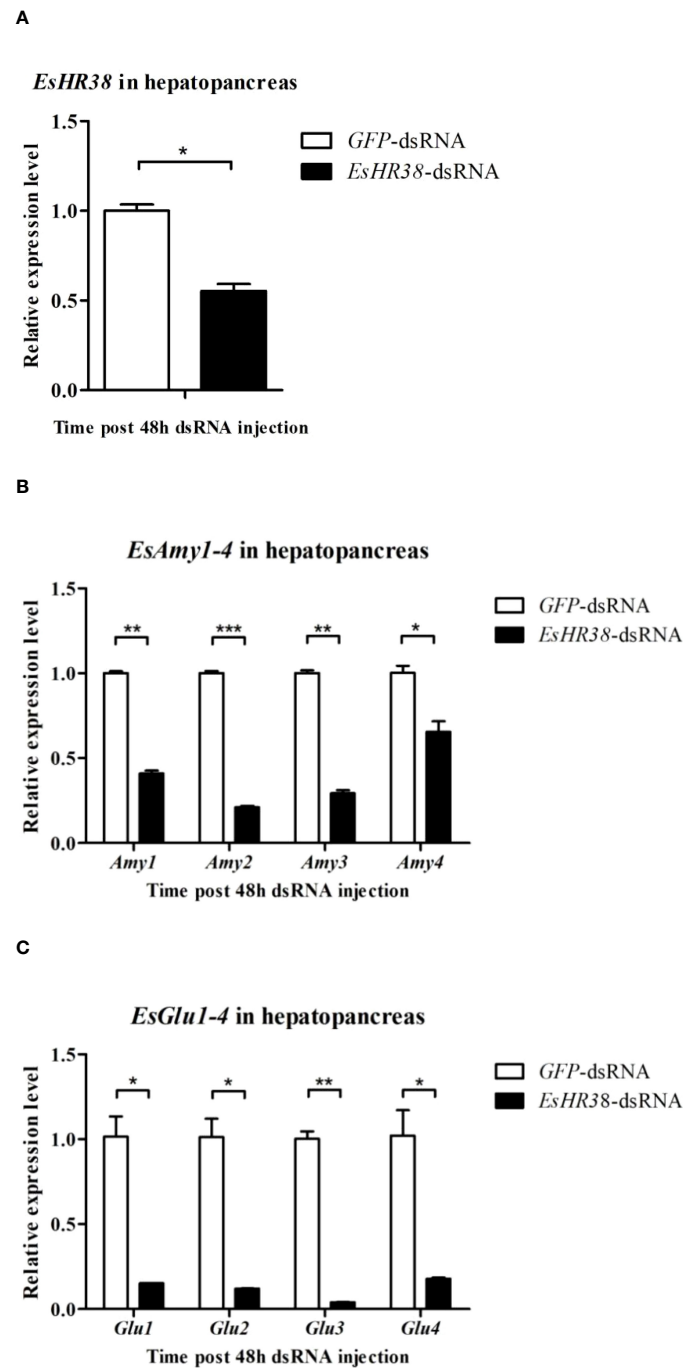


FIGURE 5

Knockdown of *EsHR38* gene inhibited the expressions of amylases and cellulases in the hepatopancreas of healthy crabs. (A) The expression level of *EsHR38* in the hepatopancreas of *EsHR38*-dsRNA-injected and GFP-dsRNA-injected crabs. (B) The expression levels of *EsAmy1-4* in the hepatopancreas of *EsHR38*-dsRNA-injected and GFP-dsRNA-injected crabs. (C) The transcriptional levels of *EsGlu1-4* in the hepatopancreas of *EsHR38*-dsRNA-injected and GFP-dsRNA-injected crabs. Injection of GFP-dsRNA was set as control group. Three random crabs were selected to eliminate individual differences. Asterisks indicate the significant differences (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ) compared with the values of the control. The data represents the mean  $\pm$  SD of three biological repeats.

carbohydrate metabolism by regulating the synthesis of amylases and cellulases in crabs. The decreased expression of *EsHR38* in the crabs with HPND may be one of the causes of carbohydrate metabolic abnormalities.

