Transcriptomes and molecular pathway analysis of the liver in the red-eared slider turtle *Trachemys scripta elegans* under chronic salinity stress (#31053)

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Transcriptomes and molecular pathway analysis of the liver 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 investigation has 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 discover the gene expression profiles and pathways that responded to elevated salinity in the liver of *T. s. elegans* exposed to 0, 5, or 15 psu (practical salinity units) for 30 days. A total of 157.21 million reads were obtained and assembled into 205,138 unigenes with an average length of 620 bp and N50 of 1,535 bp. Of these, 1,019 and 1,194 genes were significantly differentially expressed in the 0 vs 5 psu and 0 vs 15 psu groups, respectively. GO analysis identified 122 and 106 significantly enriched GO terms in 0 vs 5 psu and 0 vs 15 psu groups, respectively. Major GO terms and important genes related to osmotic adjustment among these significant enrichments were selected in order to understand their implications for salinity adaptation. KEGG analysis also illuminated immunity-related pathways as being involved in salinity adaptation including cell adhesion molecules, antigen processing and presentation, phagosome, hematopoietic cell lineage, and natural killer cell mediated cytotoxicity. The expression patterns of nine differentially expressed genes were validated by quantitative real-time PCR (qRT-PCR) yielding mean correlation coefficients of 0.743 and 0.862 in 0 vs 5 and 0 vs 15 psu groups, respectively. 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 investigation has 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 ~~discover~~, the gene expression profiles and pathways ~~that responded to elevated salinity in the liver of *T. s. elegans* exposed to 0, 5, or 15 psu (practical salinity units) for 30 days.~~ A total of 157.21 million reads were obtained and assembled into 205,138 unigenes with an average length of 620 bp and N50 of 1,535 bp. Of these, 1,019 and 1,194 genes were significantly differentially expressed in the 0 vs 5 psu and 0 vs 15 psu groups, respectively. GO analysis identified 122 and 106 significantly enriched GO terms in 0 vs 5 psu and 0 vs 15 psu groups, respectively. Major GO terms and important genes related to osmotic adjustment among these significant enrichments were selected in order to understand their implications for salinity adaptation. KEGG analysis ~~also illuminated~~ immunity-related pathways as being involved in salinity adaptation including cell adhesion molecules, antigen processing and presentation, ~~phagosome, hematopoietic cell lineage,~~ and natural killer cell mediated cytotoxicity. The expression patterns of nine differentially expressed genes were validated by quantitative real-time PCR (qRT-PCR) yielding mean correlation coefficients of 0.743 and 0.862 in 0 vs 5 and 0 vs 15 psu groups, respectively. 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.

Subjects Ecology, Physiology, Zoology, Invasive biology

Keywords ~~Transcriptome, salinity stress,~~ gene expression, invasive species, ~~*Trachemys scripta elegans*~~

INTRODUCTION

The red-eared slider turtle (*Trachemys scripta elegans*) has been introduced into diverse aquatic habitats worldwide (including many countries in Africa, Asia, Europe and 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 ~~2~~ states of northeastern Mexico (*Mittermeier et al., 2015*) but has recently been found to lay eggs in the low salinity (0.1-26 ‰) estuary of the Nandu River in Hainan province, China (*Liu et al., 2011; Yang & Shi 2014*). ~~However,~~ 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. ~~The amount of fresh water comprises only 2.5% of the available water resources on the planet (*Foundation 1994*),~~ therefore the invasion potential and the mechanism of response to salinity adaptation of *T. s. elegans* is of serious

concern.

Changing levels of salinity is a crucial environmental stress factor for aquatic species ~~and~~ can disrupt electrolyte balance, cell energetics, and various other physiological responses, including activating stress hormones (*Li et al., 2015; Lushchak 2011*). Species show altered composition and osmolality of body fluids in response to changing salinity (*Charmantier et al., 2011*) and adaptation to salinity change typically involves two physiological solutions: 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 synthesis (*Wright et al., 2004*). Accumulation of intracellular free amino acids via hepatic protein dehydration or 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) (*Shu et al., 2012*). Our previous studies have shown that *T. s. elegans* can increase blood osmotic pressure by balancing the entry of sodium chloride with a decrease in secretion of aldosterone, and by accumulating plasma urea for osmoregulation effects 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 use 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. ~~The~~ results provide insights into the molecular mechanisms underlying osmoregulation in *T. s. elegans* and ~~comment on~~ the potential for this species to further invade and spread through new aquatic and brackish territories.

MATERIALS AND METHODS

Animals

Healthy *T. s. elegans* were obtained from a local turtle farm in Hainan, China and were acclimated in three cement pools half-filled with freshwater for ~~2~~ weeks. After this time, nine healthy *T. s. elegans* (BW: 424-478g, 2 years old) were divided into ~~3~~ groups in pools (190 cm × 65 cm × 32 cm) of differing salinity: one in freshwater serving as the control (0 practical salinity units, psu), and the other two challenged with 5 ‰ (5 psu) or 15 ‰ (15 psu) saltwater. Turtles were fed a commercial diet each Monday and Thursday and 24 h after feeding, unused feed was siphoned out followed by replacement of one-third of the water in each tank volume. Water salinity was measured every day and then adjusted to the proper salinity as needed. Other water quality parameters were monitored 2-3 times a week with steady values of pH 7.5-7.9, total ammonia

99 nitrogen of <0.02 mg L^{-1} and temperature 26-28℃. Photoperiod was 12h: 12h L: D throughout.

100 After eight weeks of acclimation to the three experimental conditions, the three turtles from
101 each group were subjected to a 24 h, then anesthetized by storing at -20℃ cryo-anesthesia for
102 0.5-1 h. Experimental animal procedures had the prior approval of the Animal Research Ethics
103 Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal
104 University (permit no. HNECEE-2017-002). Following euthanasia, livers were sampled, flash
105 frozen in liquid nitrogen, and stored at -80°C until used for RNA extraction. Total RNA was mixed
106 from each group of 3 turtles for RNA-seq analysis. Another set of 3 turtles from each pool were
107 used to provide liver samples for quantitative real-time PCR (qRT- PCR). The University
108 Committee on the use and care of laboratory animals.

109 Total RNA Extraction, library construction and sequencing

110 Extraction of total RNA from liver samples used TRIzol® Reagent, following manufacturer's
111 instructions. Total RNA purity and concentration were determined using a NanoDrop 2000. The
112 sequencing library was then constructed from high-quality RNA ($OD_{260/280}$=1.8-2.2,
113 $OD_{260/230}$≥1.5, RIN≥8.0, 28S:18S≥1.0, >10 μg). RNA-seq analysiswas provided by Novel
114 Bioinformatics Co., Ltd using the Sanger/Illumina method. Subsequently, cDNA libraries were
115 made using the Hiseq4000 Truseq SBS Kit v3-HS using 5 μg total RNA, following manufacturer's
116 instructions. Poly(A) mRNA was isolated with Dyabeads (Life Technologies, USA), fragmented
117 with RNaseIII and purified. The fragmented RNA was added and ligated with ion adaptor. Then
118 double-stranded cDNA was synthesized and purified using magnetic beads. The molar

119 concentration of the purified cDNA in each cDNA library was then quantified with a TBS-380
120 fluorometer using Picogreen. The paired-end RNA-seq library was then sequenced with an
121 Illumina HiSeq 4000. The accession number of RNA-Seq is GSE117354
122 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117354).

