The Role of Antioxidant Enzymes of the Disk Abalone (*Haliotis discus discus*) and their Transcriptional Responses to Physical and Biological Stress

Mahanama De Zoysa, Jehee Lee

School of Applied Marine Sciences
Jeju National University
Republic of Korea

17th June 2009

제주대학교

Out line

1. Identification of disk abalone antioxidant genes from cDNA library (ESTs)

2. Functional characterization and different roles of abalone antioxidant genes

3. Transcriptional responses of abalone antioxidant enzymes to physical stress and biological stress

4. Future focusing and applications

Abalone 전복 *(Haliotis discus discus*)

- Belongs to phylum mollusk
- Approximately 100 species
- Commercially important in Korea, China, Japan, USA, etc.
- Production decrease associated with

  1. Disease outbreaks

  2. Unfavorable environmental conditions

- Important to study stress-immune interactions

Environmental Effects and ROS Impact

Environmental Changes

1. Ultraviolet radiation (UV)
2. Extreme temperature
3. High solar radiation
4. High salinity
5. Toxic chemicals (PCBs, PAHs)
6. Heavy metals (As, Hg, Cd, Pb)
7. Pathogenic infection

Increase Intercellular ROS level

Positive Impacts

- ✓Immune response
- ✓Cell signaling

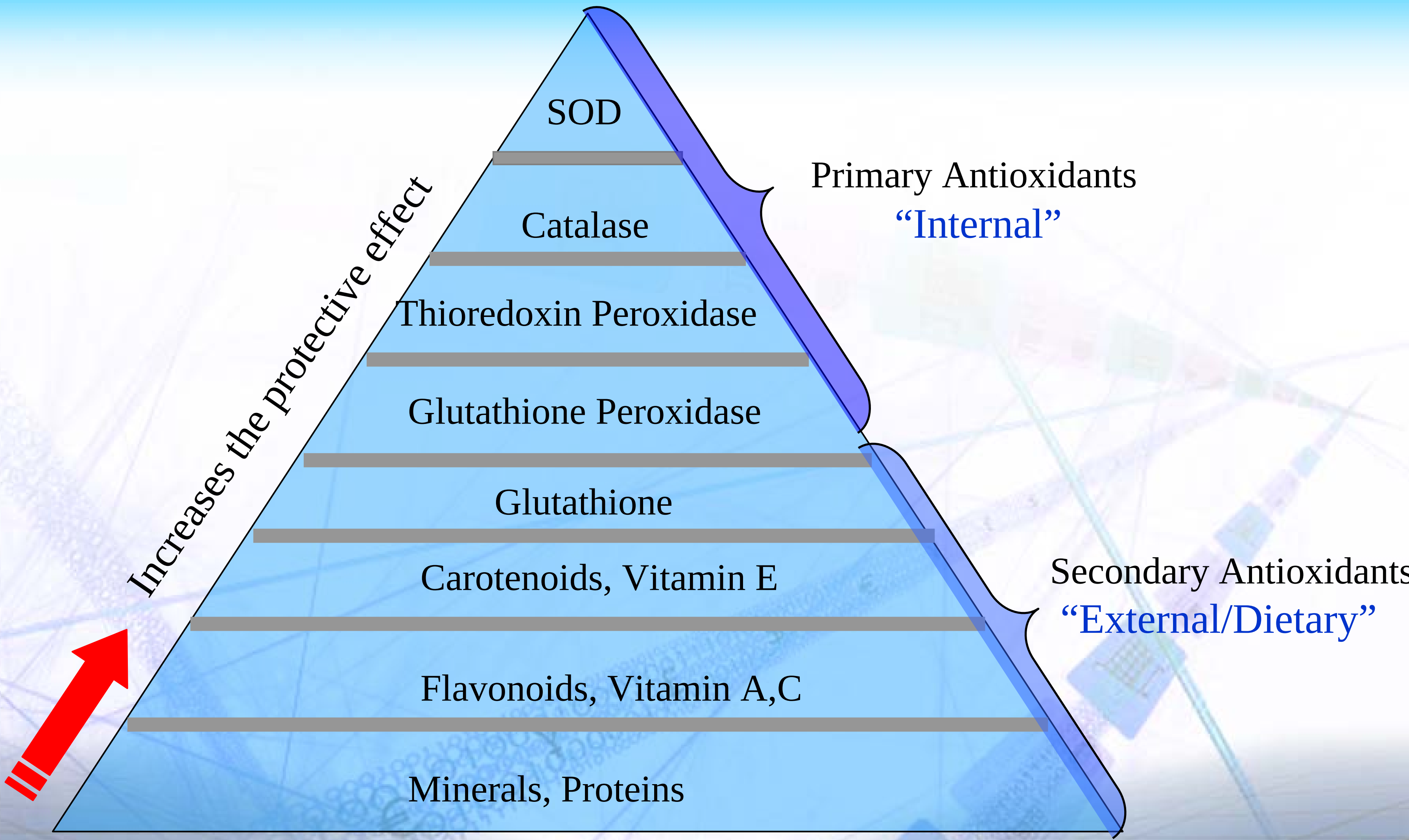
Negative Impacts

- ✓Oxidative stress
- ✓Lose of cell function
- ✓Apoptosis/necrosis
- ✓Diseases
- ✓Aging

Change of Redox status

Antioxidant Defense → ROS Detoxification

Antioxidant Defense System



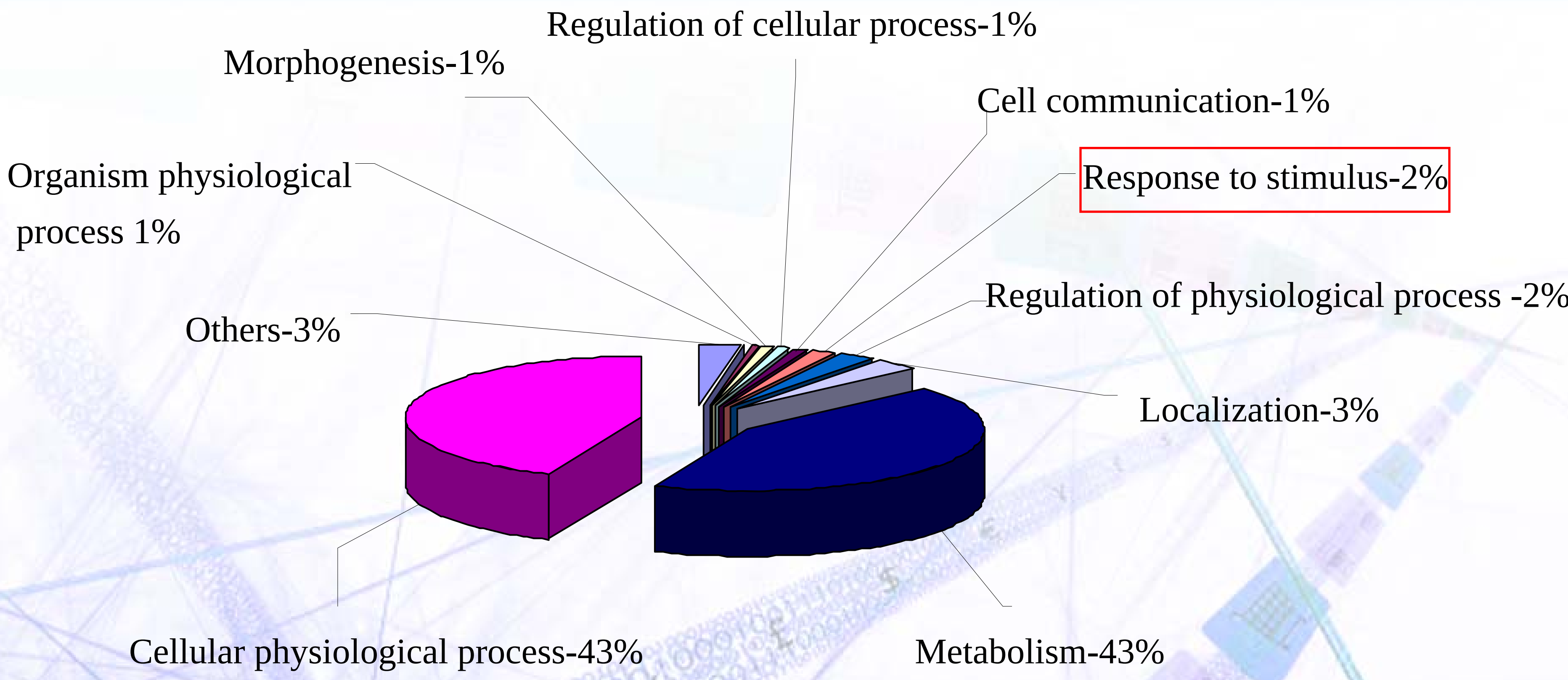
Disk abalone normalized cDNA library (ESTs)

cDNA library: constructed using RNA isolated from whole abalone tissues

Total number of ESTs sequenced	6720
Total number of ESTs analyzed	5996
Range of insert size	0.5- 3.5 kb
Number of contigs/sequence assembled	1019/2653
Singletons	3343
Unique genes	4362/5996 (72.7%)
Known genes[a]	2275
No hit	2087

a: Sequence with an E value of less than 10^{-5} was considered as a known gene

Classification of the normalized cDNA ESTs by cellular process



Gene selection for characterization

- Functionally important genes, which showing high identity to known genes has selected for individual characterization

