Part I

Zooplankton diversity and its long-time variation in the China Seas: implications of climate change

Part II

Comprehensive transcriptome study to develop molecular resources of the copepod *Calanus sinicus* for their potential ecological applications

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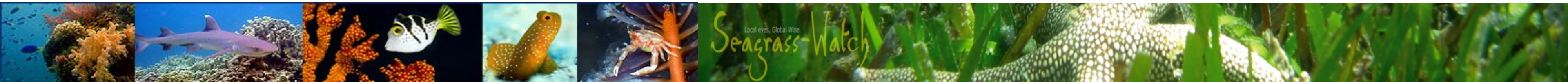
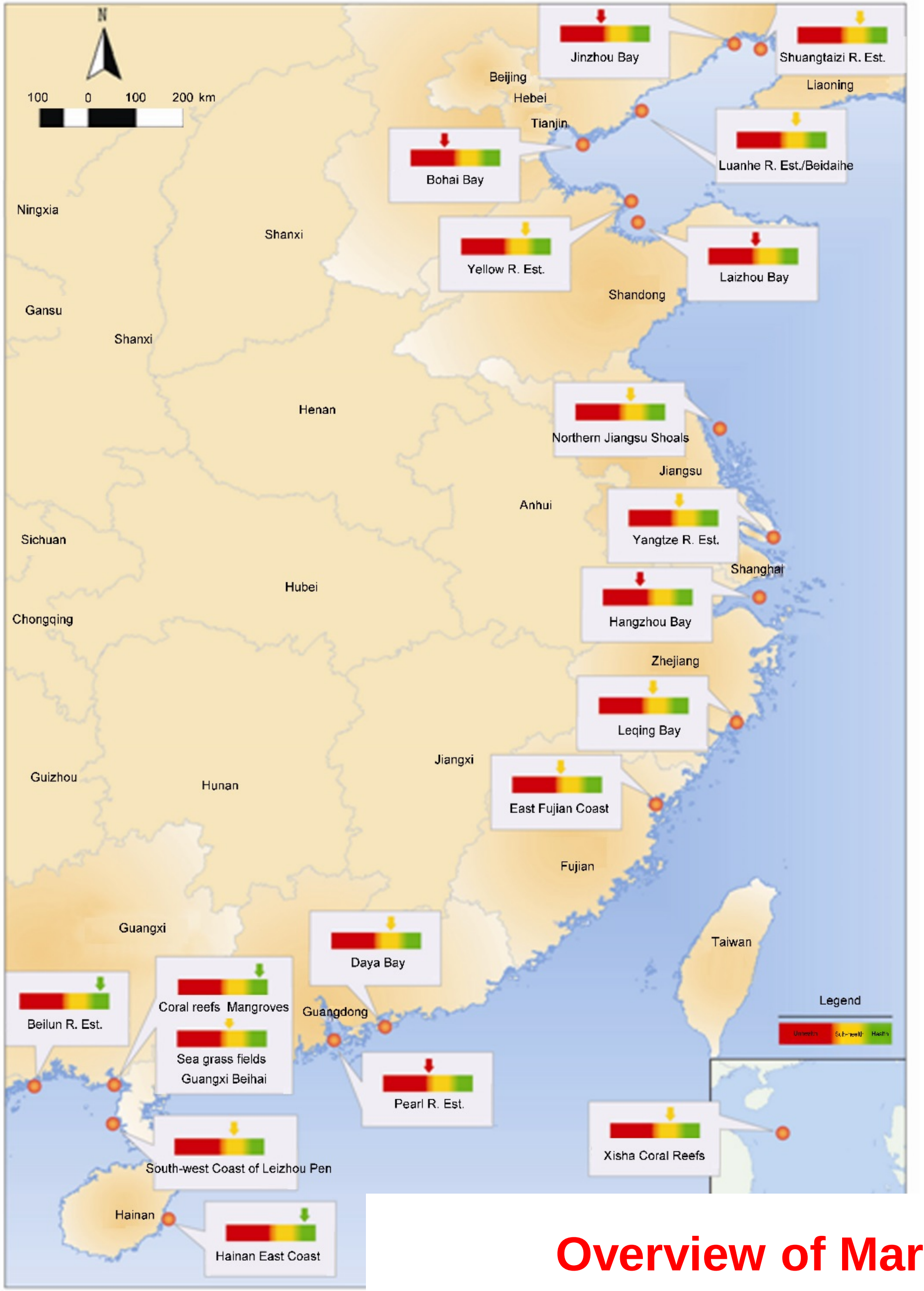
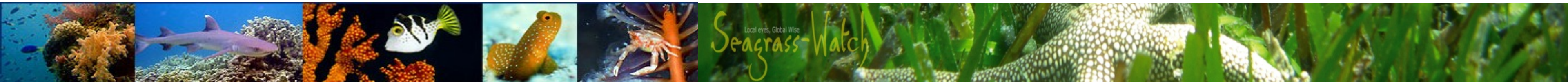
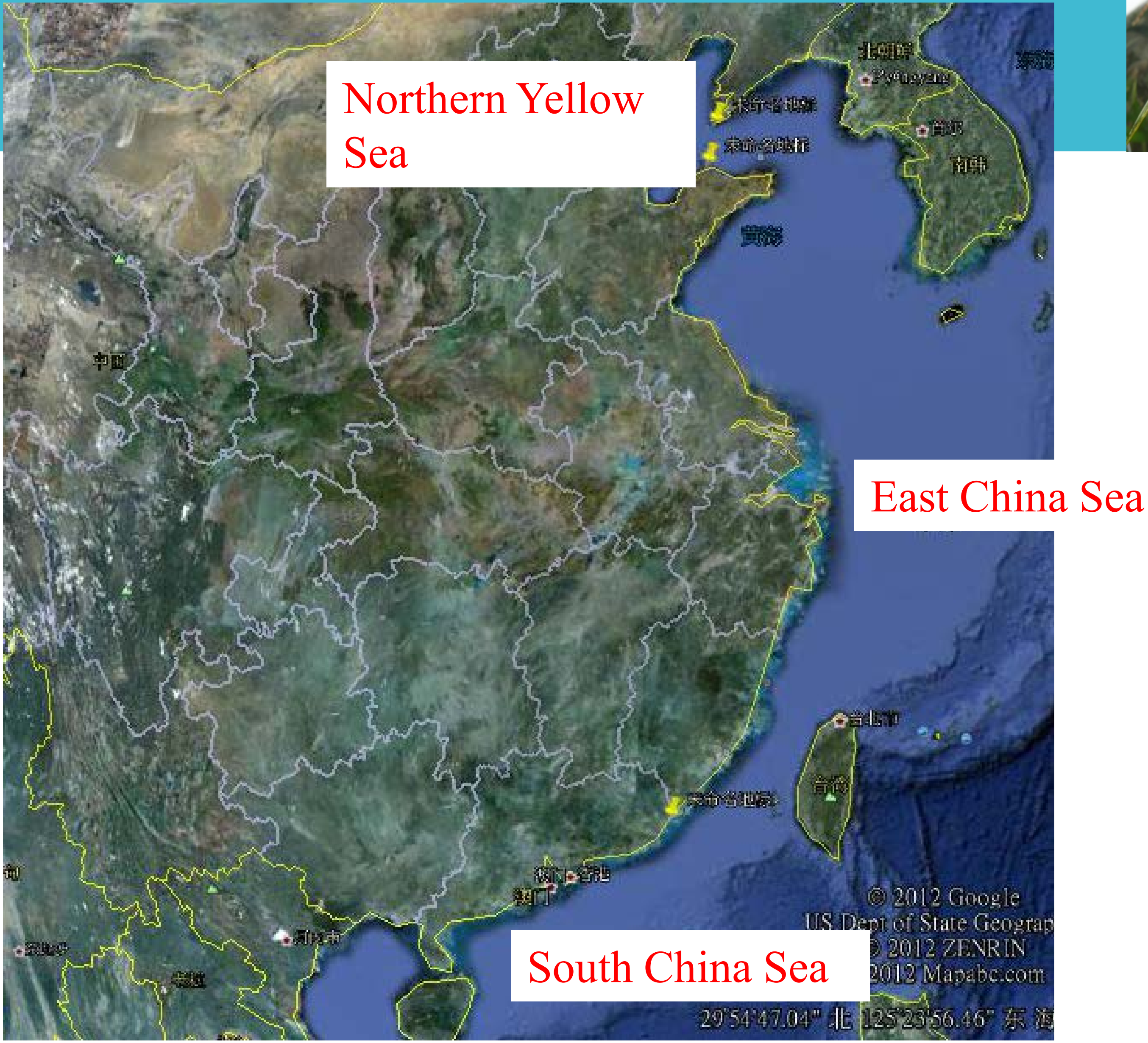
2014.10.23



