Comparative analysis of the liver transcriptome in the red-eared slider turtle *Trachemys scripta elegans* under chronic salinity stress

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The red-eared slider (*Trachemys scripta elegans*), identified as one of the 100 most invasive species in the world, is a freshwater turtle originally from the eastern United States and northeastern Mexico. Field investigations have shown that *T. s. elegans* can survive and lay eggs in saline habitats. In order to understand the molecular mechanisms of salinity adaptation, high-throughput RNA-Seq was utilized to identify the changes in gene expression profiles in the liver of *T. s. elegans* in response to elevated salinity. We exposed individuals to 0, 5, or 15 psu (practical salinity units) for 30 days. A total of 157.21 million reads were obtained and assembled into 205138 unigenes with an average length of 620 bp and N50 of 964 bp. Of these, 1019 DEGs (differentially expressed genes) were found in the comparison of 0 vs 5 psu, 1194 DEGs in 0 vs 15 psu and 1180 DEGs in 5 vs 15 psu, which are mainly related to macromolecule metabolic process, ion transport, oxidoreductase activity and generation of precursor metabolites and energy by GO (Gene Ontology) enrichment analyses. *T. s. elegans* can adapt itself into salinity adaptation by balancing the entry of sodium and chloride ions via the up-regulation expression genes of ion transport (potassium voltage-gated channel subfamily H member 5, *KCNH5*; erine/threonine-protein kinase 32, *STK32*; salt-inducible kinase 1, *SIK1*; adiponectin, *ACDC*), and by accumulating plasma urea and free amino acid via the up-regulation expression genes of amino acid metabolism (ornithine decarboxylase antizyme 3, *OAZ3*; glutamine synthetase, *GLUL*; asparaginase-like protein 1b, *ASRGL*; L-amino-acid oxidase-like, *LAAO*; sodium-dependent neutral amino acid transporter B, *SLC6A15s*; amino acid permease, *SLC7A9*) in response to osmotic regulation. An investment of energy to maintain their homeostatic balance is required to salinity adaptation, therefore, the genes related to energy production and conversion (F-ATPase protein 6, *ATP6*; cytochrome c oxidase subunit I, *COX1*; cytochrome c oxidase subunit III, *COX3*; cytochrome b, *CYTb*;

cytochrome P450 17A1, *CYP17A1*) were up-regulated with the increase of gene expression associated with lipid metabolism (apolipoprotein E precursor, *APoE*; coenzyme Q-binding protein, *CoQ10*; high-density lipoprotein particle, *SAA*) and carbohydrate metabolism (*HK*, *MIP*). These findings improve our understanding of the underlying molecular mechanisms involved in salinity adaptation and provide general guidance to illuminate the invasion potential of *T. s. elegans* into saline environments.

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ABSTRACT

The red-eared slider (*Trachemys scripta elegans*), identified as one of the 100 most invasive species in the world, is a freshwater turtle originally from the eastern United States and northeastern Mexico. Field investigations have shown that *T. s. elegans* can survive and lay eggs in saline habitats. In order to understand the molecular mechanisms of salinity adaptation, high-throughput RNA-Seq was utilized to identify the changes in gene expression profiles in the liver of *T. s. elegans* in response to elevated salinity. We exposed individuals to 0, 5, or 15 psu (practical salinity units) for 30 days. A total of 157.21 million reads were obtained and assembled into 205138 unigenes with an average length of 620 bp and N50 of 964 bp. Of these, 1019 DEGs (differentially expressed genes) were found in the comparison of 0 vs 5 psu, 1194 DEGs in 0 vs 15 psu and 1180 DEGs in 5 vs 15 psu, which are mainly related to macromolecule metabolic process, ion transport, oxidoreductase activity and generation of precursor metabolites and energy by GO (Gene Ontology) enrichment analyses. *T. s. elegans* can adapt itself into salinity adaptation by balancing the entry of sodium and chloride ions via the up-regulation expression genes of ion transport (potassium voltage-gated channel subfamily H member 5, *KCNH5*; erine/threonine-protein kinase 32, *STK32*; salt-inducible kinase 1, *SIK1*; adiponectin, *ACDC*), and by accumulating plasma urea and free amino acid via the up-regulation expression genes of amino acid metabolism (ornithine decarboxylase antizyme 3, *OAZ3*; glutamine synthetase, *GLUL*; asparaginase-like protein 1b, *ASRGL*; L-amino-acid oxidase-like, *LAAO*; sodium-dependent neutral amino acid transporter B, *SLC6A15s*; amino acid permease, *SLC7A9*) in response to osmotic regulation. An investment of energy to maintain their homeostatic balance

is required to salinity adaptation, therefore, the genes related to energy production and conversion (F-ATPase protein 6, *ATP6*; cytochrome c oxidase subunit I, *COX1*; cytochrome c oxidase subunit III, *COX3*; cytochrome b, *CYTb*; cytochrome P450 17A1, *CYP17A1*) were up-regulated with the increase of gene expression associated with lipid metabolism (apolipoprotein E precursor, *APoE*; coenzyme Q-binding protein, *CoQ10*; high-density lipoprotein particle, *SAA*) and carbohydrate metabolism (*HK*, *MIP*). These findings improve our understanding of the underlying molecular mechanisms involved in salinity adaptation and provide general guidance to illuminate the invasion potential of *T. s. elegans* into saline environments.

INTRODUCTION

The red-eared slider turtle (*Trachemys scripta elegans*) has been introduced into diverse aquatic habitats worldwide (including many countries in Africa, Asia and Europe as well as Australia) via the pet-release pathway and, as a result, is classified as a highly invasive species by the International Union for Conservation of Nature (*Luiselli et al., 1997; Martins et al., 2014*). It is native to freshwater habitats in 19 states of the eastern United States and two states of northeastern Mexico (*Mittermeier et al., 2015*). Recently these turtles have been found to lay eggs in the low salinity (0.1-26 ‰) estuary of the Nandujiang in Hainan Province, China (*Liu et al., 2011; Yang & Shi 2014*). The extent of the saltwater adaptability of *T. s. elegans* is not fully understood. Studies of endocrine stress responses by *T. s. elegans* in the Lake Pontchartrain Basin of Louisiana suggest that these turtles may serve as a sentinel species for elevated salinity in environments where salinity is rising due to saltwater intrusion (*Thompson et al., 2011*). These studies indicate that *T. s. elegans* can invade not only fresh water, but also saline water environments. Therefore, the invasion potential and the mechanism of response to salinity adaptation of *T. s. elegans* is of serious concern.

Changing levels of salinity are a crucial environmental stress factor for aquatic species that can disrupt electrolyte balance, cell energetics, and various other physiological responses, including activating stress hormones (*Lushchak 2011*). Species show altered composition the osmolality of body fluids in response to changing salinity (*Charmantier et al., 2011*) and adaptation to salinity change typically involves the physiological solution: tolerance of elevated inorganic ion concentrations (mainly sodium and chloride) in plasma (*Gordon & Tucker 1965*),

and accumulation of organic osmolytes (e.g. urea) to counteract cell-volume changes. The most dramatic changes in urea concentration are seen in plasma and tissues such as skeletal muscle, resulting from the up-regulation of hepatic urea (*Wright et al., 2004*). Accumulation of intracellular free amino acids via hepatic protein degradation or de novo amino acid synthesis can also contribute to osmotic balance (*McNamara et al., 2004; Yancey 1985*). When subjected to ambient salinity change, *T. s. elegans* increased serum glucose levels, and the activities of creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) in liver (*Shu et al., 2012*). Our previous studies have shown that *T. s. elegans* can increase blood osmotic pressure by balancing the entry of sodium and chloride ions with a decrease in the secretion of aldosterone, and by accumulating plasma urea for osmoregulation when ambient salinity was lower than 15‰ (*Hong et al., 2014*). However the molecular basis of these adaptive responses has not been studied in *T. s. elegans*.