Ecdysone receptor (EcR) is a member of nuclear receptor superfamily, which is composed of N-terminal A/B domain, the DNA binding C domain (DBD), the D domain (hinge region), ligand binding E domain (LBD), and C-terminal F domain (Mangelsdorf

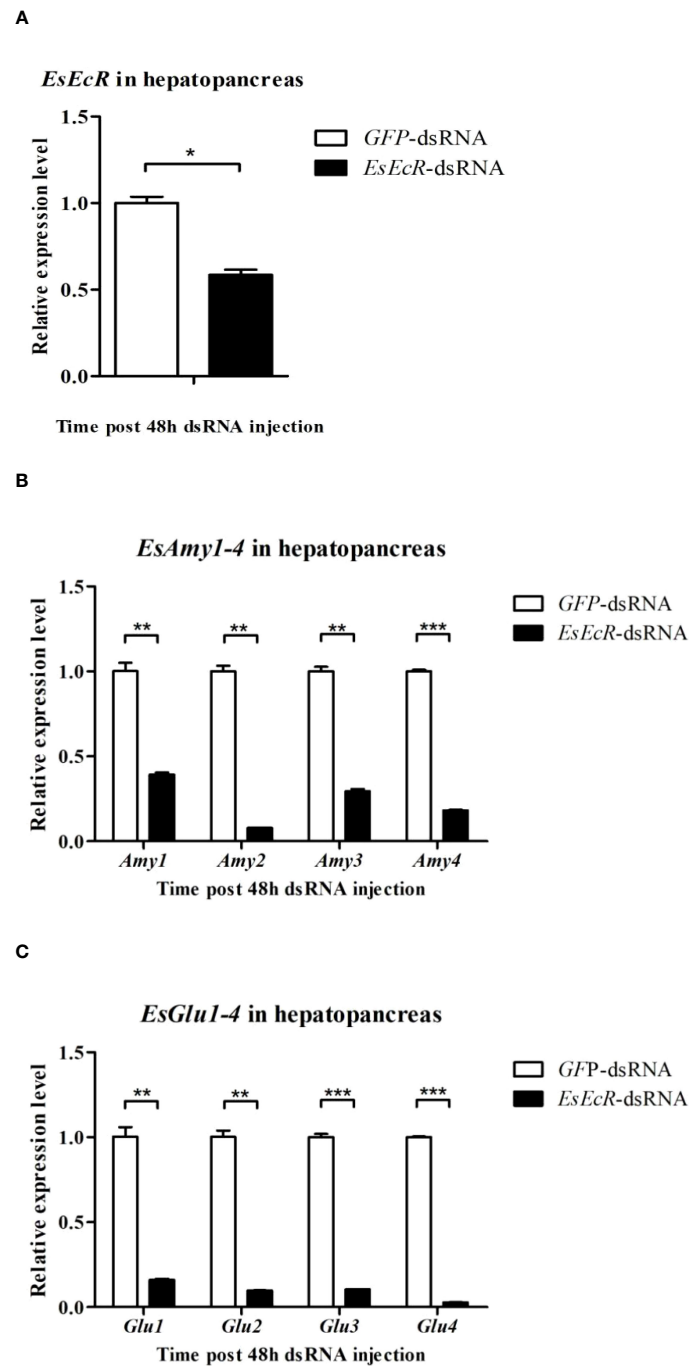


FIGURE 6

Knockdown of *EsEcR* gene inhibited the expressions of *amylases* and *cellulases* in the hepatopancreas of healthy crabs. (A) The expression level of *EsEcR* in the hepatopancreas of *EsEcR*-dsRNA-injected and GFP-dsRNA-injected crabs. (B) The expression levels of *EsAmy1-4* in the hepatopancreas of *EsEcR*-dsRNA-injected and GFP-dsRNA-injected crabs. (C) The expression levels of *EsGlu1-4* in the hepatopancreas of *EsEcR*-dsRNA-injected and GFP-dsRNA-injected crabs. Injection of GFP-dsRNA was set as control group. Three random crabs were selected to eliminate individual differences. Asterisks indicate the significant differences ( $*p < 0.05$ ,  $**p < 0.01$ , and  $***p < 0.001$ ) compared with the values of the control. The data represents the mean  $\pm$  SD of three biological repeats.

et al., 1995). As a ligand-controlled transcription factor (Panguluri et al., 2006), EcR forms heterodimers with ultraspiracle (USP) (Palli et al., 2005) and participates in key biological processes such as development and reproduction (Seliga et al., 2013). Study found that

EcR is involved in the regulation of carbohydrate metabolism, and the silence of EcR can lead to the reduction of transcription level of genes involved in carbohydrate metabolism (Hou et al., 2015). In *Drosophila*, EcR can bind to estrogen-related receptor (ERR) and

