

Cloning HSP70 and HSP90 genes of kaluga (*Huso dauricus*) and the effects of temperature and salinity stress on their gene expression

Guogan Peng¹ · Wen Zhao¹ · Zhenguang Shi¹ · Huirong Chen¹ · Yang Liu¹ · Jie Wei¹ · Fengying Gao¹

Received: 9 October 2015 / Revised: 6 December 2015 / Accepted: 8 December 2015 / Published online: 18 December 2015
© Cell Stress Society International 2015

Abstract The genes encoding HSP70 and HSP90 proteins were isolated from kaluga by homologous cloning and rapid amplification of complementary DNA (cDNA) ends (RACE). HSP70 (GenBank accession no. KP050541) and HSP90 (GenBank accession no. KP050542) cDNAs were composed of 2275 and 2718 bp and encoded polypeptides of 650 and 725 amino acids, respectively. Basic Local Alignment Search Tool (BLAST) analysis showed that HSP70 and HSP90 of kaluga shared high identities with those of *Acipenser ruthenus*, *Acipenser schrenckii*, and *Acipenser baerii* (98–99 %). Fluorescent real-time RT-PCR under unstressed conditions revealed that HSP70 and HSP90 were expressed in 11 different tissues of kaluga. Messenger RNA (mRNA) expressions of both HSP70 and HSP90 were highest in the intestine and lowest in the muscle. In addition, the patterns of mRNA expression of HSP70 and HSP90 were similar, although the level of expression was more in HSP90 than in HSP70 ($P < 0.05$). We also analyzed patterns of HSP70 and HSP90 expression in the muscle, gill, and liver of kaluga under different combinations of temperature and salinity stress, including temperatures of 4, 10, 25, and 28 °C at 0 ppt salinity, and salinities of 10, 20, 30, and 40 ppt at 16 °C, where 16 °C at 0 ppt (parts per thousand) served as the control. We found that levels of mRNA expression of both HSP70 and HSP90 were highest at 4 °C in the muscle, gill, and liver and changed little with salinity stress. These results increase understanding of the mechanisms of stress response of cold freshwater fish.

Keywords Kaluga (*Huso dauricus*) · HSP70 · HSP90 · Cloning and expression · Temperature · Salinity

Introduction

Heat shock proteins (HSPs) are an essential part of the immune system and play an important role during internal and external environmental stress. Both eukaryotes and prokaryotes have HSPs (Kiang and Tsokos 1998; Sørensen et al. 2003). HSP expression can be induced by a great variety of stressors including, but not limited to, temperature (Basu et al. 2002; Dong et al. 2008; Boone and Vijayan 2002), salinity (Hamer et al. 2004; Werner 2004), heavy metal exposure (Downs et al. 2001), hypoxia (Mu et al. 2013; Airaksinen et al. 1998; Ni et al. 2014; Gamperl et al. 1998), and microorganism infections (Bausinger et al. 2002). HSPs are a superfamily that contains the HSP90 family (molecular weight approximately 83–110 kD), the HSP70 family (molecular weight approximately 66–78 kD), the HSP60 family, and the small molecular weight family (smHSP; molecular weight approximately 12–43 kD) (Morimoto et al. 1990). Current research on the HSP families is focused on HSP70 and HSP90.

Temperature and salinity are important abiotic factors that affect survival, growth, and reproduction of fish (Dong et al. 2008; Elliott 1995). It is important to study the effects of temperature and salinity stress on fish, as fish often experience these stresses during migration. HSPs act as molecular chaperones during the refolding of denatured proteins, preventing denatured protein degradation and maintaining cell homeostasis under temperature and salinity stress (Ellis 1993; Georgopoulos and Welch 1993; Welch 1993). Therefore, it is important to research the mechanisms of stress response.

✉ Wen Zhao
zhaowen_1963@163.com

¹ College of Fisheries and Life Science, Dalian Ocean University, No.52 Heishijiao Street, Shahekou district, Dalian 116023, Liaoning, People's Republic of China

Kaluga is a cold freshwater fish belonging to the *Acipenser* form family that inhabits the main stream and tributaries of the Amur River in China. With a maximum recorded weight of more than 1000 kg, kaluga is the largest freshwater fish in the world. Normal temperature ranges of kaluga approximately from 0 to 25 °C in its natural environment, and it spends some of its life in salt water but most of its life in freshwater. Economic development has resulted in the decline of wild kaluga, making protection imperative for its survival. Currently, the little research that exists on kaluga has focused on its comparative biology, nutrition, growth, development, reproduction, and genetics (Shmigirilov et al. 2007; Lu et al. 2011; Yin et al. 2004; Zhu et al. 2012; Krykhtin and Gorbach 1996; Andoh et al. 2000; Vasil'ev et al. 2009).

This study describes the cloning and characterization of the HSP70 and HSP90 genes in kaluga. Fluorescent real-time quantitative PCR was used to determine the gene expression profiles of HSP70 and HSP90. This is the first study to clearly define molecular regulation of the HSP70 and HSP90 genes in kaluga, both in general and under differing levels of temperature and salinity stress. These data will supply information for further study of the biology of kaluga to conserve this vulnerable species.

Materials and methods

Experimental species and fish holding conditions

Kaluga was obtained from Amur Group Co Ltd., China. Fish were acclimated for 20 days in culture tanks (100 cm × 80 cm × 60 cm). On average, fish weighed 70 ± 7 g and were 26 ± 3 cm in length (mean ± SD). In the experiment, a device “controlled and ecological tank system” (Huixin Tianium, Dalian, China) was used for holding water. The system is consisted of temperature controlling system, filtration system, sterilization system, aeration system, water recycle system, biological oxidation system, and microcomputer system. Holding water was 16.0 ± 0.5 °C, with DO >6 mg/L, ammonia <0.01 mg/L, and pH 7.0–7.4 in freshwater. Fish were fed 2 % of their body weight twice a day. One fourth of the volume of the tank was changed daily. Temperature, salinity, dissolved oxygen, ammonia, and pH were tested once daily.

Fish were starved for 24 h before beginning temperature and salinity stress treatments. Fish were divided into nine groups of six individuals. Temperature stress treatments (at 0 ppt salinity) included 4, 10, 25, and 28 °C, and salinity stress treatments (at 16 °C) included 10, 20, 30, and 40 ppt. Different temperature level was controlled by the system and different salinity level was obtained with table salt. Fish were stressed for 2 h and then allowed to recover for 1 h in freshwater at 16 °C. Four of the nine groups received temperature stress treatments, four received salinity stress treatments, and the last

group served as the control group and was kept in 16 °C water with a salinity of 0 ppt. From each group, three random fish were sampled. To anesthetize the fish, 50 mg/L tricaine methane sulfonate (MS-222) was put in the water, immediately after which the tissues were collected, frozen in liquid nitrogen, and stored at –80 °C until analysis. Tissues included muscle, gill, brain, beard, heart, liver, spleen, stomach, intestine, swim bladder, and gonads.

Total RNA extraction and reverse transcription

Tissues were extracted with RNAiso reagent (Takara, Kyoto, Japan) following the manufacturer's protocol. Quality control was performed using a Nanodrop-NV3000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) based on the 260/280 nm and 260/230 nm absorbance ratios. Sample concentrations were determined to ensure sufficient homogeneous RNA for complementary DNA (cDNA) synthesis. First-strand cDNA was synthesized from 1 µg total RNA from each sample using random primers and Reverse Transcriptase M-MLV (Takara, Japan) in a 10-µL reaction.

Isolation of conserved fragments

The conserved fragments of the HSP70 and the HSP90 cDNA were synthesized using the PCR product generated above (cDNA) as a template and HSP70F/R and HSP90F/R (Table 1) as primers. The PCR protocol involved predenaturation for 10 min at 94 °C, followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, ending with 10 min at 72 °C for extension and incubation at 4 °C. The PCR products were separated by 1.2 % agarose gel and purified using the EasyPure Quick Gel Extraction Kit (TransGen Biotech, Beijing, China), cloned into the pEASY-T3 vector (TransGen Biotech), and transformed into *Escherichia coli* strain DH5α (TransGen Biotech). Cells were then grown on an Amp/LB plate, and clones were sequenced using the ABI3730XL sequencer (Applied Biosystems, Foster City, CA, USA).