Recently, high-throughput next-generation sequencing techniques have allowed researchers to broadly explore the extent and complexity of the transcriptomes of a wide range of eukaryotic species including to gain novel information about the gene responses of aquatic species to physiological and environmental stresses (*Wang et al., 2009*). RNA-seq is an efficient technique to probe the gene responses to physiological stress (*Li et al., 2013; Smith et al., 2013; Xia et al., 2013*), particularly when working with species that do not have a sequenced genome. The present study used RNA-seq to analyze the transcriptomic response of *T. s. elegans* to salinity stress and identify the genes (and their metabolic functions) that are involved in salinity adaptation of turtles challenged by a brackish water environment. These results provide insights into the

85 molecular mechanisms underlying osmoregulation in *T. s. elegans* and address the potential for
86 this species to further invade and spread through new aquatic and brackish territories.

87 **MATERIALS AND METHODS**

88 **Animals**

89 Healthy *T. s. elegans* were obtained from a local turtle farm in Hainan Province, China and
90 were acclimated in three cement pools half-filled with freshwater for two weeks. After
91 acclimatization, nine healthy *T. s. elegans* (BW: 424-478g, 2 years old) were divided into three
92 groups in pools (190 cm × 65 cm × 32 cm) of differing salinity: one in freshwater serving as the
93 control (0 practical salinity units, psu), and the other two challenged with 5 ‰ (5 psu) or 15 ‰
94 (15 psu) saltwater. Turtles were fed a commercial diet each Monday and Thursday and 24 h after
95 feeding, unused feed was siphoned out followed by replacement of one-third of the water in each
96 pool. Water salinity was measured every day and adjusted to the proper salinity as needed. Other
97 water quality parameters were monitored 2-3 times a week with steady values of pH 7.5-7.9,
98 total ammonia nitrogen of < 0.02 mg L^{-1} and temperature 26-28℃. Photoperiod was 12 h:12 h
99 L:D throughout.

100 After 30 days of exposure to the three experimental conditions, the three turtles from each
101 group were subjected to a 24 h fast, then anesthetized by cryo-anesthesia moving turtles to -20℃
102 for 0.5-1 h. Experimental animal procedures had the prior approval of the Animal Research
103 Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan
104 Normal University (permit no. HNECEE-2014-004). Following euthanasia, the liver of each

individual was sampled and divided into two sections, flash frozen in liquid nitrogen, and stored at -80°C until used for RNA extraction. One liver section was used for quantitative real-time PCR (qRT- PCR), and the other was mixed from each group of three turtles for RNA-seq analysis.

Total RNA extraction, library construction and sequencing

Extraction of total RNA from liver samples used TRIzol® Reagent, following manufacturer's instructions. Total RNA purity and concentration were determined using a NanoDrop 2000. The sequencing library was then constructed from high-quality RNA ($OD_{260/280}$=1.8-2.2, $OD_{260/230}$≥1.5, RIN≥8.0, 28S:18S≥1.0, >10 μg). RNA-seq analysis was provided by Novel Bioinformatics Co., Ltd using the Sanger/Illumina method. Subsequently, cDNA libraries were made using the Hiseq4000 Truseq SBS Kit v3-HS using 5 μg total RNA, following manufacturer's instructions. Poly(A) mRNA was isolated with Dyabeads (Life Technologies, USA), fragmented with RNaseIII and purified. The fragmented RNA was added and ligated with ion adaptor. Then double-stranded cDNA was synthesized and purified using magnetic beads. The molar concentration of the purified cDNA in each cDNA library was then quantified with a TBS-380 fluorometer using Picogreen. The paired-end RNA-seq library was sequenced with an Illumina HiSeq 4000. The RNA-Seq data were deposited in the NCBI with accession number GSE117354 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE117354).

De novo assembly and annotation

Raw reads were trimmed and quality controlled using SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) using default parameters. High-quality trimmed sequences were used for sequence assembly with Trinity (https://github.com/trinityrnaseq/trinityrnaseq/wiki) (*Grabherr et al., 2011*). Q20, Q30, GC-content and sequence duplication level of the clean data were all calculated. After that, All assembled transcripts were identified by using BLASTX against the databases of NR (NCBI non-redundant protein sequence), Swissprot (A manually annotated and reviewed protein sequence database), Pfam (Protein family), GO (Gene ontology), COG (Clutsters of Orthologous Groups of proteins) and KEGG (Kyoto Encyclopaedia of Genes and Genomes). The BLAST2GO (http://www.blast2go.com/b2ghome) program (*Conesa et al., 2005*) was used to obtain gene ontology (GO) annotations of unique assembled transcripts for describing biological processes, cellular components, and molecular functions.

Analysis of differential expression and functional enrichment

Expression levels of transcripts were calculated as fragments per kilobase of exon per million mapped reads (FPKM). RNA-Seq by Expectation-Maximization (RSEM; http://deweylab.biostat.wisc.edu/rsem/) was used to quantify gene transcripts, and DEGseq (http://www.bioconductor.org/packages/release/bioc/vignettes/DEGseq/inst/doc/DEGseq.pdf) was used to conduct differential expression analysis. The resulting p values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted p value < 0.05 found by DEGseq were assigned as differentially expressed. GO

144 enrichment analysis of the DEGs was implemented by the GOseqR packages based on Wallenius
145 non-central hyper-geometric distribution (*Xie et al., 2011*), which can adjust for gene length bias
146 in DEGs.

147 **Experimental validation by qRT-PCR**

148 Eighteen genes identified as significantly expressed from the GO terms related to osmotic
149 regulation were selected to understand the gene expression levels in different groups, and also
150 used for validation by qRT-PCR. Table 1 shows the specific primers used. TRIzol® Reagent
151 (Invitrogen, Carlsbad, USA) was used to extract total RNA from liver, followed by reverse-
152 transcription using First-strand cDNA Synthesis Kit (Invitrogen, Carlsbad, US). The qRT-PCR
153 for gene expression was analyzed by an Applied Biosystems 7500 Fast Real-Time PCR System
154 in 96-well plates with a 20 μl reaction volume containing 1×SYBR Green qPCR Master Mix,
155 gene-specific forward and reverse primers (0.4 μM) and cDNA (8 ng). The cycling conditions
156 were 95℃ for 2 min followed by 40 cycles of 95℃ for 5 s and 60℃ for 30 s. *β-actin* was chosen
157 as the reference gene, as it was expressed in the three groups and unaffected under salinity stress,
158 and relative fold changes were determined using Relative Expression Software Tool v.2009
159 based on the cycle threshold (Ct) values generated by qRT-PCR.

160 The mRNA expression levels were expressed as mean ± standard error. Statistical analyses
161 were conducted with SPSS 19.0. After testing the homogeneity of variance, statistical difference
162 between treatments and controls were determined by one-way analysis of variance (ANOVA).
163 LSD multiple comparison tests were carried out when the variances were homogeneous.

Significant differences were set at $p < 0.05$.

RESULTS

Analysis of sequenced data quality

Because we haven't got the genome of *T. s. elegans* yet, a transcriptome was used as a reference to identify the differentially expressed genes induced by salinity exposure. Therefore, a mixed RNA pool from the samples of the control and salinity treatment groups was sequenced as the reference transcriptome. The data qualities from each sample were shown in Table 2. A total of 157.21 million reads and 23.58 billion bases were obtained from the liver transcriptome of *T. s. elegans*, including 50.68 million in 0 psu, 57.91 million in 5 psu and 48.62 million in 15 psu groups. After filtering low quality sequences by, trimming sequencing adapters/poly-N and removing poor quality reads, there were 152.52 million clean reads (97.02% of raw reads) were retained including 48.92 million in 0 psu, 56.50 million in 5 psu and 47.10 million in 15 psu. Subsequently, 205138 unigenes with an average length of 620 bp and N50 of 964 bp were obtained by de novo assembly. The largest and smallest unigenes were 22866 bp and 201 bp, respectively (Table 3).