Overview of Marine Environment Monitoring in China







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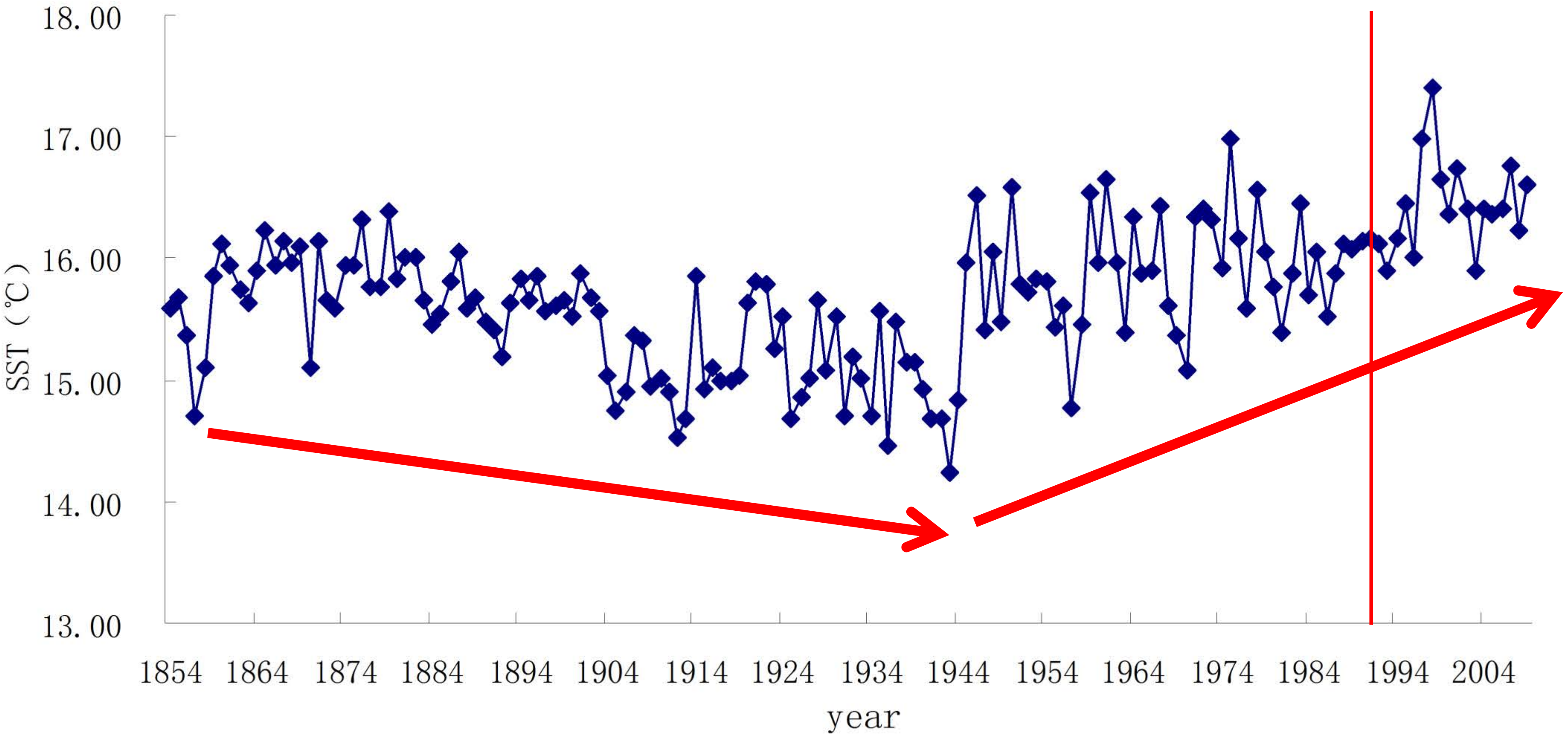
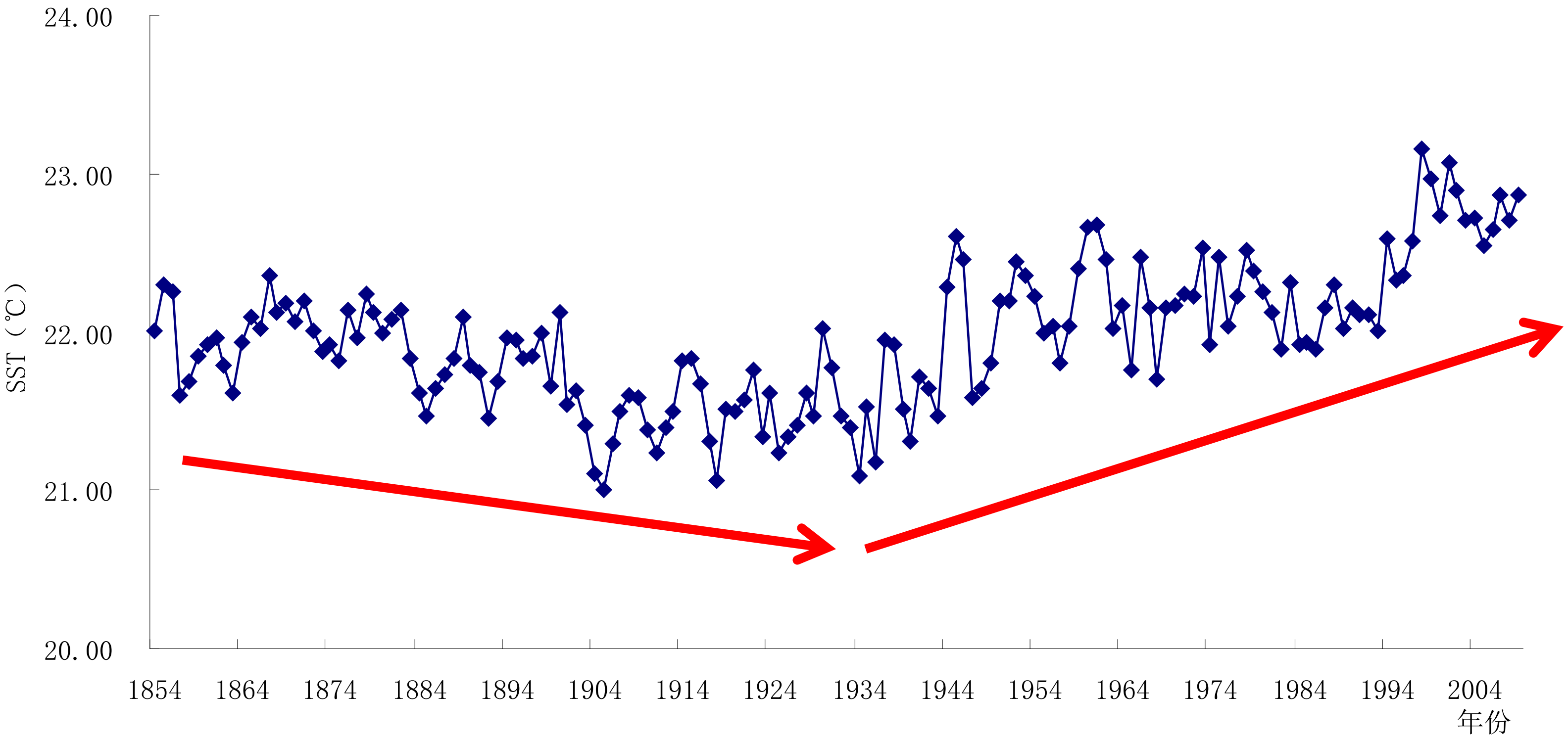
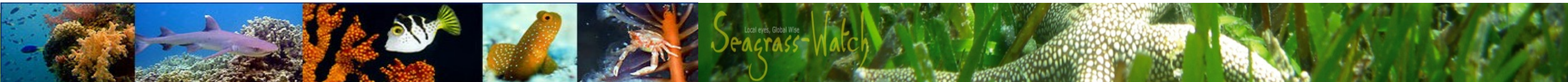
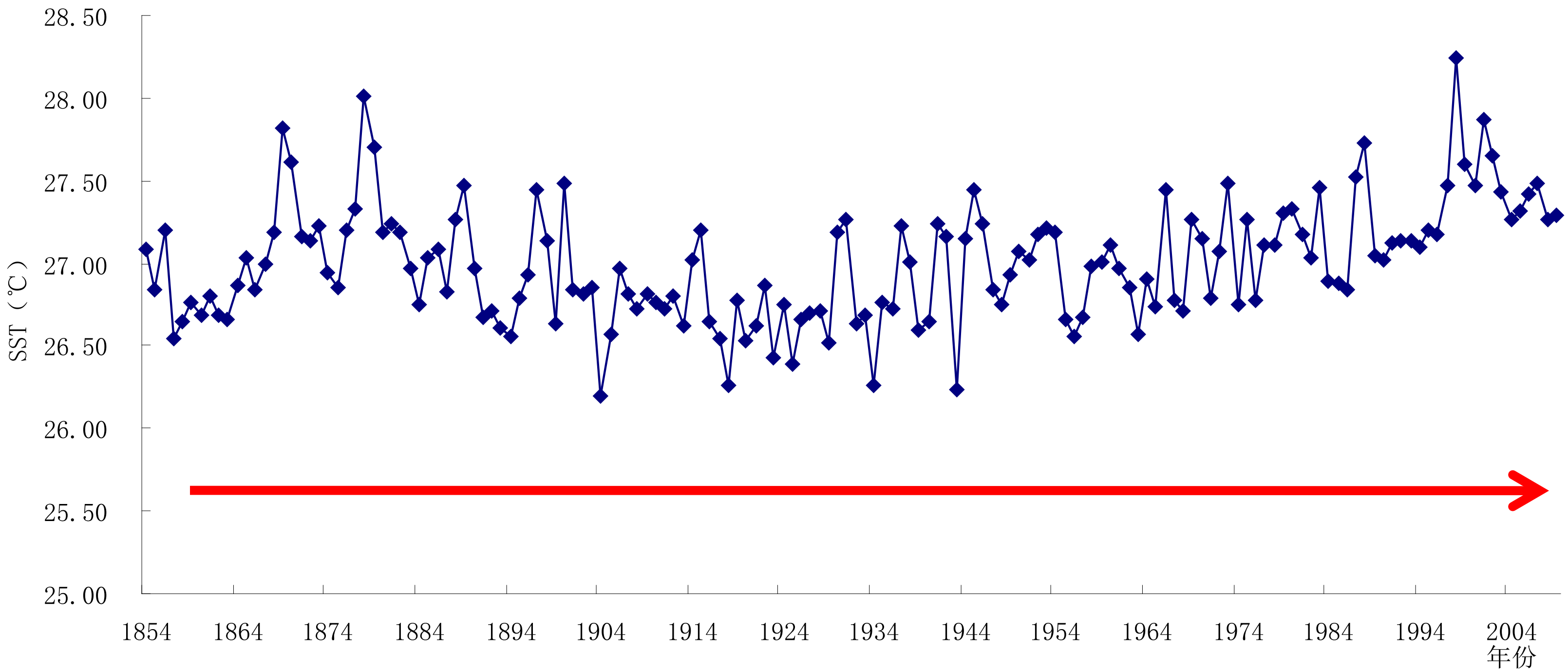


Mean annual sea surface temperature (SST) of Northern Yellow Sea in the past 100 years

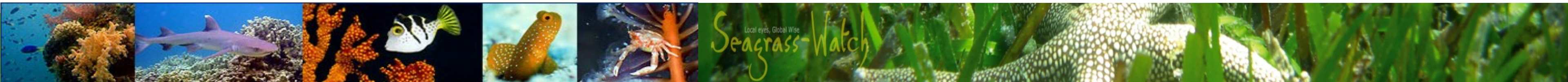


Mean annual sea surface temperature (SST) of East China Sea in the past 100 years





Mean annual sea surface temperature (SST) of South China Sea in the past 100 years

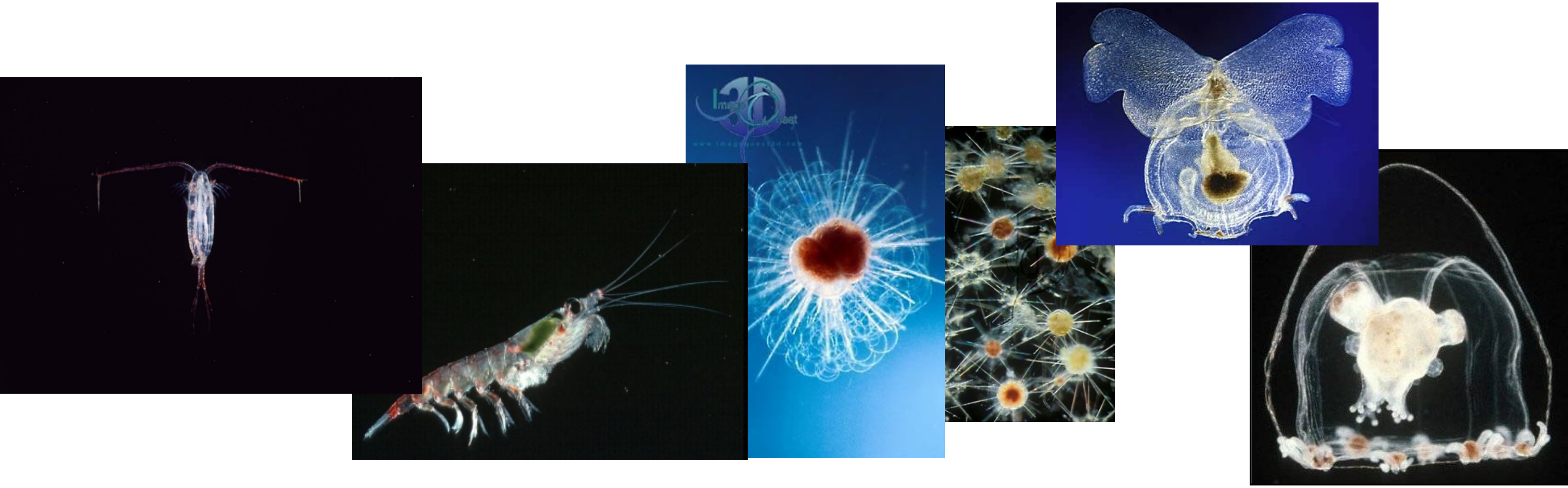


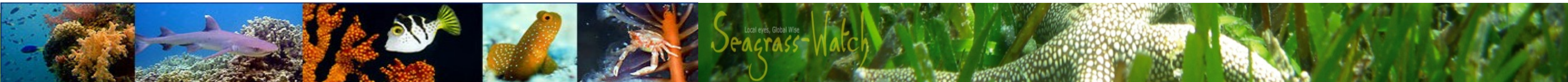
❖Zooplankton-good indicator of climate change

1. Few Zooplankton species have been commercially exploited.
2. Short life history.
3. Dramatic changes in distribution due to free floating character.

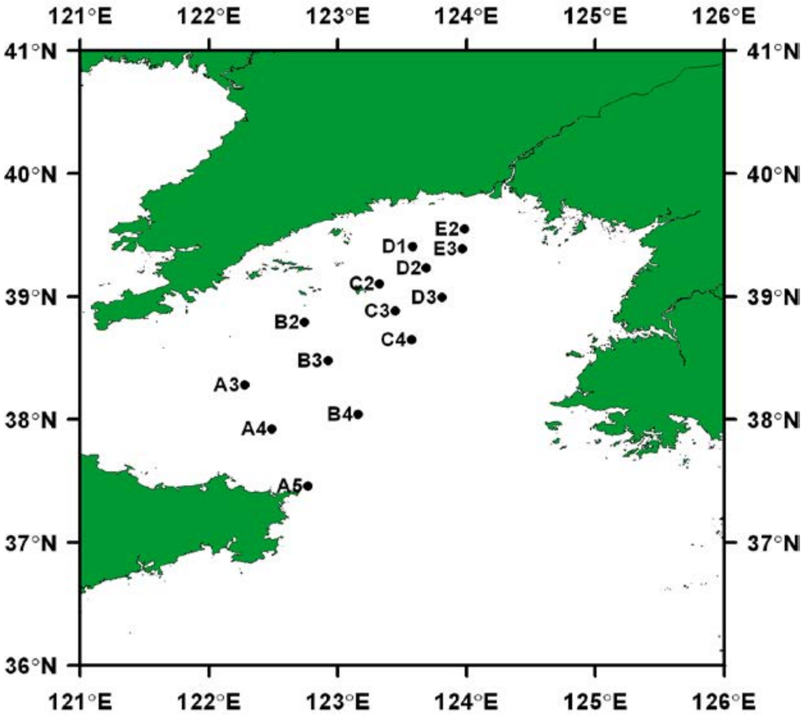
Nonlinear responses of biological communities

→amplify environmental perturbations?