123 ## De novo assembly and annotation

124 Raw reads were trimmed and quality controlled using SeqPrep (https://
125 github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) ~~with~~ default
126 parameters. High-quality trimmed sequences were used for sequence assembly ~~by~~ Trinity
127 (https://github.com/trinityrnaseq/trinityrnaseq/wiki) after trimming of raw reads (*Grabherr et al.,*
128 *2011*). All assembled transcripts ~~were searched~~ against the NCBI protein nonredundant (NR),
129 String, Swissprot, and KEGG databases ~~by BLASTX to assign identities to the transcripts and then~~
130 ~~retrieve function annotation~~. A typical cut-off E-value was set at <1.0×10-5. The BLAST2GO
131 (http://www.blast2go.com/b2ghome) program (*Conesa et al., 2005*) was used to obtain ~~GO~~
132 annotations of unique assembled transcripts for describing biological processes, cellular
133 components and molecular functions. KEGG pathway analysis used KEGG databases
134 (http://www.genome.jp/kegg/)(*Kanehisa & Goto 2002*).

135 ## Differentially expressed analysis and functional enrichment

136 Expression level of transcripts were calculated as fragments per kilobase of exon per million
137 mapped reads (FPKM). RNA-Seq by Expectation-Maximization (RSEM;
138 http://deweylab.biostat.wisc.edu/rsem/) was used to quantify gene transcripts, and edgeR

(http://www.bioconductor.org/packages/2.12/ bioc/html/edgeR.html) (*Robinson et al., 2009*) was used to conduct differential expression analysis. For functional-enrichment ~~analysis~~ we used GO (using ~~Go~~atools https://github.com/tanghaibao/GOatools) and KEGG (using KOBAS http://kobas.cbi.pku.edu.cn/home.do) (*Xie et al., 2011*). Statistical ~~analysis~~ of enrichment was carried out through Fisher's exact tests. Corrected p-values less than 0.05 for GO or KEGG analysis was considered significant enrichment.

Experimental validation by qRT-PCR

Nine genes identified as significantly expressed from the KEGG pathways were randomly selected and used for validation by qRT-PCR. Table 1 shows the specific primers used. TRIzol® Reagent (Invitrogen, Carlsbad, USA) was used to extract total RNA from liver, followed by reverse-transcription using First-strand cDNA ~~synthesis kit~~ (Invitrogen, Carlsbad, US). The qRT-PCR for gene expression was analyzed by an Applied Biosystems 7500 Fast Real-Time PCR System in 96-well plates in a 20 μl reaction volume containing 1×SYBR Green qPCR Master Mix, gene-specific forward and reverse primers (0.4 μM) and cDNA (8 ng). The cycling conditions were 95℃ for 2 min followed by 40 cycles of 95℃ for 5 s and 60℃ for 30 s. The reference gene was *β-actin* and relative fold changes were determined using Relative Expression Software Tool v.2009 based on the cycle threshold (Ct) values generated by qRT-PCR.

RESULTS

Sequencing and de novo assembly

158 A total of 157.21 million reads and 23.58 billion bases were obtained from the liver
159 transcriptome of *T. s. elegans*, including 50.68 million in 0 psu, 57.91 million in 5 psu and
160 48.62 million in 15 psu groups (Table 2). After ~~quality trimming and adapter clipping,~~ 152.52
161 million reads (97.02% of total reads) were ~~obtained~~ including 48.92 million in 0 psu, 56.50
162 million in 5 psu and 47.10 million in 15 psu (Table 2). Subsequently, 205,138 unigenes
163 (average length 620 bp) were obtained by de novo assembly ~~with Trinity software after~~
164 ~~splicing and removal of redundant sequences~~. The largest and smallest unigenes were 22,866
165 bp and 201 bp, respectively (Table 3).

166 ## Annotation and differential expression of genes

167 ~~Analysis of the annotated sequences revealed a total of 15,565 (7.59%), 16,603 (8.09%),~~
168 ~~12,825 (6.25%), 23,298 (11.36%) and 35,572 (17.34%) sequences that were unambiguous~~
169 ~~alignments relative to the reference after BLASTx searches against the Pfam, KEGG, String,~~
170 ~~Swissprot or NR databases, respectively~~ (Table 4).

171 In total, 2203 genes were significantly up- or down-regulated (FDR < 0.05 & log2|FC|
172 ⩾1), including 1019 in 0 vs 5 psu and 1194 genes in 0 vs 15 psu. In the 0 vs 5 psu group
173 445 genes were significantly up-regulated and 574 genes were significantly down-regulated
174 (Fig. 1A). Similarly, 526 genes were significantly up-regulated and 668 genes were
175 significantly down-regulated in the 0 vs 15 psu group (Fig. 1B). Further analysis revealed,
176 374 genes increased and 423 decreased ~~in common in both salinity treatments~~.

Gene Ontology

Gene Ontology (GO) annotation by BLAST2GO was used to identify differentially expressed genes. In total, 1251 and 1064 GO terms were obtained in 0 vs 5 psu and 0 vs 15 psu groups, respectively. In the 0 vs 5 psu comparison, there were 929 (74.26%) biological process terms, 112 (8.95%) cellular component terms, and 210 (16.79%) molecular function terms. In the 0 vs 15 psu comparison, there were 752 (70.68%) biological process terms, 108 (10.15%) cellular component terms, and 204 (19.17%) molecular function terms.

There were 122 and 106 significantly enriched GO terms in 0 vs 5 psu and 0 vs 15 psu groups, respectively. As for osmotic adjustment, there were 15 GO terms that showed significant enrichment related to osmotic function in liver of 0 vs 5 psu (Table 5) and 12 in the 0 vs 15 psu comparison (Table 6) turtles. Commonly enriched GO terms in both salinity treatments were ion binding (GO: 0043167), hydrogen ion transmembrane transporter activity (GO: 0015078), hydrogen ion transmembrane transport (GO: 1902600), hormone activity (GO: 0005179), monovalent inorganic cation transport (GO: 0015672), anion binding (GO: 0043168) and ion transport (GO: 0006811). Important genes in relation to osmotic adjustment in these significantly enriched GO terms included: *TAT* (tyrosine aminotransferase), *STK* (serine/threonine-protein kinase), *SIK* (salt inducible kinase), *APOE* (apolipoprotein E), *glnA/GLUL* (glutamine synthetase), *NPPA* (natriuretic peptide A), *ACDC* (adiponectin), *SCN1B* (voltage-gated sodium channel type I beta), *KCNH5* (potassium voltage-gated channel Eag-related subfamily H member 5), *INSRR* (insulin receptor-related receptor), *GCK* (glucokinase), *CYP17A* (steroid 17 alpha-monooxygenase

197 /17 alpha-hydroxyprogesterone deacetylase), *ASS1* (argG, argininosuccinate synthase), *GST*
198 (glutathione S-transferase) and *OAZ3* (ornithine decarboxylase antizyme 3).

199 KEGG pathway

200 Differentially expressed genes identified via the KEGG database fell into five branches: (a)
201 metabolism, (b) genetic information processing, (c) environmental information processing,
202 (d) cellular processes and (e) organismal systems (Fig. 2). Within the organismal systems
203 branch, a subset of genes were involved in categories of metabolism of other amino acids
204 (111 genes), lipid metabolism (577 genes), glycan biosynthesis and metabolism (349 genes),
205 energy metabolism (532 genes), carbohydrate metabolism (468 genes), and amino acid
206 metabolism (596 genes), respectively. In addition, 2171, 1012, 1089 and 1159 genes were
207 involved in signal transduction, transport and catabolism, endocrine system, and immune
208 system, respectively.

