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FAO Expert Workshop on Sustainable Use and Management of *Artemia* Resources in Asia

Tianjin, China, 7 – 9 November 2016

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Status of world aquaculture and global aquafeed requirement with special notes on *Artemia*

Tianjin, China, 7 – 9 November 2016

Mohammad R. Hasan
Fisheries and Aquaculture Department
FAO, Rome



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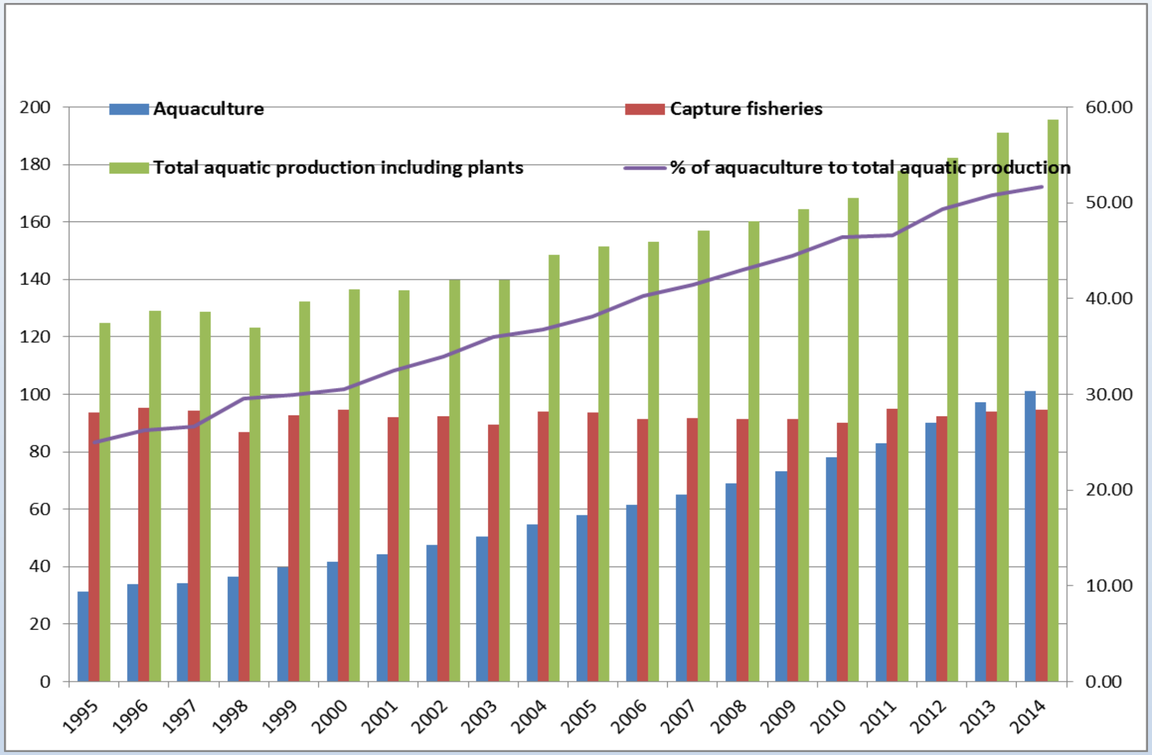
Aquaculture's contribution to global aquatic production increased from 25% in 1995 to nearly 52% in 2014

Global trends in contribution of aquaculture to fisheries production (1995-2014)

In 2013, 97.2 million tonnes, 158.5 billion USD

In 2013, 70.3 million tonnes of aquatic products excluding plants



In 2014, 101.1 million tonnes, 166 billion USD

In 2014 73.8 million tonnes of aquatic products excluding plants

In 2014, global aquaculture production reached 101.1 million tonnes, growing at an annual rate of 6.7% since 1995

Rapid growth of aquaculture has been due to technological advances in equipment and feed and access to greater areas under aquaculture

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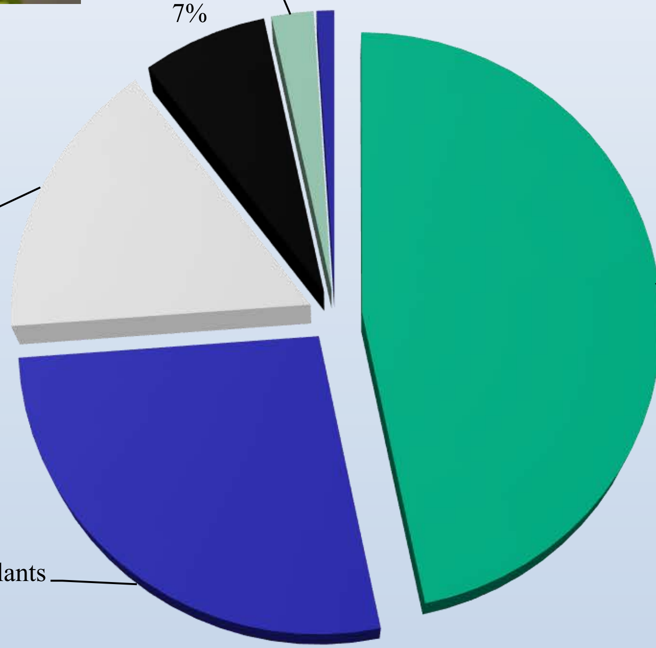
Total global aquaculture production in 2013 –101.1 million tonnes

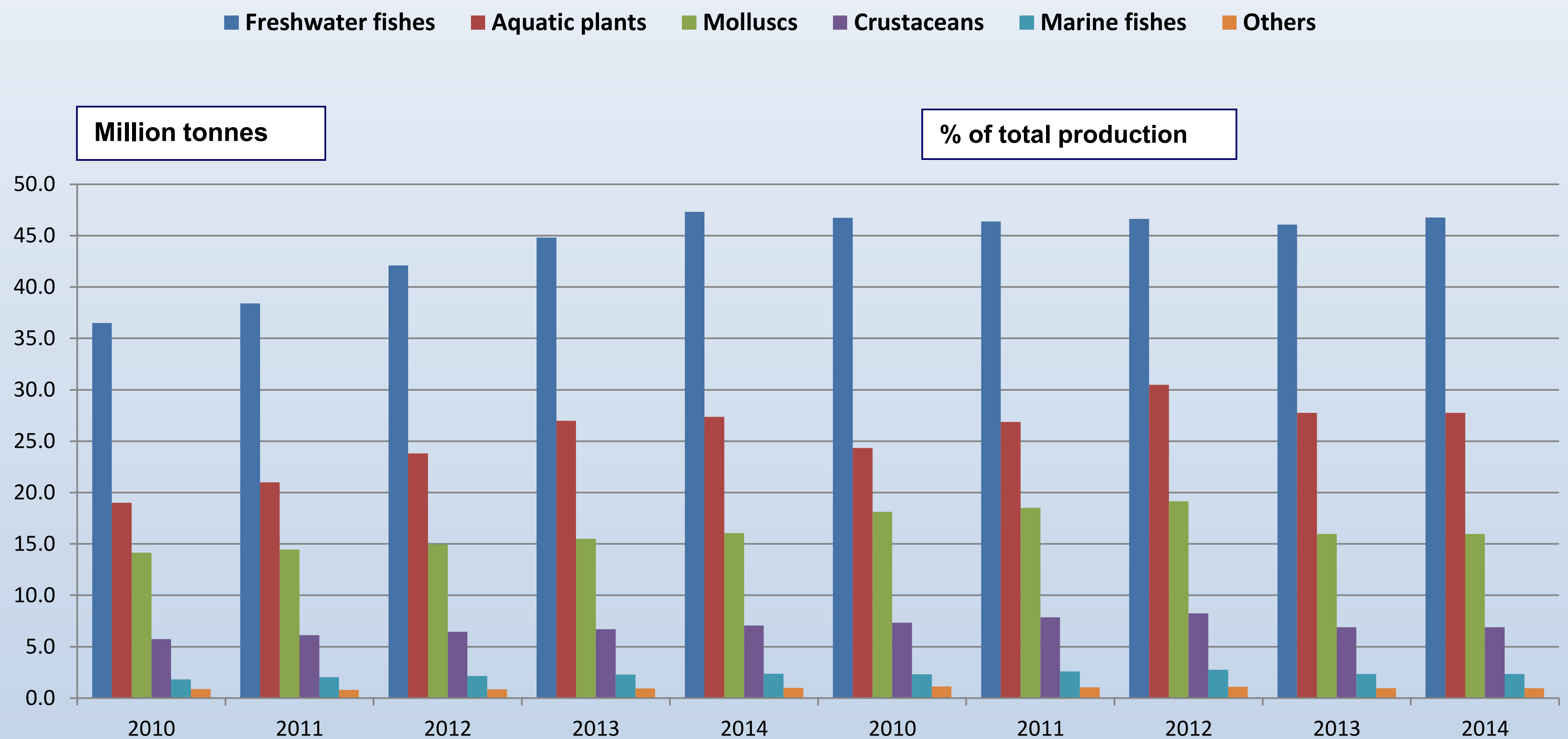
over 200 species or species/species-groups of aquatic animals and plants



Aquatic plants	27.3 Mt
Crustaceans	7.0 Mt
Freshwater fishes	47.3 Mt
Marine fishes	2.4 Mt
Mollusks	16.0 Mt
Others	0.99 Mt

Total fish & crustaceans in 2014: 56.7 million tonnes

Global output of aquaculture in volume and in percentage during 2010 and 2014 by species group



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Fed aquaculture species production – 2014 (commercial feeds, farm-made feeds, fresh feeds)

CRUSTACEANS
7.0
million tonnes

In 2014, fed species (including Indian carps) contributed to 48.5 million tonnes or 48% of total global aquaculture production

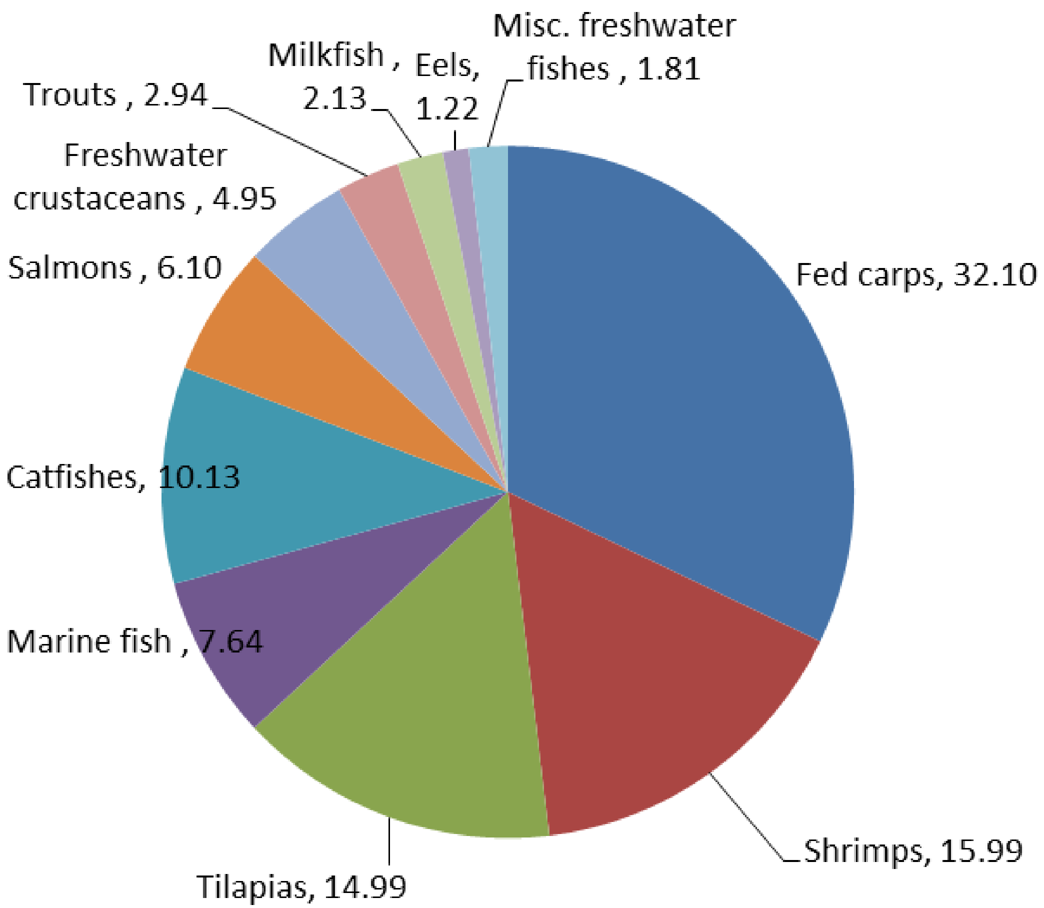
In 2014, non-fed species (silver and bighead) production 8.2 million tonnes

In 2014, total fish & crustaceans 56.7 million tonnes
Fed species: 48.5 million tonnes or 48% of total global aquaculture production in 2014

GLOBAL AQUAFEED PRODUCTION

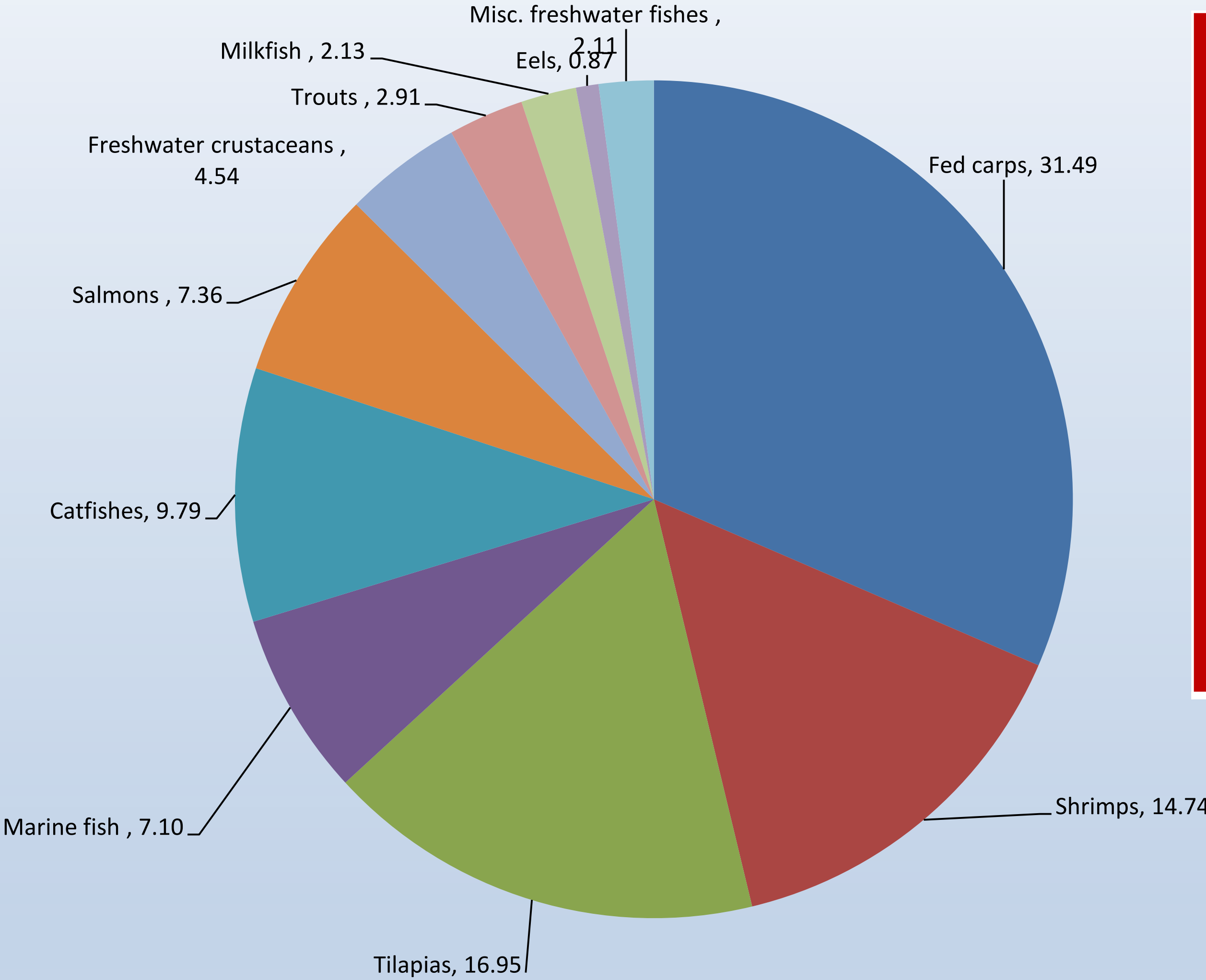
- Total industrial compound aquafeed production has increased from 7.6 million tonnes in 1995 to 34.1 million tonnes (almost 4.5-fold) in 2010, 40.3 million tonnes (5.3 fold) in 2012 and 44.3 million tonnes (almost six-fold) in 2014 with 348%, 430% and 483% increase since 1995.
- Industrial aquafeed production growing at an average rate of 11.0% or so per year.

Estimated global production of commercial aquaculture feeds by major species groups in 2010: 34.1 million tonnes



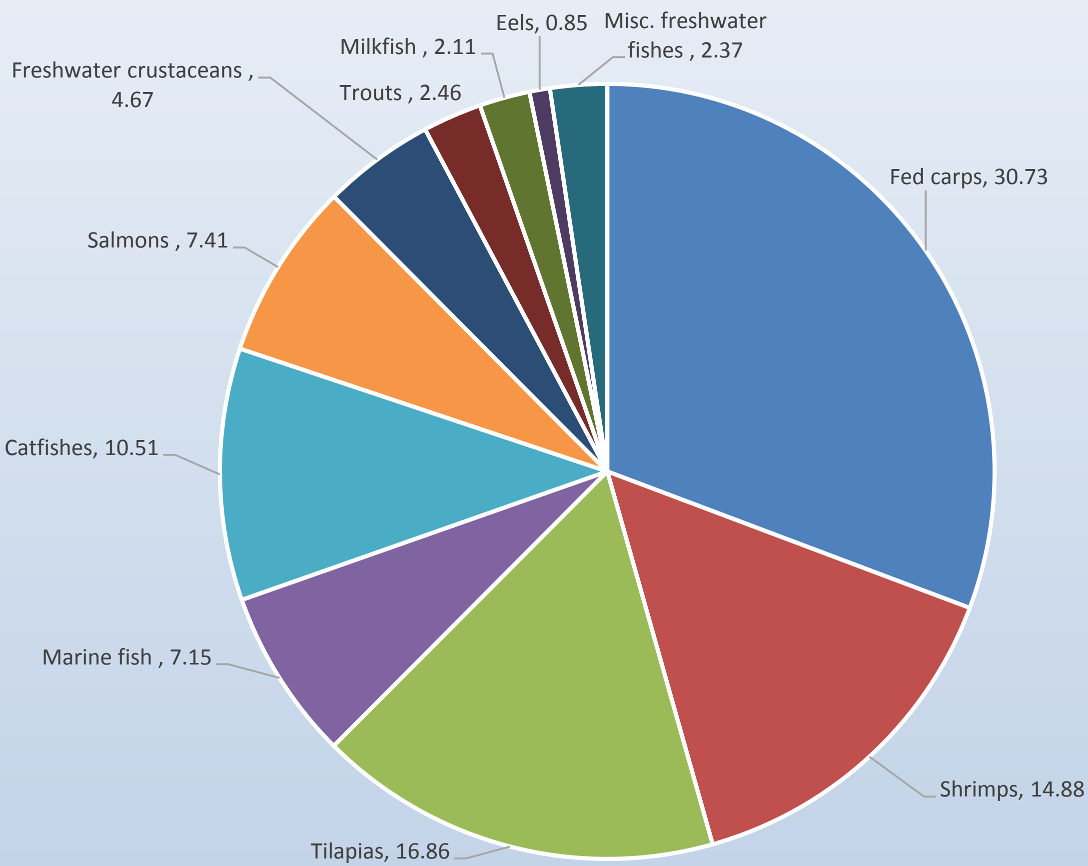
Fed carps	10.89 Mt
Shrimps	5.46 Mt
Tilapias	5.12 Mt
Catfishes	3.46 Mt
Marine fishes	2.61 Mt
Salmons	2.08 Mt
FW crustaceans	1.69 Mt
Trouts	1.00 Mt
Milkfishes	730 Tt
Misc FW fishes	620 Tt
Eels	420 Tt
TOTAL	34.1 Mt

Estimated global production of commercial aquaculture feeds by major species grouping in 2012: 40.3 million tonnes (MT)



Fed carps	12.69 Mt
Shrimps	5.94 Mt
Tilapias	6.83 Mt
Catfishes	3.94 Mt
Marine fishes	2.86 Mt
Salmons	2.97 Mt
FW crustaceans	1.83 Mt
Trouts	1.17 Mt
Milkfish	860 Tt
Misc FW fishes	850 Tt
Eels	351 Tt
TOTAL	40.29 Mt

Estimated global production of commercial aquaculture feeds by major species grouping in 2014: 44.3 million tonnes (MT)



Fed carps	13.62 Mt
Shrimps	6.60 Mt
Tilapias	7.47 Mt
Catfishes	4.66 Mt
Marine fishes	3.17 Mt
Salmons	3.28 Mt
FW crustaceans	2.07 Mt
Trouts	1.09 Mt
Misc FW fishes	1.05 Mt
Milkfish	940 Tt
Eels	380 Tt
TOTAL	44.32 Mt

Status of live food production and use

- Although there are precise estimate of global aquaculture production and subsequently the data of industrial feed used/produced for global aquaculture, there has been dearth of information on production and use feed/live food for larval stages for many of the freshwater/brackish water fish and shell fish.
- Most of the aquaculture fish/shell fish species require specialized feed specifically the live food in their larval stage after absorption of egg yolk. Also often the broodstock require specialized diet or live food to meet their special requirement which cannot be met by normal dry feed.

Status of live food production and use

- Commonly used larval food are phytoplankton/algae and zooplankton (e.g. rotifer).
- Although *Artemia* are the ideal live food for fish and shell fish larvae, its use has been limited by its volume of production and subsequently the cost.
- In most of marine finfish and crustacean hatcheries, phyto and zooplankton are used and then weaned to specialized dry diet.
- Often the use of the phyto and zooplankton result sub-optimal nutrition of the larvae resulting in low survivability and then weaning to specialized larval dry diet will increase the cost of production.

History of global *Artemia* production

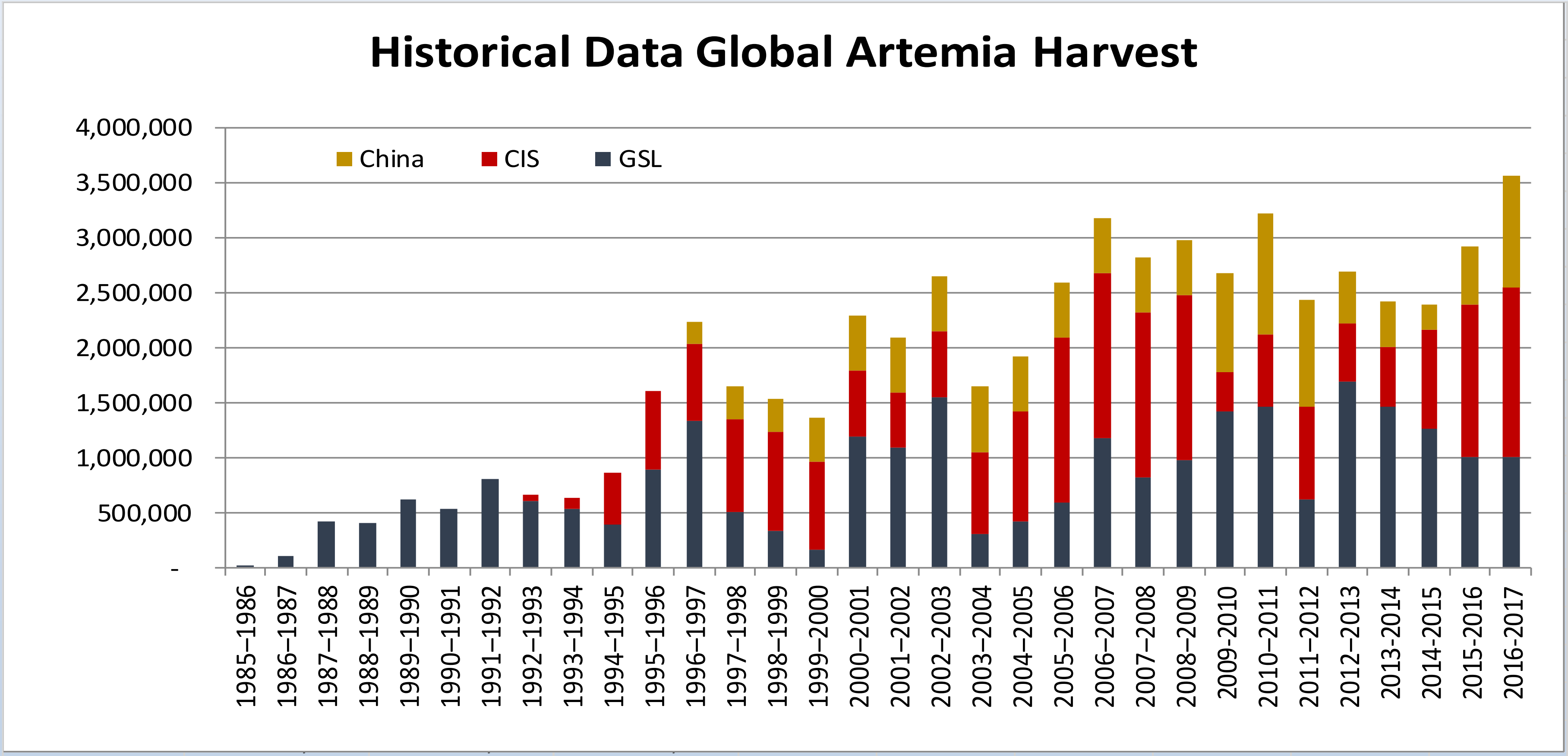
- Early 1960s commercial availability of Artemia cysts reported from salt lakes in the San Francisco Bay (SFB) area
- From 1970s new commercial source of *Artemia* were available from the Great Salt Lake (Utah, USA): much larger quantities (over 100 tonnes) as compared to SFB (around 10 tonnes)
- For many years the Great Salt Lake (Utah, USA) has been the main source of *Artemia* cysts, however, due to seasonal conditions harvests were very variable.
- From 1980s new sources (but small quantities: around 10 tonnes) were available from Australia, Brazil and China.

History of global *Artemia* production

- From mid 1990s new quantities of *Artemia* (over 100 tonnes) from Central Asia (primarily Turkmenistan, Siberia, Kazakhstan).
- Presently some 1 500 tonnes of tonnes per year enter into the market from countries of Central Asia.
- Expansion of cyst products from China as several new sources were tapped (coastal salt pans and inland salt lakes) (Aibi Lake and Bohai Bay area); at times over 500 tonnes per year.
- Significant drop was recorded in recent years; where China was a net exporter of cysts, they now import large quantities, mainly from Central Asia.

History of global *Artemia* production

- At present the world production of *Artemia* cysts is estimated at over 4,000 tonnes per year with about 1/3 each from Great Salt Lake, Central Asia and China.



Courtesy INVE Aquaculture SA, Belgium

Status of global *Artemia* consumption

- Biggest consumption of *Artemia* cysts is in China with maybe over 50 percent of what is available worldwide.
- Estimated annual consumption of *Artemia* cysts: low in the 1970s (few tonnes) and in the 1980s (just over 100 tonnes).
- Fast expansion of consumption in 1990s parallel with the fast expansion of the hatchery sector (especially of shrimp): 1 500 tonnes in the late 90s, more than 3 000 tonnes as of 2010.

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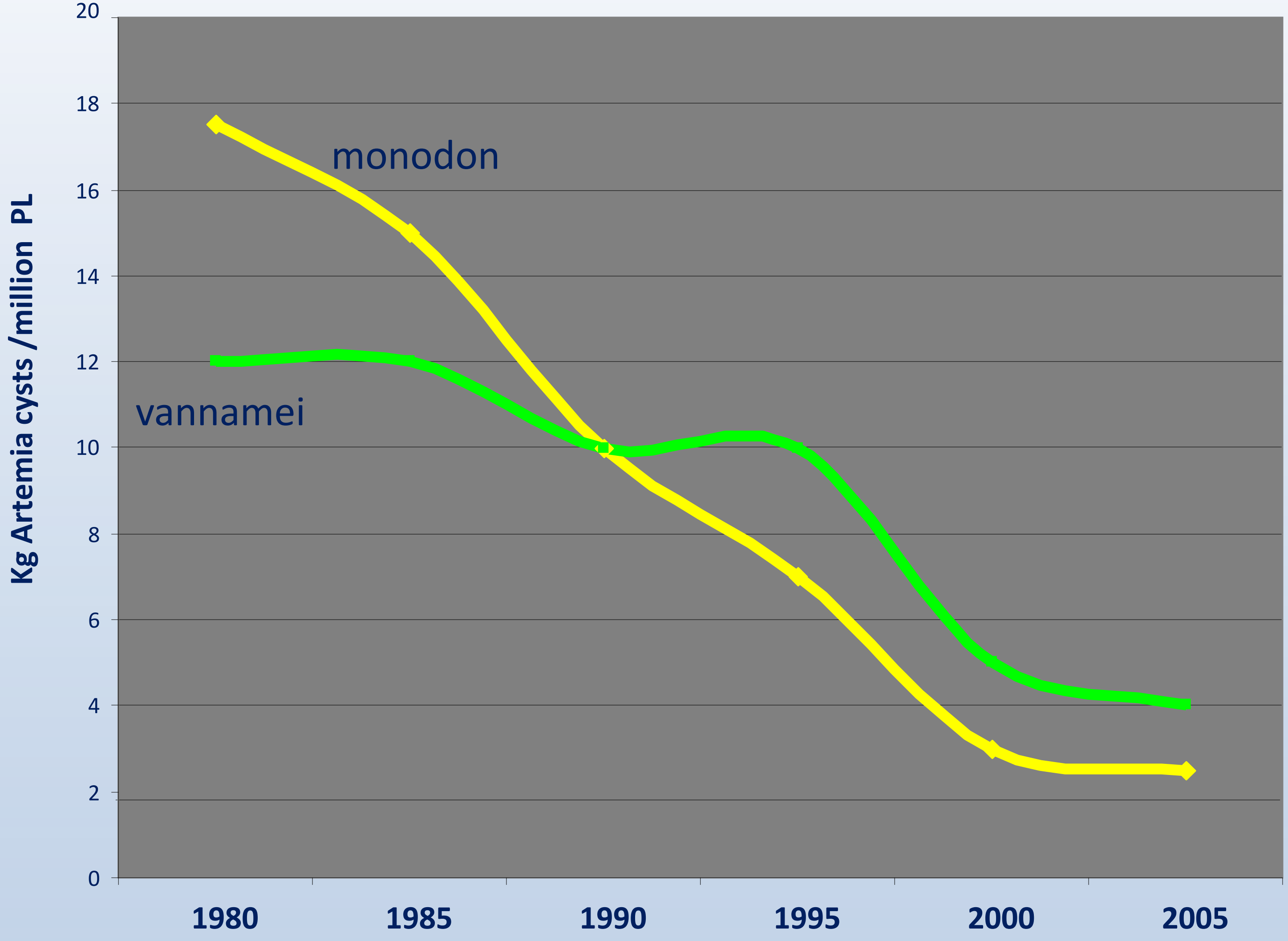
Status of *Artemia* consumption

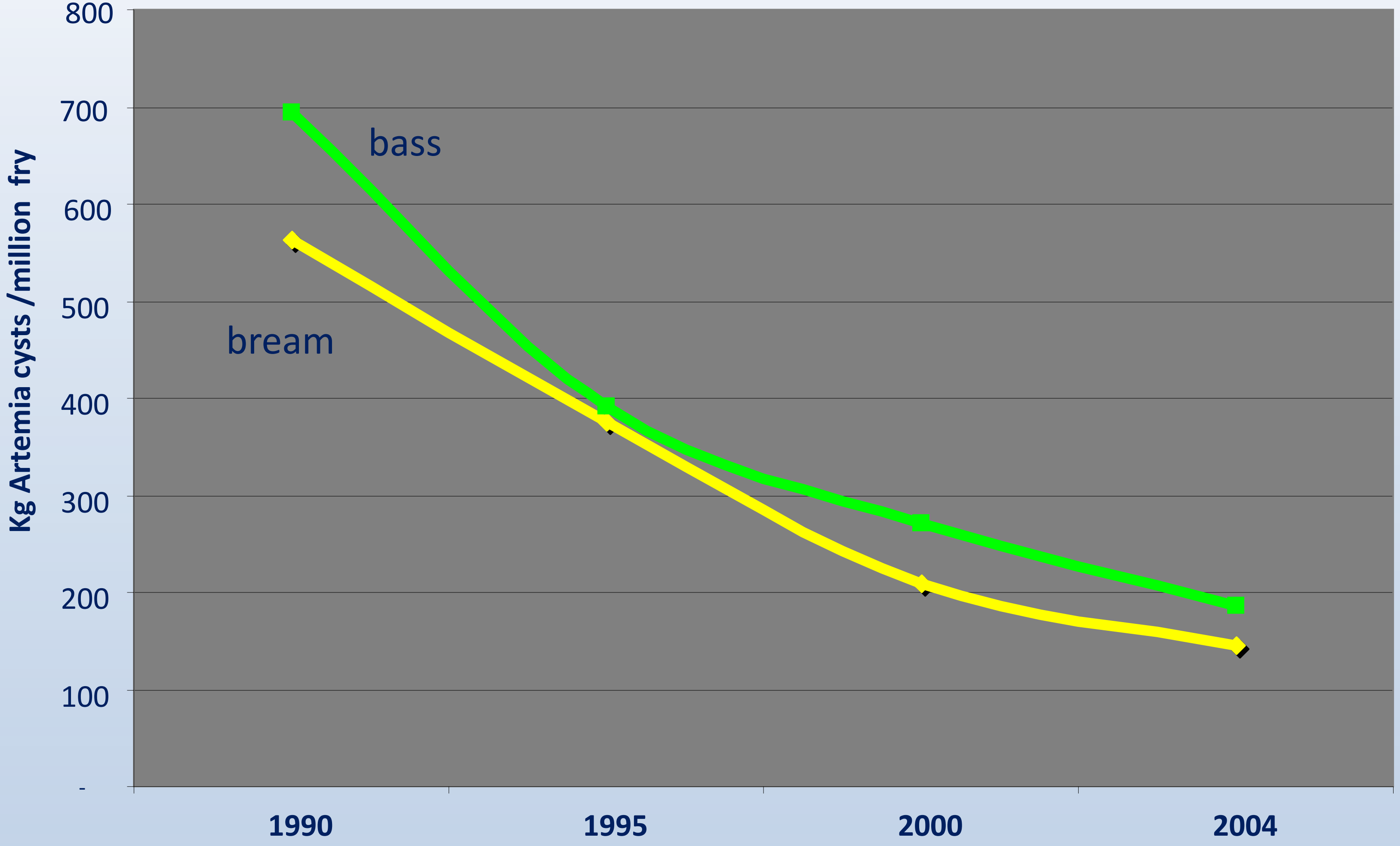
- Although *Artemia* can be considered as an ideal larval live food, its consumption is limited mostly to
  - Marine shrimp (whiteleg shrimp, *Litopenaeus vannamei* and black tiger shrimp, *Penaeus monodon*) (*85 percent of all cyst consumption is in shrimp hatcheries with 3 to 6 kg of cysts needed for the production of 1 million PLs)*
  - Selected high value marine finfish (European seabass, *Dicentrarchus labrax* and Gilthead seabream, *Sparus aurata*).

Average consumption of *Artemia* cysts in marine shrimp



Courtesy INVE Aquaculture SA, Belgium

Average *Artemia* consumption in gilthead seabream and European seabass



Courtesy INVE Aquaculture SA, Belgium

CONCLUSION

- An additional 27 million tonnes of aquatic food will be required by 2030 considering the projected population growth and to maintain the per caput consumption.
- Availability of feed will be one of the most important inputs if aquaculture has to maintain its sustained growth to meet its challenge of increased production.
- Aquafeed production is expected to continue growing at a similar rate to 49.7 million tonnes by 2015 and 69.0 million tonnes by 2020.
- If this growth is to be sustained then feed ingredient and feed input supply must grow at a similar rate.
- Similarly live food for hatchery will remain a critical factor, *Artemia* being the most important live food.

CONCLUSION

- Although production and use of *Artemia* are increasing, demand cannot match the production/ availability.
- Based on FAO production statistics (2016) data and Market survey, Inve Aquaculture calculated global shrimp PL stocking in 2014 to be 607 billion for whiteleg shrimp and 41 billion for black tiger shrimp and estimated the *Artemia* cysts requirements of 2 278 tonnes for these two species alone.
- There has been no coordinated effort in production, use and management of this resources unlike that of industrial aquafeed.
- Therefore there are need for global/regional efforts for sustainable production, use and management of this important resources.
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Thank You

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Forty years of research and use of brine shrimp *Artemia* spp.

Patrick Sorgeloos
Laboratory of Aquaculture and Artemia Reference Center
Ghent University, Ghent, Belgium

Asian sea bass larva feeding on *Artemia*

shrimp postlarva feeding on *Artemia*

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First commercial sources of *Artemia* cysts in the 1960s

REPORT of the FAO TECHNICAL CONFERENCE ON AQUACULTURE

FAO Fisheries Report No. 188

Experience Paper FIR:AQ/Conf/76/E.77 — Sorgeloos, P. — The brine shrimp *Artemia salina*: A bottleneck in mariculture?

Kyoto, Japan, 26 May - 2 June 1976

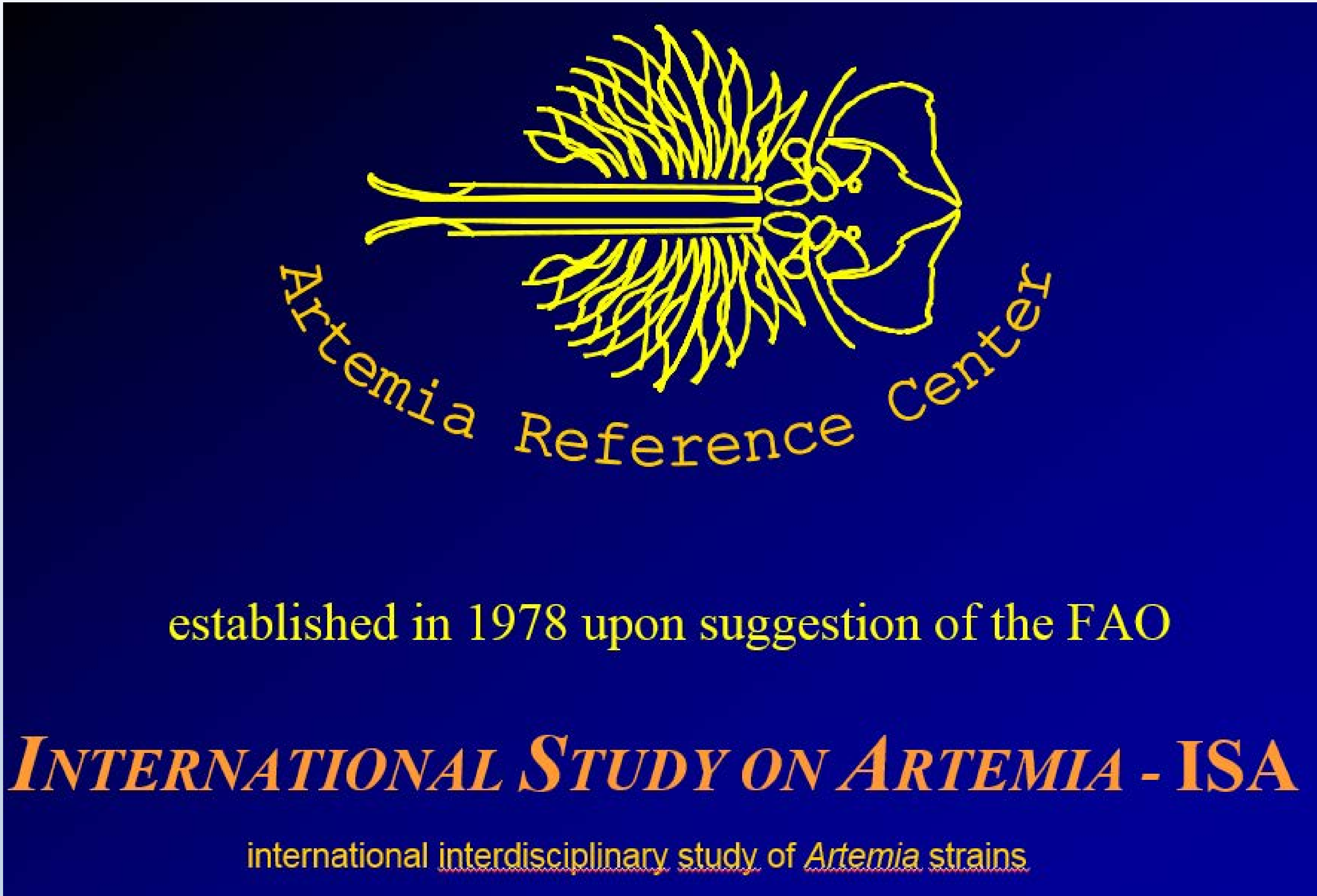
POTENTIAL IMPROVEMENTS FOR CRITICAL *ARTEMIA* SITUATION

- exploitation of more natural resources
- transplantation and inoculation of suitable habitats
- improved techniques for cyst
  - harvesting
  - processing
  - storage
  - hatching
- use of juvenile/adult Artemia biomass as food source

SouthEast Asian Development Center

1976-78 study stays at SEAFDEC

Herminio Rabanal


Artemia Reference Center
established in 1978 upon suggestion of the FAO
INTERNATIONAL STUDY ON ARTEMIA - ISA
international interdisciplinary study of *Artemia* strains

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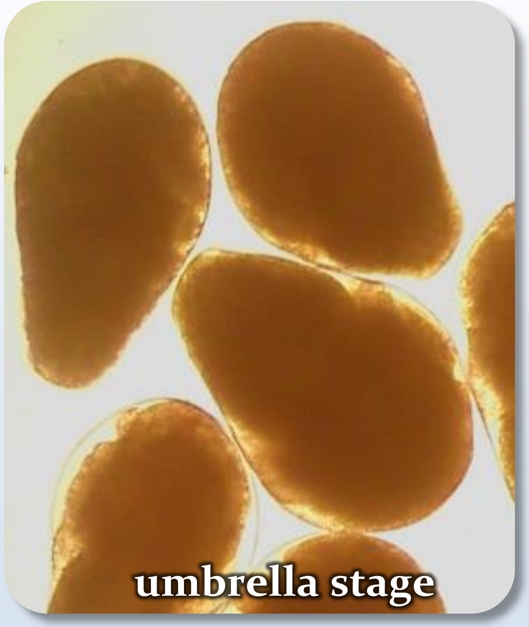
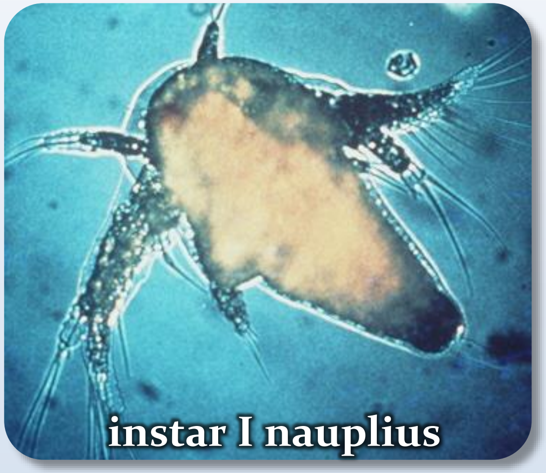
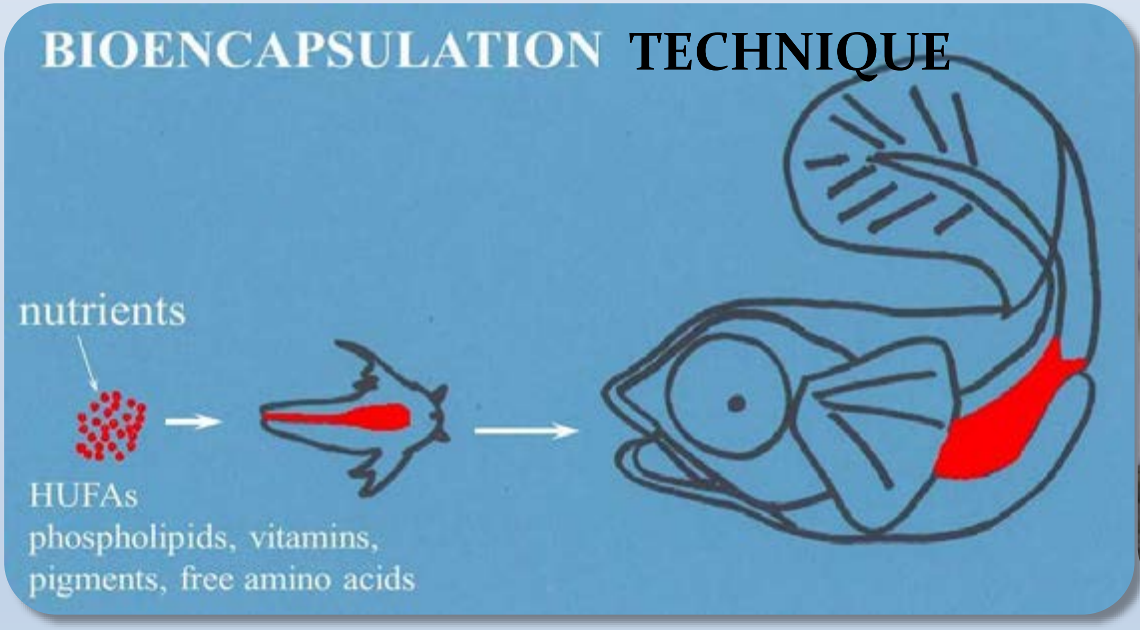
Natural distribution of *Artemia*

Improved commercial availability of *Artemia* cysts as of early 1980's


decapsulated cysts


umbrella stage


instar I nauplius


BIOENCAPSULATION TECHNIQUE
nutrients
HUFAs
phospholipids, vitamins,
pigments, free amino acids


enriched metanauplii


19

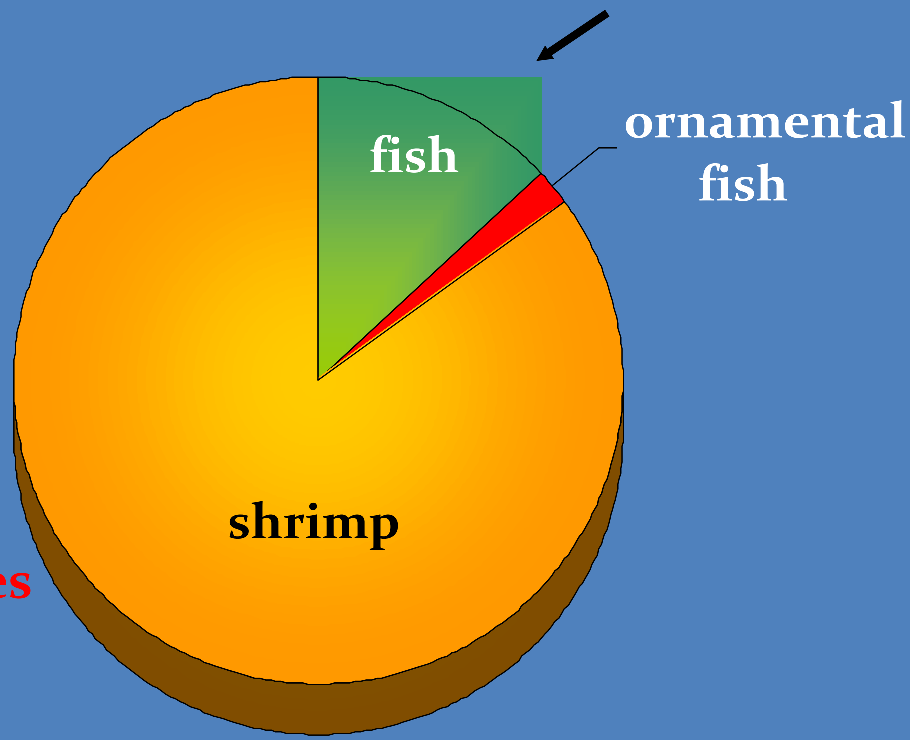
Artemia cyst consumption

1970 < 1 tonne

1980 < 100 tonnes

1990 > 1,500 tonnes

2010 > 3,000 tonnes



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Artemia required for 1 million PL's

30 kg
Mud-crab

10 kg
Prawn

3 kg
Tiger shrimp

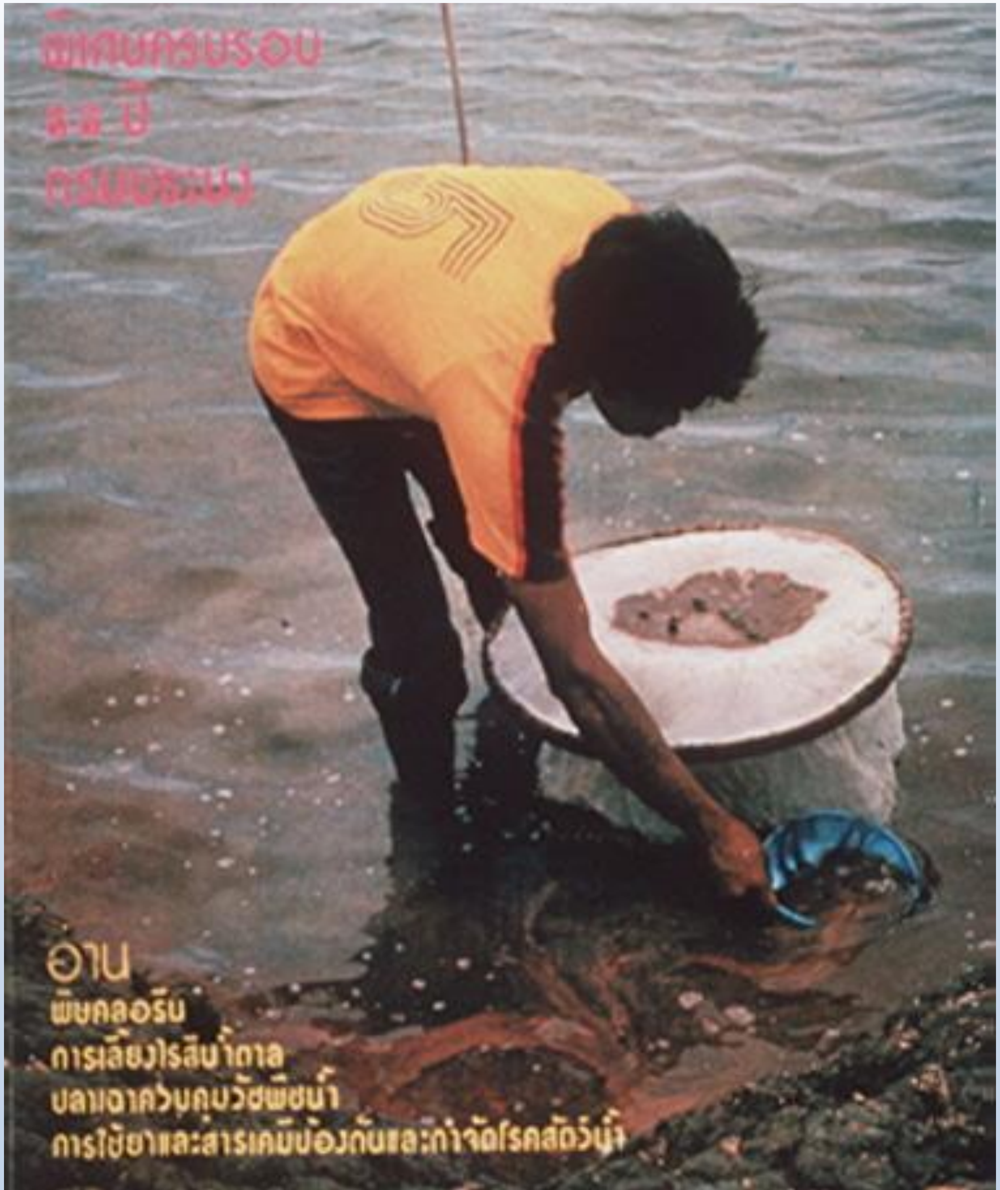
3 kg
White leg

3 kg
for 5 000 cobia
of 60-day fingerlings

Brazil : first successful *Artemia* inoculation (end 70'ies)

Seasonal salt production in SE Asia

No natural occurrence of *Artemia* in SE Asia



First *Artemia* cyst production in saltponds in Thailand(1979)

Important socio-economic benefits of integrating seasonal salt production with *Artemia* farming

> 500 saltfarmers in S-Vietnam produce 40 tonnes of cysts per dry season

total income in *Artemia* production: 4-7 500 US $ per ha

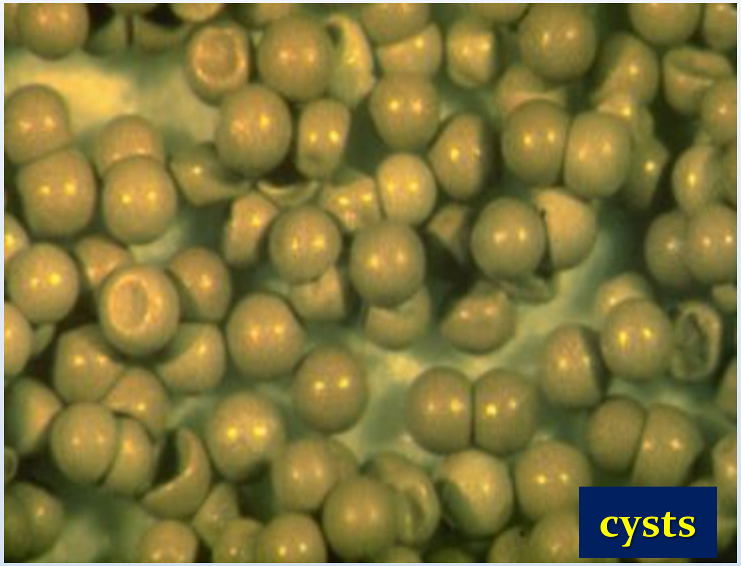
household income x 2.5-3 compared to salt production only

Courtesy College of Aquaculture and Fisheries
Can Tho University, Viet Nam

Many thousand poor households in SE Asia depend on marginal income from solar salt production

New projects planned in Bangladesh, Cambodia, Laos, Myanmar, …


Biomass


cysts

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Brine shrimp *Artemia* as human food

partial replacement in shrimp/fish/crab cakes

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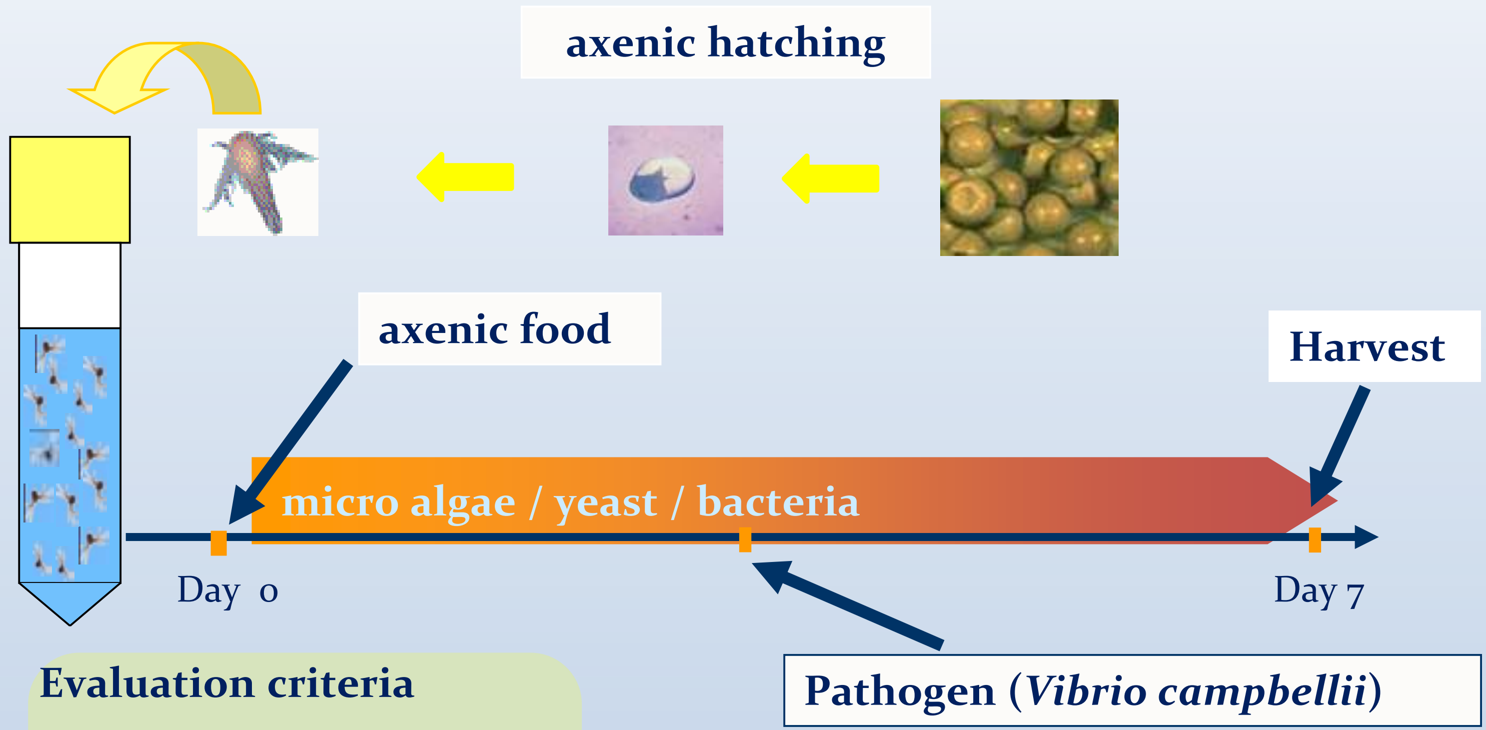
Gnotobiotic culture of *Artemia*



Evaluation criteria

- survival
- length
- total biomass
- immune parameters

Artemia as model system in larviculture research

- **host-microbe interactions**

→ *Influencing microbial numbers or activity*

☑ quorum sensing / quorum quenching

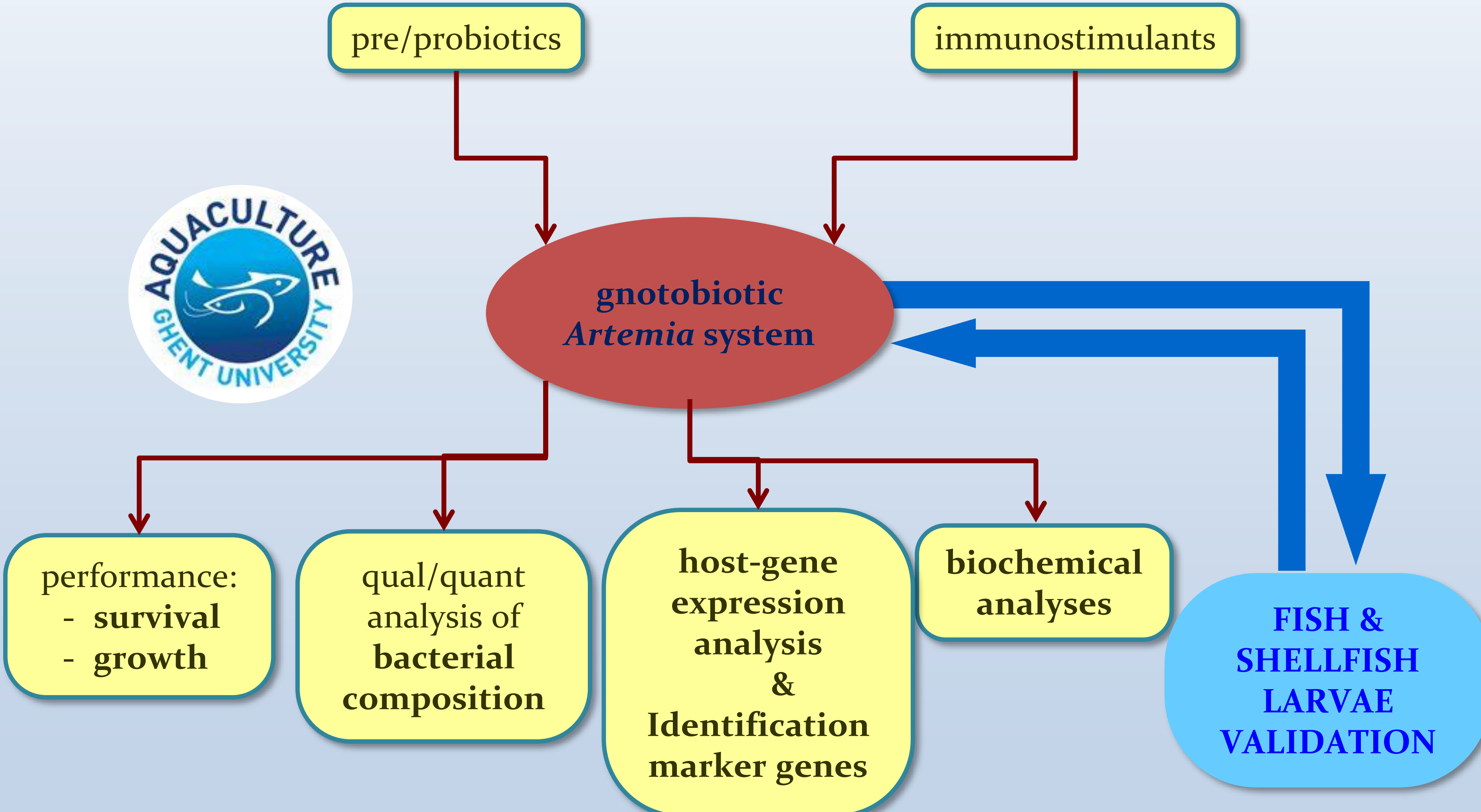
❑ Poly-β-hydroxybutyrate

→ *Stimulating the host's immune response*

❑ heat shock proteins

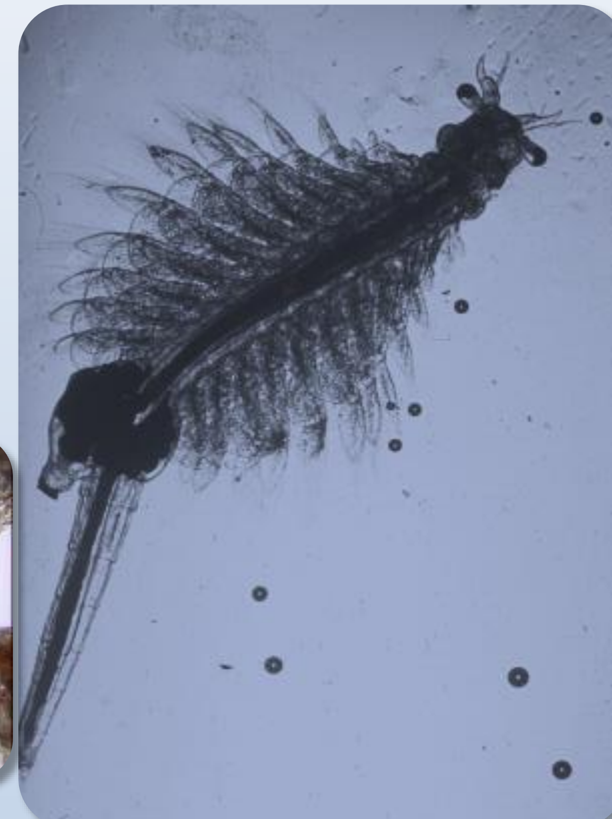
❑ yeast cell wall-bound glucan

Development of innovative microbial management systems



Brine shrimp *Artemia* as model organism in aquaculture/biology research

- host-microbe interactions
- breeding studies
- epigenetics
- nutrition studies – bioflocs
- immunology
- cell cycle studies



UGent Aquaculture R&D Consortium

Ghent University, Belgium

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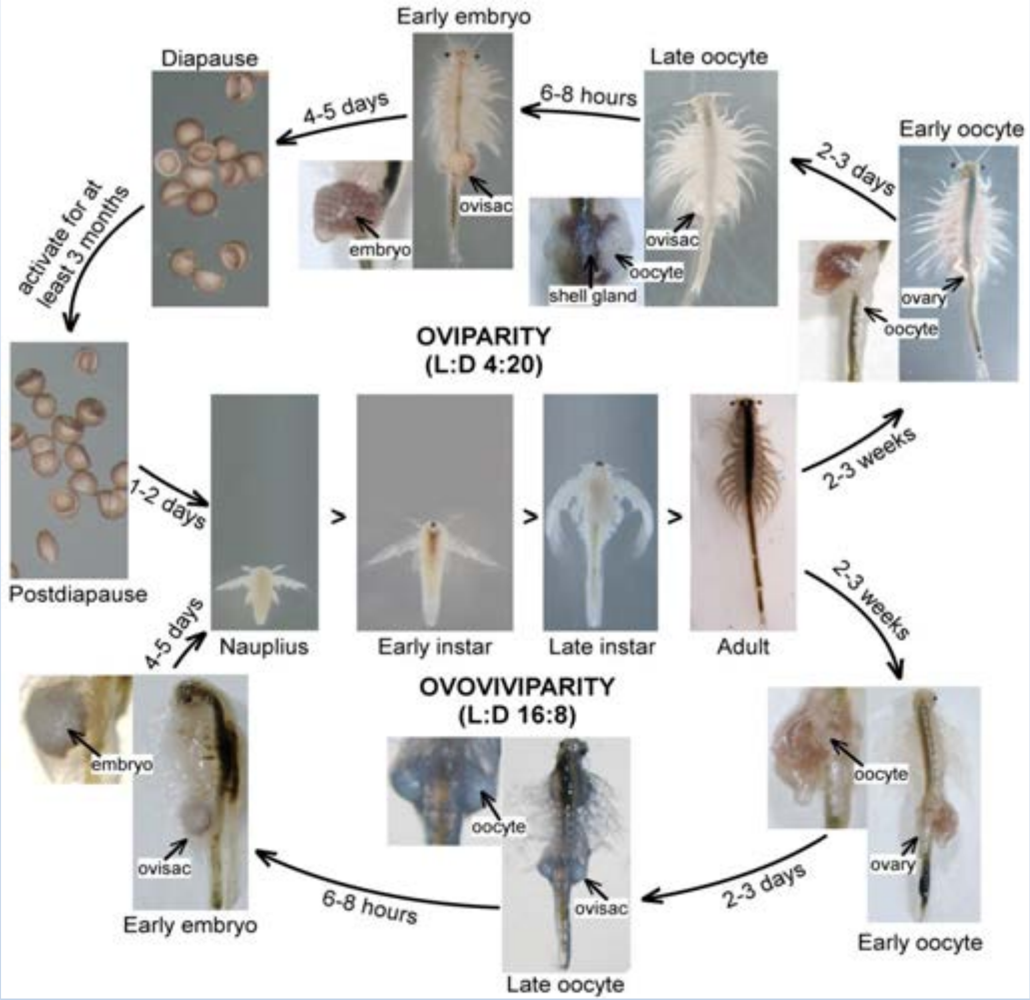
Use of *Artemia* model in biomedical research

Weijun Yang
Institute of Cell and Developmental Biology,
College of Life Sciences, Zhejiang University,
Hangzhou, Zhejiang, China

Study on stem cell quiescence
A model system *Artemia*

- — extreme salinity
- — high UV radiation levels,
- — high pH
- — anoxia
- — large temperature differences
- — intermittent dry conditions

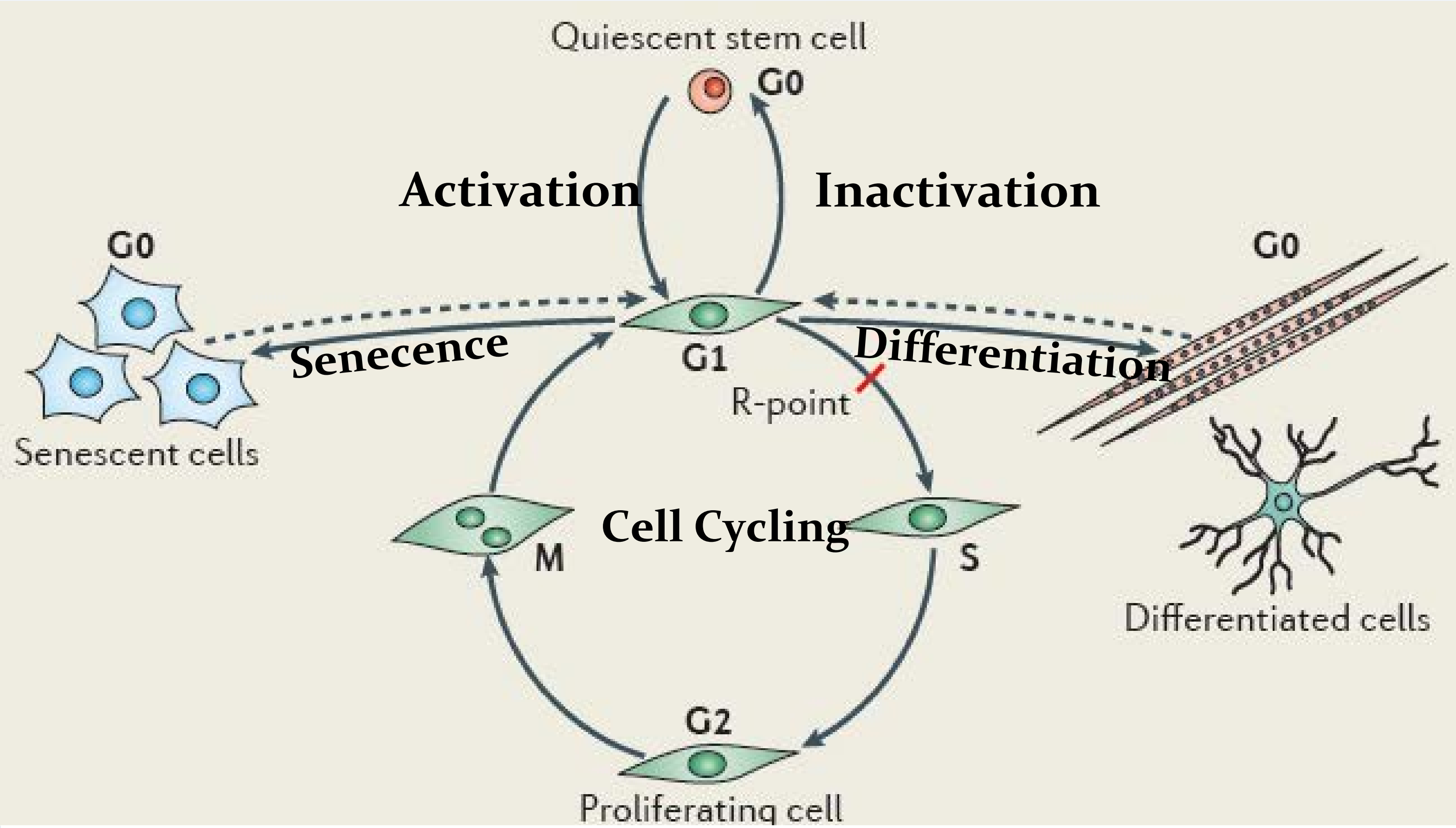


Stem cell quiescence

- Development, tissue renewal and long term survival of multi-cellular organisms is dependent upon the persistence of stem cells that are quiescent, but retain the capacity to re-enter the cell cycle to self-renew, or to produce progeny that can differentiate and re-populate the tissue.
- Deregulated release of these cells from the quiescent state, or preventing them from entering quiescence, results in uncontrolled proliferation and cancer. Conversely, loss of quiescent cells, or their failure to re-enter cell division, disrupts organ development and prevents tissue regeneration and repair.
- Understanding the quiescent state and how cells control the transitions in and out of this state is of fundamental importance.