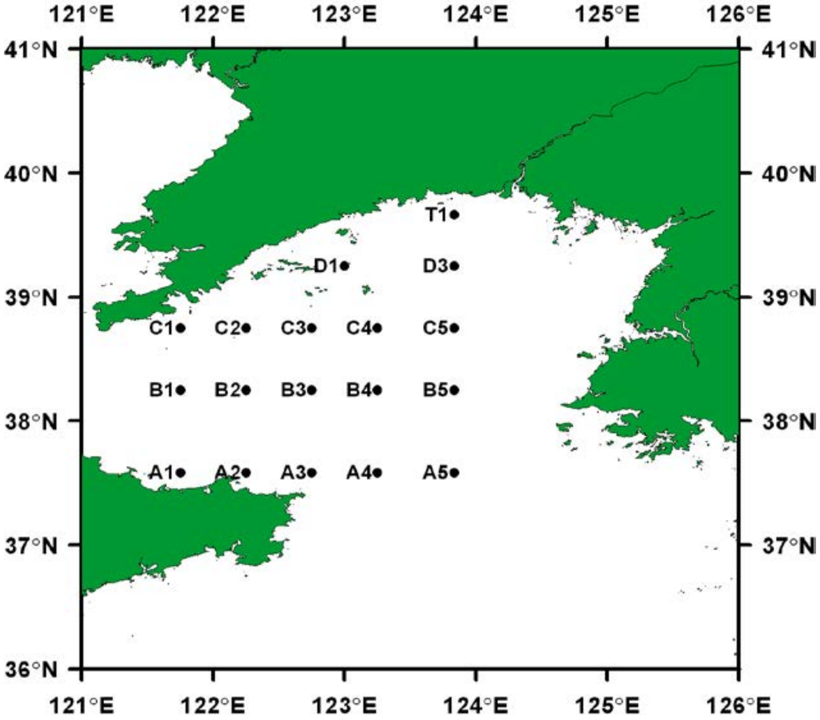




Effects of climate change on zooplankton diversity



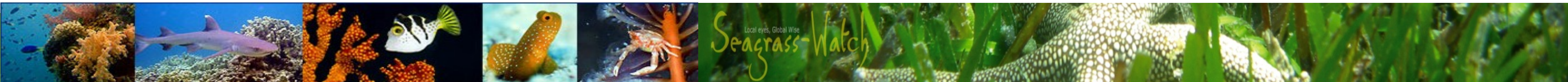
Vs.



Year 1959

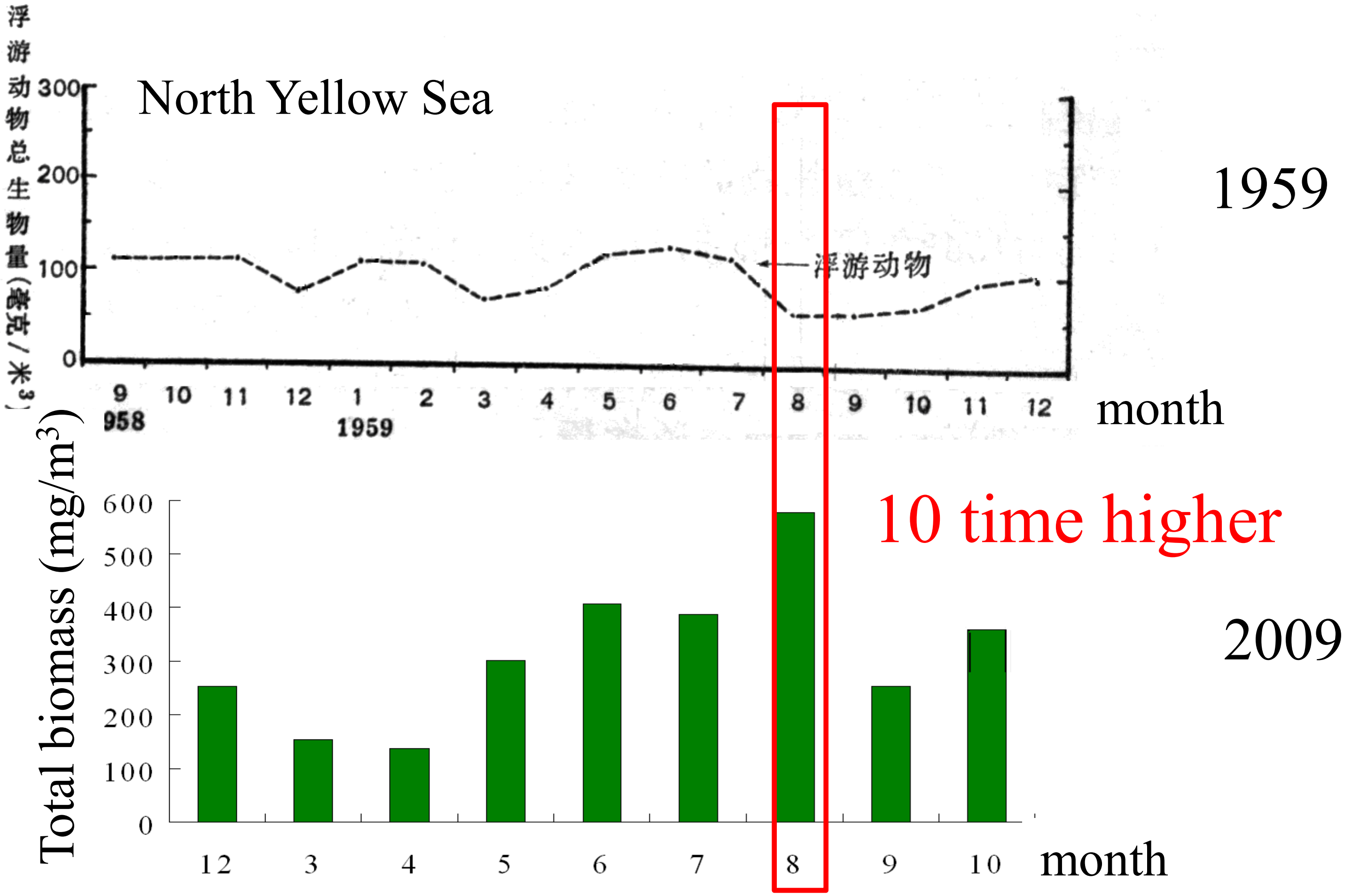
Year 1982, 2009

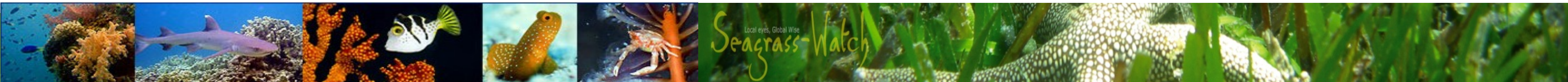
Zooplankton monitoring stations in Northern Yellow Sea



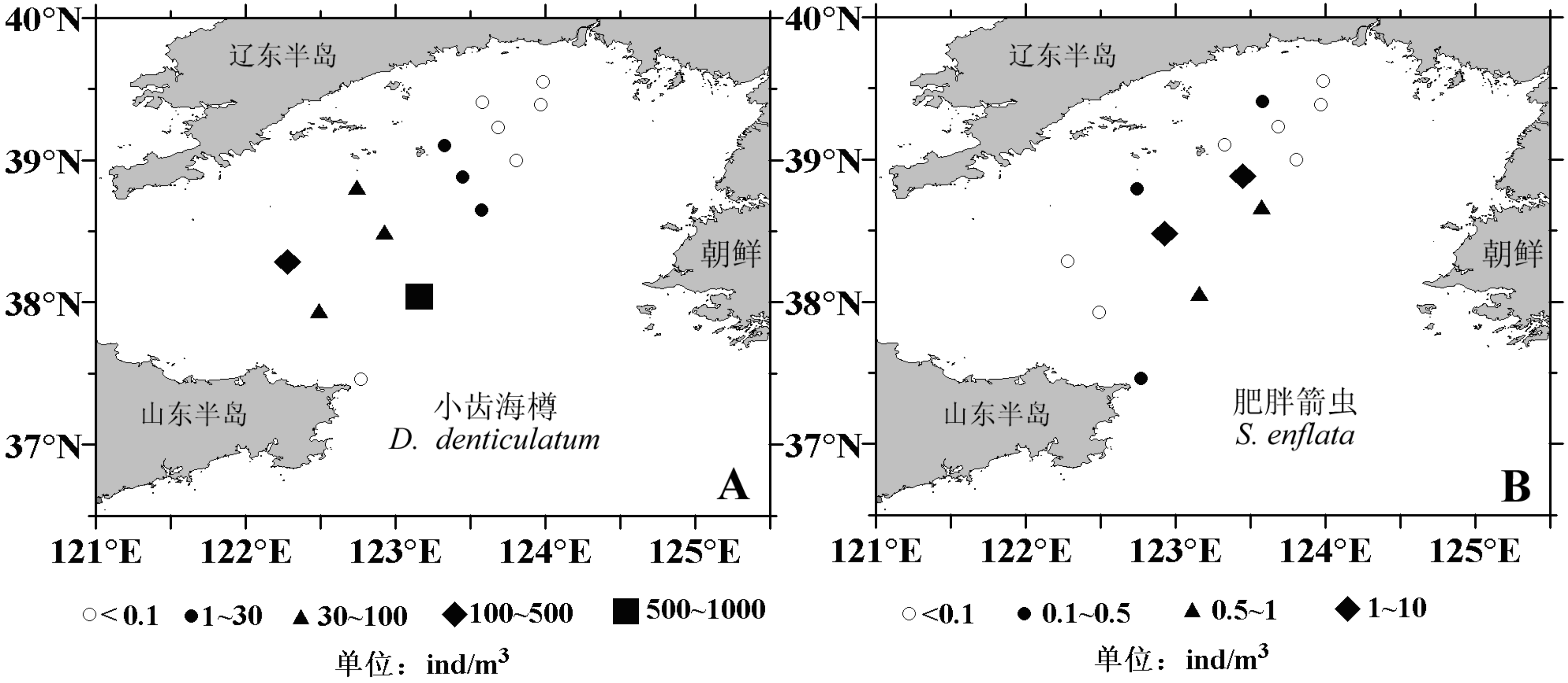
Zooplankton diversity comparision (1959 vs. 2009)

Zooplankton total biomass increased and season variation pattern changed.