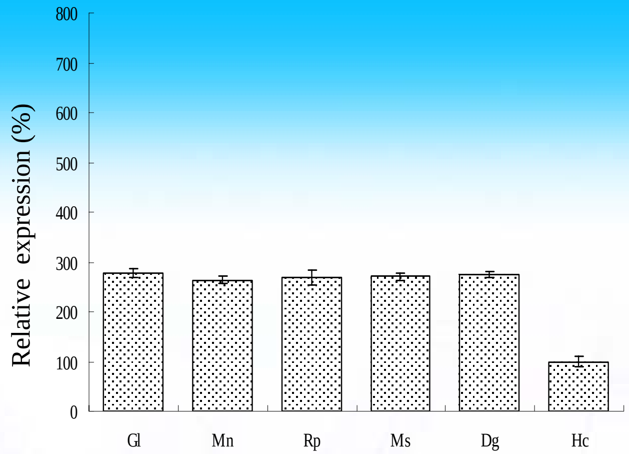
- Genes associated with specific functional group has characterized together to understand intra-gene functional relationships

  Ex: Antioxidant enzymes; Innate Immune response genes etc.

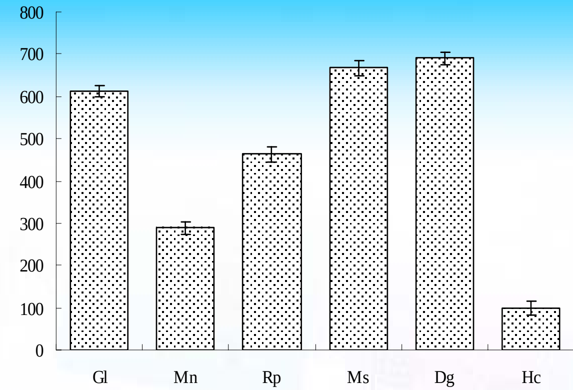
Abalone antioxidant enzymes

Enzyme	Conserved Motifs	Amino acids/Mw	Identity %
Mn-SOD (Mitochondrial) Accession: DQ821491	Mn-SOD Signature	226 =25 kDa	64 *Biomphalaria glabrata*
Cu,Zn-SOD (Cytoplasm) DQ821492	Cu,Zn-SOD Signature	154 =16 kDa	79 *H. d. supertexta*
Catalase DQ821496	Heme-ligand Signature	501=56 kDa	96 *H. discus discus*
Thioredoxin Peroxidase (TPx-1) EF103376	TPx Signature	251=28 kDa	76 *Biomphalaria glabr ata*
Thioredoxin Peroxidase (TPx-2) EF103377	TPx Signature	199=22 kDa	77 *H. discus hannai*
Mitochondrial Thioredoxin-2 (Mt-TRx-2) EF103378	WCGPC- Active site mitochondrial Signature	173=19 kDa	43 *Xenopus laevis*
Se-Glutathione Peroxidase (Se-GPx) EF103379	Selenocysteine (Sec) GPx Signature	225=25 kDa	46 *Ixodes scapularis*

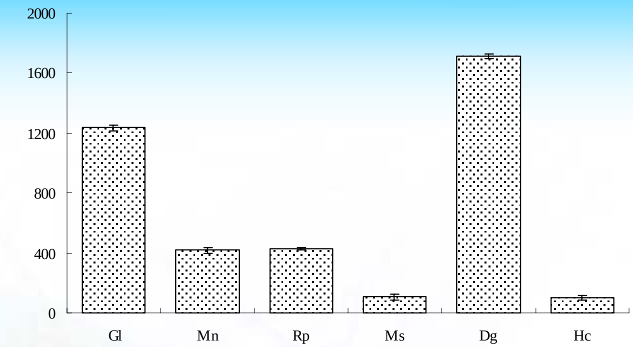
Tissue specific expression of antioxidant enzymes



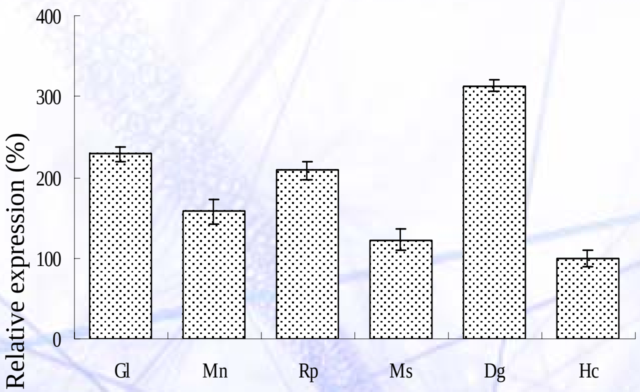
Mn-SOD



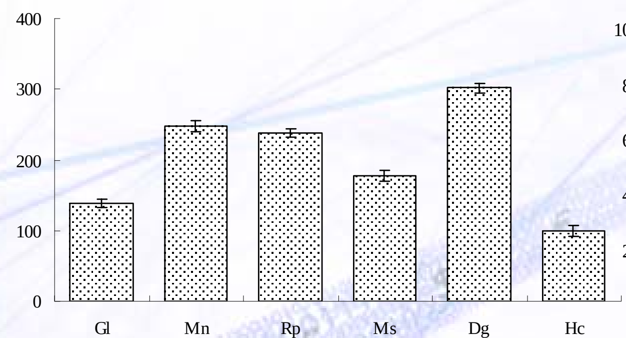
CuZn-SOD



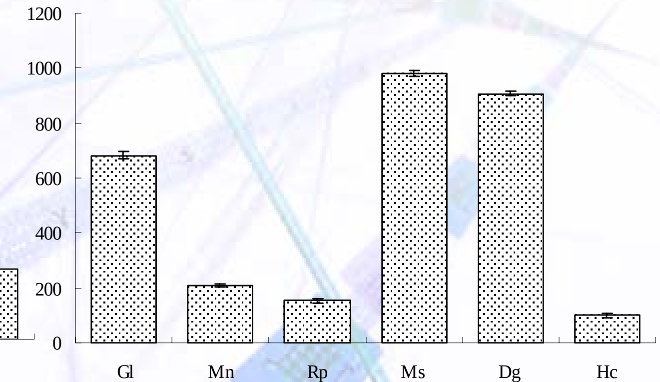
Catalase



TPx



Se-GPx



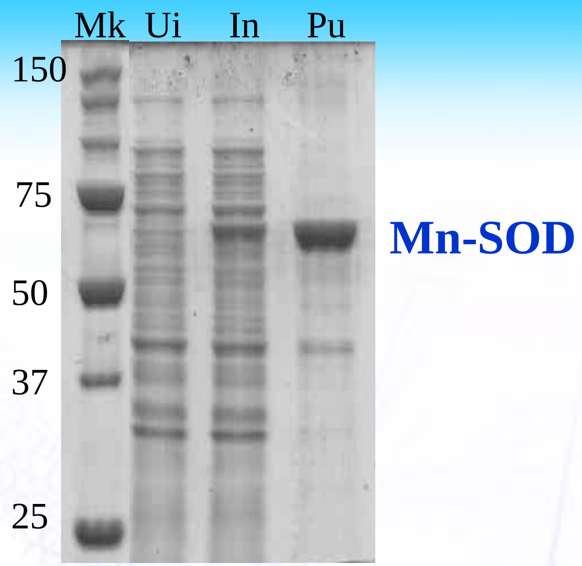
Mit-TRx-2

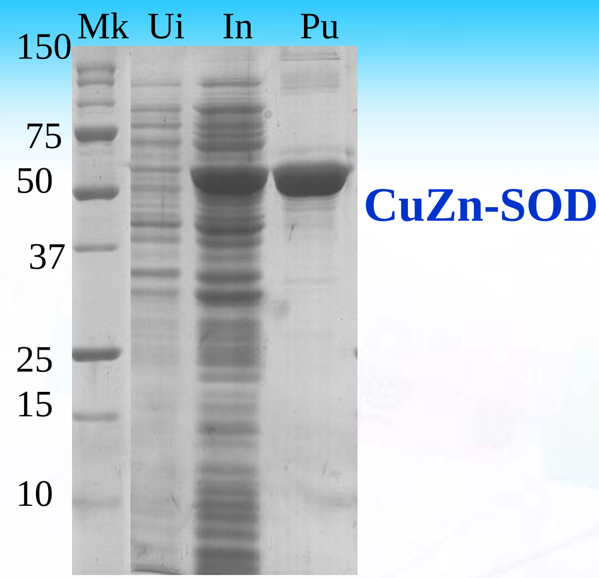
Gl-Gills; Mn-Mantle; Rp-Reproductive; Ms-Muscle; Dg-Digestive; Hc-Hemocytes