However, there is no study model for stem cell quiescence.

***Artemia* may be the a peculiar model of stem cell quiescence!**



Tom H. Cheung and Thomas A. RandoMol. Cell Bio., 2013

Stem cell quiescence is a condition in which cells cease dividing but are poised to re-enter the cell cycle in appropriate environmental conditions

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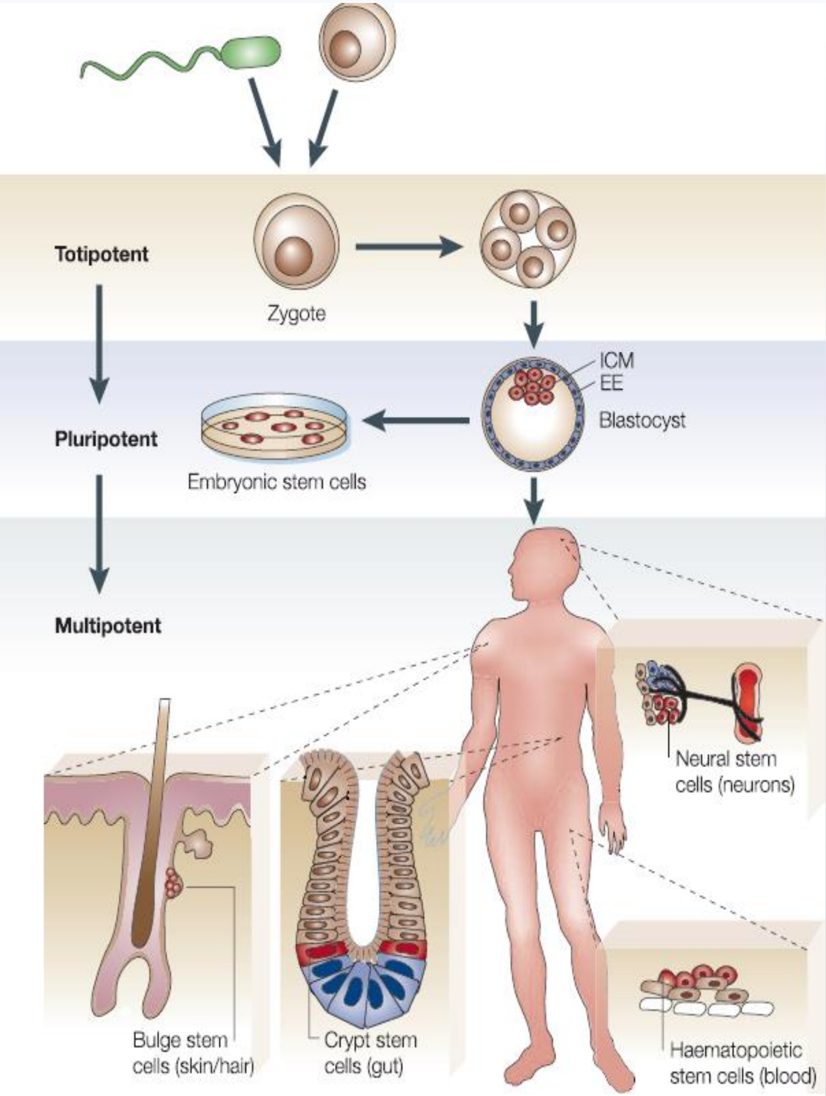
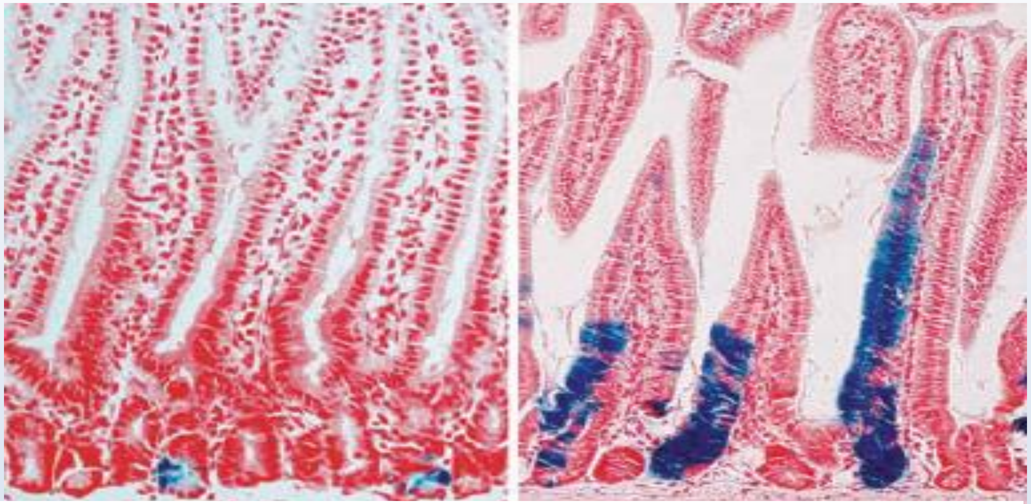
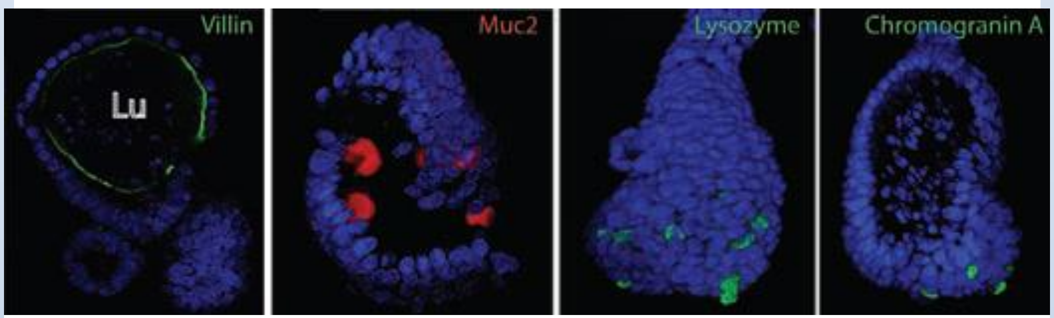


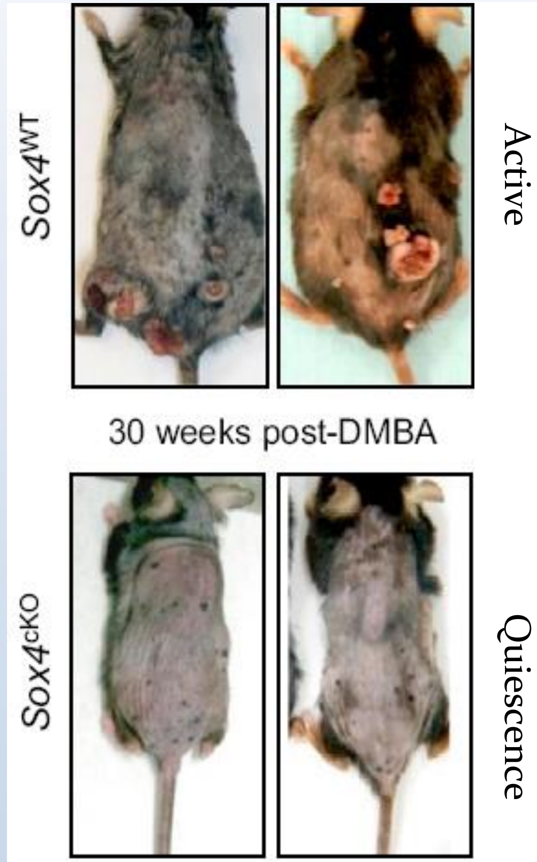
The stem-cell hierarchy Nat Rev Mol Cell Biol. 2005 Sep;6(9):726-37



Lineage-tracing studies of the intestinal stem cells (Lgr5) at the base of crypts. N. Barker et al., 2007, Nature 449:1003



Single Lgr5-expressing intestinal stem cells build crypt-villus structures. T. Sato et al., 2009, Nature 459:262



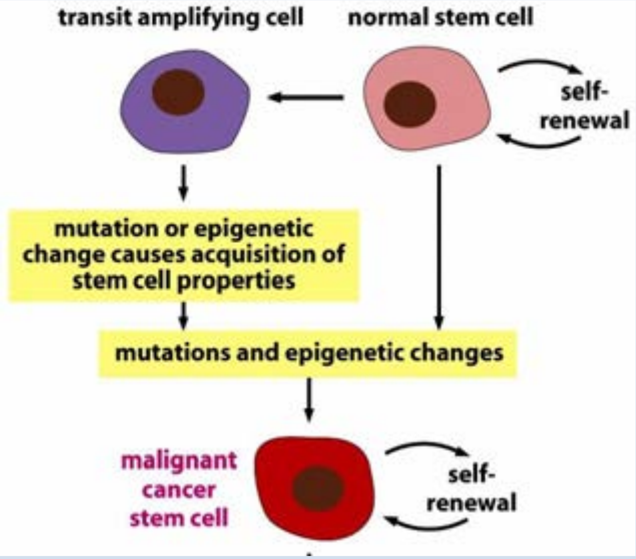
Cell Reports 8, 487–500, 24, 2014

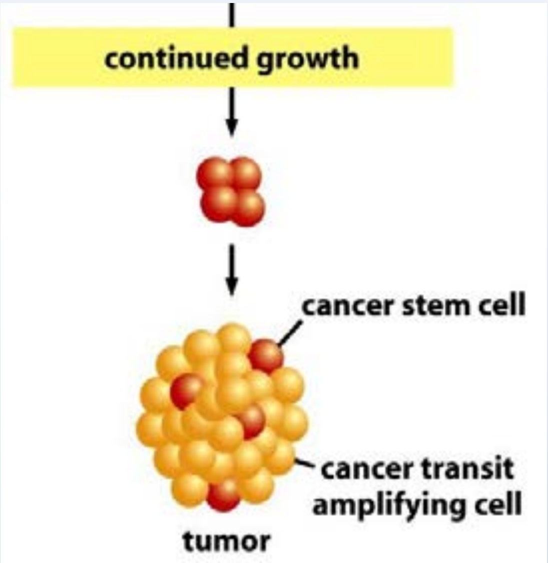
Tissue Stem Cell (SC)

Active: tissue homeostasis or to repair tissue damage, but may tumorigenesis.

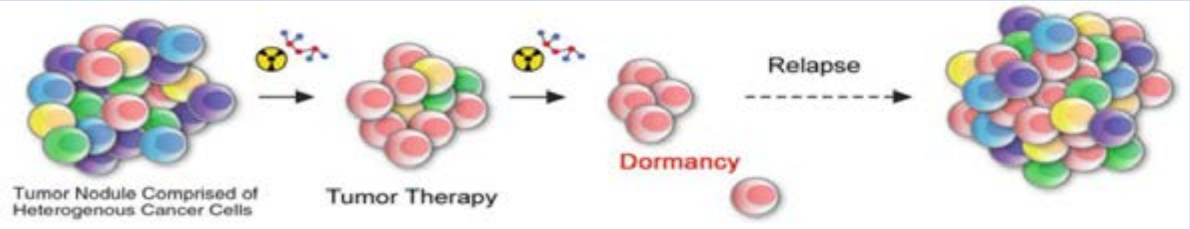
Quiescence: no tumorigenesis, but dysfunction of tissue.

Tumorigenesis: mutations and epigenetic changes in stem cell





A model of Tumor Recurrence/Relapse



Cancer Stem Cell (CSC)
Quiescence: no tumor recurrence, but anticancer treatments.
Active: sensitive to clinic treatments, but tumor recurrence.

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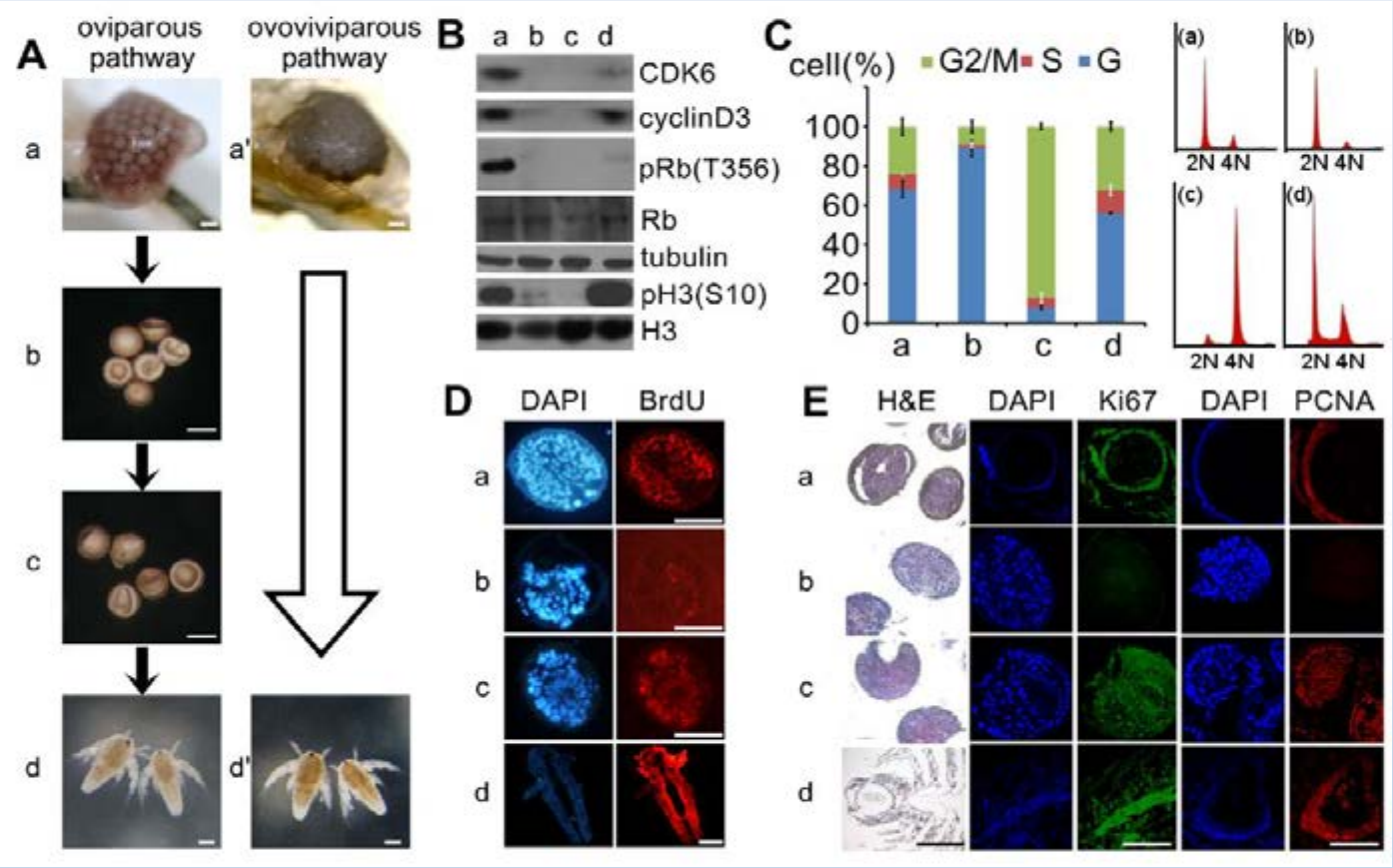
Progression and characterization of cell quiescence during *Artemia* diapause embryo formation and termination



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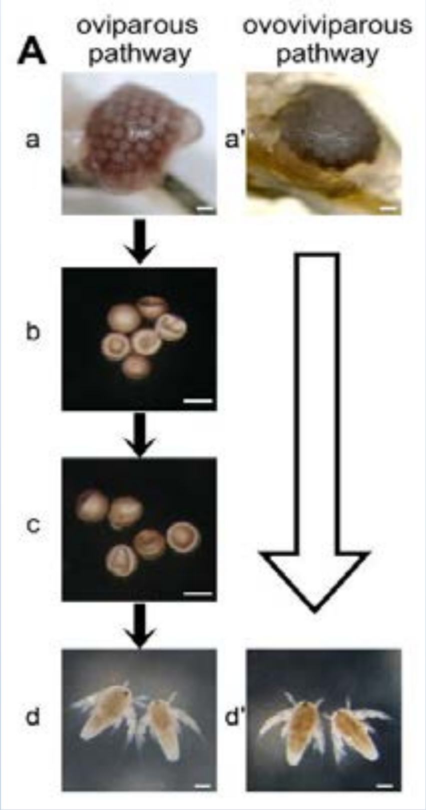
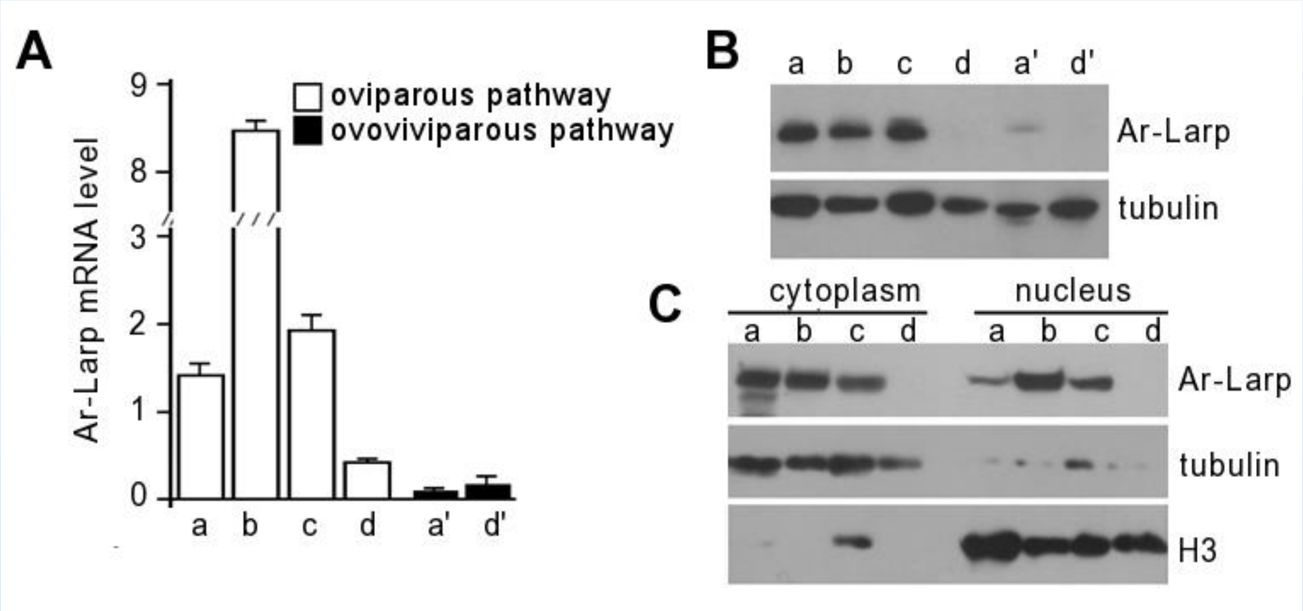
Artemia is an excellent model for the study of mechanism of biochemical and biophysical adaptation to extreme environments in general and cell quiescence regulation especially

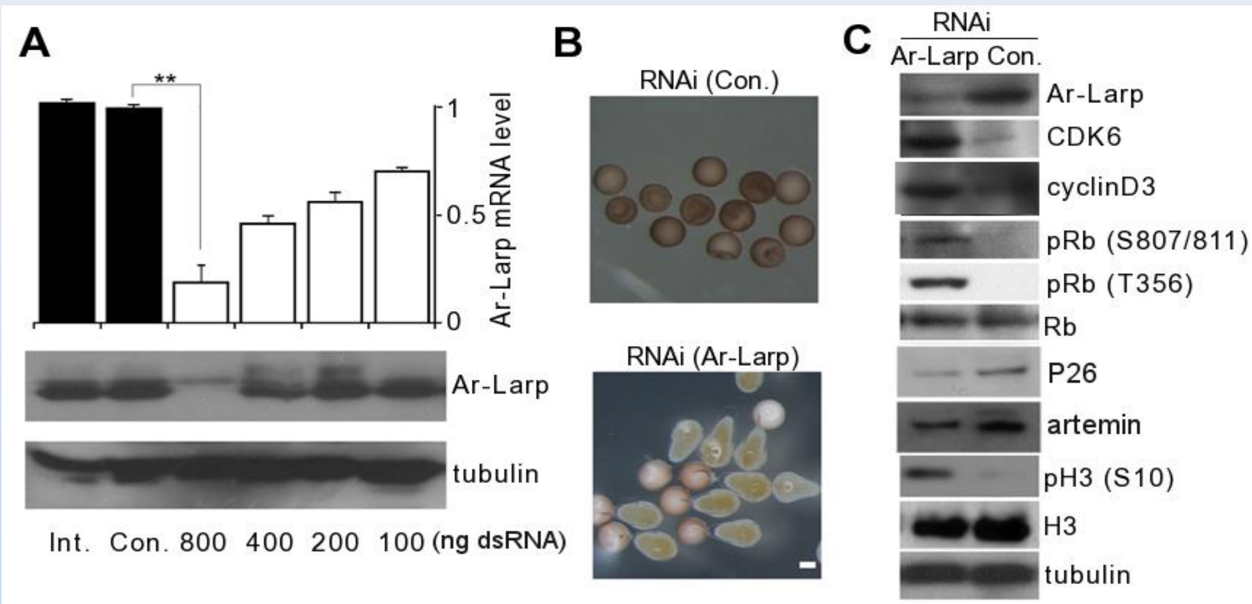
development stage		cell division status
oocyte		meiosis
↓	development	↓
pre-diapause		mitosis
↓	cellular quiescence	↓
diapause		quiesced at G_0
↓	activation	↓
post-diapause		arrested at G2/M
↓	development	↓
nauplius		mitosis

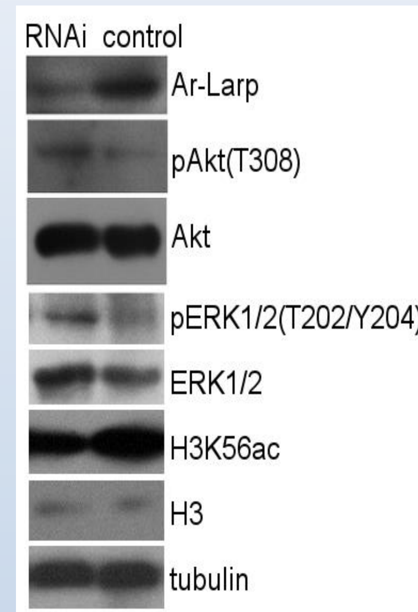
Characterization of Ar-Larp gene expression in each development stage of *Artemia* in the diapause-destined or directly developing pathways



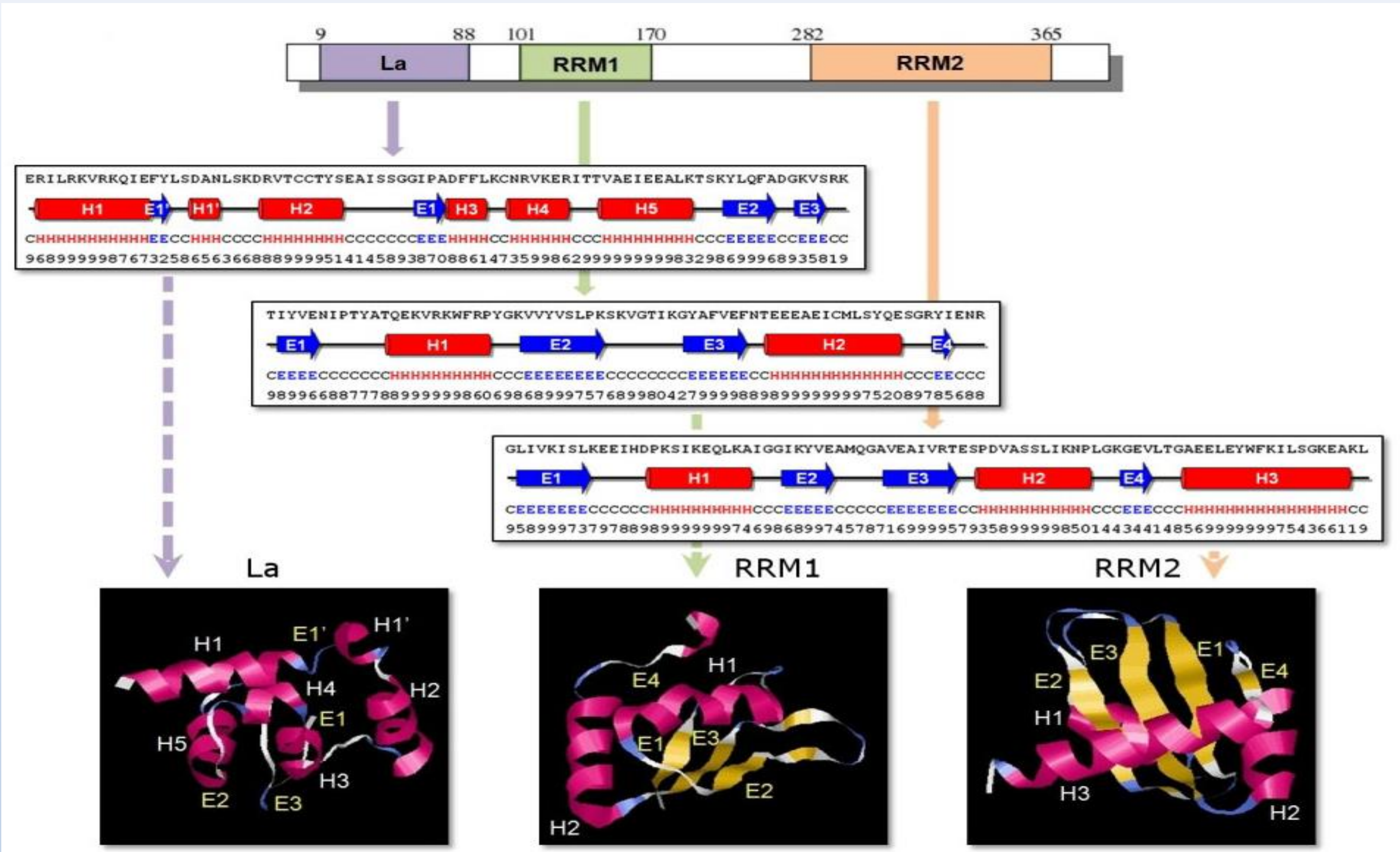


RNA interference (RNAi) of Ar-Larp in *Artemia*

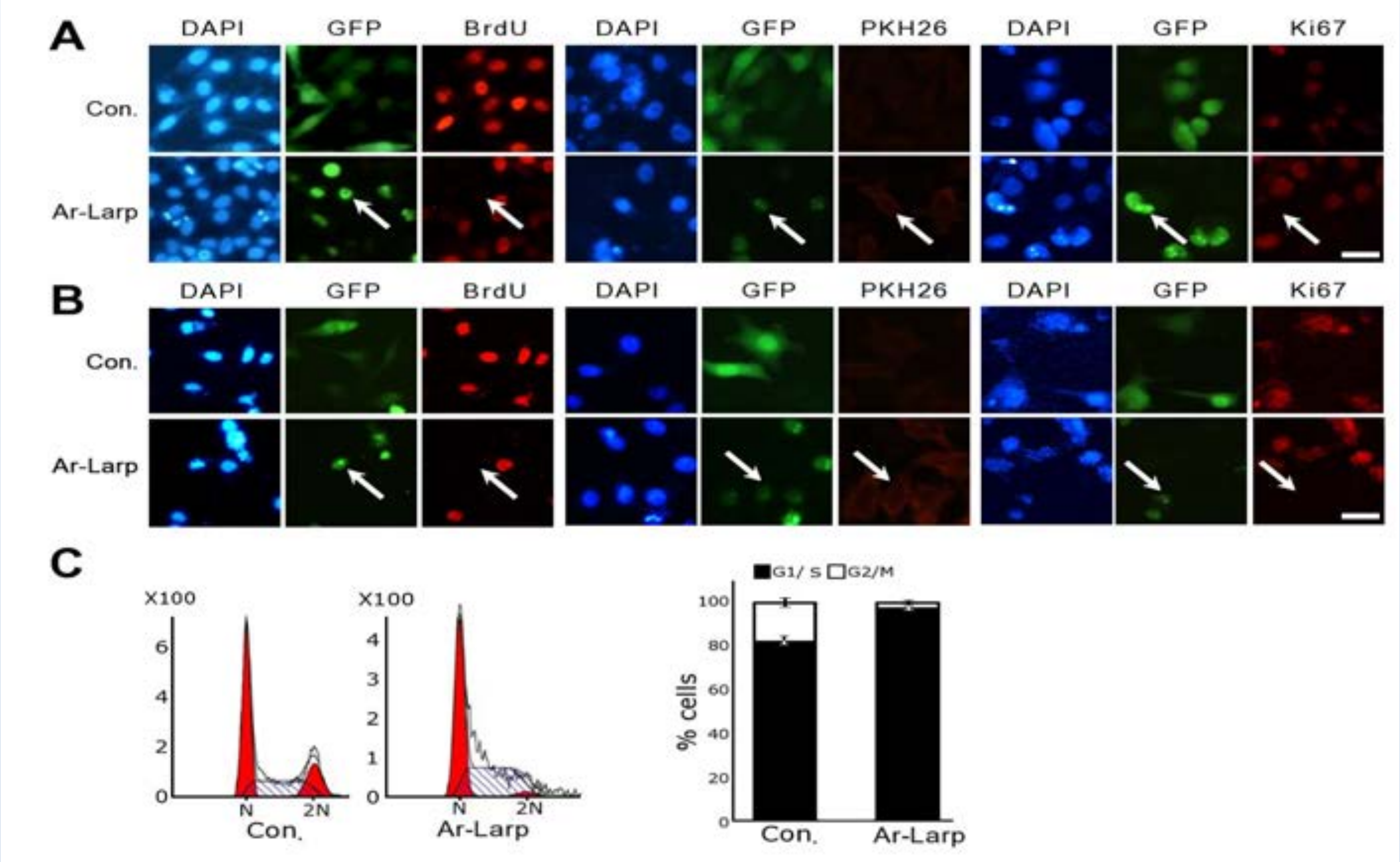




Structural predictions for the Ar-Larp



Ar-Larp overexpression results in cell cycle arrest in HeLa and MKN45 cells. Transient transfection of Ar-Larp in (a) HeLa and (b) MKN45 cells



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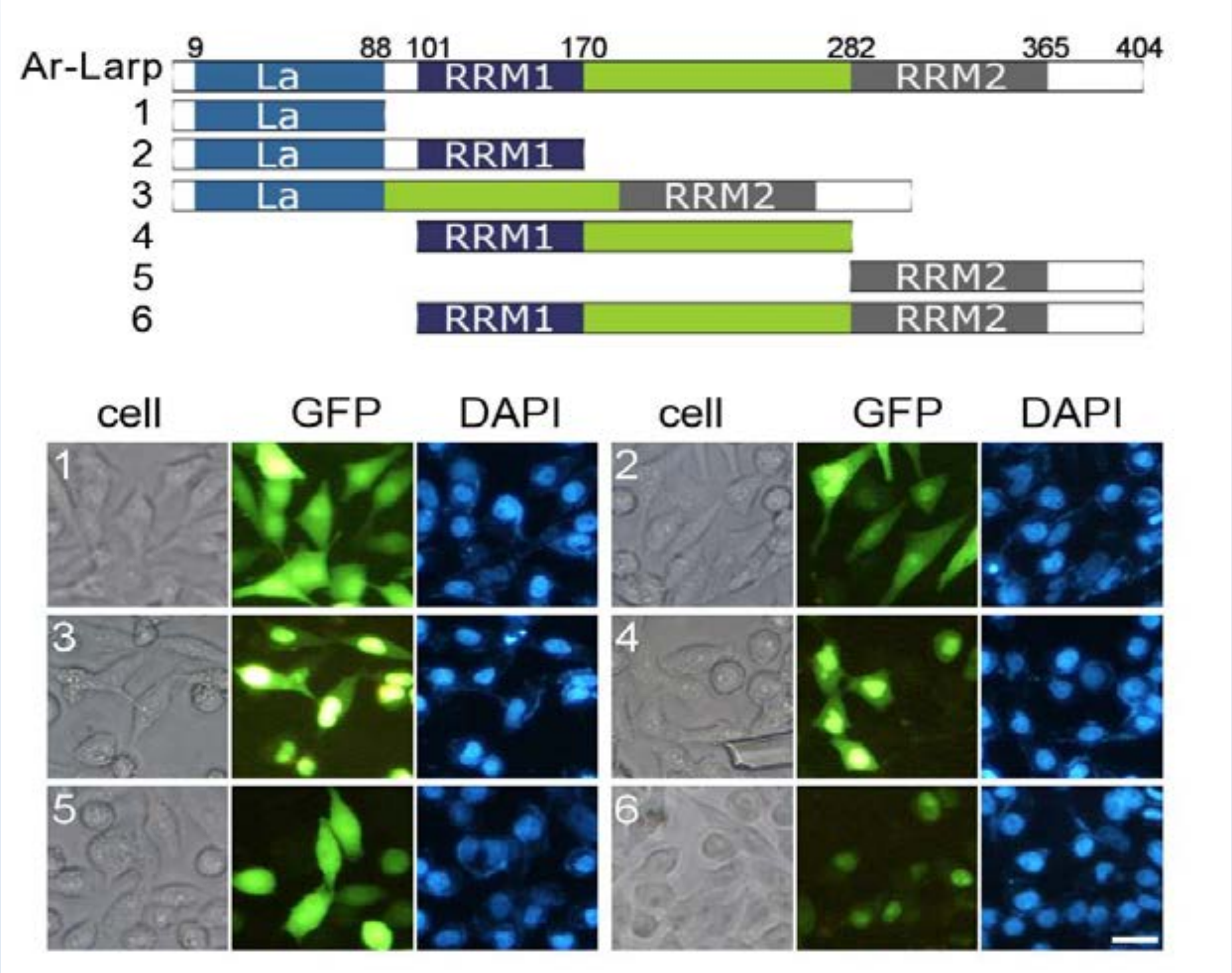
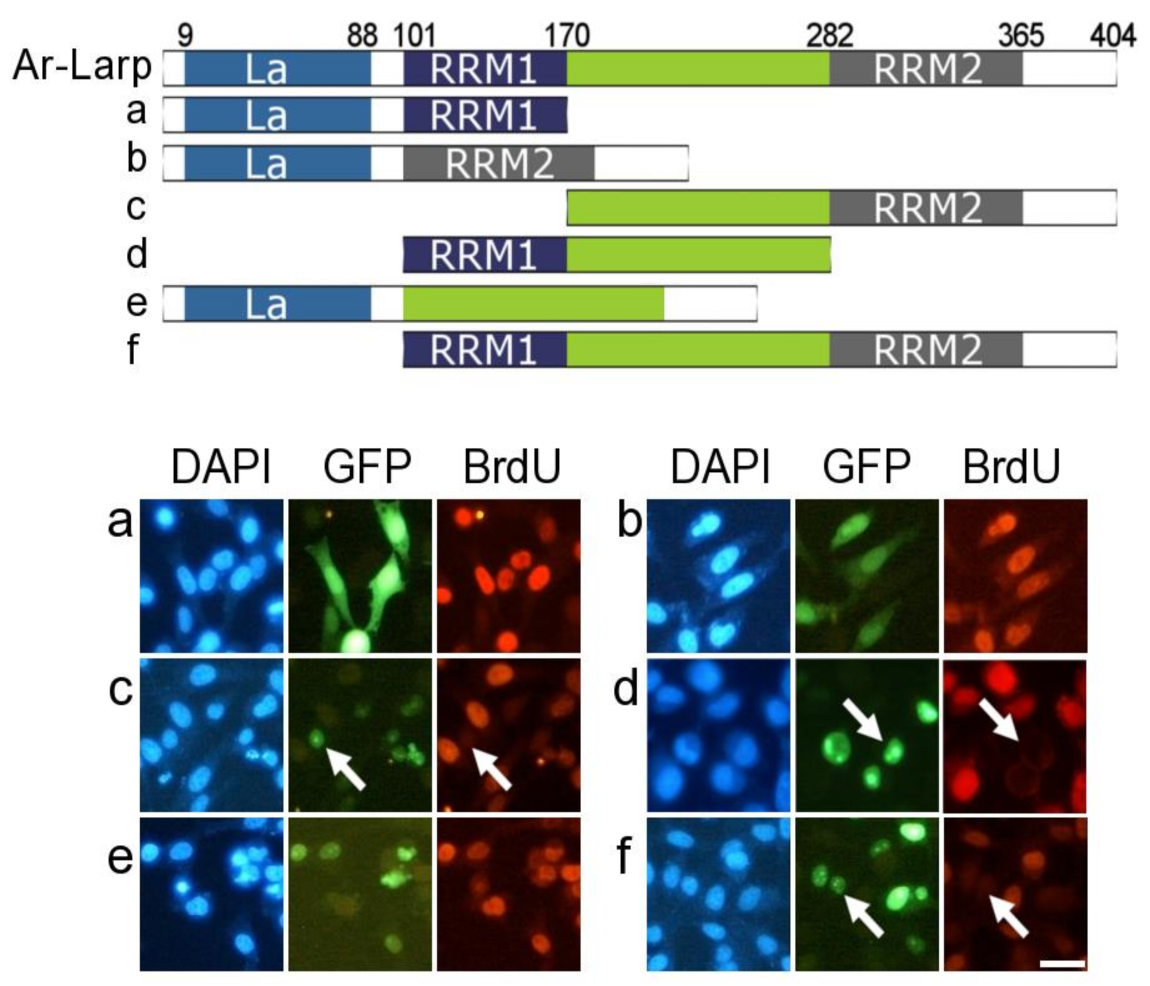
Tianjin University of Science & Technology

Analysis of subcellular locations of Ar-Larp mutantsin HeLa cell



Analysis of mitosis of HeLa cells overexpressing Ar-Larp mutants



Food and Agriculture Organization of the United Nations

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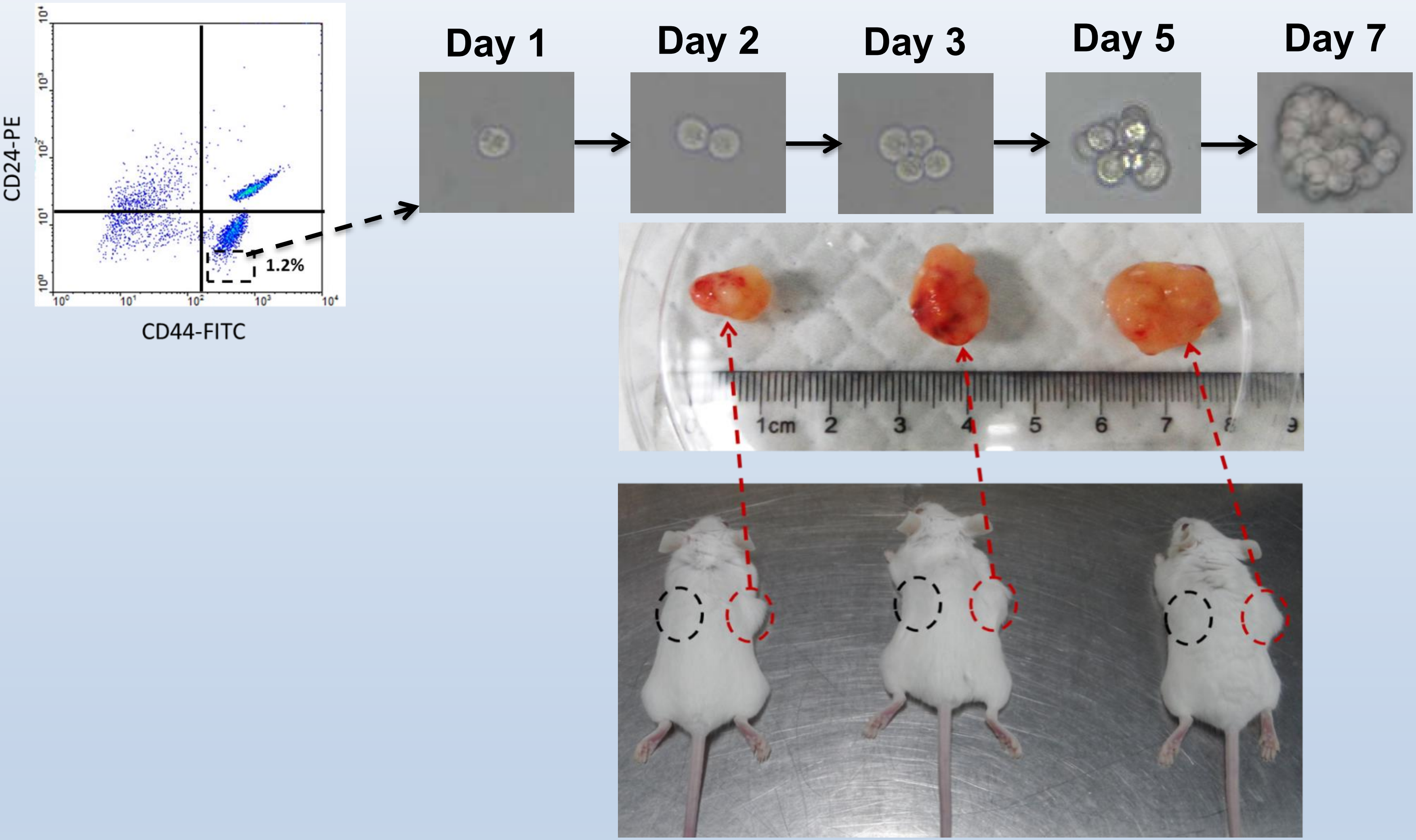
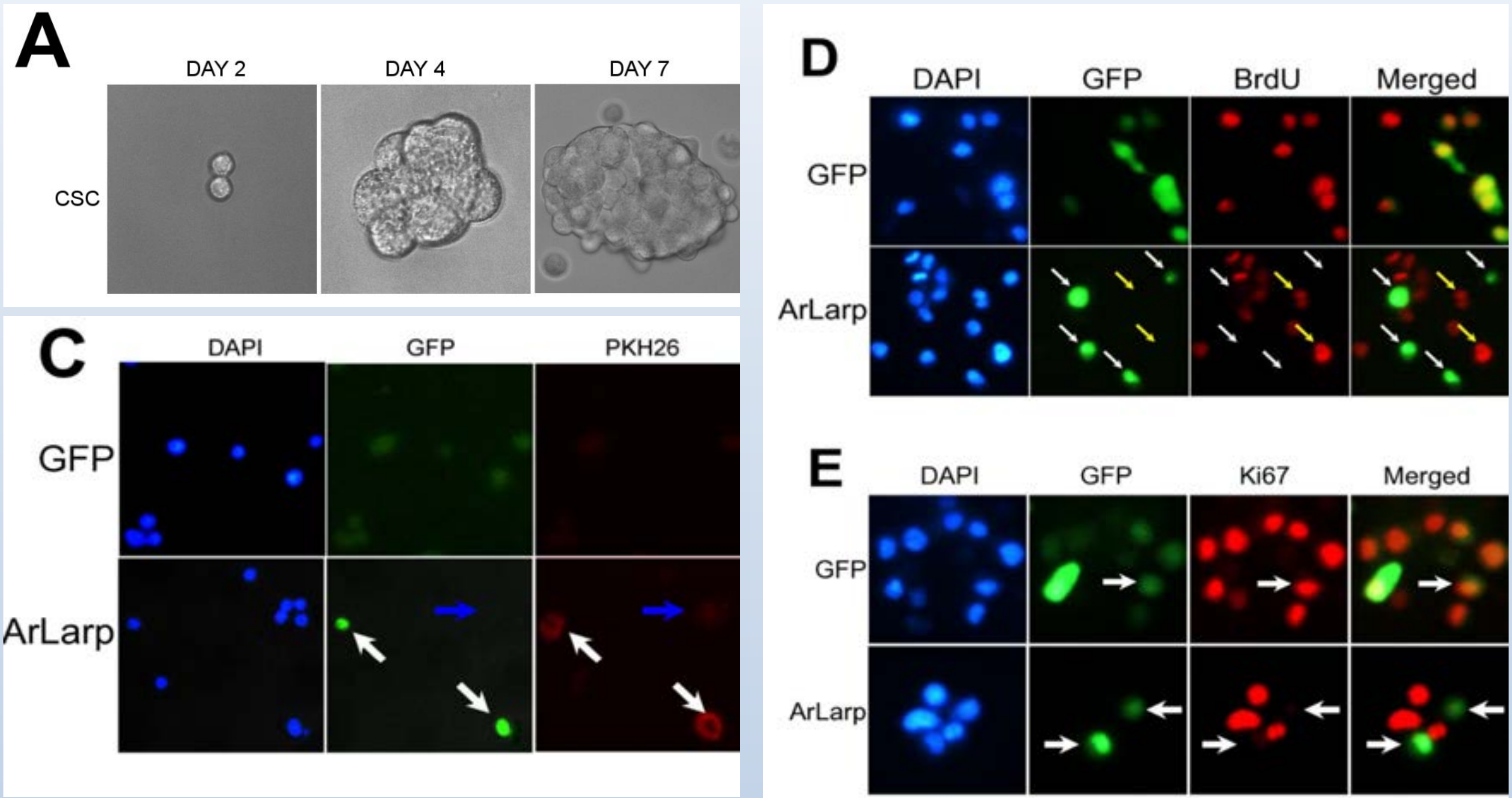
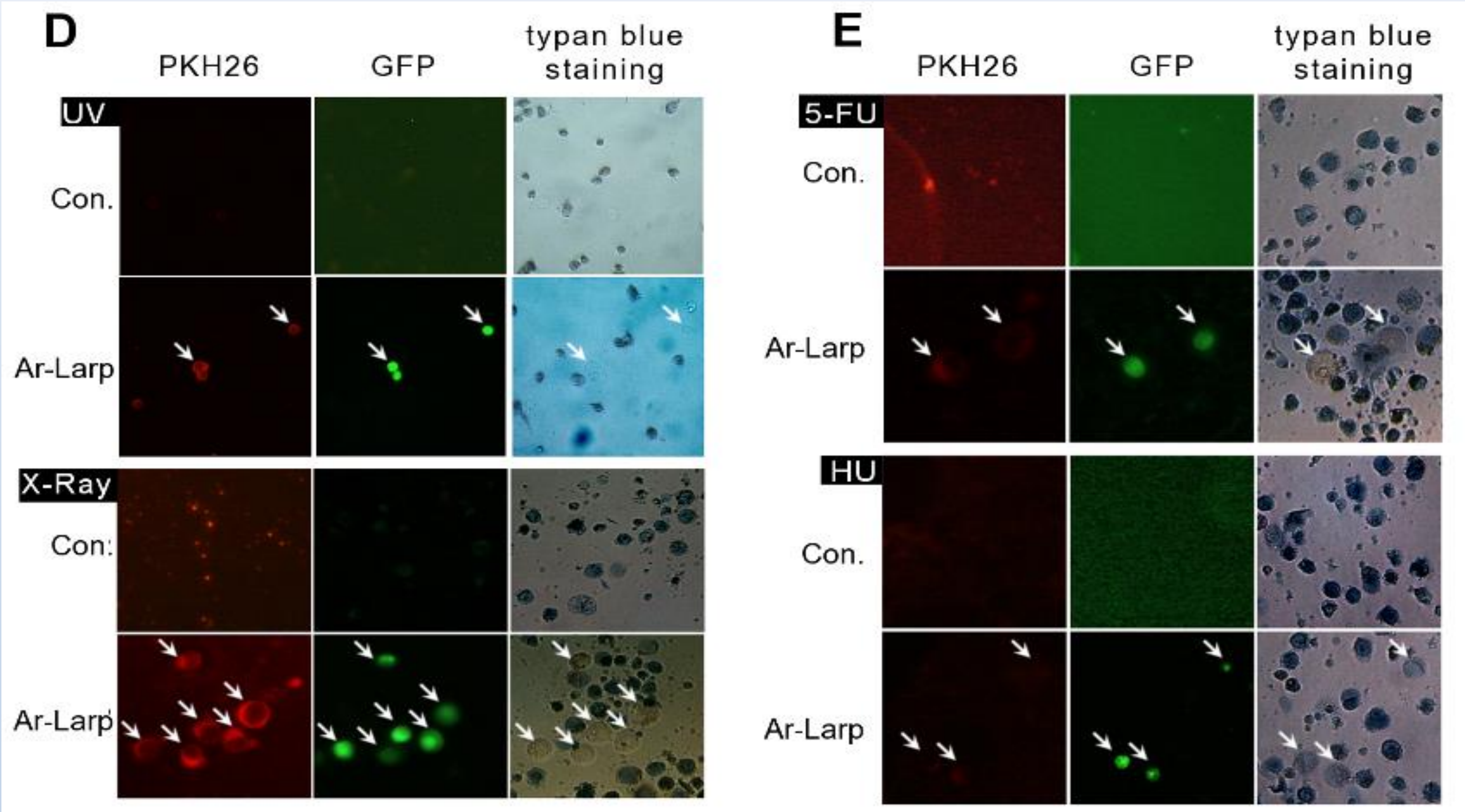
Isolation characterization of cancer stem cells from gastric tumor



Ar-Larp induces the quiescence of cancer stem cells



Ar-Larp induces the quiescence of cancer stem cells and resistance to drug and radiation treatments



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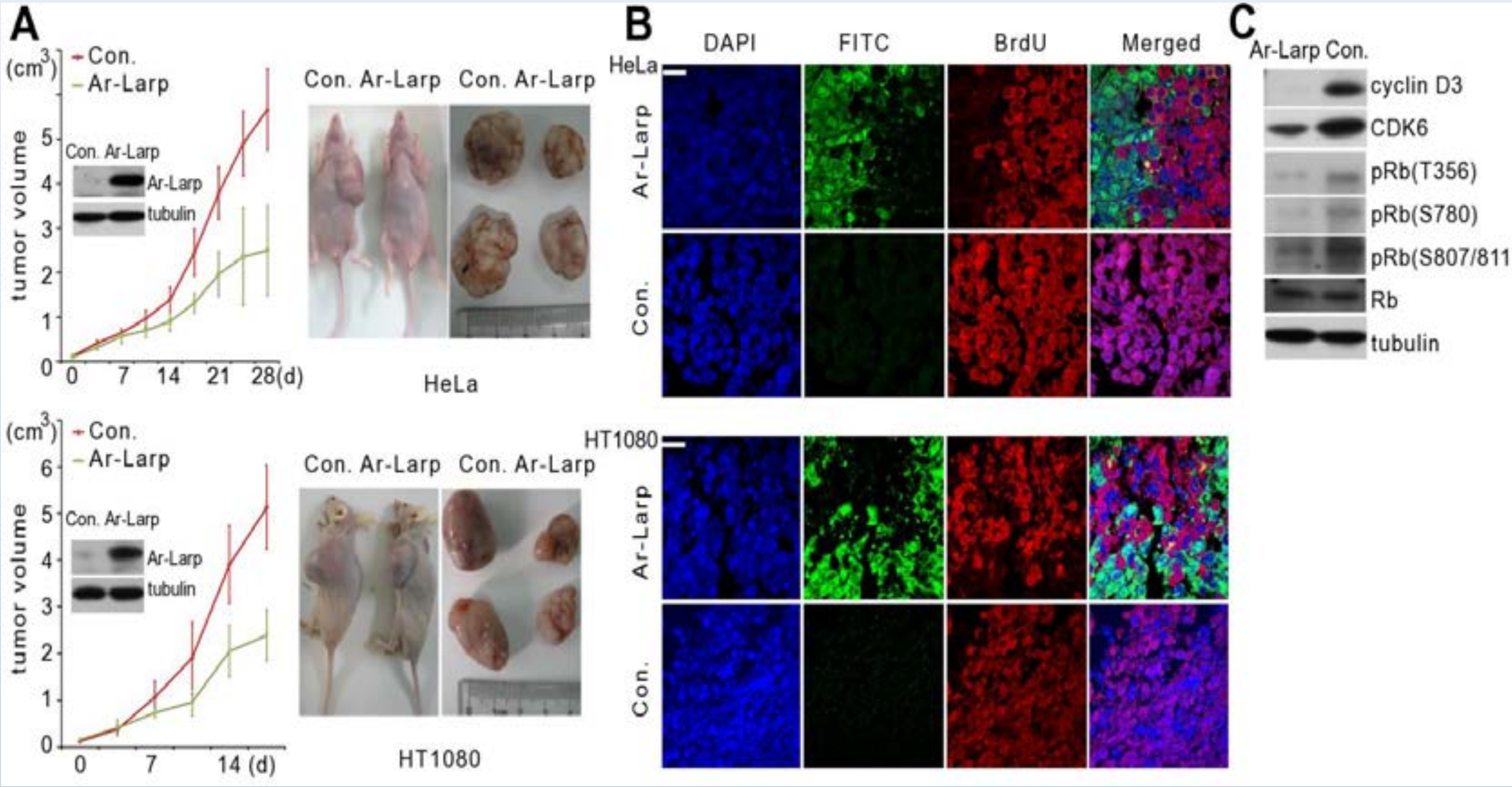
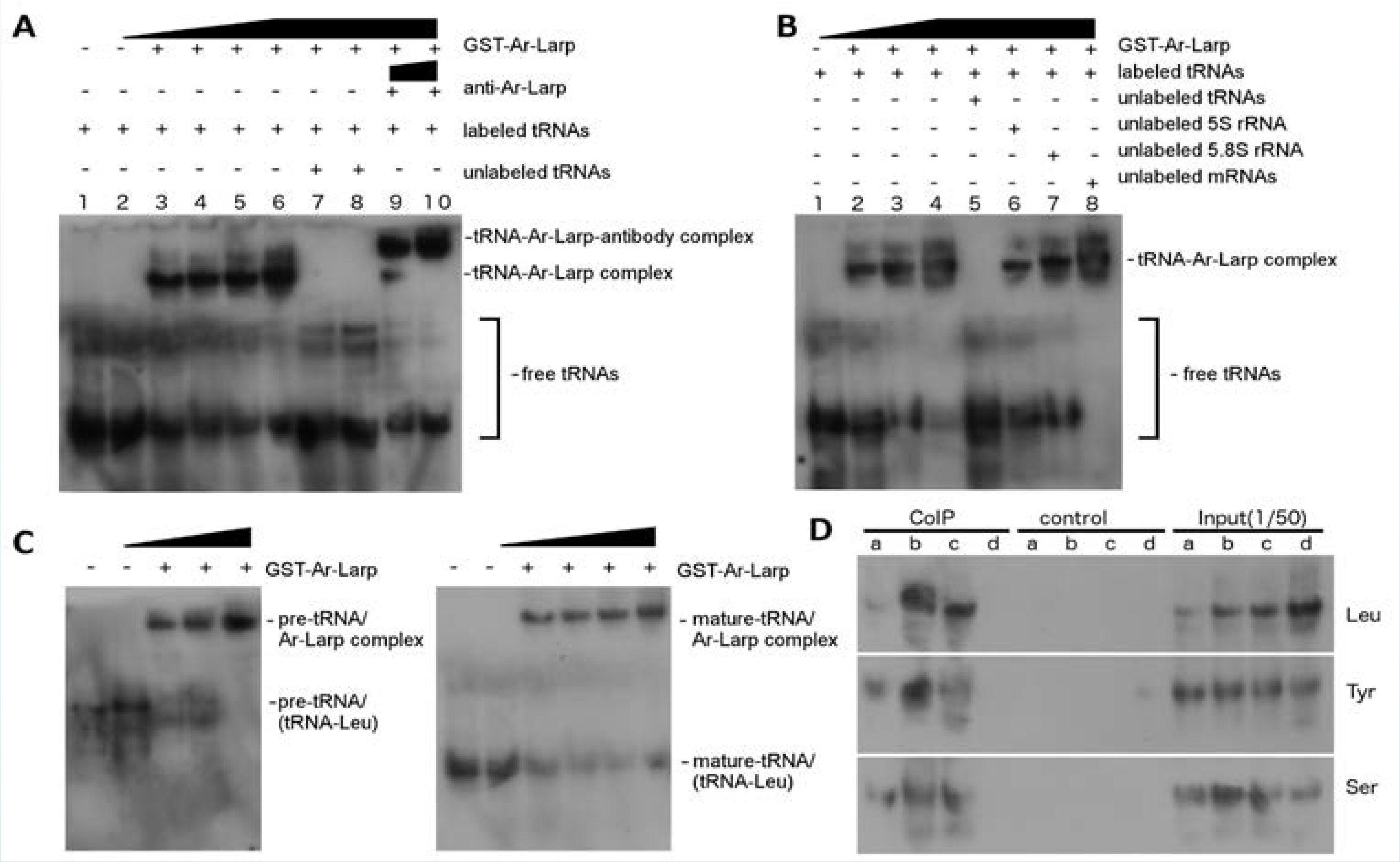
Ar-Larp inhibits mitosis of HeLa and HT1080 cells in mouse xenograft tumor models



Analysis of tRNA binding activity of Ar-Larp



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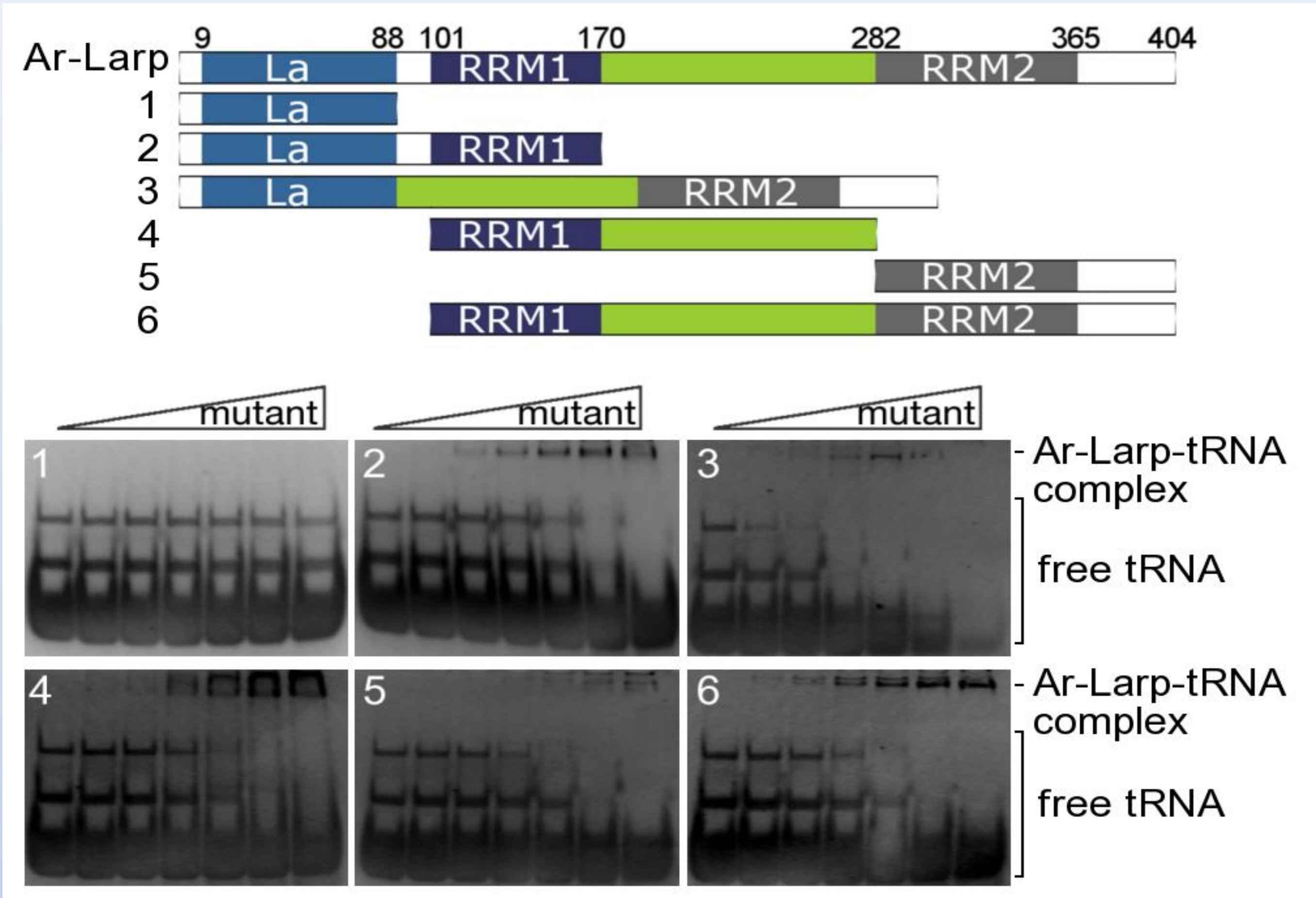
RRM1 and RRM2 of Ar-Larp exhibits tRNA binding activity



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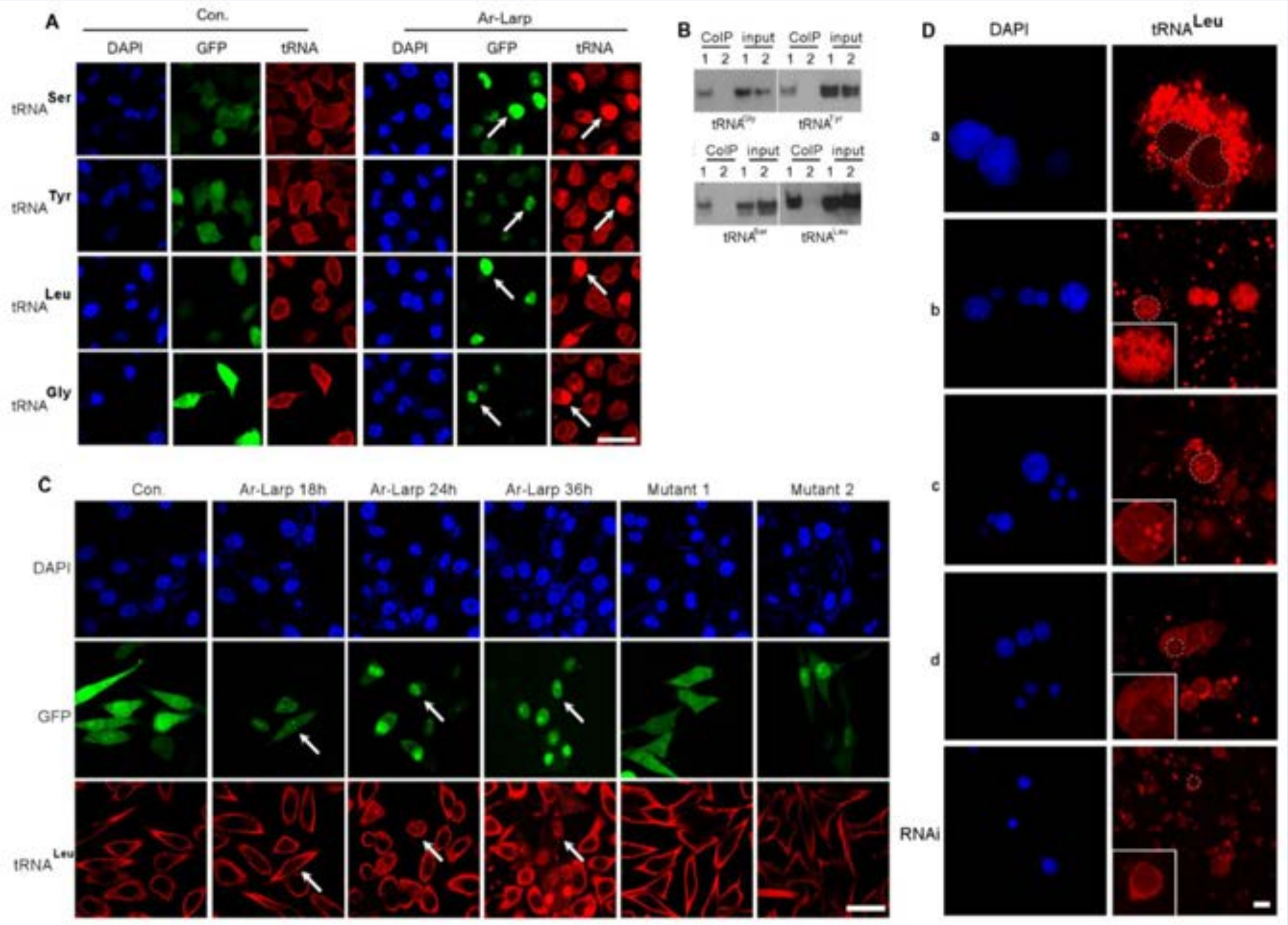
Ar-Larp exhibits tRNA binding activity and accumulates tRNAs in the cell nucleus



Conclusions

- Using *Artemia* as a peculiar model of stem cell quiescence, a La-related protein from *Artemia*, named Ar-Larp, was found to bind to tRNA and accumulate in the nucleus, leading to cell quiescence and controlling the onset of diapause formation in *Artemia*.
- Ar-Larp could also induce cell quiescence in cancer stem cell and suppress tumor growth in a xenograft mouse model, similar to the results obtained in diapause embryos of *Artemia*.
- Our study of tRNA trafficking indicated that Ar-Larp controls cell quiescence by binding to tRNAs and influencing their retrograde movement from the cytoplasm to the nucleus.