Rapid amplification of 5' and 3' cDNA ends (RACE)

5' and 3' RACE reactions were performed using the SMART™ RACE cDNA amplification kit (Clontech Laboratories, Palo Alto, CA, USA). The primers and nested primers are shown in Table 1. A combination of touchdown and nested PCR was used for 5' and 3' amplification. Touchdown PCR involved five cycles of 30 s at 94 °C and 3 min at 72 °C, then five cycles of 30 s at 94 °C, 30 s at 70 °C, and 3 min at 72 °C, and, finally, an additional 25 cycles of 30 s at 94 °C, 30 s at 68 °C, and 3 min at 72 °C. Nested PCR involved 25 cycles of 30 s at 94 °C, 30 s at 68 °C, and

Table 1 Nucleotide sequences of the primers used for the HSP70 and HSP90 genes

Primers	Sequence (5'-3')	Position	Usage
RT-PCR			
HSP70F	CCGATATGAAGCACTGGCCATTC	370–392	Degenerate primer
HSP70R	AGGTCTGGGTCTGCTTGG	1381–1398	Degenerate primer
HSP90F	ATCAAGGAGAAGTACATYGACC	882–903	Degenerate primer
HSP90R	GCCTTCATGATBCKCTCCATGTT	1869–1891	Degenerate primer
5', 3'RACE			
HSP70-5-R1	TGTGGCCTGGCGCTGGGAGTCGTTGAA	564–590	5'RACE primer
HSP70-5-R2	TGGTCTCCCCTTTGTACTCGACCTCGA	424–450	5'RACE nested primer
HSP70-3-R1	TGTGCAGGACCTGCTGCTGCTGGACGT	1277–1303	3'RACE primer
HSP70-3-R2	GGGCATTGAGACCGCCGGTGGGGTCAT	1319–1345	3'RACE nested primer
HSP90-5-R1	GTCAAAGGGAGCACGTCGTGGAATG	1070–1094	5'RACE primer
HSP90-5-R2	GGTCTCCCAGTCATTGGTGAGGCTCT	985–1011	5'RACE nested primer
HSP90-3-R1	TGCGCAAGCGTGGCTTTGAGGTG	1576–1598	3'RACE primer
HSP90-3-R2	ACCGCTTGGTCTCCTCTCCCTGCTGCA	1813–1839	3'RACE nested primer
qPCR			
HSP70-F	GGCAAGTTTGAGCTGACTGG	1485–1504	qPCR primer
HSP70-R	AGGCGTCCTTTGTCATTGGTG	1625–1645	qPCR primer
HSP90-F	CTTGAGATCAATCCAGACCAC	1943–1964	qPCR primer
HSP90-R	CATCATCAATGCCAAGACCGAG	2124–2145	qPCR primer
18S-F	CTGAGAAACGGCTACCACATCC	–	Reference primer
18S-R	GCACCAGACTTGCCCTCCA	–	Reference primer

RT-PCR reverse transcription PCR, *RACE* rapid amplification of cDNA ends, *qPCR* quantitative PCR

3 min at 72 °C for extension. The target PCR products were separated, purified, cloned, and sequenced as above.

Phylogenetic and sequence analyses

The search for similarities in nucleotide and amino acid sequences was performed using the Basic Local Alignment Search Tool (BLAST) program (<http://www.ncbi.nlm.nih.gov/blast>). Amino acid sequence information was analyzed with the Expert Protein Analysis System (<http://www.expasy.org/>). Phylogenetic trees were constructed using MEGA 4 software (Tamura et al. 2007) and neighbor-joining, and bootstrap analysis (1000 replicates) evaluated the reliability of the phylogenetic trees (Saitou and Nei 1987).

Fluorescence quantitative PCR

Gene expression of HSP70 and HSP90 in various tissues under different levels of temperature and salinity stress was measured using quantitative PCR (qPCR). Real-time PCR assays (25 µL) were carried out using the SYBR® *Premix Ex Taq*™ II (Takara, Japan) performed on the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, USA). Each individual sample was run in triplicate wells. The primers used are listed in Table 1. Comparison of 18S

rRNA, β-actin, and GAPDH demonstrated that the expression of 18S rRNA was more stable and, thus, it was used as the reference gene. A standard curve was generated using fivefold serial dilutions of cDNA. The result was analyzed using the comparative C_T method (Livak and Schmittgen 2002).

Statistics

All analyses were performed using ANOVA followed by Duncan's multiple range test ($P < 0.05$). All data were expressed as the mean ± standard error.

Results

Molecular cloning and sequence analysis of HSP70 and HSP90 cDNA from kaluga

The full-length cDNA was assembled by overlap of intermediate fragments and 5' and 3' end fragments using the DNAMAN software. The full-length HSP70 cDNA from kaluga (GenBank accession no. KP050541) was 2275 bp and contained an 85-bp 5'-terminal untranslated region (UTR) and a 237-bp 3'-terminal UTR. The open reading frame (ORF) was 1953 bp and encoded a protein of 650 amino acids. The

theoretical isoelectric point and molecular weight (pI/Mw) of the HSP70 was 5.215/71131.33. Three highly conserved signatures, including 9–16 (IDLGTTY S), 197–210 (IFDLGGGTFDVSIL), and 334–345 (IVLVGGSTRIPK), as well as the cytoplasmic characteristic motif EEVD, were identified in HSP70 (Fig. 1). The full-length HSP90 cDNA from kaluga (GenBank accession no.KP050542) was 2718 bp and

