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Effects of low salinity on hemolym h osmolality an t ansc i tome of the wa a i oyste *Crassostrea nippona*

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Ε

Keywords: Crassostrea nippona ansc i tome emolym h osmolality ow salinity Crassostrea nippona is a in of oyste s with eat e elo ment al e as it can e e i le in s mme fo its late e o cti e e io alinity is one of the im o tant limitin a iotic facto s to the s i al an ist i tion of this stenohaline s ecies o ette n e stan the hysiolo ical an imm nolo ical es onse of C. nippona to a vin en i onmental salinities the effects of low salinity on the hemolym h osmolality an ill t ansc i tome we e in esti ate in this st y he osmolality of hemolym h in i o an s o n in wate we e assesse e la ly o e one wee at e test salinities an in f om s μ to s μ hey eache osmotic e ili i m within ho s a o e sµ t emaine hy e osmotic at an sµ fo the whole sam lin e io h o h $com\ a\ ati\ e\ t\ ansc\ i\ tome\ analysis\ the\ e\ we\ e\ less\ iffe\ entially\ e \\ \\ esse\ enes\ E\ s\ in\ ai\ wise\ com\ a$ E en ichment analysis i enti e i itin SU S su than in sµ s oteolysis an mitochon ial a o tosis athway s eci cally en iche at sμ his st y aine com ehensi e insi hts on the low salinity es onse of C. nippona at the molec la le el which o i e a theo etical asis fo n e stan in the imm ne mechanism n e low salinity st ess

1. Introduction

oastal ecosystems a e the most ecolo ically an socio economically ital on the lanet e to the in ences of ti es f eshwate in ts e a o ation an inc easin ly anth o o enic contaminants this a ea is th s mo e e ose to salinity ct ations he salinity of est a ies ns f om st a o e sµ to as hi h as sµ the mo e it is elie e that seawate salinity in the f t e will ec ease a ally eca se of lo al wa min inc ease ainfall an ti es

Crassostrea nippona is nat ally ist i te in shallow wate alon an has eat otential fo la e scale oyste coasts of East sia fa min t elon s to the en s Crassostrea he lea in moll scan s ecies y the antity o ce is oyste s an s ecies of Crashe comme cial ice of C. nippona is sostrea cont i te the most estimate at e fol s than that of the C. gigas in a an ni e a o elicio s taste an ma eta ility in s mme when C. gigas is ine i le e to tili in lyco en to e elo ona s owe e com a e with othe s ecies in the family Crassostrea ist i te in inte ti al ones with la e salinity ct ation C. nippona is a in of stenohaline s ecies e io s st ies ha e shown that low salinity affecte the owth an s i al of its la ae an

t is necessa y to lea n mo e a o t the molec la es onse in the o anism n e low salinity st ess

t is nown that e ase t ansc i tome analysis e elo e a i ly an has een wi ely se in il in st ess es onsi e ene e ession o les of many a atic animals e a in *Crassostrea* oyste s com a ati e t ansc i tomics has een a lie to cha acte i e the low salinity es onsi e enes in *C. gigas — C. virginica C. hongkongesis* an *C. ariakensis* to ate ecently mo e ela o ate an tho o h esea ch has een ca ie o t in i al es es ecially in hemocytes of m ssels

n this st y the hemolym h osmolality o e time an ill t an sc i tome of C. nippona e ose to a iant salinities we e e amine his co l o i e mo e com ehensi e info mation on low salinity es onse in C. nippona

2. Materials and methods

2.1. Experimental samples and design

o imately two yea ol *C. nippona* shell hei ht \pm cm shell len th \pm cm we e collecte f om shan ay

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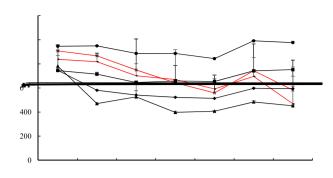


Fig. 1. emolym h osmolality in oyste s at test salinities at a e esse as mean al es of thee e licates fo each sam lin time an salinity a s e esent a ositi e stan a e iation

Table 1
mma y of ll mina sho t ea s f om the ill t ansc i tome of *C. nippona*am le aw ea s im ate a ate