***Artemia* may be the a peculiar model of stem cell quiescence!**

Acknowledgement

Zhejiang University

Dr. Dian-Fu Chen, Dr. Ran Li

Dr. Cheng Lin, Dr. Rong Zhou

UCDAVIS AGGIES

Prof. James S. Clegg

DALHOUSIE UNIVERSITY *Inspiring Minds*

Prof. Thomas H. MacRae

UNIVERSITEIT GENT

Prof. Patrick Sorgeloos

東京大学 THE UNIVERSITY OF TOKYO

Prof. Hiromichi Nagasawa

Thank You

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Food and Agriculture
Organization of the
United Nations

History and Current Status of *Artemia* Research and Applications in Bohai Bay Area

Liying Sui
College of Marine and Environmental Sciences
Tianjin University of Science and Technology
and
Naihong Xin
Salt Research Institute of CNSIC, Tianjin, China

ARTEMIA GENE BANK

Sea Salt Production in Bohai Bay Saltworks

- The major sea salt production site: 40 million tonnes in 2015 , surface area 1 500 km²

Artemia Production in Bohai Bay Saltponds

- The commercialization of *Artemia* cysts started from 1980s.
- ***Artemia* species** : *Parthenogenetica* + *A. franciscana* +?
- Bohai Bay ***Artemia* cysts** are known as better hatchability and nutritional value, thus it is highly demanded in market.
- The cysts production dropped dramatically from 800-1 000 tonnes DW/y to 200-300 tonnes DW/y in a decade.
- Harvest of ***Artemia* biomass** is several hundred tonnes/year, used as a supplemental feed for local shrimp hatchery.

Artemia Research in SRI, CNSIC

- Initiated **in 1985**, collaborated with Ghent University, under the support of UNDP and EEC (EU).
- **Major achievements** in 1985-1995:

---- Successful inoculation of SFB *Artemia* at Tanggu and Nanpu saltworks.

---- *Artemia* resources survey in China: > 30 salt lakes and coastal saltworks

---- *Artemia* strain characterization: hatchability, biometrics, nutritional value, etc.

---- Demonstration and extension of *Artemia* enrichment techniques to local hactheis

---- Improvement of cysts processing techniques

TUST Artemia Task Force

--- Bilateral cooperation project "Integrated utilization of concentrated brine after seawater desalination " supported by MOST of China

--- Pilot project "The management of the salt ecosystem in the Bohai Bay" supported by Province of East-Flanders, Belgium

--- National Natural Science Foundation of China

--- Projects supported by Tianjin Municipal Science and Technology Commission

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ĐẠI HỌC CẦN THƠ

Hangu Saltworks Co., Ltd.

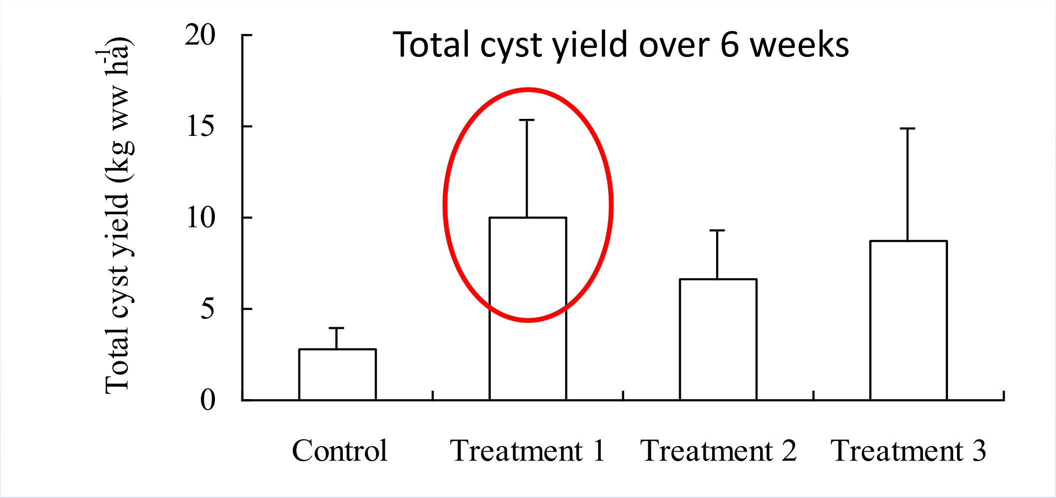
Chengkou Salt Chemicals Co., Ltd.

Hebei Xinhai Aquatic Biotech. Co., Ltd.

- Ecological investigation, focusing on impact of brine acidification on *Artemia* population in saltponds (2009-2013): Chengkou Saltworks and Hangu Saltworks

- *Artemia* inoculation（2010-2011）: Chengkou Saltworks

- Improve the pond *Artemia* production through microbial management (Biofloc Technology)



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Tianjin University of Science & Technology

TUST Artemia Task Force

- Preserved seven *Artemia* species and more than 130 cysts samples collected from inland salt lakes and coastal solar saltworks, mainly in China, Russia and central Asia countries.
- The sampling time span from late 1980's to now.

ARTEMIA GENE BANK

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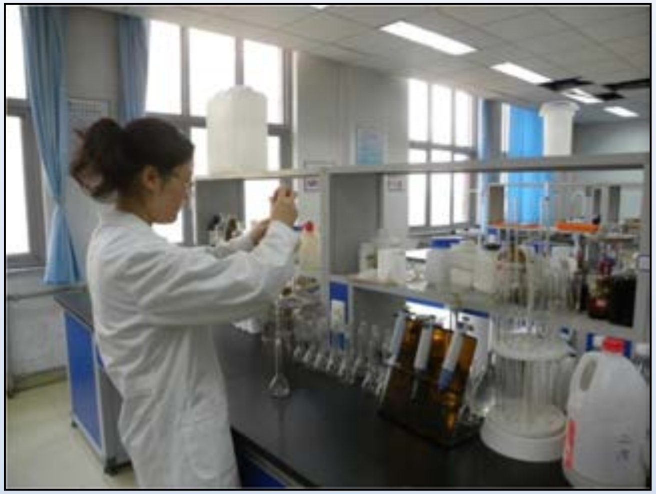
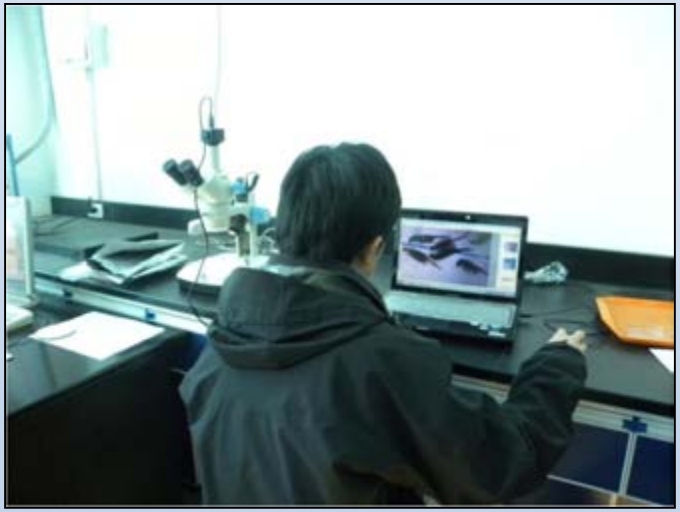
Tianjin University of Science & Technology 天津科技大学

TUST Artemia Task Force

Strain characterization

- Cyst and naupliar biometrics
- Adult morphometrics and morphology
- Hatching quality
- Diapause characteristics
- Temperature and salinity tolerance
- Life history traits and reproductive characteristics
- Nutritional value: HUFA, protein, lipid, etc
- Genetic fingerprinting





ARTEMIA GENE BANK

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TUST Artemia Task Force

ARTEMIA GENE BANK

TUST Artemia Task Force

providing network query.

Challenge of *Artemia* Production in Bohai Bay

The drop of cysts harvest during last decade

- Occupation of salt ponds due to the rapid development of the industry: more and more saltworks has been replaced by airport, freight yard,….
- Discharge of desalination and bromine extraction effluents into the saltponds: destroy the saltpond ecosystem
- Extensive shrimp culture in low salinity saltponds (3-5%) ?
- Pollution of seawater ?

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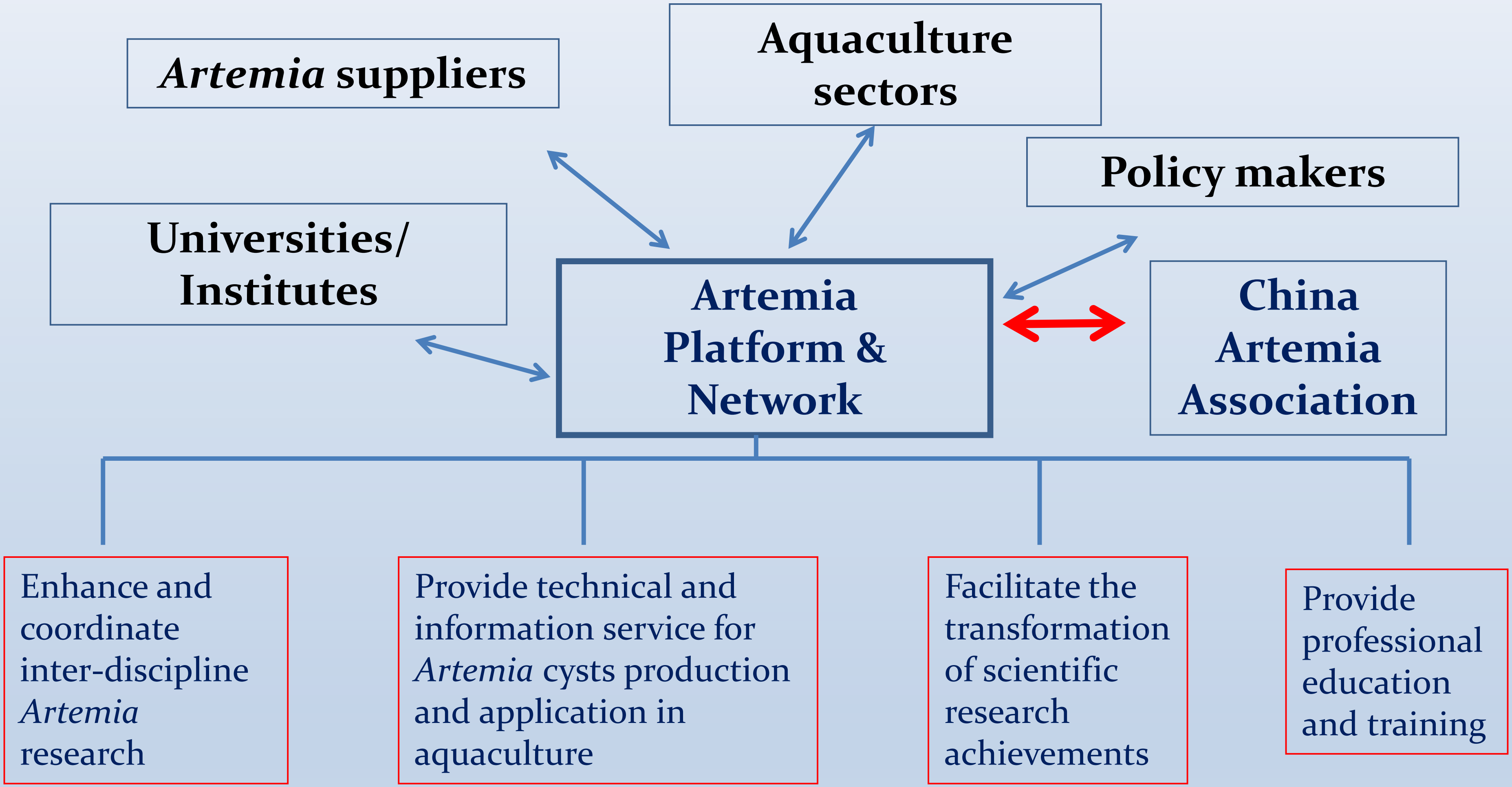
International Workshop on Brine Shrimp *Artemia* in Solar Salt Works: Functional Role and Sustainable Resource

(April 26-28th, 2013 TEDA, China)

Recommendations of the "International workshop on brine shrimp Artemia in solar saltworks: functional role and sustainable resource"

- ***Artemia* cysts and biomass production is below capacity due to insufficient management**: Expertise should help in improving Artemia production through continuous and stepwise guidance.
- ***Artemia* as an element of extractive aquaculture**: The production of *Artemia* in aquaculture effluents in condition of understanding more about nutrient dynamics in saline ecosystems.
- ***Artemia* Biodiversity**: aquaculture application and conservational issues
- **Establishment of a Chinese Artemia Reference Center under the auspices of FAO**

Need for Regional Artemia Reference Center in China



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Role and history of the Artemia Reference Center, Ghent University

Gilbert Van Stappen and Patrick Sorgeloos
Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Ghent, Belgium

Food and Agriculture Organization of the United Nations

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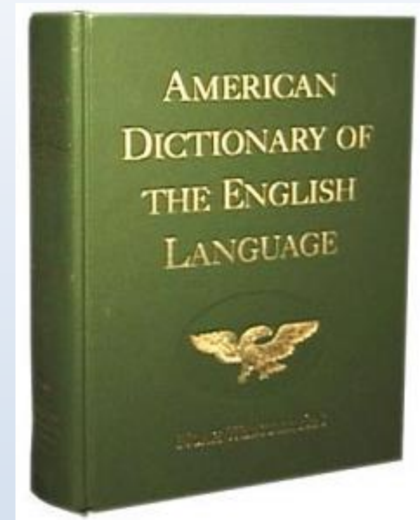
Tianjin University of Science & Technology

“Artemia Reference Center”



1. an act or instance of referring
2. a mention; allusion
3. something for which a name or designation stands; denotation.
4. a direction in a book or writing to some other book, passage, etc.
5. a book, passage, etc., to which one is directed.
6. reference mark
7. material contained in a footnote or bibliography, or referred to by a reference mark.
8. use or recourse for purposes of information
9. a person to whom one refers for testimony as to one's character, abilities, etc.
10. a statement, usually written, as to a person's character, abilities, etc.
11. relation, regard, or respect:

GHENT UNIVERSITY

History

1970: Start research on *Artemia* culture biology at Ghent University by Patrick Sorgeloos

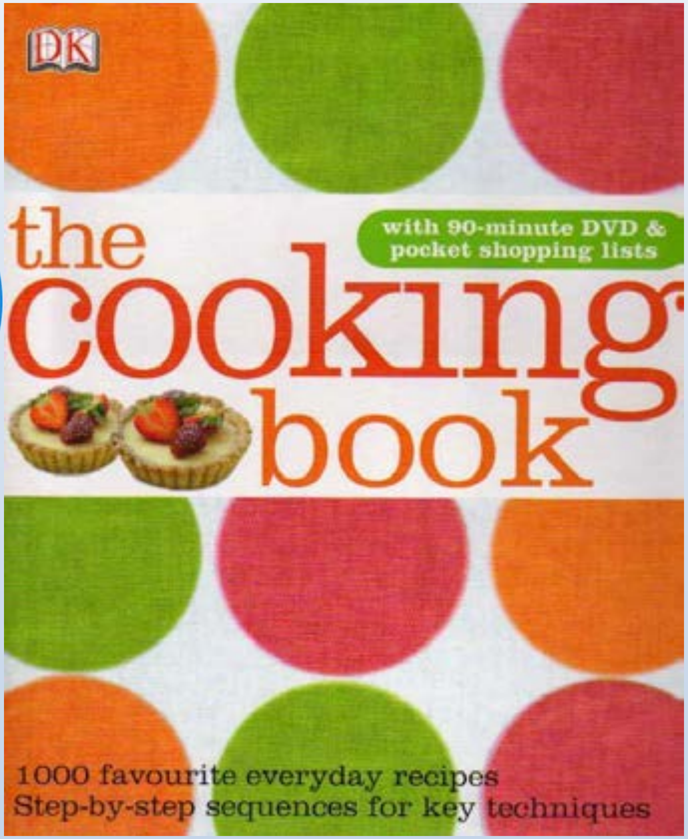
1978: Artemia Reference Center established upon suggestion of the FAO

1989: Laboratory of Aquaculture & Artemia Reference Center (ARC)

2007: establishment of the UGent Aquaculture R&D Consortium

2016:
- 3 professors + 3 post docs
- 3 senior scientists
- 15 PhD (mostly international)
- 7 lab technicians + 2 secretary staff
- since 1991 > 380 MSc alumni from 50 countries
- since 1983 > 80 PhD alumni from 21 countries

How to build/cook an Artemia Reference Center ?



Knowledge transfer: 8 *Artemia* training courses (1978 – 1996)…

Larviculture newsletter (early 1990s – 2011)…

Most Visited Getting Started

Artemia Reference Center

Home

- About the lab
  - Staff
  - History
- Research
  - About our research
  - Projects
  - Publications
  - Accomplished PhD's
- Education
- Services & Resources
  - Literature & Publications
  - In the media
  - News
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  - Database
  - Agenda
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Contact

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Laboratory of Aquaculture & Artemia Reference Center

LARVICULTURE NEWSLETTER

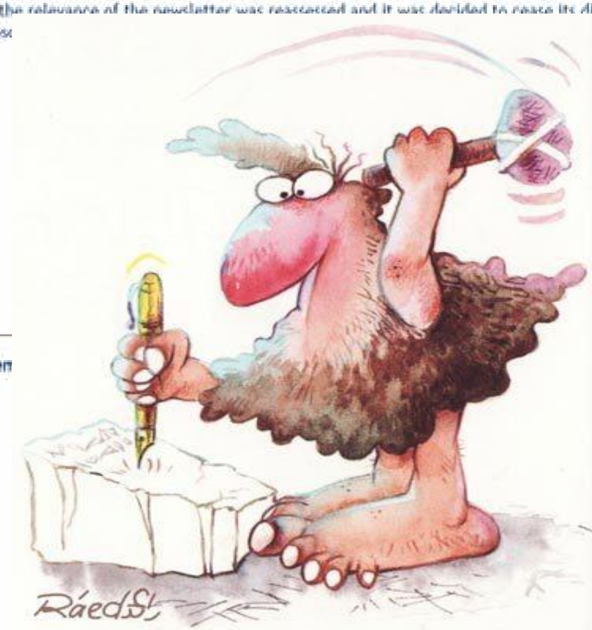
This page was last updated on 19-Sep-11

History

Responding to a need for information beyond purely scientific publications, the ARC started to distribute a paper version of a monthly newsletter in the early nineties. The newsletter covered grey literature, information on new commercial developments or products, interesting excerpts of e-mail discussions or discussion lists, etcetera. As form 1995, the newsletter appeared bi-monthly in a online format only, available through a modest subscription. Due to shifting streams of communication, the relevance of the newsletter was reassessed and it was decided to cease its distribution in July 2011. The back issues are now available without subs

Issues

- 1995
- 1996
- 1997
- 1998
- 1999
- 2000
- 2001
- 2002
- 2003
- 2004
- 2005
- 2006
- 2007
- 2008
- 2009



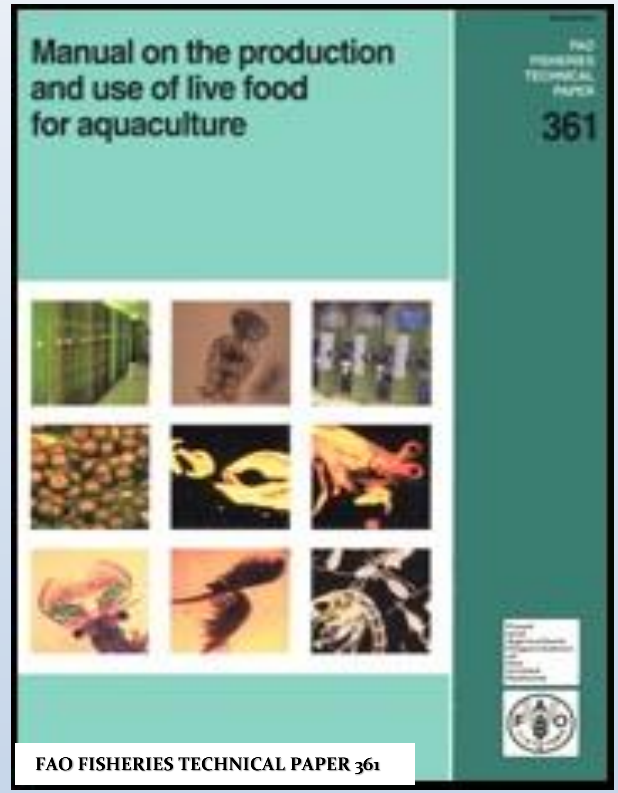
ENG 16:10

Manuals…

1986

Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture

1996



Manual on the Production and Use of Live Food for Aquaculture

Food and Agriculture Organization of the United Nations

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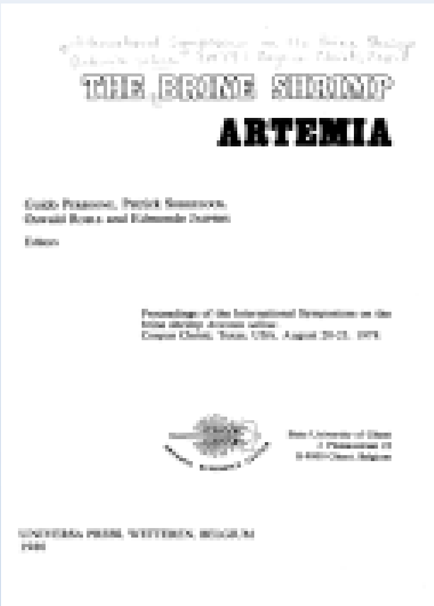
Tianjin University of Science & Technology

Symposia, proceedings and books…

1980

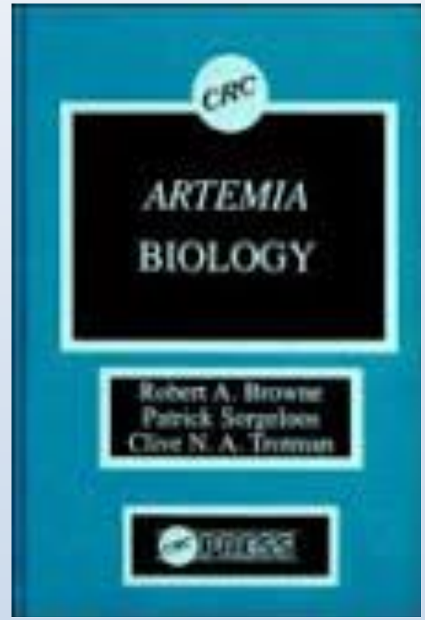


The Brine Shrimp *Artemia*: Proceedings of the International Symposium on the Brine Shrimp *Artemia salina*, Corpus Christi, Texas, USA, August 20-23, 1979 (3 volumes)

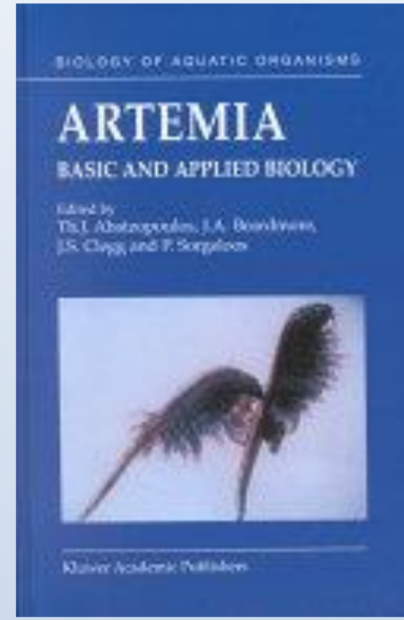
1987

Artemia Research and its Applications: Proceedings of the Second International Symposium on the Brine Shrimp *Artemia*, Antwerp, Belgium (3 volumes)

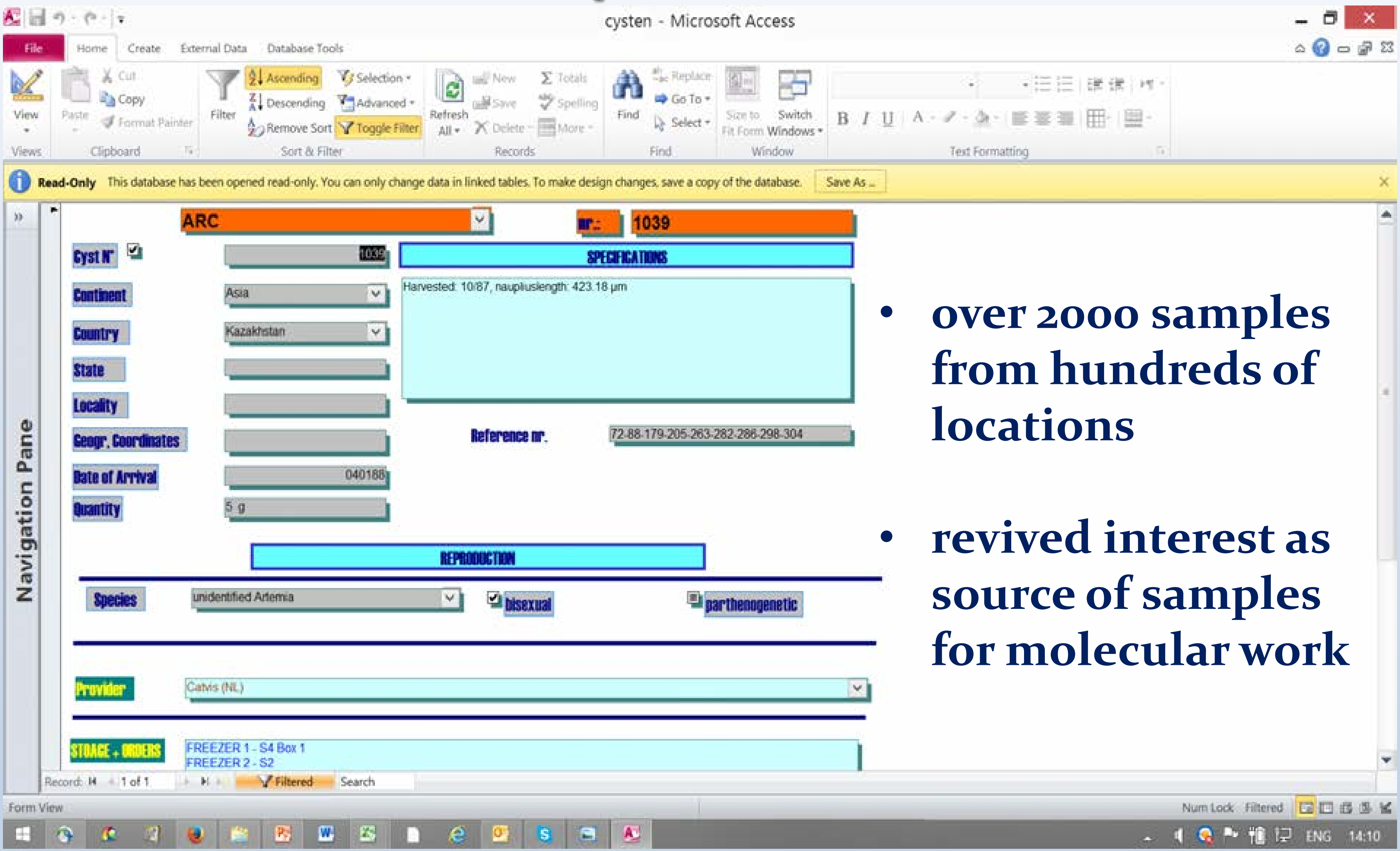
1990



2002



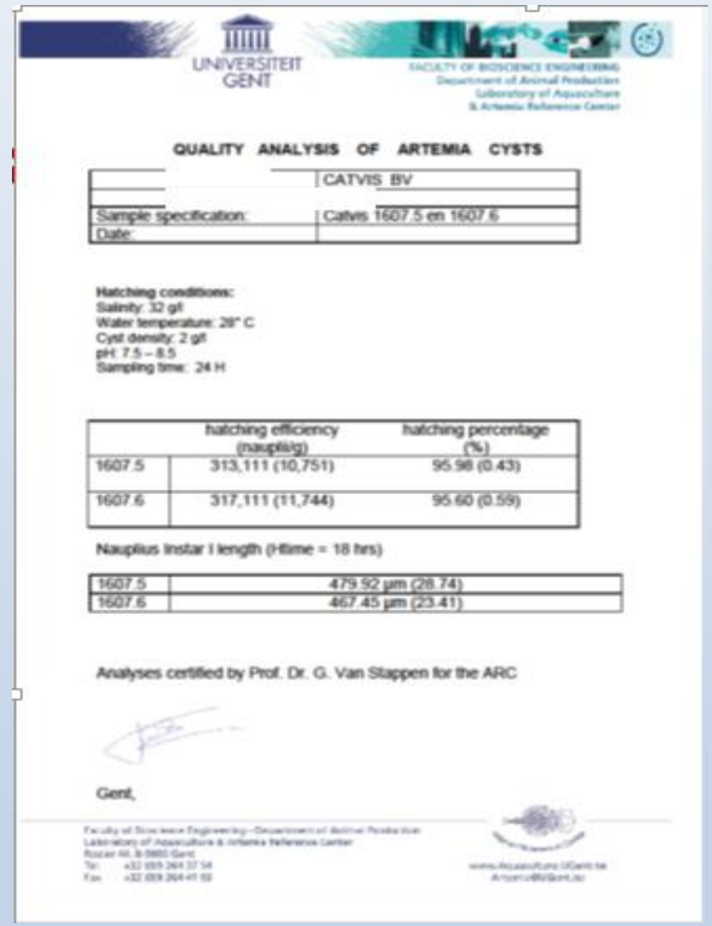
Cyst bank...



- over 2000 samples from hundreds of locations
- revived interest as source of samples for molecular work

Quality control analysis of *Artemia* cysts and other aquaculture-related samples

Hatching quality
Biometry
Proximate analysis
Highly Unsaturated Fatty Acids
Water Content
Molecular analysis
.....



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FACULTY OF BIOSCIENCE ENGINEERING
Department of Animal Production
Laboratory of Aquaculture
& Artemia Reference Center

QUALITY ANALYSIS OF ARTEMIA CYSTS

	CATVIS BV
Sample specification:	Catvis 1607.5 en 1607.6
Date:	

Hatching conditions:
Salinity: 32 g/l
Water temperature: 28° C
Cyst density: 2 g/l
pH: 7.5 – 8.5
Sampling time: 24 H

	hatching efficiency (nauplii/g)	hatching percentage (%)
1607.5	313,111 (10,751)	95.98 (0.43)
1607.6	317,111 (11,744)	95.60 (0.59)

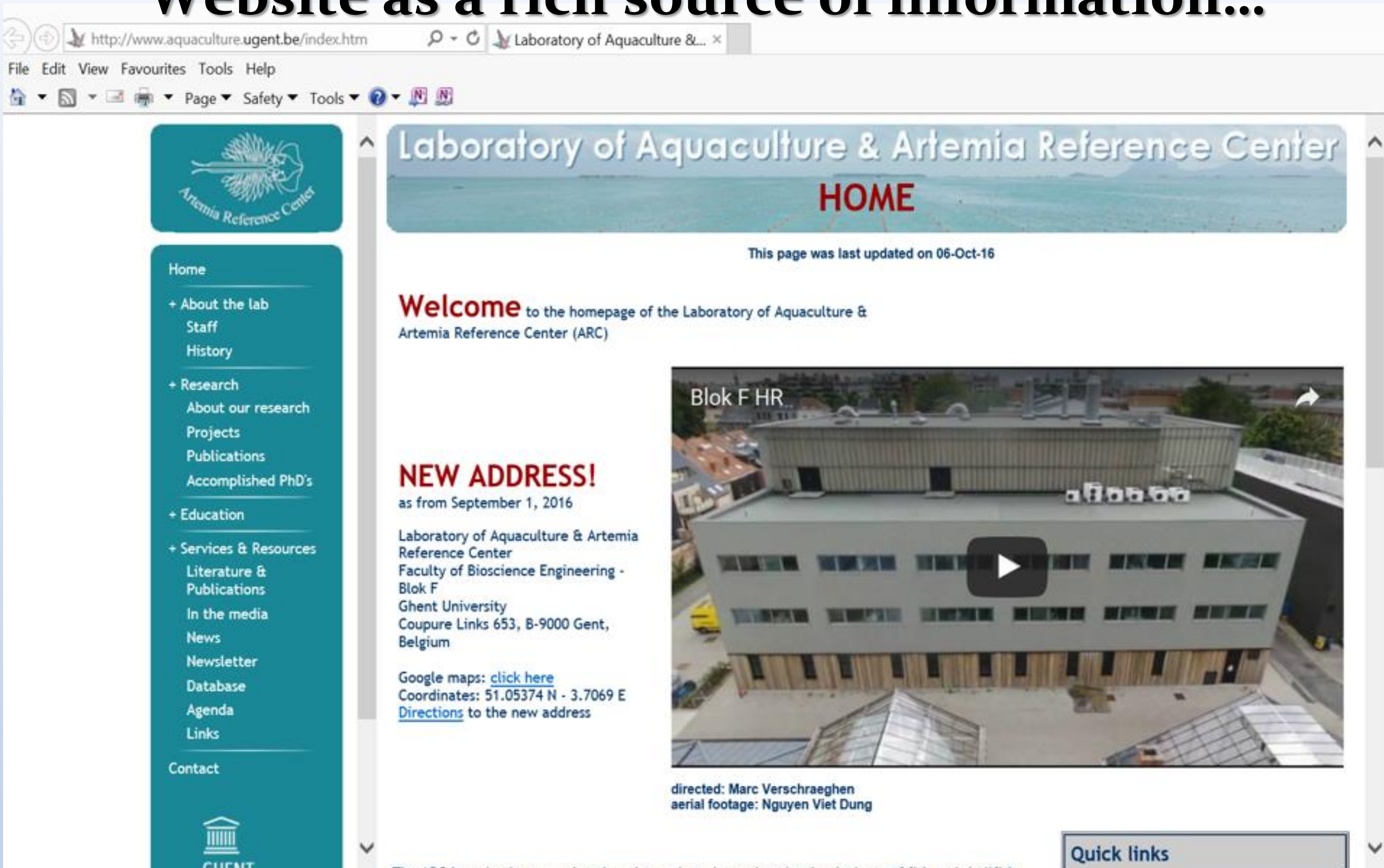
Nauplius Instar I length (Htime = 18 hrs)

1607.5	479.92 µm (28.74)
1607.6	467.45 µm (23.41)

Analyses certified by Prof. Dr. G. Van Stappen for the ARC

Gent,

Website as a rich source of information…



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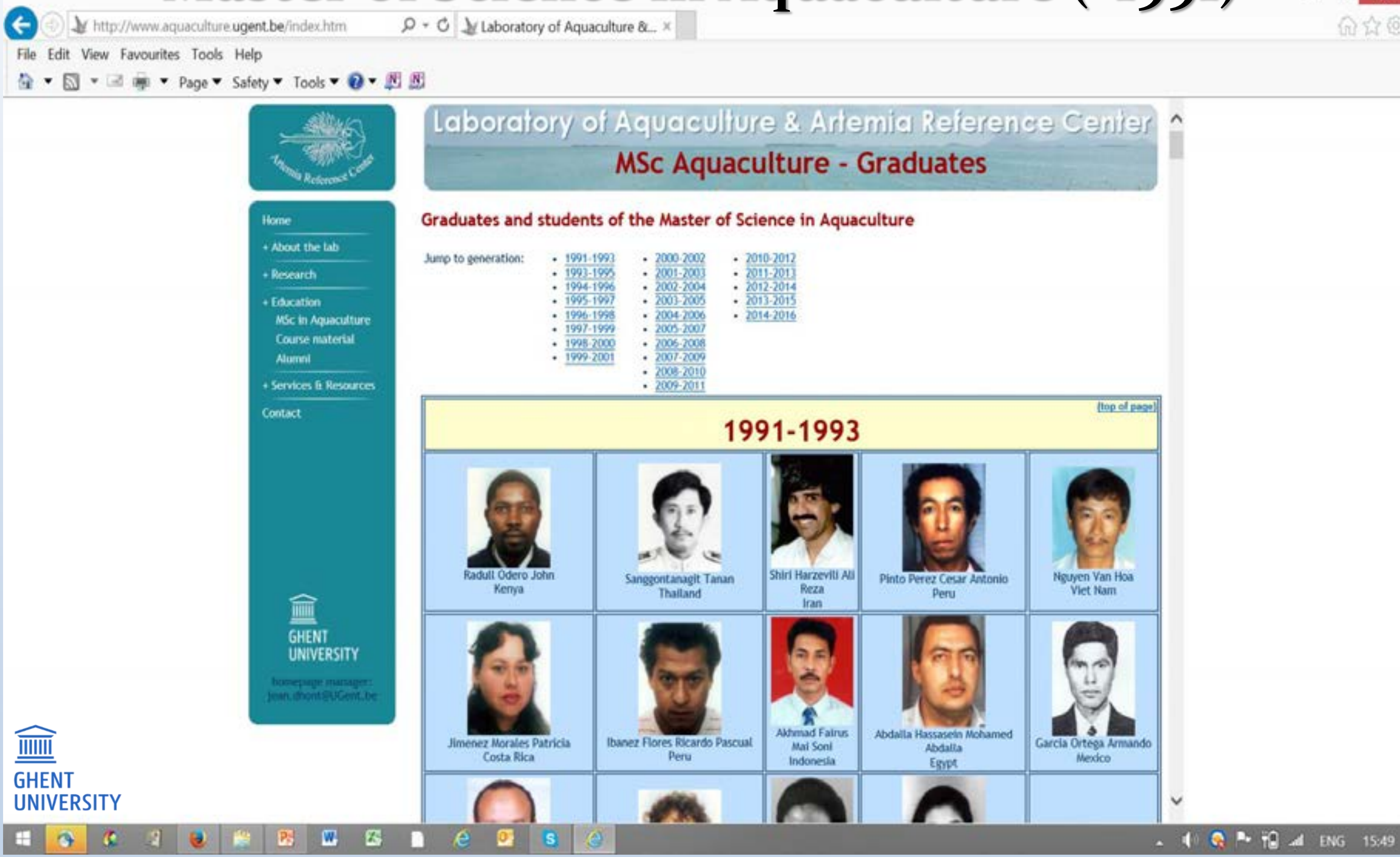
Tianjin University of Science & Technology

Master of Science in Aquaculture (°1991)



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Larviculture congresses (starting 1991)…

Collaboration and networking…

KNOWLEDGE TRANSFER

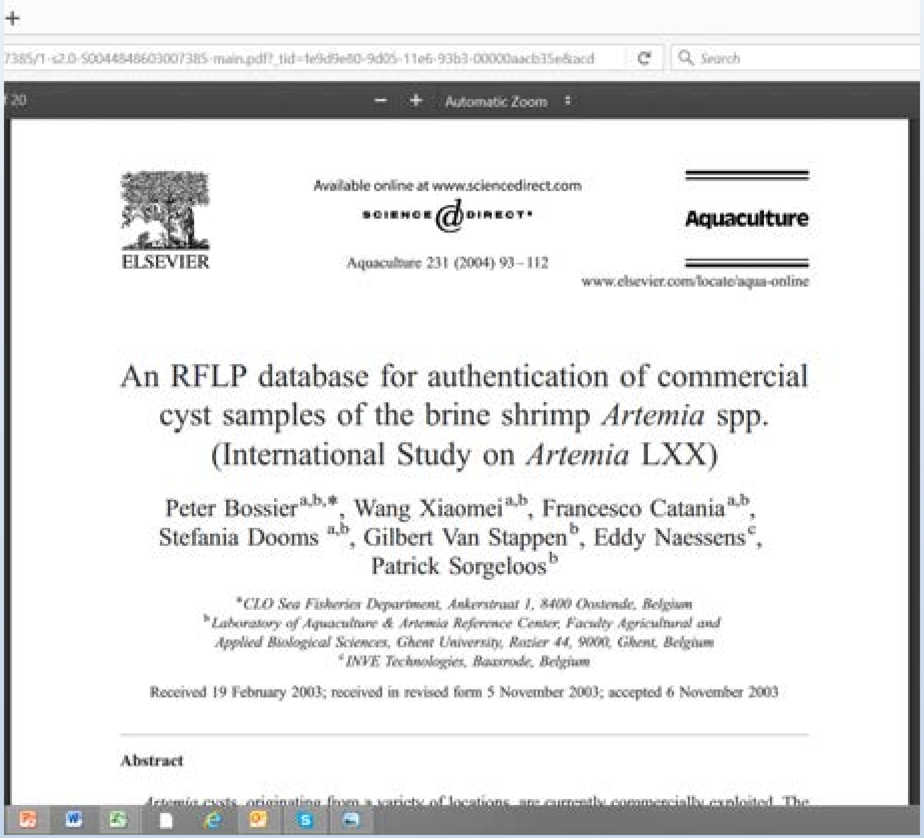
CAPACITY BUILDING

JOINT RESEARCH

International Study of *Artemia* (ISA)

- informal framework of collaboration across continents and disciplines
- about 70 publications in period 1978-2004

Trust and voluntarism: 1 + 1 > 2 !!



Available online at www.sciencedirect.com
SCIENCE @ DIRECT
ELSEVIER
Aquaculture 231 (2004) 93–112
Aquaculture
www.elsevier.com/locate/aqua-online

An RFLP database for authentication of commercial cyst samples of the brine shrimp *Artemia* spp. (International Study on *Artemia* LXX)

Peter Bossier[a,b,*], Wang Xiaomei[a,b], Francesco Catania[a,b], Stefania Dooms[a,b], Gilbert Van Stappen[b], Eddy Naessens[c], Patrick Sorgeloos[b]

[a] CLO Sea Fisheries Department, Ankerstraat 1, 8400 Oostende, Belgium
[b] Laboratory of Aquaculture & Artemia Reference Center, Faculty Agricultural and Applied Biological Sciences, Ghent University, Rozier 44, 9000, Ghent, Belgium
[c] INVE Technologies, Baasrode, Belgium

Received 19 February 2003; received in revised form 5 November 2003; accepted 6 November 2003

Abstract

EU International cooperation project on *Artemia* Biodiversity (2002-2004)

español

The European Commission Community Research

Artemia Biodiversity

Current Global Resources and their Sustainable Exploitation

Project objectives

The INCO project on Artemia Biodiversity (project number ICA4-CT-2001-10020;project period 01/01/02-31/12/04) is a Concerted Action-type project, funded by the INCO-DEV, the International Scientific Cooperation Programme with Developing Countries of the European Commission, Directoral General for Research.

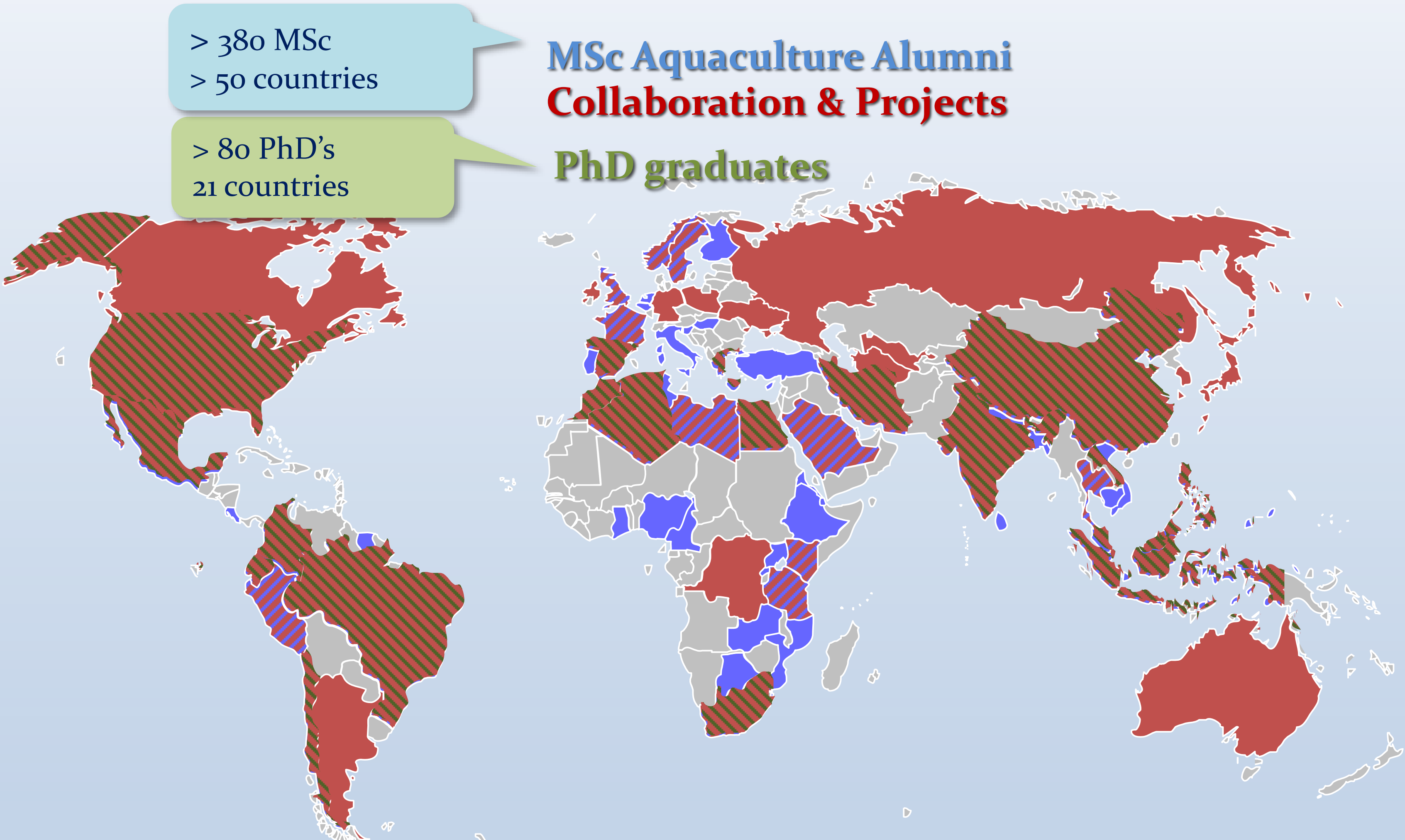
This project groups a consortium of 15 universities and governmental research institutes from EU, Latin America, Africa and Asia, and is coordinated by the Laboratory of Aquaculture & Artemia Reference Center of the Ghent University, Belgium.

This global concerted Artemia-study consists of a programme of workshops and study visits, aiming at technical intercalibration, integration of current ecological and evolutionary concepts, and uniformous methods to assess population dynamics. Guidelines will be issued on sustainable exploitation and strain introduction, and these may be tools for authorities to protect biodiversity and to avert overexploitation and extinction of strains.

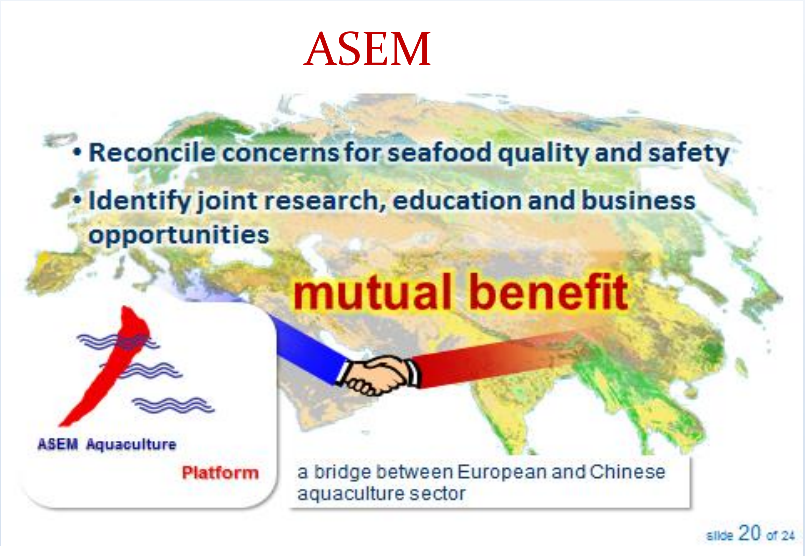
Project details

- Consortium members
- Workshops:
    1. Ghent global workshop, Belgium, February 5-7, 2002
    2. Beijing regional workshop, PR China, September 23-26, 2002
    3. Puerto Varas regional workshop, Chile, November 16-20, 2003
    4. Urmia regional workshop, Iran, September 21-25, 2004
- Action plans & Partnerships
- Reports
- ISA publications
- Cysts database
- Artemia sites database (access limited to project partners)
- Artemia Biodiversity: Protocols and Guidelines for Study and Sustainability (access limited to

Collaboration and networking…



Collaboration and networking…



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Artemia collaboration 3.0 the *Artemia* genome

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Collaboration and networking…

Aquatic veterinary medicine

Morphological development

Nutritional research

Life cycle analysis

Genomics

Environmental assessment

Microbial management

Complex and diverse challenges

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UGent Aquaculture

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- Biochemical and Microbial Technology
- Food Chemistry and Human Nutrition
- Applied Ecology and Environmental Biology
- Applied Analytical & Physical Chemistry
- Agriculture Economics
- Crop Protection
- Organic Chemistry

FACULTY OF VETERINARY MEDICINE

- Pathology, Bacteriology and Poultry Diseases
- Virology, Parasitology & Immunology
- Morphology

3 Faculties
12 professors
10 research groups
100 researchers

FACULTY OF SCIENCES

- Biochemistry, Physiology and Microbiology
- Marine Biology
- Evolutionary Morphology of Vertebrates
- Vertebrate Morphology & Developmental Biology
- Protistology & Aquatic Ecology
- Molecular Genetics

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- identifying needs and knowledge gaps
- flexibility and open-mindedness
- interdisciplinarity and team spirit

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Use of molecular tools in the study of *Artemia* biodiversity

Gonzalo Gajardo
Laboratory of Genetics, Aquaculture and Biodiversity,
University Los Lagos, Osorno, Chile

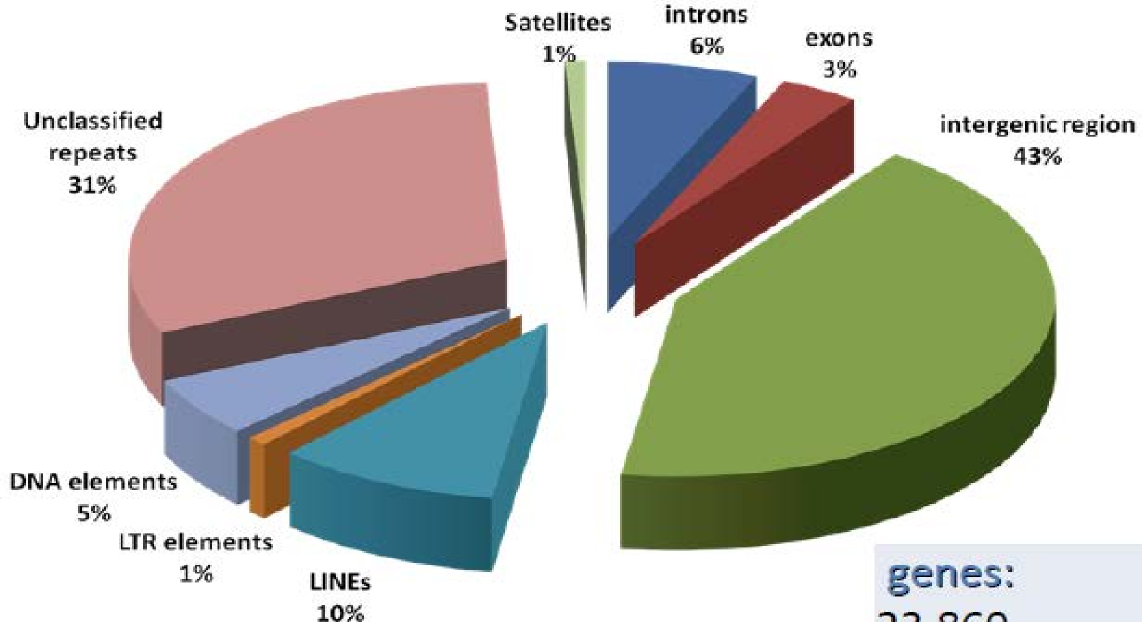
Content

- To highlight the problems/questions molecular tools have helped to clarify regarding *Artemia* biodiversity, roughly
- species number, and differences between them. What makes *A. sinica* different from *A. tibetiana*?
- To review data on biodiversity at the molecular level and share this information to stakeholders.
- To discuss how *Artemia* biodiversity (genetic diversity) can be wisely (or sustainably) used.
- How to comply with international agreements to protect (*Artemia*) biodiversity (CBD), and hypersaline environments (FAO ecosystem-approach).
- To identify/discuss threats to *Artemia* biodiversity, i.e., the ability of species or population to persist?

Available molecular markers covering different genome sectors

Annotation

Components of the *Artemia* genome



genes:
23,860
av. length.genes:
1,171 bp

De Vos et al. 2011
Artemia genome meeting, Ghent

Molecular markers and *Artemia* biodiversity: policy framework (Convention on Biological Diversity, CBD)

Strategic Plan for Biodiversity 2011–2020 and the Aichi Targets

"Living in Harmony with Nature"

General Assembly declared 2011-2020 as the United Nations Decade on Biodiversity

- Biological diversity underpins ecosystem functioning, "health = resilience"
- Provides ecosystem services essential for human well-being.
- provides food security
- contributes to local livelihoods, economic development, and poverty reduction.
- Since biodiversity continues to be lost, countries and stakeholders should (vision): **value, conserve, restore and wisely use biodiversity**

Strategic Goal C: *Improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity*

13 By 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity.

Artemia biodiversity: different levels of analysis

Level	Importance of variability	Importance of quantity	Importance of distribution
Genes	Ultimate source of variability for evolution and adaptive change.	Influences evolution, affecting how new variants establish and spread through populations.	Different environments allow the evolution of local adaptation, resistance and resilience.
Species	Irreplaceable, unique units with combinations of traits from long and independent evolution. Intrinsic value.	Provisioning and regulating services may depend on quantity; e.g. food, fresh water. Long-term viability.	Local provisioning and regulating services; e.g. structural roles, pollinators. Community and ecosystem stability arises through the co-occurrence of species.
Populations	Local populations retain local adaptations.		

Source: Mace (2005)

Molecular tools allow hypotheses testing, or to solve practical problems

- Hypothesis: how many species are out there? *Artemia* has few regionally endemic species, but does aquaculture use of *Artemia* affect this pattern?
- Do species maintain their long history of allopatric divergence (geographic separation) in a context of species translocation for aquaculture purposes?
- How to preserve/conserve species or locally adapted populations?
- How genetic diversity can be wisely and sustainably used for aquaculture?