209 Significant enrichments of pathways with differentially expressed genes were obtained from
210 KEGG pathways. Only four and two pathways of immunity-related genes were significantly
211 enriched (Corrected $P < 0.05$) in 0 vs 5 psu and 0 vs 15 psu, respectively (Table 7). The immunity-
212 related pathways found were cell adhesion molecules (CAMs), phagosome, and antigen processing
213 and presentation in the 0 vs 5 psu group, and hematopoietic cell lineage in the 0 vs 15 psu group,
214 as well as natural killer cell mediated cytotoxicity in both groups. In this last category, expression
215 of the gene *PRF1* (*perforin 1*) increased when *T. s. elegans* was subjected to low salinity stress,
216 but decreased under high salinity stress (Supplemental Fig. 1).

qRT-PCR to Validate RNA-seq

Nine genes were randomly selected for qRT-PCR analysis in order to validate the differentially expressed genes that were identified by RNA-Seq and gain further quantitative information on their differing expression patterns. All genes tested revealed a single product as assessed by melting-curve analysis and, in all cases, fold changes from qRT-PCR compared very favorably with the RNA-seq expression results (Fig. 3). The qRT-PCR results were significantly correlated with the RNA-seq results with correlation coefficients of 0.743 in 0 vs 5 psu and 0.862 in 0 vs 15 psu groups ($p < 0.05$).

DISCUSSION

Key genes related GO terms

Osmoregulation in ~~amphibians, crustaceans and some reptiles~~ can be a complex process because ~~animals frequently~~ must deal with ~~a range of increased or decreased salinities~~ in their natural habitats, often on a daily or seasonal basis. For example, resistance to hyperosmotic environmental conditions in the Chinese mitten crab (*Eroicheir sinensis*) and crab-eating frog (*Fejervarya cancrivora*) is generally linked to the elevation of inorganic ion concentrations in plasma and the accumulation of urea in plasma and all tissues, urea resulting from an up-regulation of the hepatic urea synthesis machinery (*Rathmayer & Siebers 2001; Yong et al., 2015*). As a normally freshwater species, the red-eared slider *T. s. elegans*, ~~a reptile without salt glands,~~ requires osmoregulation to survive when entering environments of higher salinity (e.g. brackish or estuarine water). In our previous ~~studies, these turtles were~~ shown to increase blood osmotic

237 pressure by balancing the entry of NaCl with the decreased secretion of aldosterone, and
238 accumulating blood urea and free amino acids. These adaptations allowed them to survive for at
239 least three months in water with elevated salinity as long as ambient salinity was 15‰ or lower.
240 Turtles died with prolonged exposure to 25‰, a value lower than the average global ocean salinity
241 of 35.5‰ (*Hong et al. 2014*). Crab (*E. sinensis*), frog (*F. cancrivora*) and turtle (*T. s. elegans*)
242 species that move between freshwater and brackish environments may have functionally
243 convergent genes that are expressed to aid adaptation to salinity (*Gordon & Notar 2015*).

244 In this study, high salt responsive genes were differentially expressed in turtle liver in
245 response to 5 or 15 psu (Table 5 and 6). Many of these were identified as encoding proteins
246 involved in ionic and osmotic regulation. For example, *KCNH5* (www.ncbi.nlm.nih.gov/gene/27133)
247 and *SCN1B* (*Qin et al., 2003*) are involved in potassium/sodium voltage-gated ion channels and
248 fluid balance that control arterial blood pressure by altering blood electrolyte composition and/or
249 volume. Natriuretic peptide A (*NPPA*) is well known to regulate body fluid levels and electrolytic
250 homeostasis and has natriuretic, diuretic and vasodilatory actions (*Espiner et al., 2014*). It is highly
251 expressed and associated with H_2O/Na^+ absorption and protein Ser/Thr phosphatases (*Espiner et*
252 *al. 2014*). The salt inducible kinase (*SIK*) acts to modulate adrenocortical function particularly in
253 response to high plasma Na^+, K^+, ACTH or stress (*Wang et al., 1999*). *SIK1* also has a role in
254 steroidogenesis whereas *SIK2* is implicated in gluconeogenesis regulation in liver; both belong to
255 the AMPK subfamily of serine/threonine kinases (*Berggreen et al., 2012*). The AMP-activated
256 kinase is a crucial regulator of cellular energy levels (*Hardie & Ashford 2014*) and under stress
257 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 gene *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*). Other differentially expressed genes including *ATP6*, *MTATP6* and *ATPeFOA* were enriched, which are associated with the energy needed for the synthesis and operation of these transport-related proteins to drive iono- and osmoregulatory processes (*Lee et al., 2003*).

When the ~~osmolality~~ of plasma and extracellular fluids rise increases, water is drawn out of cells, increasing plasma volume, and cells shrink; in the extreme this can cause damage to subcellular architecture and molecular crowding in the cytoplasm (*Burg 2000*). Organisms can ~~defend~~ cell volume by synthesizing organic osmolytes (e.g. free amino acids, urea) that provide a colligative defense against intracellular water loss (*Hong et al. 2014*). In our study, some of the differentially expressed genes were associated with cellular amino acid metabolism including *GLUL* (glutamine synthetase), *OAZ3* (ornithine decarboxylase antizyme 3), *STK33* (serine/threonine kinase), and *GST* (glutathione S-transferase). 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 ~~production~~ is a well-known defense against ammonia ~~toxicity, synthesized from the ATP-dependent conjugation of ammonia to glutamate~~ (*Cooper & Plum 1987; Essexfraser et al., 2005*). When salinity returns to normal, glutamine pools can be utilized as precursors for a variety of important cell molecules (e.g. purines, pyrimidines, mucopolysaccharides) or, in the presence of glutaminase, glutamine can be deaminated for direct excretion of ammonia in the kidney or used

for urea synthesis in liver before excretion.

The accumulation of urea in response high salinity can be due to both urea retention and elevated rates of urea synthesis (*Dépêche & Schoffeniels 1975*). Indeed, ureogenesis facilitates the ~~transfer~~ from freshwater to seawater in diamondback terrapins (*Malaclemys terrapin*) ~~that~~ are known to inhabit brackish water (*Dantzler & Schmidt-Nielsen 1966*). Similarly, under dehydration stress the desert tortoise, ~~*Gopherus agassizii,*~~ increases the osmolality of its blood principally via elevating urea concentration (*Dantzler & Schmidt-Nielsen 1966*). In ~~seawater-acclimated frogs,~~ ~~*Rana* *cancrivora*~~, plasma and muscle urea concentrations are also elevated through urea synthesis via the ornithine-urea cycle, uricolysis of uric acid, or hydrolysis of arginine (*Gordon & Tucker 1965*). Ureogenesis and uricogenesis are also activated under hyperosmotic conditions in crustaceans exposed to high salinities (*Chen & Chen 1996; Lee & Chen 2003*). In *T. s. elegans*, *OAZ3* was differentially expressed under high salinity environments suggesting that ureogenesis may also be activated in response to hyperosmotic conditions in this species. Potential interactions of the differentially expressed genes that responded to high salinity in *T. s. elegans* are shown in Figure. 4.