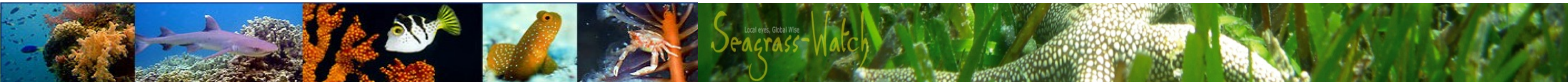


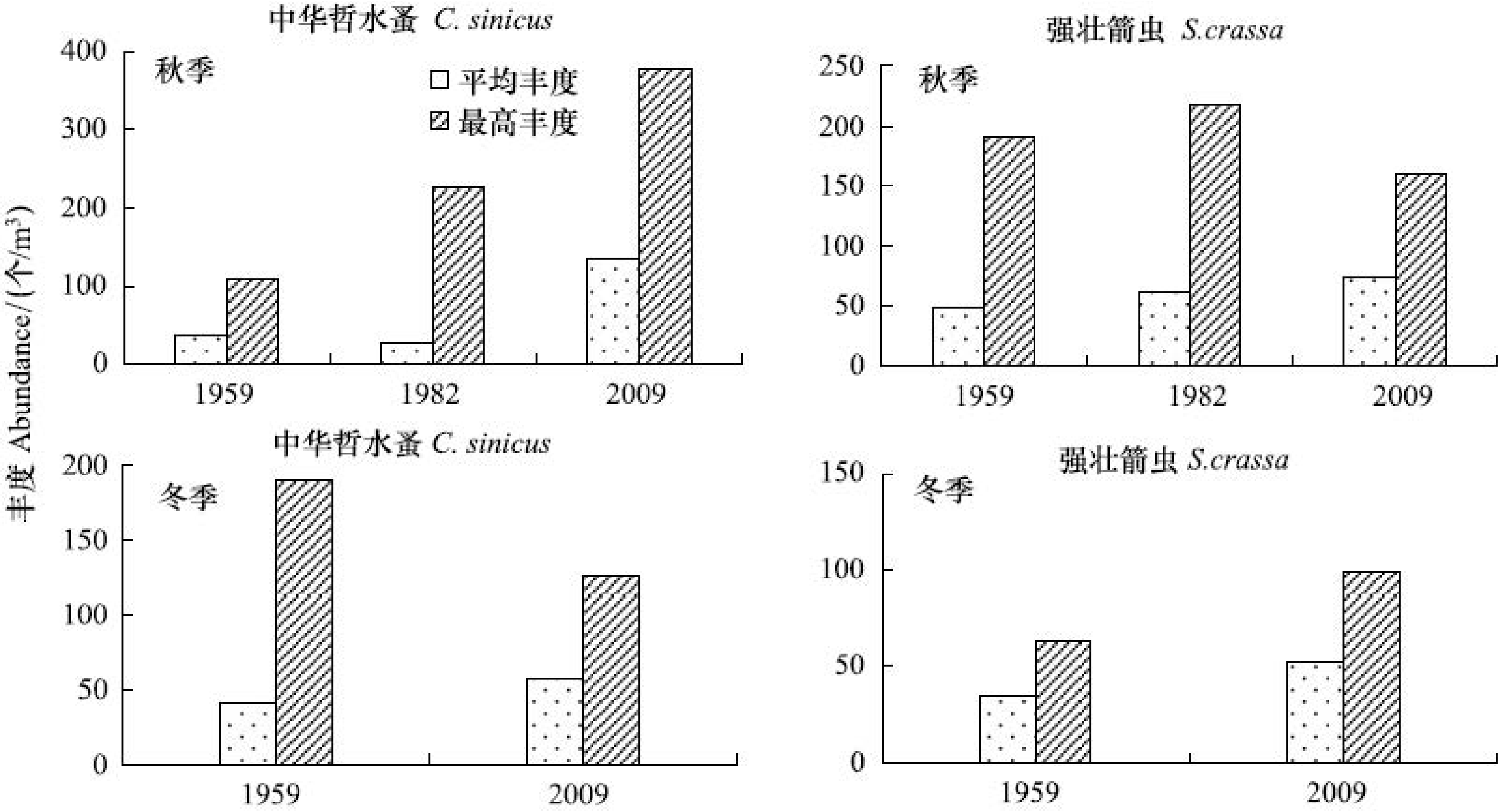


Zooplankton community structure changed, with warm-temperate species moving northword.

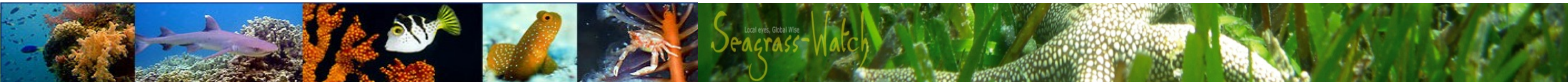


Compared with 1982, their distribution boundary both moved northword in 2009.



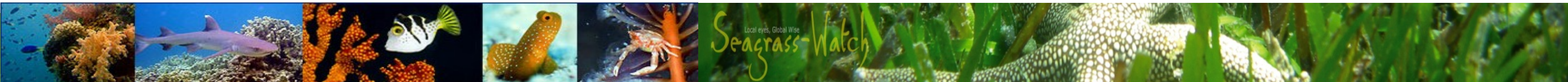



The abundance of the main warm-temperate species has increased significantly in the NYS over the past 50 years.

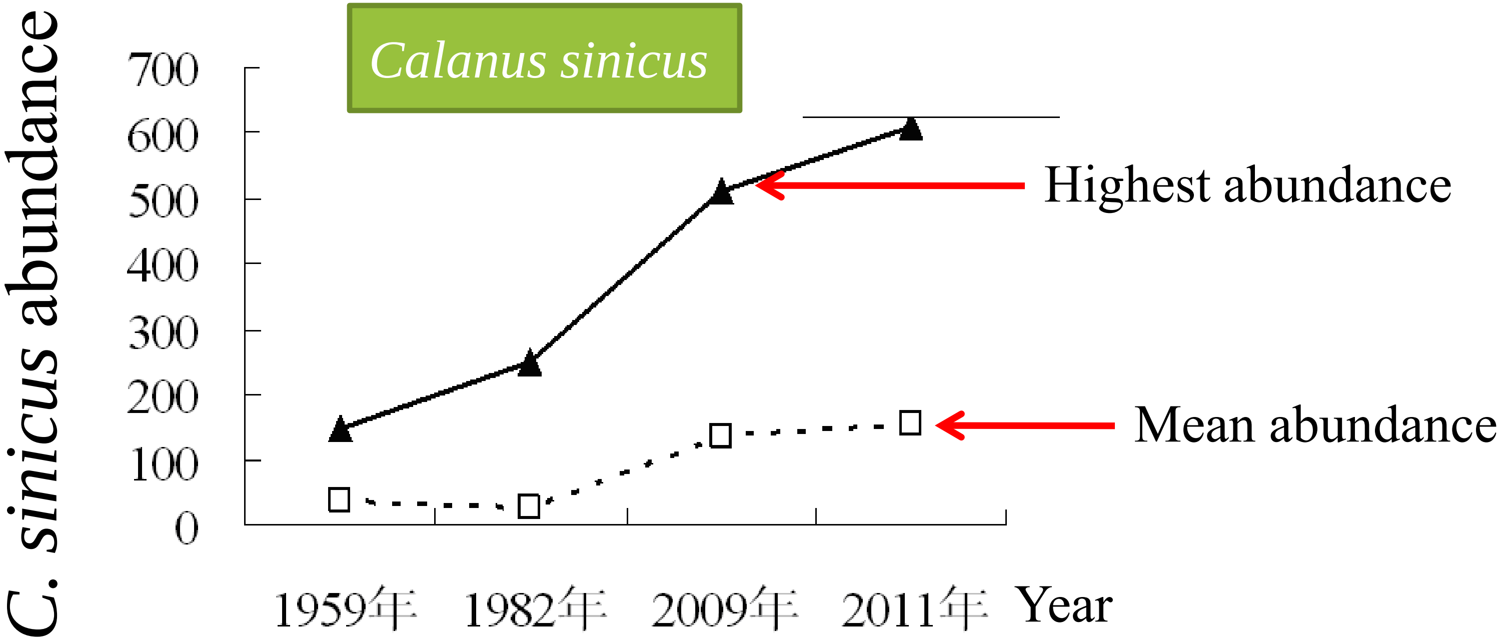


Abundance of two warm-temperate species in 1982 and 2009.

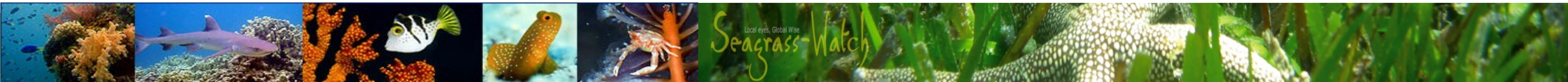
Species	Year	Mean abundance (ind/m^3)	Highest abundance (ind/m^3)
Doliolum denticulatum	1982	62.38	571.42
	2009	81.00	817.50
Sagitta enflata	1982	0.01	0.39
	2009	0.24	2.50

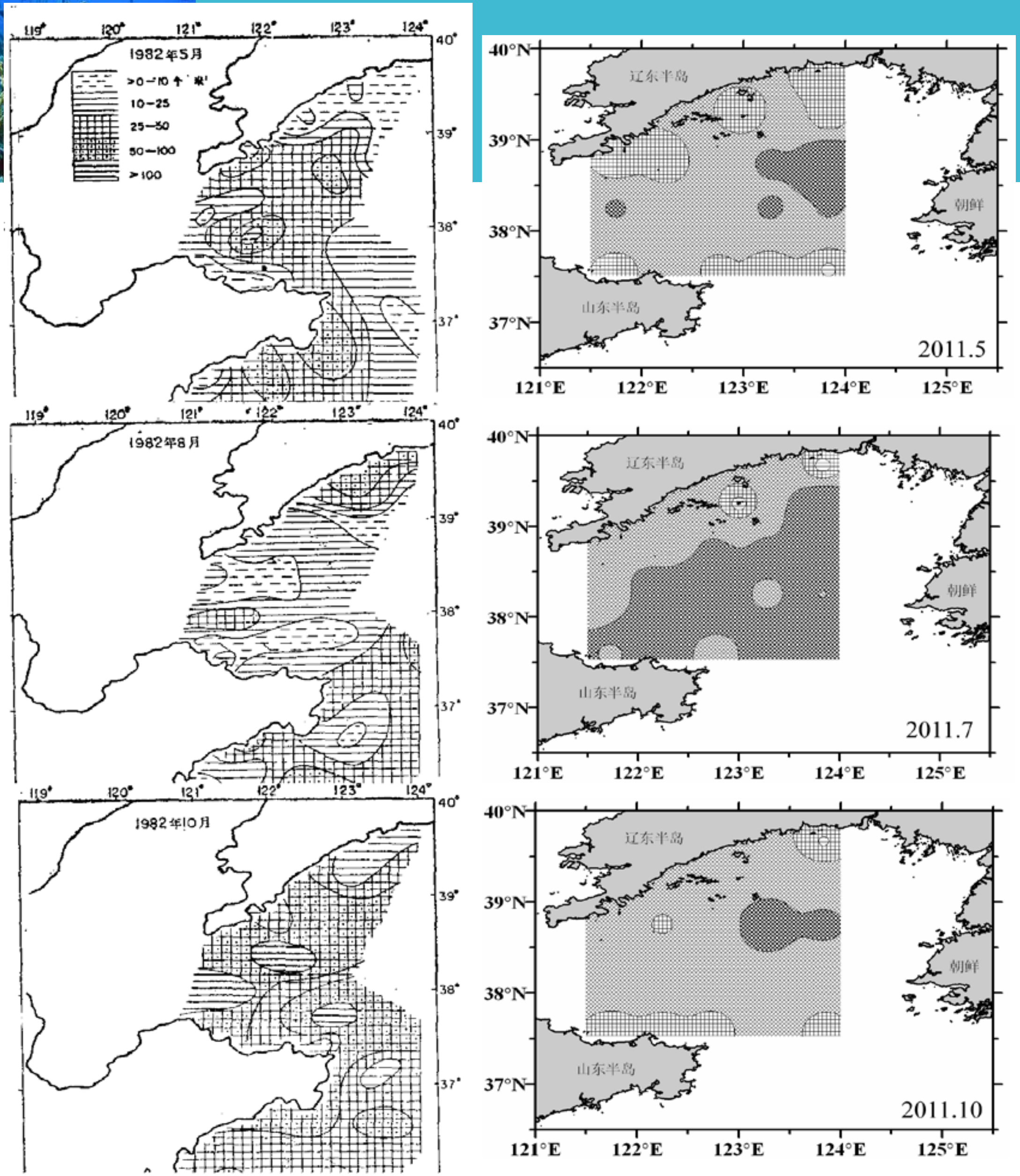


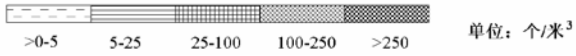
- Dominant species in 1959, 1982 and 2009:



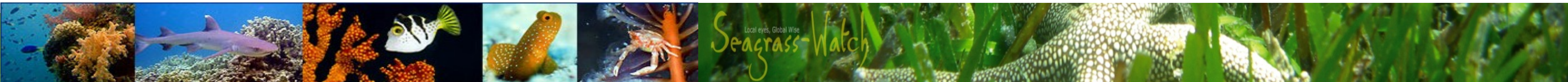
Abundance of *Calanus sinicus* in 1959, 1982 and 2009.





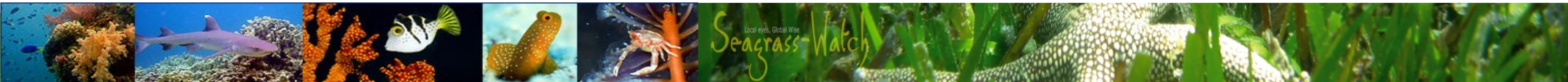


Spatial distribution of *Calanus sinicus* in 1982 and 2011.