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Massive increase in molecular tolos since 2002

2002

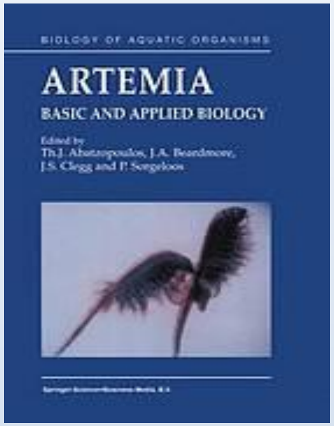

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Table 2. Genetic markers used for species identification and/or characterisation in *Artemia*

1. **Allozymes**	A protein solution is electrophoresed through a gel matrix. An enzyme-specific reaction reveals allozymes coding-loci and alleles (migrate according to charge) at a given locus.
2. **RFLPs** (Restriction Fragment Length Polymorphisms)	DNA is cut with restriction enzymes, electrophoresed (agarose gels), blotted to membranes, and probed with cloned radiolabelled DNA that binds to a single locus. Alleles differing in the presence or absence of nearby restriction sites will produce different fragment sizes.
3. **AFLPs** (Amplified Fragment Length Polymorphisms)	The methods are similar to RFLPs but more time-consuming and expensive. Detecting a higher number of loci and polymorphism is significantly higher.
4. **RAPDs** (Randomly Amplified Polymorphic DNA)	An arbitrary oligonucleotide of about 10 bases used in a PCR reaction will usually anneal well enough to serve as both forward and reverse primer at 3–10 sites. The products are electrophoresed through agarose and stained. Bands present in one individual may not be present in another for a variety of reasons, chiefly variation in the primer annealing sites. RAPD is a rapid, precise and sensitive method of detection of nucleotide variation. Good for taxonomic investigations (populations, species, genera).

- RAPD: Randomly Mmplified Polymorphic DNA
- RFLP: Restriction Fragment Length Polymorphism
- AFLP. Amplified Fragment Length Polymorphism
- Partial sequencing of nuclear and mit DNA markers/genes
- Total mit DNA: 15,822 bp mitochondrial genome sequence [Valverde et al. 1994; Stillman et al. 2008
- *Alu*I sequences
- Single nucleotide polymorphism (SNPS)
- Transcriptomics, allow to identify key genes involved in key *Artemia* functions (adaptation and speciation; cyst production, etc)
- Sequencing of the *Artemia* genome: to be revealed.

Basic knowledge provided by molecular tools

Species differentiation: how many species are out there?

Calibration of divergence time (molecular clock)

- *A. persimilis* (Chile and Argentina): diverged from ancestral species some 80 Million Years Ago (MYA)
- *A. salina* (Mediterranean area): ≈*40 MYA*
- *A. franciscana* (North, Central and South America): ≈ *32 MYA*
- *A. sinica* (China): ≈ *19.9 MYA*
- *A. urmiana* (Lake urmia), *A. tibetiana*, and EHC shared a common ancestor in the late Pliocene (*5.41 MYA*).
- The diversification within *A. urmiana* and EHC lineages occurred in the Pleistocene (*1.72 MYA*) and Holocene (*0.84 MYA*), respectively.

Data from: Kappa *et al.* (2006); Eimanifar *et al.* (2015)

Basic knowledge provided by molecular tools

RAPD (dominant): Randomly amplified polymorphic DNA. (the very first marker used)

Phylogenetic relationships, 14 populations from the Americas, Mediterranean and China. 86 RAPD markers produce 4 clusters: —the American *A. franciscana* and *A. persimilis*, the Mediterranean *A. salina*, and the species from China. (Badaracco *et al.*, 1995).

- mitochondrial cytochrome c oxidase subunit I (COI)
- nuclear internal transcribed spacer1 (ITS1)

Molecular Phylogenetics and Evolution 79 (2014) 392–403
Contents lists available at ScienceDirect
Molecular Phylogenetics and Evolution
journal homepage: www.elsevier.com/locate/ympev
ELSEVIER

Artemia biodiversity in Asia with the focus on the phylogeography of the introduced American species *Artemia franciscana* Kellogg, 1906

Amin Eimanifar[a,*], Gilbert Van Stappen[b], Brad Marden[c], Michael Wink[a]

COI: Asia harbors a diverse group of sexual and asexual *Artemia* species, including the invasive *Artemia franciscana*

A haplotype complex of parthenogenetic lineages (39 inland localities.)

A. franciscana (n 31 geographical localities, southern and eastern coastal regions of Asia)

Three sexual species (*A. sinica*, *A. tibetiana* and *A. urmiana*) have a restricted distribution

ITS: inconsistencies with the COI tree:

Asian *A. franciscana* showed higher haplotype diversity as compared to the source population from the Great Salt Lake (USA)????,: multiple introductions by mass dispersal in Asia via human activities could explain this.

The invasive success of *A. franciscana* in Asia could lead to a long-term biodiversity disturbance of the autochthonous *Artemia* species on the continent

Authentification of commercial samples. RFLP pattern of mitochondrial rDNA fragment (1500 bp)

An RFLP database for authentication of commercial cyst samples of the brine shrimp *Artemia* spp. (International Study on *Artemia* LXX)

Peter Bossier[a,b,*], Wang Xiaomei[a,b], Francesco Catania[a,b], Stefania Dooms [a,b], Gilbert Van Stappen[b], Eddy Naessens[c], Patrick Sorgeloos[b]

[a] CLO Sea Fisheries Department, Ankerstraat 1, 8400 Oostende, Belgium
[b] Laboratory of Aquaculture & Artemia Reference Center, Faculty Agricultural and Applied Biological Sciences, Ghent University, Rozier 44, 9000, Ghent, Belgium
[c] INVE Technologies, Baasrode, Belgium

Received 19 February 2003; received in revised form 5 November 2003; accepted 6 November 2003

182 RFLP polymorphic band classes were scored.
"The developed method allows to assign samples to these clusters, facilitating their authentication at the species level".

53 samples (world distribution) clustered in 5 groups (5 sexual species)

- *A. parthenogenetic* group
- *A. franciscana* group
- *A. persimilis* group
- *A. sinica* group
- *A. salina* group

These groups coincide to a great extent with the currently accepted species or species complexes. Within each cluster diversity between samples is still considerable, reflecting the genetic diversity within each species.

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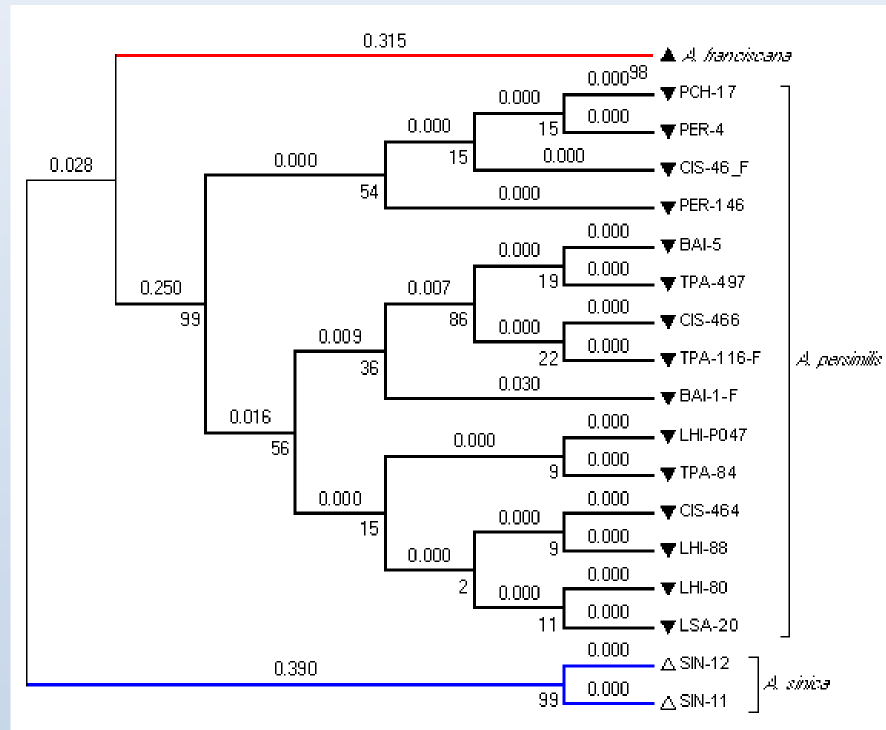
AluI sequence separates *A. franciscana* and *A. persimilis*: the new world species (Chile study)

- *Alu* elements are short (~300 bp) and mobile sequence repeats interspersed in eukaryotic genomes.
- Can be recurrently amplified and retrotransposed impacting genome structure, function and evolution
- Reiteration frequency of approximately 6 x 10^5 copies/haploid genome (Badaracco et al. 1987).
- *Artemia* exhibits AluI sequences (110 bp sequence) which represent about 3-5% of the *A. franciscana* genome



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AFLP markers

OPEN ACCESS Freely available online — PLOS | ONE

A first AFLP-Based Genetic Linkage Map for Brine Shrimp *Artemia franciscana* and Its Application in Mapping the Sex Locus

Stephanie De Vos[1,2,3], Peter Bossier[1], Gilbert Van Stappen[1], Ilse Vercauteren[2,3], Patrick Sorgeloos[1], Marnik Vuylsteke[2,3]*

- Identification of sex-linked markers: 11 chromosomes,
- haploid genome size estimated: 0.93 Gb (flow cytometry)
- WZ–ZZ sex-determining system confirmed
- mapping genomic loci underlying phenotypic differences among *Artemia* species

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Transcriptome analysis

Marine Genomics 18 (2014) 151–154

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

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Genomics/Technical resources

Sex-dependent transcriptome analysis and single nucleotide polymorphism (SNP) discovery in the brine shrimp *Artemia franciscana*

Diego Valenzuela-Miranda [a], Cristian Gallardo-Escárate [a,*], Valentina Valenzuela-Muñoz [a], Rodolfo Farlora [a], Gonzalo Gajardo [b,*]

- 36 896 high quality contigs obtained, 13 749 sequences were annotated with arthropod sequences.
- Just 4.5% matched against previously reported sequences for *Artemia* spp.
- Evidence of sex-related transcriptional responses.
- Furthermore, 221 and 534 putative SNPs were identified exclusively in males and females, respectively.
- Important to track genes underlying critical traits.

To be discussed….

- Markers are important tools to assess variability across the *Artemia* genome
- Use of markers depend on the problem addressed, but to a great extent they have produced roughly similar results.
- The critical problem of *Artemia* is that few species regionally endemic exist and they represent a long history of independent evolution (need to be conserved,
- Genetic diversity is distributed in different local populations (“eggs put in different baskets”) that retain local adaptations.
- Aquaculture use of *Artemia* has allowed translocation of species and populations risking their genetic integrity.
- Need to discuss a sort of code of conduct to protect species, populations and their evolutionary potential, as well as hypersaline ecosystems, in line with international agreements (CBD, FAO).
- New advances will allow to establish which genes express differentially in locally adapted, or what genes make-up species difference .

Thank You

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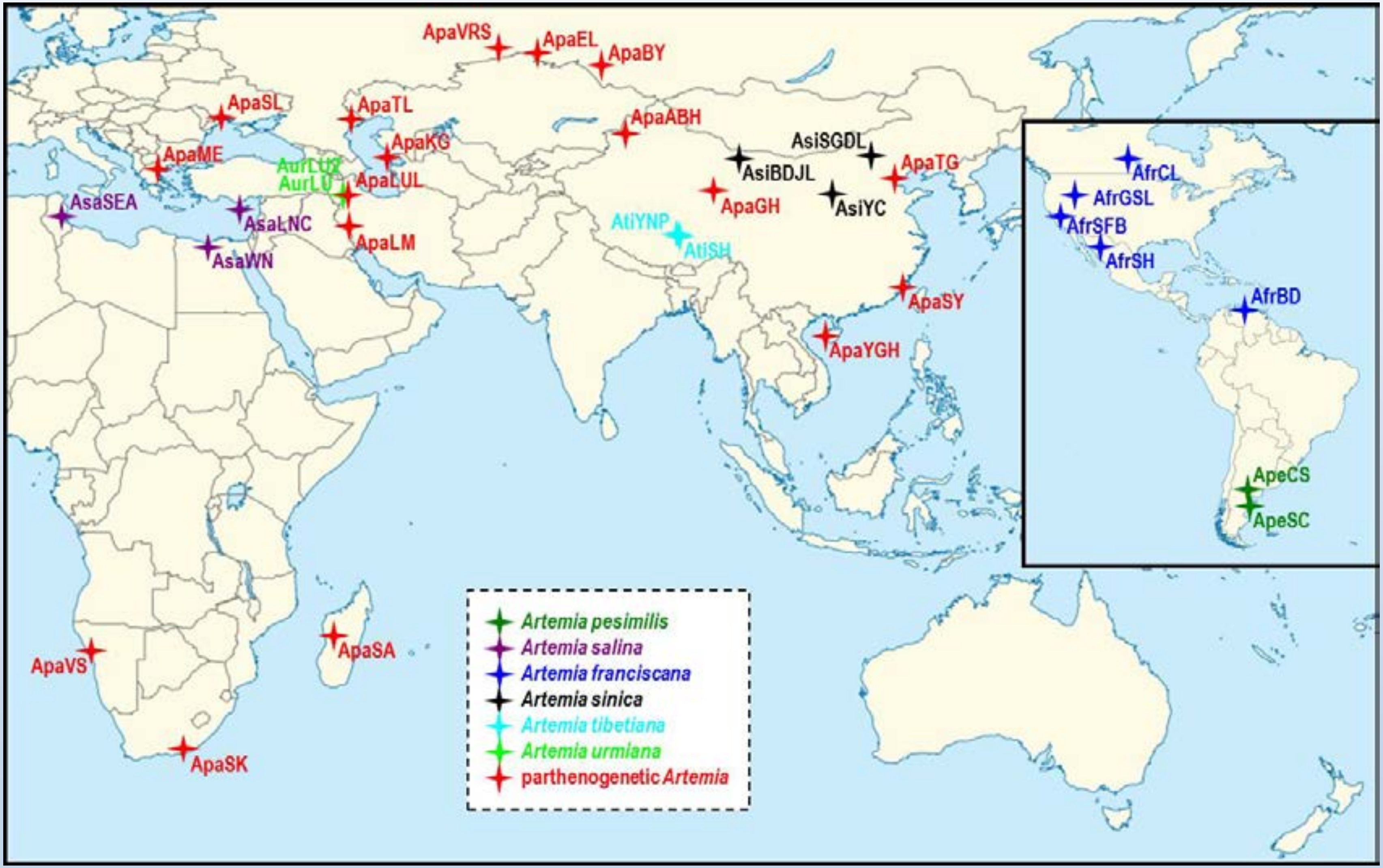
Biodiversity and biogeography of *Artemia* spp. from China and other places around the world

Jinshu Yang
Institute of Cell and Developmental Biology,
College of Life Sciences, Zhejiang University,
Hangzhou, Zhejiang, China

Geographic distributions of *Artemia* strains



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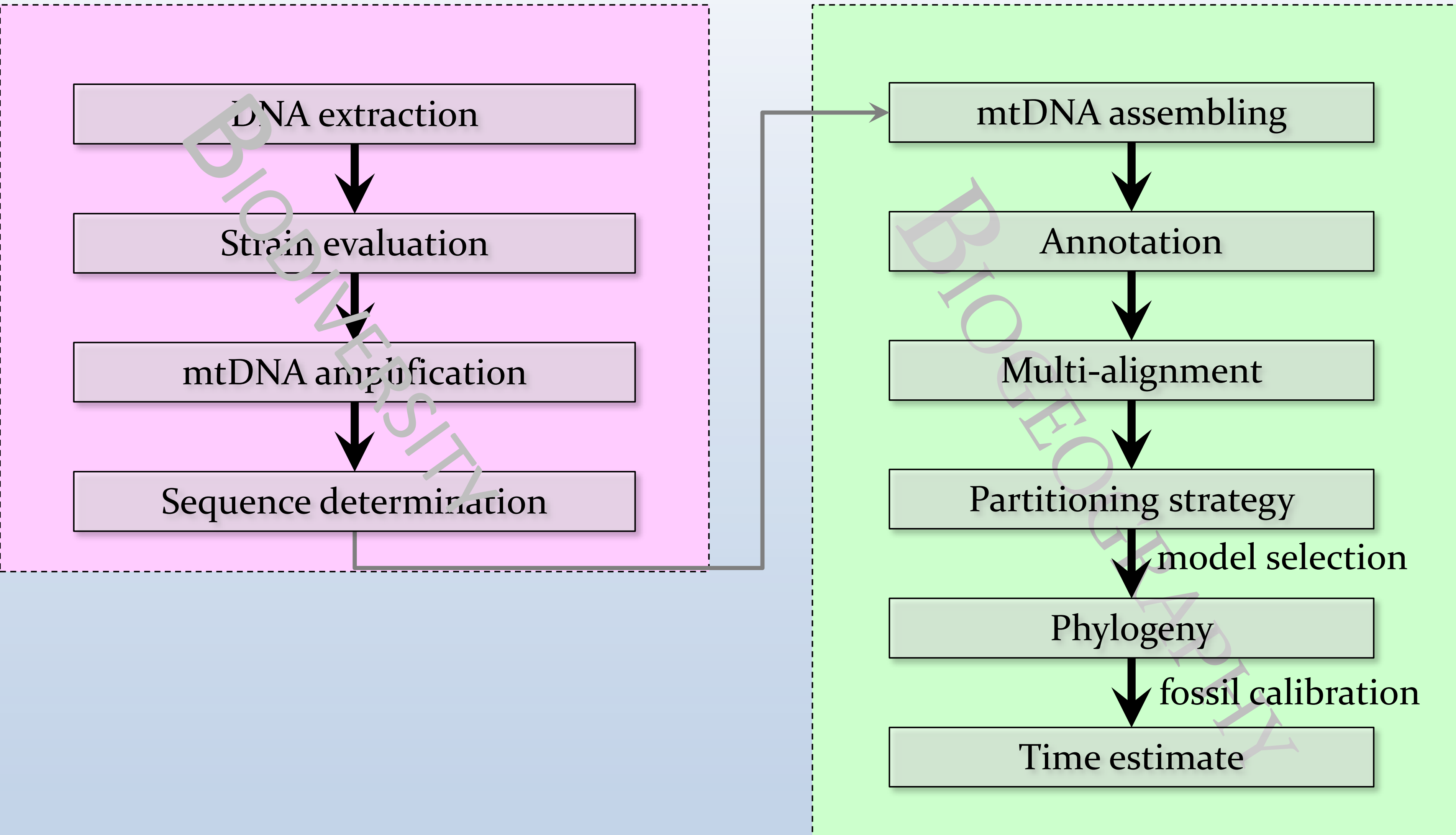
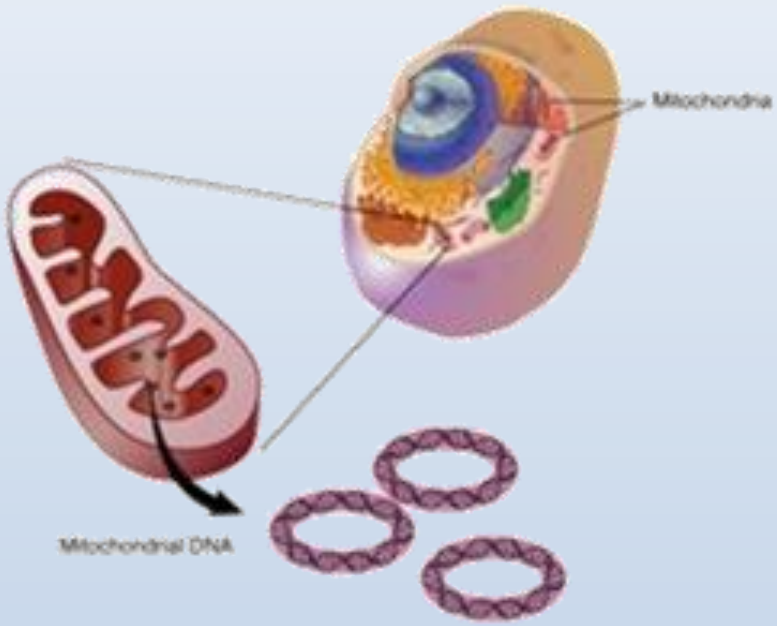
Summary of methodology

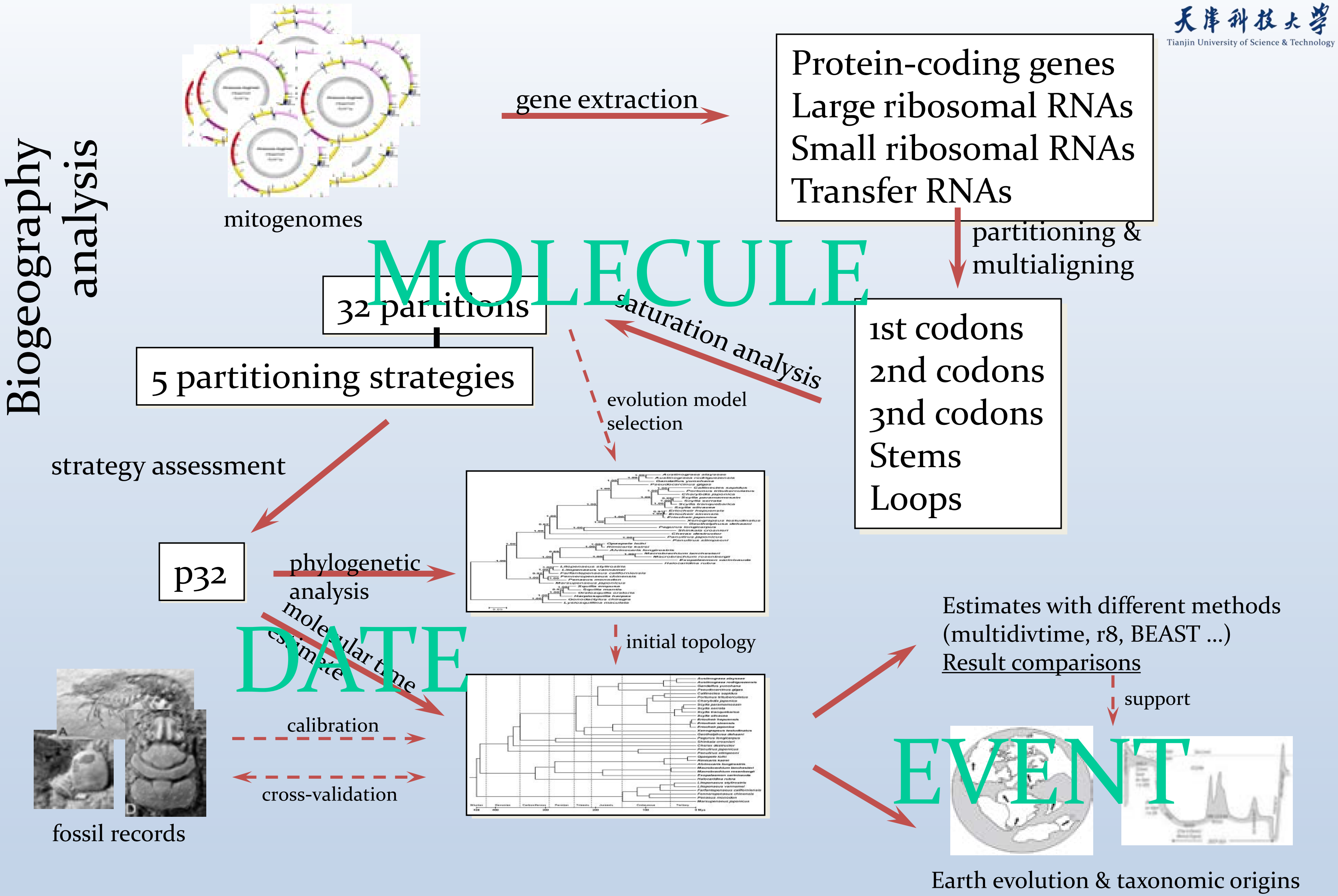


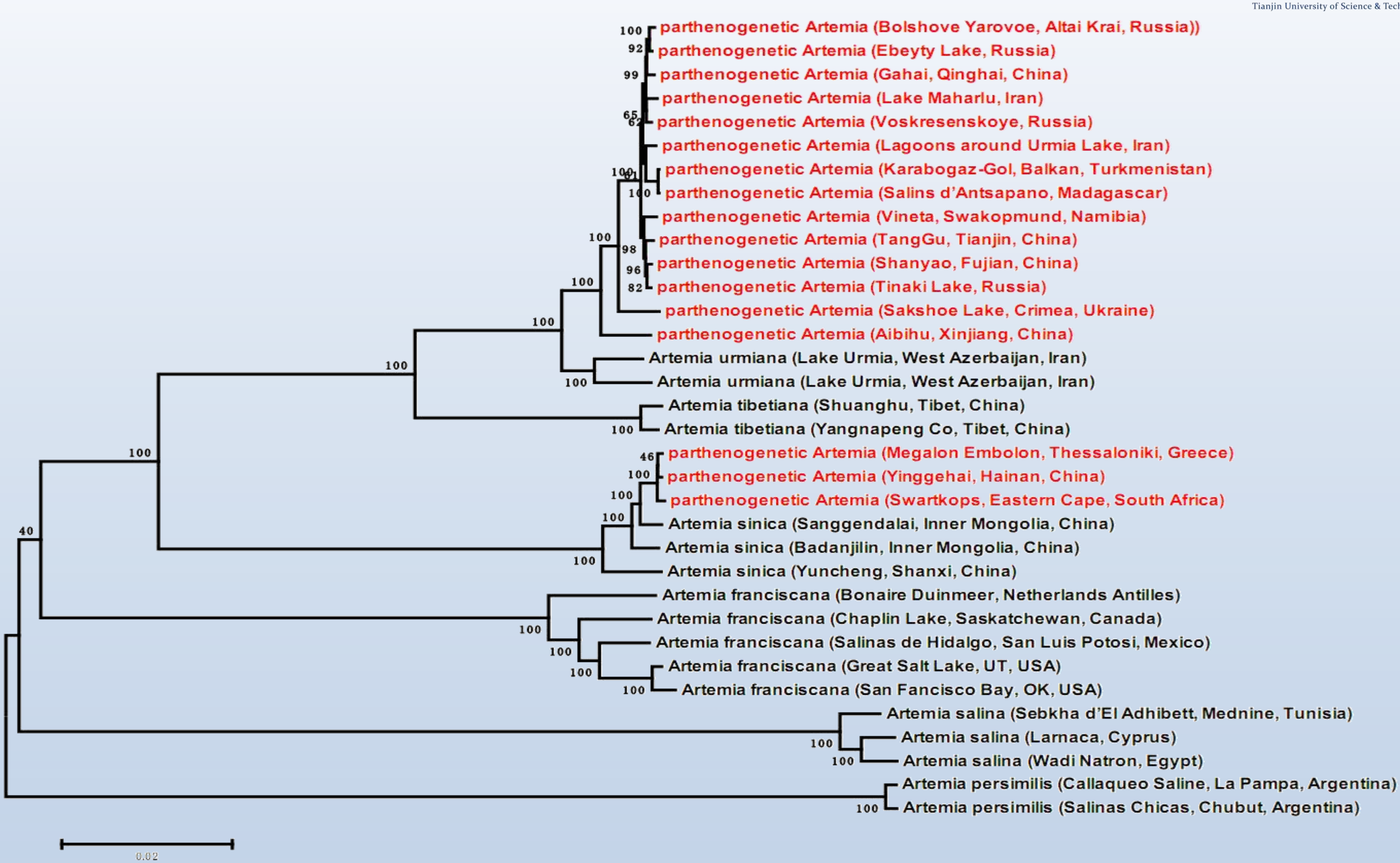
Mitochondrial genome (mtDNA)

- Chromosome-independent genome
- Self-replicable
- Small size (15-16 kb in metazoans)
- High copy number
- High evolutionary rate
- Strictly maternal inherence
- Lacking of recombination
- An ideal model for all levels of evolutionary analysis

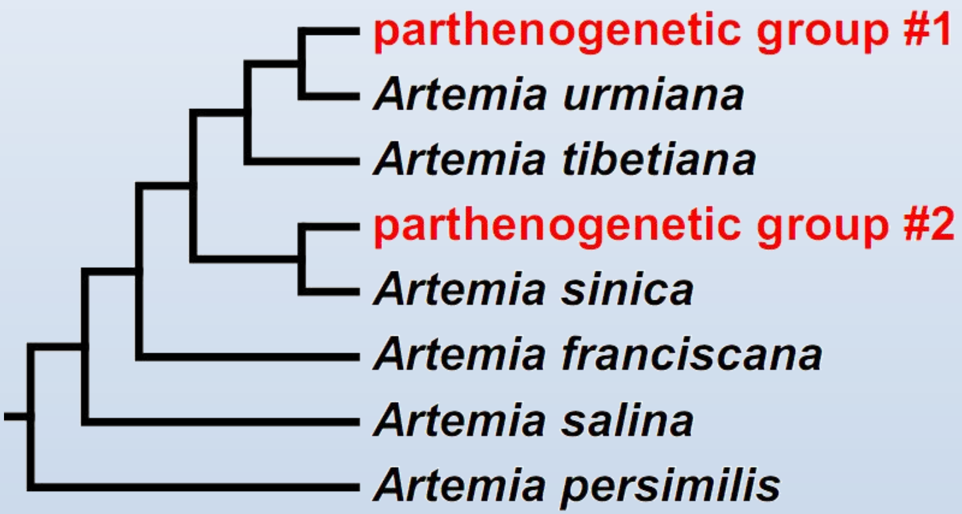


(http://www.genome.gov/glossary/?id=129)

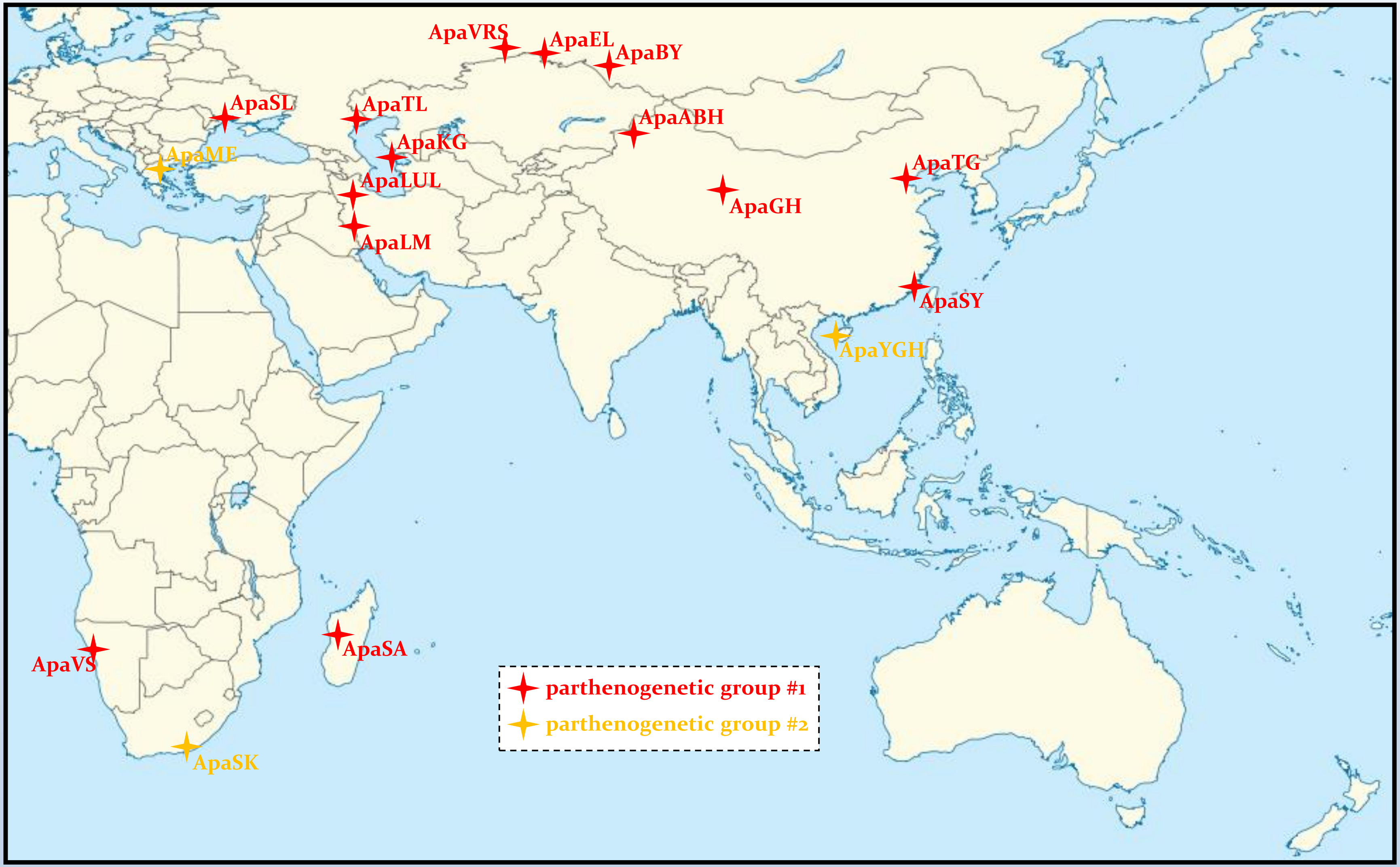

Biogeography analysis
mitogenomes
gene extraction
Protein-coding genes
Large ribosomal RNAs
Small ribosomal RNAs
Transfer RNAs
partitioning & multialigning
1st codons
2nd codons
3nd codons
Stems
Loops
MOLECULE
32 partitions
saturation analysis
5 partitioning strategies
evolution model selection
strategy assessment
p32
phylogenetic analysis
initial topology
DATE
molecular time estimate
calibration
cross-validation
fossil records
Estimates with different methods (multidivtime, r8, BEAST …)
Result comparisons
support
EVENT
Earth evolution & taxonomic origins


parthenogenetic Artemia (Bolshove Yarovoe, Altai Krai, Russia))
parthenogenetic Artemia (Ebeyty Lake, Russia)
parthenogenetic Artemia (Gahai, Qinghai, China)
parthenogenetic Artemia (Lake Maharlu, Iran)
parthenogenetic Artemia (Voskresenskoye, Russia)
parthenogenetic Artemia (Lagoons around Urmia Lake, Iran)
parthenogenetic Artemia (Karabogaz-Gol, Balkan, Turkmenistan)
parthenogenetic Artemia (Salins d'Antsapano, Madagascar)
parthenogenetic Artemia (Vineta, Swakopmund, Namibia)
parthenogenetic Artemia (TangGu, Tianjin, China)
parthenogenetic Artemia (Shanyao, Fujian, China)
parthenogenetic Artemia (Tinaki Lake, Russia)
parthenogenetic Artemia (Sakshoe Lake, Crimea, Ukraine)
parthenogenetic Artemia (Aibihu, Xinjiang, China)
Artemia urmiana (Lake Urmia, West Azerbaijan, Iran)
Artemia urmiana (Lake Urmia, West Azerbaijan, Iran)
Artemia tibetiana (Shuanghu, Tibet, China)
Artemia tibetiana (Yangnapeng Co, Tibet, China)
parthenogenetic Artemia (Megalon Embolon, Thessaloniki, Greece)
parthenogenetic Artemia (Yinggehai, Hainan, China)
parthenogenetic Artemia (Swartkops, Eastern Cape, South Africa)
Artemia sinica (Sanggendalai, Inner Mongolia, China)
Artemia sinica (Badanjilin, Inner Mongolia, China)
Artemia sinica (Yuncheng, Shanxi, China)
Artemia franciscana (Bonaire Duinmeer, Netherlands Antilles)
Artemia franciscana (Chaplin Lake, Saskatchewan, Canada)
Artemia franciscana (Salinas de Hidalgo, San Luis Potosi, Mexico)
Artemia franciscana (Great Salt Lake, UT, USA)
Artemia franciscana (San Fancisco Bay, OK, USA)
Artemia salina (Sebkha d'El Adhibett, Mednine, Tunisia)
Artemia salina (Larnaca, Cyprus)
Artemia salina (Wadi Natron, Egypt)
Artemia persimilis (Callaqueo Saline, La Pampa, Argentina)
Artemia persimilis (Salinas Chicas, Chubut, Argentina)
0.02

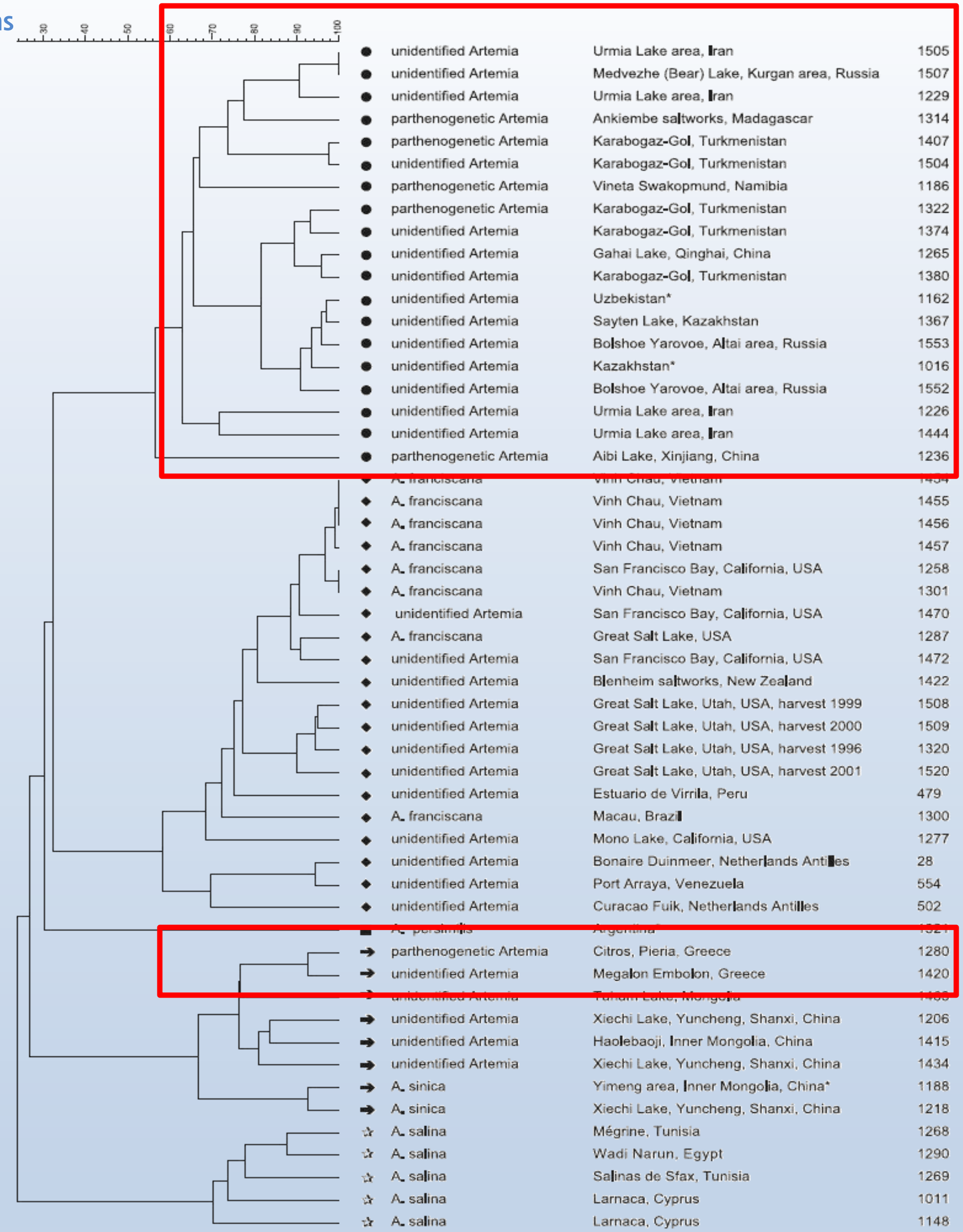
Topology of *Artemia*-tree



Two parthenogenetic groups



Evidence from RFLP

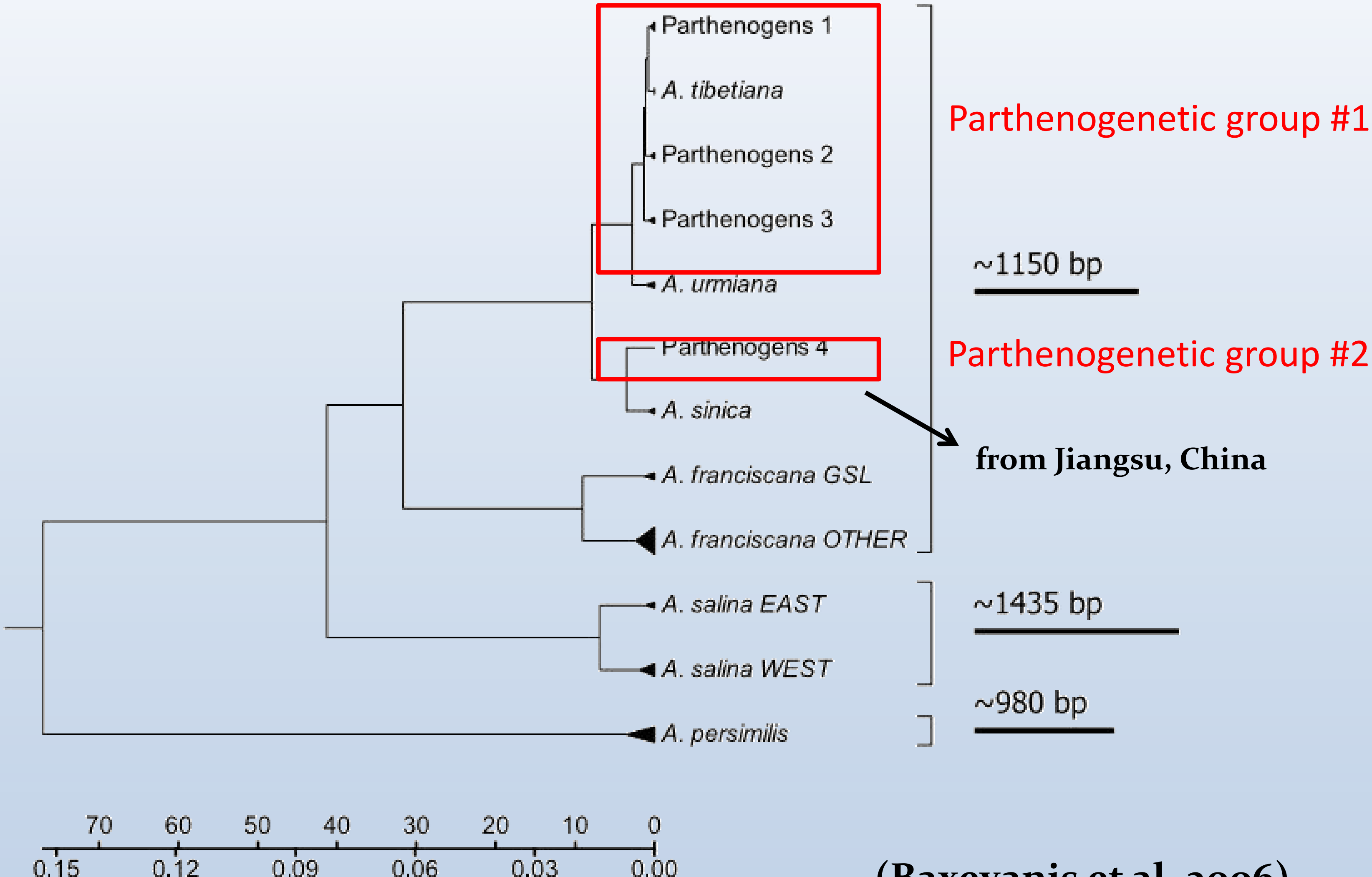


Parthenogenetic group #1

Parthenogenetic group #2

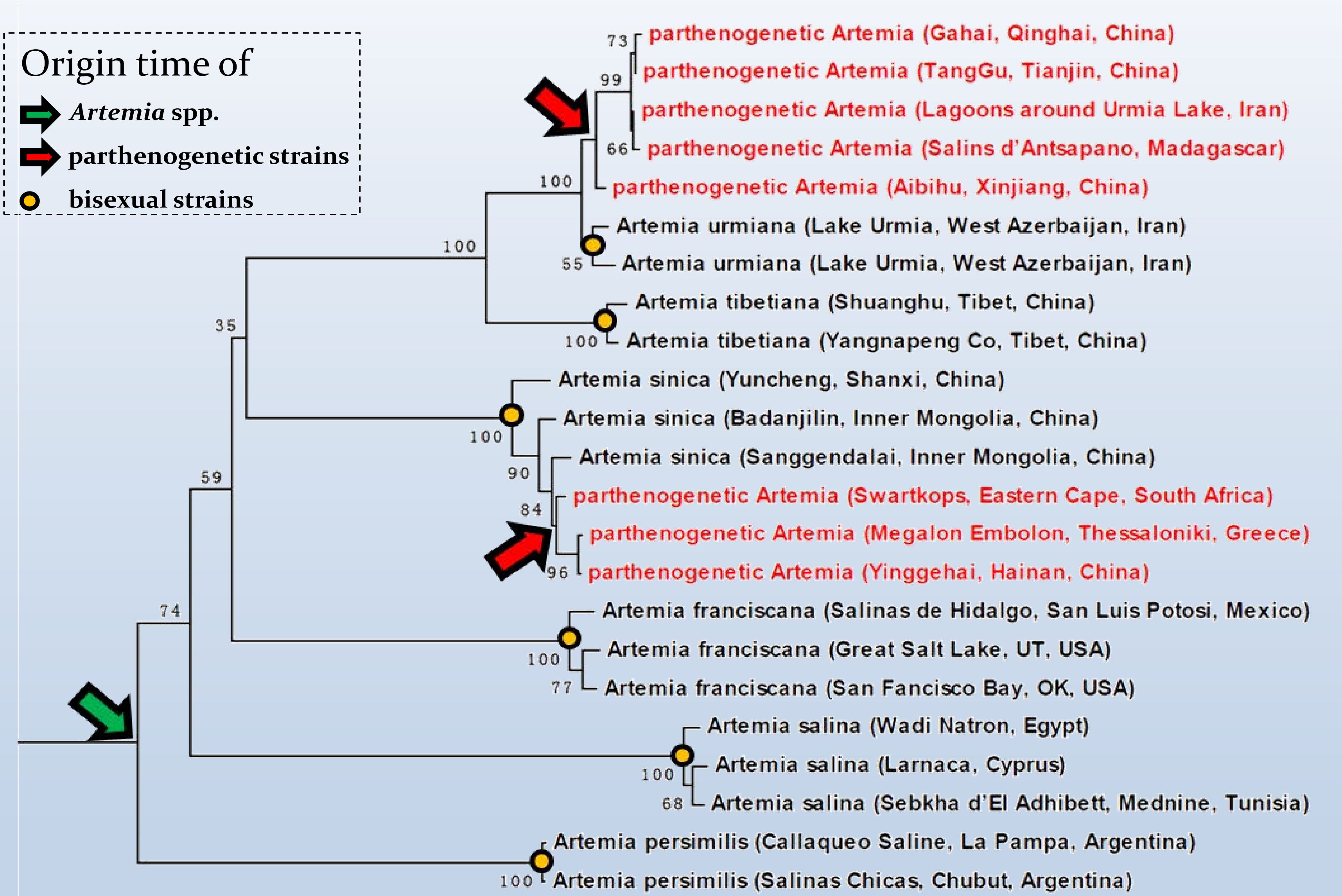
(Bossier et al. 2004)

Evidence from ncDNA

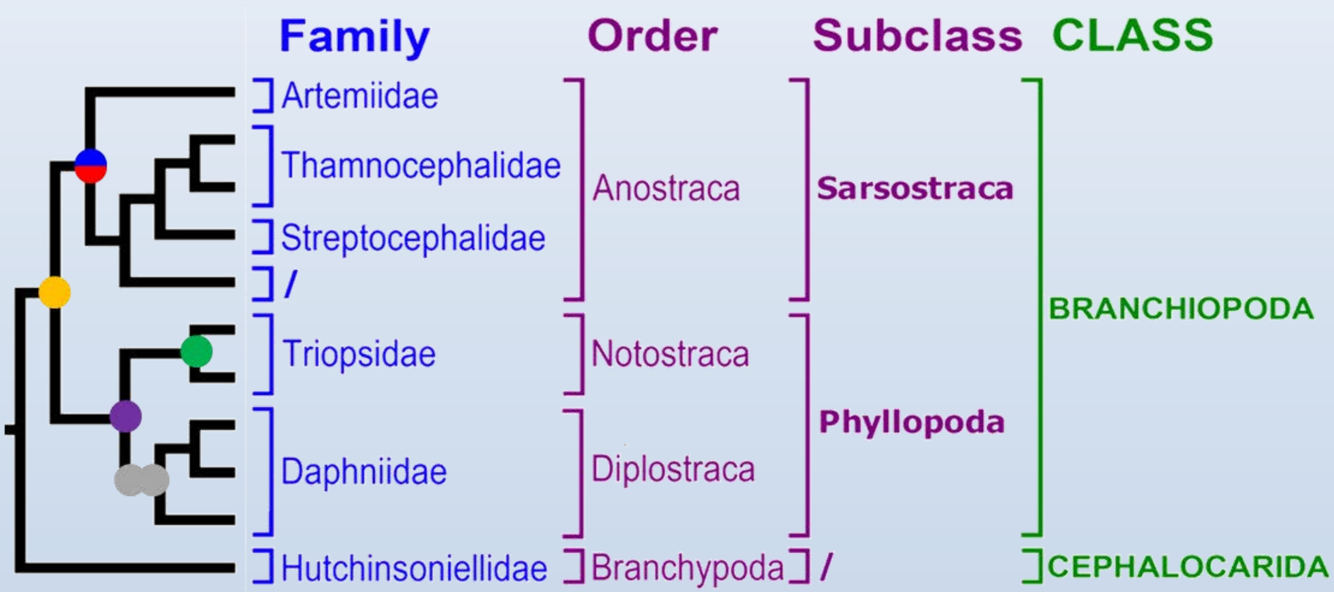


(Baxevanis et al. 2006)

Frame-tree for time estimation



Taxonomy tree and fossil calibrations



Summary

- Biodiversity of over 40 *Artemia* spp. from China and other places in the world have been evaluated.
- Complete mtDNAs of 34 *Artemia* spp. have been determined.
- Two evolutionarily distinct parthenogenetic groups are consisted of different geographic origins.
- Time estimation (evolution vs. geology) of different lineages is being conducted.

Acknowledgement

And many others who provided samples…

Thank You

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Artemia resources in Iran, Azerbaijan and Turkmenistan

Naser Agh
Artemia and Aquaculture Research Institute, Urmia Lake Research Institute, Urmia University, Urmia, Iran

Artemia and its Strategic importance for development of Aquaculture

Strategic for

- Shrimp culture
- Sturgeon Fish culture
- Marine Fish culture
- Aquarium Fish culture

Artemia Cysts required in Fish and Shrimp Hatcheries in Iran

	Cysts used in 2015-2016 (tonnes)	Cyst requirements in 2016-2017 (tonnes)	Biomass requirements in 2016-2017 (tonnes)
Shrimp Hatcheries	13.3	15	
Sturgeon Hatcheries	1.3	0.5	
Aquarium Fish	7	7	
Marine Fish	0.5	3.6	
Total	22.1	26.1	60-80

Data from Iranian Fishery Organization

Artemia Resources in Iran

West and East Azerbaijan Provinces

	Name of the Biotope	Reproductive Mood	Geographic Coordination	Present status
1	Urmia Lake	Bisexual: (*A. urmiana*) Parthenogenetic	37°20'E-45°40N	Drying up Endangered
2	Lagoons: Zanbil	Parthenogenetic	37°20'E-45°40'N	Dried up
3	Lagoons: Rashakan	parthenogenetic	37°15'E-45°85'N	Dried up
4	Lagoons: Fesendooz	Parthenogenetic	37°15'E-45°85'N	Exist
5	Lagoons: Dashte Tabriz	Parthenogenetic	37°50'E-46°40'N	Dried up

Fars Province

	Name of the Biotope	Reproductive Mood	Geographic Coordination	Present Status
6	Maharlu Lake 275 Km2	Parthenogenetic Bisexual: (*A. franciscana*)	29°57'E-52°14'N	Dried up Endangered
7	Bakhtegan Lake 85000 ha	Parthenogenetic	29°40'E-53°50'N	Dried up Endangered
8	Tashk Lake 41000 ha	Parthenogenetic	29°60'E-53°50'N	Dried up Endangered

Golestan, Sistan and Baluchestan, Khorasan Provinces

	Name of the Biotope	Reproductive Mood	Geographic Coordination	Present Status
9	Incheh Lake	Parthenogenetic	37°25'E-54°41'N	Dried up Endangered
10	Shor Lake	Parthenogenetic	37°24'E-54°36'N	Dried up Endangered
11	Varmal wetland	Parthenogenetic	30°80'E-61°50'N	Dried up Endangered
12	Kale Shoor Gonabad	Parthenogenetic Bisexual: *A. franciscana*	35°10'E-57°50'N	Exists

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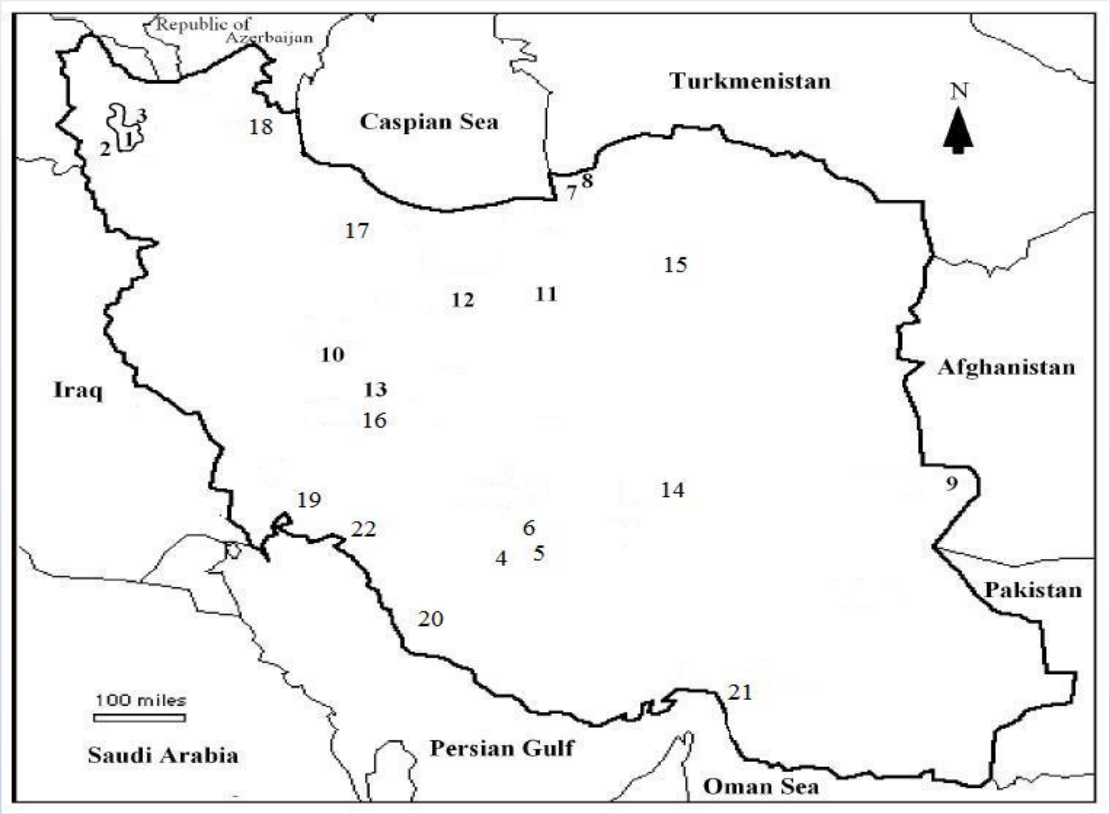
Qom, Markazi, Isfahan and Tehran Provinces

	Name of the Biotope	Reproductive Mood	Geographic Coordination	Present Status
13	Qom Lake	Parthenogenetic	34°40'E-51°80'N	Endangered
14	Hoze Soltan	Parthenogenetic	34°50'E-51°20'N	Exist
15	Mighan Lake	Parthenogenetic	34°20'E-49°80'N	Commercial production
16	Gaav Khooni wetland	Parthenogenetic	32°20'E-52°58'N	Endangered

Kerman, Khorram Abad and Ardabil Provinces

	Name of the Biotope	Reproductive Mood	Geographic Coordination	Present Status
17	Kale shoor Hashtgerd	Parthenogenetic	35°90'E-50°78'N	Endangered
19	Lake shoor Khorram Abad	Parthenogenetic	32°40'E-48°54'N	Endangered
20	Shorabil Lake	Parthenogenetic	38°25'E-48°55'N	Extinct

Distribution of *Artemia* resources in Iran

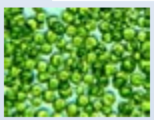


Lake Urmia, most important biotope of *Artemia* in Iran

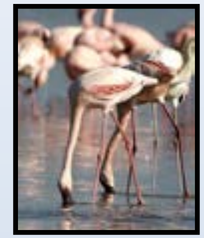
Artemia urmiana & *Parthenogenetic Artemia*

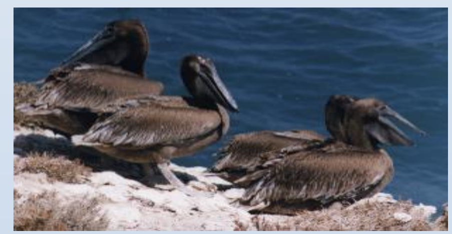




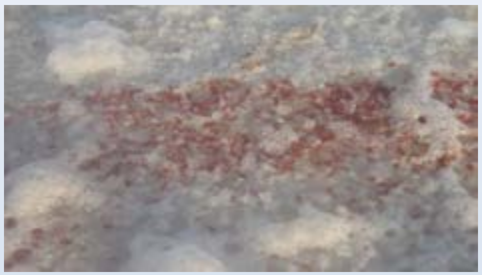







Surface Area: 5 500 km²
Annual production of *Artemia* until 1995:
300 000 tonnes of Biomass
30 000 tonnes of cyst
(Data from ARC)

Lake Urmia drying since 20 years


Death of *Artemia*


Formation of salt deserts and salt and sand storms


Situation in Sept. 2015 2% water volume 10% surface area


Death of birds


Death of mammals

Monthly field studies to determine the water depth and water quality of the Lake Urmia

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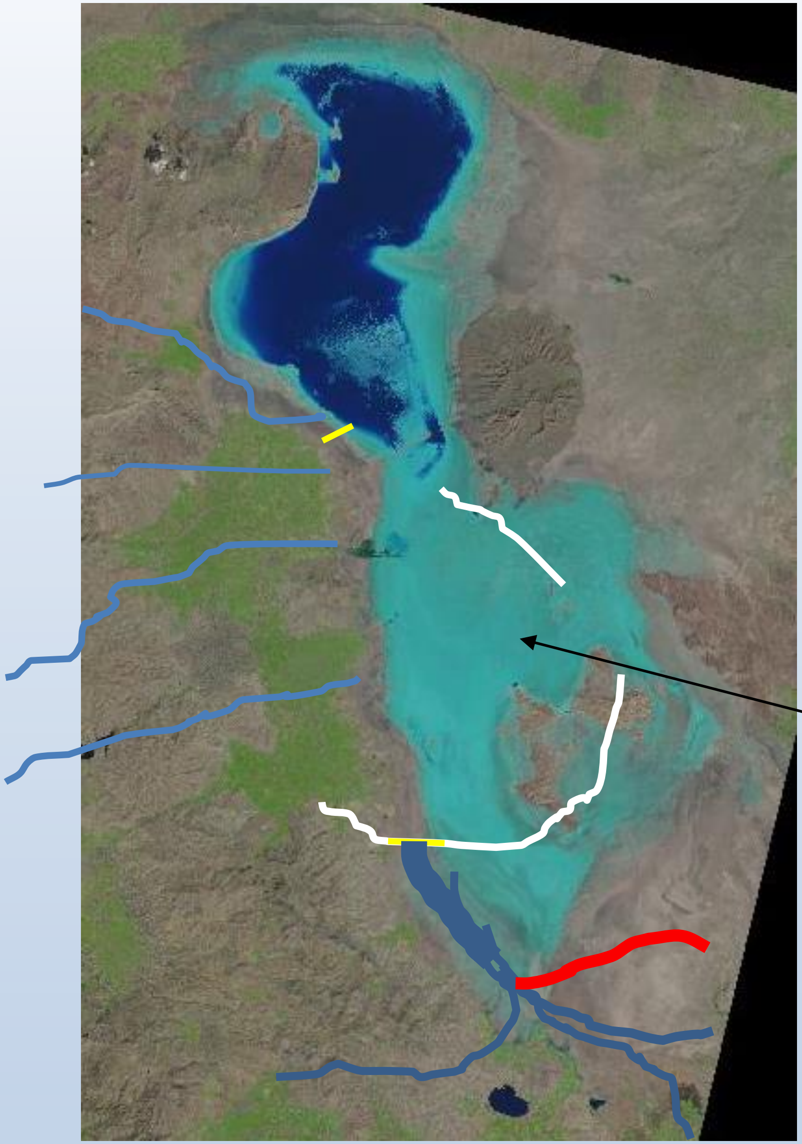

We propose phased restoration of the Lake Urmia

It is possible to save south part of the Lake within 2-3 years if phased management of the Lake is adopted

1600 Km² south wing with *Artemia* populations could be restored to normal ecosystem with annual production of above 1 000 tonnes of *Artemia* cysts

Water shortage = Drying of the lakes

Pond culture of *Artemia*
only alternative for disappearing natural biotopes of *Artemia*

Artificial pond culture projects

	Name of the city	Cultured species	Geographic Coordination	Present Status
1	Minab	Bisexual: (*A. franciscana*)	56°49'E-27°06'N	Pilot project
2	Bushehr	Bisexual: (*A. franciscana*)	53°30'E-26°56'N	Pilot project
3	Mahshahr	Bisexual: (*A. franciscana*)	49°06'E-30°31'N	Pilot project
4	Hendijan and Choeibdeh	Bisexual: (*A. franciscana*)	49°05'E-30°06'N 48°35'E-30°04'N	Pilot project
4	Nogh Rafsanjan	*A. franciscana*		In production
5	Fesenduz	Parthenogenetic		In production

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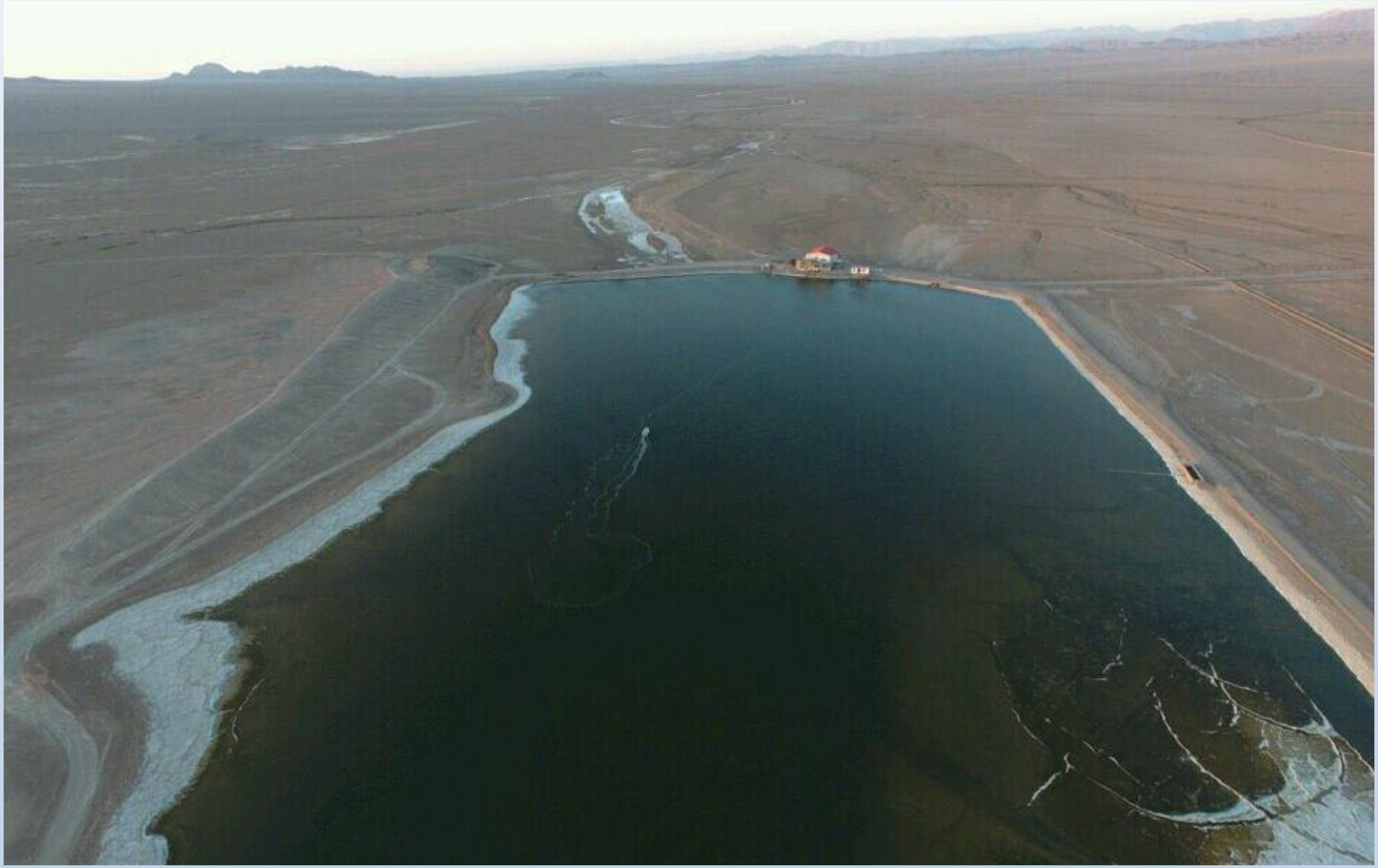
Kerman Province: Nogh Rafsanjan (50 hectare)



Current production: 1 tonne cyst and 5 tonnes biomass
Aim: 8 tonnes cyst and 50 tonnes biomass



West Azerbaijan: Fesenduz

Pilot project 13 hectare, whole project: 1 000 hectare

Aim: 100 tonnes cyst + 200 tonnes biomass

East Azerbaijan:
Ghobadlu and Rahmanlu sites 55 hectare
Aim: 8 tonnes cyst, 20 tonnes biomass

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Bushehr Province: Integrated culture of Algae, *Artemia* & Shrimp

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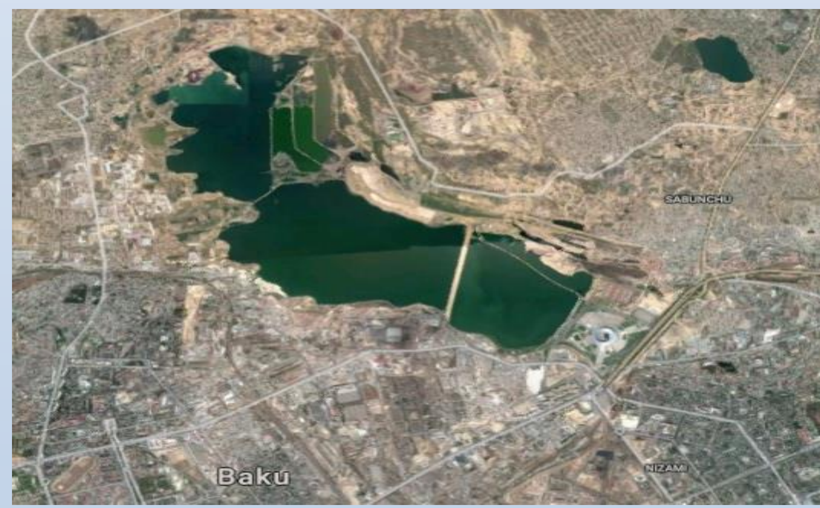
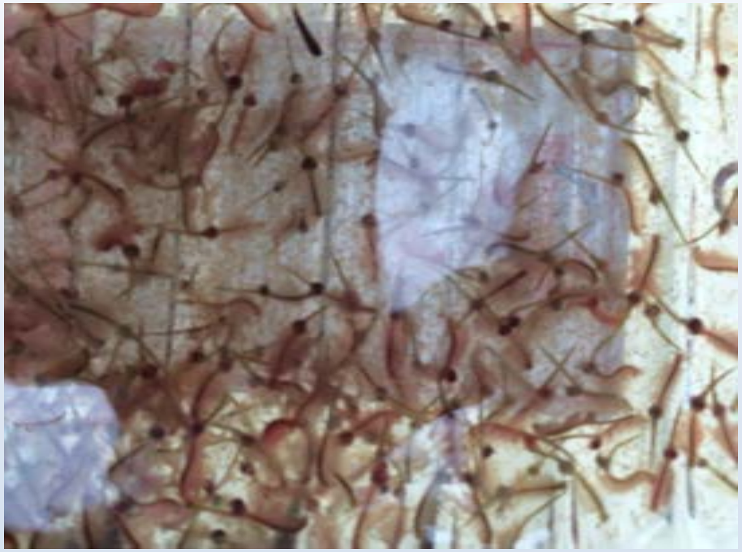
Azerbaijan Republic: many salt lakes and ponds.
Operating currently, 300 hectare, production 10 tonnes
Aim: 20 tonnes next year



Azerbaijan Republic



Turkmenistan Republic
Karabogaz Lake: 18 000 km²,
Parthenogenetic *Artemia*
Water level reduced, salinity over 300 ppt,
No *Artemia*

Crimea: Many salt lakes with *Artemia* populations

Looking forward to a sustainable exploitation from Crimean Salt Lakes

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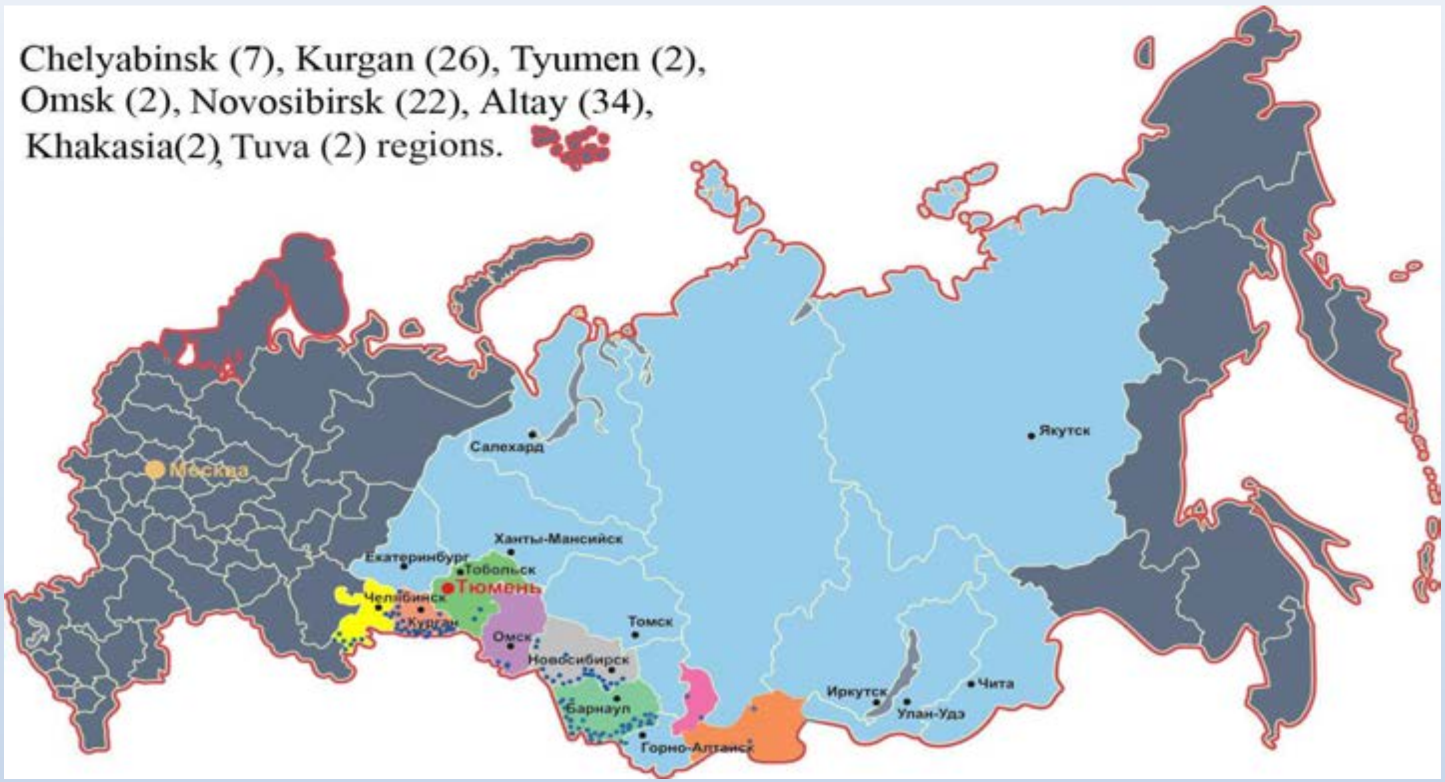
Artemia resources in Russia and Kazakhstan

Lyudmila Litvinenko
the State Scientific and Production Centre for Fisheries «Gosrybcenter», Northern Trans-Ural State Agricultural University, Tyumen, Russia

The territory of Russia under the jurisdiction of the FSBSI «Gosrybcenter»
Regions (number of *Artemia* lakes):



Our researches: about 70 salt lakes in the period 1995-2016

Annual monitoring was held in 5 lakes for 4 year (2000-2003).