Immunity related KEGG pathways

In our study, no turtles died during the chronic salinity stress (5 or 15 psu), which supports the contention that the salinities experienced were well tolerated by *T. s. elegans*. Therefore, we conclude that the current results for the transcriptome responses by *T. s. elegans* under 15 psu salinity stress represent adaptive physiological responses by the turtles. Interestingly, only

299 immunity related pathways were significantly enriched when the data ~~as~~ assessed by KEGG
300 analysis.

301 The major histocompatibility complex class (MHC) genes *MHC-I* and *MHC-II* were both
302 differentially expressed pathways when *T. s. elegans* was subjected to salinity stress. The cell
303 surface glycoprotein MHC-I is expressed in most nucleated cells and has a key role in the
304 regulation of the cytotoxic effector functions of T cells and natural killer (NK) cells (*Höglund &*
305 *Brodin 2010*). NK cells can distinguish target cells that differ only in their expression of MHC I
306 molecules (*Ulianich et al., 2011*), and can also have regulatory roles; for example, they are
307 significant early producers of interferon-γ (IFNγ), which can influence T-cell responses (*Martín-*
308 *Fontecha et al., 2011*). The MHC II pathway activates CD4+ helper T cells through the recognition
309 of antigens on the surface of the professional antigen-presenting cells (*Xiang et al., 2005*), which
310 stimulate the proliferation and differentiation of T cells and B lymphocytic cells, and activate
311 macrophages (*Robinson & Delvig 2002*). In our study, the pathway of natural killer cell mediated
312 cytotoxicity was differentially expressed in both 0 vs 5 psu and 0 vs 15 psu, suggesting that *T. s.*
313 *elegans* might activate the immunity pathways to adapt to the salinity change. However, the
314 expression of the gene *PRF1* (perforin 1) increased when subjected to low salinity stress but
315 decreased under high salinity stress, which may be the effect of hormesis (*Calabrese 2008*) .

316 # CONCLUSIONS

317 In this study, we report the first transcriptome analysis of *T. s. elegans* under salinity stress.
318 Significant enrichment of gene expression in response to elevated salinity included transcripts of
319 proteins involved in ion binding, hydrogen ion transmembrane transports, hormone activity,

monovalent inorganic cation transport, anion binding, and ion transport. In addition, altered expression of gene transcripts related to osmotic adjustment, metabolism, signaling, cell activities and immunity also occurred. Immunity-related genes identified in *T. s. elegans* under salinity stress included cell adhesion molecules (CAMs), as well as genes involved in functions including hematopoietic cell lineage, antigen processing and presentation, phagosome, and natural killer cell mediated cytotoxicity. Based on these findings, we now have a blueprint of the types of gene responses that are up-regulated in response to elevated salinity by red-eared sliders. The pathways and processes that are targeted provide us with valuable information about the molecular mechanisms that underlie high salinity tolerance and greatly aid our ability to assess the invasion potential of this widely-introduced species.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Mei Ling Hong conceived and designed the experiments, analyzed the data, prepared figures and tables, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.
- Ai Ping Jiang designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Na Li, Wei Hao Li, and Hai Tao Shi assisted the experiments, authored or reviewed drafts of the paper, approved the final draft.
- Kenneth B. Storey conceived and designed the experiments, analyzed the data, reviewed drafts of the paper, approved the final draft.
- Li Ding conceived and designed the experiments, contributed reagents/ materials/analysis tools, prepared figures and tables, reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (approving animal): Ethical clearance for this study was obtained from the Animal Research Ethics Committee of Hainan Provincial Education Center for Ecology and Environment, Hainan Normal University.

Data Availability

The following information was supplied regarding data availability:

The raw data has been supplied as Supplemental Dataset File.

Supplemental Information

Table S1 Comparison of relative fold changes between RNA-seq and qRT-PCR results in *T. s. elegans.*

Table S2 The raw of RNA-Seq.

Table S3 Affidavit of Approval of Animal Research Ethics Committee.

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Tables captions

Table 1 Sequences of primers for qRT-PCR validation

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Table 5 Significant enrichment of GO terms related to osmotic regulation in 0 vs 5 psu groups

Table 6 Significant enrichment of GO terms related to osmotic regulation in 0 vs 15 psu groups

Table 7 Differentiation of pathway analysis in the liver of *T. s. elegans* in 0 vs 5 psu and 0 vs 15 psu groups

Figure caption

Figure 1 Visualization of differentially expressed genes in liver from turtles exposed to 0 vs 5 psu ~~(A)~~, 0 vs 15 psu ~~(B)~~. The X axis shows differential fold changes of genes ($\log_2$ FC) and the Y axis shows -log P value for the differentially expressed genes. Red points show the genes with markedly

501 significant up-regulation, orange points indicate significant up-regulation, dark blue points
502 indicate markedly significant down-regulation, light blue points indicate significant down-
503 regulation, and black points with no significant difference.

504

505 **Figure 2 KEGG annotation.** KEGG metabolic pathways of differentially expressed Genes can
506 be divided into five branches: (A, orange) Metabolism, (B, yellow) Genetic Information
507 Processing, (C, green) Environmental Information Processing, (D, blue) Cellular Processes, (E,
508 purple) Organismal Systems). The vertical axis lists the KEGG metabolic pathways and the
509 horizontal axis shows the percentage of total genes in each category. The number of genes in each
510 category is listed to the right of each bar.

511

512 **Figure 3 Comparison of relative fold changes between RNA-seq and qRT-PCR results in *T.***
513 ***s. elegans.* (A) 0 vs 5 psu; (B) 0 vs 15 psu.** Fold changes are expressed as the ratio of gene
514 expression after salinity challenge to the control group as normalized with *β-actin* gene.

515

516 **Figure 4 Interactions of positively selected genes and differentially expressed genes involved**
517 **in the adaptation of *T. s. elegans* to high salinity.** (1) *NPPA* plays roles in the regulation of body
518 fluid levels and electrolytic homeostasis pathways, whereas *ADPN* and *SIK* are in the pathway of
519 lipid (glucose) metabolism under regulation by AMPK; (2) *GLUL* (glnA), *ASS1*, *STK33* and *OAZ3*
520 play roles in the accumulation of free amino acids and urea, while *KCNH5* and *SCN1B* are involved
521 mainly in the process of H_2O/Na^+ absorption; (3) *ATP6*, *MTATP6* and *ATPeFOA* are v-type-H+

522 ATPases and participate in Na+ uptake as energizers.

523

524 **Supplemental Figure 1 Natural Killer cells mediate cytotoxicity pathway response to**
525 **ambient salinity stress in the liver of *T. s. elegans*.** Natural Killer cells mediate cytotoxicity
526 pathway response to ambient salinity stress in the liver of *T. s. elegans*. The overall pathway was
527 built using Ingenuity Pathway Analysis software. White objects denote measured genes with no
528 significant change, red-bordered objects denote up-regulation, and green-bordered objects down-
529 regulation in expression relative to the control. (a) 0 vs 5 psu; (b) 0vs 15 psu

Table 1(on next page)