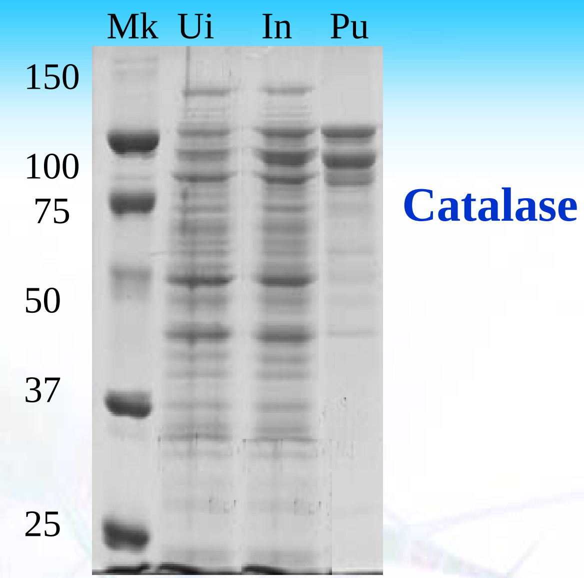
- Antioxidant enzyme expression levels are different in tissue specific manner:

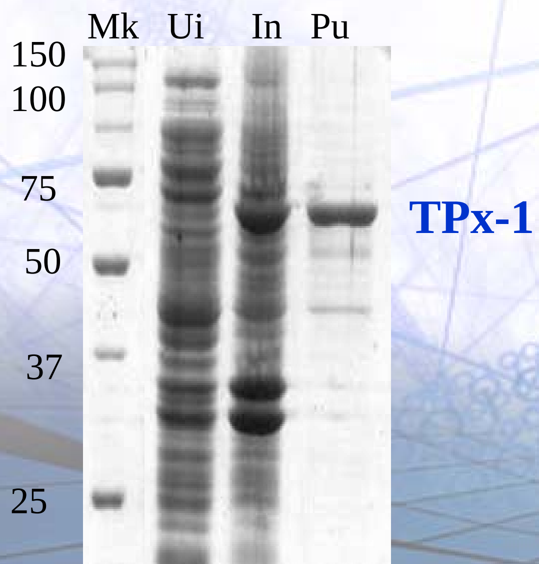
“Tissue specific variation”

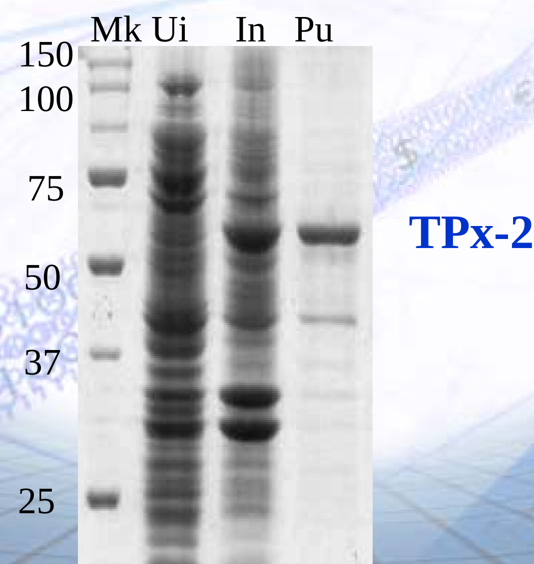
Purification of recombinant antioxidant enzymes

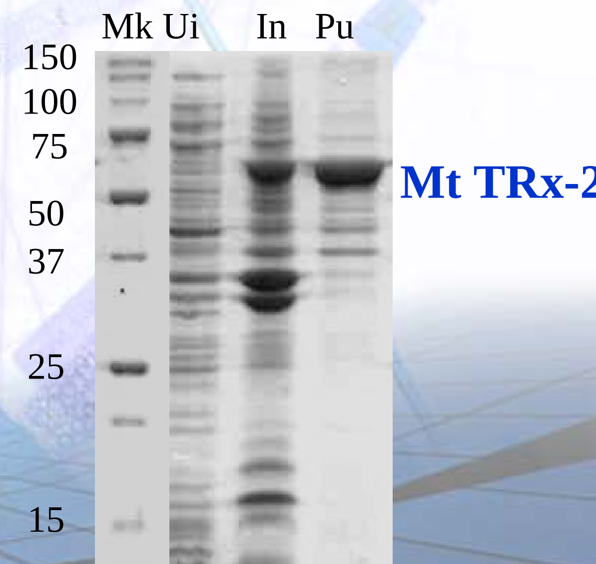








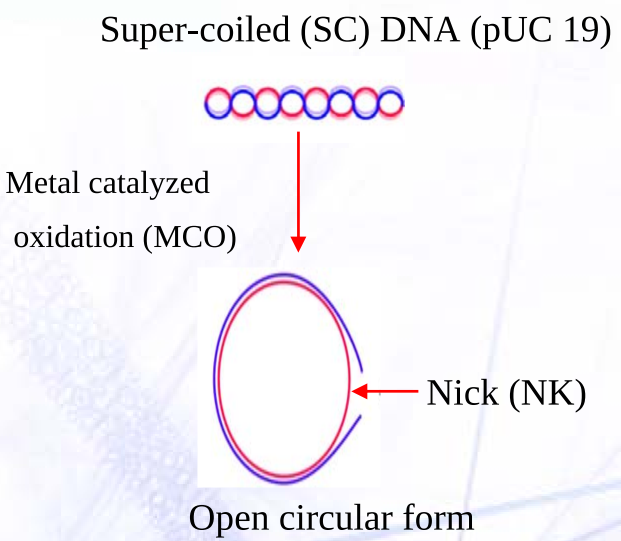


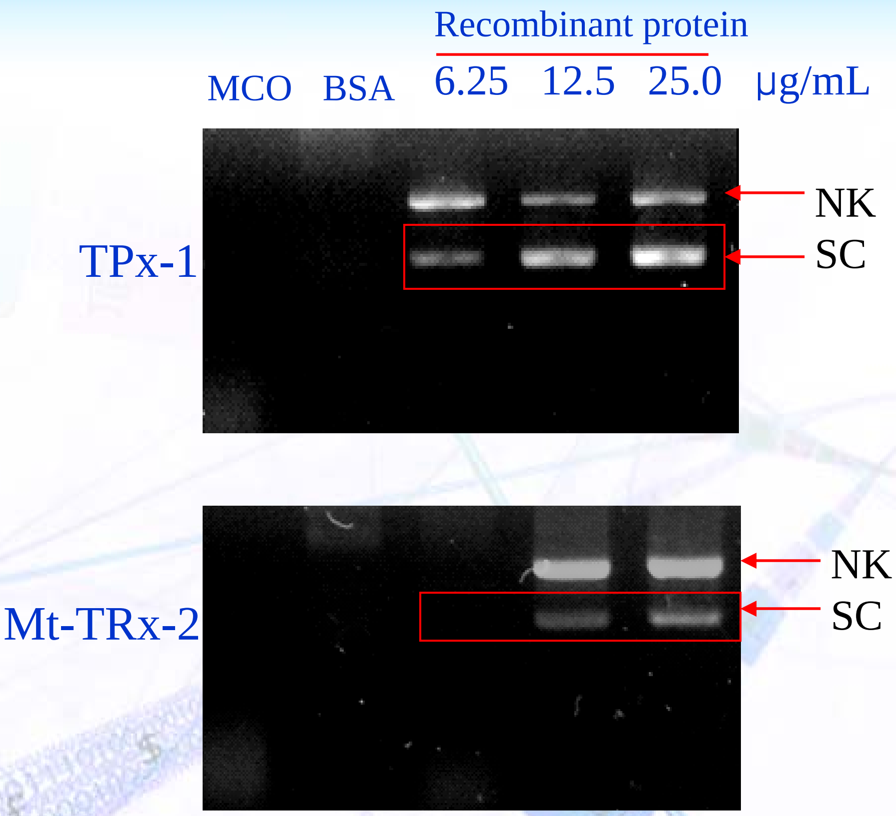


Functional characterization of recombinant antioxidant enzymes

Enzyme	Activity assay	Specific activity U/mg	Optimum Conditions
Mn-SOD	Xanthine oxidase Nagai et al., 2003	2781	T = 37 ^{o}C pH = 3.5-6.5
Cu,Zn-SOD	Xanthine oxidase Nagai et al., 2003	2461	T = 37 ^{o}C pH = 3.5-6.5
Catalase	Reduction H_2O_2 Muller, 1985	30,000	T = 30-37 ^{o}C pH = 4.5-10.5
Thioredoxin Peroxidase 1	Reduction of H_2O_2 Reduction of BHP Lim et al., 1993	2.55 0.25	T = 37 ^{o}C pH = 8.0
Thioredoxin Peroxidase 2	Reduction of H_2O_2 Reduction of BHP Lim et al., 1993	2.65 0.18	T = 37 ^{o}C pH = 8.0
Mitochondrial Thioredoxin-2	Reduction of insulin disulfide Holmgren 1979	1.825	T = 30-37 ^{o}C pH = 10.0-12.0