contained a 44-bp 5'-terminal UTR and a 497-bp 3'-terminal UTR. The ORF was 2178 bp, encoding a protein of 725 amino acids. The theoretical isoelectric point and molecular weight (pI/Mw) of the HSP90 was 4.846/83623.73. Only one highly conserved signature (32–41: YSNKEIFLRE) and the cytoplasmic characteristic motif EEVD were identified in HSP90 (Fig. 2).

```

agtttgcggagcggagacagcagcagctcttaaaagaacagcactttaattctatttatatttcataaacattaaggaacc 85
ATGCTAAGGGAACAGCTGTTGGCATTGATCTGGGAACCACTACTCCTGCGTAGGTGCTTTCCAGCATGCCAAAGTTGAAATCATTGCCAACGCCAGGGTAACAGGACCAACCCAGC 205
M S K G T A V G IDLGTTY $\text{S}$  C V G V F Q H G K V E I I A N D Q G N R T T P S 40
TATGTAGCCTTACCAGCTCAGAGGGCTGATCGGCGATGCTGCAAGAACAGGTTGCAATGAATCCCAACACAGTGTTCGATGCTAAGCGTCTGATTGGCCGAGATCGAAGAC 325
Y V A F T D S E R L I G D A A K N Q V A M N P T N T V F D A K R L I G R R F E D 80
GCAGTGTCCAGTCCGATATGAAGCACTGGCCATTCAACGTCGTGAGTGATGGTGGCCGTCCTCAAACTCGAGGTCGAGTACAAAGGGGAGACCAAGTCTTCTACCTGAGGAAGTCTCT 445
A V V Q S D M K H W P F N V V S D G G R P K L E V E Y K G E T K S F Y P E E V S 120
TCTATGGTCTGACCAAGATGAAGAAATTCAGAAGCTTACCTCGAAAGTCTGTGACCAACGCTGTGTAAGTCTGCCAGCATACTTCAACGACTCCAGCCAGGCCACAAAGGAT 565
S M V L T K M K E I A E A Y L G K S V T N A V V T V P A Y F N D S Q R Q A T K D 160
GCTGGTACAATAGCTGGCCTAATGTCTCGAATCATCAATGAACCACTGCTGCTGCTATTGCTTATGGCTGGACAAGAAGTGGAGTGAAGAAATGTGCTCATTTCGATCTG 685
A G T I A G L N V L R I I N E P T A A A I A Y G L D K K V G V E R N V L IFDL 200
GGCGGTGGCACTTTCGATGCTCCACTGACTATTGAAGATGGAATCTTTGAAGTAAATCCACCGCTGGCGACCCCATCTCGGTGAGAAGACTTTGACAACCGCATGGTCAACCAC 805
GGGTFDVSIL T I E D G I F E V K S T A G D T H L G G E D F D N R M V N H 240
TTCATTGCAGAGTCAAGCCAAAGTACAAGAAGACATCAGTGACAACAAGAGAGTGTTCGCCGCTCCGACCCGCTGTGAAAGGGAAAGCGCACCCCTTCTTCCAGCACCCAGGCC 925
F I A E F K R K Y K K D I S D N K R A V R R L R T A C E R A K R T L S S S T Q A 280
AGTATTGAAATCGACTCCCTGTACGAGGGGATCGATTTTACACTCCATCACCAGGGCTCGTTTTGAGGAGCTGAACGAGACCTGTTCCGTGGTACTCTGGACCCCGTGGAGAAGTCC 1045
S I E I D S L Y E G I D F Y T S I T R A R F E E L N A D L F R G T L D P V E K S 320
CTCCGTGATGCCAAGATGGACAAGGCCAGATCCAGACATGTGTGCTGGGAGGATCTACCCGATCCCAAGATCCAGAAGCTGCTGAGGATTTCTTCAACGGGAAGGAGCTCAAC 1165
L R D A K M D K A Q I H D IVLVGGSTRIPK I Q K L L Q D F F N G K E L N 360
AAGCATCAACCAGATGAGGCTGTGCTATGGAGCAGCTGTGCAGGCTGCCATCCTGTCTGGGACAAGTCTGAGAATGTGCAGGACCTGCTGCTGGAGTCACTCCCTGTCT 1285
K S I N P D E A V A Y G A A V Q A A I L S G D K S E N V Q D L L L L D V T P L S 400
CTGGCATTGAGACCGCGTGGGTCATGACTGTGCTGATCAAGCGTAACACCACTATCCCAAGCAGACCCAGACCTTACCACCTACTCTGACAACCGCCGGTGTGCTCATC 1405
L G I E T A G G V M T V L I K R N T T I P T K Q T Q T F T T Y S D N Q P G V L I 440
CAGTCTATGAAGTGAGCGACCATGACCAAGGACAACAATGTCTGGGCAAGTTGAGCTGACTGGTATCCCCCGCGCCCGTGGTGTCTCAGATCGAGTCACTTCGATATT 1525
Q V Y E G E R A M T K D N N L L G K F E L T G I P P A P R G V P Q I E V T F D I 480
GATGCCAAGCAGTCTGAACTGCTGCAAGTGAAGAGCAGTGGCAAGGAGAACAAGATCACCATCACCATGACAAGGACGCTGAGCAAGGAGGATATGAGCGCATGGTCCAG 1645
D A N G I L N V S A V D K S T G K E N K I T I T N D K G R L S K E D I E R M V Q 520
GAAGCAGAGAAGTACAAGTCTGAGGATGATGTCAGCGTGAAGGCTCCTCCAAGATGCCCTGGAGTCTACGCTTCAACATGAAGTCAACTGTGAGGATGAGAAGTGGAGGGC 1765
E A E K Y K S E D D V Q R E K V S S K N A L E S Y A F N M K S T V E D E K L E G 560
AAGATCAGCAATGAGGACAAGCAGAAGATCTTGGAGAAGTGCAACGAGATCATCGGCTGGTGAAGAACCAGACTGCTGAGAAGGAGGATGAGCACCATCAGAAAGAACTGGAG 1885
K I S N E D K Q K I L E K C N E I I G W L D K N Q T A E K E E Y E H H Q K E L E 600
AAGGTGTGCAACCCATCATCAACAGCTGTACCAAGCGCTGGCGGATGCCAGGTGGCATGCCAGGCGGTATGCCAGGGGGCTTCCAGGGGGTGGTGTCTCCCTCCGAGGTGGC 2005
K V C N P I I T K L Y Q G A G G M P G G M P G G M P G G F P G A G A A P S G G G 640
TCATCAGGCCCTACCATCGAGGAGTTCATTAagaacttcgtctcaagaatcgttaccgaagaccatctgtaagccaacgctggtcattggctctccaacctctccaagcc 2125
S S G P T I E E V D * 650
atagctgctatgctgtttgatgctggtgacttgaatccactgcgtaactgcagtgtagttgactgttgctggcaatacatttgagtcaggtgaataaacctacttgaaaat 2245
ccaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 2275

```

Fig. 1 The full-length cDNA nucleotide sequence and corresponding amino acid sequence of HSP70 from kaluga. Signature sequences of the HSP70 family are shown in boxes. The nuclear localization signal