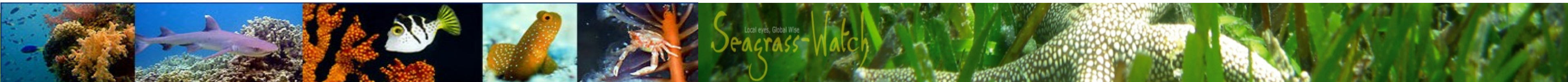
Possible causes

- Climate change (warming)
- Fishing presure
- Pollution input
- Invasion of alien species

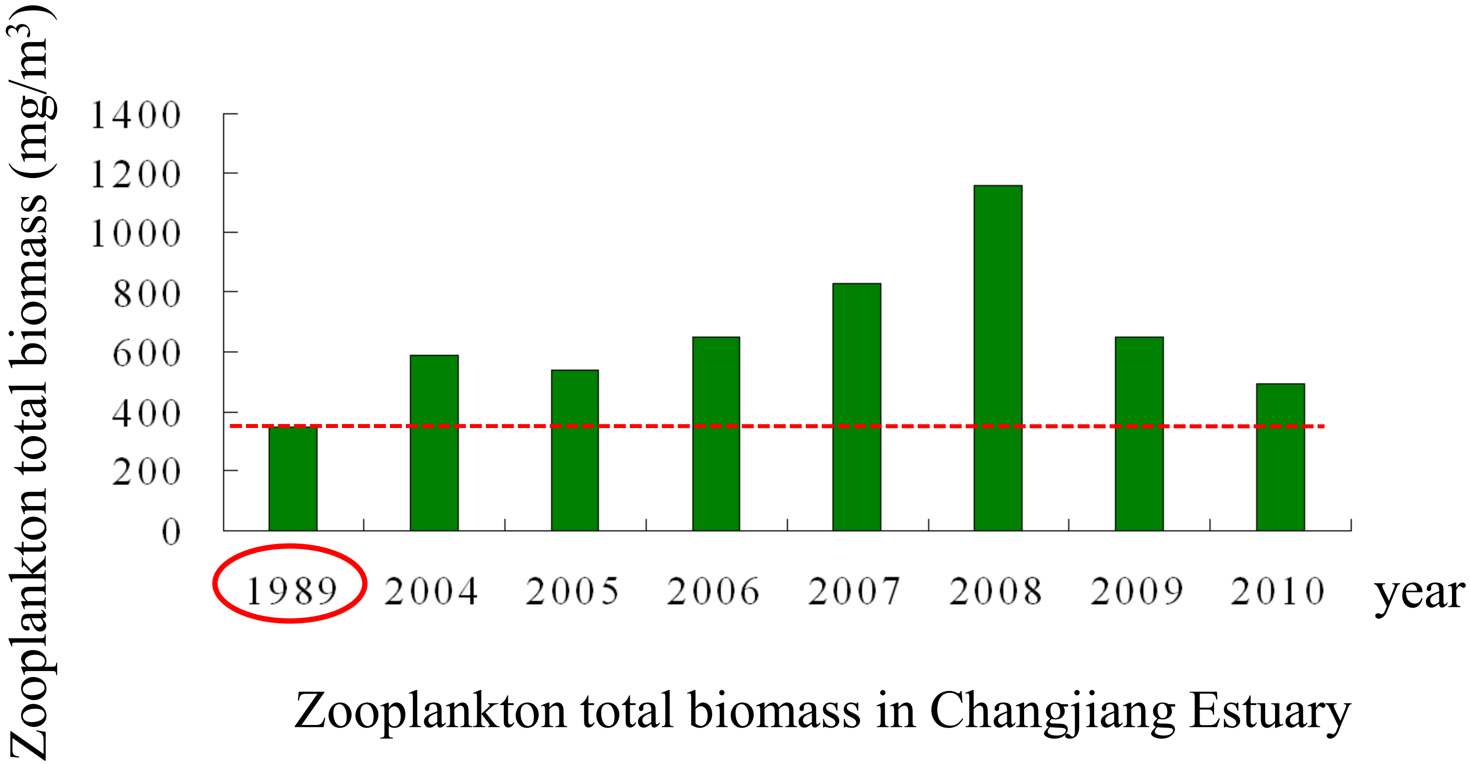


Evidence from East China Sea

In the past 50 year, the total biomass of zooplankton in the Changjiang Estuary increased in response to climate change; zooplankton community structure changed, with warm water species abundance increased and warm temperate species abundance reduced; replacement of main group and dominant species is not obvious; copepods percentage showed a downward trend, while jellyfish percentage increased; zooplankton community structure and ecological patterns changed significantly.