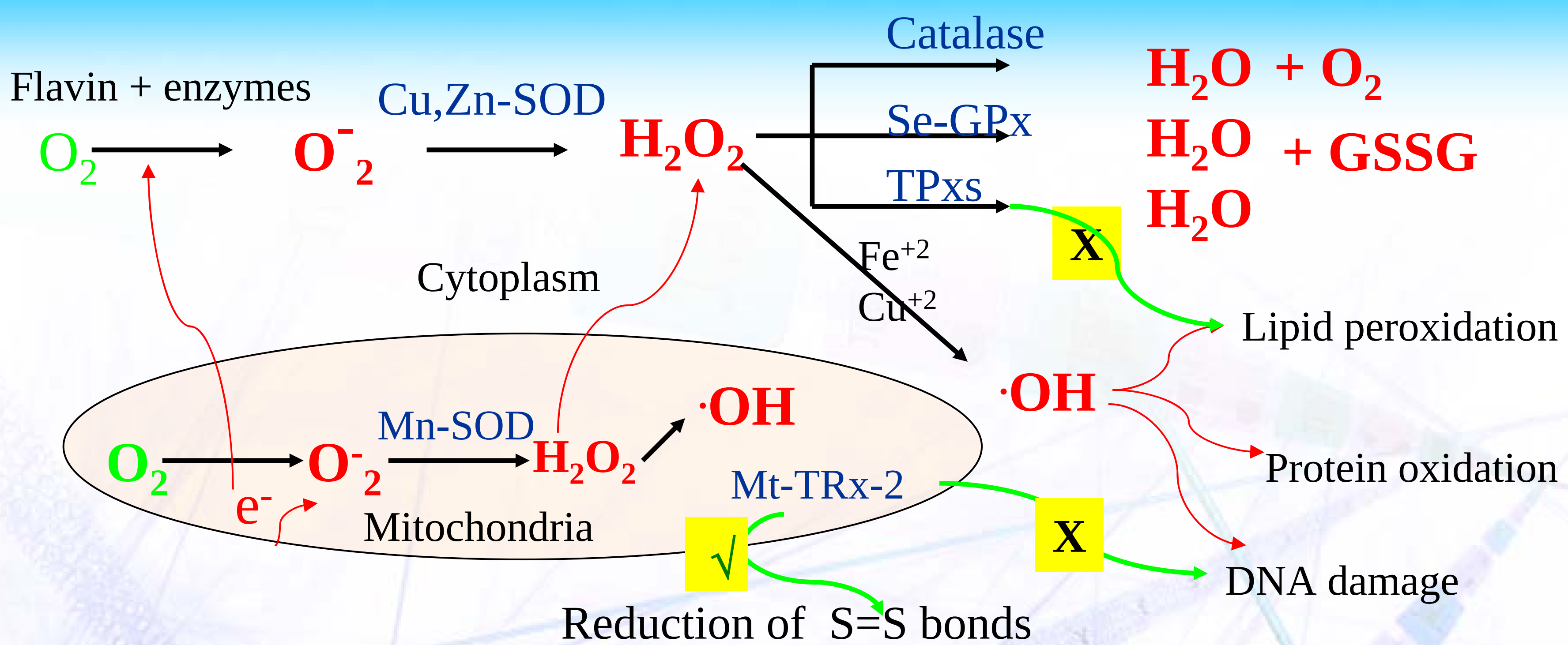
DNA protection activity of recombinant TPx and Mt-TRx-2





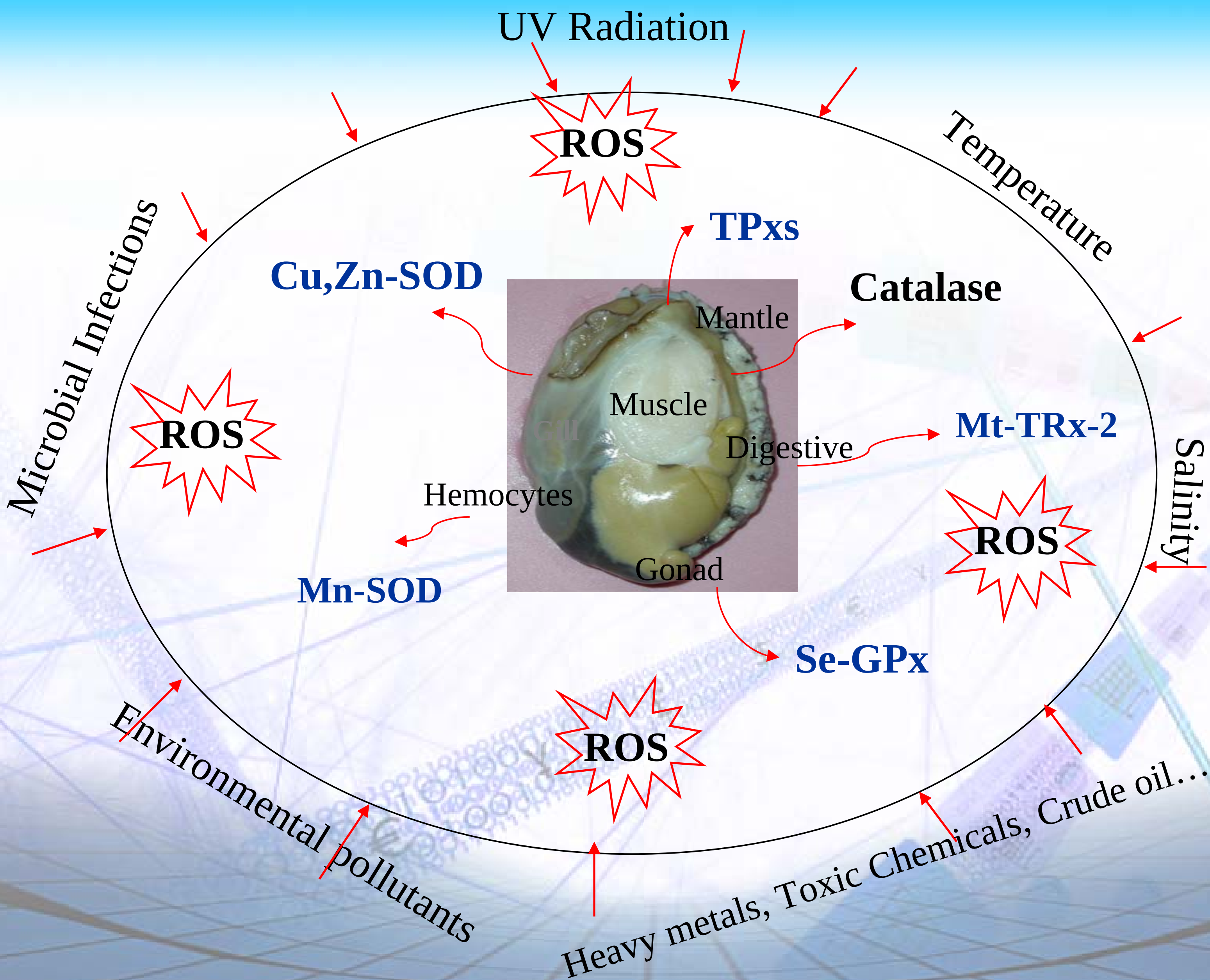
- TPx and Mt TRx-2 are able to reduce DNA damage induced by metal catalyzed oxidation (MCO)

Summery of abalone antioxidant system



- Disk abalone has almost all important primary antioxidant enzymes to maintain efficient antioxidant defense
- Recombinant antioxidant enzymes showed their respective functions of ROS detoxification

Antioxidant Potential of Abalone Antioxidant Enzymes



Abalone exposed to stresses

- Physical stress conditions:

YSI-Handheld Multiparameter

  - High temperature :28 ^{o}C
  - Low-salinity: 25 ‰
  - Hypoxia stress: Low water level without oxygenation

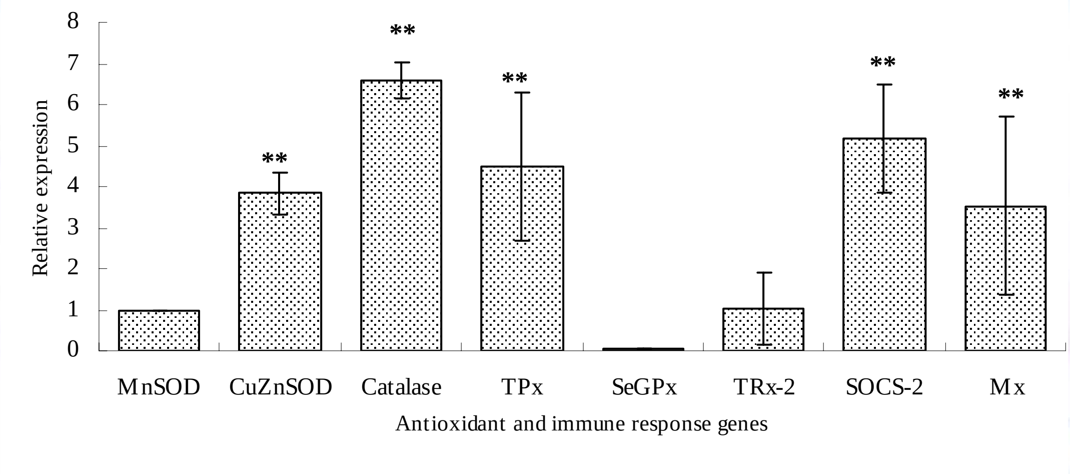
- Biological stress
  - Virus :VHSV (Viral haemorrhagic septicaemia virus):