'- ° ' an ° '- ° ' E han on hina an tans of the to the aiyith action acclimate a constant at a constant a

in e e iment they we e an omly i i e into o s with salinities of an actical salinity nit s E ally in i i als we e c lti ate in each tan he low salinity wate was e a e y il tin ae ate an san lte e seawate with ta wate he fee in st ate y is as s al as efo e alf of the seawate was enewe at s e e y ay

2.2. Hemolymph osmolality

ai e hemolym h an wate sam les we e ta en f om each tan at an h t was sam le y with awin μ f om the a cto m scle sin a m sy in e with a a e nee le Each sam le was analy e n e a mic osco e to ens e hemocytes we e esent an no contamination with tiss e e is a o o® a o ess e osmomete esco nc was se to ete mine the osmolality of the hemolym h an co es on in wate

2.3. RNA extraction and Illumina sequencing

ill tiss es of the oyste s in

lent echnolo ies

collecte an imme iately lace into li i nit o en to f ee e an then sto e at - ° fo f the t ansc i tome analysis otal was e t acte f om the sam les sin ol ea ent n it o en acco in to the man fact e 's inst ctions he ity an inte ity of total we e ete mine sin ano o s ect o hotomete he mo an ilent io naly e i

an

total of nine sam les we e se fo li a y

sµ at

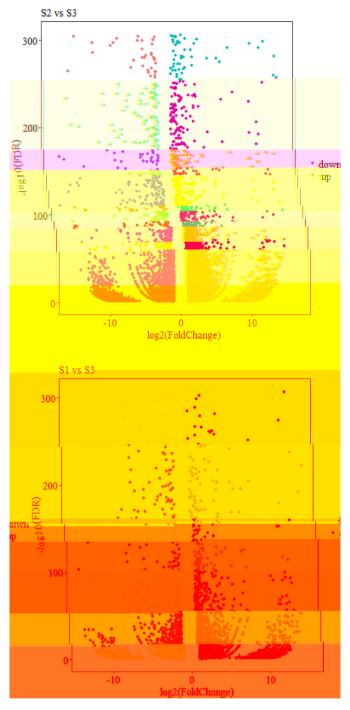


Fig. 2. olcano lot fo the iffe entially e esse enes shows the estimate lo fol chan e a is a ainst its statistical si ni cance y a is etween low salinity o s an an cont ol o ene that a e si ni cantly e late an own e late a e in een an in es ecti ely

const ction fte $\,$ am li cation the $\,$ li a y was then se ence ia ll mina ise TM

2.4. Functional annotation and enrichment analysis of differentially expressed genes (DEGs)

h we e

en an accession n m e sin owtie y efa lt a amete s afte wa s he ea s n m e ma e to each ene was estimate y E he e ession le el of each t an sc i t was t ansfo me sin lo + he e ession fol chan e of a t ansc i t was estimate y E e he stan a fo consi e in E s is lo ol han e > an al e < he ational ente fo iotechnolo y nfo mation non n ant n cleoti e se ences t an non e n ant otein we e se to annotate the E s y ences en ichment analysis was im lemente with last o ontolo y he It a eomet ic test was se to etect si ni cantly en iche te ms in E s com a e with the t ansc i tome ac o n athways of E s we e annotate a ainst the yoto Encyclo e ia of enes an enomes E ata ase sin En iche athways we e i enti e acco in to the same fo m la se in the analysis

$2.5. \ \ Quantitative \ reverse-transcription \ PCR \ (qRT-PCR) \ verification$

se in the analysis was isolate with the same metho se in the se eci c ime s we e esi ne sin E was se as the inte nal cont ol he analysis was e fo me sin i ht ycle ® inst ment oche wit e lan with anti o aTM een it ia en e many he mi t e containe μ c μ een aste i μ fo wa e e se ime s an μ istille wate in a nal ol me of μ yclin con itions we e $^{\circ}$ fo min followe y cycles $^{\circ}$ fo s an $^{\circ}$ fo s an then a melt c e sta e afte the cyclin sta e he elati e e ession of selecte enes ase on cycle th eshol C_T al es was calc late sin the "c" ac a e htt