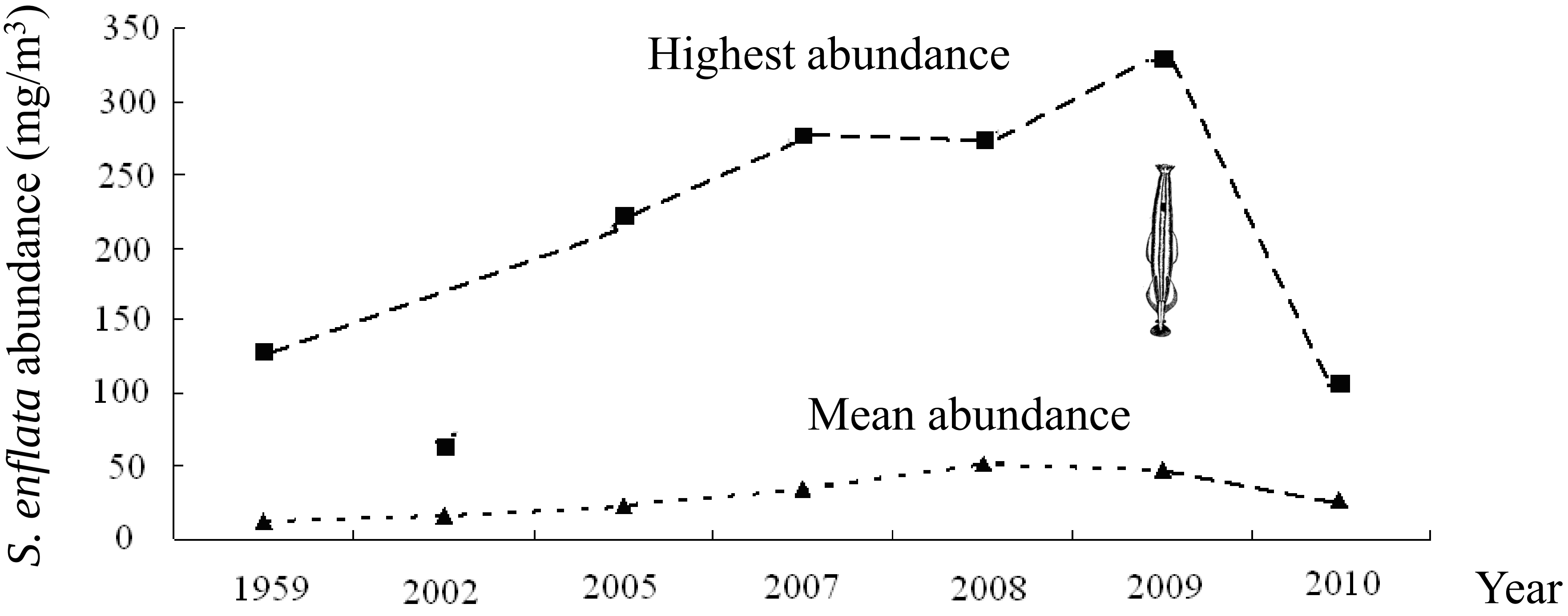


Zooplankton total biomass increased

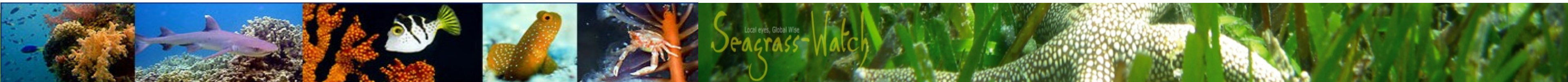


Zooplankton total biomass in Changjiang Estuary

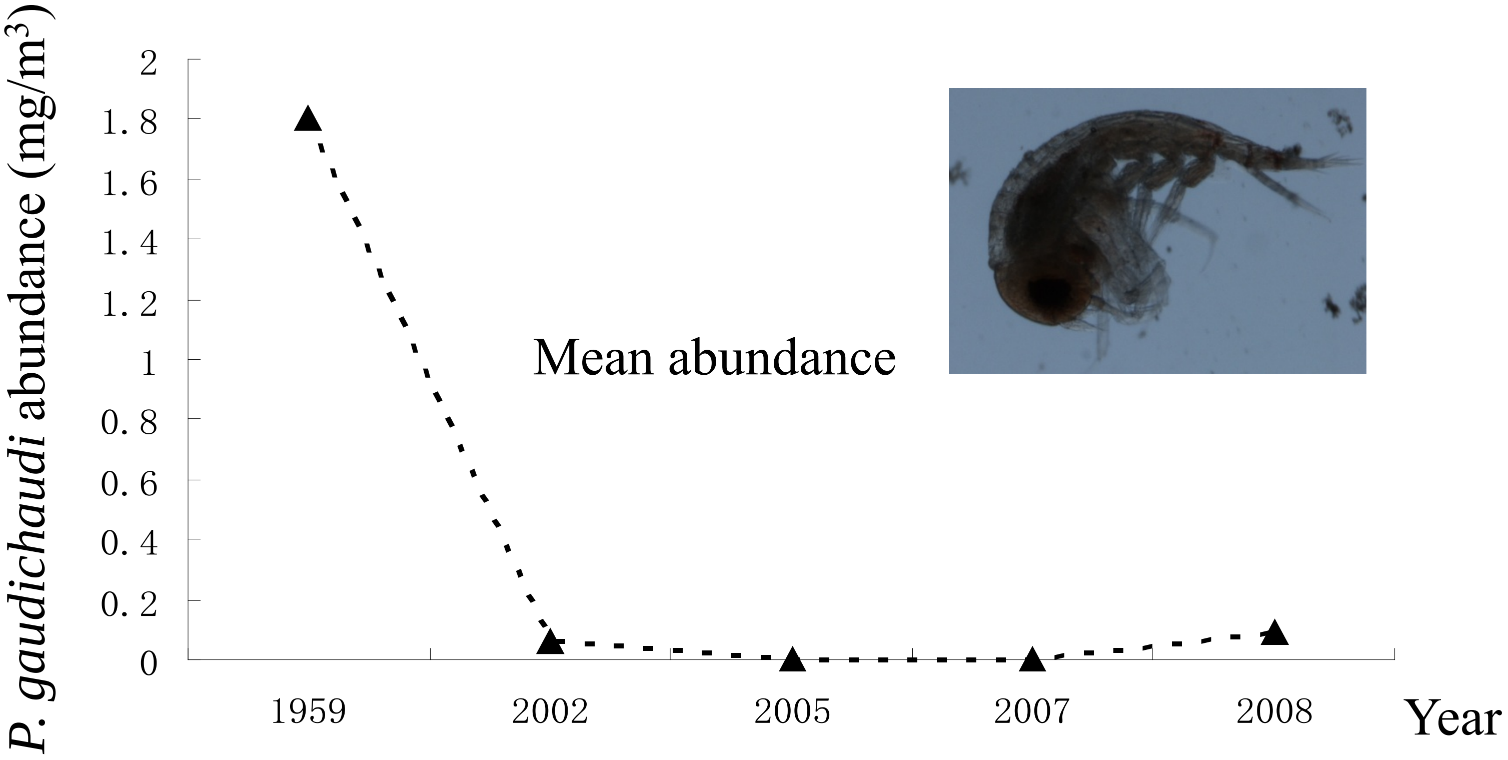
Warm water species abundance increased



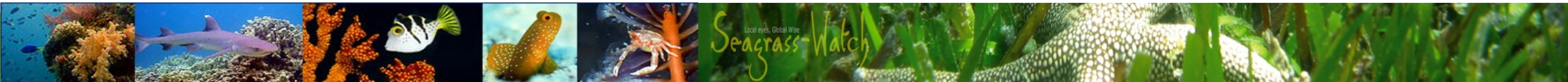
Sagitta enflata abundance in summer



Warm temperate species abundance reduced

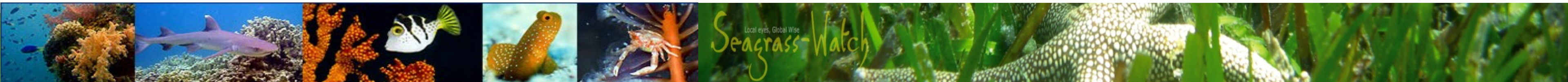


Parathemisto gaudichaudi abundance in summer



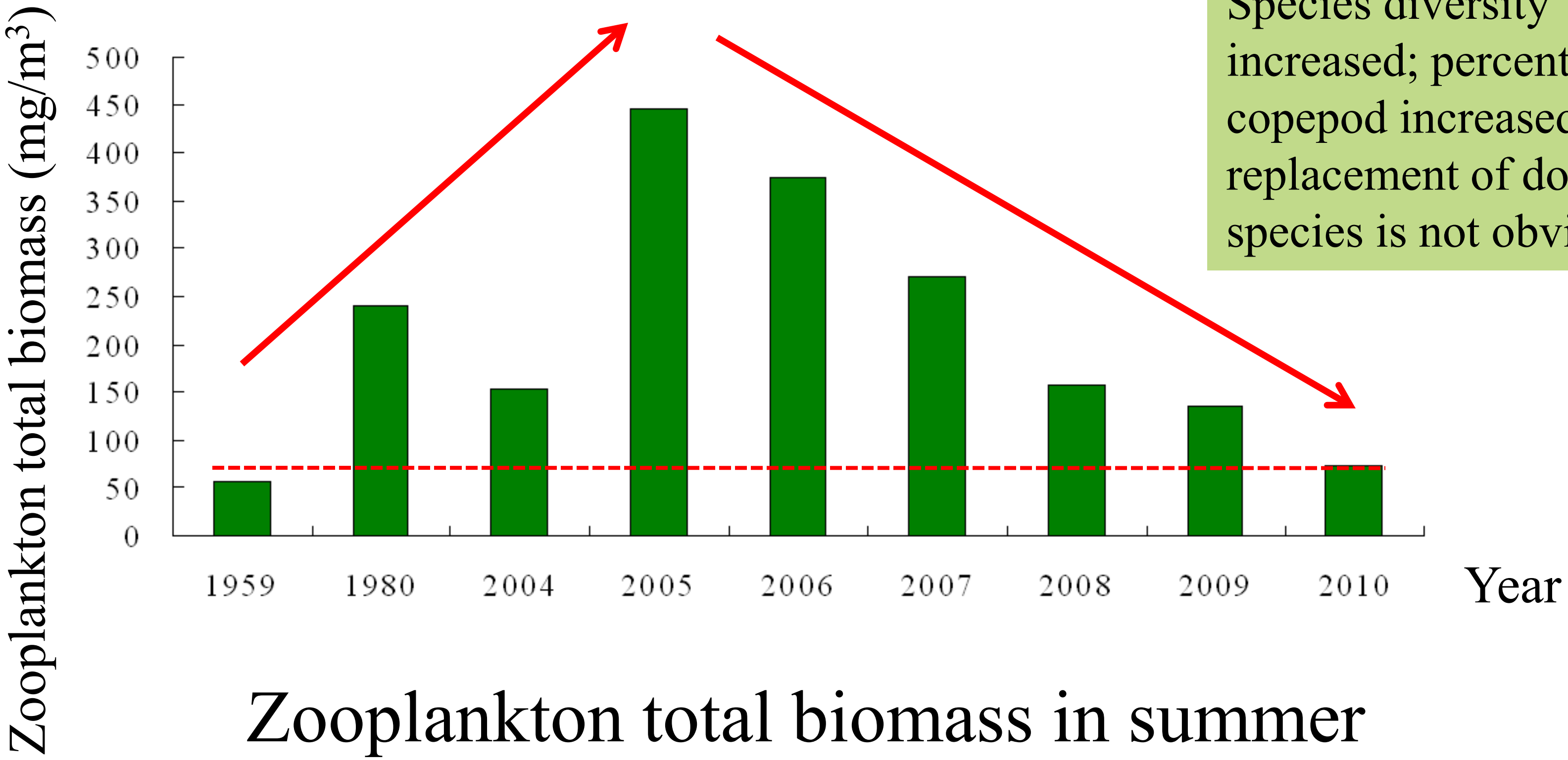
Evidence from South China Sea

- In the past 50 years, zooplankton total biomass firstly increased and then decreased to the level of 1959 in 2009;
- Zooplankton species diversity in spring and summer showed an upward trend;
- The percentage of copepod showed an increasing trend;
- The replacement of dominant species has not occurred.

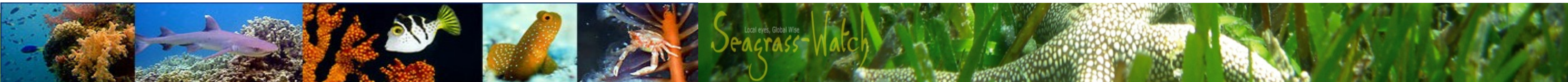


Zooplankton total biomass firstly increased and then decreased

Species diversity increased; percentage of copepod increased; the replacement of dominant species is not obvious.



Zooplankton total biomass in summer



Part II

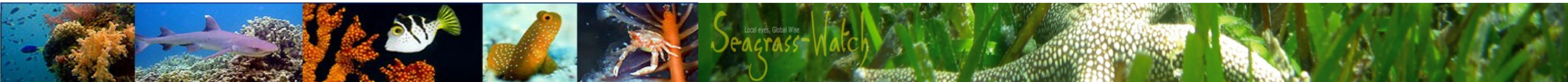
Comprehensive transcriptome study to develop molecular resources of the copepod *Calanus sinicus* for their potential ecological applications

Calanus sinicus Brodsky (Copepoda, Crustacea) is a dominant zooplanktonic species widely distributed in the margin seas of the Northwest Pacific Ocean. In this study, we sequenced the transcriptome to develop molecular resources for this species.

The transcriptome is the total set of transcripts, mRNA and non-coding RNA, in one or a population of cells under specific conditions. The transcriptome analysis lays the foundation for gene structure and function research. Based on new generation sequencing technology, RNA-Seq found its applications in many research fields.

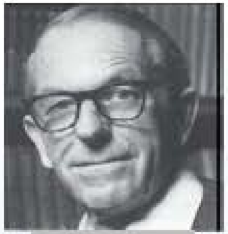
Calanus sinicus

Transcriptome



Brief History of Sequencing Technology Development

First-generation technology


1977

Sequence length, sequence quality, throughput, cost

Next-generation technology

2005

2006

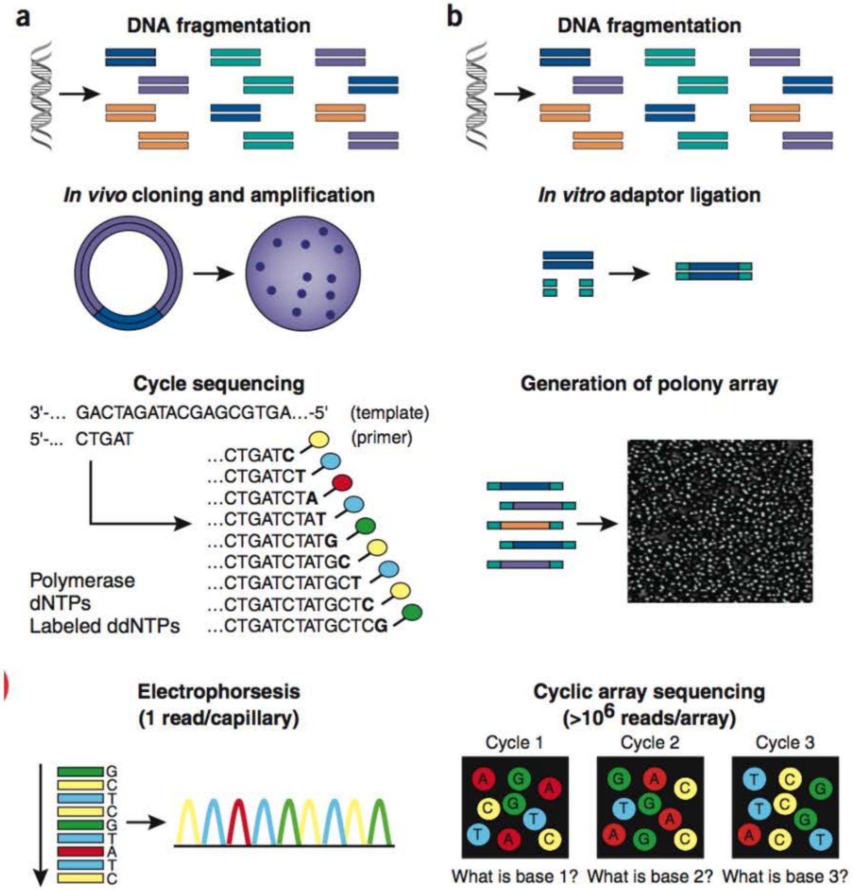
2007

Third-generation technology


2008

Next Generation Sequencing – Next Wave of Revolution

ecological genomics

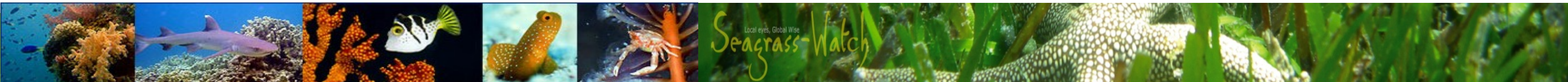


Lego Model

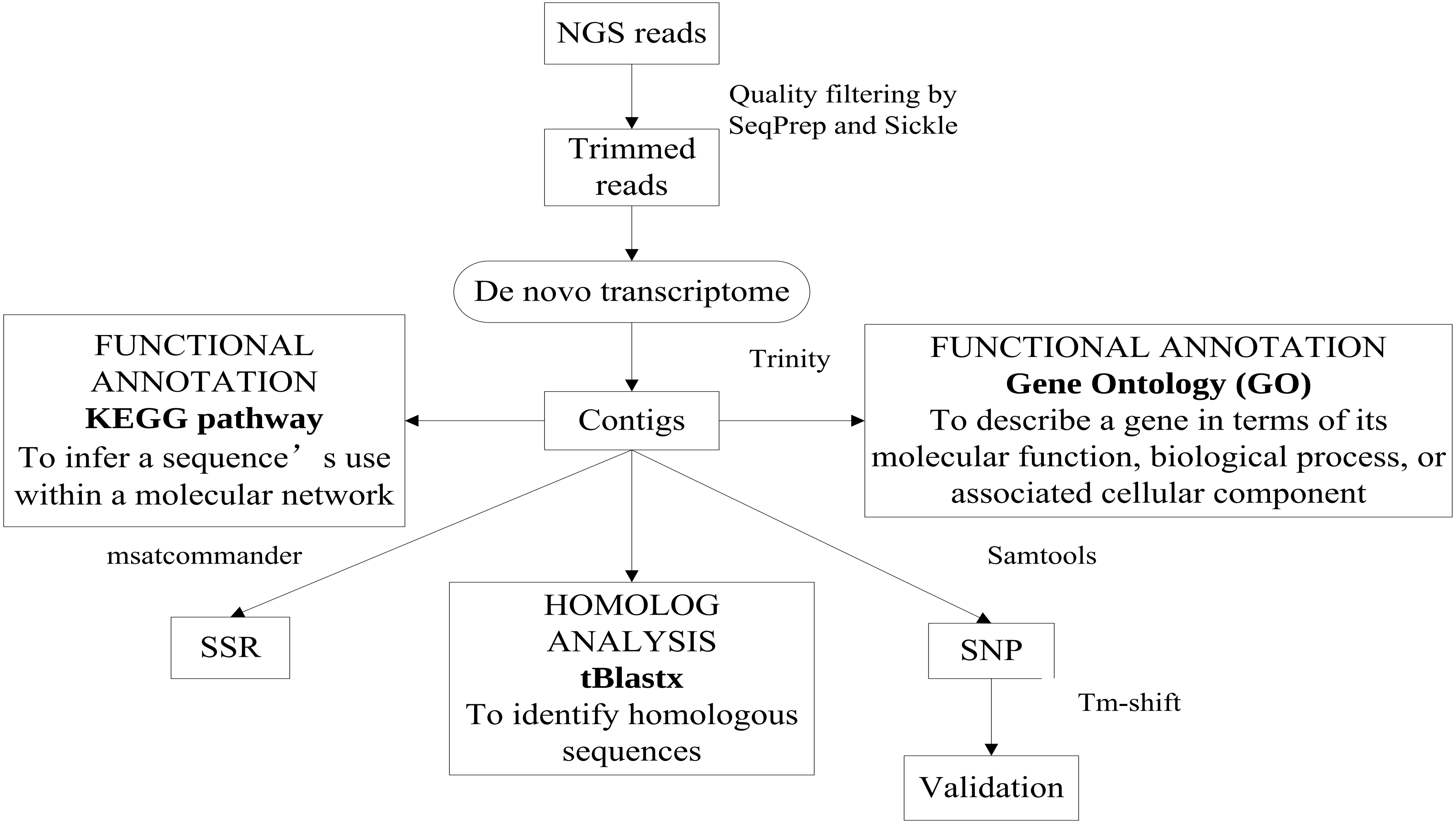
Sample collection and transcriptome sequencing

Calanus sinicus samples for transcriptome sequencing were collected from the Yellow Sea (38° 45′N, 121° 45′E) with a 500 µm mesh zooplankton net in May 2013. Zooplankton were preserved in fresh seawater temporarily and transported to the Zooplankton Ecology Lab of National Marine Environmental Monitoring Center (Dalian, China). *C. sinicus* were manually picked up with the aid of a stereomicroscope and preserved at −80^{o}C pending RNA extraction.