    50 µL/1 X 10^{8} pfu/mL/abalone

- Control animals

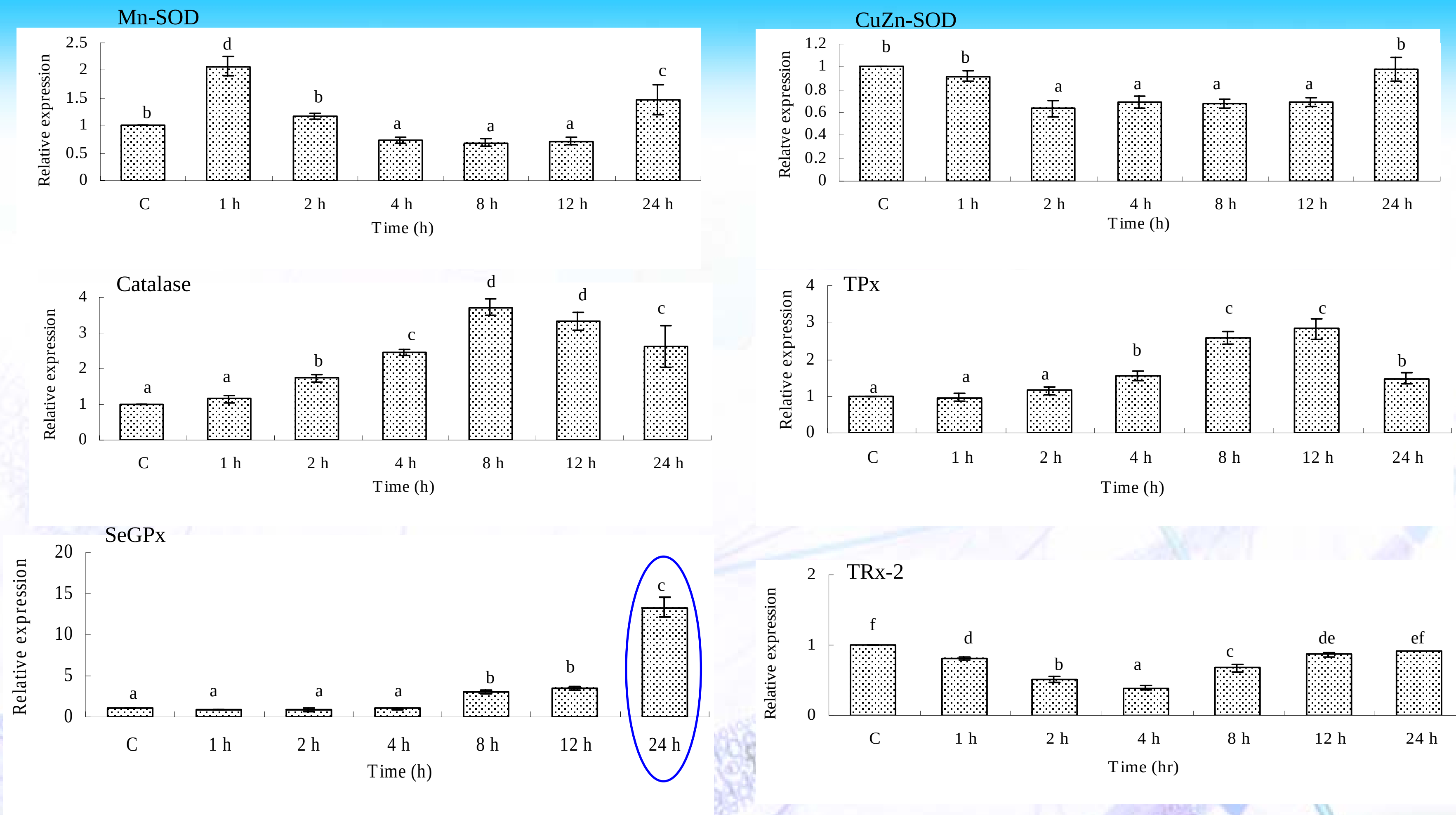
Temperature: 20 ^{o}C; Salinity :35 ‰; Dissolved oxygen:>95%; pH:8.1

Transcriptional expression of antioxidant enzymes and immune relevant genes



- Constitutive expression of all the genes
- Catalase showed the highest expression (6.6 times higher than the Mn-SOD)
- SeGPx showed the lowest expression

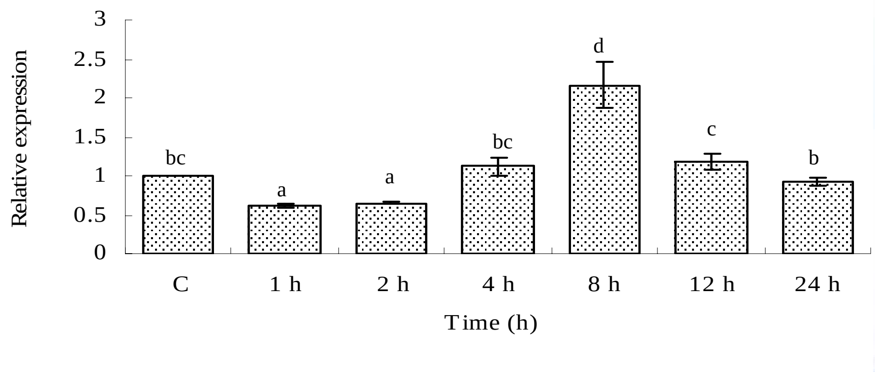
Transcriptional expression of antioxidant enzymes after thermal stress



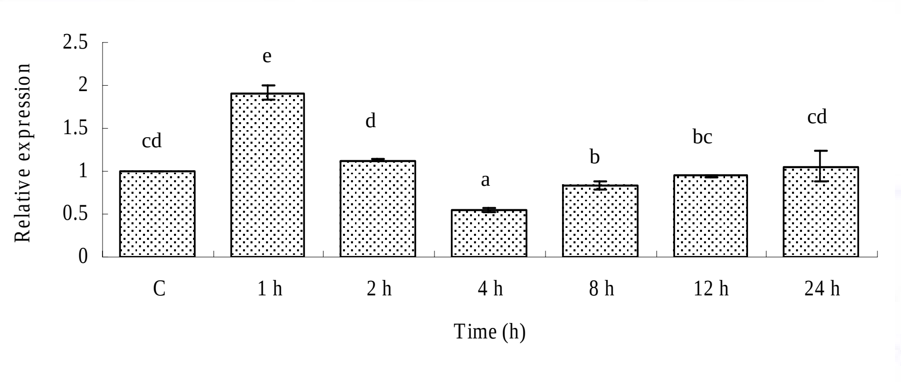
- Catalasse, TPx and Se-GPx transcripts were significantly induced
- CuZn-SOD and TPx-2 were not induced against heat stress
- Se-GPx has the highest induction (13.3-fold)

Transcriptional expression of SOCS-2 and Mx after thermal stress

SOCS-2



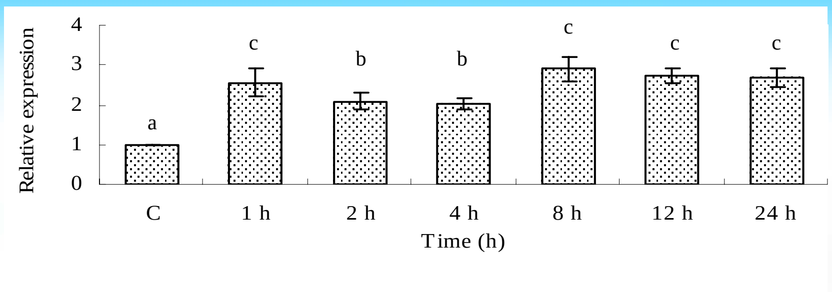
Mx



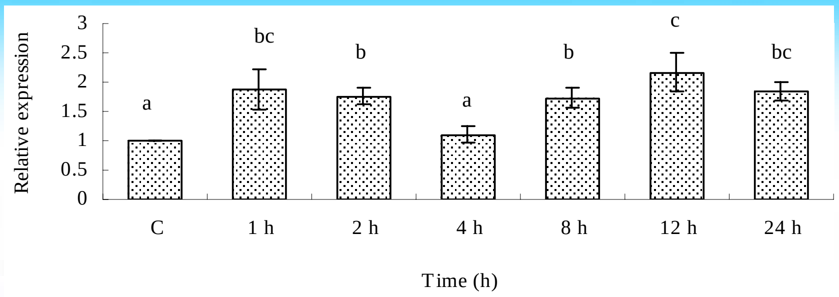
- SOCS-2 was down regulated over the first 2 hours and then up-regulated to maximum level at 8 h
- Mx was either down regulated or at basal level (except at 1 h up-regulation)