Regular monitoring was conducted in 5 regions of the Ural & Siberia: Chelyabinsk, Kurgan, Tyumen, Omsk, Novosibirsk.

In Altai, Khakassia, Tuva only 1-3 expeditions were done in the period 2000-2004

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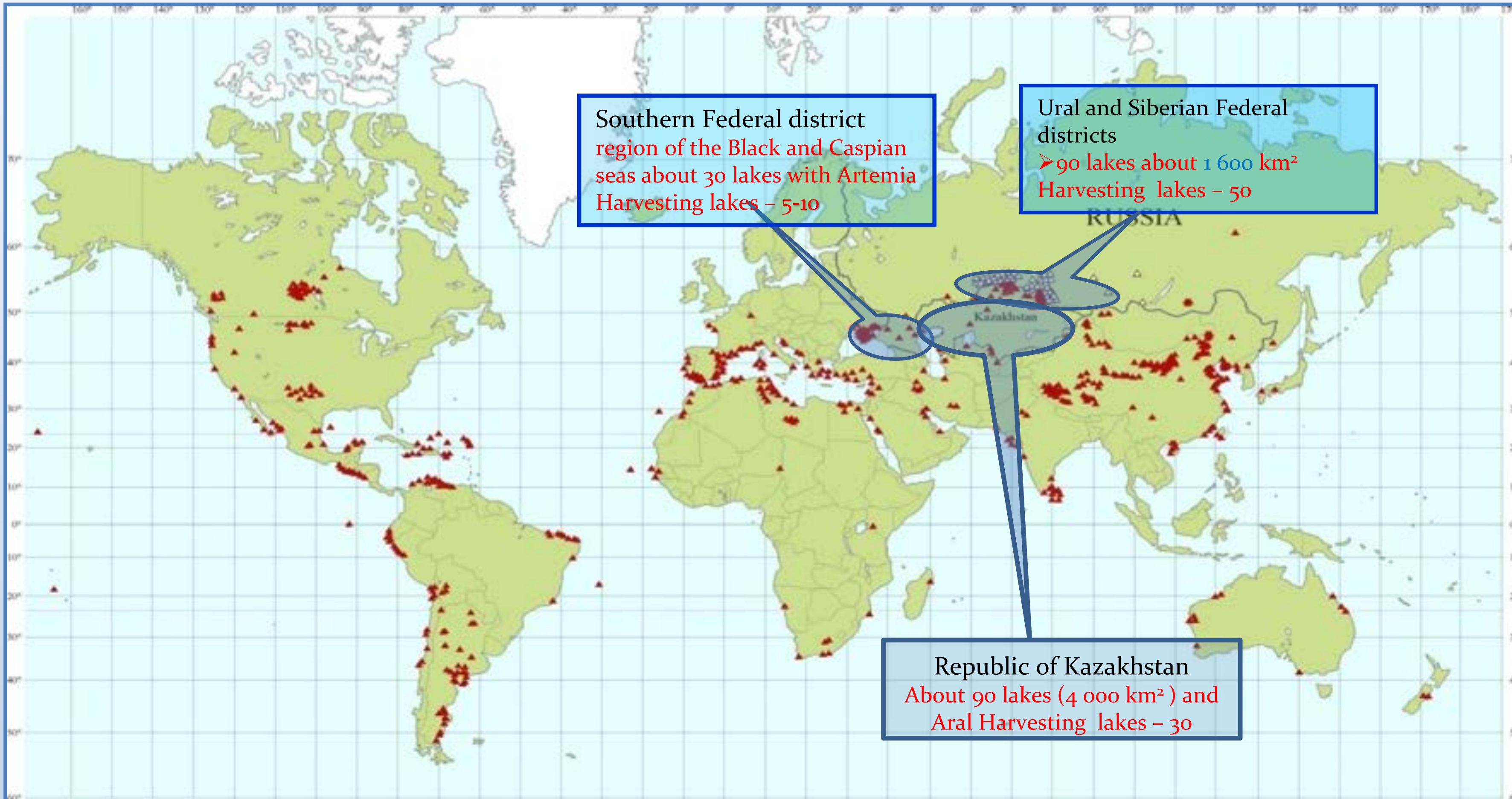
The world distribution of *Artemia*



△ - our data

Coordinates of habitats are according to Vanhaecke *et al.* (1987); Van Stappen (2002); Cohen (2012); Zheng and Sun (2013); Ubaskin and Volf (2005); Shadrin and Anufrieva (2012); Shadrin *et al.* (2012); Litvinenko *et al.* (2009).

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Species of *Artemia* living in the area of Russia & Kazakhstan

1 -Crimean lakes: Koyashskoe and Terekly-Konradskoe, pond near of Tobechik
2- Crimean lakes: Shtormovoye, Chersonessus, Sasik-Sivash
3 - Khakass lake: Svatikovo (Dus-Chol) (our data)

4 -After Pilla and Beardmore (1994). Samples from ARC Ghent (Catvis, Kazakhstan, C.I.S., 1988, A.R.C. № 1039)

5- Crimea: Popovskoe и Bolshoe Otar-Moinakskoe in 1967;
Altai: Solenoe in 1981-1985; Petuchovo in 1996-1997, Malinovie in 2003, Tanatar, Khakassia: Tus – in 2000, 2004 (our data)

6- The southern part of Russia from the Crimea to Sayan, including Kazakhstan (within 30°-90° E & 40°-55° Artemia parthenogenetica

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Differentiation of *Artemia* by morphometric characteristics of shrimps

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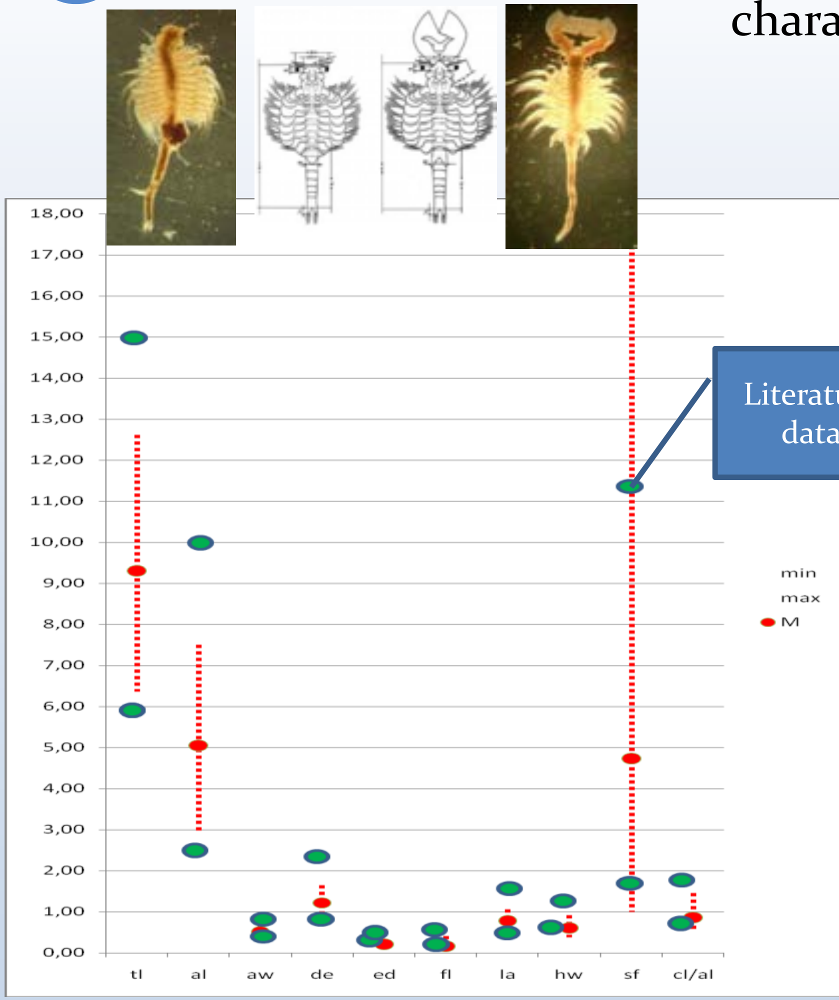
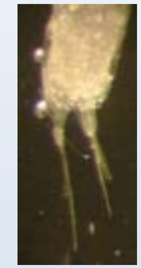


Literature data

salinity 198 g/l



salinity 113 g/l

Table-Morphometric parameters of shrimps (female)

Annually about 30 populations were surveyed (total sum was nearly 264).

		min	max	M	m	CV,%	min-max*
tl	mm	6.36	12.61	9.31	0.062	11	5.98-15.0
al	mm	2.93	7.51	5.05	0.046	14	2.49-10.0
aw	mm	0.23	0.87	0.51	0.007	23	0.25-0.84
de	mm	0.76	1.65	1.22	0.011	14	0.85-2.24
ed	mm	0.10	0.31	0.21	0.002	18	0.19-0.37
fl	mm	0.04	0.41	**0.16**	0.005	48	0.18-0.49
la,	mm	0.50	1.06	0.78	0.008	16	0.56-1.58
hw	mm	0.38	0.92	**0.60**	0.007	18	0.65-1.22
sf	setaes	1.00	17.53	4.72	0.202	68	1.47-11.2
cl/al	-	0.55	1.46	0.86	0.010	19	0.73-1.7
al/tl	-	0.41	0.65	0.54	0.003	8	0.38-0.67
tl/al	-	1.55	2.46	1.86	0.001	9	1.73-2.70

Note. * Literature data on different world populations: Gajardo *et al.* (1998); Triantaphyllidis *et al.* (1997); (1998); Abatzopoulos *et al.* (2009); Naceur (2011); Mejia et al. (2013)

Literature and our data on the morphometry of shrimps are shown in the table and figure. You can see that basically the average sizes of *Artemia* from Siberian populations beyond the boundaries known in the literature with the exception of furca length and width of the head. These morphometric characteristics are slightly less for shrimps of Siberian and Ural populations. Also the fluctuation in the number of setaes on furca in Sib. Populations is bigger.

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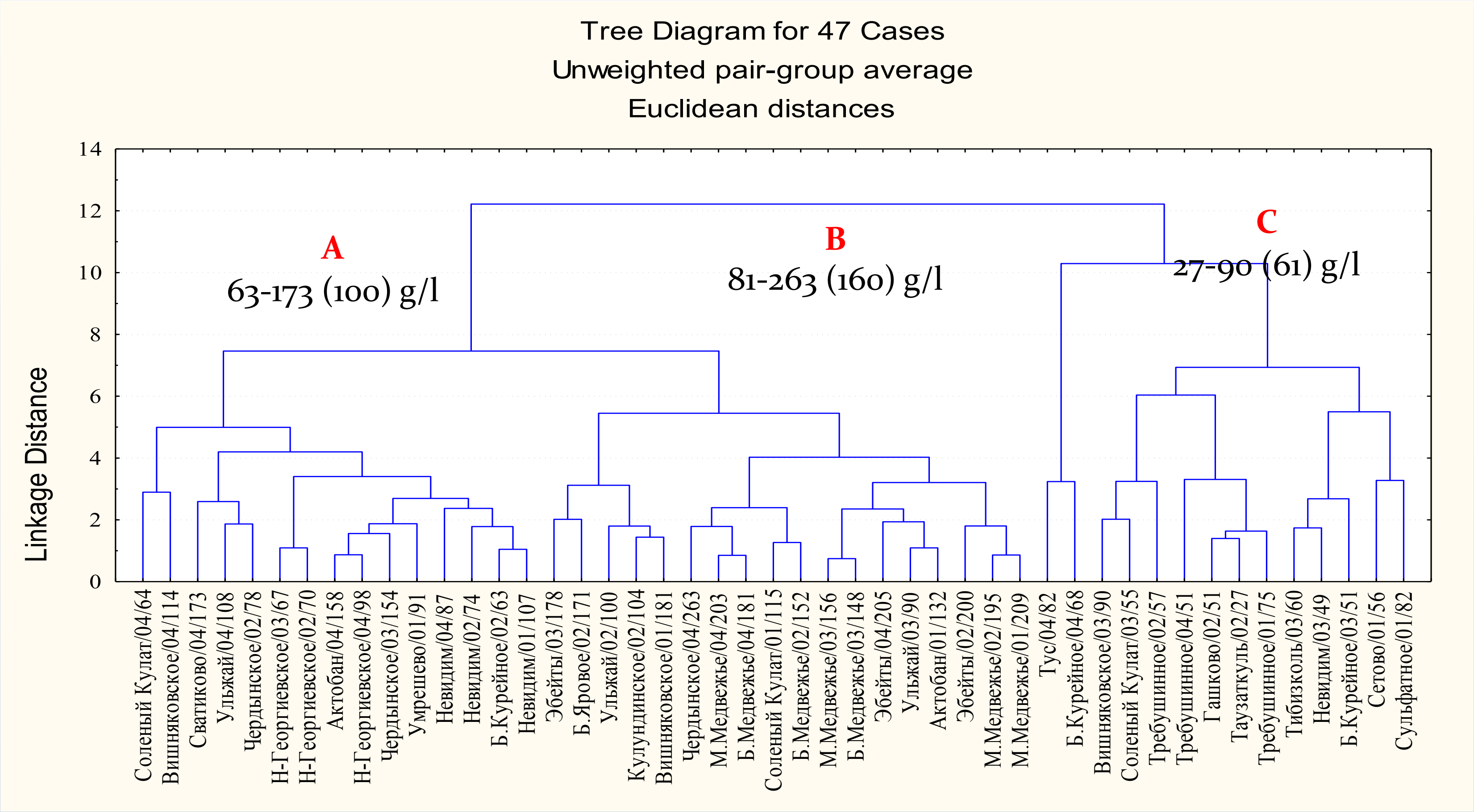
The dendrogram of similarity built on the basis of morphometric data for 12 parameters



--Cluster analysis of morphometric parameters of adult shrimps during fourth-term period of investigations showed the presence of three significantly different groups according to the salinity of lakes: high (cluster B), medium (cluster A) and low level of mineralization (cluster C).

--These data confirm the defining influence of salinity on the proportions of *Artemia* body.

--In addition, *Artemia* populations of the same lakes in different seasons (due to different salinity) belonged to different clusters.

--The division of clusters does not show the influence of geographical factor, although analyzed reservoirs are at considerable distance from each other (from the Ural to Tuva about 2 000 km).

Differentiation of *Artemia* by morphometric characteristics of cysts

Comparison of 12 populations in one year
(2002, about 200-400 cysts/lake, 30 nauplii/lake)

	Siberian population	Literature data*
The diameter of cysts, μm	240-289	220-330
The diameter of decapsulated cysts, μm	218-264	207-296
The thickness of the chorion, μm	3.9-12.5	2.7-15.6
Length of just hatched nauplii, μm	432-502	428-560

Note. *-Vanhaecke, Sorgeloos, 1980; Sorgeloos, Lavens, Leger et al., 1986;Соловов, Студеникина, 1990; Pilla, Beardmore, 1994; Amat et al., 2005; Abatzopoulos et al., 2006; Вольф, 2011; Ben Naceur H. et al., 2012, Shadrin et al., 2015

-Comparative analysis of cysts and nauplii from Siberian populations with the literature data presented in table 1 showed that the size of cysts and nauplii of Artemia from Siberian populations occupy an intermediate position.
-Comparison of average population values for years showed that (table 2) the fluctuation of the averages for the season diameter of cysts from Siberian populations ranges from 230 μm to 290 μm, in one lake the fluctuation ranges from 10 to 40 μm, average population over the years - from 240 to 278 μm.
- Frankly speaking, sizes of *Artemia* cysts in the most part of main lakes, especially such as Medvezhie and Ebeity, are the same. Therefore, identification of commercial harvests of cysts from different Siberian populations with use of only sizes of cysts in many cases is very difficult task.
-Thus, using literature data we can conclude that the Siberian populations of Artemia are well separated from the species *A. tibetiana* with rather large the cysts and nauplii (up to 330 and to 667, respectively) and to some extent from the species *A. salina, A. persimilis, A. franciscana* – with small cysts (average of 220 to 240).
-The mentioned data showed basically the impossibility of identifying *Artemia* species only with help of morphometric parameters. Therefore, for these purposes genetic methods necessary to attract.

Comparison of mid-season values of 24 populations for 9 years (1995-2004)

	M, μm	min-max	CD
B. Medvezhie	253±2	250-260	0.005
M. Medvezhie	253±2	250-260	0.005
Nevidim	261±4	240-270	0.011
Sulfatnoe	245±5	**230**-260	0.014
N- Georgievskoe	266±2	260-270	0.005
Cherdynskoe	258±2	250-260	0.005
Trebushinoe	253±3	240-260	0.008
Sobachie	265±2	260-270	0.007
B. Kureinoe	255±2	250-260	0.005
Gaskovo	244±3	**230**-250	0.009
Vishnyakovskoe	256±2	250-260	0.005
Actoban	**278**±6	250-**290**	0.019
Filatovo	255±4	240-270	0.013
Setovo	243±3	**230**-250	0.010
Borky	245±2	240-250	0.007
Umreshevo	**240**±5	**230**-250	0.014
Voskresenskoe	253±2	250-260	0.006
Lavrushino	245±2	240-250	0.007
Tauzatkul	250	250	
Salt Kulat	254±2	250-260	0.005
Salenoe 18	**240**	240	
Ulzhai	245±3	**230**-250	0.008
Ebeity	253±2	250-260	0.005
Tus	260	260	
Svatikovo	**240**	240	

Characteristics of *Artemia* biocenosis

Parameter	units	fluctuation limits	M±m	Cv,%	n
Abiotic factors					
Salinity	g/l	1.1-463.0	141.5±2.5	57	1137
pH		5.9-9.6	8.1±0.001	6	1023
Cl^-	g/l	0.4-186.1	65.2±1.1	56	1023
SO_4^{-2}		0.1-147	27.0±1.0	94	983
Cl^-/SO_4^{-2}		0.1-296	6.1±0.4	94	979
$N\text{-}(NO_3^-+NO_2^-)$	mg/l	0.0-2.2	0.1±0.001	305	202
PO_4^{3-}	mg/l	0.0-2.9	0.5±0.001	88	219
Fe_{total}	mg/l	0.0-2.5	0.2±0.001	217	38
T	°C	-15 - +35	18.0±0.3	53	864
O_2	mg/l	0.1-15.0	4.2±0.1	65	736
Biotic factors					
$B_{phitoplankton}$	mg/l	0.0-116.7	2.0±0.3	458	286
$B_{zooplankton}$	mg/l	0.0-2181.5	4.9±2.5	1665	784
$N_{видов\ фитопланктона}$		1.0-12.0	5.0±0.1	49	106
$N_{видов\ зоопланктона}$		0.0-6.0	1.9±0.01	59	1010
Production characteristics of *Artemia*					
$N_{shrimps}$	shrimps/l	0-5164	43.4±7.2	532	1034
$B_{shrimps}$	mg/l	0-807	25.6±1.8	223	1047
$N_{plancton.cysts}$	cysts/l	0-6757	128.4±11.4	285	1002
$N_{bentic\ cysts}$	thousand cysts/m²	0-18594	641.0±45.8	230	819
B_{cysts}	kg/ha	0-570 (1240)	78.0±5.9	129	285
$N_{female\ with\ cysts}$	%	0-100	73.8±0.8	36	608
$N_{female\ with\ eggs}$	%	0-100	25.1±0.8	99	553
$N_{female\ with\ nauplii}$	%	0-41.4	1.2±0.1	319	427
$N_{cysts\ in\ ovisak}$	Cysts/ovisak	0-90	21.8±0.5	68	593
$N_{eggs\ in\ ovisak}$	eggs /ovisak	0-84	16.9±0.4	73	503
$N_{nauplii\ in\ ovisak}$	nauplii /ovisak	0-41	4.7±0.3	186	230
The duration of maturation female in lakes	days	21-35			
The duration of maturation female in culture	days	14-21			
Number of clutches		2-10	4.54±0.37		
Weight of cysts	mg	0.007 – 0.013	0.01		
Weight of nauplii	mg	0.013-0.018	0.015		
Weight of adult females	mg	0.8-14.2	4.1±0.1	45	313
Weight of adult males	mg	0.5-7.0	3.0±0.01	42	110

-Statistics on the limits of the parameter, on average, in variation of the characteristic and others are important in a comparative analysis from a scientific point of view.

-These data are also important in predictive work productivity *Artemia* reservoirs.

-The table shows data for all the years of research on abiotic and biotic factors on the productivity of *Artemia* in lakes.

Ratio between salinity and Artemia shrimp weight (A), general number of brood per female (B), *Artemia* shrimps biomass (C), benthonic (D) and planktonic cysts number (I) in average for populations

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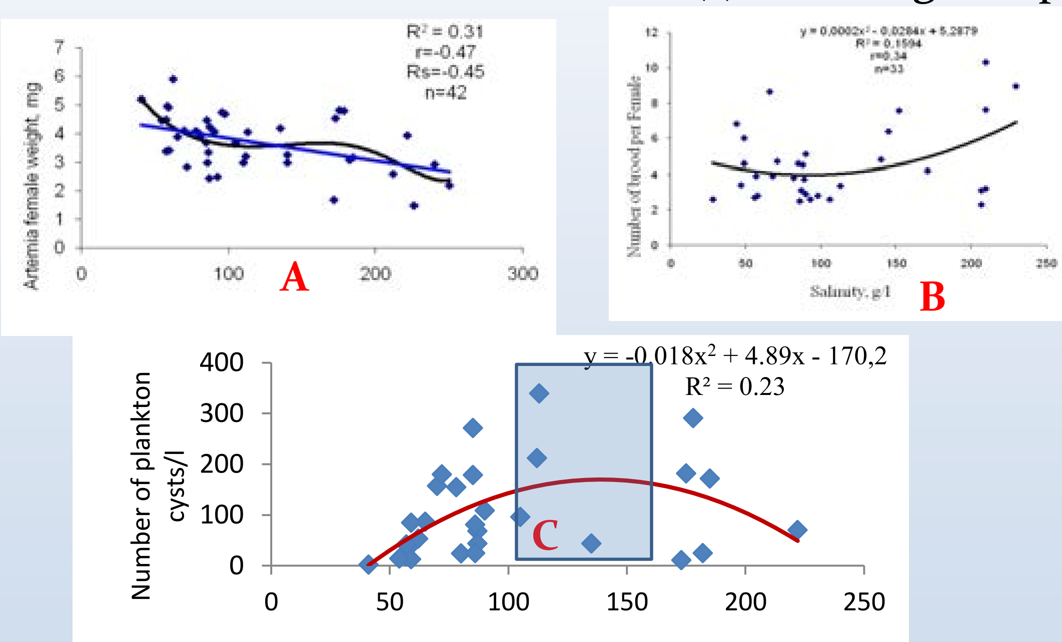
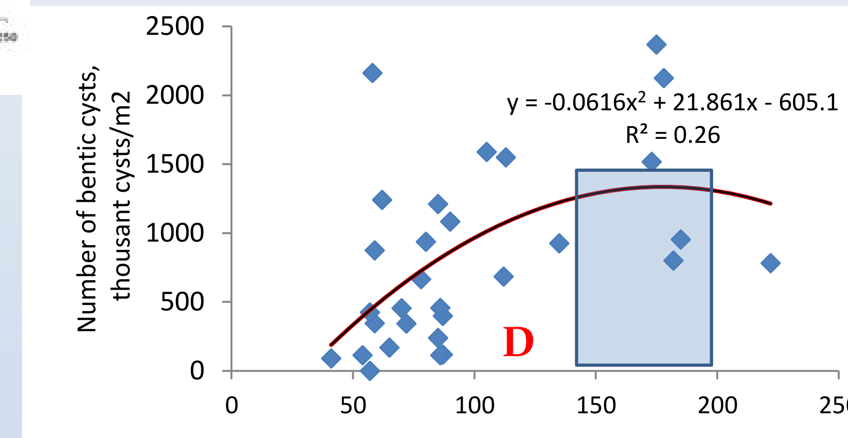
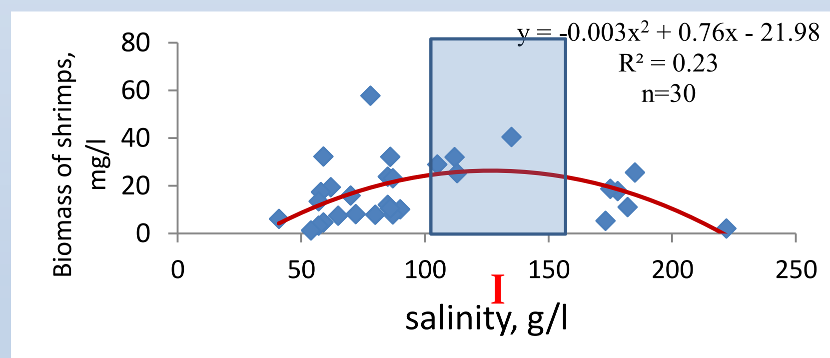




Salinity border (g/l) determining the livelihoods of *Artemia* shrimps

Natural populations	
34-380 (400)	**Border occurrence of shrimps**
70-230	**Population normally exist**
100-150	**Optimum conditions for number population**
110-170	**Optimum for planktonic cysts**
150-200	**Optimum for benthic cysts**



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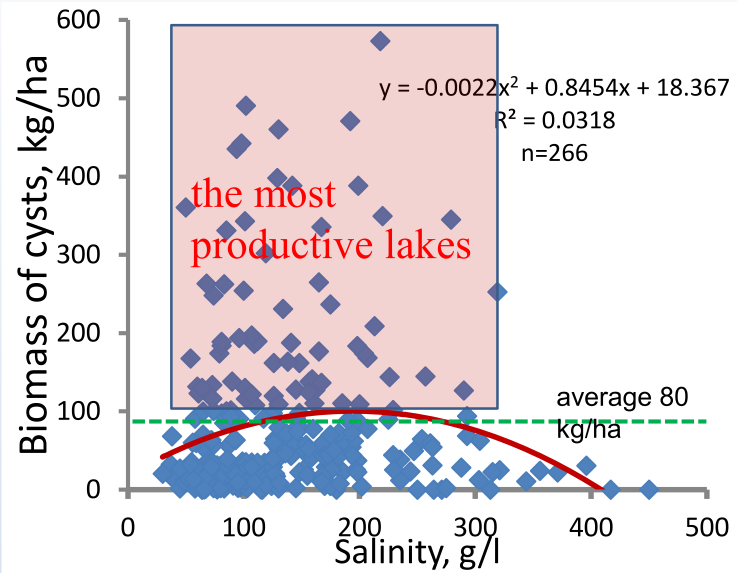
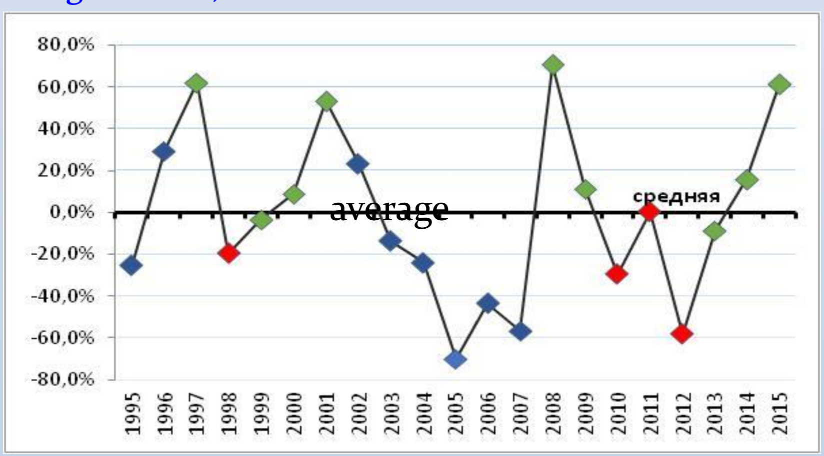
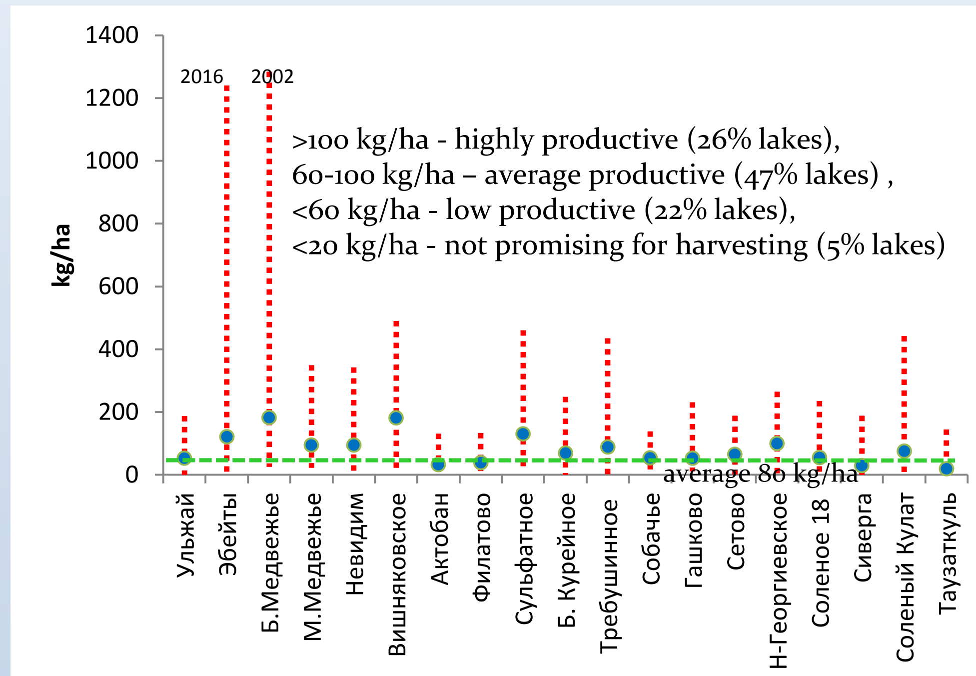


Long-term (1995-2015) dynamics of Artemia cyst stocks (n= 31 lakes), expressed through the ratio of actual to average annual, %



marker color: red - dry year, blue-wet year , green –average-water year

Productivity of lakes (biomass cysts, kg/ha) depending on salinity



Productivity of lakes (biomass cysts, kg/ha)

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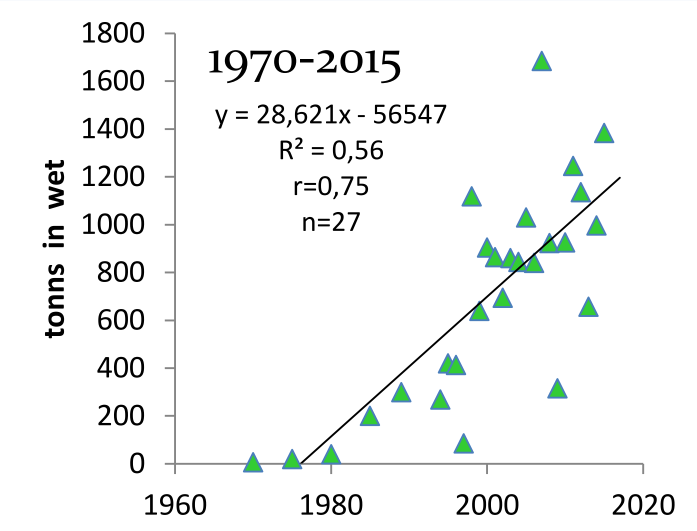
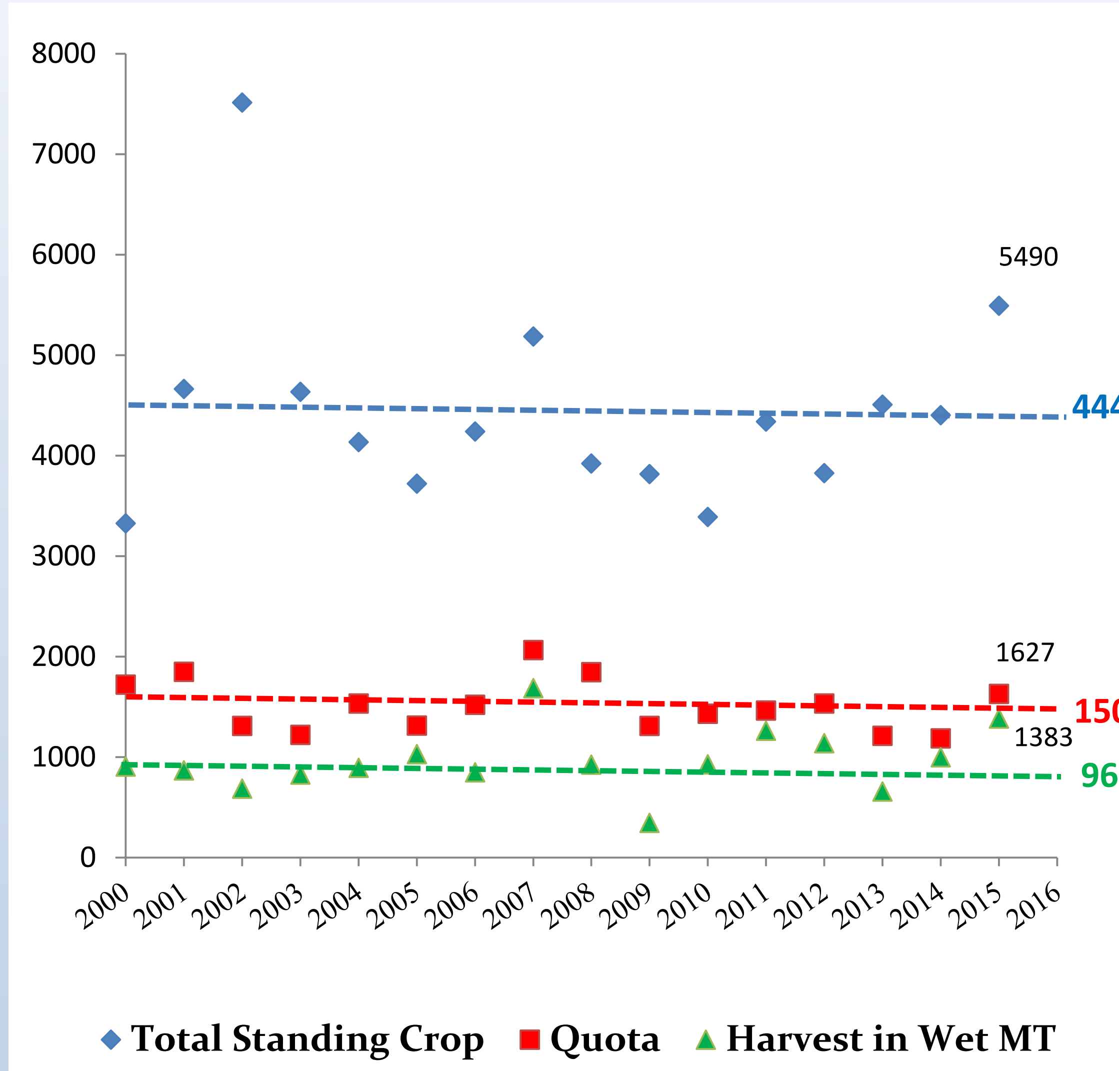
Results of cyst harvesting in Russia





-Figure 1 illustrates harvests since the 70-ies of last century. We can see that for 45 years of harvesting volumes have been increasing significantly and they are growing now. If before 80-ies last century the volumes of harvested raw material were used only in Russia, since 90-ies large part of them was exported abroad. The maximum amount of harvest in volume 1620 t was in 2007.

-Figure 2 shows the analysis of cyst catch in Russia since 2000. According to this figure stocks of cysts were affected by the greatest fluctuations; their wet weight consisted of 4 443 tonnes in average. The quota or volume of available catch consists of 1 508 tonnes in averages. Average harvest is 960 tonnes.

- Low Figure shows the ratio of harvest volumes in the Ural, Siberia and Altai. We can see that from 2000 to 2008 the share of the Altai was about 80%. Starting from 2009 to 2015 this proportion decreased to 55%. It was due to the harvest restrictions in 2009 and 2013 in the Altai.

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The Volume of Available Catch (VAC) of *Artemia* cysts for 2015-2016; their correction on the current status (in brackets) and results of harvesting in tonnes wet weight

Region (the number of harvesting lakes)	2015		2016	
	VAC (correction)	Harvest	VAC (correction)	Harvest***
Russia				
Ural & Siberia Federal districts (50)	1772 (2282)	1383	1745	1596
Southern Federal district (5)	0.4	0.4	100	468
Total Russia	1772 (2282)	1383	1845 (2750)	2064!
Kazakhstan*				
North Kazakhstan (12)	190	-	-	
Kyzylorda (2 Bays of Aral)	123.7	-	-	
Pavlodar (14)	807	-	-	
Total Kazakhstan	1350.7	about 1000**		about 1000

* - https://zakon.uchet.kz/view/122639/; **- analysis of the custom data of Kazakhstan ; ***-data from territorial departments of Federal Agency of Fishery

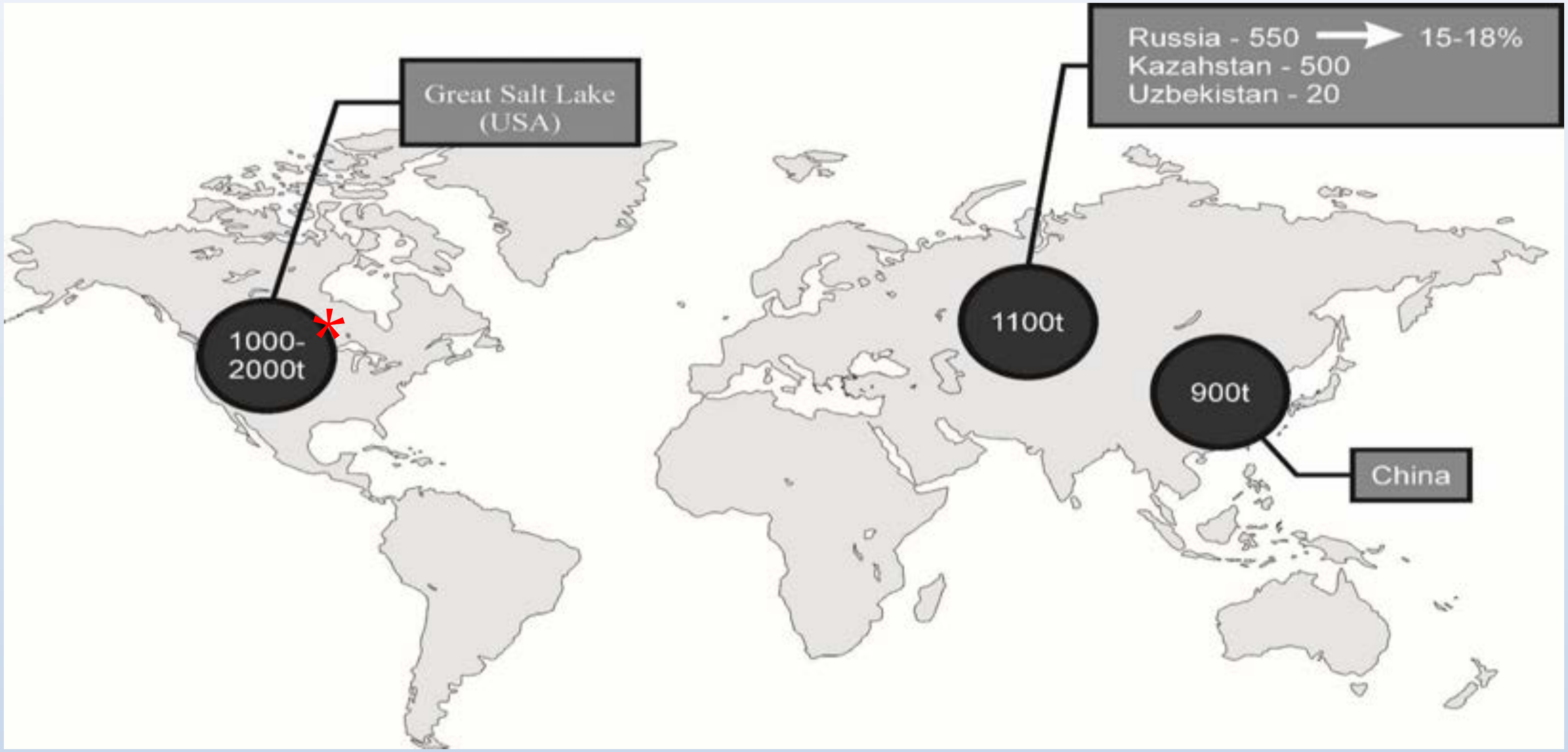
Cyst export from Russia and Kazakhstan

- Analysis of the custom data of Kazakhstan and Russia for three years showed that approximately the quantity of exported cysts per year
- from Russia is about 800-900 t in wet weight and 100-200 t cysts in dry weight
- from Kazakhstan – about 1000 t in wet weight
- from Uzbekistan – about 50-150 t in wet

Export country	Import country	The share of the total, %
Russia	China	72.4
	Thailand	20.1
	Germany	5.2
	Turkey	1.1
	Madagascar, Viet Nam, Taiwan, India	0.1-0.2
Kazakhstan	China	97.5
	Germany	2.5

Artemia cyst production in Russia

published in Chinese journal of Oceanology and Limnology (CJOL) Vol. 33 №6, P 1436-1450, 2015.

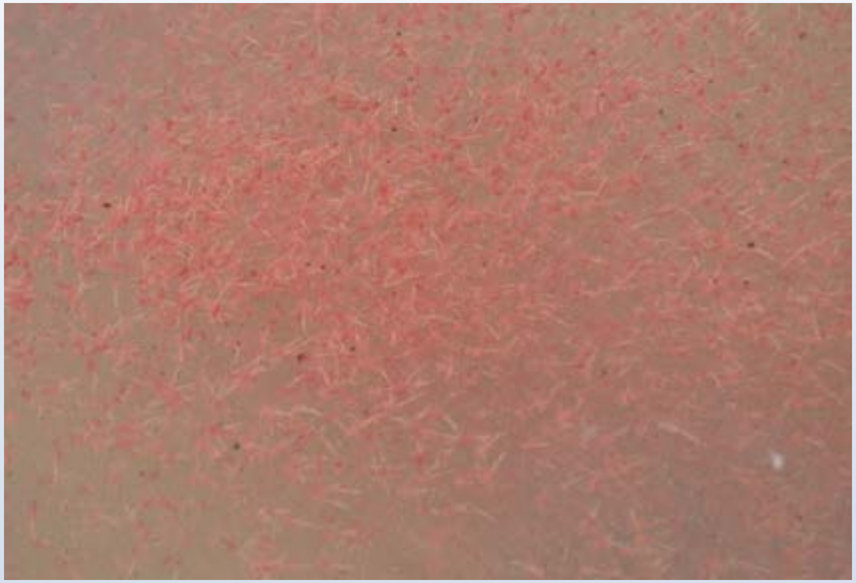


*- From Lavens and Sorgeloos (2000) and the long-term trend of the DWR data (UT-DWR,2014)

-The analysis of literature data and data from the various competent persons showed that the world produces about 3-4 thousand tonnes of dry cysts. According to official data in Russia harvest of cysts is about 500 t annually.

- That is way, share of Russia in the world market of cysts is about 15-20%. Approximately the same share is for Kazakhstan.

Monitoring of *Artemia* in Russia



Sampling on the lake

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Impact of environmental changes on the ecology and *Artemia* cyst production of Aibi Lake in China

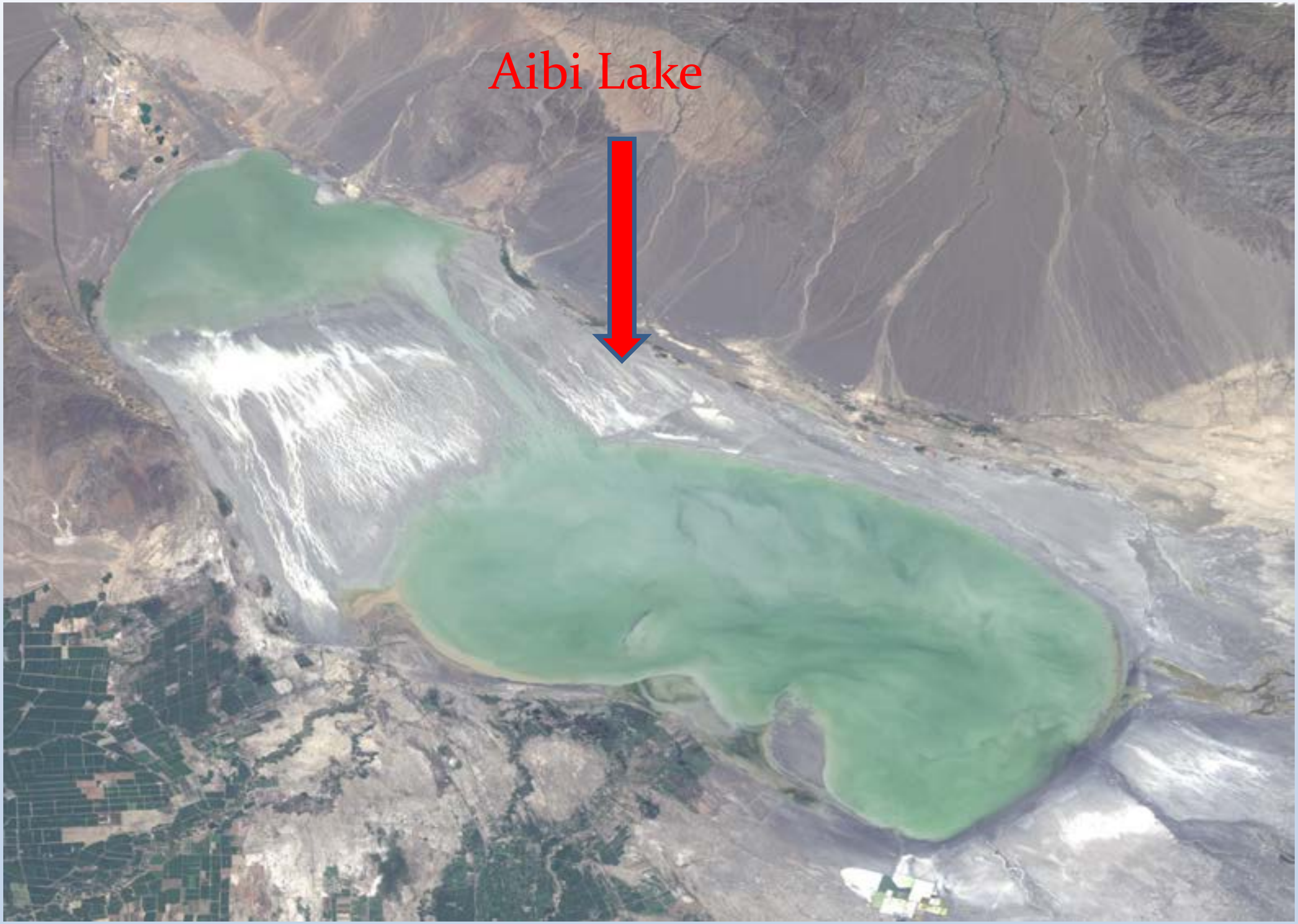
Yan Guo
Xinjiang Fisheries Research Institute,
Urumqi, Xinjiang, China

Main Contents

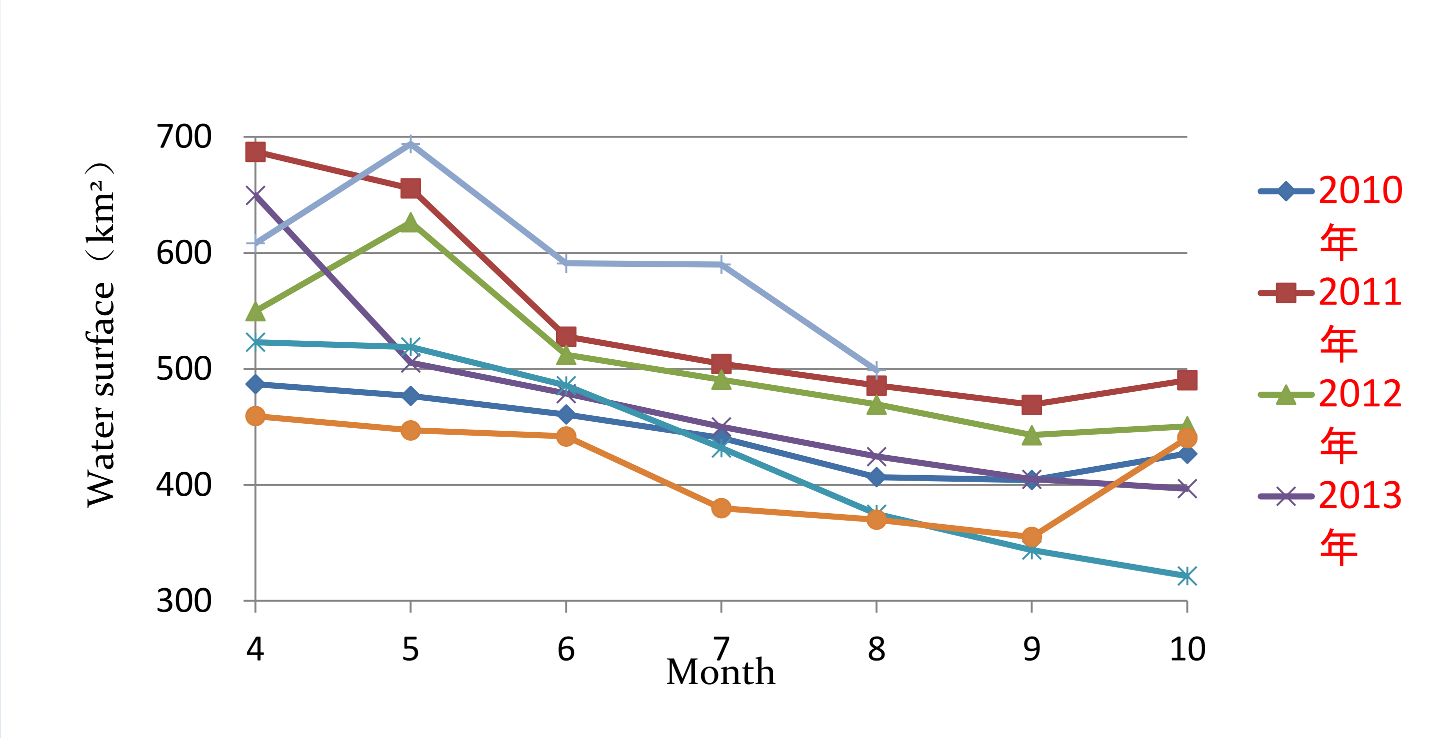
- General information on Aibi Lake
- Environmental changes and cysts production in Aibi Lake in recent five years.
- Impact environmental condition on *Artemia* cysts industry of inland salt lakes.

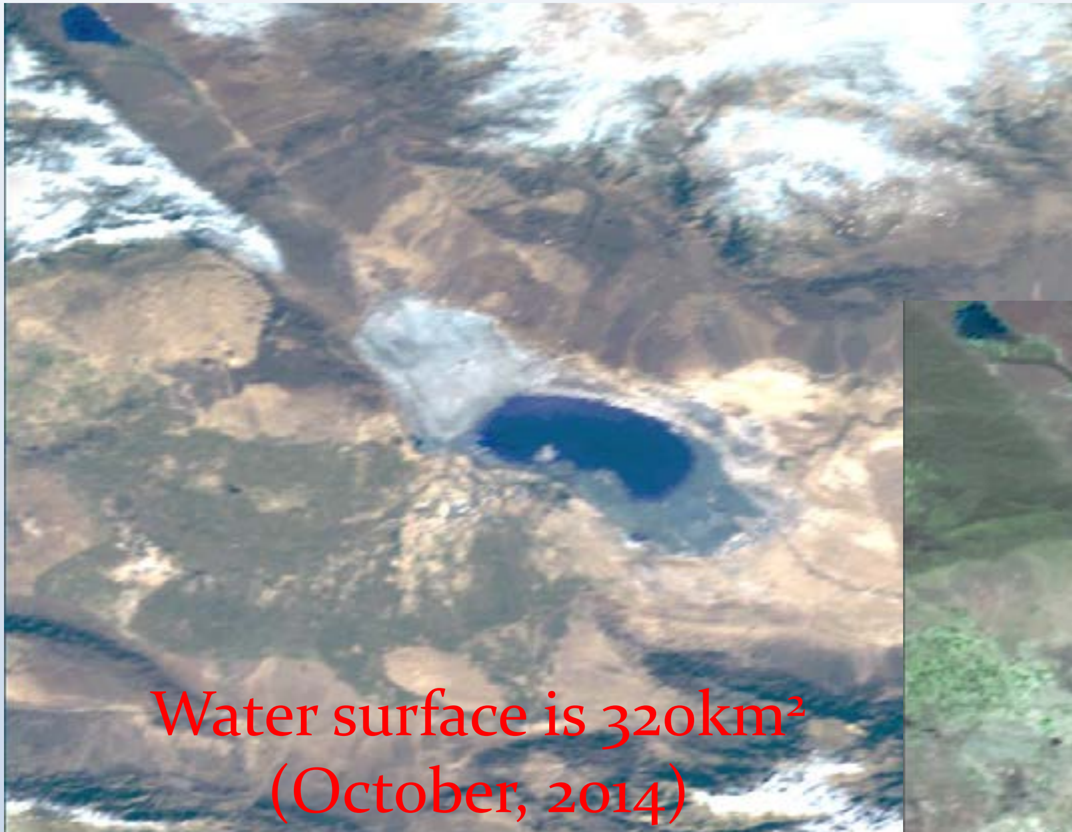
General Information

- Aibi Lake is located in the lowest point (194 m above sea level) of Junggar Basin in Xinjiang.
- At present Aibi Lake is the largest salt lake in China, with water surface fluctuating between 300-800 km^2.
- There are more than 20 rivers in the drainage basin, but only 2 rivers flow into the lake throughout the year.
- Annually there are about 168 windy days (> 6 level). The average evaporation is 1662 mm, and average precipitation is 90.9 mm.
- In 2015, the water salinity was 55-65 ppt in spring, 90-180 ppt in summer, 100 ppt in autumn. The highest salinity is 280 ppt.
- *Artemia* in Aibi Lake was first noticed in 1983. It is parthenogenetica strain.