Total RNA was extracted from a pool of about 50 individuals using RNeasy Mini Kit (Qiagen, Germany) following the manufacturer’s instruction. After poly(A) mRNA was enriched by beads with Oligo (dT), a fragmentation buffer was added for shearing mRNA to short fragments (200–700 bp). Taking these short fragments as templates, a random primer was used to synthesize the first-strand cDNA, and then the second-strand was amplified. The double-stranded cDNA was purified with the Qiagen PCR extraction kit, and the short fragments were connected with sequencing adaptors. After KAPA quantitation and dilution, the library was sequenced on an Illumina HiSeq 2000 instrument.



Transcriptome analysis



NGS reads

Quality filtering by SeqPrep and Sickle

Trimmed reads

De novo transcriptome

Trinity

Contigs

FUNCTIONAL ANNOTATION
KEGG pathway
To infer a sequence’ s use within a molecular network

FUNCTIONAL ANNOTATION
Gene Ontology (GO)
To describe a gene in terms of its molecular function, biological process, or associated cellular component

msatcommander

SSR

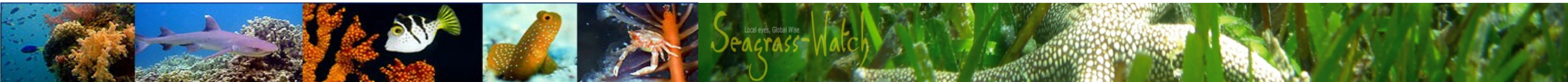
HOMOLOG ANALYSIS
tBlastx
To identify homologous sequences

Samtools

SNP

Tm-shift

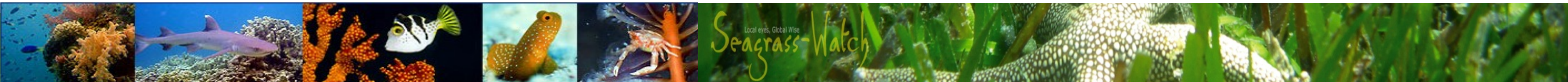
Validation

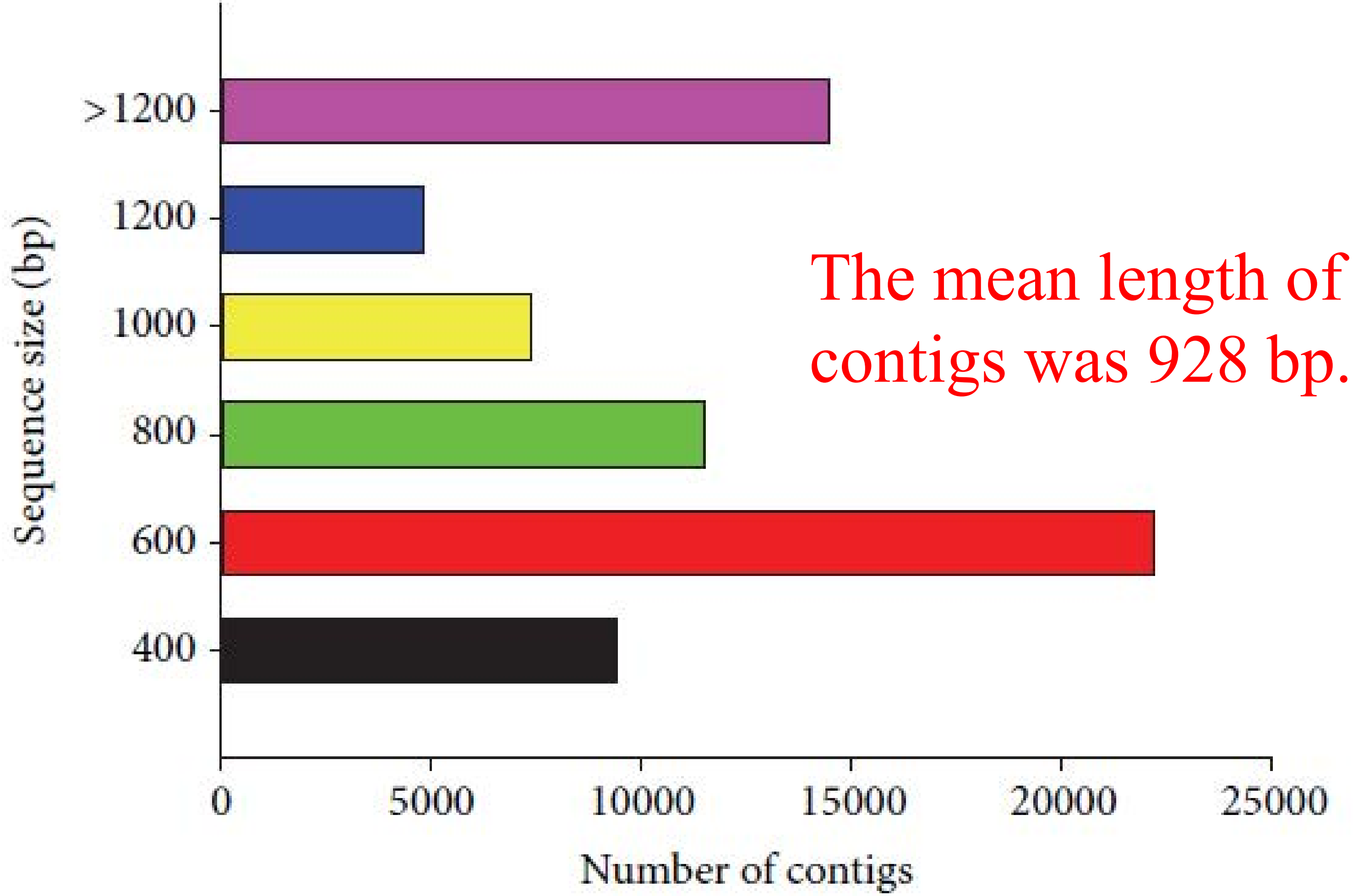


Results

Table 1: Summary of RNA-Seq of the copepod *Calanus sinicus* transcriptome.

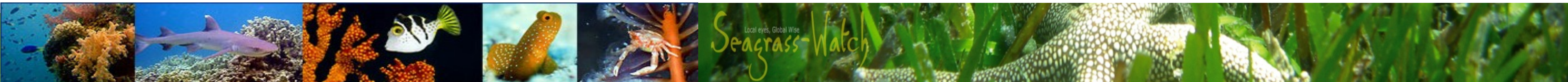
Category	Number/length
Reads from raw data	58,944,478
Average read length (bp)	100
Reads after trimming	57,773,604
Percentage retained	98.0%
Average read length after trimming (bp)	97.9
Contigs after removing redundancy	69,751
Average length (bp)	928.8
Final N50 (bp)	1,127
Unigenes	43,417

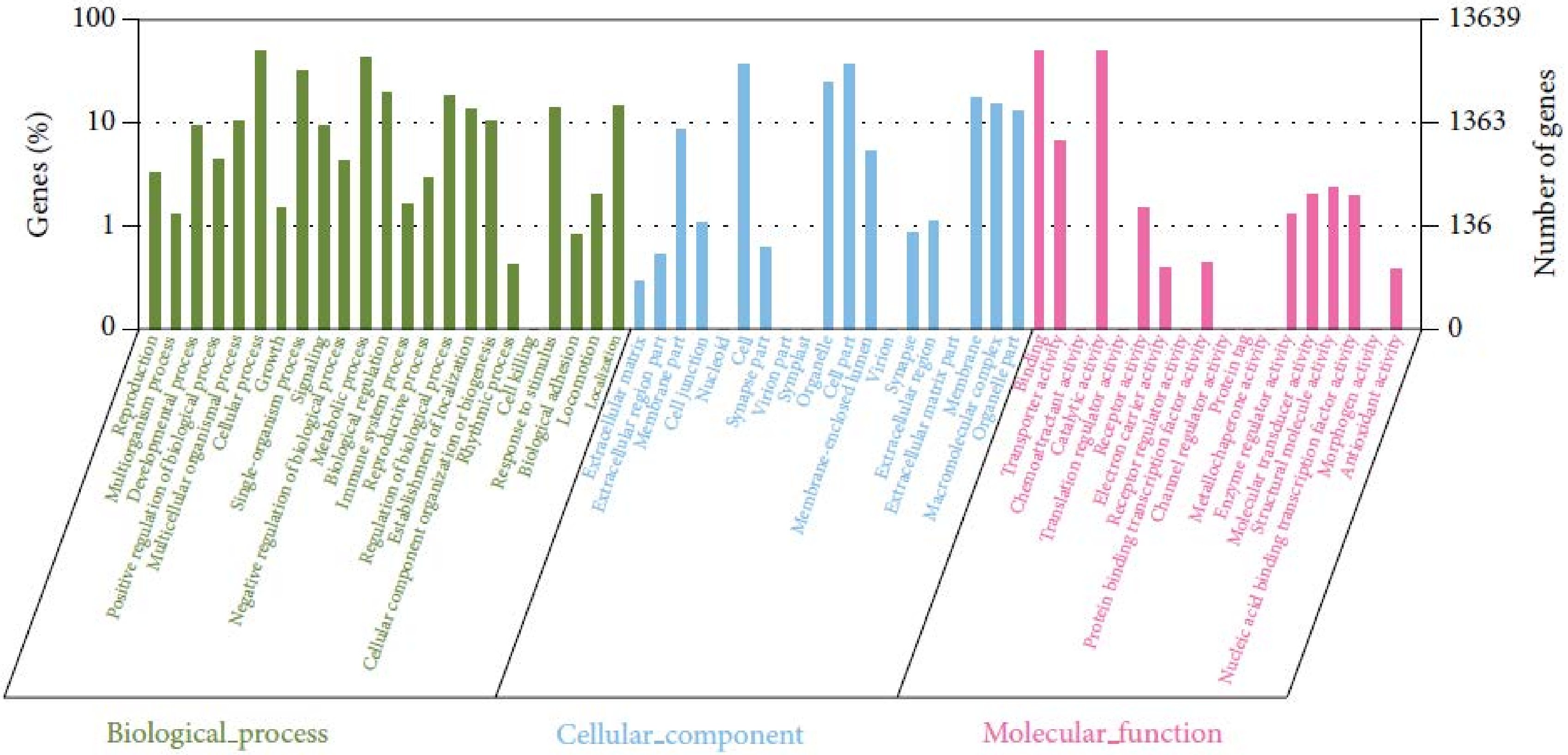




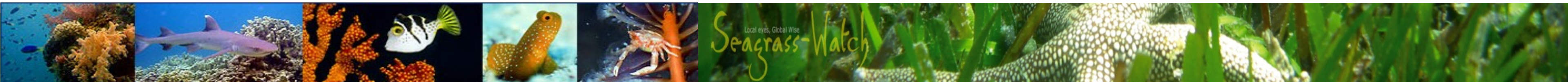
The mean length of contigs was 928 bp.

Figure 2: Size distribution of the assembled contigs in the *Calanus sinicus* transcriptome.





Gene ontology classification of assembled unigenes of *Calanus sinicus* transcriptome on biological process, cellular component, and molecular function levels.