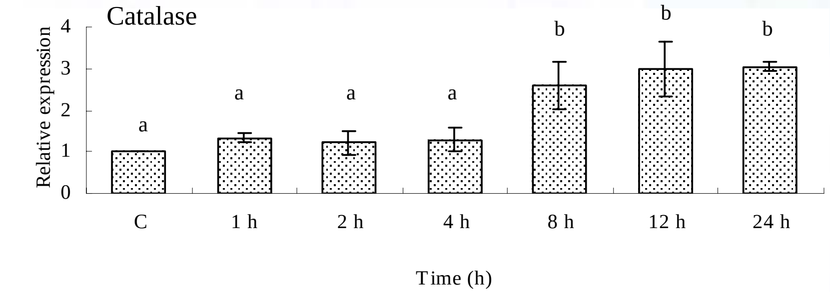
Transcriptional expression of antioxidant enzymes after low-salinity stress

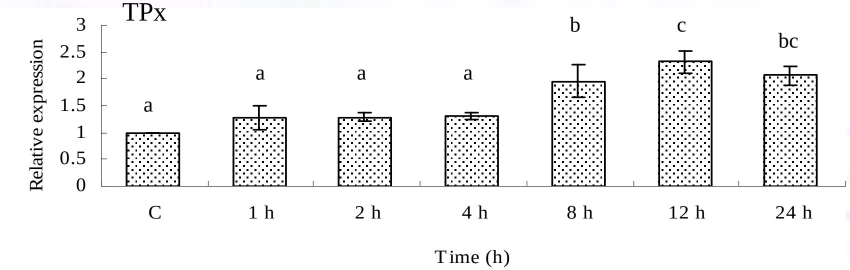
Mn-SOD

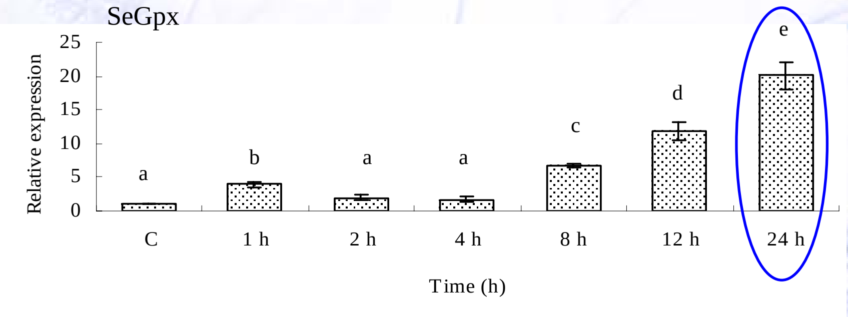


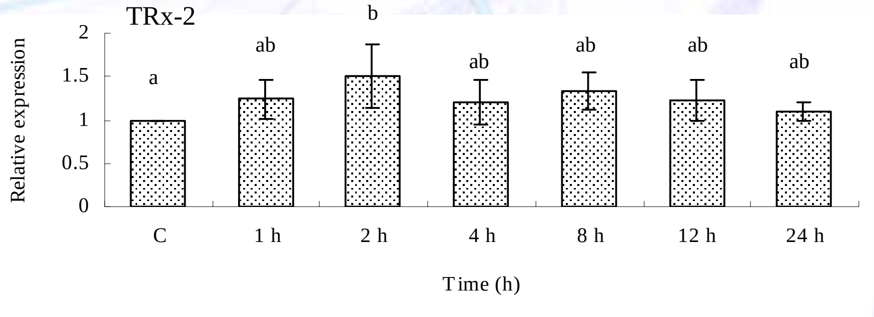
CuZn-SOD







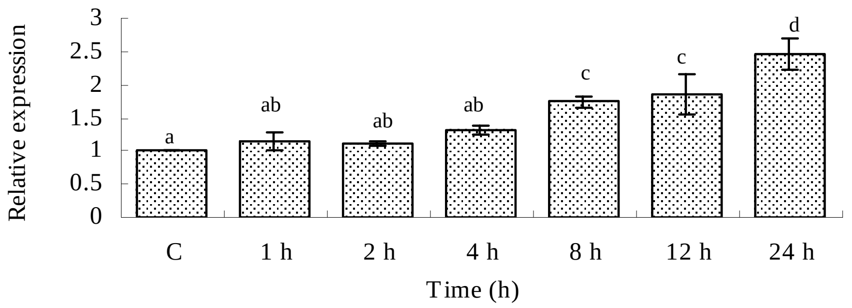




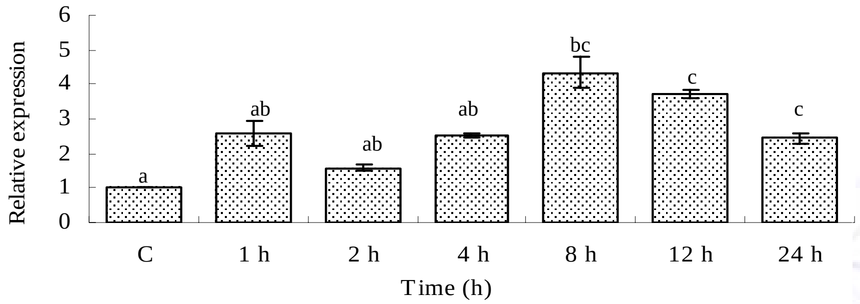
- All the antioxidant transcripts were significantly induced
- Se-GPx has the highest induction (20.1-fold)