Sequences of primers for qRT-PCR validation

1

Table 1 Sequences of primers for qRT-PCR validation

Gene	Forward primer (5' to 3')	Reserse primer (5' to 3')
KLF15	ATGTTCTTTCCCTGGCTGTA	TGTTGTCGGCTGTTCTGC
c111797_g1	GTGAAGGATGTCGTGGAT	GAAGGAACCCTGGAGTAG
c111797_g2	CACTTCGTTCCACCACTG	AAGGCTTCATTTCCAGAG
c116256_g1	AGTTCTATACAATGGCATCT	CTATCTCAGCCTCACAAG
c116256_g2	GAAGTTGTGCCGAGAAGA	CCAAACACCTGCCCATTA
c119704_g2	GGGAAGAAGCAGCCGACGAT	CTGCGATGACTTGGTGAGGAAA
c118858_g2	GAGGCACAAGACAAGACGAA	GACAGAGCTGCAAACCCAC
COX1	CACCCGAGCCTACTTTAC	TATGATGGCGAATACTGC
CYP17A	TGTGAGTTCCTTCCCGTCTG	CCATTACCACTGTATCTGAGCC
β-actin	GCACCCTGTGCTGCTTACA	CACAGTGTGGGTGACACCAT

2

Table 2(on next page)

Table 2 Summary of Illumina expressed short reads production and filtering

1

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

2

3

Table 3(on next page)

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

1

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

1

Table 4 The functional annotation of unigenes

Type	Unigene	Unigene_tot	Precent(%)
Pfam	15565	205138	7.59
KEGG	16603	205138	8.09
GO	17184	205138	8.38
String	12825	205138	6.25
Swissprot	23298	205138	11.36
NR	35572	205138	17.34

2

Table 5(on next page)

Table 5 Significant enrichment of GO terms related to osmotic regulation in 0 vs 5 psu groups

Table 5 Significant enrichment of GO terms related to osmotic regulation in 0 vs 5 psu groups

Description	*P*_bonferroni	Type	Differential genes
Ion binding	3.88E-05	molecular_function	COX1;c101564_g1;ADH4;TRIM39;CYP2P;c105226_g1;c105433_g1;c106588_g1;SNAI2,SLUG;c107478_g1;DSC1;TAT;ADCK,ABC1;TNK1;c111614_g1;ALB;K06911;c112636_g1;ACTB_G1;DDR2,TKT;KRAB;RASD2;RASD2;CDHE,CDH1;CDH3;c115683_g7;TRIM29,ATDC;c117980_g4;c119888_g1;c120156_g1;STK32,YANK;CYP2C;ADH1_7;SIK;ALPK;E1.2.1.5;CRYAA;GPX4;c156641_g1;c168787_g1;APOE;CYTB,petB;glnA,GLUL;c19896_g1;SOCS1,JAB;CRYL1;EPHA8,EEK;TEX14,SGK307;MYL7;ACADVL;c72315_g1;TUBA;COX2;E2.7.3.2;c86748_g1;KRAB;c88272_g1;c88272_g2;c90496_g1;PKD1L2;STK33;HOMER;ASPN;c99149_g1;c99623_g2
Ion transmembrane transport	0.000195	biological_process	COX1;TRPA1,ANKTM1;COX3;ATPeF0A,MTATP6,ATP6;CHRNA4;KCNH5;SLC26A9;COX2
Hydrogen ion transmembrane transporter activity	0.00029	molecular_function	COX1;COX3;ATPeF0A, MTATP6,ATP6;COX2
Hydrogen ion transmembrane transport	0.000332	biological_process	COX1;COX3;ATPeF0A,MTATP6,ATP6;COX2
Hormone activity	0.00106	molecular_function	ADM;c893_g1;NPPA;ACDC
Inorganic ion	0.00201	biological_process	COX1;COX3;ATPeF0A,MTATP6,ATP6;KCNH5;SLC26A9;COX2

transmembrane transport			
Monovalent inorganic cation transport	0.00596	biological_process	COX1;COX3;ATPeF0A,MTATP6,ATP6;KCNH5;SCN1B;COX2
Anion binding	0.00643	molecular_function	ADH4;c105433_g1;TAT;ADCK,ABC1;TNK1;c111614_g1;ALB;c112636_g1;ACTB_G1;DDR2,TKT;RASD2;RASD2;c120156_g1;STK32,YANK;SIK;ALPK;E1.2.1.5;GPX4;c156641_g1;APOE;glnA,GLUL;c19896_g1;CRYL1;EPHA8,EEK;TEX14,SGK307;ACADVL;c72315_g1;TUBA;E2.7.3.2;c88272_g1;c88272_g2;c90496_g1;STK33;HOMER;c99623_g2
Cellular amino acid metabolic process	0.00659	biological_process	GST,gst;TAT;MTHFD2;c113444_g1;c120156_g1;STK32,YANK;SIK;ALPK;E1.2.1.5;GPX4;PTS-HPR;glnA,GLUL;OAZ3;E2.3.1.7;CRYL1;E2.7.3.2;STK33;HOMER;K14165
Ion transport	0.00735	biological_process	COX1;TRPA1,ANKTM1;SLC6A5S;COX3;c1641_g1;ATPeF0A,MTATP6,ATP6;CHRNA4;KCNH5;SLC26A9;APOE;SCN1B;COX2
Ion transmembrane transporter activity	0.00816	molecular_function	COX1;TRPA1,ANKTM1;SLC6A5S;COX3;ATPeF0A, ATP6;CHRNA4;KCNH5;SLC26A9;COX2
Nitrogen compound metabolic process	0.0168	biological_process	c106312_g1;c106644_g2;GST,gst;c107959_g1;TAT;MTHFD2;FBP,fbp;c112105_g3;c112323_g1;c113444_g1;RASD2;RASD2;c115683_g7;E3.1.27.1;c120156_g1;STK32,YANK;SIK;K16330;ALPK;E1.2.1.5;c124849_g1;GPX4;c132576_g1;c1641_g1;PTS-HPR;ATPeF0A,MTATP6,ATP6;glnA,GLUL;OAZ3;c207168_g1;E2.3.1.7;CRYL1;TUBA;E2.7.3.2;STK3

			3;HOMER;K14165;c99023_g1;c99149_g1
Inorganic cation transmembrane transport	0.0338	biological_process	COX1;COX3;ATPeF0A,MTATP6,ATP6;KCNH5;COX2
Inorganic cation transmembrane transporter activity	0.0354	molecular_function	COX1;SLC6A5S;COX3;ATPeF0A,MTATP6,ATP6;KCNH5;COX2
Cation transmembrane transport	0.0378	biological_process	COX1;COX3;ATPeF0A,MTATP6,ATP6;KCNH5;COX2

1

Table 6(on next page)

Table 6. Significant enrichment of GO terms related to osmotic regulation in 0 vs 15 psu groups

1

Table 6. Significant enrichment of GO terms related to osmotic regulation in 0 vs 15 psu groups