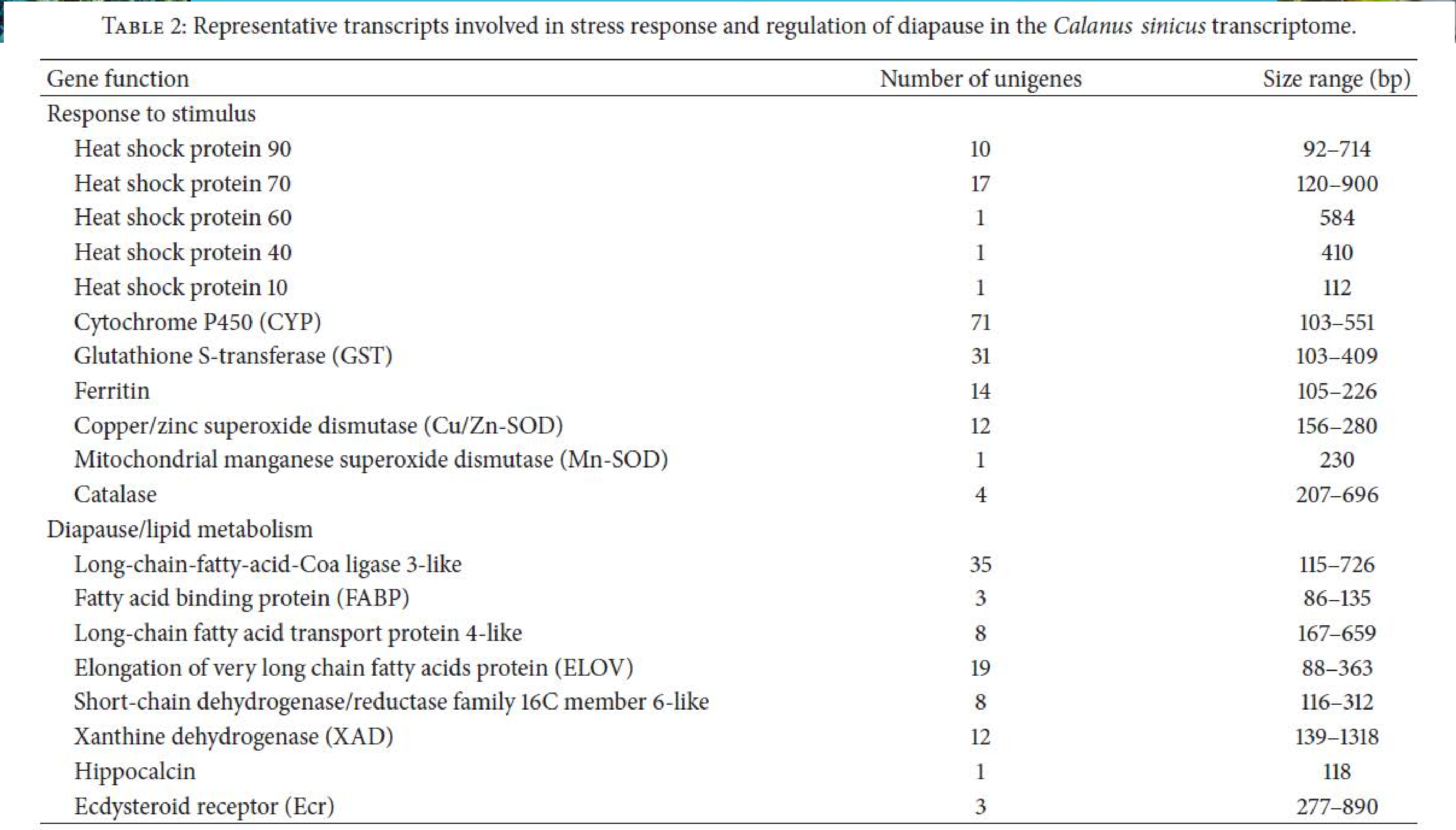
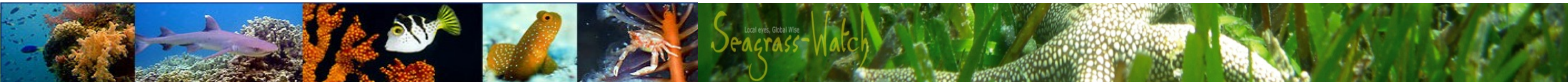
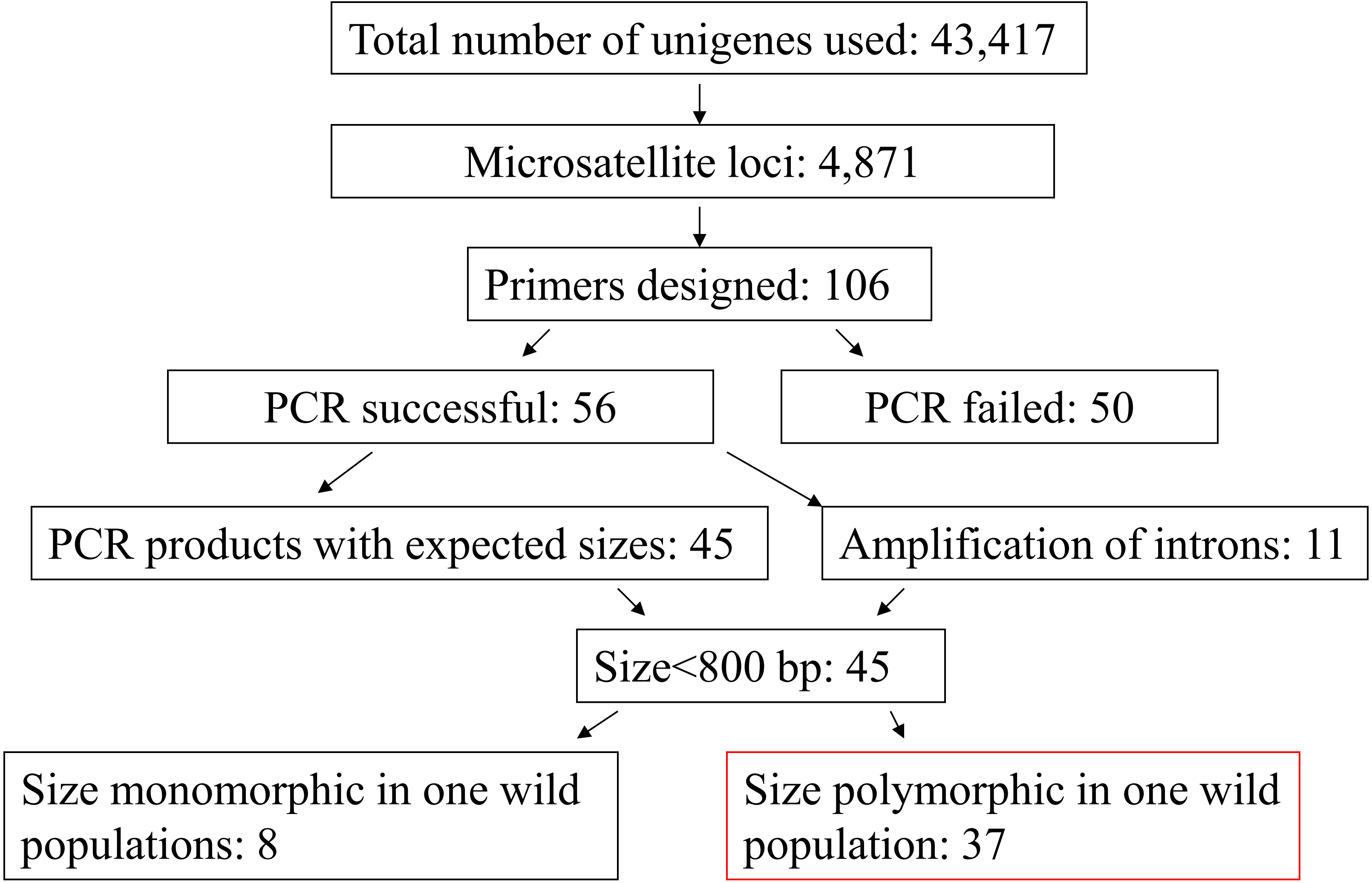


Table 2: Representative transcripts involved in stress response and regulation of diapause in the *Calanus sinicus* transcriptome.

Gene function	Number of unigenes	Size range (bp)
Response to stimulus		
Heat shock protein 90	10	92–714
Heat shock protein 70	17	120–900
Heat shock protein 60	1	584
Heat shock protein 40	1	410
Heat shock protein 10	1	112
Cytochrome P450 (CYP)	71	103–551
Glutathione S-transferase (GST)	31	103–409
Ferritin	14	105–226
Copper/zinc superoxide dismutase (Cu/Zn-SOD)	12	156–280
Mitochondrial manganese superoxide dismutase (Mn-SOD)	1	230
Catalase	4	207–696
Diapause/lipid metabolism		
Long-chain-fatty-acid-Coa ligase 3-like	35	115–726
Fatty acid binding protein (FABP)	3	86–135
Long-chain fatty acid transport protein 4-like	8	167–659
Elongation of very long chain fatty acids protein (ELOV)	19	88–363
Short-chain dehydrogenase/reductase family 16C member 6-like	8	116–312
Xanthine dehydrogenase (XAD)	12	139–1318
Hippocalcin	1	118
Ecdysteroid receptor (Ecr)	3	277–890



Microsatellite marker development



Total number of unigenes used: 43,417

Microsatellite loci: 4,871

Primers designed: 106

PCR successful: 56

PCR failed: 50

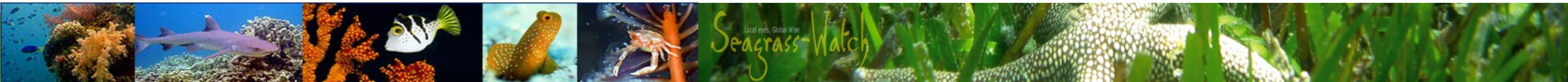
PCR products with expected sizes: 45

Amplification of introns: 11

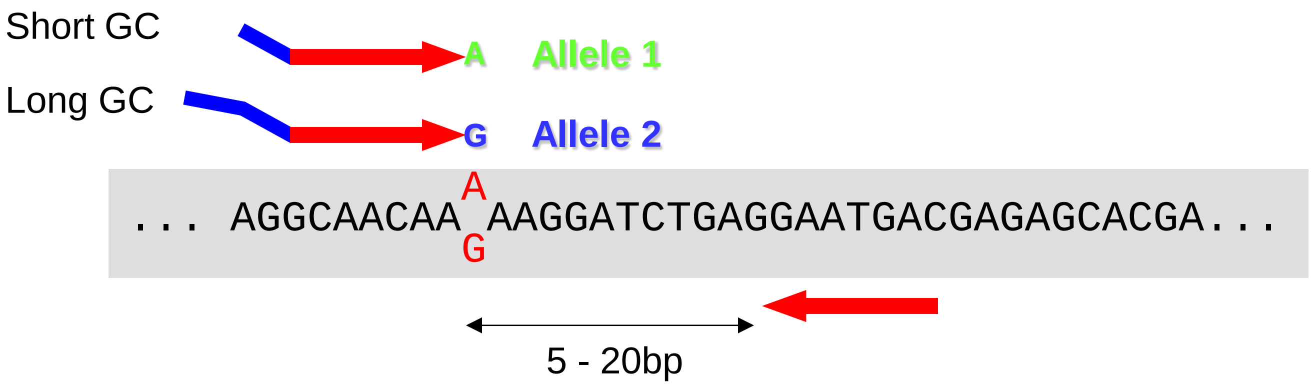
Size<800 bp: 45

Size monomorphic in one wild populations: 8

Size polymorphic in one wild population: 37



SNP marker development



Short GC

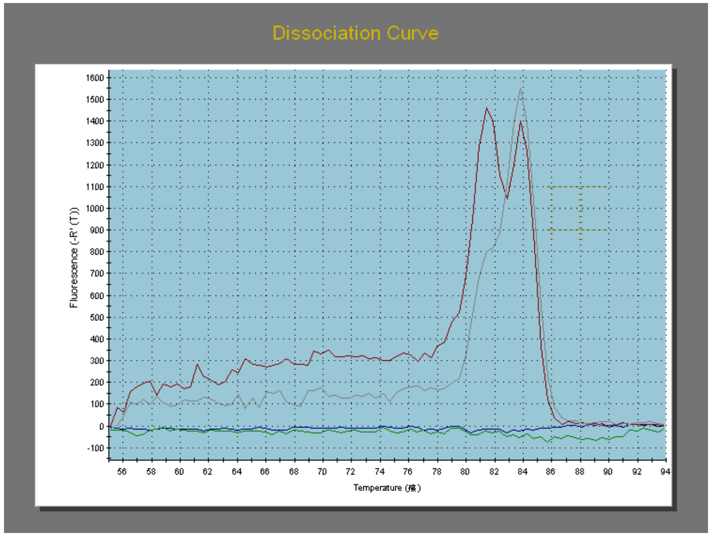
Long GC

A **Allele 1**

G **Allele 2**

```
                 A
... AGGCAACAA AAGGATCTGAGGAATGACGAGAGCACGA...
                 G
```

5 - 20bp



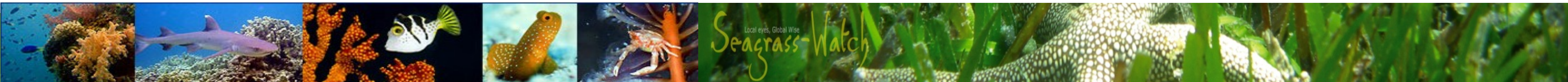
Conclusin

1. We sequenced a transriptome of *Calanus sinicus* using the Illumina HiSeq 2000 platform.

2. We obtained the full length cDNA of HSP, CYP and SOD using RACE technology.

3. We developed 37 microsatellite markers.

4. We validated 15 SNP loci by Tm-shift method.



Thank you
for your attention!