Annotation and differential expression of genes

Putative functions of proteins encoded by the 205138 genes were predicted by NR, Pfam, COG, Swissprot, GO and KEGG database. The results showed that there were 20458 (9.97%),

20421 (9.95%), 13362 (6.51%), 10673 (5.2%), 25438 (12.36%), and 38651 (18.84%) in Pfam, KEGG, GO, COG, Swissprot and NR databases, respectively (Table 4).

By GO annotation, the genes up-regulated or down-regulated were divided into three categories including biological process, cellular component and molecular function (Fig 1). Among the category of biological process, the number of differentially expressed gene was higher in the GO terms of cellular process, metabolic process, single-organism process and biological regulation. Among the category of cellular component, the number of differentially expressed gene was higher in the GO terms of cell, cell part, macromolecular complex, and membrane. In relation to molecular function, the number of differentially expressed gene was higher in the GO terms of binding, catalytic activity, and transporter activity. Moreover, there was almost the same trend in the comparisons of 0 vs 5 psu, 0 vs 15 psu, and 5 vs 15 psu.

On the basis of criteria of two fold or greater change and Q of $p < 0.05$, 3393 unigenes were identified as significant differentially expressed genes (DEGs), including 1019 (445 up-regulated and 574 down-regulated) DEGs in 0 vs 5 psu, 1194 (526 up-regulated and 668 down-regulated) DEGs in 0 vs 15 psu, and1180 (548 up-regulated and 632 down-regulated) DEGs in 5 vs 15 psu. Many DEGs were classified into some dominant categories by GO enrichment analyses, including the macromolecule metabolic process (52 DEGs), ion transport (35 DEGs), ion transmembrane transport (24 DEGs) in biological process, and nucleic acid binding (37 DEGs), oxidoreductase activity (35 DEGs), transporter activity (33 DEGs), and transmembrane transporter activity (27 DEGs) in molecular function (Table 5).

As for osmotic adjustment, there were 15 major GO terms related to osmotic function in turtle liver in the comparison of 0 vs 5 psu (Table S1) and 12 in the comparison of 0 vs 15 psu (Table S2). Based on GO enrichment analysis, a list of candidate genes involved in salinity acclimation in *T. s. elegans* were identified. Many of these were identified as encoding proteins involved in ion transport, energy production and conversion, and macromolecule metabolic process including lipid, protein and carbohydrate.

Based on the annotation of DEGs, we selected ten genes related to ion regulation, five genes related to energy production and conversion, five genes related lipid metabolism, eight genes related amino acid metabolism and six genes related carbohydrate metabolism (Table 6). Among the DEGs associated with ion regulation, five genes (adipocytokine, *ACDC*; insulin receptor-related protein, *INSRR*; serine/threonine-protein kinase 32, *STK32*; salt-inducible kinase 1, *SIK1*; potassium voltage-gated channel subfamily H member 5, *KCNH5*) were up-regulated in the comparison of 0 vs 5 psu, and four genes (salt-inducible kinase 2, *SIK2*; *SIK1*; *ACDC*; *STK32*) were up-regulated in the comparison of 0 vs 15 psu, and five genes (*SIK1*, *SIK2*, *STK33*, *ACDC*, and solute carrier family 26 member 9, *SLC26A9*) were up-regulated in the comparison of 5 vs 15 psu. All of the five DEGs (cytochrome c oxidase subunit I, *COX1*; cytochrome c oxidase subunit III,*COX3*; cytochrome b, *CYTb*; F-ATPase protein 6, *ATP6*, and cytochrome P450 17A1, *CYP17A1*) associated with energy production and conversion showed up-regulation under salinity stress, which indicated that there was a higher energy demand in response to salinity exposure. In general, lipid and carbohydrate were the main sources of energy. As for five DEGs associated with lipid metabolism, three DEGs (apolipoprotein E precursor, *ApoE*; coenzyme Q-

binding protein, *CoQ10*; high-density lipoprotein particle, *SAA*) increased with ambient salinity increased, while one DEGs (alcohol dehydrogenase 4, *ADH4*) showed down-regulation in the comparison of 0 vs 5 psu and one DEGs (fatty acid desaturase 6, *FADS6*) down-regulation in the comparison of 0 vs 15 psu. Among six DEGs related to carbohydrate metabolism, two DEGs (hexokinase, *HK* and lens fiber major intrinsic protein, *MIP*) increased with ambient salinity increased, four DEGs (glucokinase, *GCK*; tagatose 1,6-diphosphate aldolase, *LacD*; L-gulono-gamma-lactone oxidase, *GLO* and ribulose bisphosphate carboxylase small chain, *RBCs*) decreased under salinity stress. In addition, six DEGs (ornithine decarboxylase antizyme 3, *OAZ3*; glutamine synthetase, *GLUL*; asparaginase-like protein, *ASRGL*; L-amino-acid oxidase-like, *LAAO*; sodium-dependent neutral amino acid transporter, *SLC6A15s*; amino acid permease, *SLC7A9*) related to amino acid metabolism showed up-regulation and two DEGs (tyrosine aminotransferase, *TAT* and argininosuccinate synthase, *ASS1*) showed down-regulation in the comparison of 0 vs 5 psu, while four DEGs (*OAZ3*, *GLUL*, *ASRGL*, *SLC7A9*) showed up-regulation and three DEGs (*LAAO*, *TAT*, *ASS1*) showed down-regulation in the comparison of 0 vs 15 psu.

Verification of gene expression by SYBR Green qRT-PCR

Some genes related to ion transport, energy production and conversion and macromolecule metabolic process mentioned above were selected for qRT-PCR analysis in order to validate the differentially expressed genes that were identified by RNA-Seq and gain detailed quantitative information on their differing expression patterns. As shown in Table 7, the mRNA expression levels of *COX3*, *ATP6* and *CYP17A1* related to energy production and conversion in the 5 psu

group were almost 3.6-, 2.2- and 1.5-fold of the control respectively, while those in the 15 psu group were almost 2.4-, 3.4- and 2.1-fold of the control respectively. In relation to ion transport, the mRNA expression levels of *STK32* and *SIK1* in the 5 psu group were almost 3.6- and 2.1-fold of the control respectively, while those in the15 psu group were almost 3.0- and 3.5-fold of the control respectively. The mRNA expression levels of *INSRR* increased 2-fold in the 5 psu group and decreased 2.6-fold in the 15 psu group compared to the control, however, that of *STK33* decreased 2.2- and 1.5-fold in the group of 5 psu and 15 psu compared to the control. As for macromolecular metabolism, the mRNA expression levels of *APoE*, *CoQ10*, *SAA*, *HK*, *GLUL*, and *ASRGL* increased with ambient salinity increased. Especially for the genes of *CoQ10* and *SAA*, the mRNA expression levels in the 15 psu group were almost 8.4- and 15.3-fold of the control respectively. However, the mRNA expression levels of *GCK* and *ASS1* decreased with ambient salinity increased, and those in the group of 5 psu were almost 1.5- and 2.0-fold of the control, and those in the group of 15 psu were 3.7- and 9.7-fold of the control. The mRNA expression levels of *FADS6* and *TAT* in the 5 psu group were lowest among the three groups, and decreased almost 2.3- and 3.6-fold compared to the control.

The qRT-PCR results were significantly correlated with the RNA-seq results with correlation coefficients of 0.744 in 0 vs 5 psu, 0.862 in 0 vs 15 psu groups and 0.748 in 5 vs 15 psu groups ($p < 0.05$) (Fig. S1). This provides strong evidence that RNA-Seq data can be a reliable indicator of the expression patterns of the hundreds of genes identified as differentially expressed in the current study.