Transcriptional expression of SOCS-2 and Mx after low-salinity stress

SOCS-2

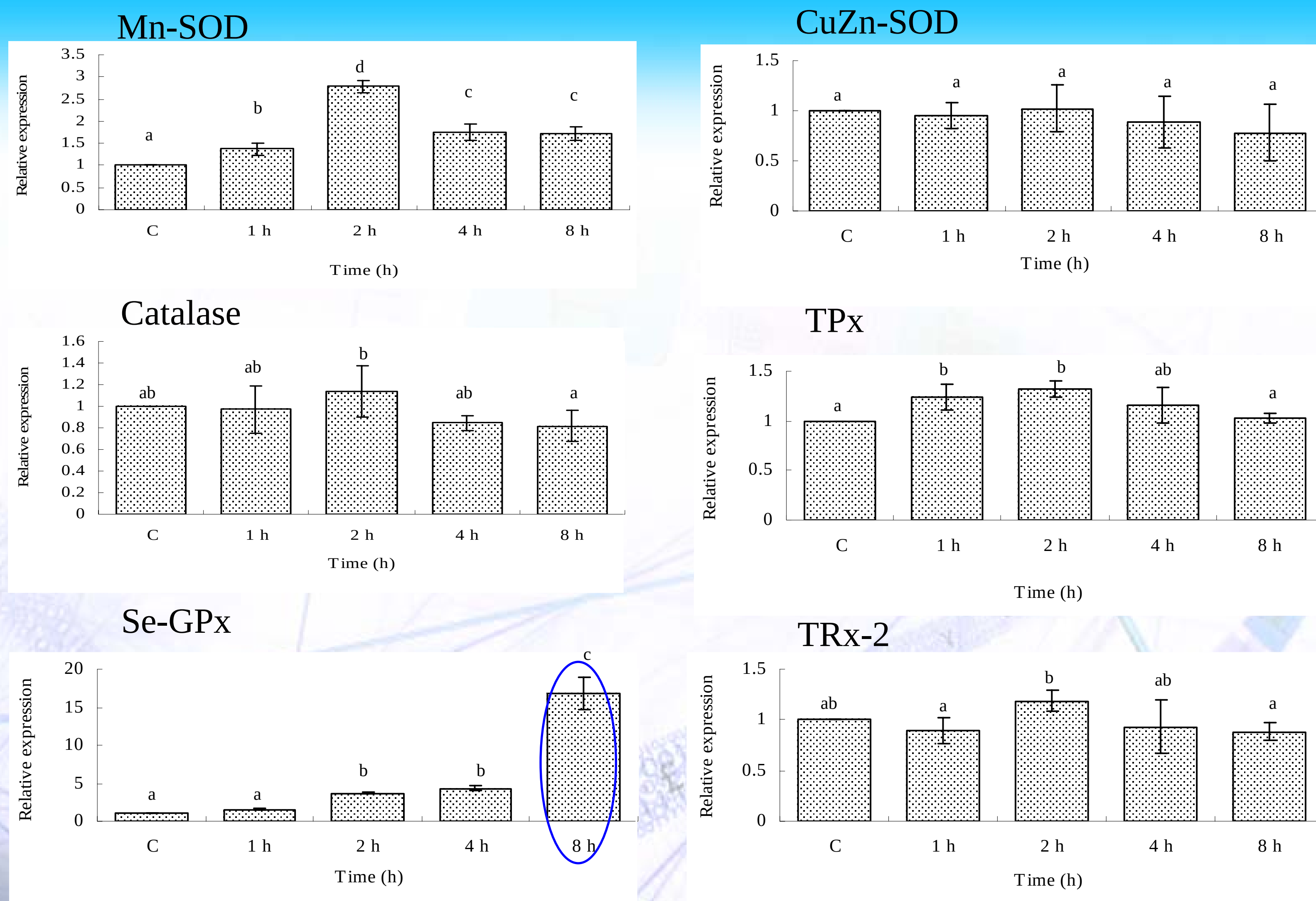


Mx



- Both SOCS-2 and Mx were induced after lowered the salinity

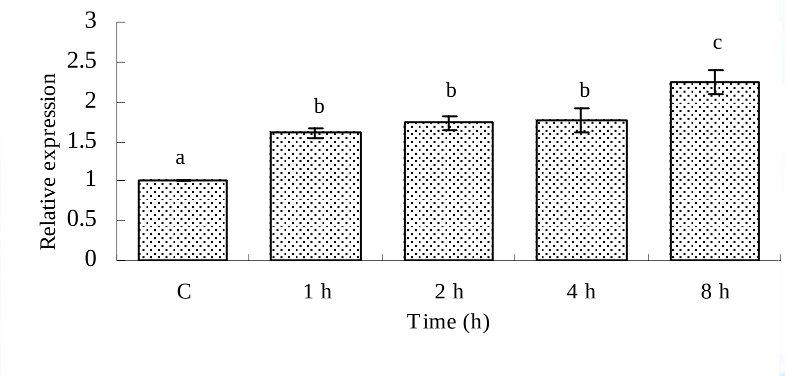
Transcriptional expression of antioxidant enzymes after hypoxia stress



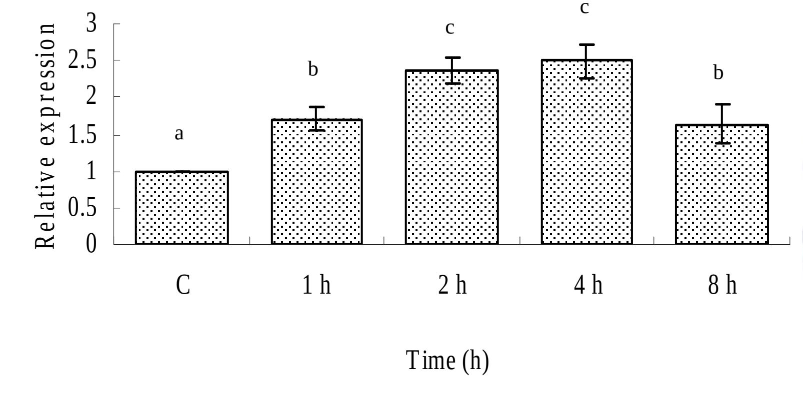
- Mn-SOD, catalase, TPx, Se-GPx were up-regulated
- CuZn-SOD was not responded
- Se-GPx has the highest induction (16.8-fold)

Transcriptional expression of SOCS-2 and Mx after hypoxia stress

SOCS-2



Mx



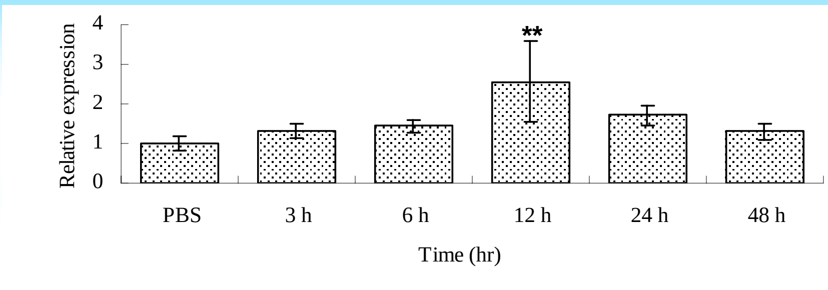
- Both SOCS-2 and Mx were induced after hypoxia stress

Comparative analysis of antioxidant and immune defense genes against thermal, low-salinity and hypoxic stress in abalone gill

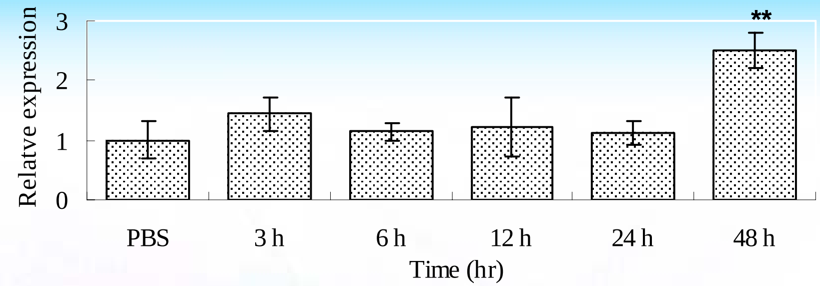
Type of transcriptional regulation	Stress type		
	Heat	Salinity	Hypoxia
1) Complete up-regulated	Catalase TPx Se-GPx	Catalase TPx Se-GPx Mn-SOD CuZnSOD TRx-2 SOCS-2 Mx	Catalase TPx Se-GPx Mn-SOD SOCS-2 Mx
2) Complete down-regulated	CuZn-SOD TRx-2	none	none
3) Mixed (Up-down) regulated	Mn-SOD SOCS-2 Mx	none	TRx-2
4) Not responded	none	none	CuZnSOD