sequence of HSP70 is *underlined*. Start and stop codons are indicated in *bold*

	aagcagtggatcaacgcagagtagatgggcaattgttttaagaaccaacaataaatcagtc	66
ATG CTGGAAGAAGCGCCCAAGAAGAGGAGGTGGAGACCTTTGCCCTCCAGGCTGAGATTGCTCAGCTTATGCTCTAATTAATACCTTTTATTCCAACAAGGAAATTTTCCTCAGG		186
M L E E A R Q E E E V E T F A F Q A E I A Q L M S L I I N T F Y S N K E I F L R		40
GAGATTATCTCTAATGCCTCTGACGCTCTGACAAAATCAGATATGAAAGCTTGACAGACCCCAAGCTGGACAGTGGAAAAGAGCTTAAAGATTGATATTATCTCAACAAGATGAG		306
E I I S N A S D A L D K I R Y E S L T D P T K L D S G K E L K I D I I P N K N E		80
CGTACCTGACACTTATGACACTGGGATGGCATGACCAAGGCGACCTCATCAACAACCTGGAAACCATGCCAAGTCTGGAACCAAGGCTCCATGGAGGCCCTGCAGGCTGGTGTCT		426
R T L T L I D T G I G M T K A D L I N N L G T I A K S G T K A S M E A L Q A G A		120
GACATATCTATGATGGTCAGTTGGTGGTCTCTACTCTGCTACCTGGTTCGAGAGAAGGTGGTGGTATACCAAGCATAACGATGATGAACAATACATCTGGGAGTCTCTGCT		546
D I S M I G Q F G V G F Y S A Y L V A E K V V V I T K H N D D E Q Y I W E S S A		160
GGAGGTTCTTCCCGTCAAAGTTGACACTGGTGGAGCCATGGCCGAGGTACCAGGTCATCTTGACCTGAAGGAAGACAGCGAATACATTGAGGACAAGAGGTCAGGAGGTT		666
G G S F T V K V D T G E P I G R G T R V I L H L K E D Q T E Y I E D K R V K E V		200
GTCAGAACAACCTCCAGTTTATTGGATACCCCATCCCTATATGTTGAAAAGAGCGTGAAGAAAGAAATCAGCGATGATGAAGCAGAAAGAGAAAAGACAGAGAAGGAGAAAAGAAA		786
V K K H S Q F I G Y P I T L Y V E K E R E K E I S D D E A E E E K T E K E E K K		240
GAAGATGAGGAGGATGAAGAAAGCCAAAATGAGGATGTTGGCTCTGATGATGAGGACTCCAAGGACAAGAAGAAGAAAAAATCAAGGAGAAGTACATTGACCAAGAG		906
E D E E G D E E K P I E D V G S D E D E D S K D K K K K K I K E K Y I D Q E		280
GAGTTAAACAAGACAAGCCTATCTGGACCAAGAAATCTGATGACATCACACCGAGGAATACGGAGAGTCTACAAGAGCCTGACCAATGACTGGGAGGACCATCTGCTGTAAAGCAC		1026
E L N K T K P I W T R N P D D I T T E E Y G E F Y K S L T N D W E D H L A V K H		320
TTTTCTGTTGAGGTCAGCTCGAGTTCCGTCTGCTTTTCATCCCAGCAGTGCACCTTTGACCTTTTGAGAACAAAAGAGGAATAACATCAAGCTGATGATGAGGAGGTT		1146
F S V E G Q L E F R A L L F I P R R A P F D L F E N <u>K K K R N</u> N I K L Y V R R V		360
TTCATCATGGACAGCTGTAAGAGCTCATCCAGAATACCTGAACTTGTTCGTTGGTGTTCGATCTGTAAGACTTGCCCTTGAACATCTCCAGAGAATGCTCAACAGAGCAAAATC		1266
F I M D S C E E L I P E Y L N F V R G V V D S E D L P L N I S R E M L Q Q S K I		400
CTGAGGTTATTCCAGAATAATAGTTAAGAATGCATGGAACCTTTGTTGAGCTGGCTGAAAGCAAAAGAACTACAAGAAGTTATATGATGGCTTCCAGAAGCTGAAGCTGGT		1386
L R V I R K N I V K K C M E L F V L E A D K E N Y K K L Y D G F S K N L K L G		440
ATCCATGAAGATTCCAGAACCCGAGGAAGCTGTCAGAGCCGTTGAGGTACCACAGCTCTCAGTCTGGGATGAGATGACCTCTCTGAGCGAGTACATCTCCCGCATGAAGAGAACCAG		1506
I H E D S Q N R R K L S E P L R Y H S S Q S G D E M T S L T E Y I S R M K E N Q		480
AAATGCATCTACTACACTCGTGAAGCAAGGACCAAGTTGCTAACTGCAATTTGTTGAGCGTGGCCGAGCGTGGCTTTGAGGATGATACATGACGGAACCCATGATGAATAT		1626
K C I Y Y I T G E S K D Q V A N S A F V E R V R K R G F E V I Y M T E P I D E Y		520
TGTGTACAGCAGCTGAAGAGTTTGTGGGAAGACTTTAGTCTCTGTCCACCAAGGAGGACTGGAACCTCCAGAGGATGAAGAGAGAAGAAAAATGGAGGAAGACAAGACTAGATT		1746
C V Q Q L K E F D G K T L V S V T K E G L E L P E D E E E K K K M E E D K T R F		560
GAGAACCTCTCAAACCTCATGAAGGAGATCCTGGACAAAAGTTGAAAAGTCAAGCTGTCACCAAGCTGGTCTCCCTCTCCCTGCTGATGTCACCAGACTTATGGCTGGACAGCA		1866
E N L C K L M K E I L D K K V E K V T V S N R L V S S P C C I V T S T Y G W T A		600
AACATGGAGCGCATCATGAAGGCTCAAGCACTTAGGATAACTTACCATGGTTACATGATGCCAAGAAGCACTGGAGATCAATCCAGACCACCAATGTTGAAACCCCTGAGGAG		1986
N M E R I M K A Q A L R D N S T M G Y M M A K K H L E I N P D H P I V E T L R Q		640
AAGGCTGAAGCAGACAAGAAATGACAGGCTGTAAGAGCCTGGTACCCTCTGTTGAGACTGCTGCTGCTCTGGGTTTTGCTAGATGATCTCAGACTCACTCAATCGCATC		2106
K A E A D K N D K A V K D L V T L L F E T A L L S S G F S L D D P Q T H S N R I		680
TACAGGATGATCAAACTCGTCTTGGCATTGATGATGATGAAGTGACAACAGAGGACCGCACTGCACCCATCTGATGAGATTCCACCTCTGAGGGGAGGATGATGCTCCCGC		2226
Y R M I K L G L G I D D D E V T T E E P A T A P I P D E I P P L E G E D D A S R		720
ATGGAGGAAGTAGATTAAGacaaagctagccttttacctcaatgtggatttattttatgtaaatagttaagtgttttaaggggcagtaactctggccaatacctgggagctctg		2346
M E E V D *		725
aaaagttgacattaacattgatgctgtatgggtataaacaccagtggtgctctctgtatgcaatgtccttttaaatgtgctgcaaatgctactgaggtgggttagcagatggcct		2466
catgcagtgcaaatgtactctctgcaactgagttattgttgaatgttaaaggagtagtgcataagtcagtagtggctcattttattttctggattcctggagttgctggataggggat		2586
gaatattcagtgcatcatgcattttgcatgggggaatatagtgctgctcttttaaatcatgctcctggaagtaagaatgccctgttctgtcttttttttaaatgaaataaac		2706
acttaagctccaaaaaataaaaaaaaaaaaaaaaa		2741

Fig. 2 The full-length cDNA nucleotide sequence and corresponding amino acid sequence of HSP90 from kaluga. Signature sequences of the HSP90 family are shown in boxes. The nuclear localization signal sequence of HSP90 is *underlined*. Start and stop codons are indicated in *bold*

Homology analysis and construction of the phylogenetic trees

BLAST analysis revealed that the amino acid sequences of the HSP70 and HSP90 genes of kaluga shared high similarity with those of other known HSP70 and HSP90 genes. The protein sequence of the HSP70 gene of kaluga was compared with other representative species, and the results were shown in Table 2. Using the amino acid sequences of the HSP70 genes of kaluga and the other species analyzed, a phylogenetic tree was constructed using the MEGA 4 software. The resulting phylogenetic tree was composed of five major groups: fish, mammals, insects, molluscs, and echinoderms (Fig. 3). Similarly, we analyzed the homology of the HSP90

cDNA sequence of kaluga with that of other species, and the results are shown in Table 3. The phylogenetic tree of the HSP90 gene was composed of six major groups: fish, mammals, insects, crustaceans, and molluscs (Fig. 4).

Expression of the HSP70 and HSP90 genes in tissues of kaluga in unstressed conditions

Fluorescence quantitative PCR results showed that HSP70 and HSP90 messenger RNA (mRNA) were widely expressed in 11 tissues of kaluga, that is, gill, brain, beard, heart, liver, spleen, stomach, intestine, swim bladder, and gonads. mRNA expression of the HSP70 and HSP90 genes in kaluga was highest in the

Table 2 The homology analysis between kaluga and other representative species for HSP70

Species	GenBank accession no.	Similarity
Fish		
<i>Acipenser ruthenus</i>	AEK81529	99 %
<i>Acipenser schrenckii</i>	AFM75819	99 %
<i>Acipenser baerii</i>	ADL40977	98 %
<i>Danio rerio</i>	AAH56709	86 %
<i>Pelophylax lessonae</i>	ACY69994	85 %
<i>Salmo salar</i>	ACN11053	84 %
<i>Fundulus heteroclitus macrolepidotus</i>	ABB17041	82 %
<i>Paralichthys olivaceus</i>	ABG56390	82 %
<i>Xiphophorus maculatus</i>	BAB72167	81 %
Mammals		
<i>Homo sapiens</i>	NP_005518	82 %
<i>Mus musculus</i>	ACC85670	82 %
Insects		
<i>Drosophila simulans</i>	EDX13300	74 %
<i>Drosophila auraria</i>	CAA55168	73 %
Molluscs		
<i>Crassostrea gigas</i>	BAD15286	74 %
<i>Crassostrea hongkongensis</i>	ACH95805	74 %
Echinoderms		
<i>Apostichopus japonicas</i>	ACJ54702	72 %
<i>Paracentrotus lividus</i>	Q06248	71 %

intestine and lowest in muscle tissue. The expression levels of HSP70 and HSP90 were significantly higher in all tissues than they were in muscle ($P < 0.05$), with the exception of HSP70 mRNA expression in the liver ($P \geq 0.05$). In addition, trends in the levels of expression were consistent between the HSP70 and HSP90 genes (Fig. 5).