DISCUSSION

Osmoregulation in some aquatic animals can be a complex process because individuals must deal with fluctuating salinity levels in their natural habitats, often on a daily or seasonal basis. As a normally freshwater species, the red-eared slider *T. s. elegans*, does not possess salt glands, and requires osmoregulation to survive when entering environments of higher salinity (e.g. brackish or estuarine water). The physiological research has shown that *T. s. elegans* can increase blood osmotic pressure by balancing the entry of NaCl with the decreased secretion of aldosterone, and accumulating urea and free amino acids in blood (*Hong et al. 2014*). In our study, many DEGs were classified into some dominant categories including the macromolecule metabolic process, ion transport and ion transmembrane transport in biological process, which provide a strong evidence for the physiological mechanism.

Under salinity stress, loss of water from the cells can cause cells shrink and potentially die. In this sense, ion regulation is important for a cell to balance osmotic change. *KCNH5* (www.ncbi.nlm.nih.gov/gene/27133) and *SCN1B* (*Qin et al., 2003*) are involved in potassium/sodium voltage-gated ion channels and fluid balance that controls arterial blood pressure by altering blood electrolyte composition and/or volume. Natriuretic peptide A (*NPPA*) is well known to regulate body fluid levels and electrolytic homeostasis and has natriuretic, diuretic, and vasodilatory actions (*Espiner et al., 2014*). *NPPA* is highly expressed and associated with H_2O/Na^+ absorption and protein Ser/Thr phosphatases (*Espiner et al. 2014*). *SIK* acts to modulate adrenocortical function particularly in response to high plasma Na^+, K^+, ACTH, or stress (*Wang et al., 1999*). *SIK1* also has a role in steroidogenesis whereas *SIK2* is implicated in

gluconeogenesis regulation in liver; both belong to the AMPK (AMP-activated kinase) subfamily of serine/threonine kinases (*Berggreen et al., 2012*). The AMPK is a crucial regulator of cellular energy levels (*Hardie & Ashford 2014*) and under stress conditions that deplete ATP, AMPK action promotes ATP-producing catabolic pathways while inhibiting ATP-consuming anabolic functions (*Rider et al., 2009; Rider et al., 2006*). Transcripts of adiponectin (*ACDC*) were also enriched in liver under both salinity stresses and this hormone participates in the pathway of fatty acid oxidation by regulating AMPK (*Chong et al., 2013*). So, in our study, five genes (*ACDC*, *INSRR*, *STK32*, *SIK1*, *KCNH5*) were up-regulated in the comparison of 0 vs 5 psu, and three genes (*ACDC*, *STK32*, *SIK2*) were up-regulated in the comparison of 0 vs 15 psu, which suggested that *T. s. elegans* can adapt itself into saline environment by increasing the expression levels of genes related to ion regulation.

Genes associated with transporting molecules related to metabolic processes were also modulated in association with an up-regulation of genes involved in ATP energy production. As salinity level increases, acclimation of *T. s. elegans* to elevated salinity conditions requires investment of energy to maintain their homeostatic balance (*Hong et al. 2014*). In our study, the DEGs related to energy production and conversion including *ATP6*, *COX1, COXIII, CYTb,* and *CYP17A1* increased with ambient salinity increased, which suggested that a requirement for more energy by *T. s. elegans* during raised salinity conditions. The result is associated with the ATP needed for the synthesis and operation of transport-related proteins that drive ion- and osmoregulatory processes (*Lee et al., 2003*). It is also documented that changes in components related to the glycolysis, fatty acid metabolism, and ATP production are often associated with

raised salinity conditions in freshwater fish (*Lavado et al., 2014; Tine et al., 2008*). Our previous study has shown that *T. s. elegans* increased serum glucose and triglyceride levels when subjected to salinity stress (*Shu et al., 2012*). This study also indicated that salinity stress influences glycolysis/gluconeogenesis and fatty acid metabolism-related genes. The mRNA expressions of *HK* and *MIP* were increased, which suggested that utilization of glycogen might be increased and more glucose might be used for energy expenditure under salinity exposure. Moreover, the gene expressions of *APoE*, *CoQ10*, and *SAA* were up-regulated while *ADH4* and *FADS6* were down-regulated, which suggested that the lipolysis increased and lipogenesis decreased to produce more energy in response to salinity stress.

The ureogenesis is a strategy for diamondback terrapins (*Malaclemys terrapin*) that are known to inhabit brackish water, and for the desert tortoise, *Gopherus agassizii* under dehydration stress (*Dantzler & Schmidt-Nielsen 1966*). The red-eared slider turtle, *T. s. elegans*, can also increase urea content in plasma and tissues by synthesizing organic osmolytes (e.g. free amino acids and urea) in order to provide a colligative defense against water loss (*Hong et al. 2014*). Some DEGs associated with cellular amino acid metabolism including *GLUL*, *ASRGL*, *LAAO*, *SLC6A15s* and *SLC7A9* were up-regulated in the comparison of 0 vs 5 psu, which indicated that amino acid metabolism and transport were strengthened by salinity stress. The up-regulated expression of *GLUL* showed the increase of glutamine synthetase. Glutamine is the most prevalent amino acid in body fluids and muscle, is mainly transported by a Na^+-dependent neutral amino acid system, and its turnover rate exceeds those of other amino acids (*Zander et al., 2015*). Glutamine is synthesized from the ATP-dependent conjugation of ammonia to glutamate,

328 and is a well-known defense against ammonia (*Cooper & Plum 1987; Essexfraser et al., 2005*).
329 When salinity returns to normal, glutamine pools can be utilized as precursors for a variety of
330 important cell molecules (e.g. purines, pyrimidines, mucopolysaccharides) or, in the presence of
331 glutaminase, glutamine can be deaminated for direct excretion of ammonia in the kidney or used
332 for urea synthesis in liver before excretion. The accumulation of urea in response to high salinity
333 can be due to both urea retention and elevated rates of urea synthesis via the ornithine-urea cycle,
334 uricolysis of uric acid, or hydrolysis of arginine (*Dépêche & Schoffeniels 1975; Gordon &*
335 *Tucker 1965*). *OAZ3*, involved in the ornithine-urea cycle, showed up-regulation under salinity
336 exposure in this study, which suggested that ureogenesis may also be activated in response to
337 hyperosmotic conditions in this species. Our result correlated well with studies in crab-eating
338 frog (*Fejervarya cancrivora*), where its resistance to hyperosmotic environmental conditions is
339 generally linked to the accumulation of urea in plasma and tissues, urea resulting from an up-
340 regulation of the hepatic urea synthesis machinery (*Gordon & Tucker 1965*). Also, the striped
341 catfish (*Pangasianodon hypophthalmus*) can counteract osmotic imbalance by triggering a
342 regulatory volume increase, an internal process that initiates a net gain in osmolytes and/or water,
343 increasing cell volume that re-establishes normal values and prevents further cell shrinkage
344 (*Nguyen et al., 2016*).

345 The current study was designed not only to generate a catalogue of differentially expressed
346 genes involved with salinity exposure, but also to allow the data to be integrated to identify the
347 relationships on the adaptive response. Based on gene ontology information and from data in
348 published studies, functional categories of differentially expressed genes were identified.

Therefore, the potential interactions of the differentially expressed genes that responded to high salinity in *T. s. elegans* are shown in Figure. 2. The pathways and processes that are targeted provide us with numerous candidate genes for future investigations about the molecular mechanisms that underlie high salinity tolerance.

CONCLUSIONS

In this study, we report the first transcriptome analysis of *T. s. elegans* under salinity stress. When *T. s. elegans* was subjected with salinity exposure, 3393 unigenes in the liver were identified as DEGs, which were classified into some dominant categories including macromolecule metabolic process, ion transport, ion transmembrane transport in biological process. And a list of candidate DEGs potentially involved in salinity acclimation in *T. s. elegans* were identified into three kinds such as ion transport, energy production and conversion, and macromolecule metabolic process including lipid, protein and carbonhydrate.