Transcriptional expression of antioxidant enzymes after VHSV infection

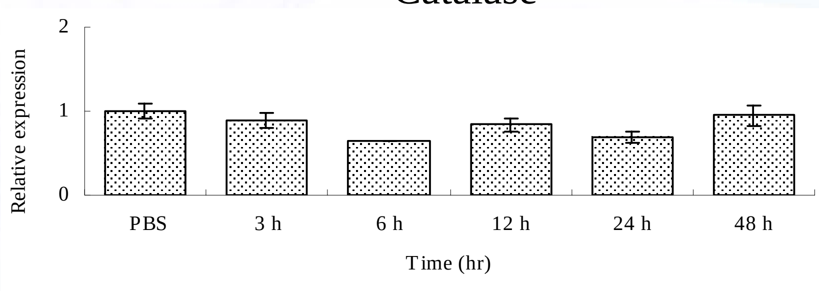
Mn-SOD



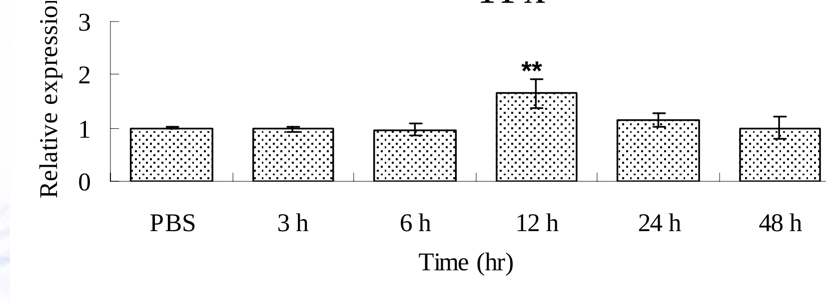
CuZn-SOD



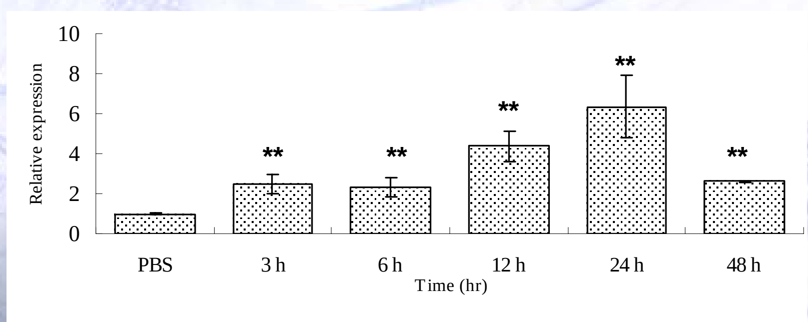
Catalase



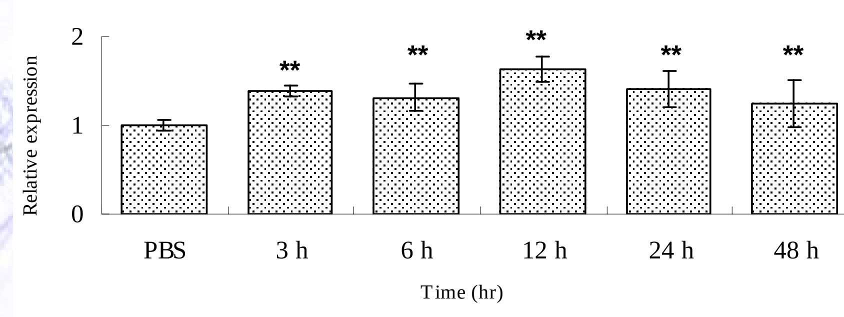
TPx



Se-GPx



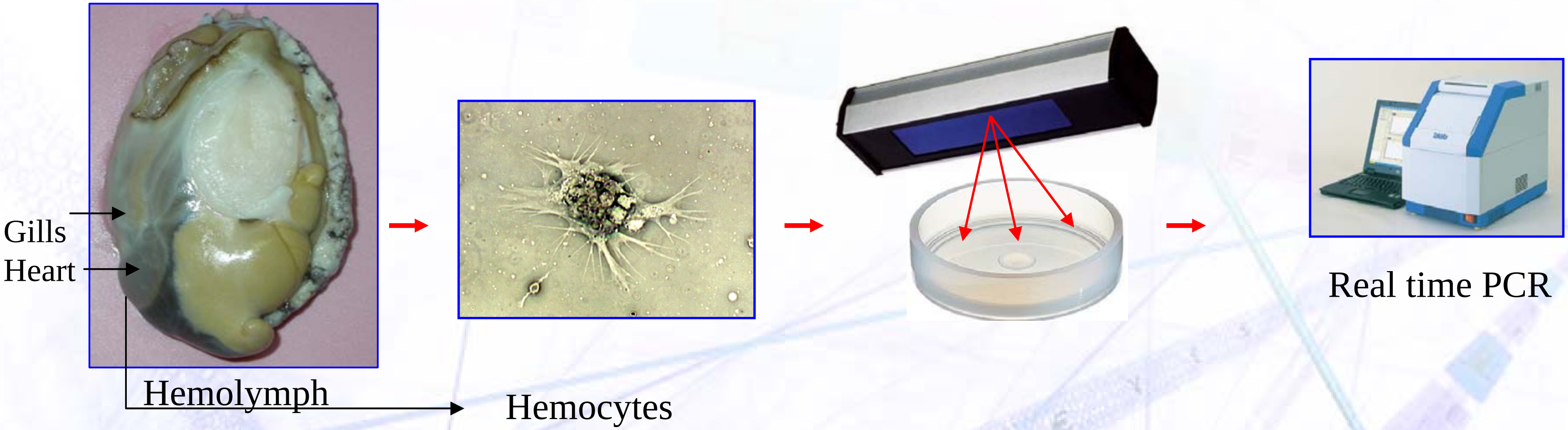
Mt-TRx-2



- All transcripts (except catalase) were up-regulated during the VHSV infection

Transcriptional analysis of antioxidant enzymes after UV-B exposure on abalone hemocytes

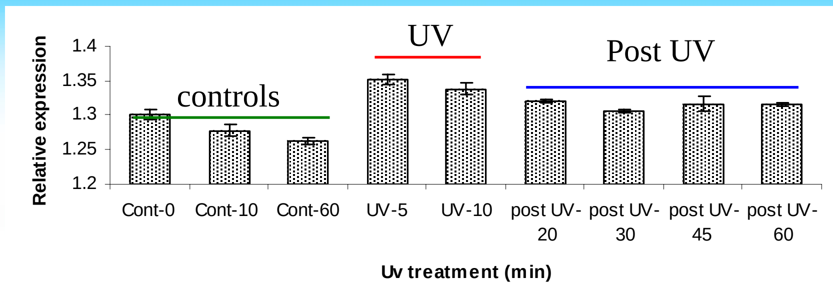
Isolation of abalone hemocytes | UV-B treatment (312 nm) | Transcriptional analysis



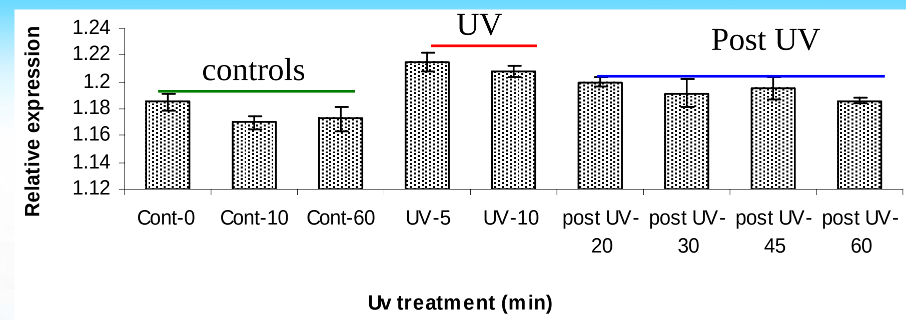
- UV-B expose for 10 min (100 mJ/cm^2) at 20 ^{o}C

Transcriptional expression analysis of antioxidant enzymes in abalone gill after UV stress

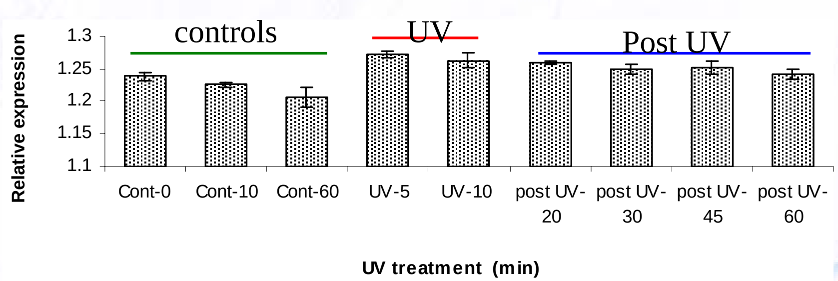
Mn-SOD



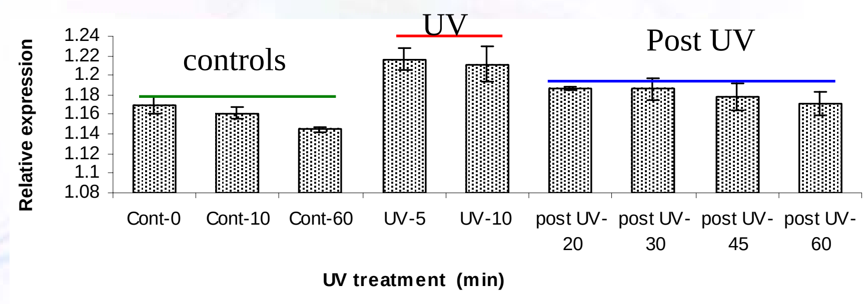
CuZn-SOD



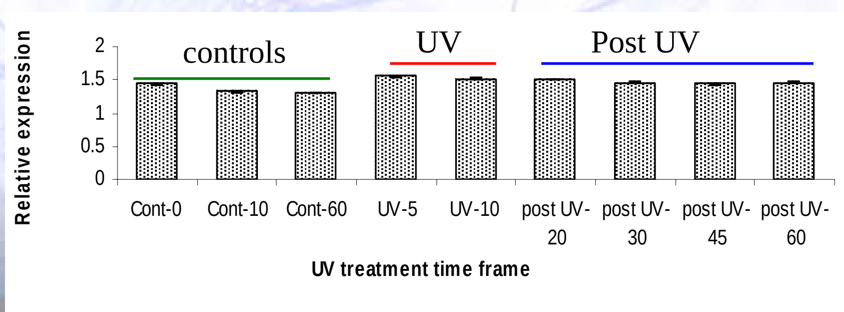
Catalase



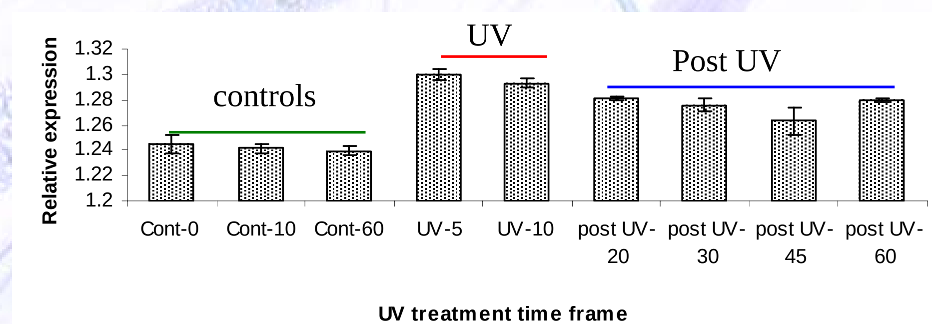
TPx



Se-GPx



Mt-TRx-2



- All transcripts showed elevated transcripts during the UV-B exposure compared to controls

Summery

- Several antioxidant enzymes such as MnSOD, CuZnSOD, catalase, TPx, SeGPx, and TRx-2 are represent classical enzymatic antioxidant defense system in abalone

- All the antioxidant transcripts exhibited significant up-regulation in response to low salinity-stress at 25 ‰

- Catalase, TPx and SeGPx transcripts were significantly up-regulated by all three physical stress conditions namely thermal (28^{o}C), salinity (25‰) and hypoxia

- Also, all the antioxidant enzymes are shown elevated transcripts after UV-B (312 nm) radiation for 10 min (100 mJ/cm2) in abalone hemocytes

- Antioxidant enzymes were induced by viral hemorrhagic septicemia virus (VHSV) infection

- Salinity, hypoxia and VHSV treatments increase the transcription of immune response SOCS-2 and Mx

Future focusing and applications

- **Transcriptional analysis of abalone antioxidant enzymes against**

  √ Chemicals stress, crude oil, etc

  √ Biological stress by pathogenic bacteria and parasites like scutica cilliate

- **Field experiment based seasonal variation and potential environmental polluted sites**

- **Selection of highly "sensitive biomarker antioxidant genes" from abalone for biomonitoring studies**

Thank You