Description	*P*_bonferroni	Type	Differential genes
Ion binding	2.96E-07	molecular_function	c100305_g1;COX1;c101564_g1;MFI2;TRIM39;CYP2K;EPHA7,EHK3,HEK11;CYP2P;KLF15;c105226_g1;c105433_g1;MDK;KRAB;c106494_g1;SNAI2,SLUG;c107478_g1;DSC1;TAT;c109536_g1;htpG,HSP90A;MYO7;TNK1;PLOD2;c111564_g1;ALB;EPHB2,ERK,DRT;K06911;MYL3;RNF170;ACTB_G1;c114593_g4;RASD2;c115288_g1;TRIB;EPHA1,EPH;LHCB1;THBS1;TRIM29,ATDC;AGXT2L1,ETNPPL;AGXT2L1,ETNPPL;c116330_g1;c116515_g1;c116999_g1;adk,AK;NR2A1,HNF4A;c117849_g1;c118484_g1;c118989_g1;c119497_g1;INSRR;ZC3H12,MCPIP;GCK;STK32,YANK;SLC25A23S;PLA2G,SPLA2;SIK;CYP17A;ARL4;c123809_g3;NPNT;c124238_g1;CRYAA;COX1;c155439_g1;c156641_g1;c168787_g1;CYTB,petB;glnA,GLUL;c19896_g1;SOCS1,JAB;CRYL1;TNNC1;TEX14,SGK307;MYL7;ACADVL;E2.7.3.2;CETN2;c86748_g1;FN1;c88272_g1;c88272_g2;c88310_g1;c90496_g1;c93858_g1;argG, ASS1;c99623_g2
Anion binding	3.90E-05	molecular_function	c100305_g1;EPHA7,EHK3,HEK11;c105433_g1;MDK;c106494_g1;TAT;htpG,HSP90A;MYO7;TNK1;PLOD2;ALB;EPHB2,ERK,DRT;ACTB_G1;c114593_g4;RASD2;TRIB;EPHA1,EPH;THBS1;AGXT2L1,ETNPPL;AGXT2L1,ETNPPL;c116999_g1;adk,AK;c117849_g1;INSRR;GCK;STK32,YANK;SIK;ARL4;ARL4;c123809_g3;c156641_g1;glnA,GLUL;c19896_g1;CRYL1;TEX14,SGK307;ACADVL;E2.7.3.2;FN1;c88272_g1;c88272_g2;c88310_g1;c90496_g1;argG,ASS1;c99623_g2
Iron ion binding	0.000159	molecular_function	COX1;MFI2;CYP2K;CYP2P;c107478_g1;PLOD2;CYP17A;c124238_g1
Calcium ion	0.000395	molecular_function	c101564_g1;c105226_g1;DSC1;c109536_g1;MYL3;THBS1;c116330_g1;c118484_g1;c119497_g1;SLC25A23

binding			S;PLA2G,SPLA2;NPNT;c155439_g1;TNNC1;MYL7;CETN2;c86748_g1;c93858_g1
Proton transport	0.00128	biological_process	COX1;COX3;c131564_g1;ATPeF0A,MTATP6,ATP6;ND2
Hydrogen transport	0.00135	biological_process	COX1;COX3;c131564_g1;ATPeF0A,MTATP6,ATP6;ND2
Transmembrane transport	0.00202	biological_process	COX1;SLC7A9,BAT1;SLC6A5S;CD79B,IGB;SLC38A2,SNAT2;MIA40,CHCHD4;TRPA1,ANKTM1;c118570_g1;SLC5A9,SGLT4;SLC25A23S;COX3;ATPeF0A, MTATP6, ATP6;c75960_g1
Hormone activity	0.00224	molecular_function	CD79B, IGB;NPPA;RLN;ACDC
Monovalent inorganic cation transport	0.00335	biological_process	COX1;CD79B,IGB;COX3;c131564_g1;ATPeF0A,MTATP6,ATP6;ND2;SCN1B
Ion transport	0.0046	biological_process	COX1;MFI2;SLC7A9,BAT1;SLC6A5S;CD79B,IGB;SLC38A2,SNAT2;TRPA1,ANKTM1;c118570_g1;COX3;c131564_g1;c1641_g1;ATPeF0A,MTATP6,ATP6;ND2;SCN1B;c75960_g1
Metal ion binding	0.0112	molecular_function	COX1;c101564_g1;MFI2;TRIM39;CYP2K;CYP2P;KLF15;c105226_g1;KRAB;SNAI2,SLUG;c107478_g1;DSC1;c109536_g1;PLOD2;c111564_g1;K06911;MYL3;RNF170;c115288_g1;LHCB1;THBS1;TRIM29,ATDC;c116330_g1;c116515_g1;NR2A1,HNF4A;c118484_g1;c118989_g1;c119497_g1;ZC3H12,MCPIP;SLC25A23S;PLA2G,SPLA2;CYP17A;NPNT;c124238_g1;CRYAA;c155439_g1;c168787_g1;CYTB,petB;SOCS1,JAB;TNNC1;MYL7;CETN2;c86748_g1;c93858_g1
Hydrogen ion	0.012	molecular_function	COX1;COX3;ATPeF0A,MTATP6,ATP6

transmembrane transporter activity			
Cation binding	0.0122	molecular_function	COX1;c101564_g1;MFI2;TRIM39;CYP2K;CYP2P;KLF15;c105226_g1;KRAB;SNAI2,SLUG;c107478_g1;DSC1;c109536_g1;PLOD2;c111564_g1;K06911;MYL3;RNF170;c115288_g1;LHCB1;THBS1;TRIM29,ATDC;c116330_g1;c116515_g1;NR2A1,HNF4A;c118484_g1;c118989_g1;c119497_g1;ZC3H12,MCPIP;SLC25A23S;PLA2G,SPLA2;CYP17A;NPNT;c124238_g1;CRYAA;c155439_g1;c168787_g1;CYTB,petB;SOCS1,JAB;TNNC1;MYL7;CETN2;c86748_g1;c93858_g1
Hydrogen ion transmembrane transport	0.0135	biological_process	COX1;COX3ATPeF0A,MTATP6,ATP6
Ion transmembrane transport	0.0369	biological_process	COX1;SLC7A9,BAT1;CD79B,IGB;SLC38A2,SNAT2;TRPA1,ANKTM1;COX3;COX1;ATPeF0A, MTATP6, ATP6
Transaminase activity	0.0406	molecular_function	TAT;AGXT2L1, ETNPPL;GPT, ALT

2

Table 7 (on next page)

Table 7 Differentiation of pathway analysis in the liver of T. s. elegans in 0 vs 5 psu and 0 vs 15 psu groups

1

Table 7 Differentiation of pathway analysis in the liver of T. s. elegans in 0 vs 5 psu and 0 vs 15 psu groups

Pathways	Id	Corrected P-Value	Differential genes
0 vs 5 psu			
Cell adhesion molecules (CAMs)	ko04514	0.000485	MHC2; MHC1; CDHE, CDH1; B7H3, CD276; MPZ;CLDN; PTPRC, CD45; PTPRM;CDH3
Antigen processing and presentation	ko04612	0.002901	MHC2; MHC1; ABCB3, TAP2
Phagosome	ko04145	0.003541	MHC2; SEC61B, SBH2; TUBA; ABCB3, TAP2; MHC1; MRC; ACTB_G1; SFTPD, SFTP4; ABCB3, TAP2
Natural killer cell mediated cytotoxicity	ko04650	0.005348	MHC1;PRF1;GZMB
0 vs 15 psu			
Hematopoietic cell lineage	ko04640	0.001151	ANPEP; IGH; CD24;CR1, CD35; IL1B; CSF3R; CSF3R; IGH; CD8A; CSF3, GCSF
Natural killer cell mediated cytotoxicity	ko04650	0.0249	PRF1; IGH; MHC1

2

3

Figure 1(on next page)

Figure 1 Visualization of differentially expressed genes in liver from turtles exposed to 0 vs 5 psu (A), 0 vs 15 psu (B).

Red points show the genes with markedly significant up-regulation, orange points indicate significant up-regulation, dark blue points indicate markedly significant down-regulation, light blue points indicate significant down-regulation, and black points with no significant difference.