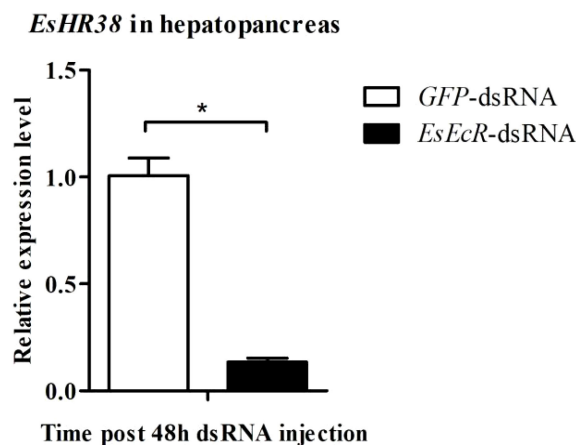


FIGURE 7

RNAi of *EsEcR* inhibited the expression of *EsHR38* in the hepatopancreas of healthy crabs. Samples from the GFP-dsRNA injection group were used as control. Three random crabs were selected to eliminate individual differences. Asterisks indicate the significant differences ( $*p < 0.05$ ) compared with the values of the control. The data represents the mean  $\pm$  SD of three biological repeats.

participate in the regulation of carbohydrate metabolism (Kovalenko et al., 2019). In the current study, RT-qPCR analysis showed that the expression of *EsEcR* was significantly decreased in the crabs with HPND, indicating that the occurrence of HPND inhibits the expression of *EsEcR* in crabs. Further studies found that knockdown of *EsEcR* remarkably down-regulated the expressions of *amylases* and *cellulases* in healthy crabs, suggesting that *EsEcR* plays a positive role in the synthesis of amylases and cellulases. These findings suggest that HPND may lead to carbohydrate metabolic abnormalities by inhibiting the expression of *EcR*. Finally, the production of amylases and cellulases in crabs was inhibited in HPND crabs.

Some studies have shown that *HR38* is a target gene of *EcR* (Dong et al., 2018), and there is a strong interaction between *HR38* and *USP* (Sutherland et al., 1995). In *Drosophila*, it has been confirmed that *HR38* can form a complex with *USP* and its DBD can interact with *EcR* (Pakula et al., 2012). Furthermore, the hinge region of *EcR* can mediate the formation of specific complexes between *EcR* and *HR38* (Zoglowek et al., 2012). Therefore, *HR38* may modulate its activity by forming complexes with two components of the *USP*-*EcR* heterodimer (Zoglowek et al., 2012). In our study, knockdown of *EsEcR* could significantly decrease the expression of *EsHR38*, indicating that *EcR* plays a positive regulatory role in the expression of *HR38* in *E. sinensis*. So, we can conclude that HPND causes the down-regulated expression of *EsEcR*. Down-regulation of *EsEcR* inhibits the transcription of *EsHR38*, and further leads to the reduced production of amylases (*EsAmy1-4*) and cellulases (*EsGlu1-4*) in crabs. The abnormal expressions of amylases and cellulases in crabs with HPND may destroy the normal carbohydrate metabolism and result in the abnormal feeding of crabs, which may be the reason why HPND crabs do not eat and are in a hungry state.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary Material](#).

## Author contributions

YZ: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, and Writing—Original Draft. XM: Validation, Formal analysis, Investigation, and Data Curation. QR: Conceptualization, Supervision, Writing—review & editing. XH: Conceptualization, Supervision, Resources, and Writing—review & editing. JG: Conceptualization, Supervision, Resources, and Writing—review & editing. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The 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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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1195099/full#supplementary-material>