Expression of the HSP70 and HSP90 genes in kaluga under temperature stress

To determine if temperature influences the expression of HSP70 and HSP90 genes in kaluga, levels of HSP70 and HSP90 mRNA in muscle, gill, and liver tissue that had been treated with various temperatures were quantified by qPCR.

Fig. 3 Phylogenetic tree of HSP70s. The phylogenetic tree was constructed using neighbor joining with 1000 bootstrappings. Numbers on the nodes indicate the confidence level of the bootstrap analyses

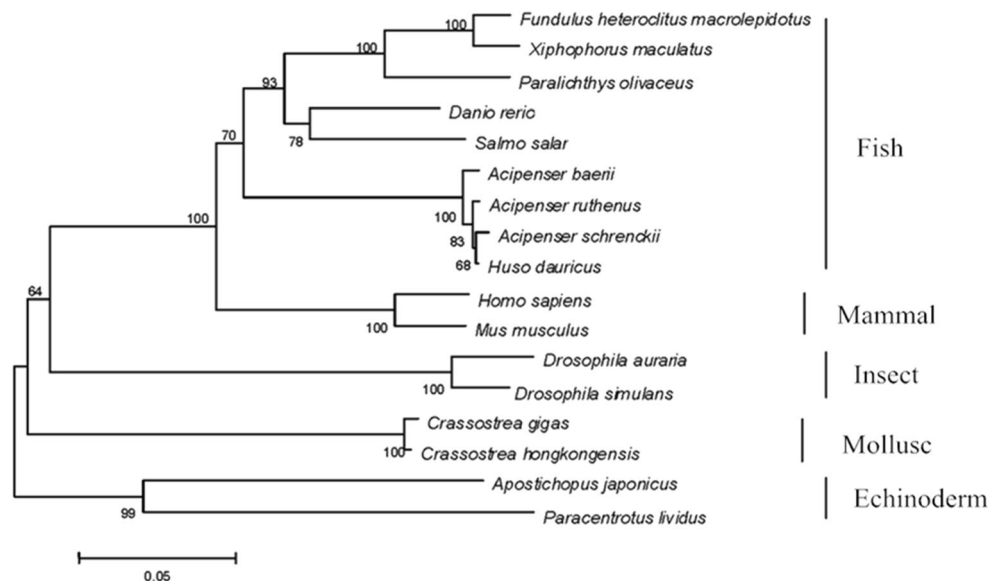
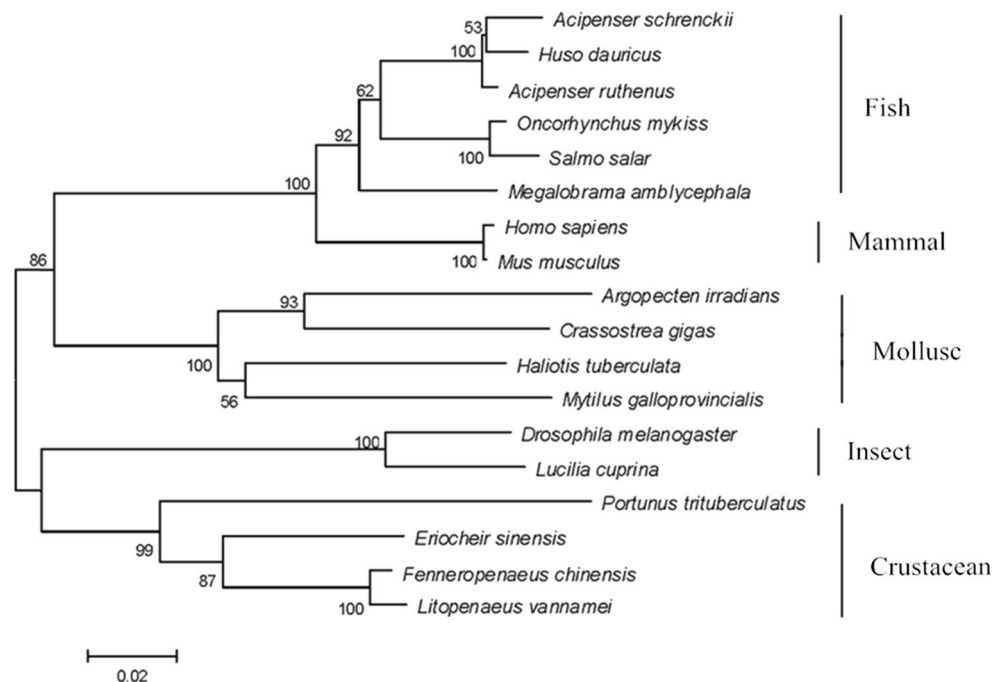


Table 3 The homology analysis between kaluga and other representative species for HSP90

Species	GenBank accession no	Similarity
Fish		
<i>Acipenser ruthenus</i>	AFA25806	99 %
<i>Acipenser schrenckii</i>	AFS88930	98 %
<i>Oncorhynchus mykiss</i>	CDQ59193	92 %
<i>Megalobrama amblycephala</i>	AGI97008	91 %
<i>Salmo salar</i>	AAD30275	91 %
Mammals		
<i>Homo sapiens</i>	NP_031381	91 %
<i>Mus musculus</i>	NP_032328	91 %
Crustaceans		
<i>Eriocheir sinensis</i>	ACJ01642	79 %
<i>Fenneropenaeus chinensis</i>	ABM92446	79 %
<i>Litopenaeus vannamei</i>	ADU03767	78 %
<i>Portunus trituberculatus</i>	ACQ90226	76 %
Insects		
<i>Drosophila melanogaster</i>	ABG94057	78 %
<i>Lucilia cuprina</i>	AEF38377	77 %
Molluscs		
<i>Haliotis tuberculata</i>	CAK95235	79 %
<i>Mytilus galloprovincialis</i>	CAE52893	78 %
<i>Argopecten irradians</i>	ABS50431	77 %
<i>Crassostrea gigas</i>	ABS18268	77 %

mRNA expression of the HSP70 and HSP90 genes after temperature stress are shown in Fig. 6. The control group at 16 °C and 0 ppt salinity was used to calibrate the relative qPCR. The

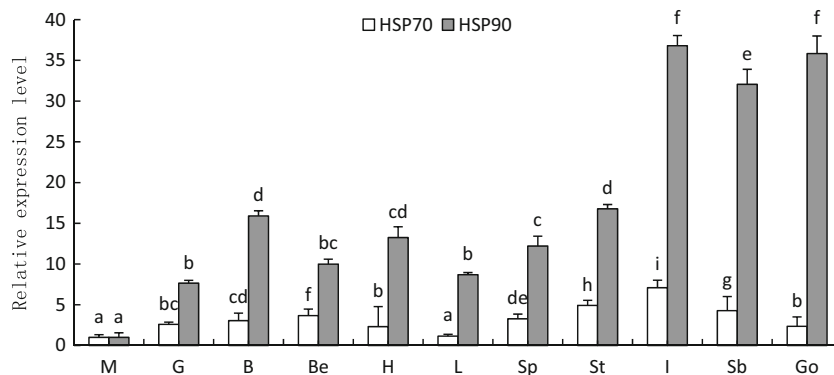
Fig. 4 Phylogenetic tree of HSP90s. The phylogenetic tree was constructed using neighbor joining. Numbers on the nodes indicate the confidence level of the bootstrap analyses

experiment was divided into cold stress (4 and 10 °C) and heat stress (25 and 28 °C). mRNA expression of HSP70 and HSP90 was highest in all tissues (muscle, gill, and liver) in the 4 °C cold stress treatment ($P < 0.05$). In the muscle, HSP70 and HSP90 gene expression increased gradually as temperatures decreased to a maximum in the 4 °C treatment (Fig. 6a, $P < 0.05$). Under heat shock, expression of HSP70 increased in the 25 °C treatment compared with the control (16 °C), but less increase in the 28 °C treatment compared to the control than at 25 °C. The levels of HSP90, however, were not significantly different from the control ($P \geq 0.05$). In gill tissue, the levels of HSP70 and HSP90 expression increased gradually under cold stress to a maximum at 4 °C (Fig. 6b, $P < 0.05$). After heat shock, HSP70 levels gradually increased, reaching the highest level in the 28 °C treatment. In addition, levels of HSP90 gene expression in the 25 and 28 °C treatments were much higher than in the control ($P < 0.05$), although there was no significant difference between the heat stress treatments (25 vs. 28 °C; $P \geq 0.05$). In the liver, the levels of HSP70 and HSP90 expression increased gradually with cold stress, reaching maximum levels at 4 °C (Fig. 6c, $P < 0.05$). During heat stress, the levels of HSP70 and HSP90 gene expressed increased gradually to maximum levels at 28 °C ($P < 0.05$).