 $o \ ect \ o$ ac $a \ e= \ c$

3. Results

3.1. Hemolymph osmolality under salinity stress

he hemolym hosmolality of *C. nippona* was hy eosmotic to the ete nal wate in the initial eos ean eacheosmotic ei li imwithin — hat an sµ elow sµ he molym hof oystes emaine hy eosmotic elatieto the ete nal en ionment tho hot the sam line io i itionally oyste motalities weeose e in tansat an sµ es ltin in eoe nesat the last sam line io

3.2. Gill transcriptomæ

total of

with the s o n in seawate hen the e ose salinity nea o eyon thei tole ance limits they chose to close thei al es to isolate the o anism f om the e te nal en i onment s sessile o an isms thi in in est a ies an inte ti al ones oyste s ene ally can tole ate wi e ct ations in salinity n the e io s st y C. virginica co 1 t ac all t eatment salinities own to sµ within wee of e os e e en tho h they cons me lon e to each isosmotic con itions at the lowe salinities owe e the hemolym h osmolality of C. nippona has little owe to a a t to an en i onment sμ onsi e in *C. nippona* can only li e in the shallow wate whe e the salinity is elati ely sta le the iffe ence in thei tole ance of salinity can

3.4. C. nippona mitochondrial apoptosis pathway analysis

he ma o mitochon ial a o tosis athway elate enes in C. nippona incl e t an as a le hese enes e fo m simila e ession t en s sin an calc lation i the "tton" of the athway in mammals is yet to e isco e e in the ata

4. Discussion

yste s li e most ma ine animals a e ty ically consi e e $\,$ to $\,$ e osmoconfo me s $\,$ hat means that thei $\,$ e $\,$ t acell $\,$ la $\,$ i $\,$ is isosmotic

cià cot tosis watihway" e io s e e iments ha e showirmathat a atic animals s ally n e o chan es in imm ne stat s n e en i onmental nli e e te ates oyste s lac an a a ti e imm ne sys tem an th s m st ely hea ily on the innate imm ne system as a o tection f om iotic an a iotic st esses o tosis is the im o tant imm ne mechanism of oyste in a a ti e es onses to en i onmental st ess which ha e een notice to lay im o tant oles in an the clam Cyclina sinensis C. gigas t is initiate an t ans ce ia the int insic mitochon ial o the e t insic ece to En i onmental st esso s s ch as low salinity athway me iate st ess has een con me to acti ate the mitochon ial athway in he o a o totic an os i al mem e s of the cl moll scs family oteins a e the cent al e lato s he a sence of only homolo is al ea y con me in aci c oyste C. gigas the ma ita es hili ina m nila clam an the ma ine

mhws cioememeti ofleins

owie a o E ito ial meta olism an imm ne tole ance ont mm nol

an ao i an ho on han han i
on het ansc i tional es onse of the aci c oyste Crassostrea gigas a ainst
ac te heat st ess ish hell sh mm nol —
hao on i ansc i tomic es onses to salinity st ess in the
aci c oyste Crassostrea gigas o ne e
i i an han an hen i hen
an ian ansc i tome o lin of the low salinity st ess es onses in
the ills of the enile Pseudopleuronectes yokohamae om iochem hysiol
enom oteonomics
o e han elon o a mm ne an st ess es onses in
oyste s with insi hts on a a tation ish hell sh mm nol oll scan
mm nity —
an on on he oyste imm nity e om mm nol a
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i i ian i e on ill t ansc i tome analysis e eals the

iss o tosis an its f nctional si ni cance in moll scs o tosis i han an i han onse ation an i e ence of mitochon ial a o tosis athway in the aci c oyste Crassostrea gigas ell eath s e o ei a alsei o lanas ste elt an ansc i tomics of in it o imm ne stim late hemocytes f om the anila clam ita es hili ina m sin hi h th o h t se encin o ne EE hili aeme el ne o st a hieme in eisen ch ei e osenstiel assi ely a allel se encin i enti es a com le imm ne ene e e toi e in the lo hot ocho oan ytil se lis o ne eitscho f aen ele eihe e hos ho ylation ta ets cl fo i itin e en ent e a ation a lin etween the a o tosome an the oteasome athway E e — — i E eta im a amamoto sa i eacti e o y en s ecies cont of the e ession of cl family oteins y e latin thei hos ho ylation an i itination ance ci -