The genes related to macromolecule metabolic process (*OAZ3*, *GLUL*, *ASRGL*, *LAAO*, *SLC6A15s*, *SLC7A9*, *APoE*, *CoQ10*, *SAA*, *HK*, and *MIP*), ion transport (*KCNH5*, *STK32*, *SIK1*, and *ACDC*) and ATP synthesis (*ATP6*, *COX1*, *COX3*, *CYTb*, and *CYP17A1*) were up-regulated by salinity stress, which indicated that *T. s. elegans* could adapt itself into salinity stress by balancing the entry of NaCl and accumulating urea and free amino acids in blood in response to osmotic pressure with higher ATP energy production. However, some genes related to macromolecule metabolic process (*ADH4*, *FADS6*, *LacD*, *GLO*, *RBCs*, *TAT*, and *ASS1*) and ion transport (*SCN1B*, *NPPA*, *SLC26A9*, and *STK33*) were down-regulated by salinity stress. Finally, we combined the data on functional salinity tolerance genes into a hypothetical schematic model

370 that describe potential relationships and interactions among target genes to explain the molecular
371 pathways related to salinity responses in *T. s. elegans*.

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375 **Data Availability**

376 The following information was supplied regarding data availability:

377 The raw data has been supplied as a Supplemental Dataset File
378 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE117354).

379

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Supplemental Information

Table S1 Major GO terms related to osmotic regulation in 0 vs 5 psu groups

Table S2 Major GO terms related to osmotic regulation in 0 vs 15 psu groups

Fig S1 The relative fold changes (log_2FC) of RNA-seq and qRT-PCR results in the comparison of 0 vs 5 psu (A), and 0 vs 15 psu (B). The expression levels of selected genes were each normalized to that of the *β-actin* gene.

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Table 1(on next page)

Table 1 Sequences of primers for qRT-PCR validation

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Table 1 Sequences of primers for qRT-PCR validation

Gene	Forward primer (5' to 3')	Reserse primer (5' to 3')	Product length (bp)
COX3	TCACTTGAGCCCACCATAGC	AGAGCCGTACACACCATCAG	155
ATP6	CTTGATGCCCTCTTCCCGTG	TTCCTCGTTCTCCACAGCCT	145
CYP17A1	GATCGGCTTCGAGAGACACC	GGATCAGCAGAGGGGAGACA	110
APoE	GTCTGGAGCGGGCTTAGTAG	CATTCCCAGGTCTCCCACAG	117
CoQ10	GCGAGTGCTGGGCTACT	TGAGCCCTTTGCGGTAGG	119
SAA	TCTAGGCGCTGGGGATATGT	CCACTAATGCCATCCTGCCA	180
FADS6	GCTGCCATACAACGAGGACT	AGCCCTATGTCTTGCTGTCG	146
HK	TAAAGGCGTAACCAGGCTGC	AATCGCACGTCAGAGTCAGG	126
GCK	CGGGAACTGCTGAAATGCTC	GAATGTGAAGCCCAGAGGCA	104
GLUL	GTTGCCACACCAACTTCAGC	AAGCGCGGATATGGTACTGG	111
ASRGL	TAGCACCTGTTCCAGTGAGC	GCTGTGTTTGATGCAGGTCA	193
TAT	CATCCACCAGCGACTCCAAG	CATCCTGGTGCCAAGACCTG	137
ASS1	CGGGCTGTACCAGAAACCAT	GGGACCATCCTGTACCATGC	129
STK32	TCCAGTGCTAATGCCAGCTC	TGGAACACCCCTTCCTGGTT	172
SIK1	TGGTGTGGTGCTGTATGTCC	TCCACAACTAGCATCCGTCG	156
SIK2	GCTGGTCCTAGACCCATCCA	GAAGGCTCGTTCTCCTGTCC	123
INSRR	CCGAGTACCGTGATCTGCTC	GGCAGCTCCACATCTACCAC	127
STK33	AGGCAGTTTTGGGGTGGTAA	TAAGATGCTCACCTCCCGTTC	130
β-actin	GCACCCTGTGCTGCTTACA	CACAGTGTGGGTGACACCAT	190

2

Table 2(on next page)

Table 2 Summary of Illumina expression short reads production and filtering

Table 2 Summary of Illumina expressed short reads production and filtering

	Groups	Total_Reads	Total_Bases	Error%	Q20%	Q30%	GC%
Raw data	0 psu	50681398	7602209700	0.0126	96.31	92.31	49.34
	5 psu	57912616	8686892400	0.0121	96.72	92.87	49.18
	15 psu	48616356	7292453400	0.0129	96.21	91.98	49.93
Clean data	0 psu	48918412	7157032240	0.0104	98.29	95.12	49.19
	5 psu	56498176	8281257833	0.0103	98.34	95.18	49.09
	15 psu	47101028	6881004183	0.0107	98.17	94.77	49.83

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Table 3(on next page)

Table 3 Summary of *de novo* assembly results of illumina sequence data

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Table 3 Summary of de novo assembly results of Illumina sequence data

Type	unigene	transcripts
Total sequence number	205138	244815
Total sequence base	127206404	185942194
Percent GC	46.09	46.65
Largest	22866	22866
Smallest	201	201
Average	620.1	759.52
N50	964	1535
N90	249	268

2

Table 4(on next page)

Table 4 The functional annotation of unigenes

Table 4 Functional annotation of the *Trachemys scripta elegans* transcriptome

Annotated Database	Number of Unigenes	Precent(%)
Pfam	20458	9.97
KEGG	20421	9.95
GO	13362	6.51
COG	10673	5.2
Swissprot	25438	12.36
NR	38651	18.84
Total	205138	1

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Table 5(on next page)

Table 5 Summary of GO term enrichment results on ion-regulation and macromolecular metabolism in *T. s. elegans* under salinity stress

Table 5 Summary of GO term enrichment results on ion-regulation and macromolecular metabolism in *T. s. elegans* under salinity stress

GO ID	Description	Ratio in study	Ratio in pop	*p*-value (FDR)
GO:0006091	generation of precursor metabolites and energy	17/357	119/13362	4.33E-05
GO:0009060	aerobic respiration	7/357	17/13362	0.0003
GO:0045333	cellular respiration	7/357	19/13362	0.0004
GO:0015980	energy derivation by oxidation of organic compounds	8/357	40/13362	0.0065
GO:0034220	ion transmembrane transport	24/357	336/13362	0.0081
GO:0098660	inorganic ion transmembrane transport	18/357	209/13362	0.0081
GO:0098655	cation transmembrane transport	18/357	210/13362	0.0081
GO:0006119	oxidative phosphorylation	4/357	7/13362	0.0081
GO:1902991	regulation of amyloid precursor protein catabolic process	3/357	3/13362	0.0081
GO:1902992	negative regulation of amyloid precursor protein catabolic process	3/357	3/13362	0.0081
GO:1902430	negative regulation of beta-amyloid formation	3/357	3/13362	0.0081
GO:1902003	regulation of beta-amyloid formation	3/357	3/13362	0.0081
GO:0006811	ion transport	35/357	623/13362	0.0109
GO:0006812	cation transport	23/357	335/13362	0.0114
GO:1902600	hydrogen ion transmembrane transport	10/357	77/13362	0.0117
GO:1900221	regulation of beta-amyloid clearance	3/357	4/13362	0.0177
GO:0098662	inorganic cation transmembrane transport	15/357	176/13362	0.0178
GO:0050773	regulation of dendrite development	6/357	28/13362	0.0184
GO:0015672	monovalent inorganic cation transport	14/357	171/13362	0.0410
GO:0005576	extracellular region	18/357	220/13362	0.0097
GO:0044463	cell projection part	16/357	206/13362	0.0281
GO:0015078	hydrogen ion transmembrane transporter activity	11/357	76/13362	0.0047
GO:0015077	monovalent inorganic cation transmembrane transporter activity	16/357	176/13362	0.0083
GO:0022890	inorganic cation transmembrane transporter activity	18/357	236/13362	0.0167
GO:0008324	cation transmembrane transporter activity	20/357	293/13362	0.0268
GO:1902991	regulation of amyloid precursor protein catabolic process	3/357	3/13362	0.0081