## References

- Asaro, A., Paggi, R. A., Del Valle, J. C., and Lopez Mananes, A. A. (2018). Glucose homeostasis in the euryhaline crab *Cyrtograpsus angulatus*: effects of the salinity in the amylase, maltase and sucrase activities in the hepatopancreas and in the carbohydrate reserves in different tissues. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* 216, 39–47. doi: 10.1016/j.cbpb.2017.11.012
- Baker, K. D., Shewchuk, L. M., Kozlova, T., Makishima, M., Hassell, A., Wisely, B., et al. (2003). The drosophila orphan nuclear receptor DHR38 mediates an atypical ecdysteroid signaling pathway. *Cell* 113, 731–742. doi: 10.1016/S0092-8674(03)00420-3
- Chao, L. C., Wroblewski, K., Zhang, Z., Pei, L., Vergnes, L., Ilkayeva, O. R., et al. (2009). Insulin resistance and altered systemic glucose metabolism in mice lacking Nur77. *Diabetes* 58, 2788–2796. doi: 10.2337/db09-0763
- Chao, L. C., Zhang, Z., Pei, L., Saito, T., Tontonoz, P., and Pilch, P. F. (2007). Nur77 coordinately regulates expression of genes linked to glucose metabolism in skeletal muscle. *Mol. Endocrinol.* 21, 2152–2163. doi: 10.1210/me.2007-0169
- Cheng, Y., Wu, X., Yang, X., and Hines, A. H. (2008). Current trends in hatchery techniques and stock enhancement for Chinese mitten crab, *Eriocheir japonica sinensis*. *Rev. Fish. Sci.* 16, 377–384. doi: 10.1080/10641260701681698
- Chi, C., Yu, X. W., Zhang, C. Y., Liu, J. D., Ye, M. W., Zhang, D. D., et al. (2021). Acute exposure to microcystin-LR induces hepatopancreas toxicity in the Chinese mitten crab (*Eriocheir sinensis*). *Arch. Toxicol.* 95, 2551–2570. doi: 10.1007/s00204-021-03061-9
- Colombani, J., Bianchini, L., Layalle, S., Pondeville, E., Dauphin-Villemant, C., Antoniewski, C., et al. (2005). Antagonistic actions of ecdysone and insulins determine final size in drosophila. *Science* 310, 667–670. doi: 10.1126/science.1119432
- Dai, H., Sun, Y., Ren, N., and Lu, X. (2021). Investigation of Chinese hairy crab industry and analysis of development strategies. *Jiangsu Agric. Sci.* 49, 248–252.
- Ding, Z., Meng, Q., Liu, H., Yuan, S., Zhang, F., Sun, M., et al. (2016). First case of hepatopancreatic necrosis disease in pond-reared Chinese mitten crab, *Eriocheir sinensis*, associated with microsporidian. *J. Fish Dis.* 39, 1043–1051. doi: 10.1111/jfd.12437
- Ding, Z., Pan, J., Huang, H., Jiang, G., Chen, J., Zhu, X., et al. (2018). An integrated metabolic consequence of hepatospora *Eriocheir* infection in the Chinese mitten crab *Eriocheir sinensis*. *Fish Shellfish Immunol.* 72, 443–451. doi: 10.1016/j.fsi.2017.11.028
- Dong, D., Zhang, Y., Smykal, V., Ling, L., and Raikhel, A. S. (2018). HR38, an ortholog of NR4A family nuclear receptors, mediates 20-hydroxyecdysone regulation of carbohydrate metabolism during mosquito reproduction. *Insect Biochem. Mol. Biol.* 96, 19–26. doi: 10.1016/j.ibmb.2018.02.003
- Fu, Y., Luo, L., Luo, N., Zhu, X., and Garvey, W. T. (2007). NR4A orphan nuclear receptors modulate insulin action and the glucose transport system: potential role in insulin resistance. *J. Biol. Chem.* 282, 31525–31533. doi: 10.1074/jbc.M701132200
- Gao, T. H., Xu, Y., Wang, K., Deng, Y. F., Yang, Y. J., Lu, Q. P., et al. (2018). Comparative LC-MS based non-targeted metabolite profiling of the Chinese mitten crab *Eriocheir sinensis* suffering from hepatopancreatic necrosis disease (HPND). *Aquaculture* 491, 338–345. doi: 10.1016/j.aquaculture.2018.03.030
- Hou, Y., Wang, X. L., Saha, T. T., Roy, S., Zhao, B., Raikhel, A. S., et al. (2015). Temporal coordination of carbohydrate metabolism during mosquito reproduction. *PLoS Genet.* 11, e1005309. doi: 10.1371/journal.pgen.1005309
- Huang, X., Feng, Y., Duan, H., Zhao, L., Yang, C., Geng, Y., et al. (2021). Evaluation of pathology and environmental variables contributing to hepatopancreatic necrosis syndrome of Chinese mitten crab, *Eriocheir sinensis*. *Ecotoxicol. Environ. Safety.* 215, 112157. doi: 10.1016/j.ecoenv.2021.112157