Expression of the HSP70 and HSP90 genes in kaluga under salinity stress

We evaluated the effects of salinity stress on HSP70 and HSP90 expression in muscle, gill, and liver tissue. mRNA expression of the HSP70 and HSP90 genes after varying levels of salinity stress is shown in Fig. 7. Fish in the control

Fig. 5 Expression of HSP70 and HSP90 in different tissues of kaluga. *M*: muscle; *G*: gill; *B*: brain; *Be*: beard; *H*: heart; *L*: liver; *Sp*: spleen; *St*: stomach; *I*: intestine; *Sb*: swim bladder; *Go*: gonads. Values are expressed as mean \pm standard error. Different letters indicate significant differences ($P < 0.05$)



group (0 ppt) were used to calibrate the qPCR for comparison among treatments. In muscle tissue, the expression of both HSP70 and HSP90 increased gradually from 0 to 40 ppt, with maximum expression at 40 ppt (Fig. 7a, $P < 0.05$). In gill tissue, levels of HSP70 expression decreased from 0 to 10 ppt and from 20 to 30 ppt but increased from 10 to 20 ppt and again from 30 to 40 ppt, reaching maximum levels at 40 ppt (Fig. 7b, $P < 0.05$). The expression of HSP90 increased from 0 ppt to peak levels at 20 ppt ($P < 0.05$) and then decreased from 20 to 40 ppt. Liver tissue followed similar patterns of expression to gill tissue for both HSP70 and HSP90 (Fig. 7c).

Discussion

In this study, we cloned the full-length cDNA sequences of HSP70 and HSP90 from kaluga using RT-PCR and RACE methodology. Including the 5'-terminal UTR, 3'-terminal UTR, and the ORF, the HSP70 cDNA was 2275 bp and encoded a protein of 650 amino acids. Similarly, the full-length of the HSP90 cDNA was 2718 bp, encoding a protein of 725 amino acids. While the HSP70 protein contained three signature sequences of the HSP family, there was only one signature sequence in the HSP90 protein. Similarly to the HSP70 and HSP90 genes of other eukaryotes, HSP70 and HSP90 from kaluga also contained the highly conservative cytosol-specific EEVD motif in the C-terminus, indicating that HSP70 and HSP90 are mainly present in the cytosol (Demand et al. 1998; Vayssier et al. 1999; Ivanina et al. 2008; Yenari et al. 1999). Some researchers have suggested that, in many species, conserved tetrapeptide GGMP repeats in the C-terminal of the HSP70 protein may mediate the association of HSP70 and HSP90 into a multichaperone complex that is involved in the regulation of protein co-chaperone interactions (Scheufler et al. 2000; Piano et al. 2005). We found three repeats of the GGMP motif in the HSP70 of kaluga but did not find it in the HSP90 genes. Manchado et al. (2008) determined that the HSP90 family contained two subtypes, HSP90 α and HSP90 β . Expression of HSP90 α is highly inducible and plays an important role in cell growth, apoptosis,

and the cell cycle. HSP90 β is involved in maintaining normal physiological cell function through its role in cell differentiation and maintenance of cell structure and defense. A high degree of similarity indicated by BLAST analysis suggested that the HSP90 of kaluga is an HSP90 β gene.

Homology analysis showed that the amino acid sequence of the HSP70 gene of kaluga shared the highest identity (98–99 %) with that of the family Acipenseridae. In this regard, the gene must be HSP70. Similarity with the HSP70 of other vertebrates was over 81 %, and similarity with other invertebrates and specifically with *Drosophila* was nearly 73 %. Kaluga shared one branch of the phylogenetic tree with *Acipenser ruthenus*, *Acipenser schrenckii*, *Acipenser baerii*, *Danio rerio*, *Salmo salar*, *Fundulus heteroclitus macrolepidotus*, *Paralichthys olivaceus*, and *Xiphophorus maculatus* (Fig. 3). The relationship of these HSP70s as depicted by the phylogenetic tree was generally in agreement with the traditional taxonomy of these species. The amino acid sequence of the HSP90 gene of kaluga had 91 % similarity with the Acipenseridae family and 91, 77, 76, and 78 % similarity with the HSP90s of vertebrates, molluscs, crustaceans, and insects, respectively. The fish species kaluga, *A. ruthenus*, *A. schrenckii*, *Megalobrama amblycephala*, *Salmo salar*, and *Oncorhynchus mykiss* shared one branch of the phylogenetic tree. Thus, the phylogenetic relationships determined for these HSP90s were also in agreement with the traditional taxonomy of these species (Fig. 4).

Tissue expression analysis of the HSP70 and HSP90 genes of kaluga revealed ubiquitous expression in each of the 11 tissues examined in this study, indicating that both HSP70 and HSP90 were synthesized under normal, unstressed conditions. Furthermore, maximum expression of both HSP70 and HSP90 in the intestine suggests that HSP70 and HSP90 might be associated with digestive function, which was further substantiated by expression in stomach tissue. Various studies have shown that HSPs have a cytoprotective function in digestive organs that is necessary for the adaptation of cells to environmental stress (Jin et al. 1999; Otaka et al. 1997). Expression was lowest in muscle tissue, a pattern also found by Zakhartsev et al. (2005) in *Gadus morhua*. Interestingly,

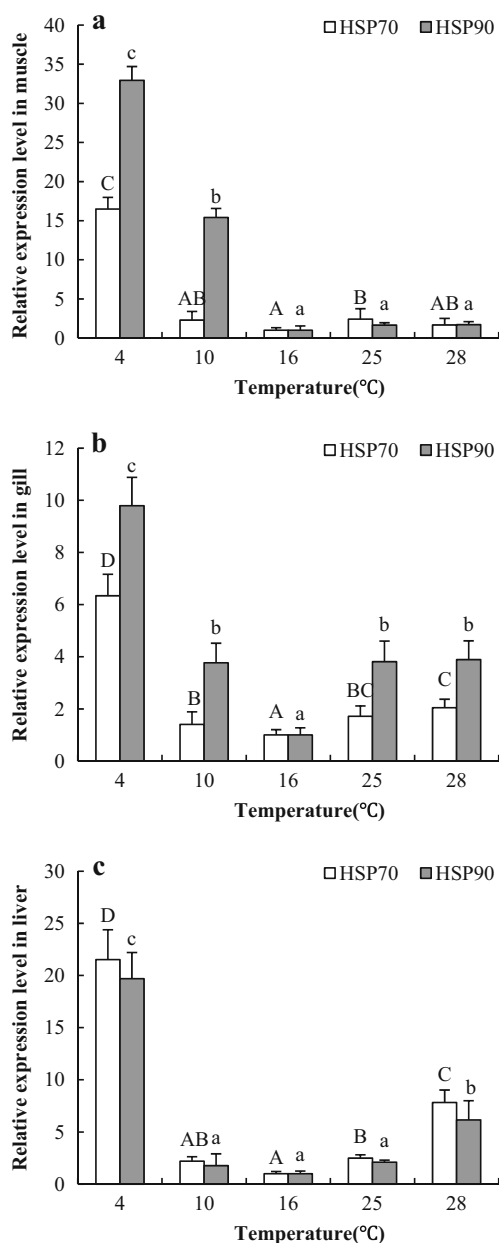


Fig. 6 The relative level of expression of HSP70 and HSP90 mRNA in muscle, gill, and liver tissue of kaluga at different temperatures. Values are expressed as mean \pm standard error. Different letters indicate significant differences ($P < 0.05$)

there was also expression of HSP70 and HSP90 in the beard and swim bladder, indicating that these genes also play a role in these sensory and balance organs (Fu et al. 2014; Lin et al. 2009; Liu et al. 2004). Wegele et al. (2004) argued that the functions of genes in the HSP70 and HSP90 families are usually closely related. The finding that patterns of expression of HSP70 and HSP90 were similar in all tissues corroborates this hypothesis and suggests that HSP70 and HSP90 have similar functions in kaluga. Levels of HSP90 expression were higher than those of HSP70 in all tissues, but it remains unclear if HSP90 serves a more important function than HSP70 in