Aibi Lake

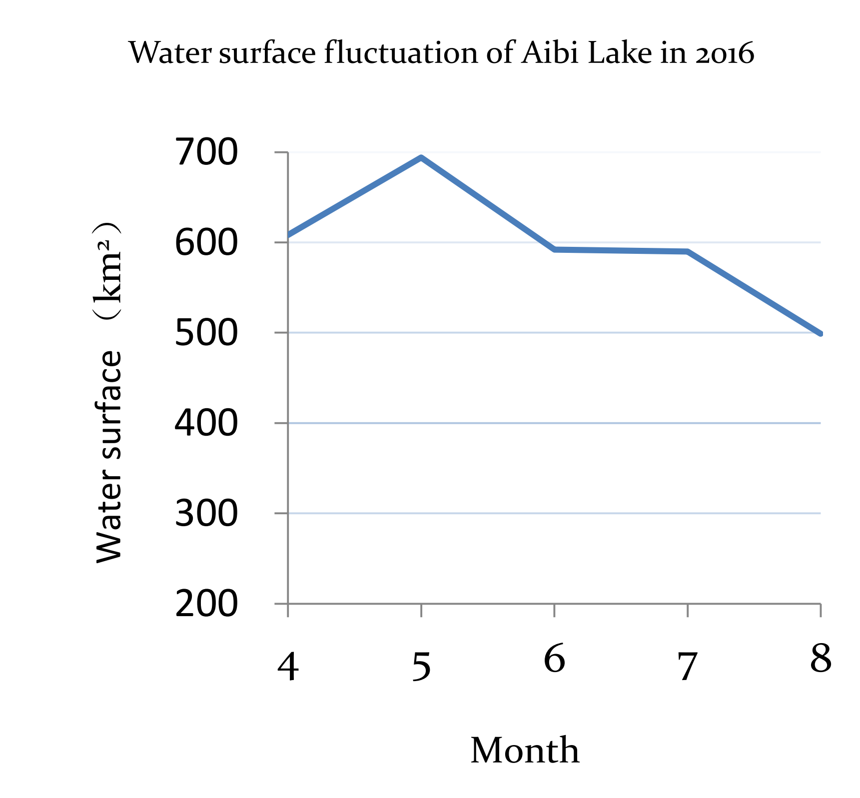
Water surface fluctuation between 2010-2016

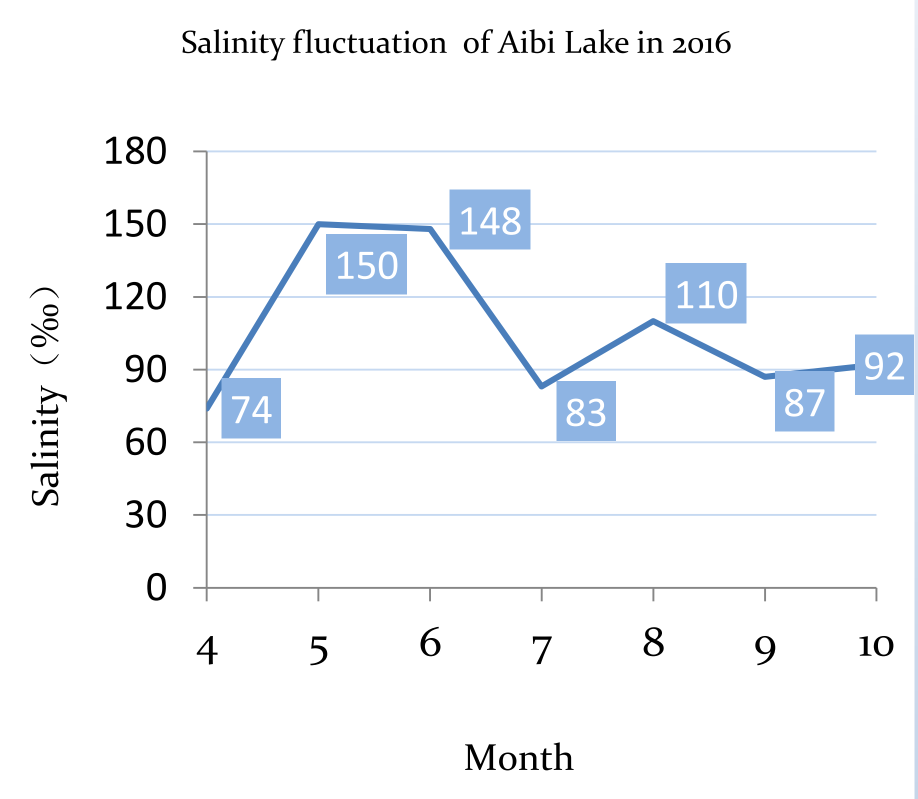


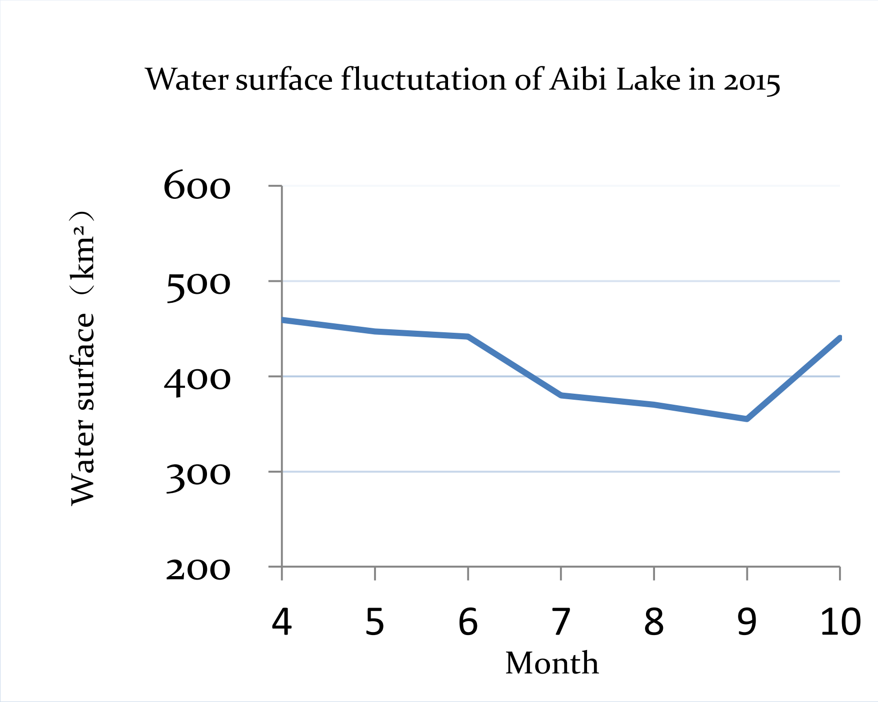

Water surface is 320km²
(October, 2014)

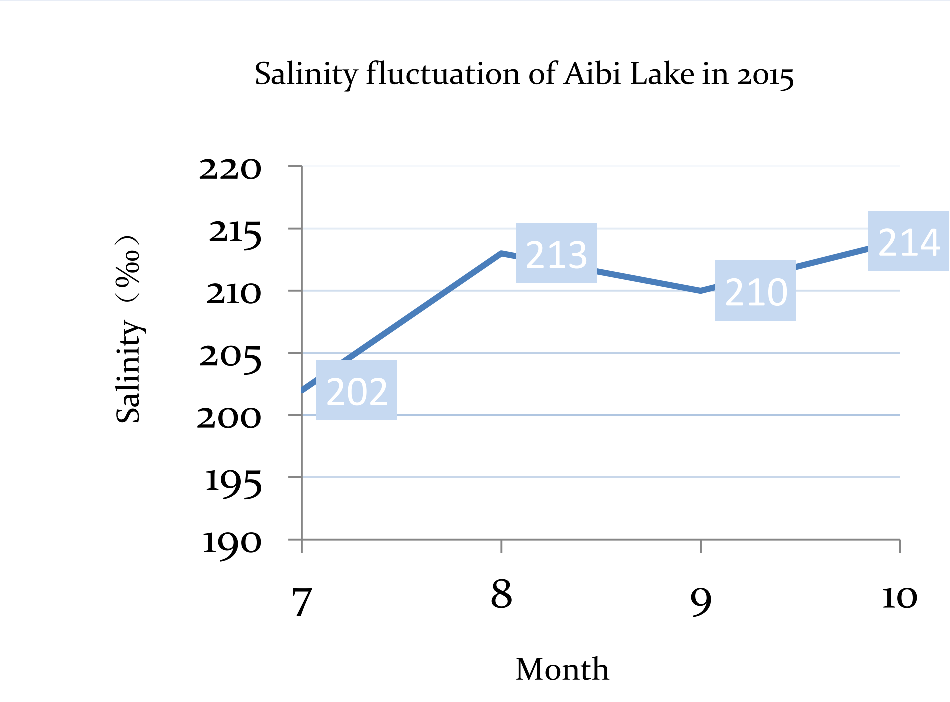

Water surface is 690km²
(June, 2016)

Environmental changes and cysts production in Aibi Lake in recent five years.


Water surface fluctuation of Aibi Lake in 2016
700
600
500
400
300
200
Water surface （km²）
4
5
6
7
8
Month


Salinity fluctuation of Aibi Lake in 2016
180
150
120
90
60
30
0
Salinity （‰）
74
150
148
83
110
87
92
4
5
6
7
8
9
10
Month


Water surface fluctutation of Aibi Lake in 2015
Water surface（km²）
600
500
400
300
200
4
5
6
7
8
9
10
Month


Salinity fluctuation of Aibi Lake in 2015
Salinity（‰）
220
215
210
205
200
195
190
202
213
210
214
7
8
9
10
Month

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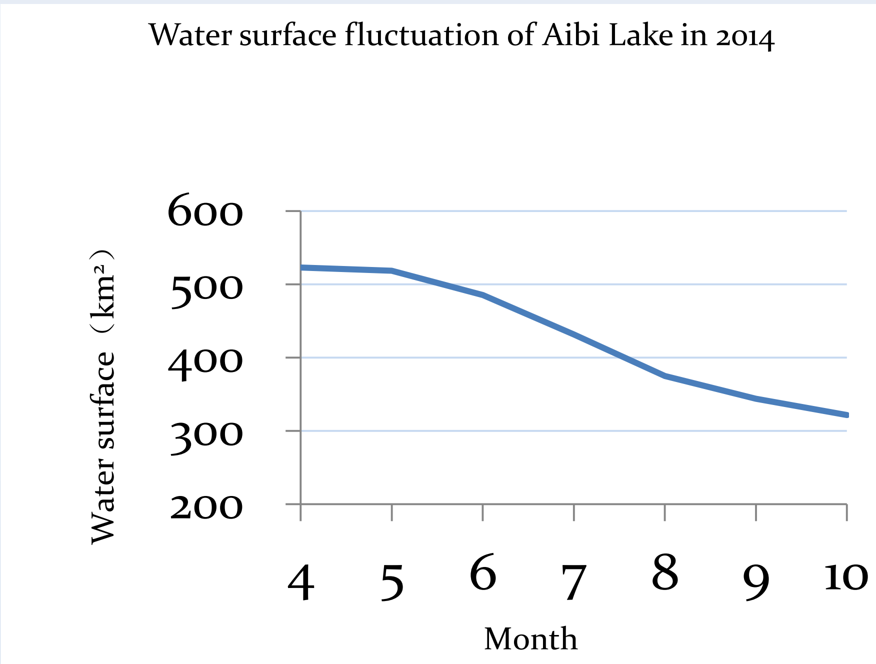
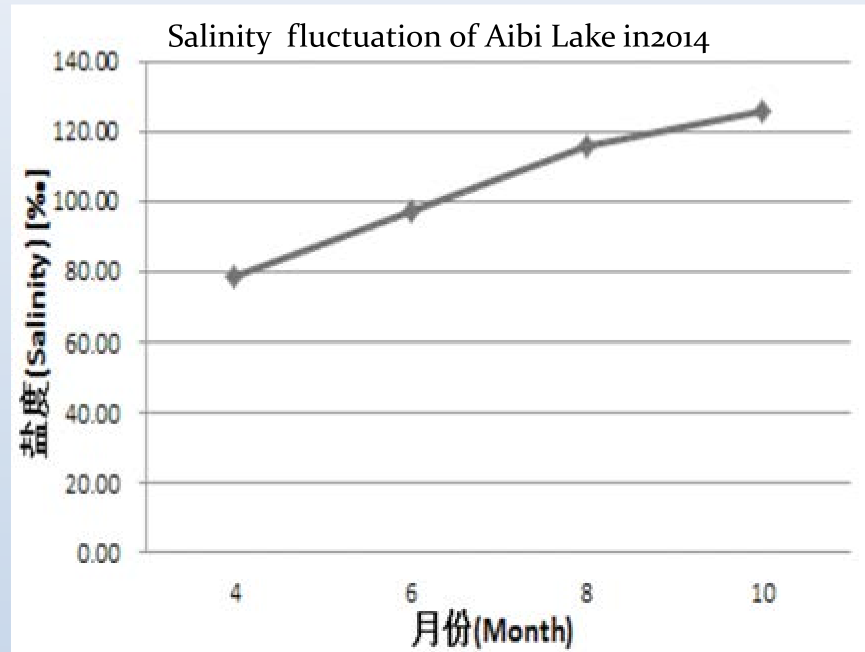
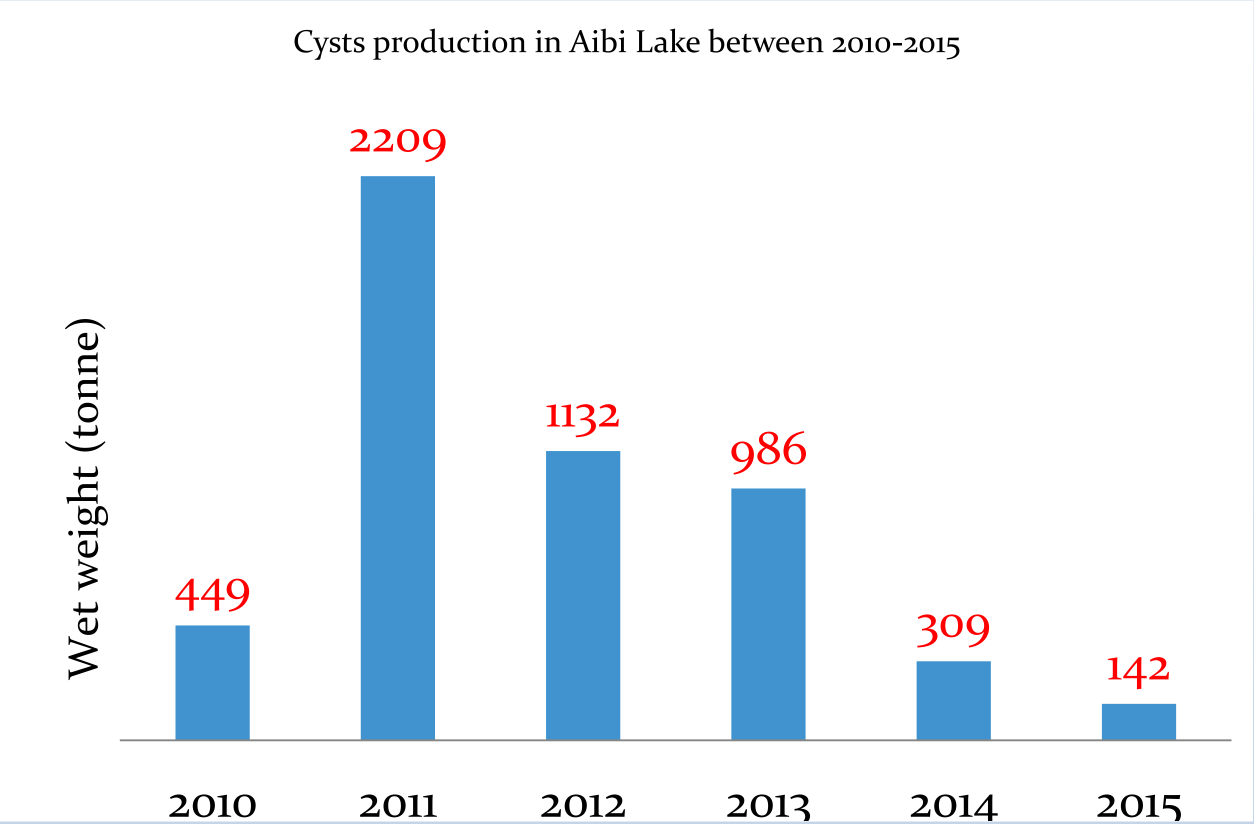





Cysts production in Aibi Lake between 2010-2015
Wet weight (tonne)
449
2209
1132
986
309
142
2010
2011
2012
2013
2014
2015

Impact of environmental condition *Artemia* cysts industry of inland salt lakes

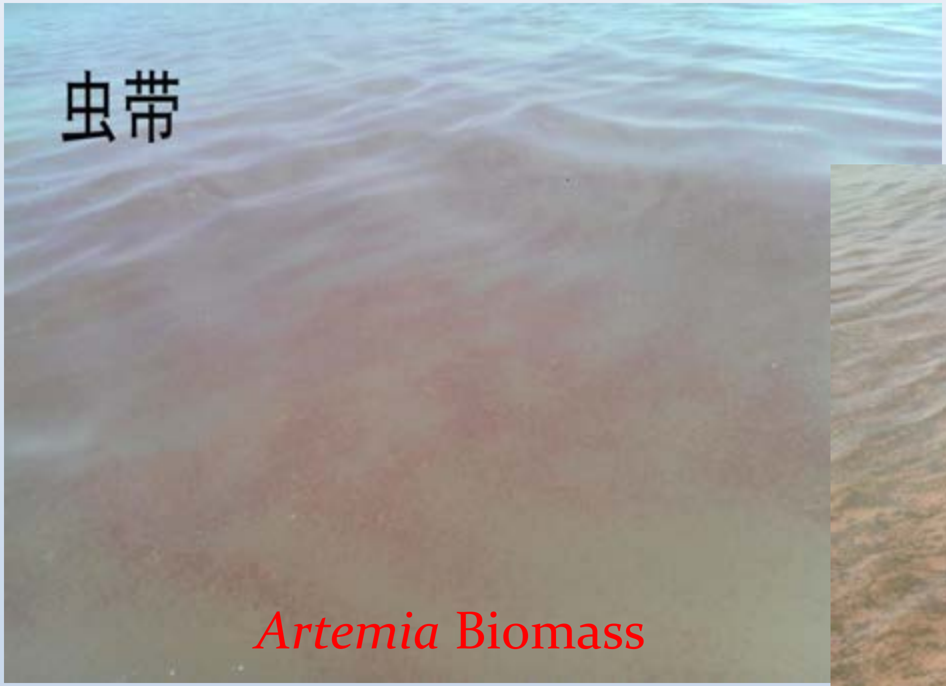
- Water salinity influences the cysts production. The optimal salinity is 120-150 ppt. Too high salinity results in lower cysts production due to:
  --- The main reproductive mode of *Artemia* shifts to viviparous and less generation.
  --- Less food availability and less population density.

- Moreover too high salinity also lead to inferior nutritional quality of the cysts, longer diapause period and lower hatching synchronism.

- Most inland salt lakes are located in the harsh environmental area, i.e. strong wind, less precipitation, and big temperature fluctuation. It is usually hot in summer and very cold in winter. So the harvest should start earlier (in August). This will reduce blow off the cysts by wind, as well as prevent the cysts pile-up on the beach.

- Notice that the hatching quality of the newly developed salt lake and a stop-redeveloped lake is not as good as the developed one. The harvest should not be restrict controlled as high intensity harvest will help to improve the cysts quality.

Artemia biomass and cysts Accumulation in Aibi Lake



Artemia cysts harvest in Aibi Lake

Artemia cysts processing on site

Thank You

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Integrated production of salt and *Artemia* in artisanal salt ponds in Viet Nam

Nguyen Van Hoa
College of Aquaculture and Fisheries,
Cantho University, Cantho, Viet Nam

Content

- Introduction
  - cyst demand in Viet Nam
  - history in Vinhchau-Baclieu
  - culture in solar saltworks
- Culture model
  - mono-culture
  - *Artemia*-salt
- Social-economy

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Artemia as feed in aquaculture

Artemia cyst required for 1 million of PL's

30 kg
mud-crab

10-13 kg
prawn

3 kg
tiger shrimp

3 kg
white leg

3 kg
5 000 cobia fry
50-day old

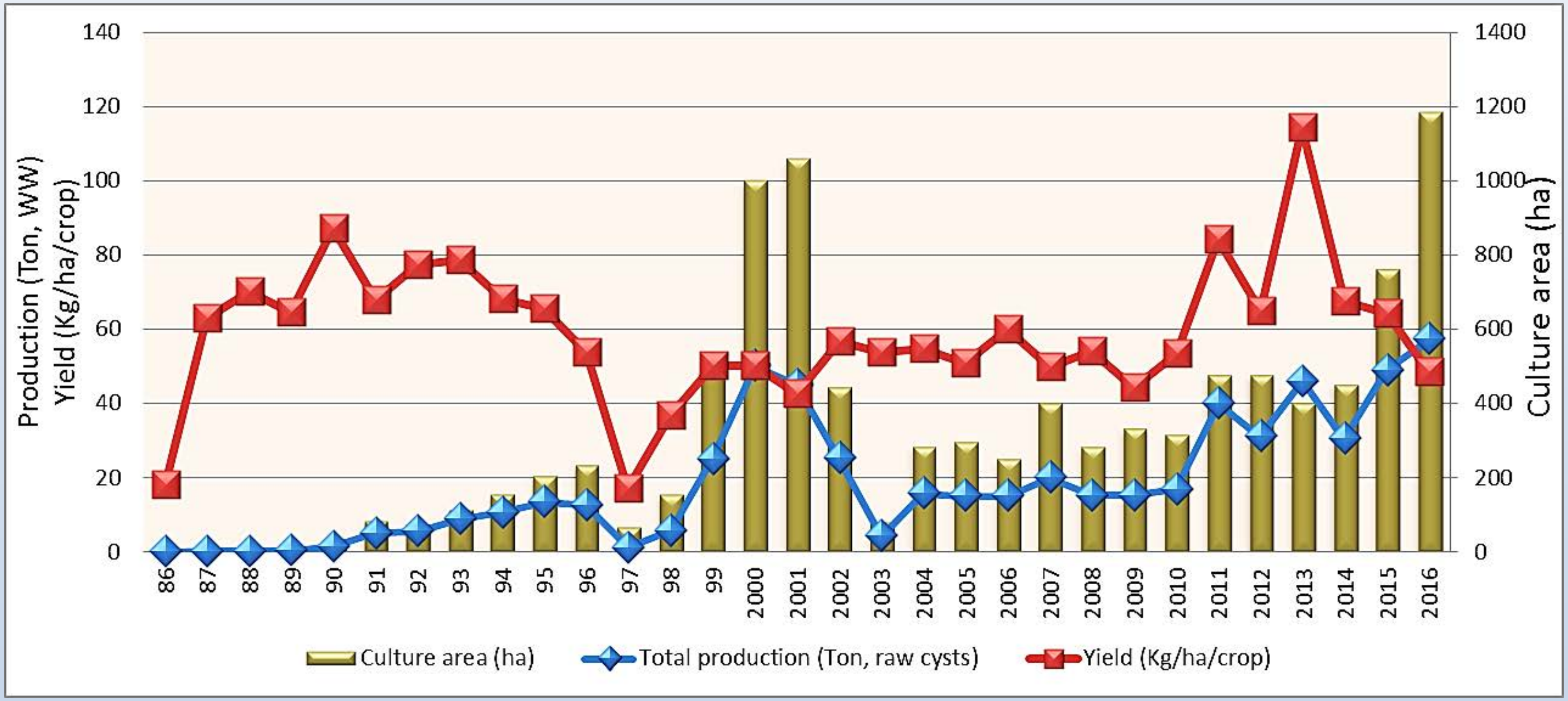
X kg

Introduction

- cyst demand in Viet Nam
  - Currently, Viet Nam has a capacity of 130 billions PLs (30 Bills for tiger shrimp and 100 billions for whiteleg shrimp)

    (Nhu Van Can, 2016; http://www.thuysanvietnam.com.vn)
  - Over 80% cysts have been used for marine shrimp hatcheries (app. 500 tonnes/year)
- history of *Artemia* culture in Vinhchau-Baclieu
- culture in solar saltworks

Artemia cyst production in Vinh chau and Bac lieu



➔ recently, Viet Nam hatcheries require *Artemia* cysts app. **400 - 500 tonnes/year (60-80 millions US$)** but the production in Vinh chau and Bac lieu covers only app. 5 % of the country's demand

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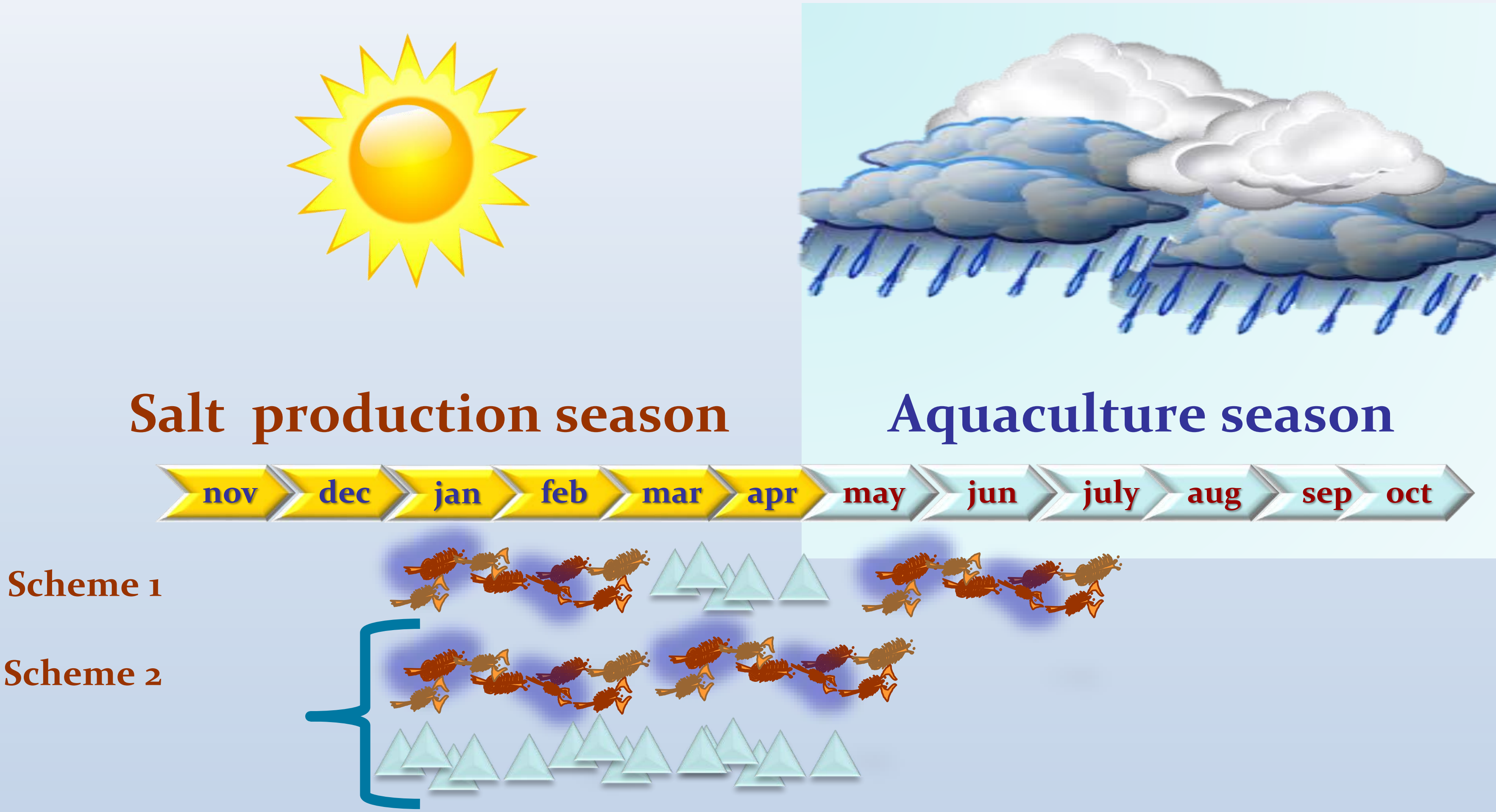
Artemia culture season in Vinh Chau and Bac Lieu



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Common *Artemia* culture models in Vinhchau-Baclieu

Type	Invest.	operation1	operation2	inoculum	product
Mono	Traditional	Stagnant	1 cycle	Hatched N	Cyst
Rotation	Intensive	Flow-	n-cycles	On-grown	Biomass
Integration					

- Market
- Farmer habit
- Climate
- Location/area
- Applied protocol
- Experiences
- Budget available
- ...

Common *Artemia* culture models in Vinhchau-Baclieu

	Culture models	Description
1	Monoculture	Saltpans to be completely converted into *Artemia* ponds
2	Rotation	*Artemia*→Salt→*Artemia*
3	Integration	*Artemia*—Salt
4	Traditional	Low density, without fertilization pond
5	Intensive	High density, fertilization pond, suplementary feeding
6	Stagnant	Static, independent unit, less water exchange
7	Flowthrough	System management, salinity gradient, typical integrated system
8	1-cycle	One stocking; population to be maintained through the season
9	N-cycle	N stocking; a cycle be completed within 1-2 generation
10	Hatched nauplii	Newly hatched nauplii as stock
11	Ongrown *Artemia*	Ongrown *Artemia* to be available (e.g. Juv, Pre-Adults, Adults)
12	Cyst	The only product
13	Biomass	The main product BUT cysts as by-product could be collected

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Common *Artemia* culture models in Vinhchau-Baclieu

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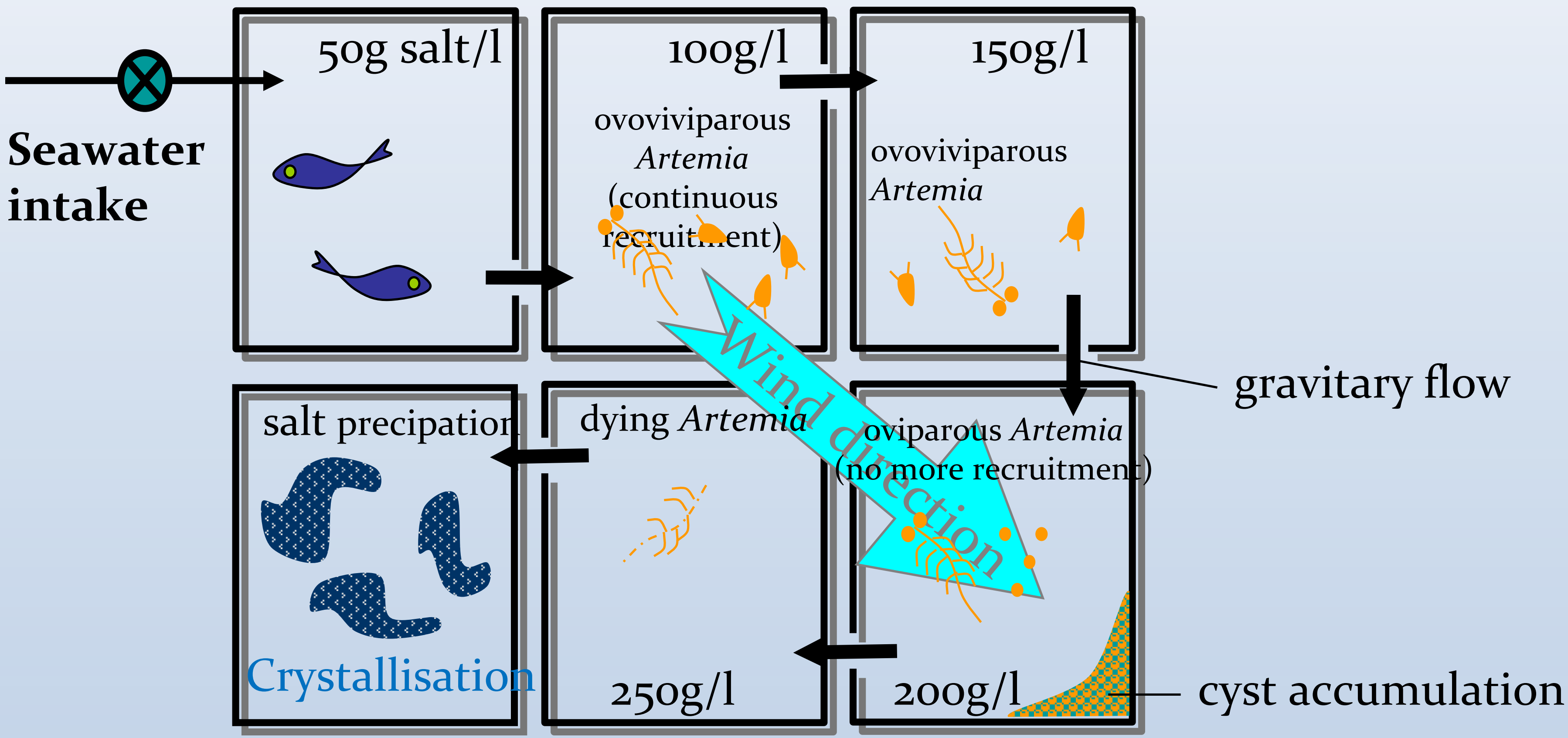
Advantage of *Artemia*-Salt integration system in Vinhchau-Baclieu

- Integrate in existing system = more cost effective
- Convertibility between salt and *Artemia*
- Enhancement of *Artemia* production (i.e. less/low production ponds be returned back to salt production)
- Balance the (traditional) salt production
- Easy to operate

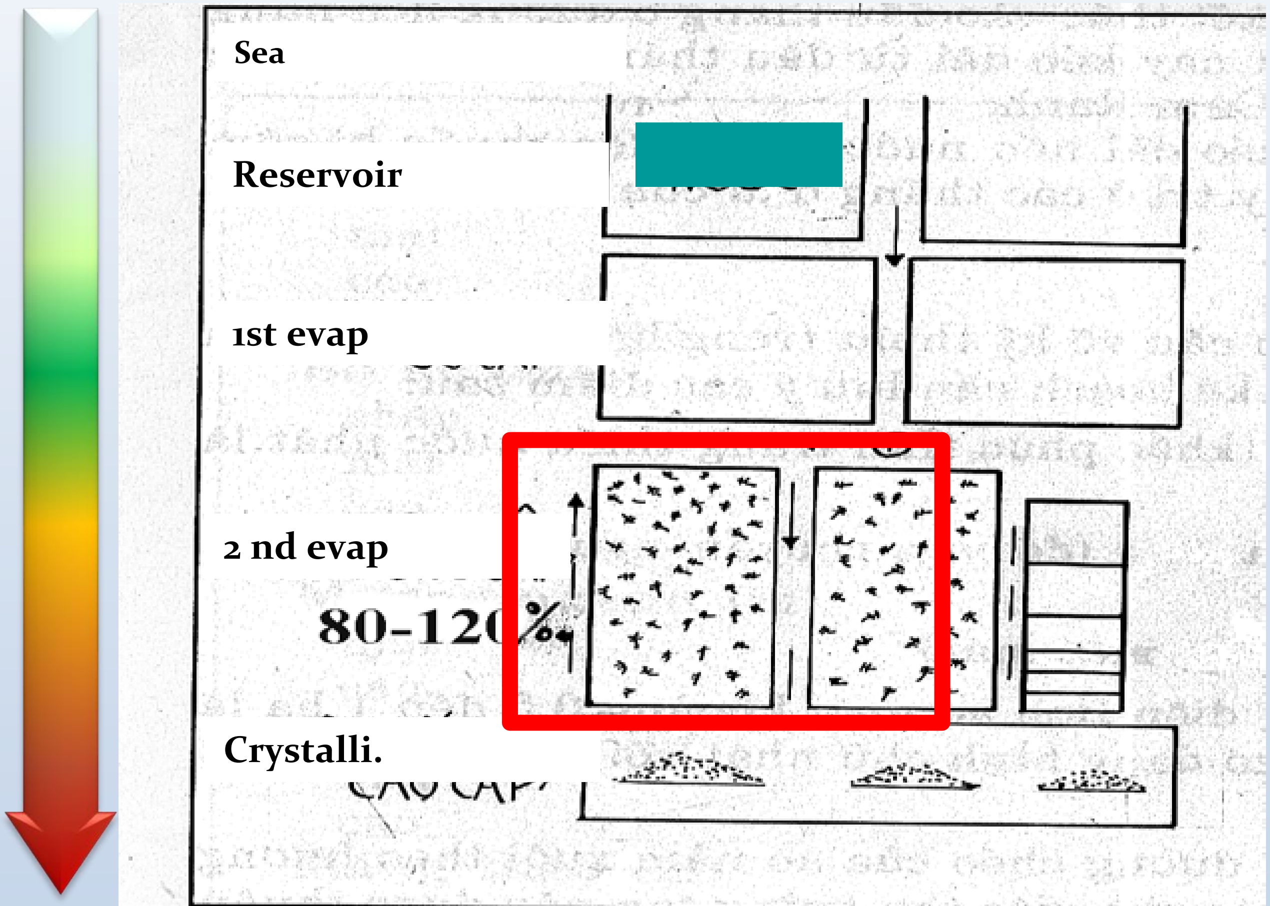
➔ ***To be considered as* "Smart system" = Salt and maximal *Artemia* production system**

Schematic diagram of a solar salt operation with natural occurrence of *Artemia*



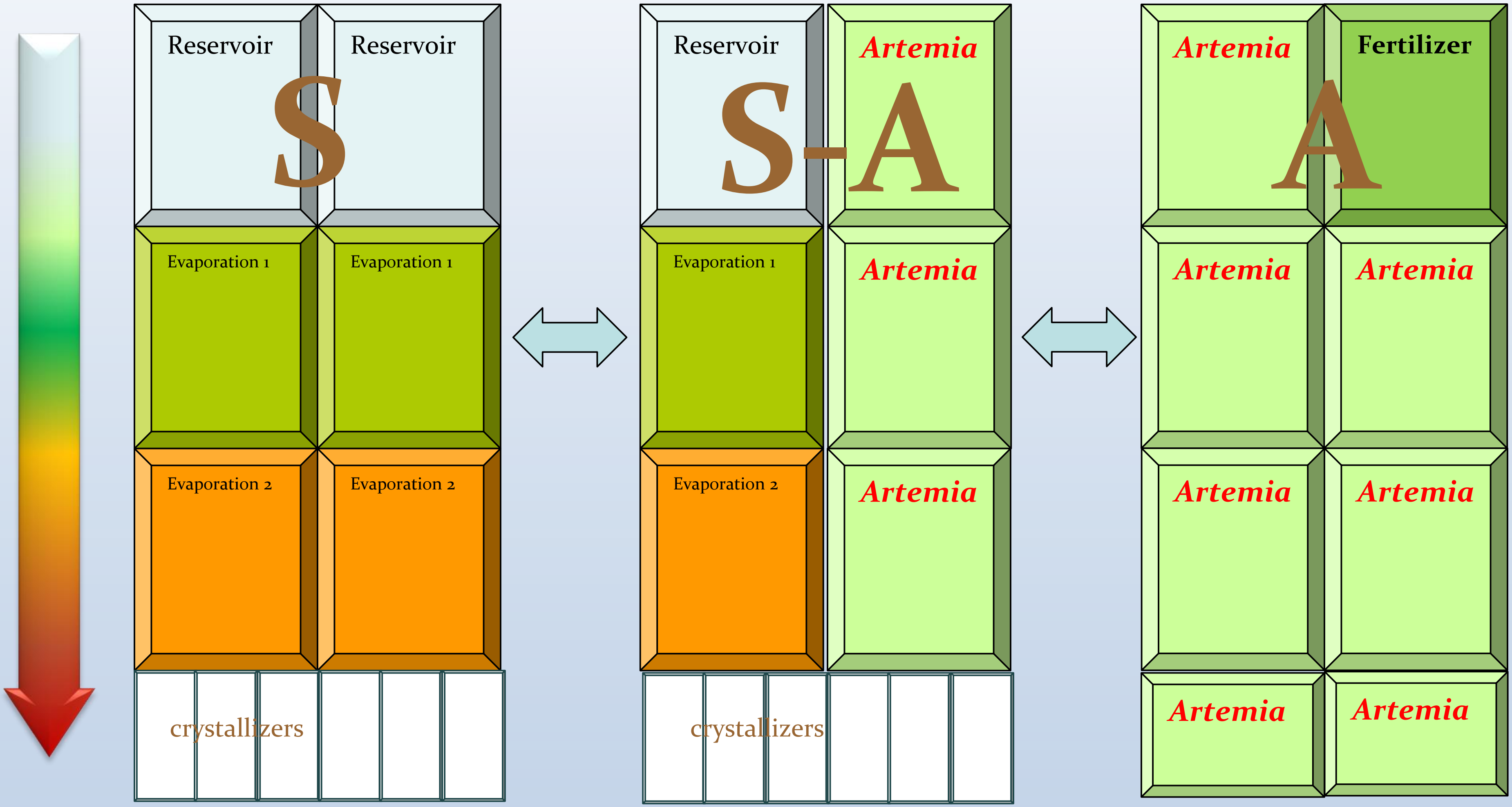
Artemia culture in solar saltworks (Sorgeloos *et al.*, 1986)

System design



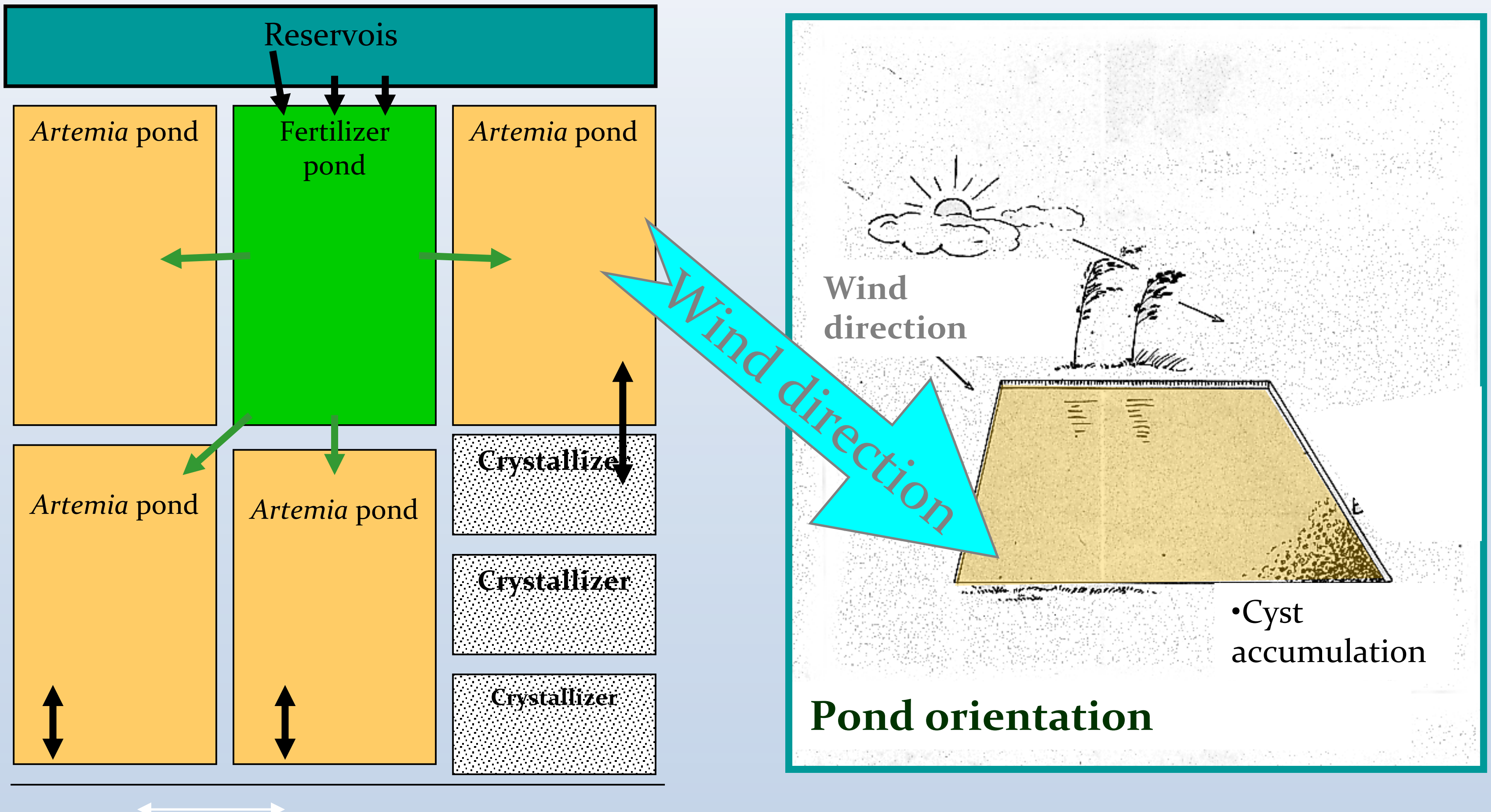
Suitable area for *Artemia* culture in salt-fields (during 90's)

System design

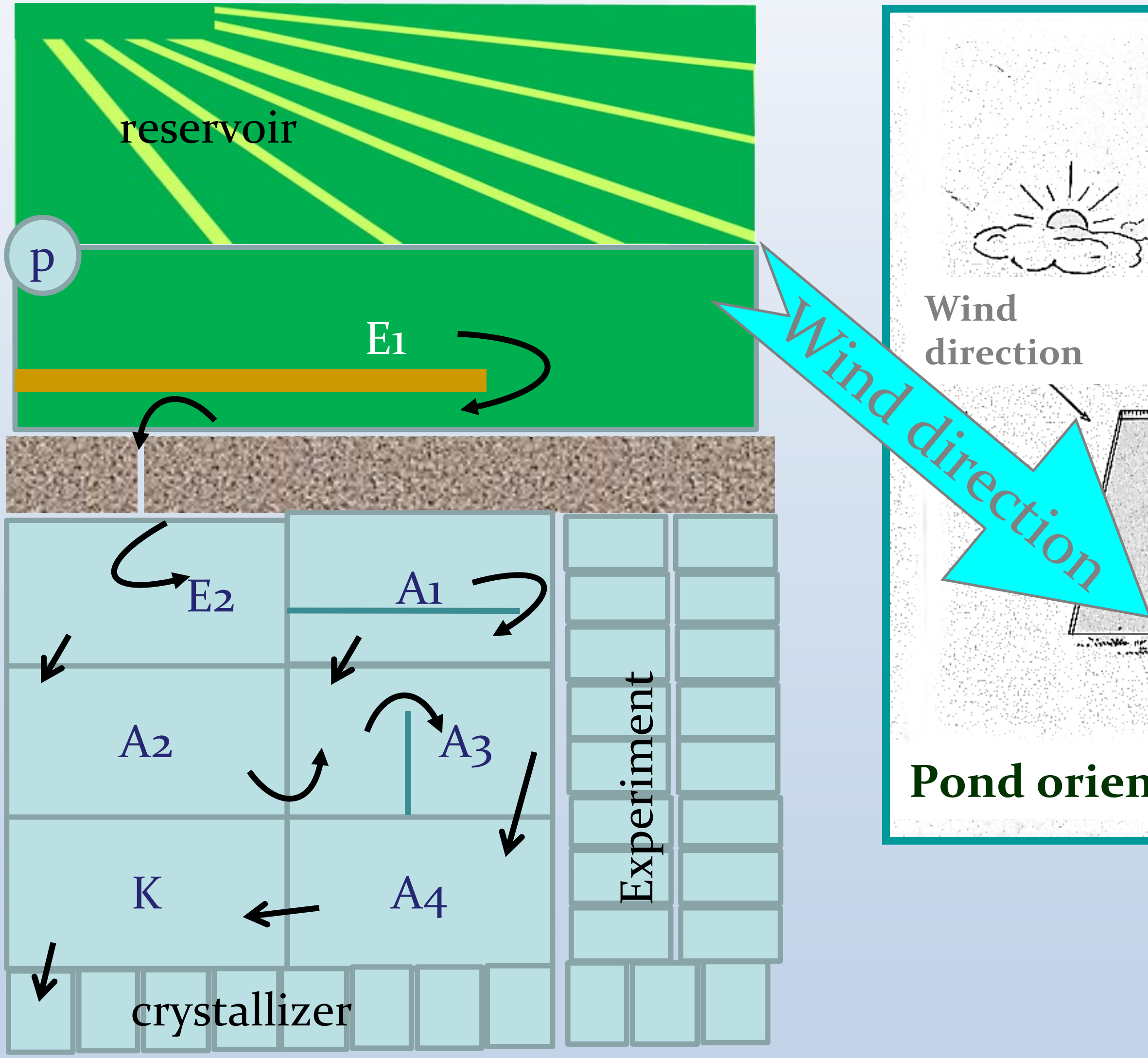


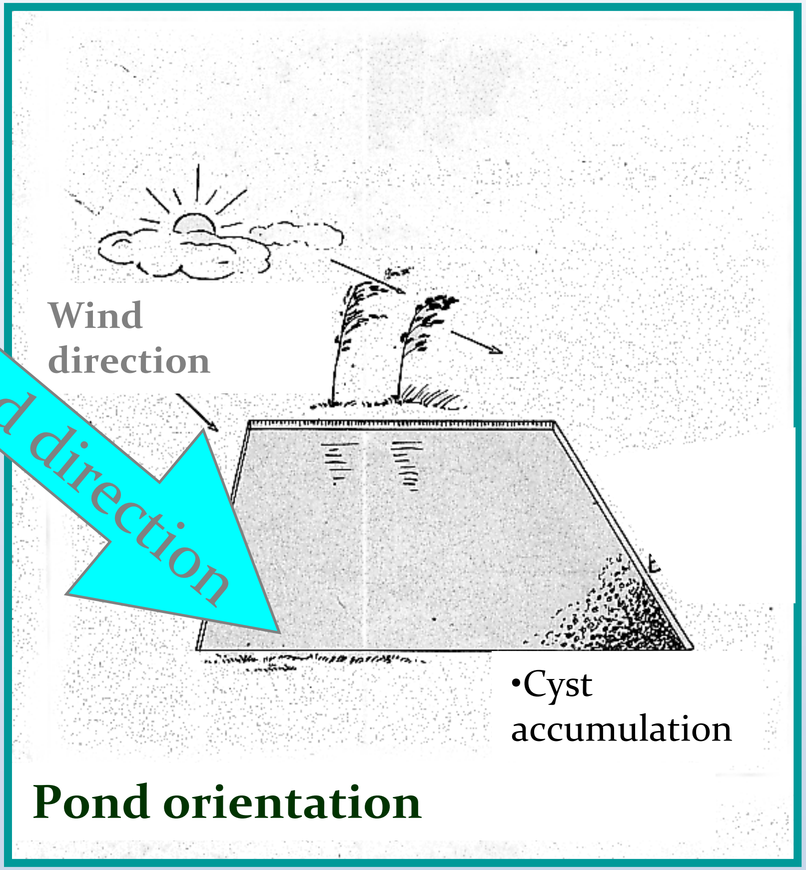
Suitable area for *Artemia* culture in salt-fields recently

Static system (system design)



Flow-through system (system design)





Climatology

- Evaporation higher than precipitation.
  - Wind velocity, temperature, relative humidity
  - Presence salt works
- Temperature !!
  - Evaporation acceleration
  - Suitability of *Artemia* (i.e. Strain selection)
- Wind
  - Prevailing wind (facilitate for cyst collecting)

Topography

- Flat land with gentle slope.
- Dug-out versus levee

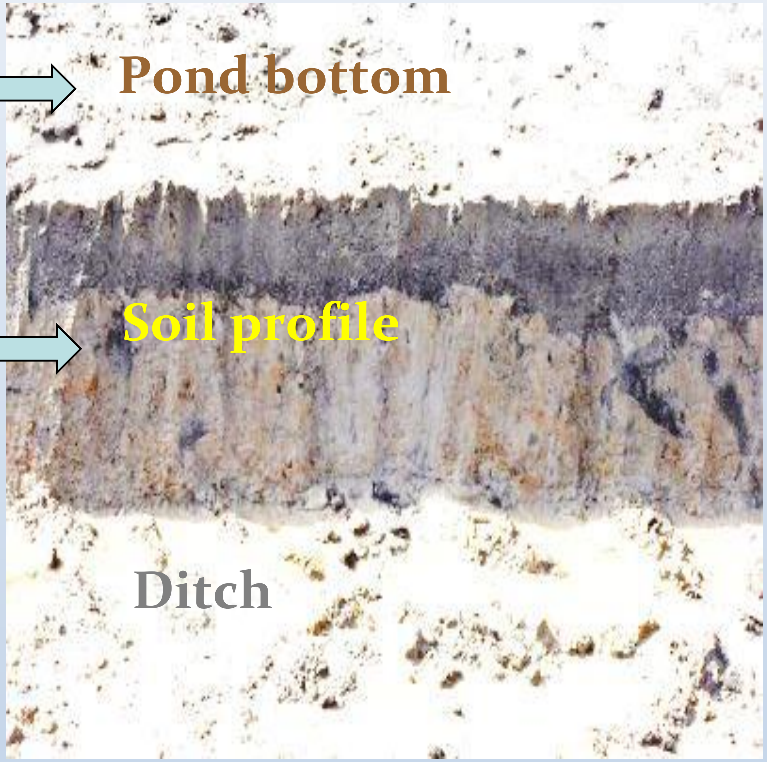
~ Type of ponds already present (cost)

⇓

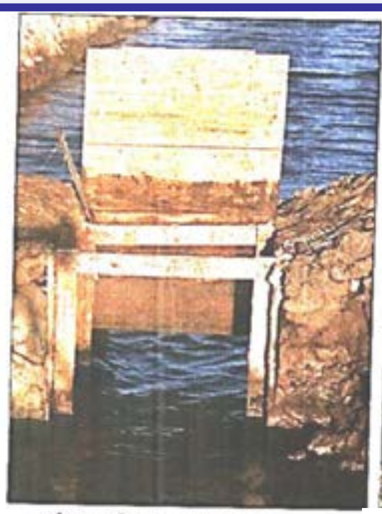
Water flow by gravity

Soil conditions

- Organic content
  - Interaction with fertilization schemes
- Acid sulfate soils (mangroves!)
- Leakage !
  - Clay proportion
  - Presence organic materials
  - Construction methods (compacting)
  - Presence digging animals (i.e. fish, crabs)



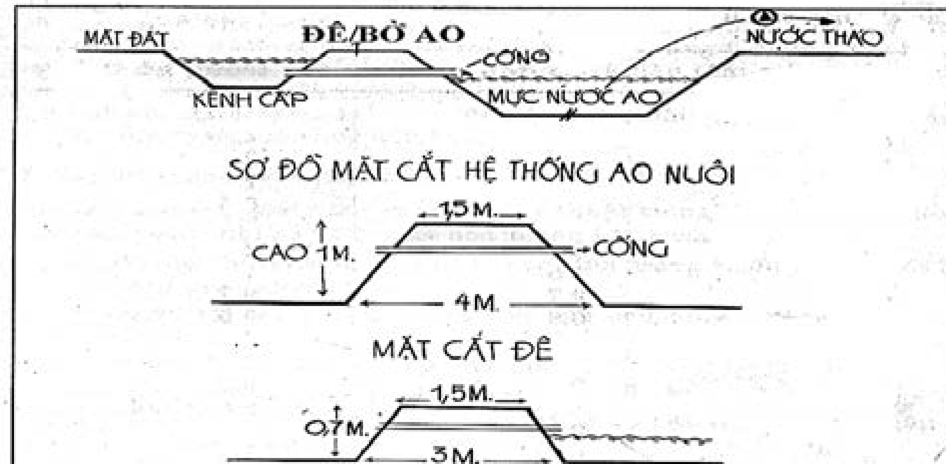
Pond construction



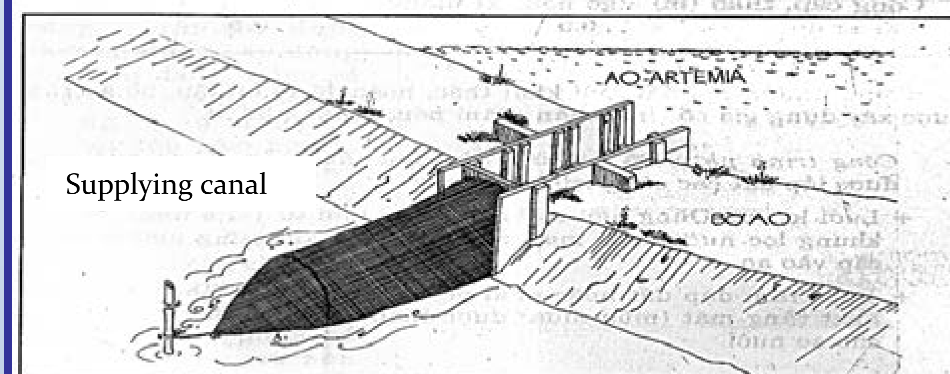
Wooden gate

Fine nylon screen

Screening SW with fine nylon net

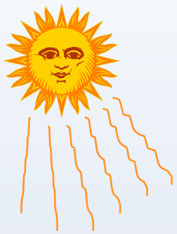


Dissection of supplying canal, pond and dike



Screening SW with fine nylon net

Nutrient management in fertilization pond



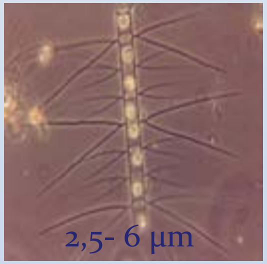
KEY NUTRIENTS

N & P
N:P ratio

→ **Primary production** →

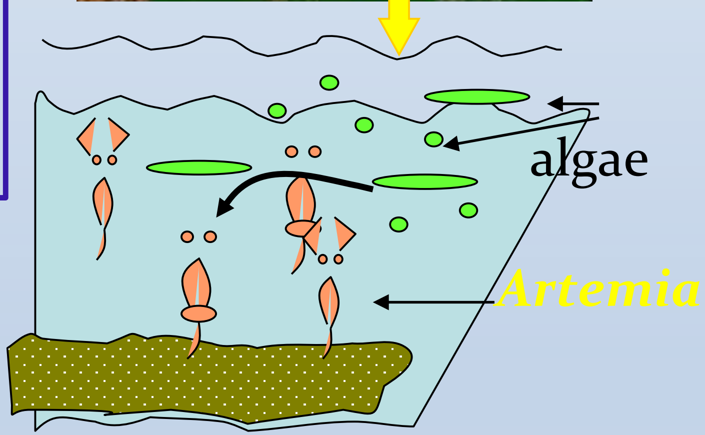
Dunaliella

Tetraselmis



Chaetoceros

Nitzschia



Microalgae are the best natural food for *Artemia*

Chicken manure and fertilizer (urea and DAP) were applied into Fertilizer pond to produce "green-water" as feed for *Artemia*

Enhancement of green water (traditional vs new formula)

Traditional	New formula
• Chicken manure (300 g/m^3) • Urea (3 g/m^3)+ D.A.P (1 g/m^3)	• Fish meal (low value) (30 g/m^3) • Urea (19 g/m^3) +D.A.P (10 g/m^3)

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Enhancement of green water (traditional vs new formula)

Day 1
- Sea water inlet
- Applying chicken manure or fish meal
- adding urea + D.A.P

Day 2
- Waiting 1 day

Day 3
- adding urea + D.A.P

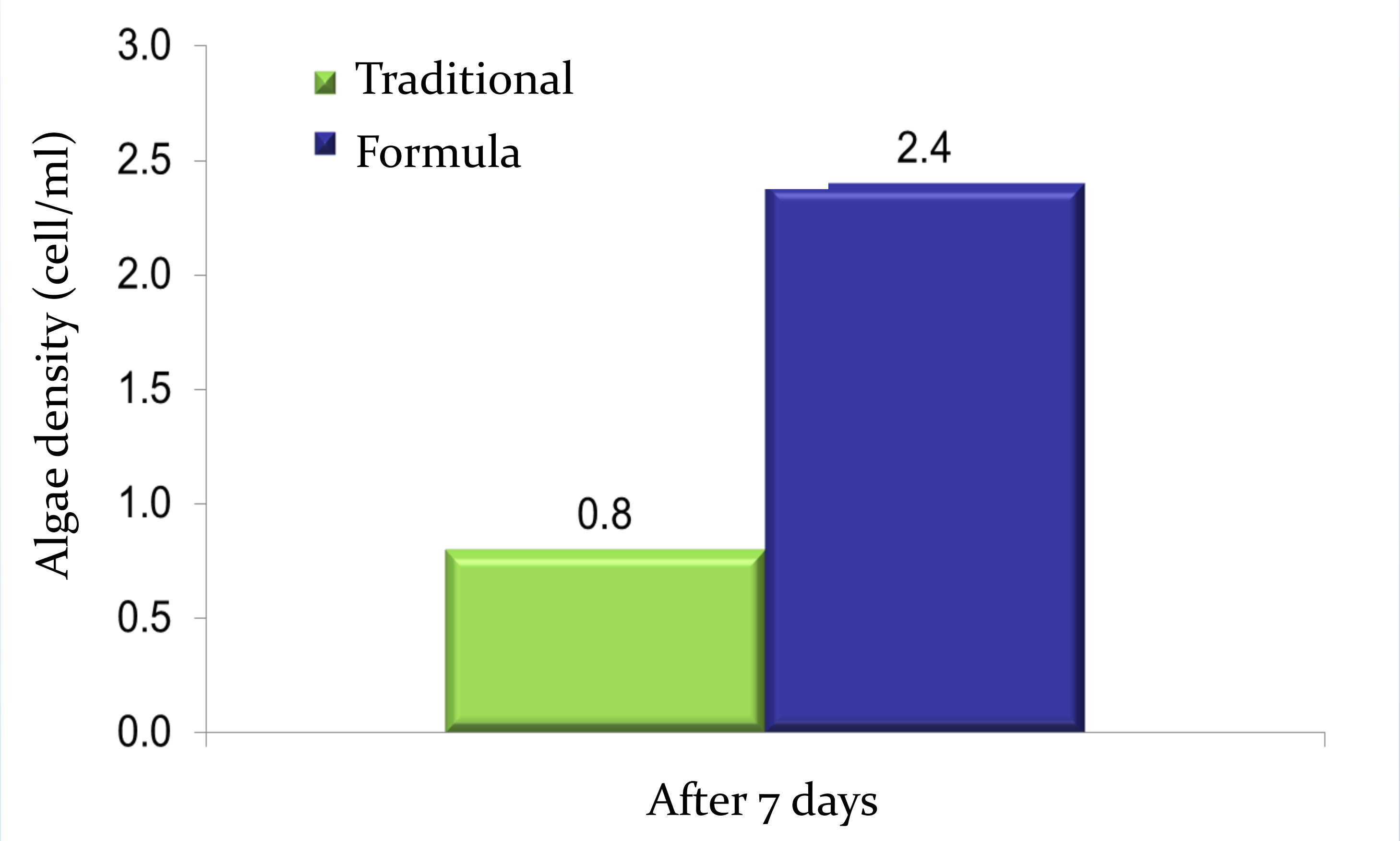
Day 4
- Pumping green water for *Artemia*

Day 5
- adding urea + D.A.P

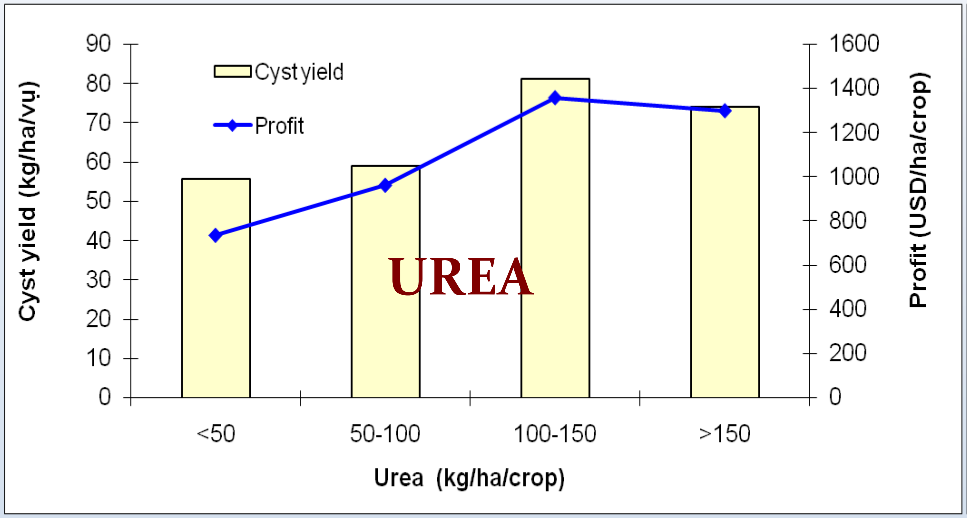
Day 6
- Pumping green water for *Artemia*