GO:1902992	negative regulation of amyloid precursor protein catabolic process	3/357	3/13362	0.0081
GO:0034364	high-density lipoprotein particle	5/357	13/13362	0.0081
GO:0032994	protein-lipid complex	5/357	16/13362	0.0129
GO:1990777	lipoprotein particle	5/357	16/13362	0.0129
GO:0034358	plasma lipoprotein particle	5/357	16/13362	0.0129

1 GO names were retained only from GO terms of levels > 2

Table 6(on next page)

Table 6 DEGs related to energy production and conversion, macromolecule metabolic process, and ion transport in the liver of *T. s. elegans*

Table 6 DEGs related to energy production and conversion, macromolecule metabolic process, and ion transport in the liver of *T. s. elegans*

Unigene ID	Description	Log_2 (5/0psu)	Log_2 (15/0psu)	Log_2 (15/5psu)
DEGs related to energy production and conversion				
c198757_g1	cytochrome c oxidase subunit I (*COX1*)	7.03	7.14	—
c205807_g1	cytochrome c oxidase subunit III (*COX3*)	5.79	5.57	—
c184332_g1	cytochrome b (*CYTb*)	6.37	6.51	—
c168776_g1	F-ATPase protein 6 (*ATP6*)	7.93	8.41	0.67
c122818_g1	cytochrome P450 17A1 (*CYP17A1*)	0.81	1.63	0.82
DEGs related to lipid metabolic process				
c183562_g1	apolipoprotein E precursor (*APoE*)	4.59	4.18	-0.40
c108401_g1	coenzyme Q-binding protein (*CoQ10*)	2.51	3.44	0.93
c106996_g1	high-density lipoprotein particle (*SAA*)	1.24	4.13	2.89
c101752_g1	alcohol dehydrogenase 4 (*ADH4*)	-3.8	—	3.81
c5275_g1	fatty acid desaturase 6 (*FADS6*)	-3.38	-1.54	1.85
DEGs related to carbohydrate metabolic process				
c184502_g1	hexokinase-1 (*HK*)	4.49	5.22	0.73
c141564_g1	lens fiber major intrinsic protein (*MIP*)	3.37	3.75	—
c121000_g1	glucokinase (*GCK*)	-0.22	-1.94	-1.85
c198426_g1	tagatose 1,6-diphosphate aldolase (*LacD*)	-5.38	-5.12	—
c139209_g1	L-gulono-gamma-lactone oxidase (*GLO*)	-5.38	-5.12	—
c44501_g1	ribulose bisphosphate carboxylase small chain (*RBCs*)	-4.38	-4.12	—
DEGs related to amino acid metabolic process				
c198734_g1	ornithine decarboxylase antizyme 3 (*OAZ3*)	6.28	7.00	0.72
c184545_g1	glutamine synthetase (*GLUL*)	4.03	4.3	0.26
c197142_g1	asparaginase-like protein 1b (*ASRGL*)	4.49	4.96	0.47
c117856_g3	L-amino-acid oxidase-like (*LAAO*)	1.62	-2.06	-3.68
c169209_g1	sodium-dependent neutral amino acid transporter B (*SLC6A15s*)	3.59	—	-1.54
c103361_g1	amino acid permease (*SLC7A9*)	1.78	5.72	3.93
c108456_g1	tyrosine aminotransferase (*TAT*)	-1.72	-1.41	0.31
c99414_g1	argininosuccinate synthase (*ASS1*)	-0.69	-1.1	-0.41
DEGs related to ion transport				
c182997_g1	potassium voltage-gated channel subfamily H member 5 (*KCNH5*)	3.5	—	-3.5
c121057_g1	serine/threonine-protein kinase 32 (*STK32*)	1.95	1.80	—
c121806_g3	salt-inducible kinase 1 (*SIK1*)	1.23	2.20	0.97
c123793_g3	salt-inducible kinase 2 (*SIK2*)	—	0.27	0.40
c114797_g4	adiponectin (*ACDC*)	0.60	2.12	1.52

c119528_g1	insulin receptor-related protein (*INSRR*)	0.30	-1.70	-2.00
c95283_g1	serine/threonine-protein kinase 33 (*STK33*)	-1.5	-0.65	0.85
c58533_g1	sodium channel subunit beta-1 (*SCN1B*)	-5.31	-5.31	—
c94169_g1	natriuretic peptides A-like (*NPPA*)	-5.72	-5.72	—
c95302_g1	solute carrier family 26 member 9 (*SLC26A9*)	-3.55	-0.44	3.11

Note: The values above zero show up-regulation of gene expression, while the values below zero show down-regulation. "—" means that the level of gene expression is so low that it could not be detected.

Table 7 (on next page)

Table 7 The expression levels of some genes in relation to osmotic adjustment in the liver of *T. s. elegans* by qRT-PCR

Table 7 The expression levels of some genes in relation to osmotic adjustment in the liver of *T. s. elegans* by qRT-PCR