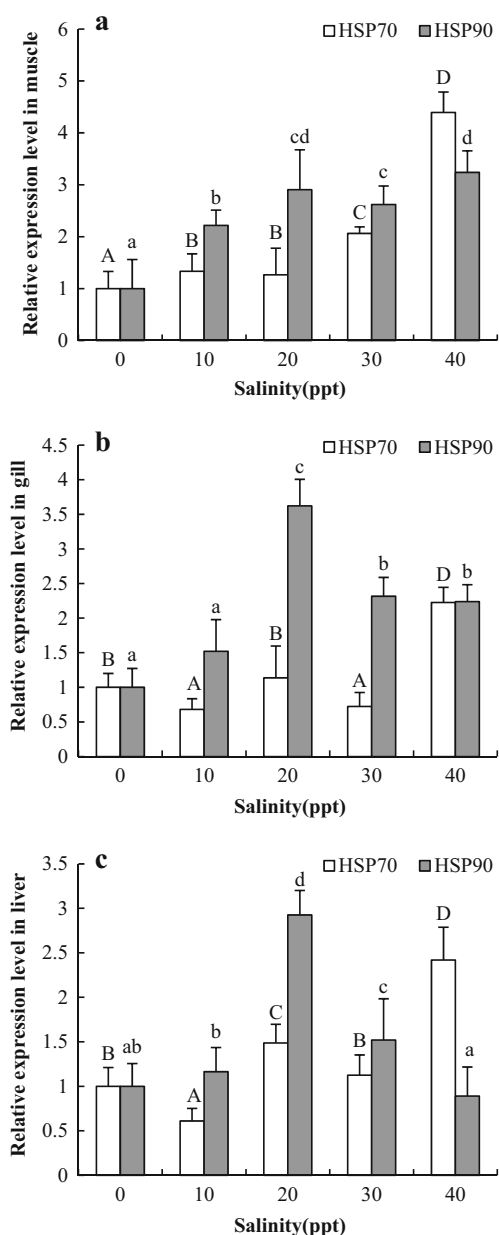


Fig. 7 The relative level of expression of HSP70 and HSP90 mRNA in muscle, gill, and liver tissue of kaluga under different magnitudes of salinity stress. Values are expressed as mean \pm standard error. Different letters indicate significant differences ($P < 0.05$)

kaluga. Expression of HSP70 and HSP90 proteins occurs in both normal and stressful conditions and is related to the transcription level (Wagner et al. 2010; Wan et al. 2007).

Temperature and salinity are important factors defining suitable fish habitat, and fish commonly experience temperature and salinity stress during transportation. We found that temperature and salinity stress significantly altered levels of expression of the HSP70 and HSP90 genes in the muscle, gill, and liver tissue of kaluga compared with the unstressed control group (Figs. 6 and 7, $P < 0.05$). Similarly to other studies (Palmisano et al. 2000; Manzerra et al. 1997; Lang et al.

2000), we found that gene expression in response to temperature and salinity stress is tissue specific. Levels of HSP70 and HSP90 expression were highest in the 4 °C treatment, indicating that they play an important role during cold stress. Wild populations of Kaluga in the Amur River in northeastern China survive beneath a frozen surface layer in the winter. Thus, these results indicate that HSP70 and HSP90 are important for survival during harsh winters. Moreover, there was little expression of HSP70 and HSP90 during heat stress, indicating that the genes are more sensitive to cold stress than heat shock. We suggest that this is because kaluga has adapted to the cold water in which it predominantly lives. HSP70 and HSP90 expression changed little during salinity stress. Deane and Woo (2004) also found insignificant expression of HSP70 in the liver and kidneys of *Sparus sarba* during salinity stress, and Palmisano et al. (2000) found that levels of HSP90 expression in the gill and liver of *Oncorhynchus tshawytscha* changed little during salinity stress. Thus, kaluga may be better able to respond to changes in osmotic stress. In aquaculture, fish are usually disinfected at salinities of 30–50 ppt, and fish are generally transported in low-salinity waters (Carneiro and Urbinati 2001). The present study revealed the molecular adaptation of kaluga to saline water. Moreover, these data clarify the effect of temperature and salinity stress on the gene expression of HSP70 and HSP90 in kaluga and point to the need for further research into the interaction of these stressors on gene expression of HSPs.

In conclusion, we isolated and characterized the HSP70 and HSP90 genes of kaluga for the first time. HSP70 and HSP90 gene expression was detected in 11 different tissues, and patterns of HSP70 and HSP90 expression were determined under different levels of temperature and salinity stress in muscle, gill, and liver tissue. This cloning and expression analysis of the HSP70 and HSP90 genes in kaluga provides more information on the HSPs in other teleost fishes. Thus, these data are useful for further study of HSP expression under environmental stress, and they further our understanding of the mechanisms of HSP70 and HSP90 stress response in cold freshwater fish.