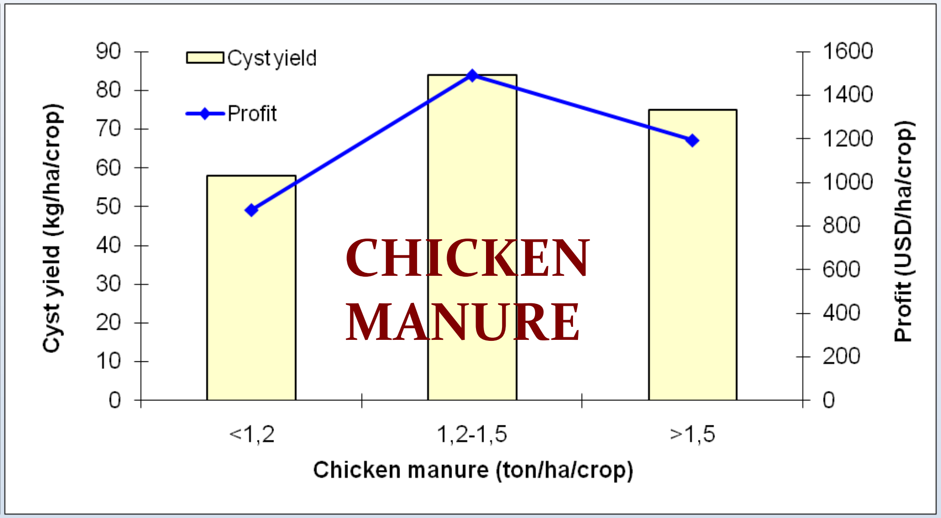
Day 7
- Sea water inlet
- Applying chicken manure or fish meal
- adding urea + D.A.P

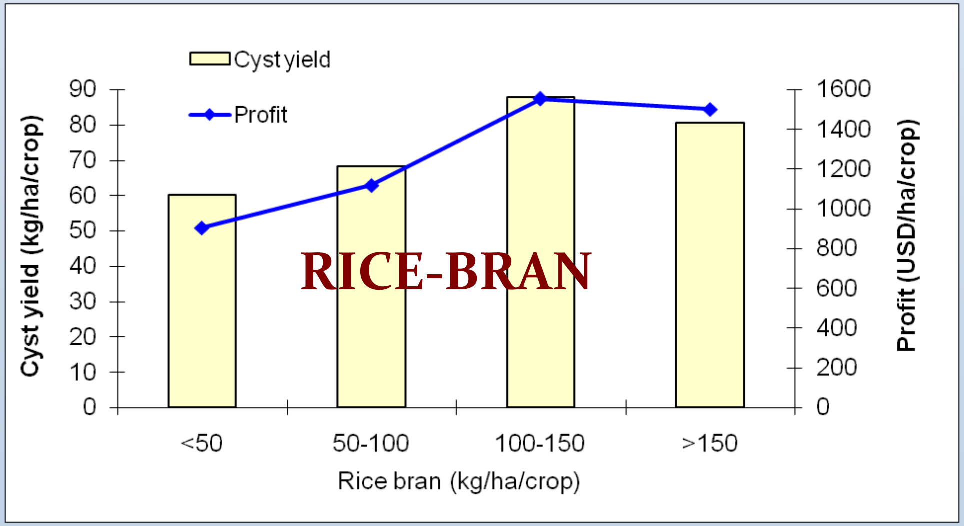
Alga concentration

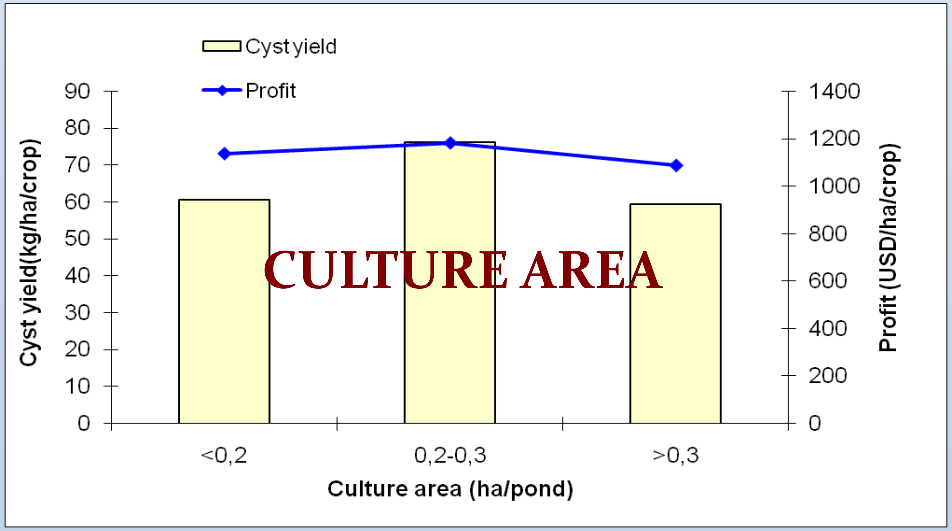


Effect of feed quantity/culture area on cyst yield and profit



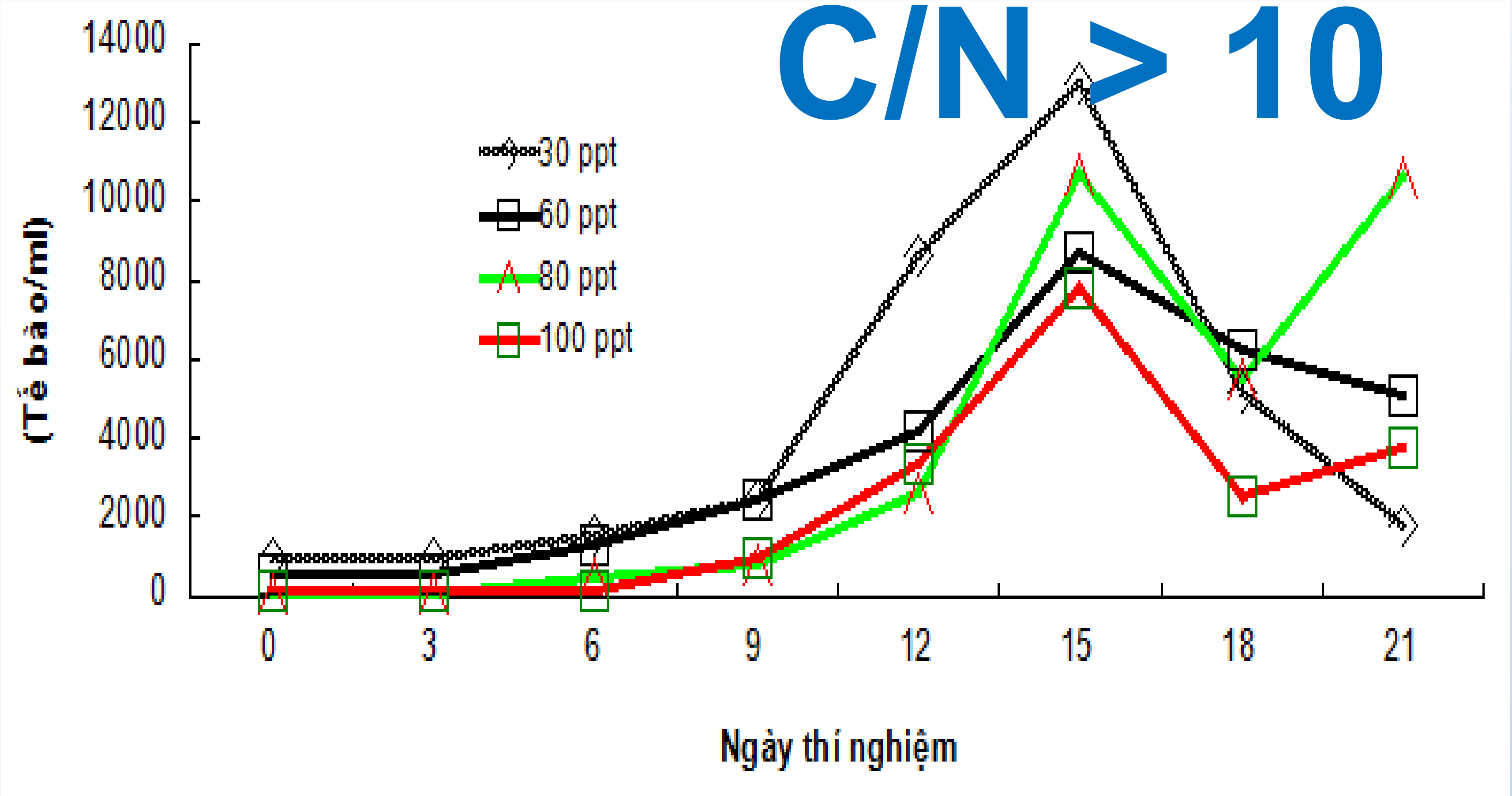






Biofloc as feed for *Artemia*

Total bacteria



Vibrio, Baccillus, Nitrosomonas and Nitrobacter, in which Bacillus is dominated group

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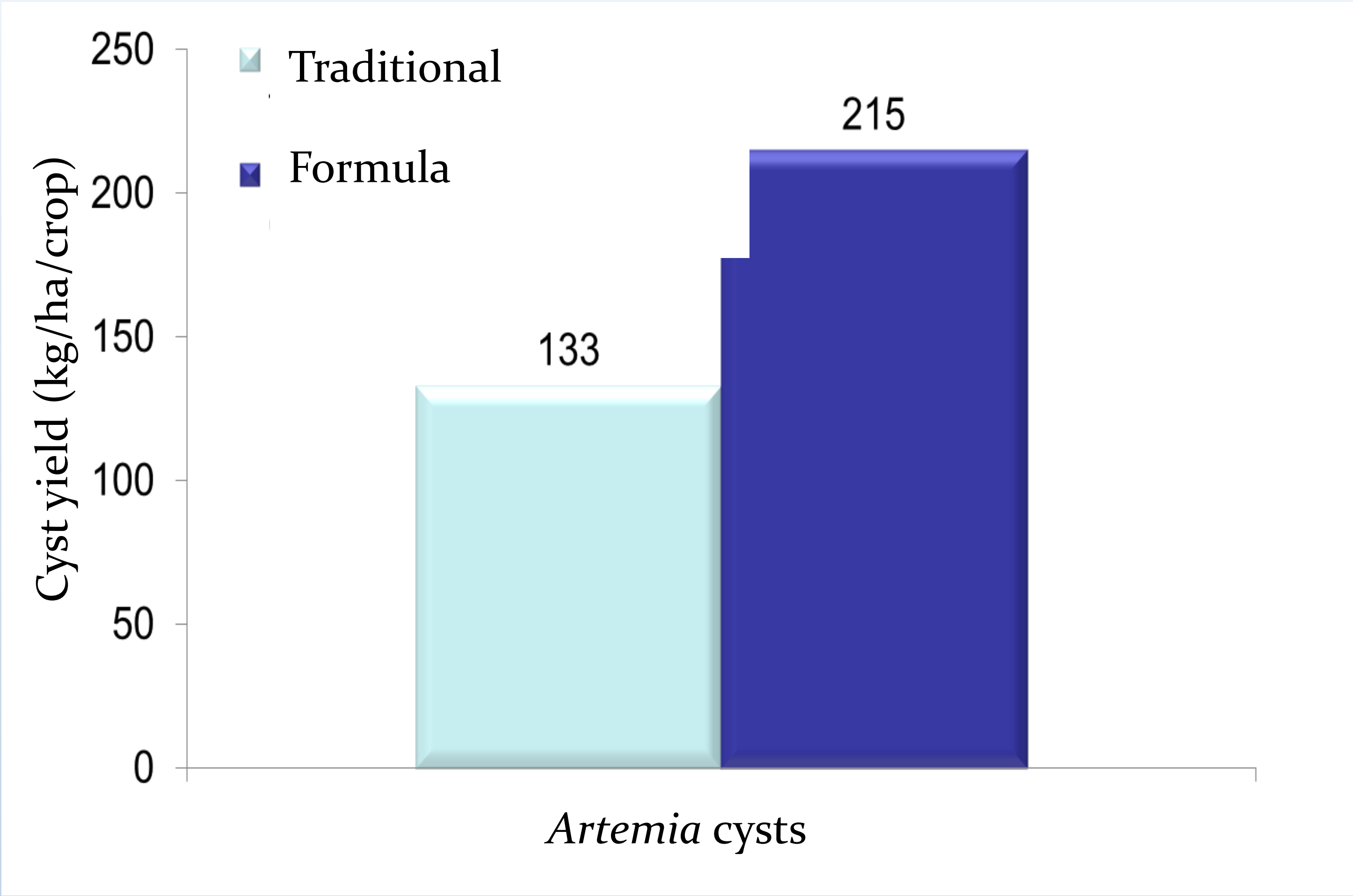
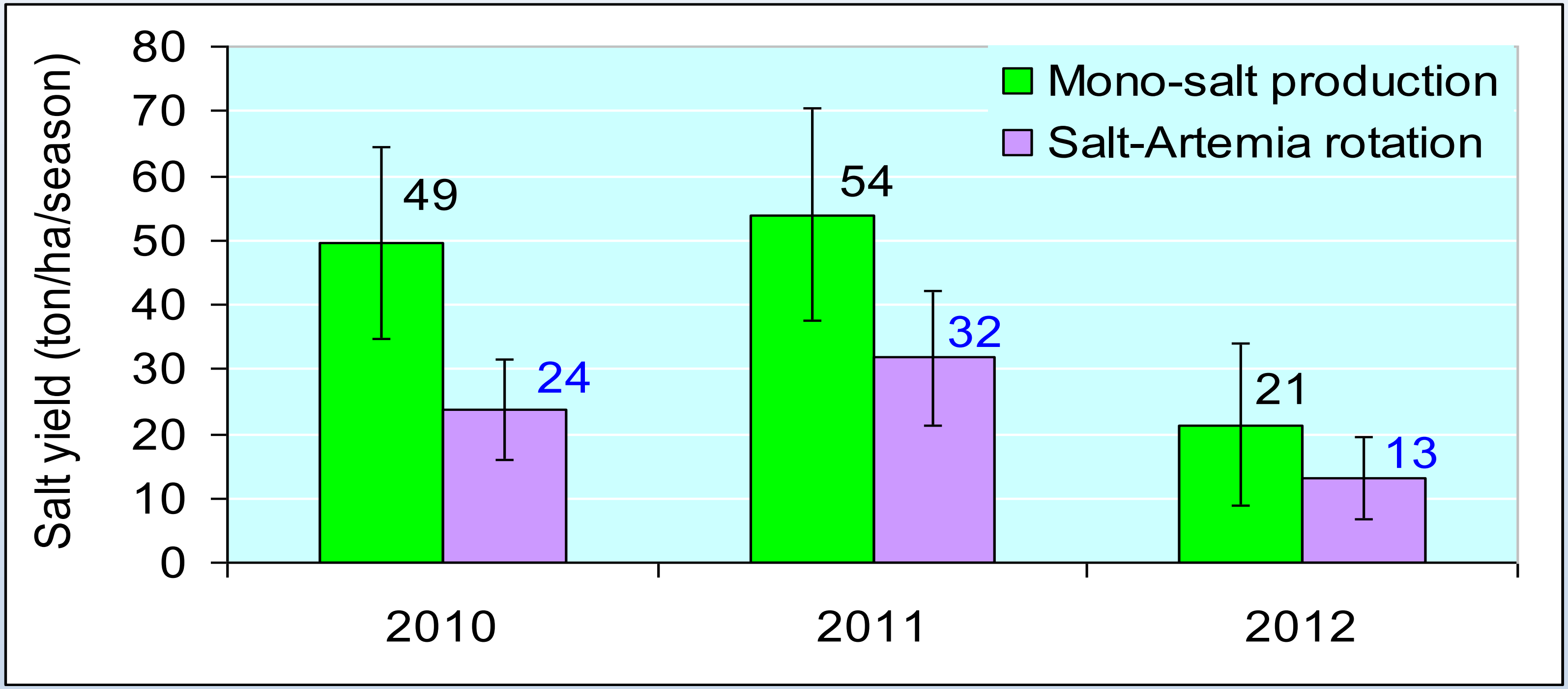
Cyst yield



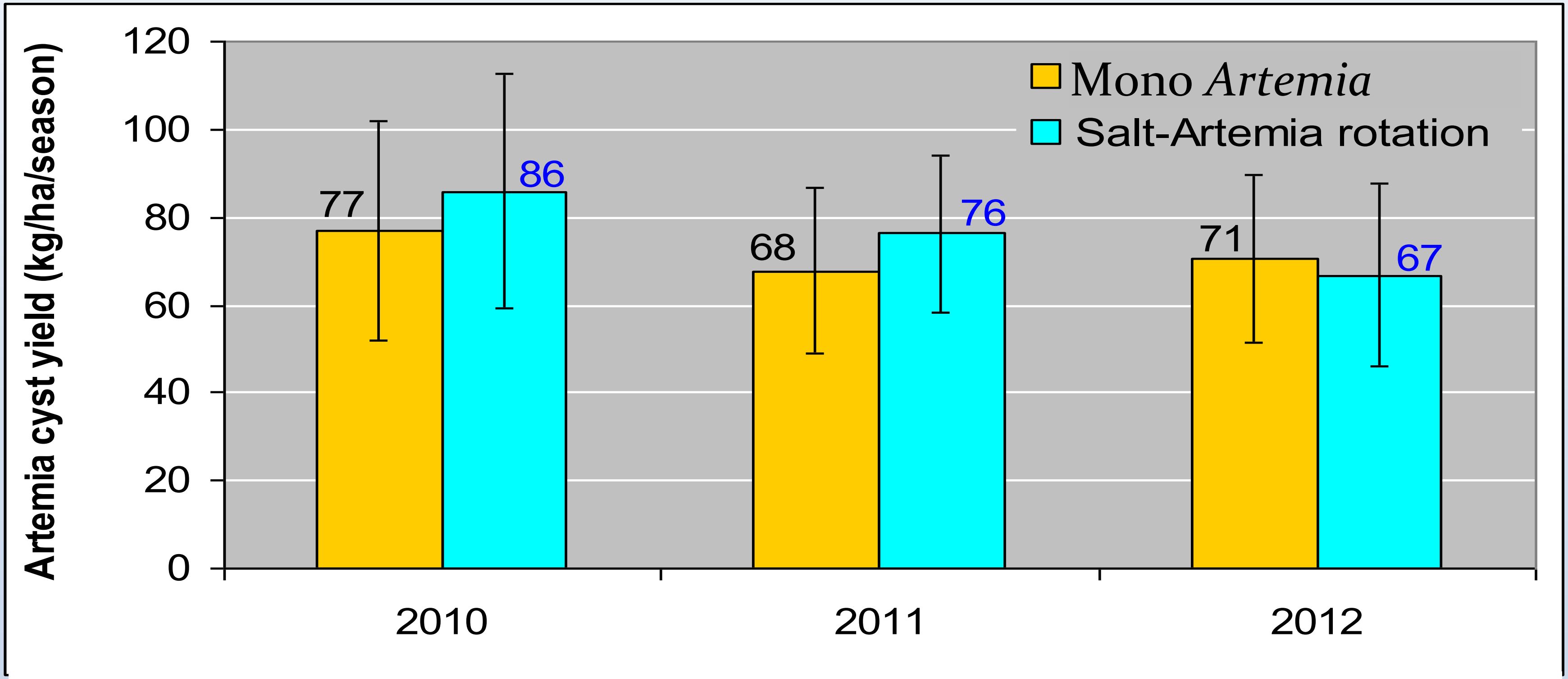
Salt production



Lower salt yield in rotative system compared to mono-system
Low salt yield in 2012: due to raining occur in-between dry season

(Nguyen Thi Ngoc Anh, 2013)

Artemia cyst production



Cyst yield in rotative system comparable to monosystem due to acceptable salinity range maintained at the beginning of the rainy season

(Nguyen Thi Ngoc Anh, 2013)

Salt and *Artemia* cyst productivity and yield

Description	Soc Trang (n=40)		Bac Lieu (n=30)		Medium (n=70)	
	Artemia (n=22)	*Artemia*-salt (n=18)	*Artemia* (n=15)	*Artemia*-salt (n=15)	*Artemia* (n=37)	*Artemia*-salt (n=33)
1. Productivity						
- *Artemia (kg)*	57.34±20.27	60.12±33.11	34.49±21.86	41.4±41.69	28.07±23.55	51.35±38.42
- Salt (tonne)		69.11±25.33		50.85±23.26		60.81±25.74
2. Yield						
- *Artemia (kg)*	100.32±42.06	124.44±75.94	59.93±30.49	74.27±73.38	83.95±42.39	101.63±77.87
- Salt (tonne)		146.39±66.64		138.13±75.57		142.64±69.82

Hong Thi Hai Yen (2014)

Income, profit and ratio profit

Unit: Million VND/ha/crop

Description	Soc Trang (n=40)		Bac Lieu (n=30)		Medium (n=70)	
	Artemia (n=22)	*Artemia*-salt (n=18)	*Artemia* (n=15)	*Artemia*-salt (n=15)	*Artemia* (n=37)	*Artemia*-salt (n=33)
Income	68.61±35.56	119.73±43.06	29.98±16.21	93.51±45.34	39.75±16.56	107.81±45.39
Profit	41.48±34.31	84.85±48.14	7.73±20.2	68.61±39.94	15.52±17.54	77.47±2.97
Ratio profit	1.67±1.28	3.17±2.55	1.3±3.38	2.74±1.35	1.08±2.17	2.97±2.72

Hong Thi Hai Yen (2014)

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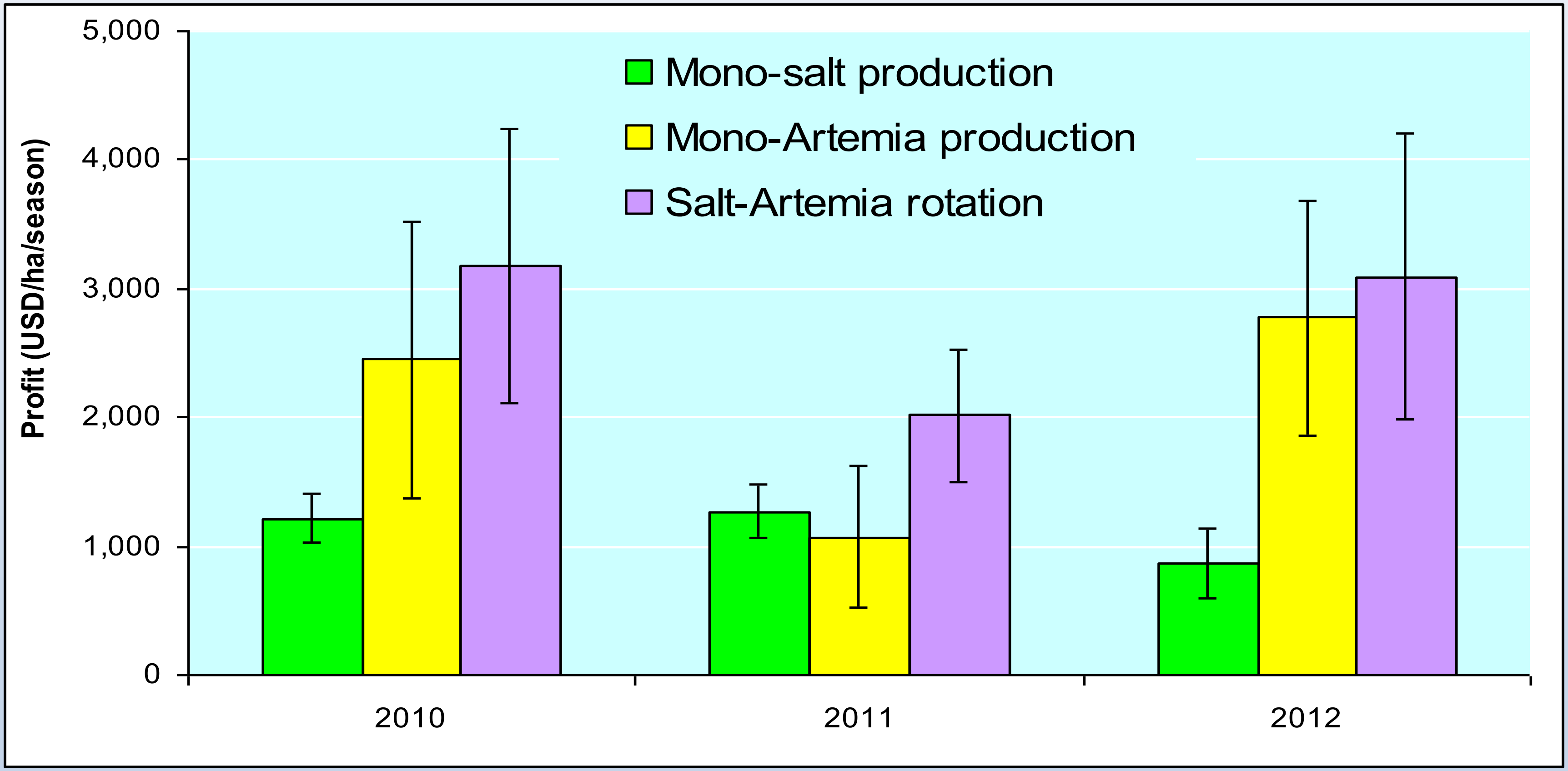
Net profit



Conclusions

- **Vinhchau solar salt works:** suitable biotopes for rotational/integration production of salt and *Artemia* in dry season

- Rotational/integration system:

  - ✓ helps to enhance *Artemia* production as farmers could maintain only the high production ponds, the rest could be kept for salt production
  - ✓ obtained higher profits than the mono production systems → improve the use of land resources and income for farmers
  - ✓ could adapt to climate changes;

- Feeding management is the main concern and improvement of fertization and application of biofloc, formulated feed helped to enhance and sustain of cyst production.

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Selective breeding research with *Artemia*: a possible model for commercial crustacean species?

Sheng Luan, Zhiwei Zhang and Jie Kong
Yellow Sea Fisheries Research Institute, CAFS,
Qingdao, Shandong, China

An model animal for quantitative genetics

- ***Artemia sinica*** is only found in China and named as an unique bisexual species.

※The advantages for *Artemia sinica* as the animal model

Short life cycle: **multiple generations;**
Small body size: **low cost of rearing families;**
Dormant cysts: **short period of family production, easily stop at an assigned generation**

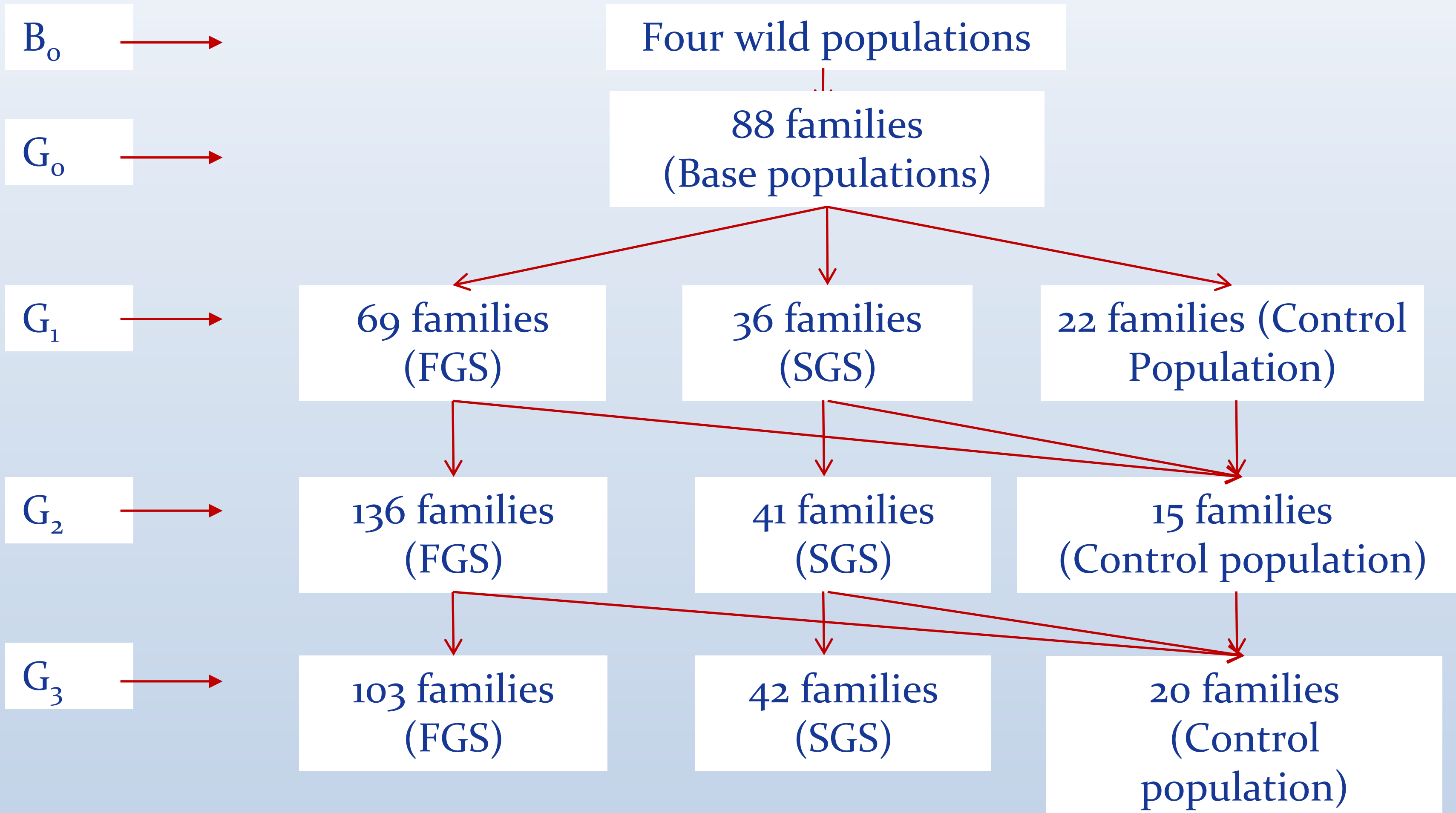
1. Breeding program

- 1.1 Breeding goal

  Fast-growing strain (FGS), Slow-growing strain (SGS)

- 1.2 Founder populations

1.3 Production of families

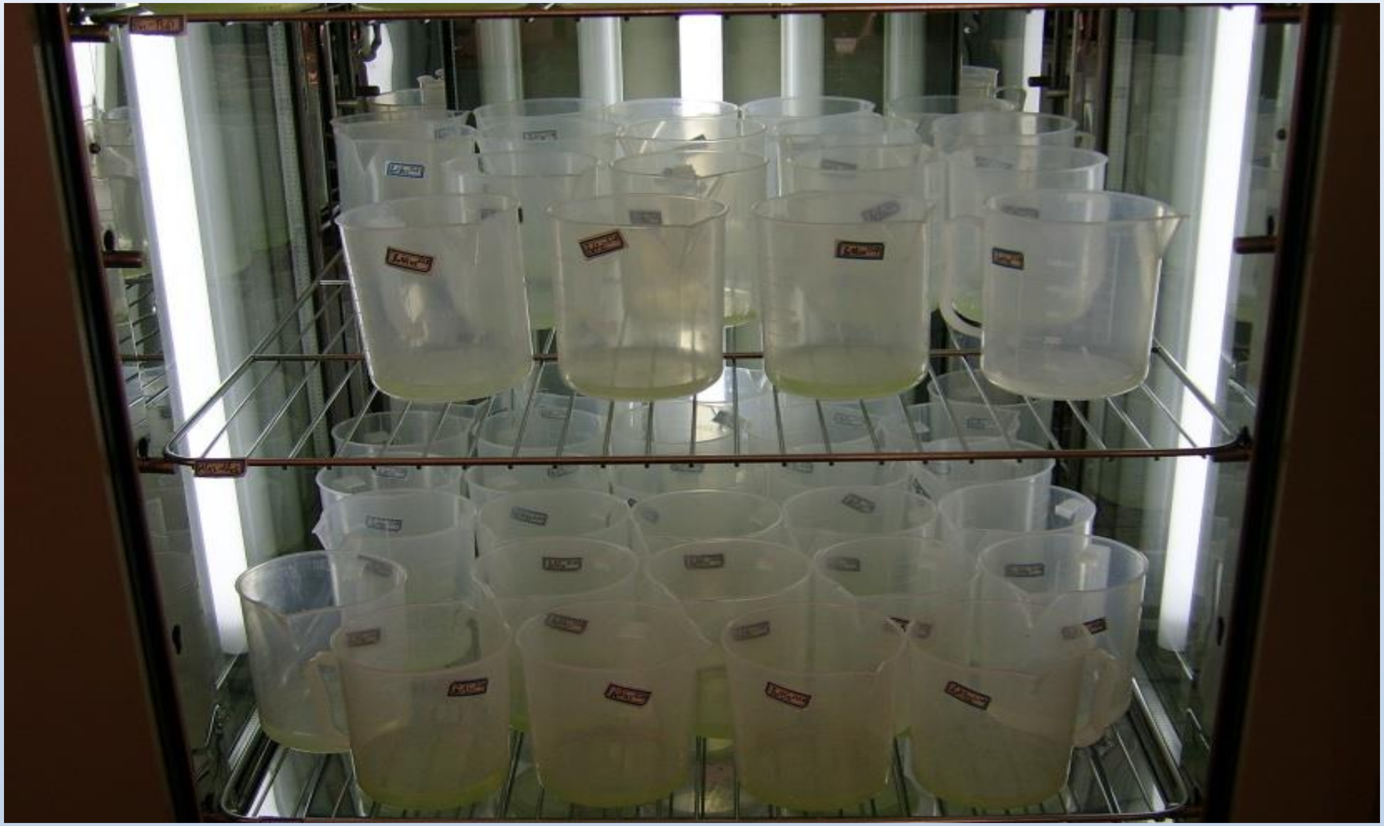


The facilities of eggs hatching

400 L illumination incubator

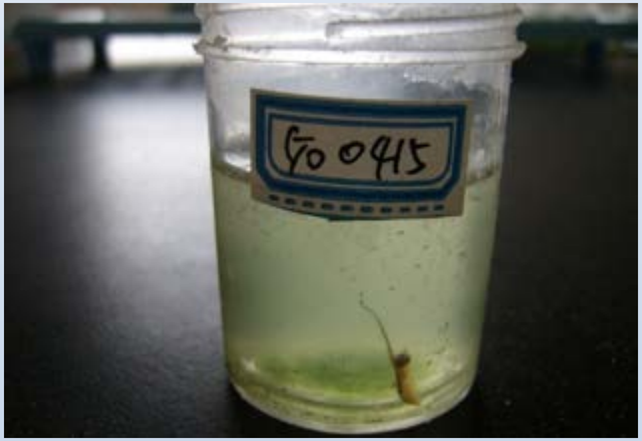
culture dish

- After 36 hours hatching, nauplius per family were transferred to one 500ml plastic beaker and reared separately for 5d at 26℃ in 77 g/L seawater.



1.4 Growth and survival test

- Each Larva in each family was transferred to one 50 ml plastic vial which containing 35 ml seawater at 5th day.
- Body length, body weight and survival was measured at 25th day.

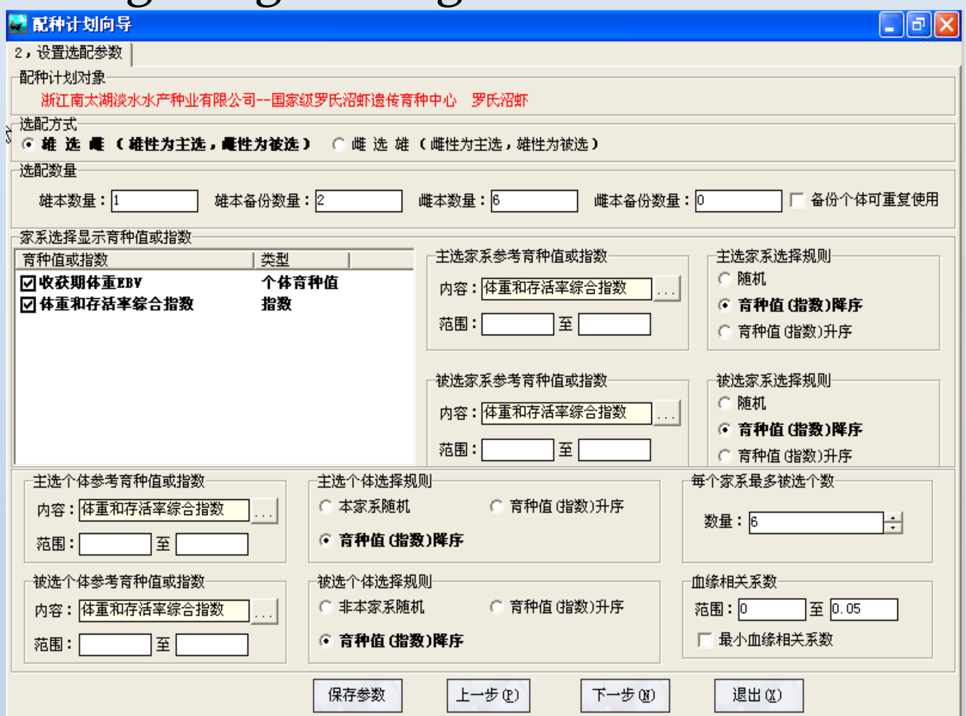


1.5 Evaluation of genetic parameter

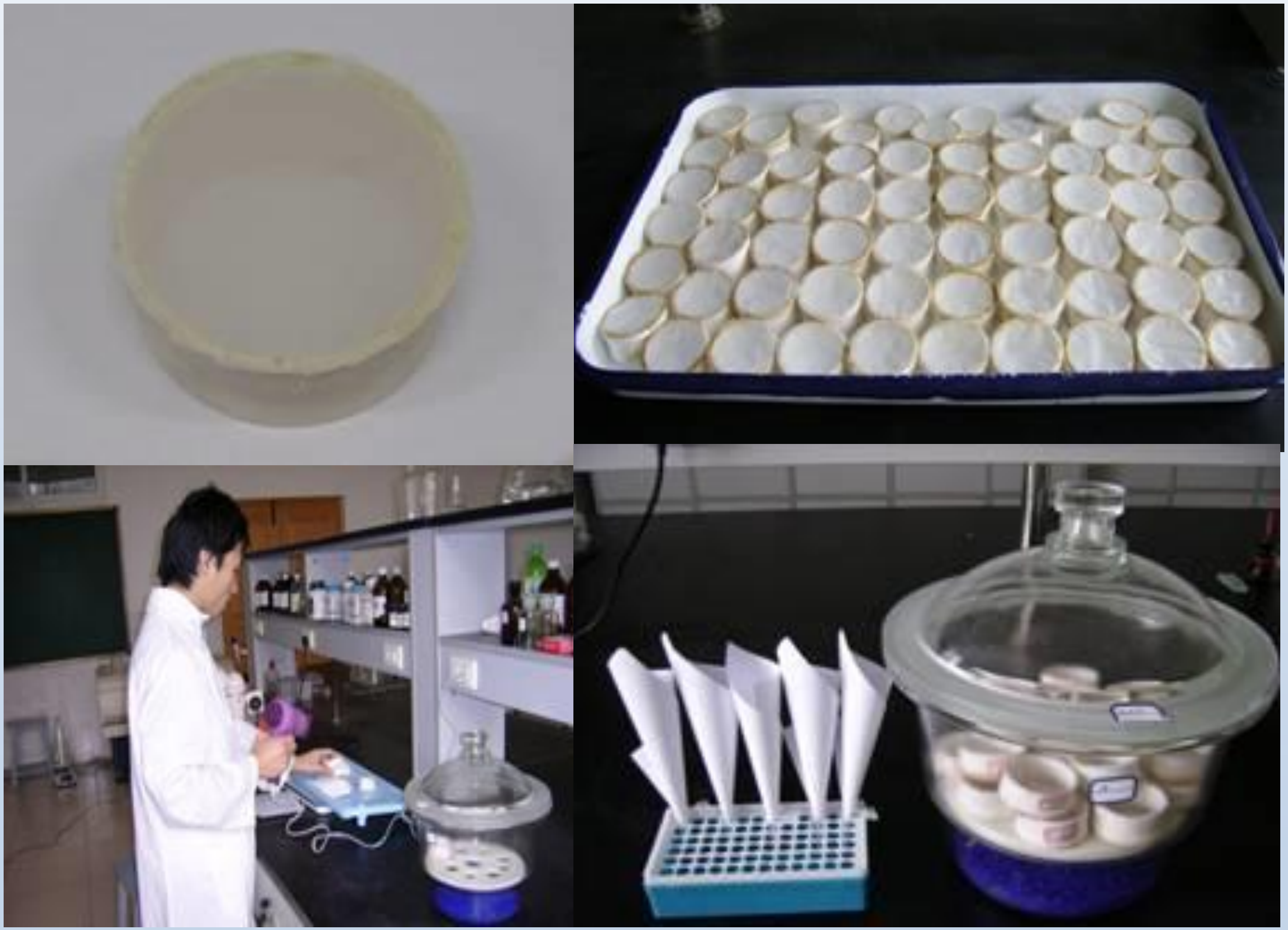
- Genetic parameters
  - Heritability (h^2)
  - Common environmental effects (c^2)
  - Genetic, phenotypic and environmental correlations
- Models and Methods
  - Animal model for growth trait, sire and dam model for survival
  - REML: Restricted Maximum Likelihood
  - BLUP: Breeding value of each animal
  - The complete pedigree

1.6 Selection of new breeders

- Animals with high/low selection index will be selected as new breeders
- Rational mating scheme:
  - Limiting the inbreeding coefficient in each generation
  - Maximizing the genetic gain



1.7 Collection and drying of dormant cysts



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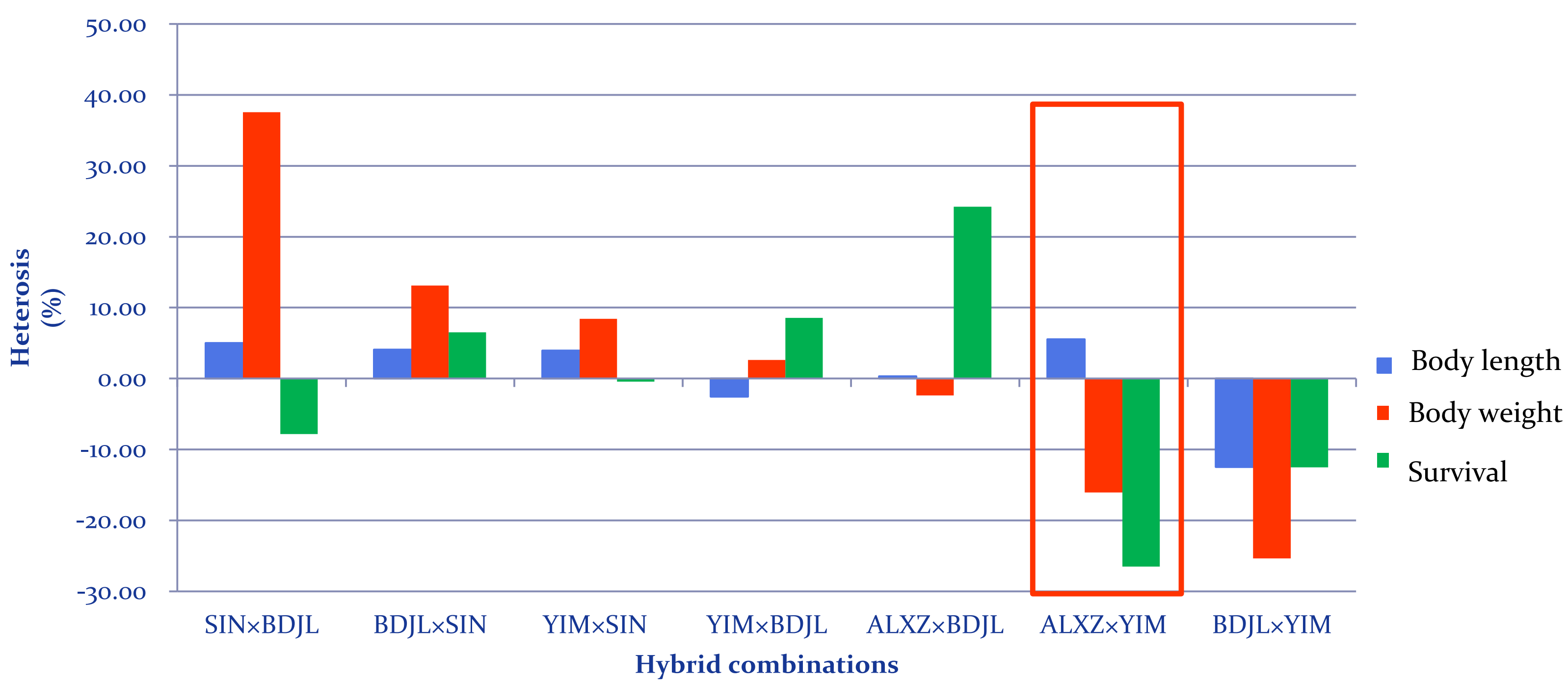
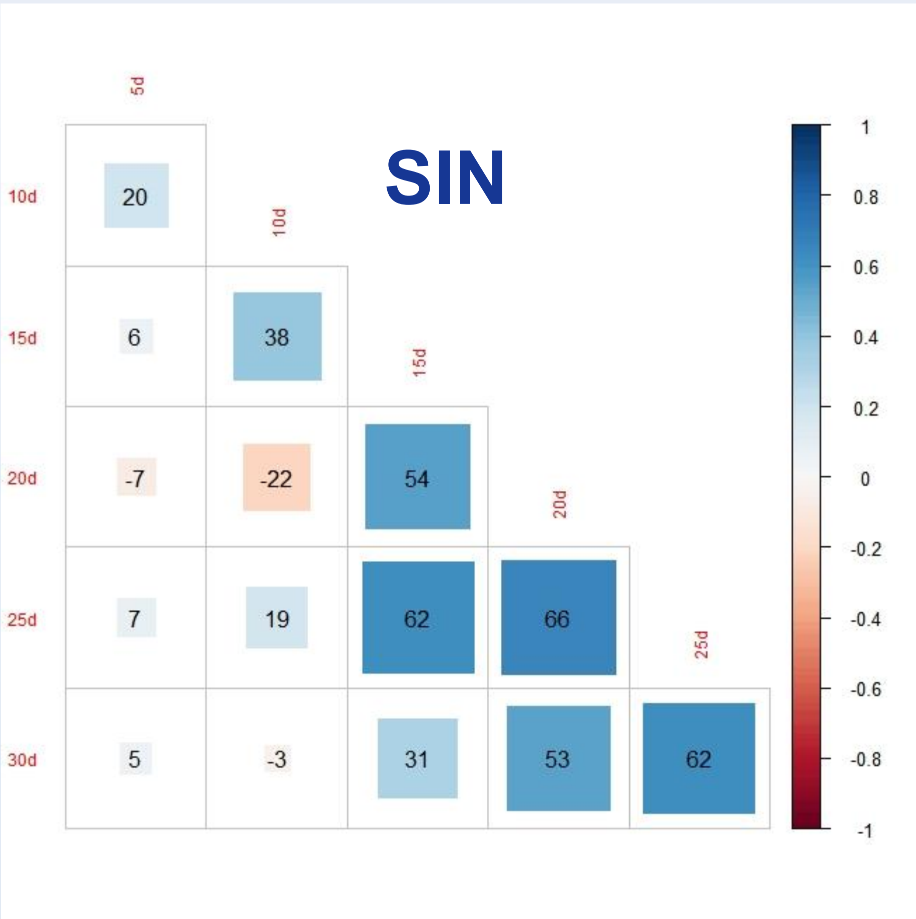
2. Results

2.1. Analysis of heterosis for four strains



2.2 Correlation coefficients of total length at six developmental stages

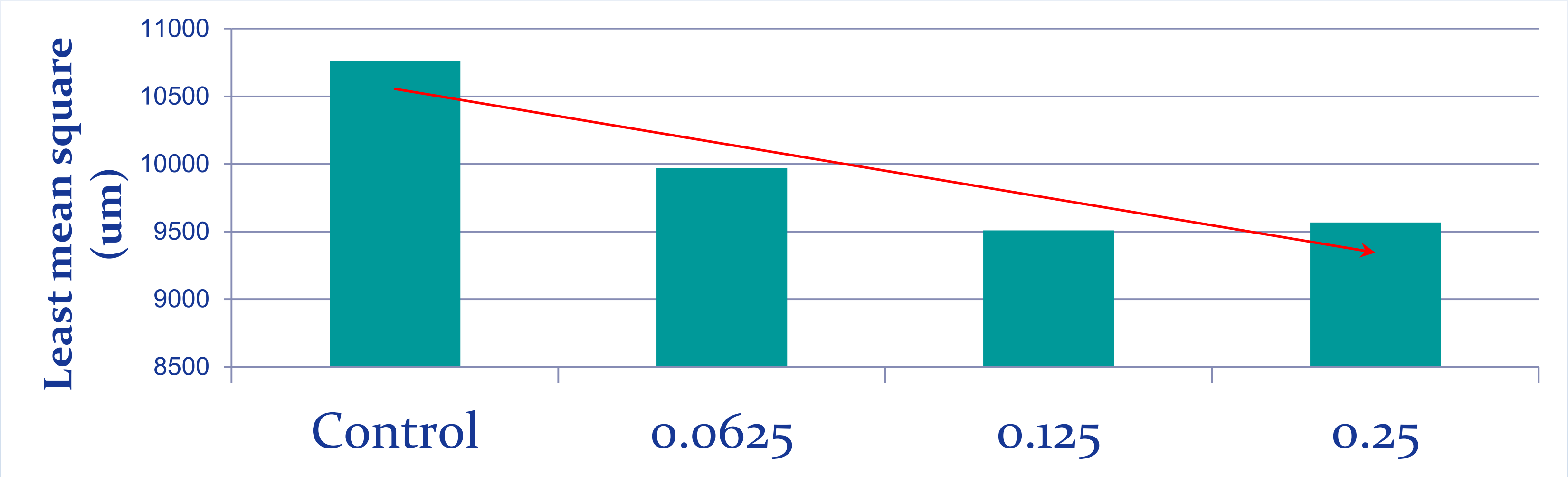


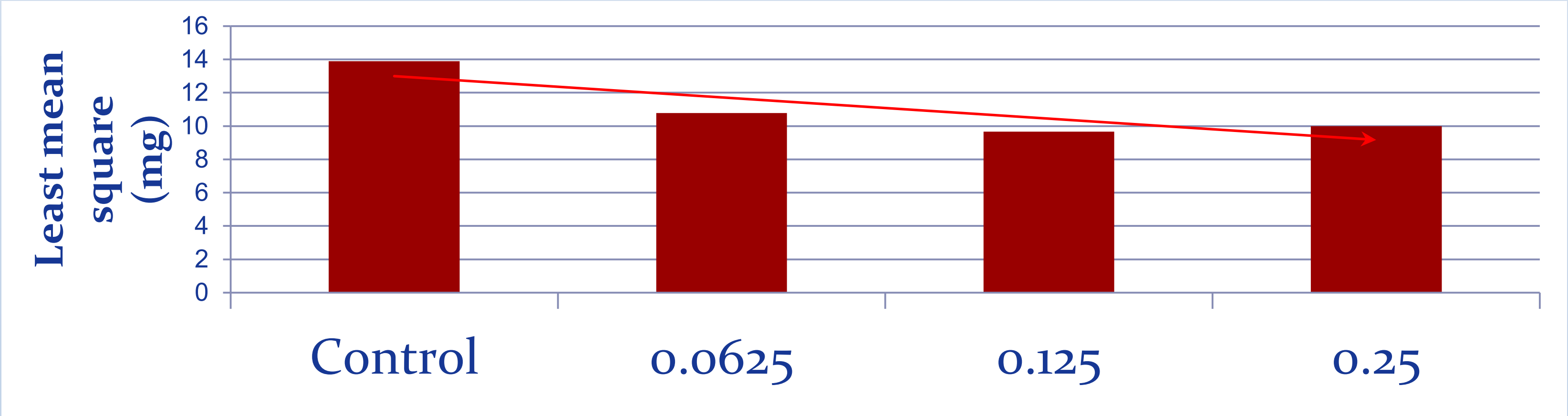
2.3 Genetic parameters

Estimates of the heritability, common environment coefficients for harvest body length of *Artemia sinica* across generation in FGS and SGS

Line	Body length		Survival	
	$h^2 \pm$S.E.	$c^2 \pm$S.E.	$h^2 \pm$S.E	$c^2 \pm$S.E.
FGS	0.28±0.036	0.11±0.015	0.34±0.051	0.26±0.087
SGS	0.21±0.074	0.13±0.038	0.43±0.06	0.17±0.092

2.4 Inbreeding effect on growth traits





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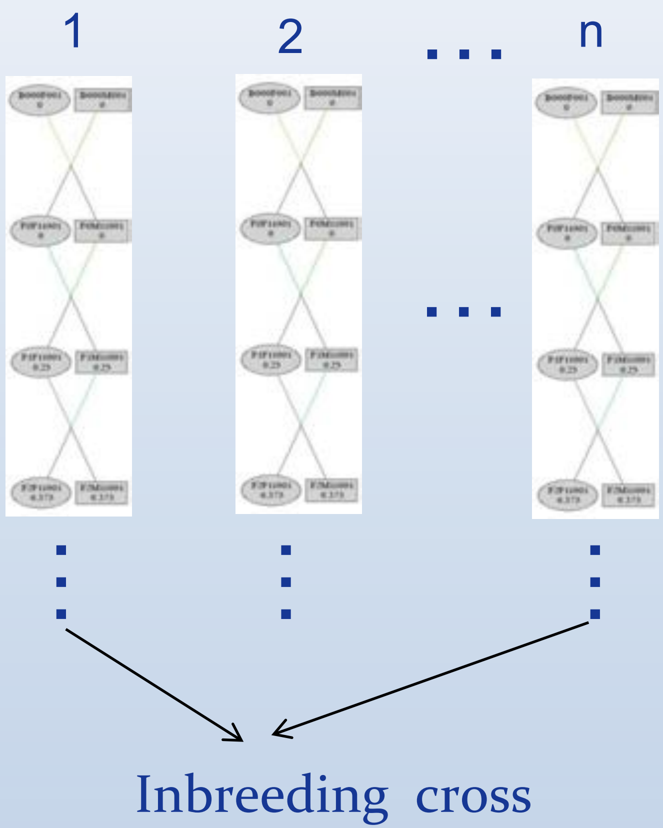
3. The genetics of inbreeding depression and heterosis



Inbreeding depression and heterosis are predominantly caused by **the presence of recessive deleterious mutations** in populations (Nature Reviews Genetics, 2009).

- Evidence for deleterious mutation:
  - GWAS analyses of inbreeding depression
  - Epigenetics and differential gene expression
  - Tests for balancing selection using DNA sequence diversity
- Possible causes of overdominance.
  - The mechanisms that cause homozygosity for particular alleles to result in lower survival and reproductive capacities.

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4. Genotype and environment interaction at genomic level

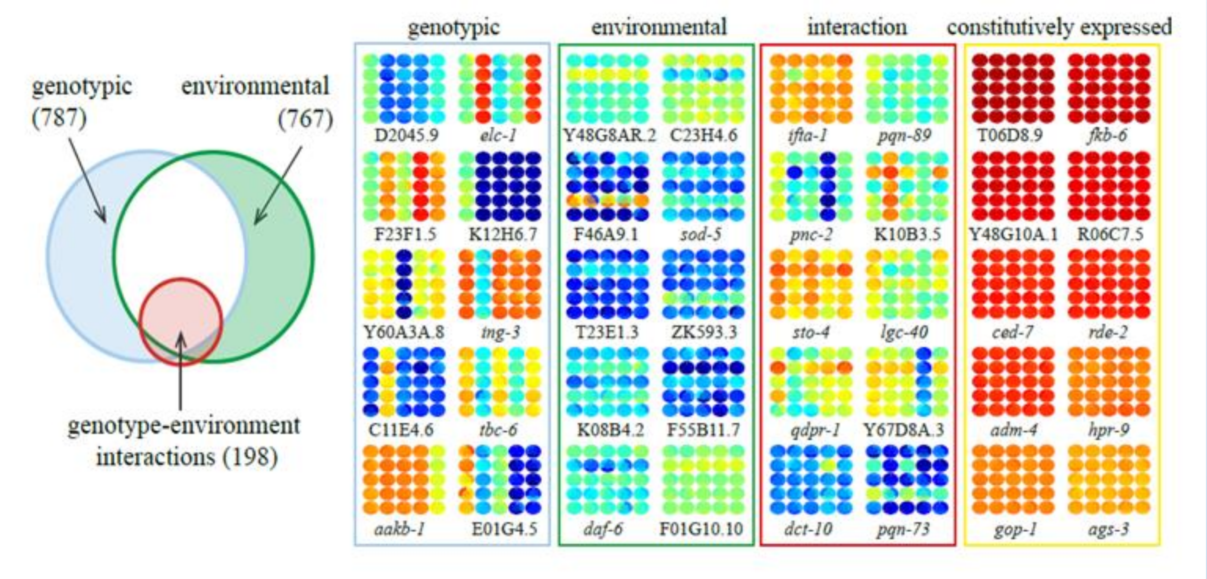
Production of families (40 to 60)

↓

Test of performance for families (density; salinity; temperature)

↓ **Performance evaluation by BLUP**

Genotype by environment correlations analysis (gene; allele frequencies)



(*Molecular Systems Biology*, 2012)

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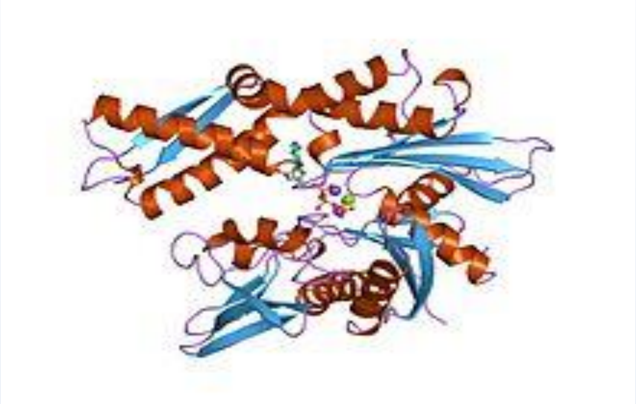
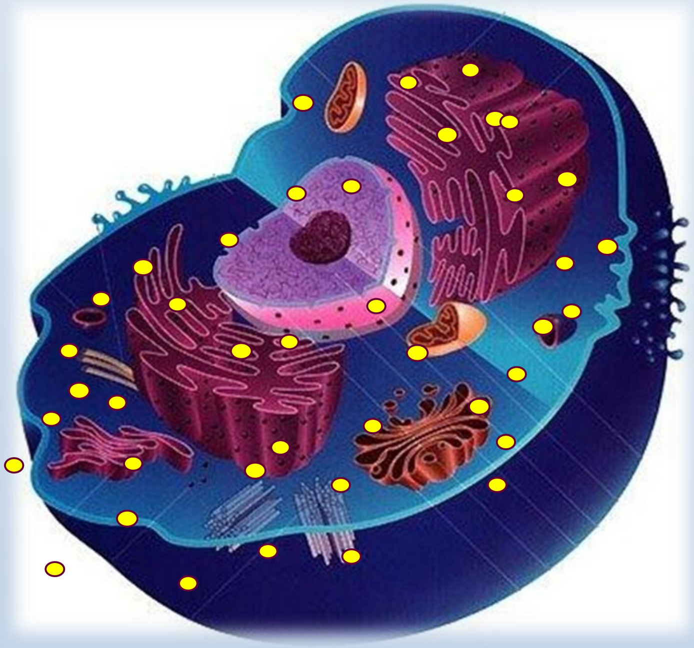
Heat shock proteins in *Artemia*: role and applications in aquaculture

Yeong Yik Sung
University Malaysia Terengganu, Malaysia

Heat shock protein



Human Hsp70



- Proteins synthesized constitutively in all living cells (heat shock cognates)

- HS and many other stresses induce transcription of sets of proteins referred to as **heat shock protein (Hsp).**

- Intracellular functions
  - protein chaperones
  - repair damage/aggregated protein
  - translocation of protein
  - targets protein degradation

Classes of Hsps

Approximate molecular weight (kDa)	Prokaryotic proteins	Eukaryotic proteins
10 kDa	GroES	Hsp10
20-30 kDa	GrpE	Hsp20
40 kDa	DnaJ	Hsp40
60 kDa	GroEL	Hsp60
70 kDa	DnaK	Hsp70
90 kDa	HtpG, C62.5	Hsp90
100 kDa	ClpB, ClpA, ClpX	Hsp100

Hsp70 has a conserved amino-terminal ATP inding/hydrolysis domain (NBD) connected by a hydrophobic flexible linker to a variable, carboxyl-terminal substrate binding domain (SBD) capped by a lid structure of unknown function

Induce thermo tolerance(ITT)

- Coho salmon (Arkush *et al*., 2008) *Acta Zoologica* 89; 331-338)
- *M. rosenbergii* (Rahman *et al*., 2004) *Aquaculture* 230; 569-579)
- Oysters (Brown *et al*., 2004) *J. Shellfish Research* 23; 135-141)
- *Artemia* (Sung *et al*., 2008) *Cell Stress & Chaperones* 13; 59-66)
- *Cyprinus carpio* (Sung *et al*., 2012) J. Fish Diseases

confer protection against other abiotic stresses

- Salinity (Rahman *et al*., 2004)
- Desiccation (Ma *et al*., 2005)
- Heavy metals (Boone & Vijayan, 2002)
- Ammonia (Sung *et al*., 2012)

ELSEVIER

Fish & Shellfish Immunology 22 (2007) 318–326

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

Non-lethal heat shock protects gnotobiotic *Artemia franciscana* larvae against virulent Vibrios

Yeong Yik Sung [a,b,*], Els J.M. Van Damme [c], Patrick Sorgeloos [a], Peter Bossier [a]

[a] *Laboratory for Aquaculture & Artemia Reference Center, Faculty of Bioscience Engineering, Ghent University, Rozier 44, 9000 Gent, Belgium*

[b] *Department of Fishery and Aquaculture, Faculty of Agrotechnology and Food Science, University College of Science and Technology Malaysia (KUSTEM), 21030 Kuala Terengganu, Malaysia*

[c] *Laboratory of Biochemistry and Glycobiology, Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Gent, Belgium*

Received 21 March 2006; revised 18 May 2006; accepted 28 May 2006
Available online 8 June 2006

Abstract

Brine shrimp *Artemia* were exposed under gnotobiotic conditions to a non-lethal heat shock (NLHS) from 28 to 32, 37 and 40 °C. Different recovery periods (2, 6, 12 and 24 h) and different heat-exposure times (15, 30, 45 and 60 min) were tested. After these NLHS, *Artemia* was subsequently challenged with *Vibrio*. Challenge tests were performed in stressed and unstressed nauplii at concentrations of 10^7 cells ml^{-1} of pathogenic bacteria, *Vibrio campbellii* and *Vibrio proteolyticus*. A NLHS with an optimal treatment of 37 °C for 30 min and a subsequent 6 h recovery period resulted in a cross-protection against pathogenic *Vibrio*. A 100% increase in the larval survival ($P < 0.05$) was observed. We have also demonstrated by Western blot that a NLHS increases the expression of HSP-70 in heat-shocked (HS) treated animals. This report is the first to reveal a cross protection of a NLHS against deleterious bacterial challenges in living crustaceans. The putative role of heat shock proteins (HSPs) in this process is discussed.