- Huang, P., Zhang, X., Ame, K. H., Shui, Y., Xu, Z., Serwadda, A., et al. (2019). Genomic and phylogenetic characterization of a bunya-like virus from the freshwater Chinese mitten crab *Eriocheir sinensis*. *Acta Virologica* 63, 433–438. doi: 10.4149/av\_2019\_410
- Janecek, S., Svensson, B., and MacGregor, E. A. (2014).  $\alpha$ -amylase: an enzyme specificity found in various families of glycoside hydrolases. *Cell. And Mol. Life Sci. CMLS* 71, 1149–1170. doi: 10.1007/s00018-013-1388-z
- Kh, S. D., and Keshan, B. (2021). The feeding status regulates the action of insulin and 20-hydroxyecdysone on haemolymph trehalose level in bombyx larvae. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* 255, 110579. doi: 10.1016/j.cbpb.2021.110579
- King-Jones, K., and Thummel, C. S. (2005). Developmental biology. less steroids make bigger flies. *Science* 310, 630–631. doi: 10.1126/science.1120410
- Kovalenko, E. V., Mazina, M. Y., Krasnov, A. N., and Vorobyeva, N. E. (2019). The drosophila nuclear receptors EcR and ERR jointly regulate the expression of genes involved in carbohydrate metabolism. *Insect Biochem. Mol. Biol.* 112, 103184. doi: 10.1016/j.ibmb.2019.103184
- Kurakula, K., Koenis, D. S., van Tiel, C. M., and de Vries, C. J. (2014). NR4A nuclear receptors are orphans but not lonesome. *Biochim. Biophys. Acta* 1843, 2543–2555. doi: 10.1016/j.bbamcr.2014.06.010
- Linton, S. M., Greenaway, P., and Towle, D. W. (2006). Endogenous production of endo-beta-1,4-glucanase by decapod crustaceans. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 176, 339–348. doi: 10.1007/s00360-005-0056-5
- Liu, Y., Zhou, S., Ma, L., Tian, L., Wang, S., Sheng, Z., et al. (2010). Transcriptional regulation of the insulin signaling pathway genes by starvation and 20-hydroxyecdysone in the bombyx fat body. *J. Insect Physiol.* 56, 1436–1444. doi: 10.1016/j.jinsphys.2010.02.011
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)<sup>-delta delta c</sup> method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262
- Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., et al. (1995). The nuclear receptor superfamily: the second decade. *Cell* 83, 835–839. doi: 10.1016/0092-8674(95)90199-X
- Nakagawa, Y., and Henrich, V. C. (2009). Arthropod nuclear receptors and their role in molting. *FEBS J.* 276, 6128–6157. doi: 10.1111/j.1742-4658.2009.07347.x
- Pakula, S., Orłowski, M., Rymarczyk, G., Krusinski, T., Jakob, M., Zoglowek, A., et al. (2012). Conformational changes in the DNA-binding domains of the ecdysteroid receptor during the formation of a complex with the hsp27 response element. *J. Biomol. Struct. Dyn.* 30, 379–393. doi: 10.1080/07391102.2012.682215
- Palli, S. R., Hormann, R. E., Schlattner, U., and Lezzi, M. (2005). Ecdysteroid receptors and their applications in agriculture and medicine. *Insect Hormones* 73, 59. doi: 10.1016/S0083-6729(05)73003-X
- Pan, Z., Song, X., Hu, X., Xue, R., Cao, G., Sahib Zar, M., et al. (2017). Pathological changes and risk factors of hepatopancreas necrosis disease of mitten crab, *Eriocheir sinensis*. *Fish. Aquac.* J. 8, 220. doi: 10.4172/2150-3508.1000220
- Panguluri, S. K., Kumar, P., and Palli, S. R. (2006). Functional characterization of ecdysone receptor gene switches in mammalian cells. *FEBS J.* 273, 5550–5563. doi: 10.1111/j.1742-4658.2006.05545.x
- Pei, L., Waki, H., Vaitheeswaran, B., Wilpitz, D. C., Kurland, I. J., and Tontonoz, P. (2006). NR4A orphan nuclear receptors are transcriptional regulators of hepatic glucose metabolism. *Nat. Med.* 12, 1048–1055. doi: 10.1038/nm1471
- Ranhotra, H. S. (2015). The NR4A orphan nuclear receptors: mediators in metabolism and diseases. *J. Recept. Signal Transduct. Res.* 35, 184–188. doi: 10.3109/10799893.2014.948555
- Riddiford, L. M., Cherbas, P., and Truman, J. W. (2000). Ecdysone receptors and their biological actions. *Vitamins Hormones* 60, 1–73. doi: 10.1016/S0083-6729(00)60016-X