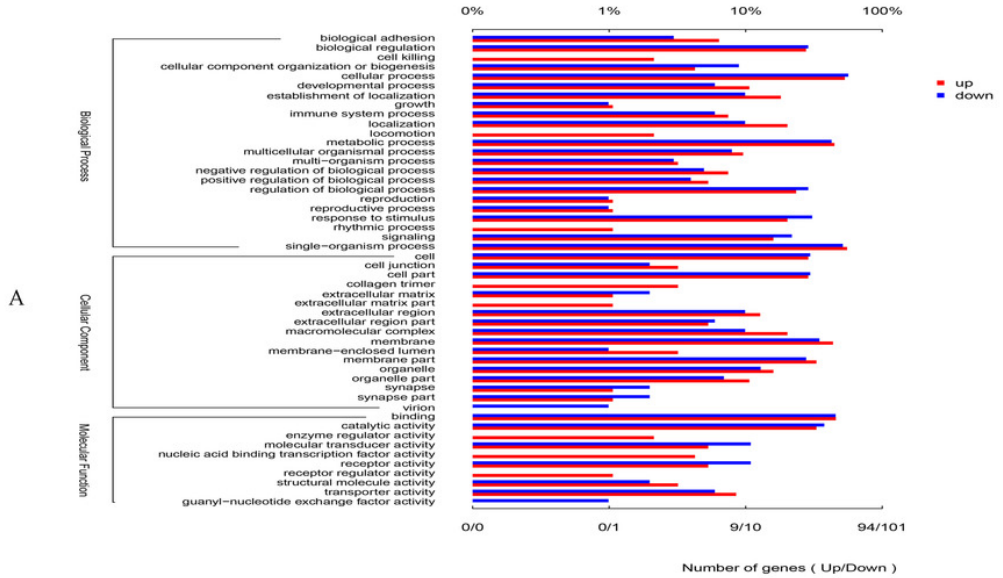
Unigene ID	Description	Control	5 psu	15 psu
Genes related to energy production and conversion				
c205807_g1	cytochrome c oxidase subunit III (*COX3*)	1.01±0.06 ^{c}	3.57±0.27 ^{a}	2.40±0.29 ^{b}
c168776_g1	F-ATPase protein 6 (*ATP6*)	1.09±0.10 ^{c}	2.42±0.09 ^{b}	3.67±0.21 ^{a}
c122818_g1	cytochrome P450 17A1 (*CYP17A1*)	2.13±0.11 ^{c}	3.15±0.22 ^{b}	4.46±0.38 ^{a}
Genes related to lipid metabolic process				
c183562_g1	apolipoprotein E precursor (*APoE*)	0.86±0.06 ^{c}	2.77±0.12 ^{a}	1.80±0.13 ^{b}
c108401_g1	coenzyme Q-binding protein (*CoQ10*)	0.64±0.08 ^{c}	2.66±0.17 ^{b}	5.39±0.23 ^{a}
c106996_g1	high-density lipoprotein particle (*SAA*)	0.48±0.06 ^{c}	1.18±0.08 ^{b}	7.32±0.27 ^{a}
c5275_g1	fatty acid desaturase 6 (*FADS6*)	2.23±0.27 ^{a}	0.97±0.23 ^{b}	1.37±0.13 ^{b}
Genes related to carbohydrate metabolic process				
c184502_g1	hexokinase-1 (*HK*)	1.37±0.09 ^{c}	2.35±0.21 ^{b}	3.27±0.22 ^{a}
c121000_g1	glucokinase (*GCK*)	1.79±0.07 ^{a}	1.19±0.22 ^{b}	0.48±0.05 ^{c}
Genes related to amino acid metabolic process				
c184545_g1	glutamine synthetase (*GLUL*)	1.82±0.14 ^{c}	5.10±0.23 ^{b}	6.00±0.19 ^{a}
c197142_g1	asparaginase-like protein 1b (*ASRGL*)	1.01±0.05 ^{b}	1.64±0.17 ^{a}	1.94±0.31 ^{a}
c108456_g1	tyrosine aminotransferase (*TAT*)	3.37±0.17 ^{a}	0.93±0.03 ^{c}	1.38±0.06 ^{b}
c99414_g1	argininosuccinate synthase (*ASS1*)	5.16±0.29 ^{a}	2.59±0.20 ^{b}	0.53±0.07 ^{c}
Genes related to ion transport				
c121806_g3	salt-inducible kinase 1 (*SIK1*)	1.19±0.13 ^{c}	2.46±0.31 ^{b}	4.16±0.15 ^{a}
c123793_g3	salt-inducible kinase 2 (*SIK2*)	1.06±0.15 ^{a}	0.85±0.09 ^{a}	1.13±0.10 ^{a}
c119528_g1	insulin receptor-related protein (*INSRR*)	2.04±0.18 ^{b}	4.13±0.26 ^{a}	0.78±0.13 ^{c}
c121057_g1	serine/threonine-protein kinase 32 (*STK32*)	1.48±0.16 ^{c}	5.39±0.24 ^{a}	4.49±0.33 ^{b}
c95283_g1	serine/threonine-protein kinase 33 (*STK33*)	3.93±0.37 ^{a}	1.79±0.16 ^{b}	2.57±0.23 ^{b}

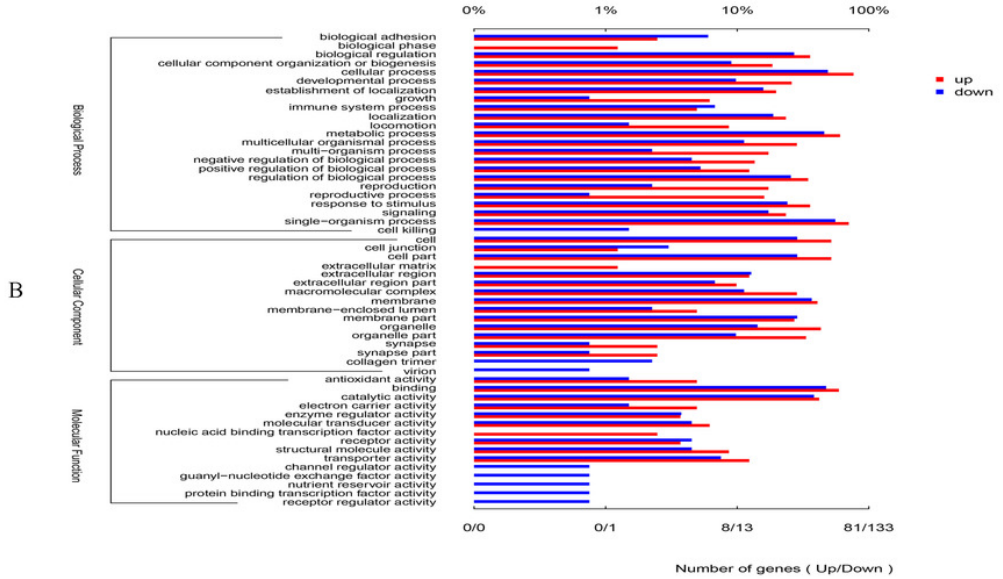
1 Different lowercase letters represent significance among different groups ($p < 0.05$).

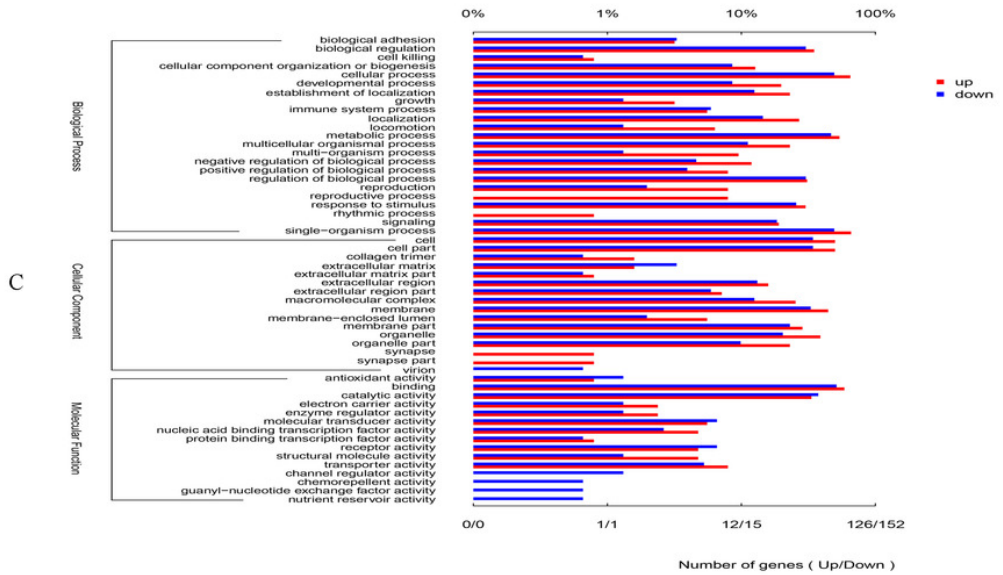
Figure 1

Figure 1 GO categories in the comparison of 0 vs 5 psu (A), 0 vs 15 psu (B), and 5 vs 15 psu (C)

All genes were divided into several functional groups within three categories: cellular component, molecular function, and biological process. The below x-axis indicated the number of genes in each category, while the above x-axis indicated the percentage of total genes in that category.