References

- Airaksinen S, Råbergh CM, Sistonen L, Nikinmaa M (1998) Effects of heat shock and hypoxia on protein synthesis in rainbow trout (*Oncorhynchus mykiss*) cells. *J Exp Biol* 201: 2543–2551
- Andoh T, Nagasawa H, Matsubara T (2000) Multiple molecular forms of glucagon and insulin in the kaluga sturgeon, *Huso dauricus*. *Peptides* 21(12):1785–1792
- Basu N, Todgham AE, Ackerman PA, Bibeau MR, Nakano K, Schulte PM, Iwama GK (2002) Heat shock protein genes and their functional significance in fish. *Gene* 295:173–183
- Bausinger H, Lipsker D, Ziylan U, Manié S, Briand JP, Cazenave JP, Muller S, Haeuw JF, Ravanat C, Hdl S (2002) Endotoxin-free heatshock protein 70 fails to induce APC activation. *Eur J Immunol* 32:3708–3713
- Boone AN, Vijayan MM (2002) Constitutive heat shock protein 70 (HSC70) expression in rainbow trout hepatocytes: effect of heat shock and heavy metal exposure. *Comp Biochem Physiol C Toxicol Pharmacol* 132:223–233
- Carneiro PCF, Urbinati EC (2001) Salt as a stress response mitigator of matrixinã, *Brycon cephalus* (Günther), during transport. *Aquac Res* 32(12):297–304
- Deane EE, Woo NYS (2004) Differential gene expression associated with euryhalinity in sea bream (*Sparus sarba*). *Am J Physiol Regul Integr Comp Physiol* 287:1054–1063
- Demand J, Lüders J, Höhfeld J (1998) The carboxy-terminal domain of Hsc70 provides binding sites for a distinct set of chaperone cofactors. *Mol Cell Biol* 18(4):2023–2028
- Dong Y, Dong S, Meng X (2008) Effects of thermal and osmotic stress on growth, osmoregulation and Hsp70 in sea cucumber (*Apostichopus japonicus Selenka*). *Aquaculture* 276:179–186
- Downs CA, Fauth JE, Woodley CM (2001) Assessing the health of grass shrimp (*Palaeomonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. *Mar Biotechnol* 3:380–397
- Elliott JA (1995) A comparison of thermal polygons for British freshwater teleosts. *Freshwater Forum* 5(3):178–184
- Ellis RJ (1993) The general concept of molecular chaperones. *Philos Trans R Soc Lond B* 339:257–261
- Fu Y, Li C, Liu F, Dong Z, Ji X, Chen H (2014) Cloning, sequencing of the HSC70 gene in *Ctenopharyngodon idella*. *Wuhan Univ J Nat Sci* 19:235–244
- Gamperl AK, Vijayan MM, Pereira C, Farrell AP (1998) β -Receptors and stress protein 70 expression in hypoxic myocardium of rainbow trout and chinook salmon. *Am J Physiol Regul Integr Comp Physiol* 274:428–436
- Georgopoulos C, Welch WJ (1993) Role of the major heat shock proteins as molecular chaperones. *Annu Rev Cell Biol* 9:601–634
- Hamer B, Hamer DP, Müller WEG, Batel R (2004) Stress-70 proteins in marine mussel *Mytilus galloprovincialis* as biomarkers of environmental pollution: a field study. *Environ Int* 30:873–882
- Ivanina AV, Sokolova IM, Sukhotin AA (2008) Oxidative stress and expression of chaperones in aging mollusks. *Comp Biochem Physiol B: Biochem Mol Biol* 150:53–61
- Jin M, Otaka M, Okuyama A, Itoh S, Otani S, Odashima M, Iwabuchi A, Konishi N, Wada I, Pacheco I (1999) Association of 72-kDa heat shock protein expression with adaptation to aspirin in rat gastric mucosa. *Dig Dis Sci* 44:1401–1407
- Kiang JG, Tsokos GC (1998) Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacol Ther* 80:183–201
- Krykhtin ML, Gorbach EI (1996) Fecundity of the kaluga *Huso dauricus* and the Amur sturgeon *Acipenser schrencki*. *J Ichthyol C/C of Voprosy Ikhtiologii* 36:56–60
- Lang L, Miskovic D, Lo M, Heikkilä JJ (2000) Stress-induced, tissue-specific enrichment of hsp70 mRNA accumulation in *Xenopus laevis* embryos. *Cell Stress Chaperones* 5(1):36–44
- Lin YQ, Zheng YC, Ji H, He QH, Huang JQ (2009) Cloning and tissue expression of HSP90 partial cDNA sequence in grass carp. *Fish Sci* 28(8):439–442
- Liu J, Yang WJ, Zhu XJ, Karouna-Renier NK, Rao RK (2004) Molecular cloning and expression of two HSP70 genes in the prawn, *Macrobrachium rosenbergii*. *Cell Stress Chaperones* 9(3):313–323
- Livak KJ, Schmittgen TD (2002) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25:402–408
- Lu HS, Liu JK, Wang YS, Shi ZG, Li WL, Han J (2011) The growth characters of 1 year-old kaluga juvenile. *J Hydroecol* 32(5):78–82

- Manchado M, Salas-Leiton E, Infante C, Ponce M, Asensio E, Crespo A, Zuasti E, Cañavate JP (2008) Molecular characterization, gene expression and transcriptional regulation of cytosolic HSP90 genes in the flatfish Senegalese sole (*Solea senegalensis* Kaup). *Gene* 416:77–84
- Manzerra P, Rush SJ, Brown IR (1997) Tissue-specific differences in heat shock protein hsc70 and hsp70 in the control and hyperthermic rabbit. *J Cell Physiol* 170:130–137
- Morimoto RI, Tissières A, Georgopoulos C (1990) Stress proteins in biology and medicine. CSHL Press, Cold Spring Harbor, pp 1–36
- Mu WJ, Wen HS, Li JF, He F (2013) Cloning and expression analysis of a HSP70 gene from Korean rockfish (*Sebastes schlegelii*). *Fish shellfish immunol* 35:1111–1121
- Ni M, Wen HS, Li JF, Chi M, Ren YY, Song ZF, Ding HM (2014) Two HSPs gene from juvenile Amur sturgeon (*Acipenser schrenckii*): cloning, characterization and expression pattern to crowding and hypoxia stress. *Fish Physiol Biochem* 40:1801–1816
- Otaka M, Okuyama A, Otani S, Jin M, Itoh S, Itoh H, Iwabuchi A, Sasahara H, Odashima M, Tashima Y (1997) Differential induction of HSP60 and HSP72 by different stress situations in rats (correlation with cerulein-induced pancreatitis). *Dig Dis Sci* 42:1473–1479
- Palmisano AN, Winton JR, Dickhoff WW (2000) Tissue-specific induction of Hsp90 mRNA and plasma cortisol response in chinook salmon following heat shock, seawater challenge, and handling challenge. *Mar Biotechnol* 2:329–338
- Piano A, Franzellitti S, Tinti F, Fabbri E (2005) Sequencing and expression pattern of inducible heat shock gene products in the European flat oyster, *Ostrea edulis*. *Gene* 361:119–126
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Scheuffler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I (2000) Structure of TPR domain–peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell* 101:199–210
- Shmigirilov AP, Mednikova AA, Israel JA (2007) Comparison of biology of the Sakhalin sturgeon, Amur sturgeon, and kaluga from the Amur River, Sea of Okhotsk, and Sea of Japan biogeographic Province. *Environ Biol Fish* 79(3–4):383–395
- Sørensen JG, Kristensen TN, Loeschcke V (2003) The evolutionary and ecological role of heat shock proteins. *Ecol Lett* 6:1025–1037
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599
- Vasil'ev VP, Vasil'eva ED, Shedko SV, Novomodny GV (2009) Ploidy levels in the kaluga, *Huso dauricus* and Sakhalin sturgeon *Acipenser mikadoi* (Acipenseridae, Pisces). *Doklady Biol Sci MAIK Nauka/Interperiodica* 426(1):228–231
- Vayssier M, Leguerhier F, Fabien JF (1999) Cloning and analysis of a *Trichinella britovi* gene encoding a cytoplasmic heat shock protein of 72 kD. *Parasitology* 119:81–93
- Waagner D, Heckmann LH, Malmendal A, Nielsen NC, Holmstrup M, Bayley M (2010) Hsp70 expression and metabolite composition in response to short-term thermal changes in *Folsomia candida* (Collembola). *Comp Biochem Physiol A Mol Integr Physiol* 157:177–183
- Wan WJ, Wang JT, Shi CB, Wu SQ (2007) Gene expression of HSP70 in green swordtail *Xiphophorus helleri* exposed to *Vibrio alginolyticus*. *J Dalian Fish Univ* 22:330–334
- Wegele H, Müller L, Buchner J (2004) Hsp70 and Hsp90-a relay team for protein folding. *Rev Physiol Biochem Pharmacol* 151:1–44
- Welch NJ (1993) Heat shock proteins functioning as molecular chaperones: their roles in normal and stressed cells. *Philos Trans R Soc Lond B* 339:327–333
- Werner I (2004) The influence of salinity on the heat-shock protein response of *Potamocorbula amurensis* (Bivalvia). *Mar Environ Res* 58:803–807
- Yenari MA, Giffard RG, Sapolsky RM, Steinberg GK (1999) The neuroprotective potential of heat shock protein 70 (HSP70). *Mol Med Today* 5:525–531
- Yin HB, Sun ZW, Sun D, Qiu LJ et al (2004) Comparison of nutritive compositions in muscles among six farmed sturgeon species. *J Dalian Fish Coll* 19:92–96
- Zakhartsev M, De Wachter B, Johansen T, Pörtner HO, Blust R (2005) Hsp70 is not a sensitive indicator of thermal limitation in *Gadus morhua*. *J Fish Biol* 67:767–778
- Zhu X, Xiao H, Zhang DZ, Tang DM, Wang W, Jian JL (2012) Culture and artificial propagation of Siberian *H. Sturgeon* (kaluga) in Central China. *Fish Sci* 31(1):46–49