Figure 1

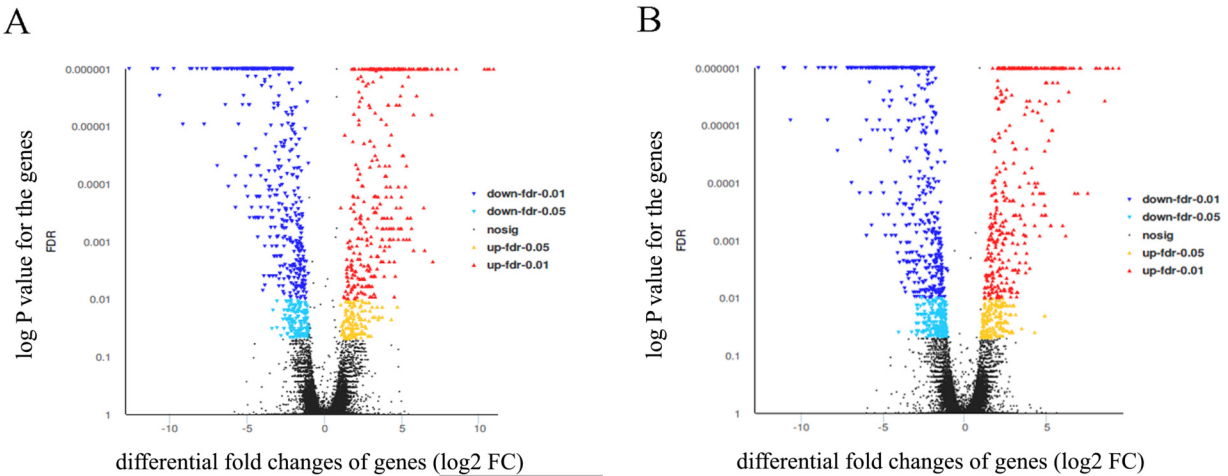

A
B
log P value for the genes
FDR
0.000001
0.00001
0.0001
0.001
0.01
0.1
1
-10
-5
0
5
10
down-fdr-0.01
down-fdr-0.05
nosig
up-fdr-0.05
up-fdr-0.01
differential fold changes of genes (log2 FC)

Figure 2(on next page)

Figure 2 KEGG annotation

KEGG metabolic pathways of differentially expressed Genes can be divided into five branches: (A, orange) Metabolism, (B, yellow) Genetic Information Processing, (C, green) Environmental Information Processing, (D, blue) Cellular Processes, (E, purple) Organismal Systems). The vertical axis lists the KEGG metabolic pathways and the horizontal axis shows the percentage of total genes in each category. The number of genes in each category is listed to the right of each bar.

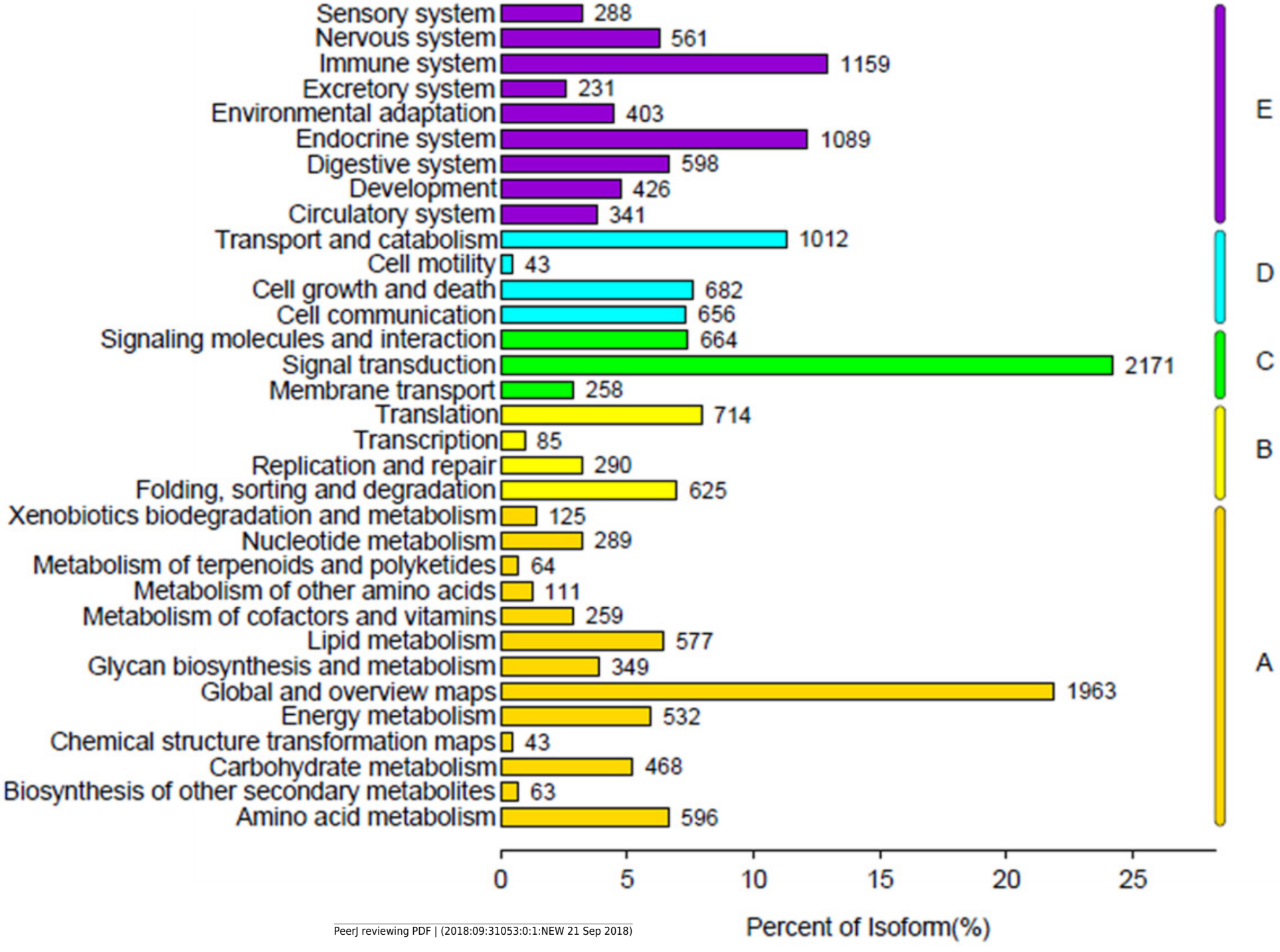

Sensory system 288
Nervous system 561
Immune system 1159
Excretory system 231
Environmental adaptation 403
Endocrine system 1089
Digestive system 598
Development 426
Circulatory system 341
Transport and catabolism 1012
Cell motility 43
Cell growth and death 682
Cell communication 656
Signaling molecules and interaction 664
Signal transduction 2171
Membrane transport 258
Translation 714
Transcription 85
Replication and repair 290
Folding, sorting and degradation 625
Xenobiotics biodegradation and metabolism 125
Nucleotide metabolism 289
Metabolism of terpenoids and polyketides 64
Metabolism of other amino acids 111
Metabolism of cofactors and vitamins 259
Lipid metabolism 577
Glycan biosynthesis and metabolism 349
Global and overview maps 1963
Energy metabolism 532
Chemical structure transformation maps 43
Carbohydrate metabolism 468
Biosynthesis of other secondary metabolites 63
Amino acid metabolism 596
E
D
C
B
A
0
5
10
15
20
25
Percent of Isoform(%)

Figure 3(on next page)

Figure 3 Comparison of relative fold changes between RNA-seq and qRT-PCR results in *T. s. elegans.* (A) 0 vs 5 psu; (B) 0 vs 15 psu.