A
0%
1%
10%
100%
biological adhesion
biological regulation
cell killing
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
growth
immune system process
localization
locomotion
metabolic process
multicellular organismal process
multi−organism process
negative regulation of biological process
positive regulation of biological process
regulation of biological process
reproduction
reproductive process
response to stimulus
rhythmic process
signaling
single−organism process
cell
cell junction
cell part
collagen trimer
extracellular matrix
extracellular matrix part
extracellular region
extracellular region part
macromolecular complex
membrane
membrane−enclosed lumen
membrane part
organelle
organelle part
synapse
synapse part
virion
binding
catalytic activity
enzyme regulator activity
molecular transducer activity
nucleic acid binding transcription factor activity
receptor activity
receptor regulator activity
structural molecule activity
transporter activity
guanyl−nucleotide exchange factor activity
Biological Process
Cellular Component
Molecular Function
up
down
0/0
0/1
9/10
94/101
Number of genes (Up/Down)


B
0%
1%
10%
100%
biological adhesion
biological phase
biological regulation
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
growth
immune system process
localization
locomotion
metabolic process
multicellular organismal process
multi−organism process
negative regulation of biological process
positive regulation of biological process
regulation of biological process
reproduction
reproductive process
response to stimulus
signaling
single−organism process
cell killing
cell
cell junction
cell part
extracellular matrix
extracellular region
extracellular region part
macromolecular complex
membrane
membrane−enclosed lumen
membrane part
organelle
organelle part
synapse
synapse part
collagen trimer
virion
antioxidant activity
binding
catalytic activity
electron carrier activity
enzyme regulator activity
molecular transducer activity
nucleic acid binding transcription factor activity
receptor activity
structural molecule activity
transporter activity
channel regulator activity
guanyl−nucleotide exchange factor activity
nutrient reservoir activity
protein binding transcription factor activity
receptor regulator activity
Biological Process
Cellular Component
Molecular Function
up
down
0/0
0/1
8/13
81/133
Number of genes (Up/Down)


C
0%
1%
10%
100%
biological adhesion
biological regulation
cell killing
cellular component organization or biogenesis
cellular process
developmental process
establishment of localization
growth
immune system process
localization
locomotion
metabolic process
multicellular organismal process
multi−organism process
negative regulation of biological process
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extracellular region
extracellular region part
macromolecular complex
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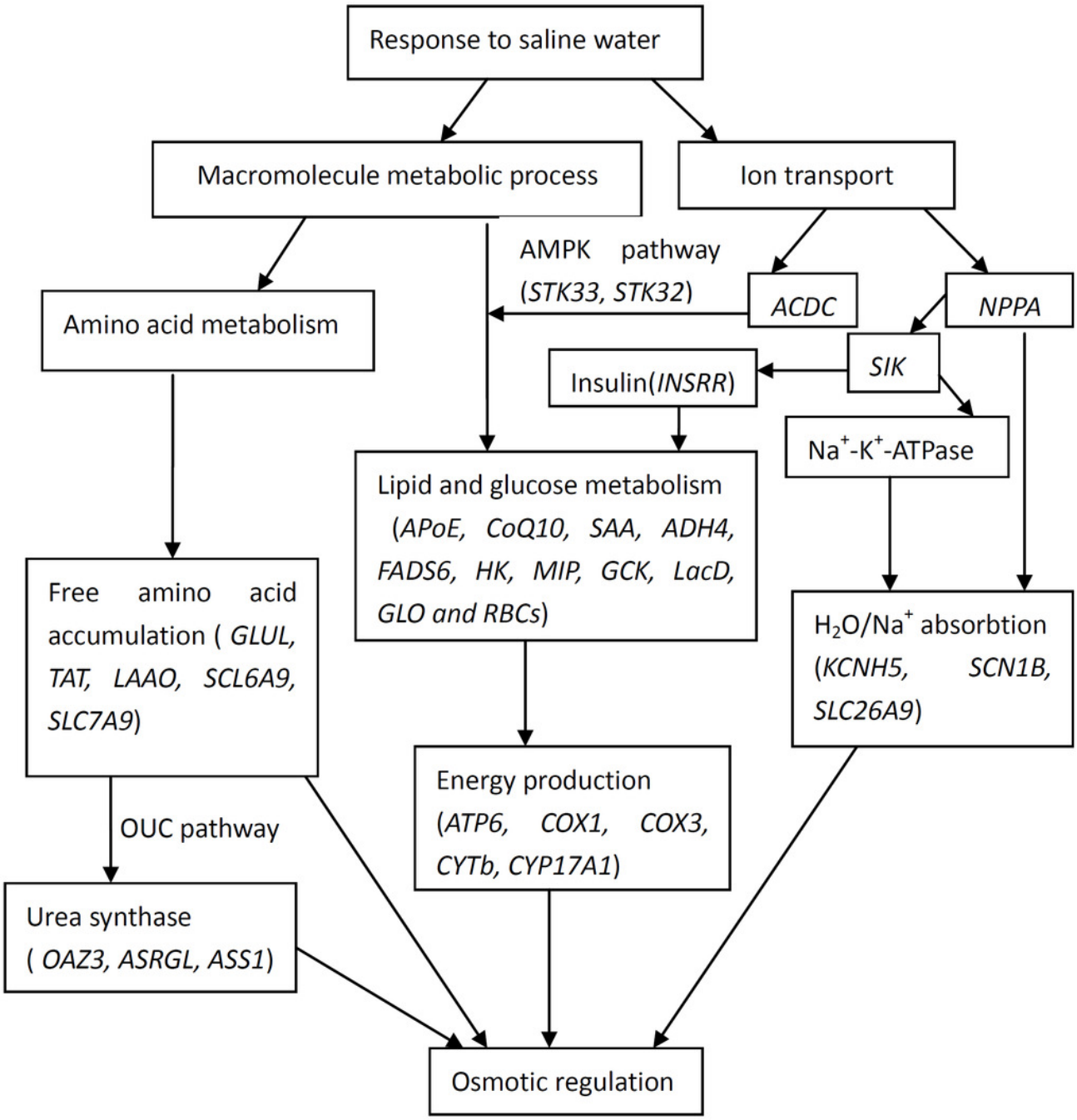
membrane−enclosed lumen
membrane part
organelle
organelle part
synapse
synapse part
virion
antioxidant activity
binding
catalytic activity
electron carrier activity
enzyme regulator activity
molecular transducer activity
nucleic acid binding transcription factor activity
protein binding transcription factor activity
receptor activity
structural molecule activity
transporter activity
channel regulator activity
chemorepellent activity
guanyl−nucleotide exchange factor activity
nutrient reservoir activity
Biological Process
Cellular Component
Molecular Function
up
down
0/0
1/1
12/15
126/152
Number of genes (Up/Down)

Figure 2

Fig 2. Interactions of positively selected genes and differentially expressed genes involved in the adaptation of *T. s. elegans* to high salinity

(1) *NPPA* plays roles in the regulation of body fluid levels and electrolytic homeostasis pathway, while *ACDC* and *SIK* in the pathway of lipid (glucose) metabolism by the regulation of *AMPK* (*STK32*, *STK33*); (2) *GLUL*, *TAT*, *LAAO*, *SCL6A9*, *SLC7A9 and OAZ3*, *ASRGL*, *ASS1* play roles in the accumulation of free amino acid and urea, while *KCNH5*, *SCN1B*, *SLC26A9* mainly in the process of H_2O/Na^+ absorption; (3) *ATP6*, *COX1, COX3*, *CYTb*, *CYP17A1* are associated with energy production and mediated by lipid and glucose metabolism (*APoE*, *CoQ10*, *SAA*, *ADH4*, *FADS6*, *HK*, *MIP*, *GCK*, *LacD*, *GLO and RBCs*).


Response to saline water
Macromolecule metabolic process
Ion transport
AMPK pathway
(STK33, STK32)
ACDC
NPPA
Amino acid metabolism
SIK
Insulin(INSRR)
Na+-K+-ATPase
Lipid and glucose metabolism
(APoE, CoQ10, SAA, ADH4, FADS6, HK, MIP, GCK, LacD, GLO and RBCs)
Free amino acid accumulation (GLUL, TAT, LAAO, SCL6A9, SLC7A9)
H2O/Na+ absorbtion
(KCNH5, SCN1B, SLC26A9)
Energy production
(ATP6, COX1, COX3, CYTb, CYP17A1)
OUC pathway
Urea synthase
(OAZ3, ASRGL, ASS1)
Osmotic regulation