- Ruaud, A. F., Lam, G., and Thummel, C. S. (2011). The drosophila NR4A nuclear receptor DHR38 regulates carbohydrate metabolism and glycogen storage. *Mol. Endocrinol.* 25, 83–91. doi: 10.1210/me.2010-0337
- Seliga, J., Bielska, K., Wieczorek, E., Orłowski, M., Niedenthal, R., and Ozyhar, A. (2013). Multidomain sumoylation of the ecdysone receptor (EcR) from drosophila melanogaster. *J. Steroid Biochem. Mol. Biol.* 138, 162–173. doi: 10.1016/j.jsbmb.2013.05.007
- Shen, Z., Kumar, D., Liu, X., Yan, B., Fang, P., Gu, Y., et al. (2021). Metatranscriptomic analysis reveals an imbalance of hepatopancreatic flora of Chinese mitten crab eriocheir sinensis with hepatopancreatic necrosis disease. *Biology* 10, 462. doi: 10.3390/biology10060462
- Sutherland, J. D., Kozlova, T., Tzertzinis, G., and Kafatos, F. C. (1995). Drosophila hormone receptor 38: a second partner for drosophila USP suggests an unexpected role for nuclear receptors of the nerve growth factor-induced protein b type. *Proc. Natl. Acad. Sci. U.S.A.* 92, 7966–7970. doi: 10.1073/pnas.92.17.7966
- Tian, L., Guo, E., Wang, S., Liu, S., Jiang, R. J., Cao, Y., et al. (2010). Developmental regulation of glycolysis by 20-hydroxyecdysone and juvenile hormone in fat body tissues of the silkworm, bombyx mori. *J. Mol. Cell Biol.* 2, 255–263. doi: 10.1093/jmcb/mjq020
- Toullec, J. Y., Chikhi, M., and van Wormhoudt, A. (1992). *In vitro* protein synthesis and alpha amylase activity in f cells from hepatopancreas of palaemon serratus (Crustacea; decapoda). *Experientia.* 48, 272–277. doi: 10.1007/BF01930474
- Vogt, G. (2019). Functional cytology of the hepatopancreas of decapod crustaceans. *J. Morphol.* 280, 1405–1444. doi: 10.1002/jmor.21040
- Vogt, G. (2021). Synthesis of digestive enzymes, food processing, and nutrient absorption in decapod crustaceans: a comparison to the mammalian model of digestion. *Zoology.* 147, 125945. doi: 10.1016/j.zool.2021.125945
- Wang, W. (2011). Bacterial diseases of crabs: a review. *J. Invertebrate Pathol.* 106, 18–26. doi: 10.1016/j.jip.2010.09.018
- Wang, Z. F., Tang, D., Guo, H. Y., Shen, C. C., Wu, L., and Luo, Y. Q. (2020). Evolution of digestive enzyme genes associated with dietary diversity of crabs. *Genetica.* 148, 87–99. doi: 10.1007/s10709-020-00090-7
- Watanabe, H., and Tokuda, G. (2001). Animal cellulases. *Cell. Mol. Life Sci. CMLS.* 58, 1167–1178. doi: 10.1007/PL00000931
- Yang, Z., Hu, K., Hou, Y., Wang, Y., Yao, Y., Lei, X., et al. (2020). Transcriptome analysis of hepatopancreas of eriocheir sinensis with hepatopancreatic necrosis disease (HPND). *PLoS One* 15, e0228623. doi: 10.1371/journal.pone.0228623
- Yang, Z., Zhang, Y., Hu, K., Liu, L., Cai, H., Zhang, F., et al. (2018). Etiological and histopathological study on hepatopancreatic necrosis syndrome in Eriocheir sinensis. *Acta Hydrobiologica Sin.* 42, 17–25. doi: 10.7541/2018.003
- Yang, Z., Zhang, Y., Liu, L., Zhang, F., Cai, H., and Yang, X. (2016). The incidence of river crab HPND in 2015–2016. *Sci. Fish Farming* 32, 54.
- Zhang, C., Zhang, B., Zhang, X., Sun, G., and Sun, X. (2020). Targeting orphan nuclear receptors NR4As for energy homeostasis and diabetes. *Front. Pharmacol.* 11, 587457. doi: 10.3389/fphar.2020.587457
- Zoglowek, A., Orłowski, M., Pakula, S., Dutko-Gwozdz, J., Pajdzik, D., Gwozdz, T., et al. (2012). The composite nature of the interaction between nuclear receptors EcR and DHR38. *Biol. Chem.* 393, 457–471. doi: 10.1515/hsz-2011-0283