Data are expressed as the ratio of gene expression in salinity challenged animals compared to controls. Expression of the β-actin gene was used for standardization.

Figure 3

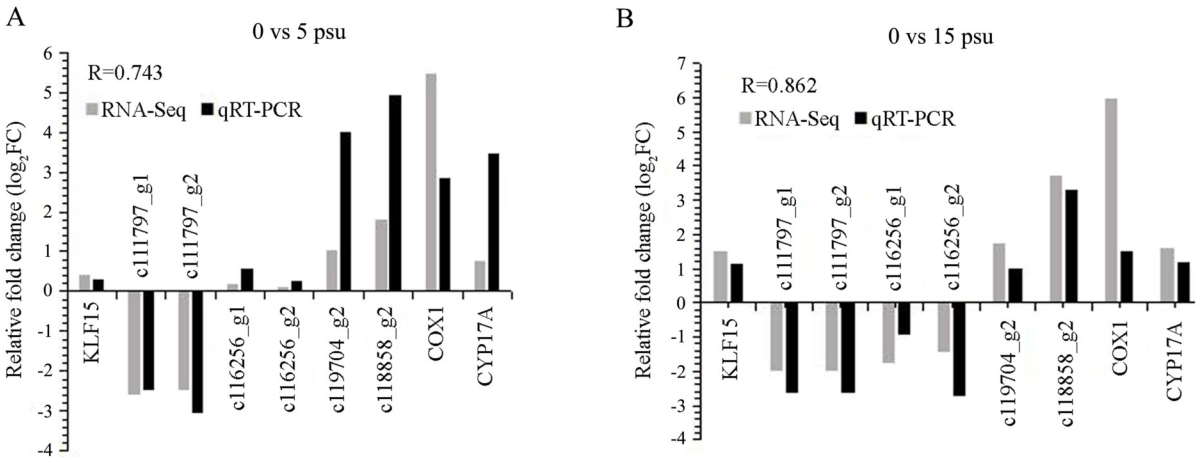
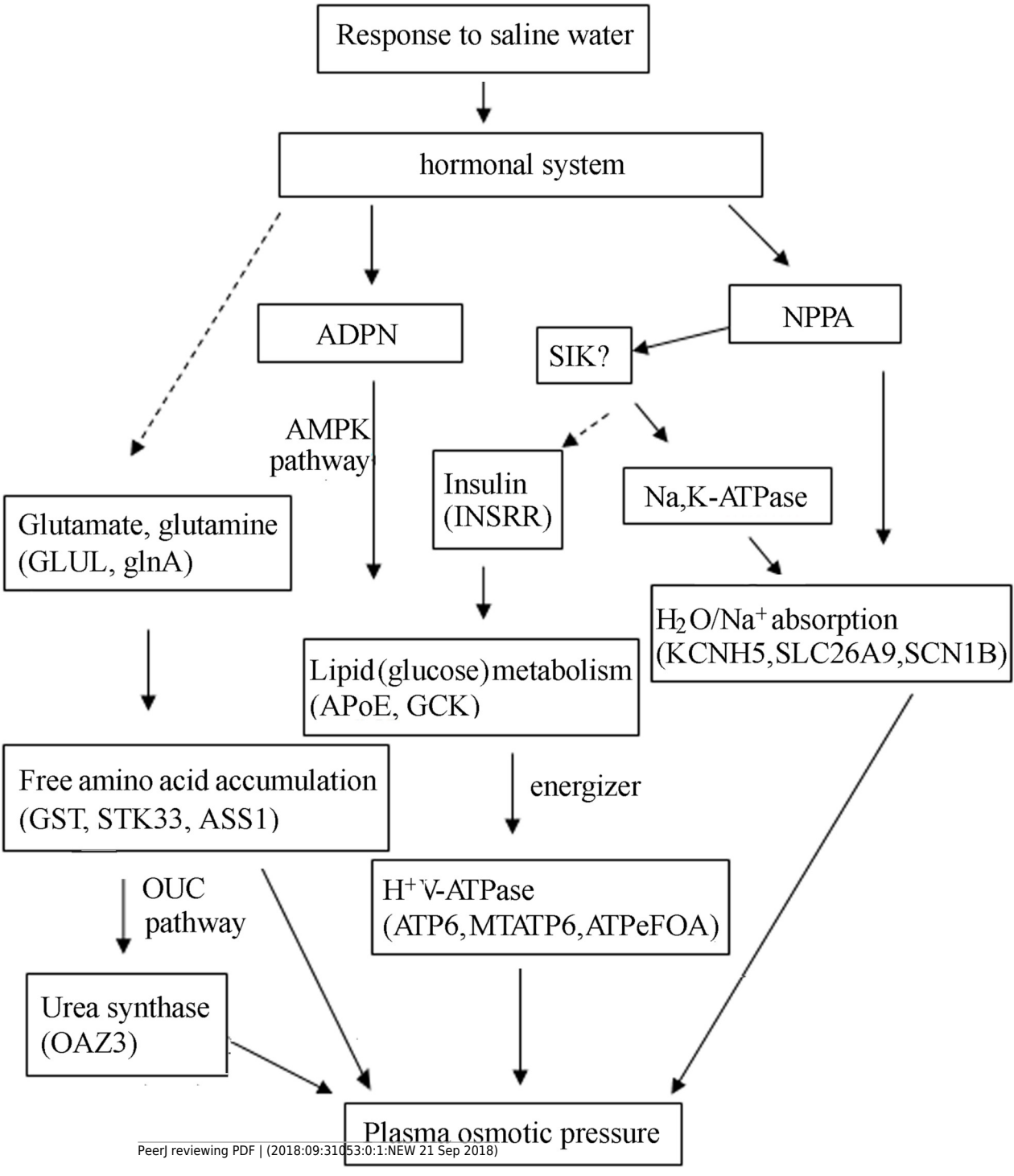

A
0 vs 5 psu
R=0.743
RNA-Seq
qRT-PCR
Relative fold change (log2FC)
KLF15
c111797_g1
c111797_g2
c116256_g1
c116256_g2
c119704_g2
c118858_g2
COX1
CYP17A
B
0 vs 15 psu
R=0.862
RNA-Seq
qRT-PCR
Relative fold change (log2FC)
KLF15
c111797_g1
c111797_g2
c116256_g1
c116256_g2
c119704_g2
c118858_g2
COX1
CYP17A

Figure 4(on next page)

Figure 4 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 body fluid regulation and electrolytic homeostasis pathways, whereas ADPN and SIK are in the pathway of lipid (glucose) metabolism under regulation by AMPK; (2) GLUL (glnA), GST, ASS1, STK33 and OAZ3 play roles in the accumulation of free amino acids and urea, whereas KCNH5 and SCN1B are involved mainly in the process of H_2O/Na^+ absorption; (3) ATP6, MTATP6 and ATPeFOA are v-type-H^+ ATPases.


Response to saline water
hormonal system
ADPN
NPPA
SIK?
AMPK pathway
Insulin (INSRR)
Na,K-ATPase
Glutamate, glutamine (GLUL, glnA)
H_2O/Na^+ absorption (KCNH5,SLC26A9,SCN1B)
Lipid(glucose)metabolism (APoE, GCK)
Free amino acid accumulation (GST, STK33, ASS1)
energizer
OUC pathway
H^+ V-ATPase (ATP6,MTATP6,ATPeFOA)
Urea synthase (OAZ3)
Plasma osmotic pressure