Keywords: Non-lethal heat-shock; Heat shock proteins; *Artemia franciscana*; Immune response; Challenge test; *Vibrio campbellii*; *Vibrio proteolyticus*

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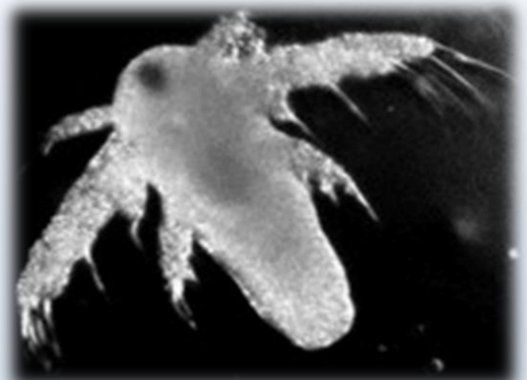
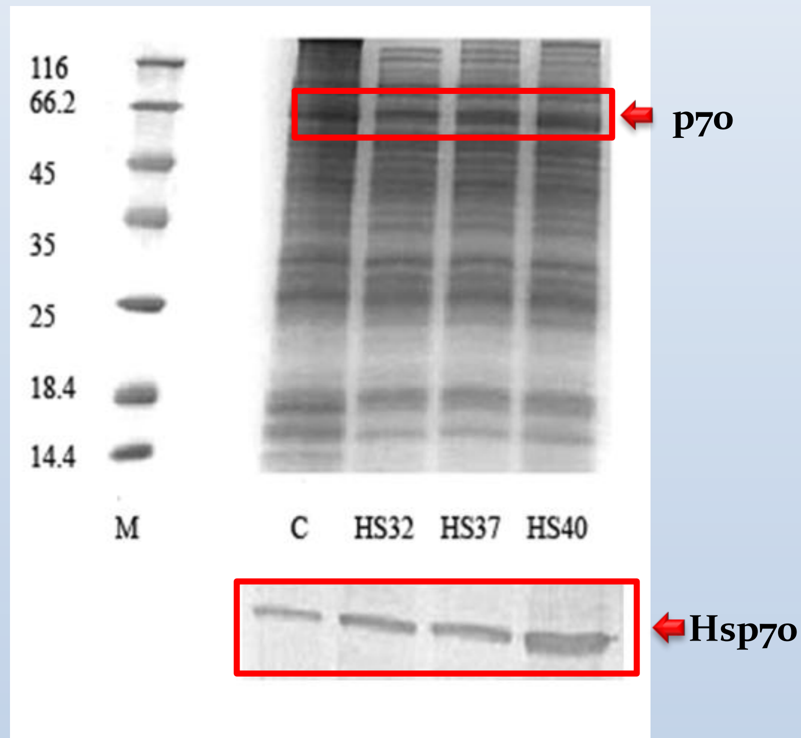
Protection against vibriosis



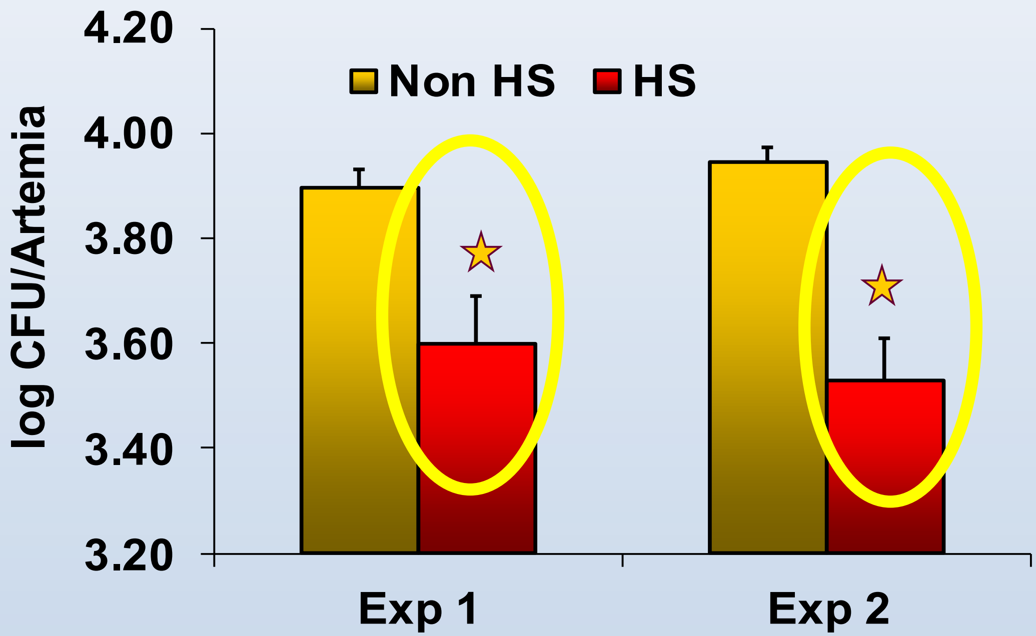
A non-lethal HS at 37°C for 30 minutes with 6 hour recovery (induced Hsp70 expression) protects brine shrimp *Artemia* against Vibriosis

HS treatments (ºC)	24h Survival (%)
Non-HS	36 ± 4[a]
HS 32	**65 ± 2[b]**
HS 37	**70 ± 7[b]**
HS 40	**68 ± 8[b]**



Sung *et al.* (2007) *Fish Shellfish Immunol.*

Vibrio campbelii colonization in gnotobiotic *Artemia*



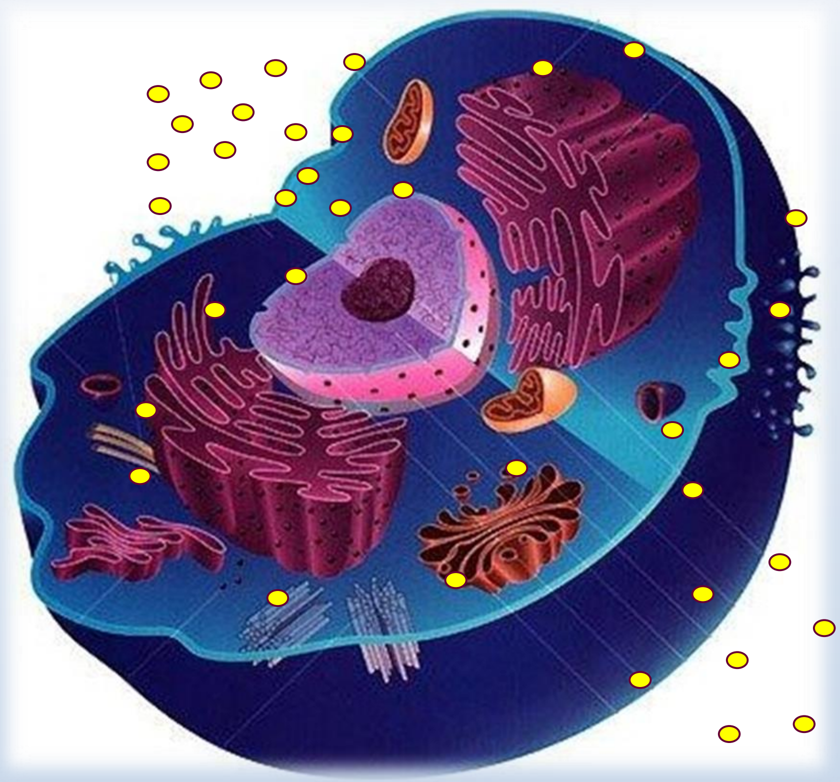
- Reductions of approximately 49 and 61% after HS

Sung *et al.* (2008) *Cell Stress & Chaperones*

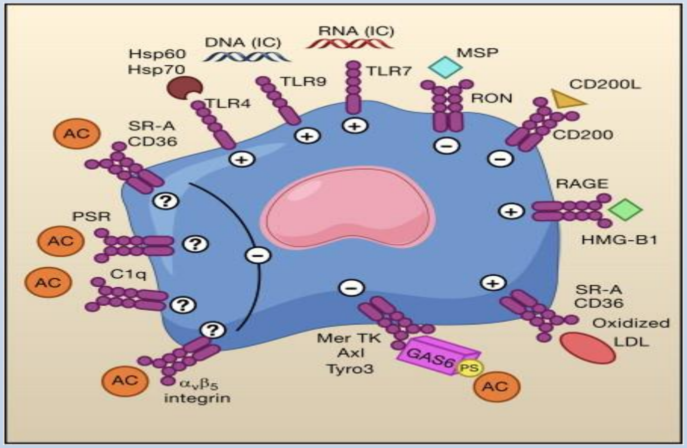
- Enhanced survival is due to enhanced ProPO activity

Baruah *et al.* (2011) *Fish and Shellfish Immunol.*

Extracellular functions



- **Play significant role in immune system**
  - Mediate production of cell surface peptides – recognize disease cells
- Transduce inflammatory danger signal to immune cells
 (e.g. Toll-Like receptor)



What happens if we knock-down Hsp70 in *Artemia*??

RNA interference (RNAi)

Therapeutic strategy based on highly specific and efficient silencing of a target gene , triggered by dsRNA.

- RNAi research almost exclusively focused on **single-target studies**, to selectively knockdown the **function of a single gene**).
- Best approach for "proof of principle" experiments .
- The silencing spread through several generations.

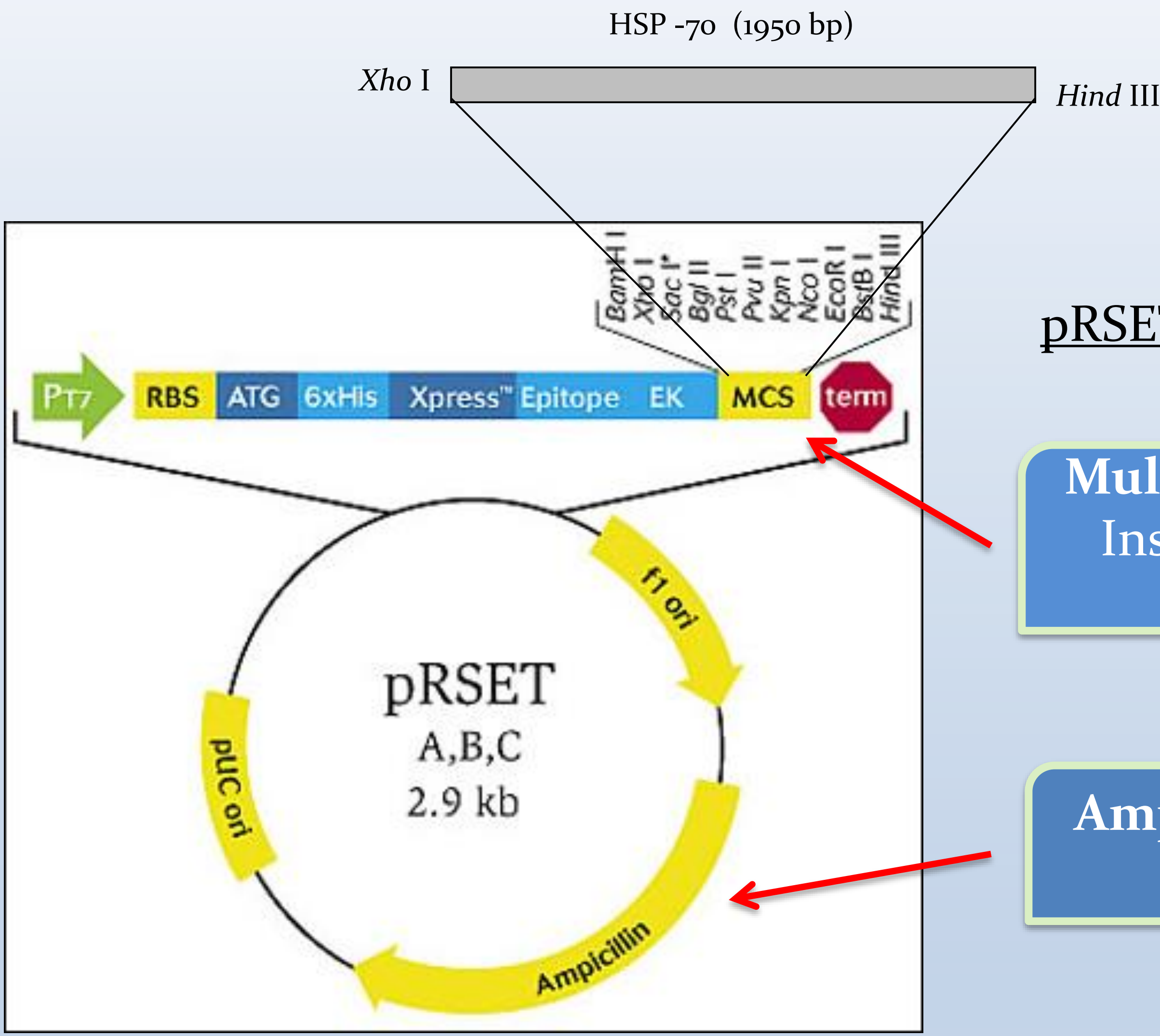
Introduction of dsRNA → **targeted mRNA Degradation of mRNA** → **Knockdown of targeted gene, prevent transcription into protein**

Objectives

To investigate roles of HSP70 in:

1) **Embryo development**
2) **Thermal resistance**
3) **Bacterial (*Vibrio*) tolerance**

Plasmid construction



pRSET-C-HSP70 plasmid

Multiple cloning site
Insertion of gene of interest

Ampicilin resistance gene

Construction of HSP70 dsRNA

pRSET-C-HSP70 plasmid

Transformation
into *E. coli* competent cells

Plasmid DNA Extraction
using miniprep kit (Sigma Aldrich)

PCR
using Taq DNA Polymerase (Invitrogen)
Forward :5'-TAATACGACTCACTATAGGGATTCTCAAAGACAAGC-3'
Reverse :5'-TAATACGACTCACTATAGGGCATAGAGCTTGGTAAT-3'

Production of HSP70 dsRNA
using MEGAscript RNAi Kit (Ambion)

GFP dsRNA (control)

Forward :5'-TAATACGACTCACTAAGGGAGACACATGAAGCAGCACGACCT-3'
Reverse :5'-TAATACGACTCACTATAGGGAGAAGTTCACCTTGATGCCGTTC-3'

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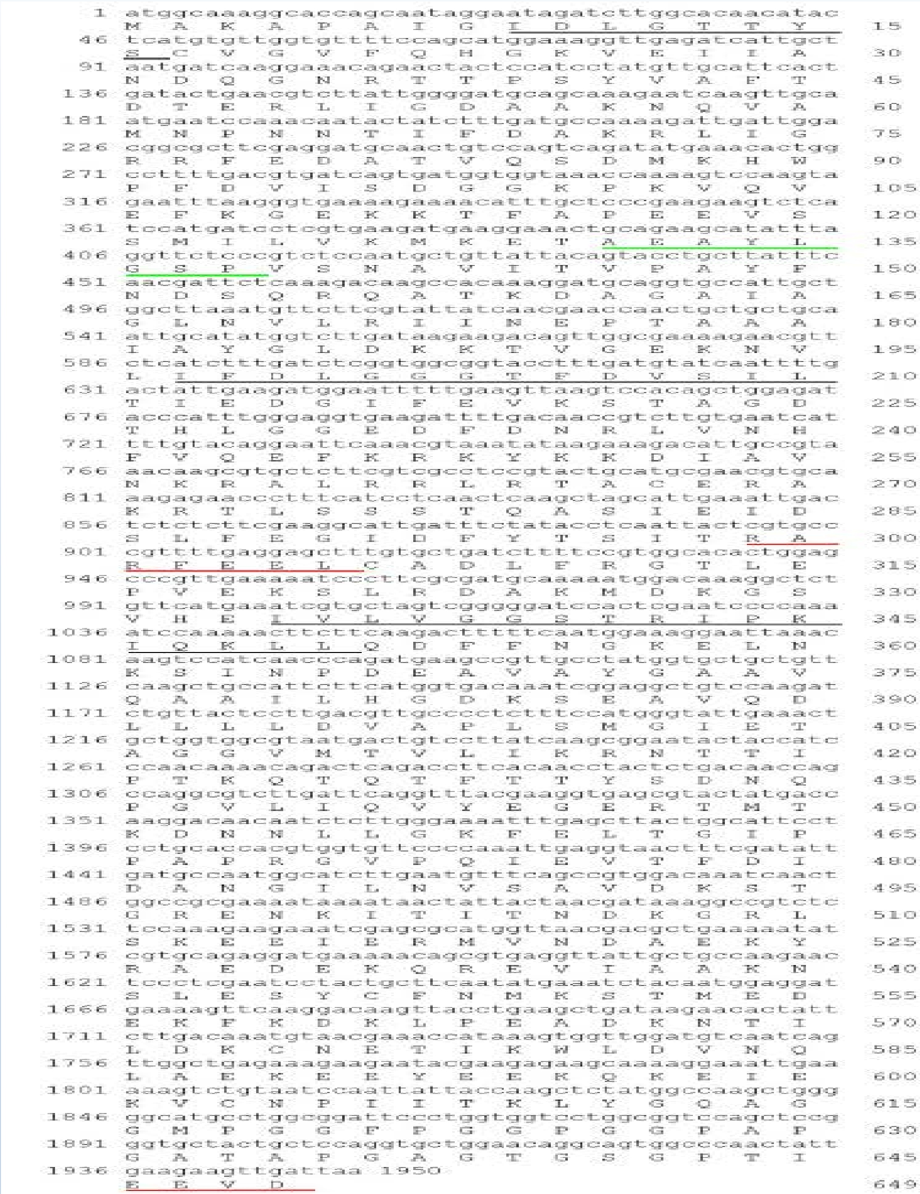


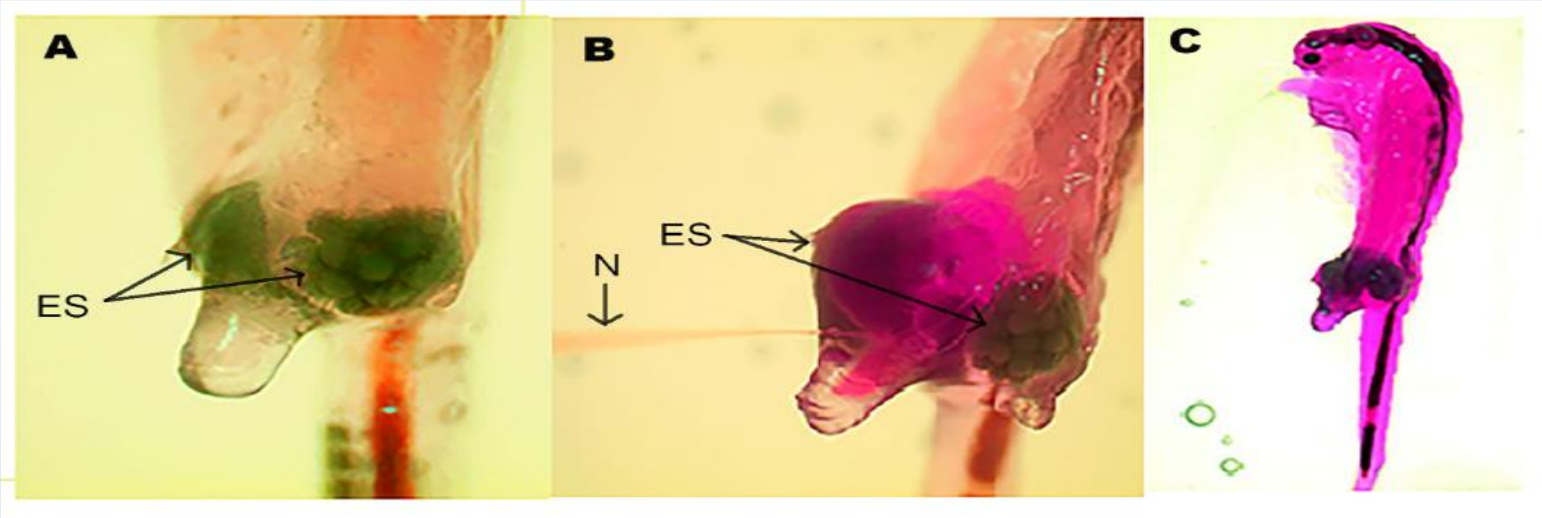
Sequence of *A. franciscana* Hsp70.

- The amino acid sequence of the *A. franciscana* Hsp70 isoform was deduced from the nucleotide sequence of the cloned *A. franciscana* cDNA used herein to make dsRNA.
- The amino acids underlined by solid black and green lines are respectively signature motifs and the ATP binding site of Hsp70 family members.
- The stress protein motif and the carboxyl-terminal EEVD motifs characteristic of Hsp70 family members are underlined by solid red lines.

Micro-injection of *Artemia* females with Hsp70-dsRNA

Injection solution
1 : 1 (v/v) ratio
dsRNA : 0.5% phenol red

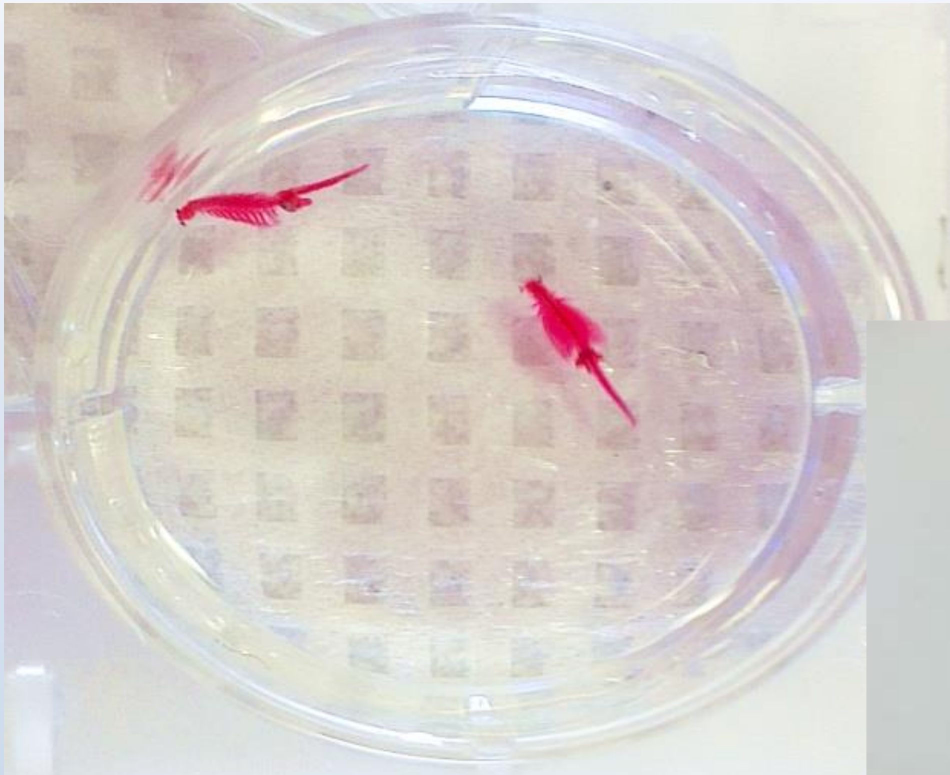
Adult female immobilized on cooled 3% agar dish



Unfertilized egg sacs **Micro-injection** **Post-injection**

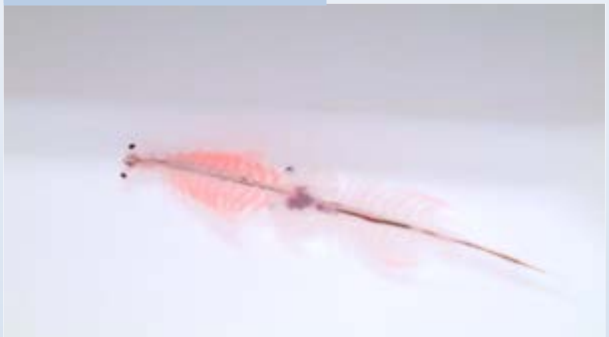
250nl (80 ng) of dsRNA solution were injected into egg sacs

* Adult were monitored for 2 h. Those exhibiting normal swimming and dye retention were used in experiment.

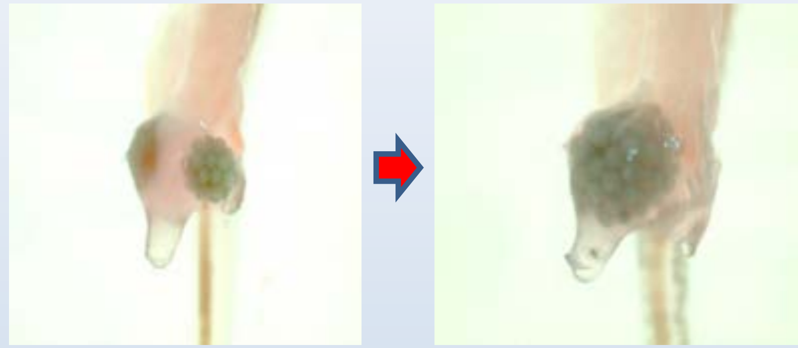


Observation of embryo development within *Artemia* females

Post-injection



Mating



Fertilization : fusion of egg sacs

Post-fertilization

or

Time to release (of brood) post-fertilization were recorded.

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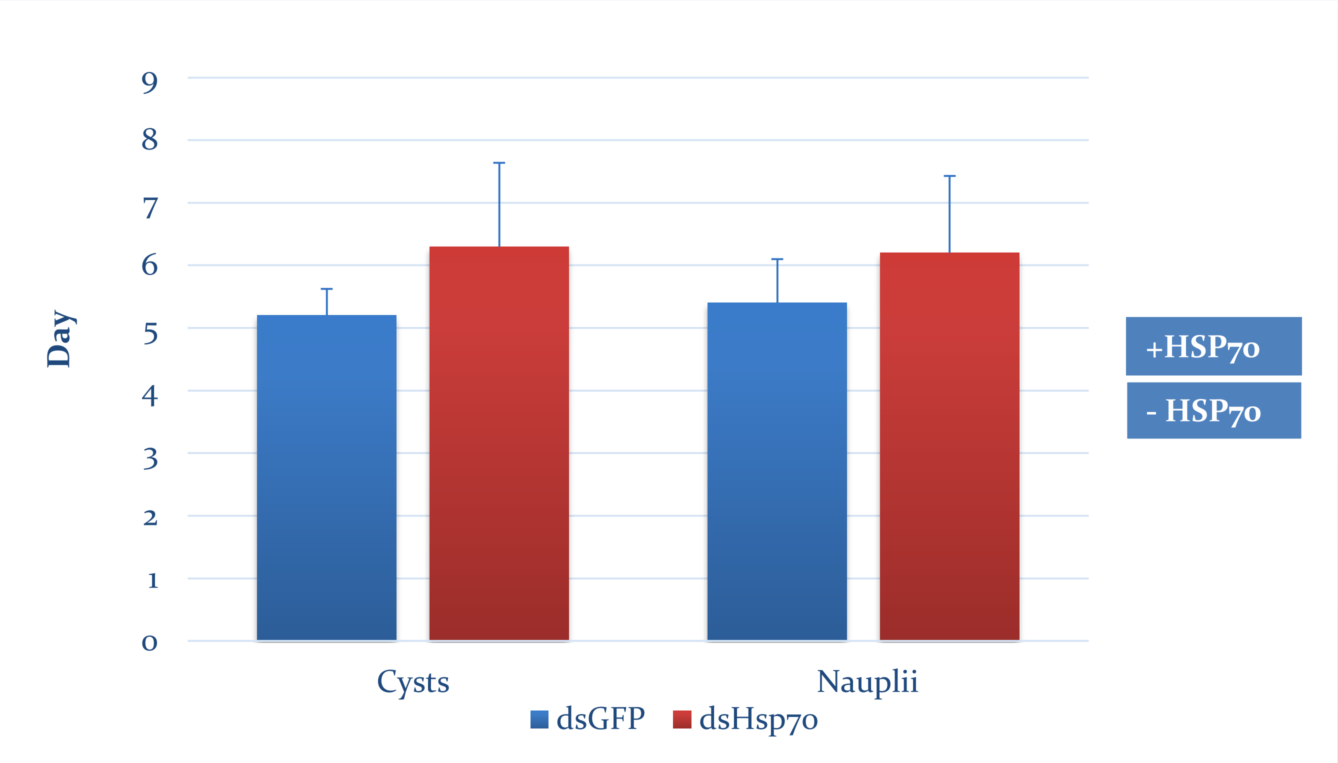
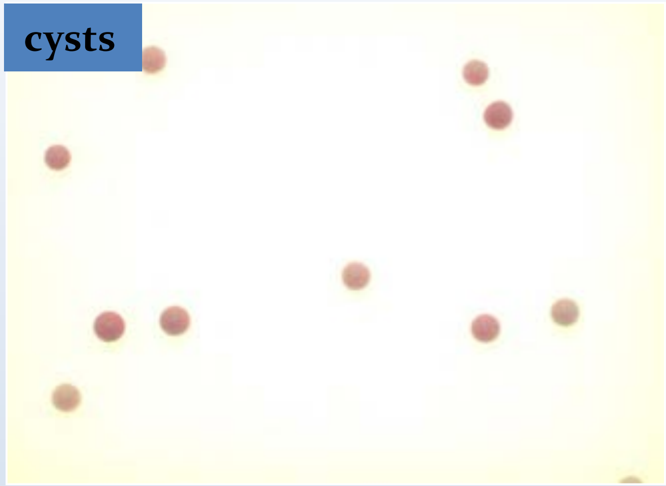
Time to release (post-fertilization)



Elimination of HSP70 showed no significant effect on embryo development

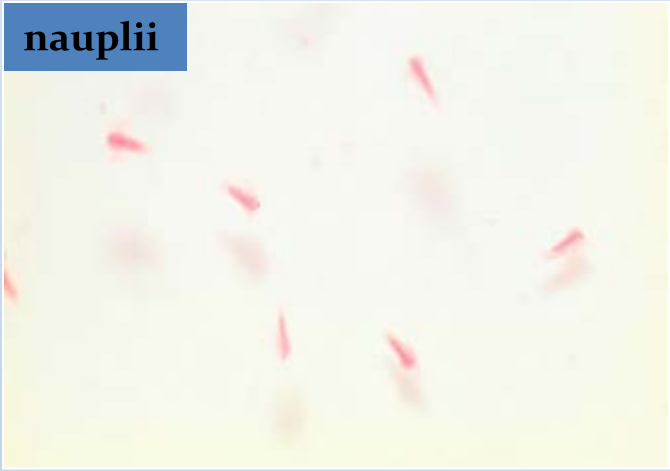
Hsp70 knockdown does not affect embryo development



Detection of HSP70 mRNA (RT-PCR)

Detection of HSP70 (SDS-Page & WB)

Diapause Termination and Cyst Hatching
- 90 days incubation in seawater prior to hatching
- Hatching experiment: 7 days



Detection of HSP70 mRNA (RT-PCR)

Detection of HSP70 (SDS-Page & WB)

Stress Tolerance
Thermal tolerance : Heat shock at 38ºC
Bacterial tolerance : *V. campbellii* (10^8)

Detection of HSP70 mRNA and HSP70

DNA gel

Western Blot

in cysts

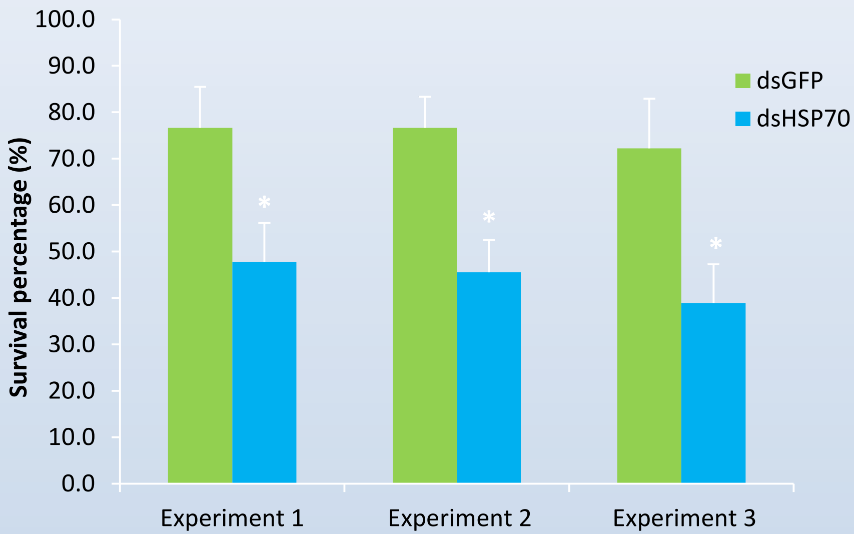
in nauplii

Lane 1 : Ladder
Lane 2 : GFP dsRNA (ctrl)
Lane 3 : HSP70 dsRNA

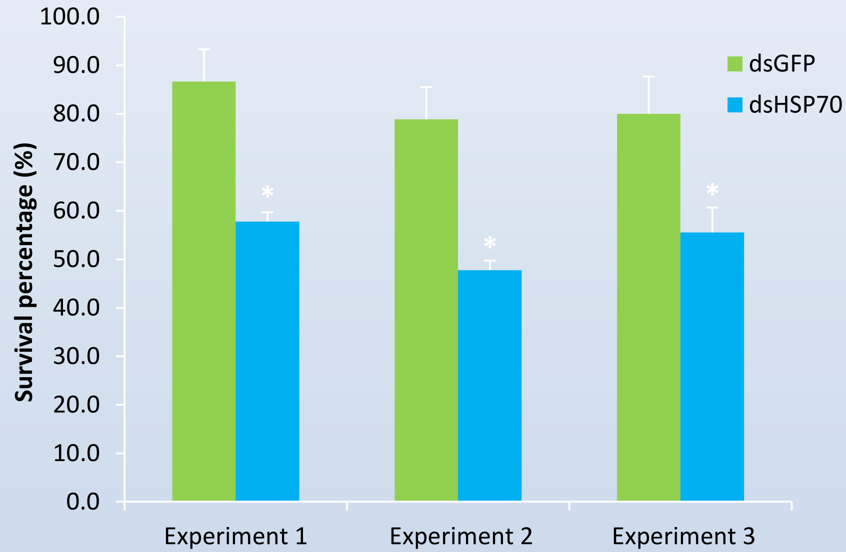
Injection of HSP70 dsRNA prevented the synthesis of HSP70 mRNA and protein in cyst and nauplii

Nauplii tolerance against thermal stress and vibrio challenge

Heat shock (38ºC)



V. campbellii **(10^8)**



HSP70 knockdown nauplii were **less resistant** to heat perturbation and pathogenic *Vibrio campbellii*, with survival percentage reduced approximately 31% and 28%.

HSP70 is required for protection against heat and pathogenic *Vibrios*

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HSP70 is highly conserved!

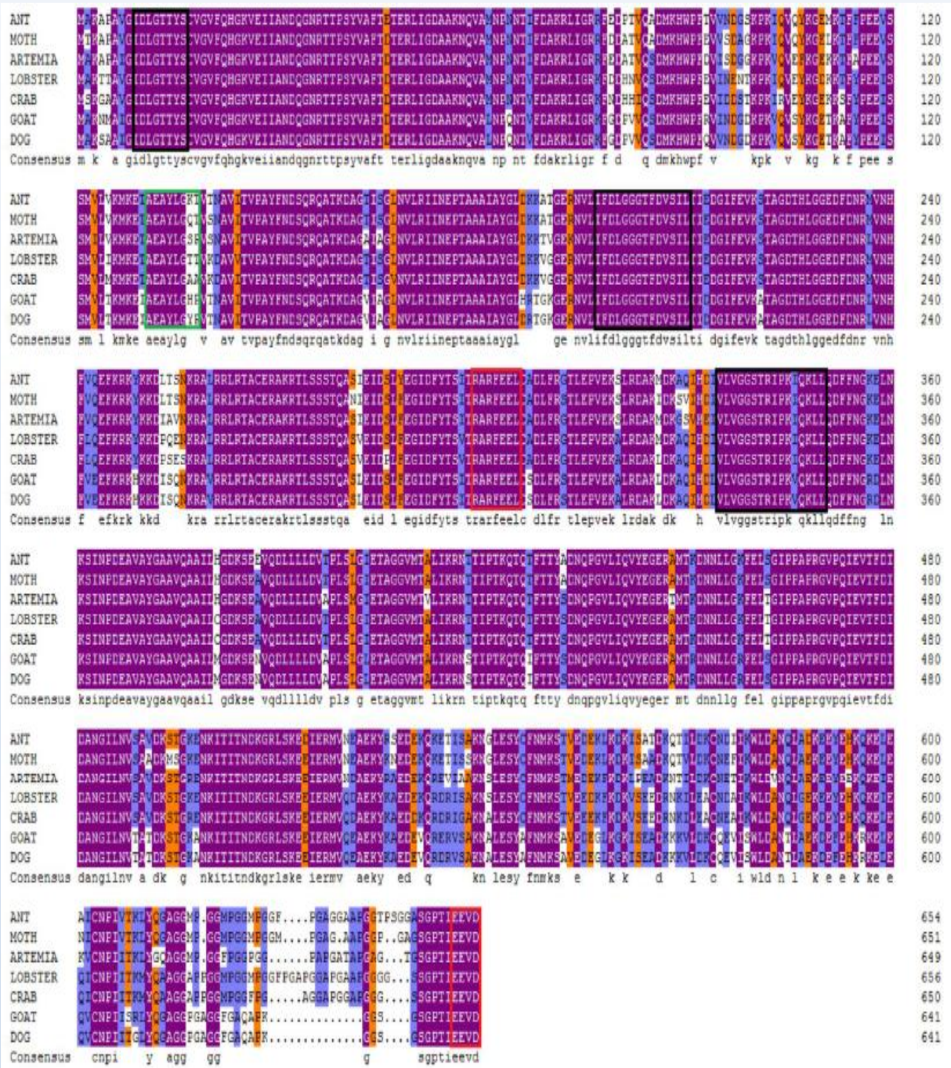

Table 1. HSP70 sequence comparisons

	Amino Acids	
Organism	Similarity (%)	Identity (%)
Ant	92	86
Moth	92	86
Lobster	90	83
Crab	90	84
Goat	87	81
Dog	87	81

The *A. franciscana* HSP70 amino acid sequence was compared to the sequence of Hsp70s from Ant (*Cerepachys ant biroi*), XP_011340138; Moth (*Cotesia Vestalis*), AGF34718; Lobster (*Homarus americanus*),ABA02165; Crab (*Scylla paramamosain*), AFX62578; goat (*Ovis aries*), AEX55801; Dog (*Canis lupus famIlaris*), BAC79356.1.

Possible role and applications

- Hsp70 plays significant role in **thermal and pathogenic bacteria (disease)** tolerance in *Artemia*
- Manipulation (up-regulation) of endogenous Hsp70 expression can boost tolerance

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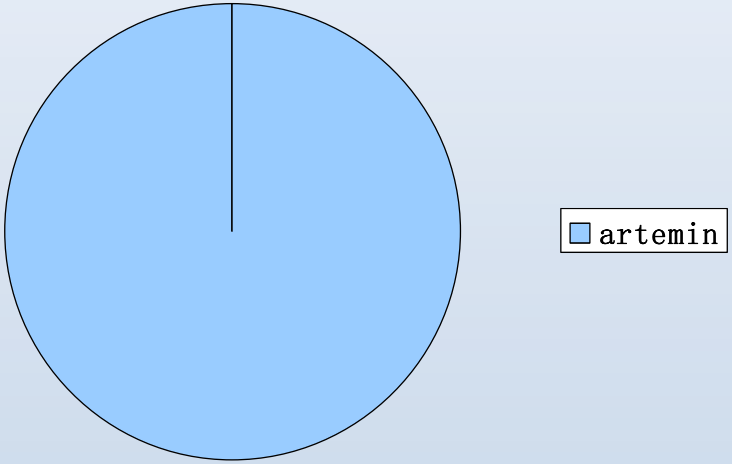
Structural view on Artemin, an important chaperon in *Artemia*

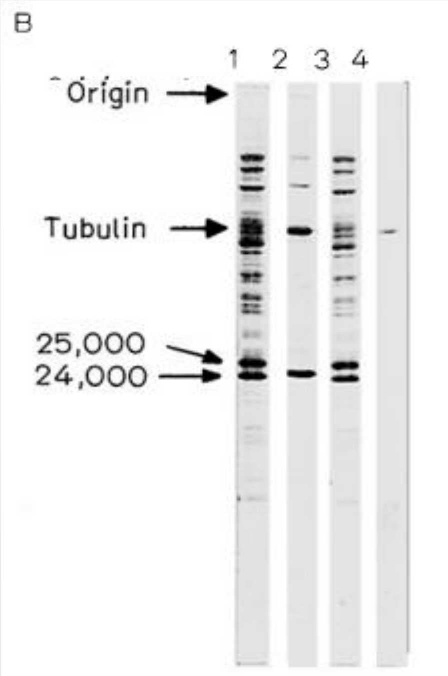
Xiang Liu,
Tianjin International Joint Academy of Biomedicine,
Tianjin, China

Artemin, a “neglected” familiar molecule

- keyword
  Artemin 3120 papers
- keyword
  Artemin+*Artemia*
  20 papers, **less than 1%**

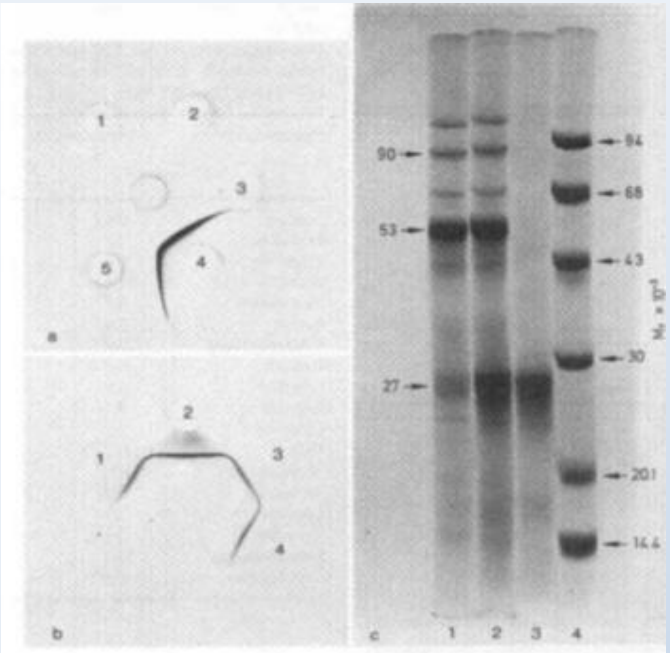


Artemin, a neglected familiar molecule



" The 'cyst-specific' proteins might have some function in the encystemnt process itself……"

Eur. J. Biochem 70,589-599 (1976)



" …… it appears very likely that 24,000-27,000 proteins detected in both laboratories are identical"

Eur. J. Biocemh 96,423-430 (1979)

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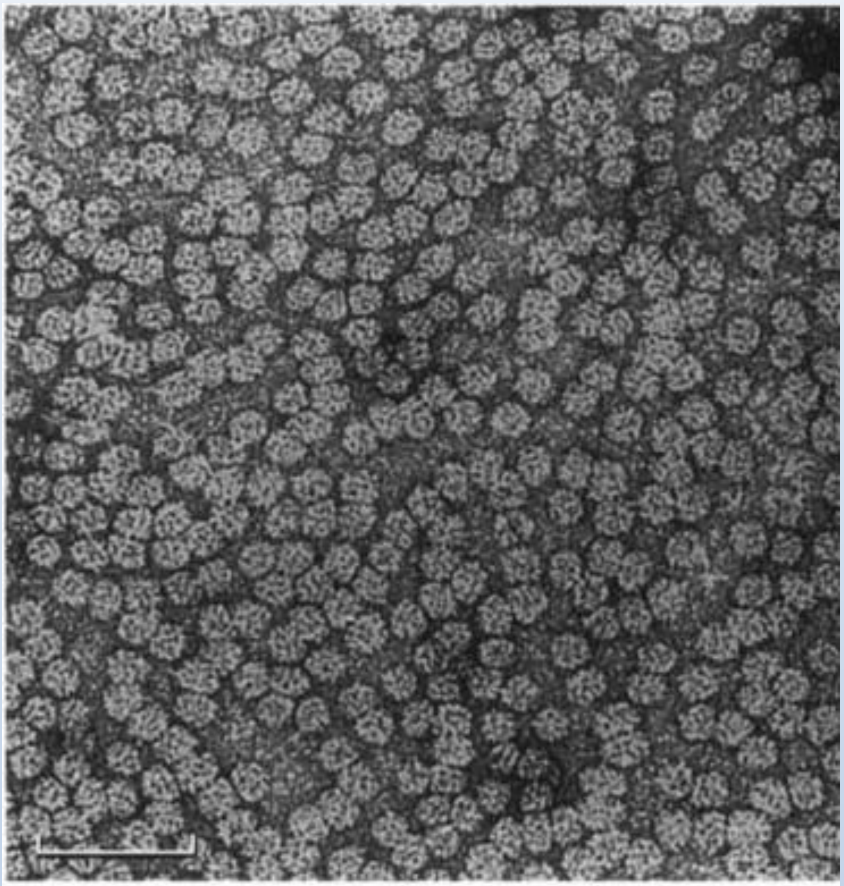
Artemin, a neglected familiar molecule



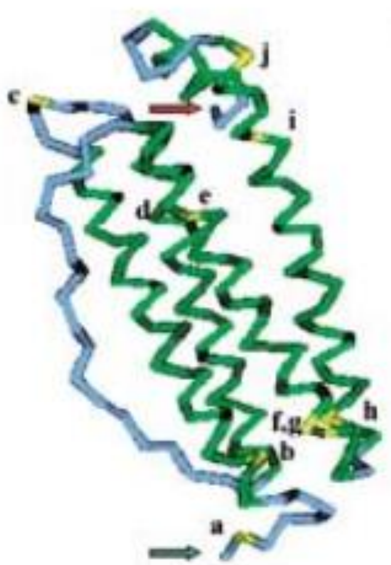
The primary structure of artemin from Artemia cysts

Jack DE GRAAF, Reinout AMONS and Wim MÖLLER
Department of Medical Biochemistry, State University of Leiden, The Netherlands

(Received March 26, 1990) — EJB 90 0339

" The protein, called **artemin** by Solbin in our laboratory......"

Artemin, ferritin-like protein



Residue Number	Location in Artemin
C21 (a)	Outside area of homology
C31 (b)	Beginning of helix A
C60 (c)	Loop between helices A and B
C70 (d)	Helix B
C118 (e)	Helix C
C135 (f)	End of helix C
C136 (g)	End of helix C
C143 (h)	Beginning of helix D
C166 (i)	Helix D
C172 (j)	End of helix D

Eur. J. Biochem. 270, 137-145 (2003)

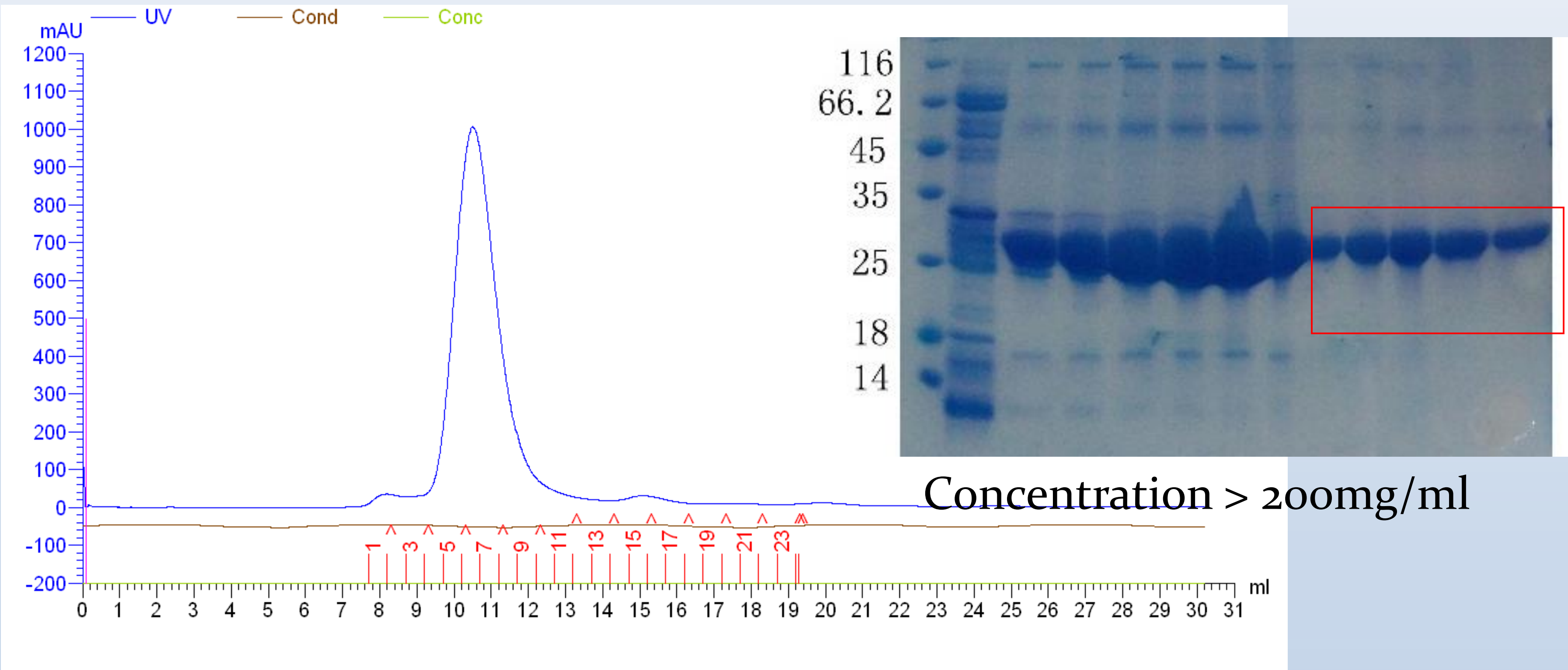
Artemin structure-model given in the paper, **not the real one**

Artemin, function? Mysterious!

- 27kDa, 24-oligomers, just like Ferritin, but cannot interact with iron
- Thermo-stable, 70℃ in vitro
- more cysteines in sequence
- Can bind to RNA

All these information summarized from past 12 years.

Artemin, production from *E.coli*



Concentration > 200mg/ml

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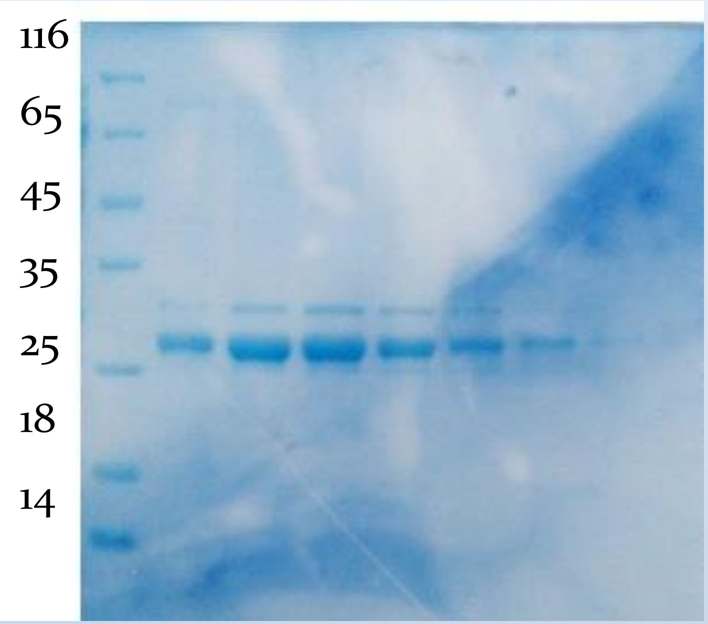
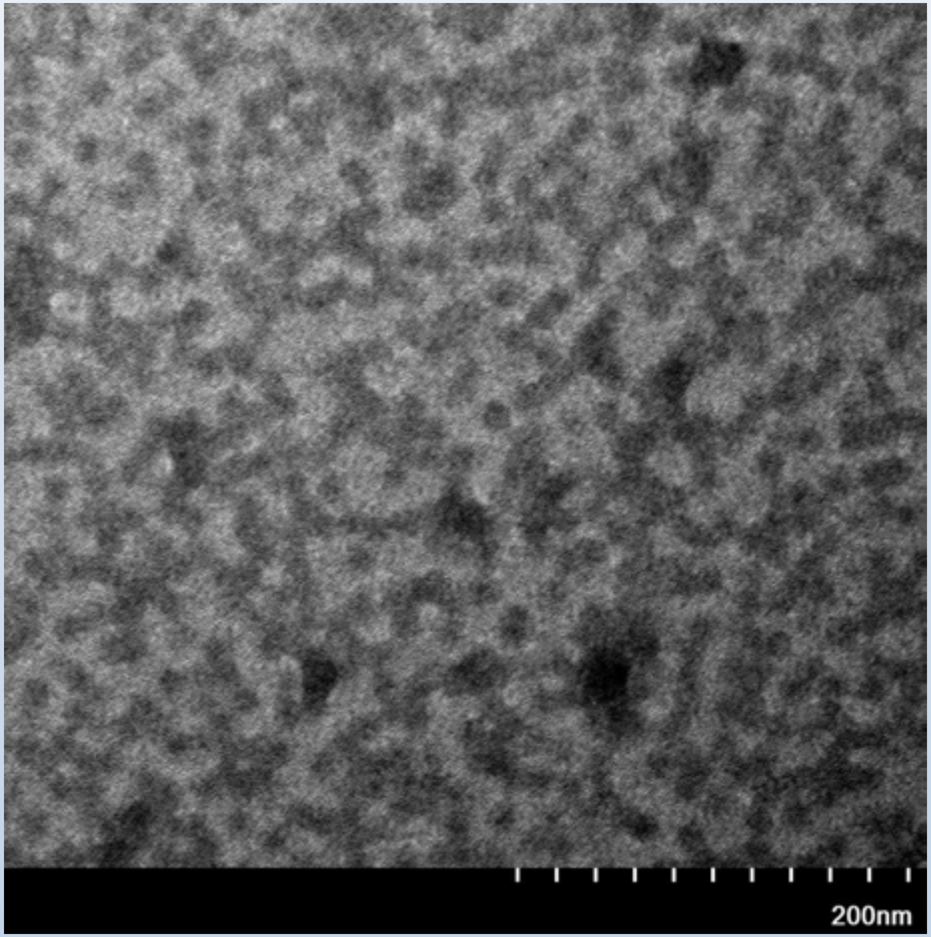
Artemin, purified from cyst



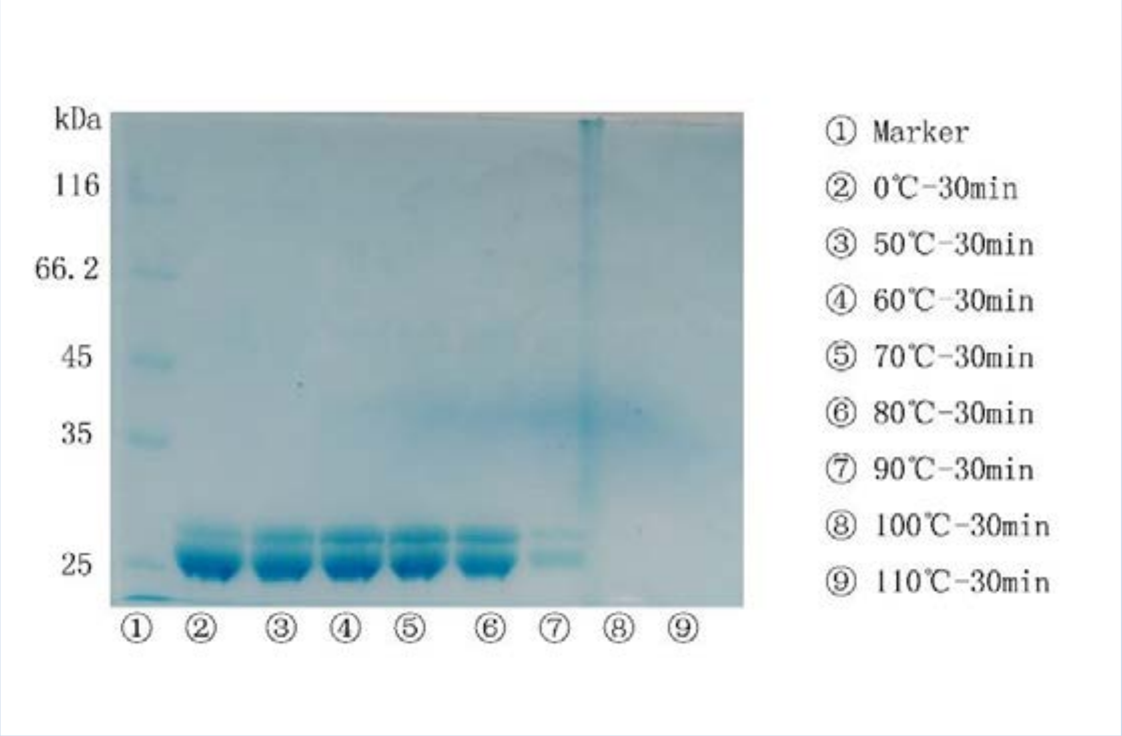
- Purified protein from cysts(5 g), about 1.5 mg in total.
- Followed protocols in published papers
- The cysts were supported by Prof. Sui

Artemin, self-assemble *in vivo*



Small particles, d~15 nm,
@TIB EM negative stain

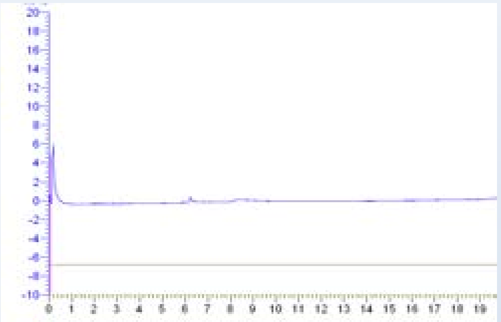
Artemin, thermo stable



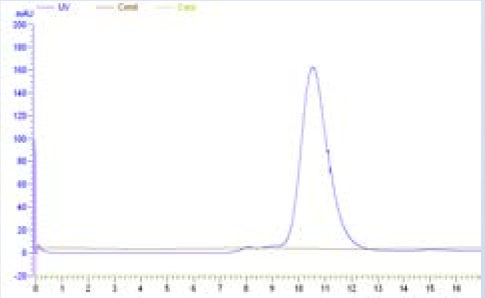
Thermo-stable @80℃, 30 minutes,

No obvious precipitants

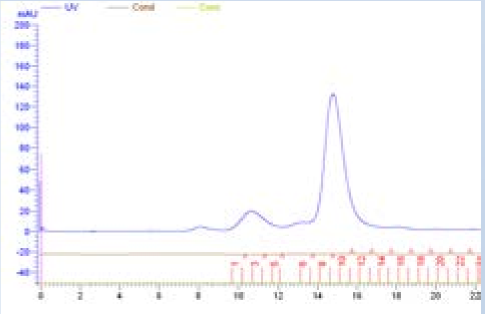
Artemin, pH depended



Low pH, insoluble, no structure information

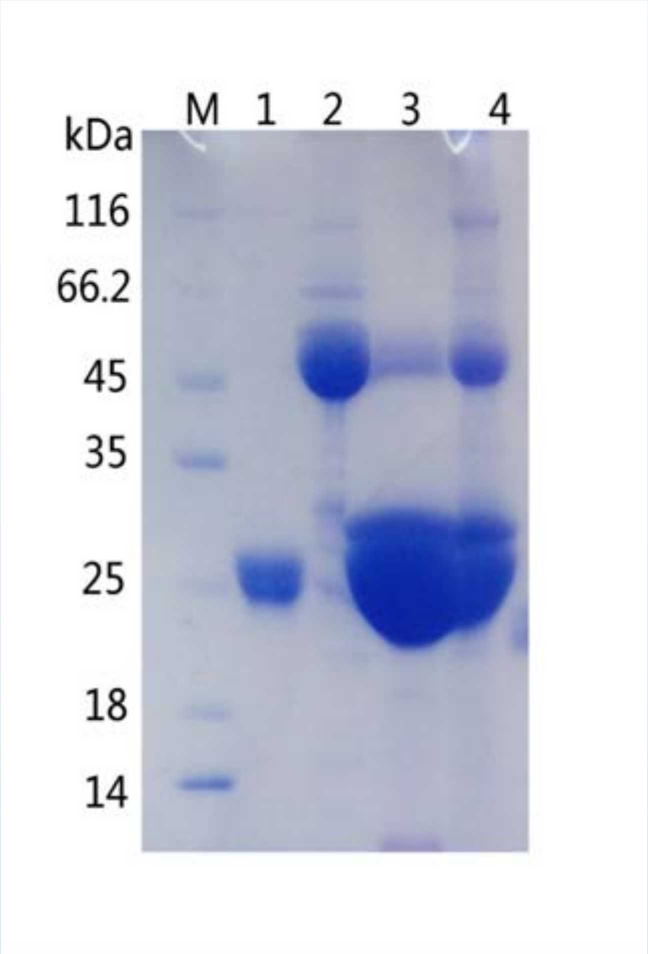


medium pH, soluble, self-assemble



high pH, soluble, monomer

Artemin, empty cavity



Artemin-GFP fusion protein can be produced from *E.coli* and be thermo-stable.

Artemin, the unique c-tail

NO	Truncation
1	Artn-C39-191
2	Artn-C35-195
3	Artn-C33-197
4	Artn-C30-200
5	Artn-C25-205
6	Artn-C23-207
7	Artn-C20-210
8	Artn-C17-213
9	Artn-C15-215
10	Artn-C5-225
11	Artn-N20-210
12	Artn-N26-204

Unstable, no-assemble

Stable, self-assemble

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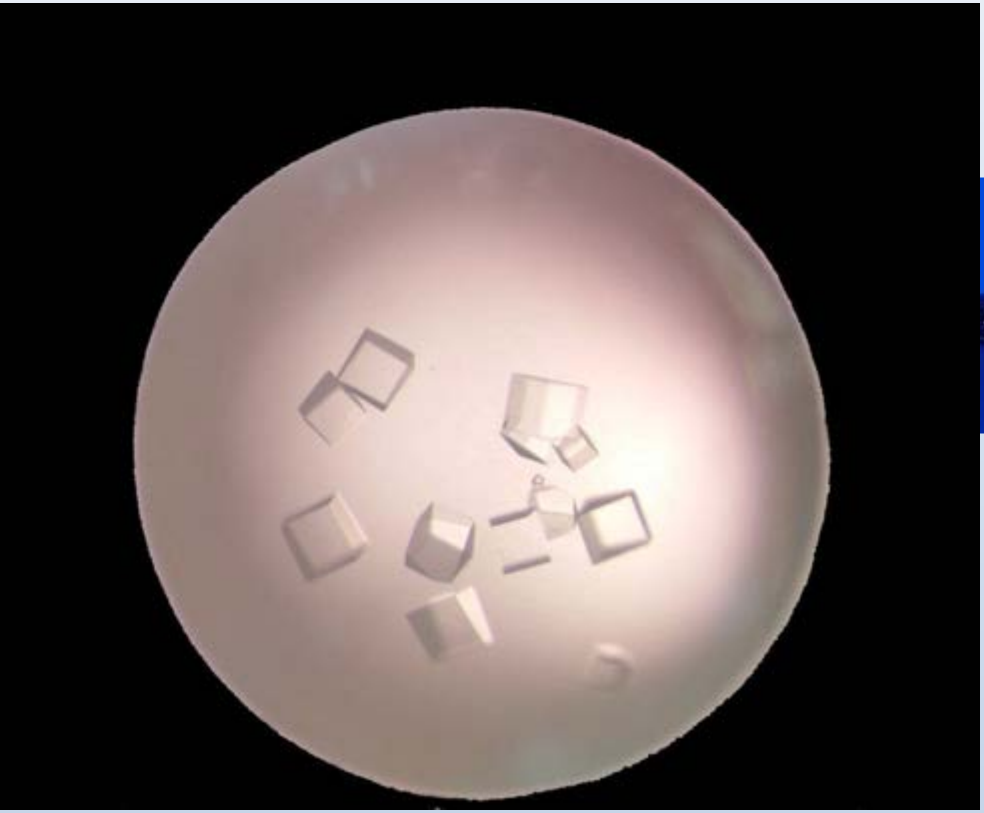
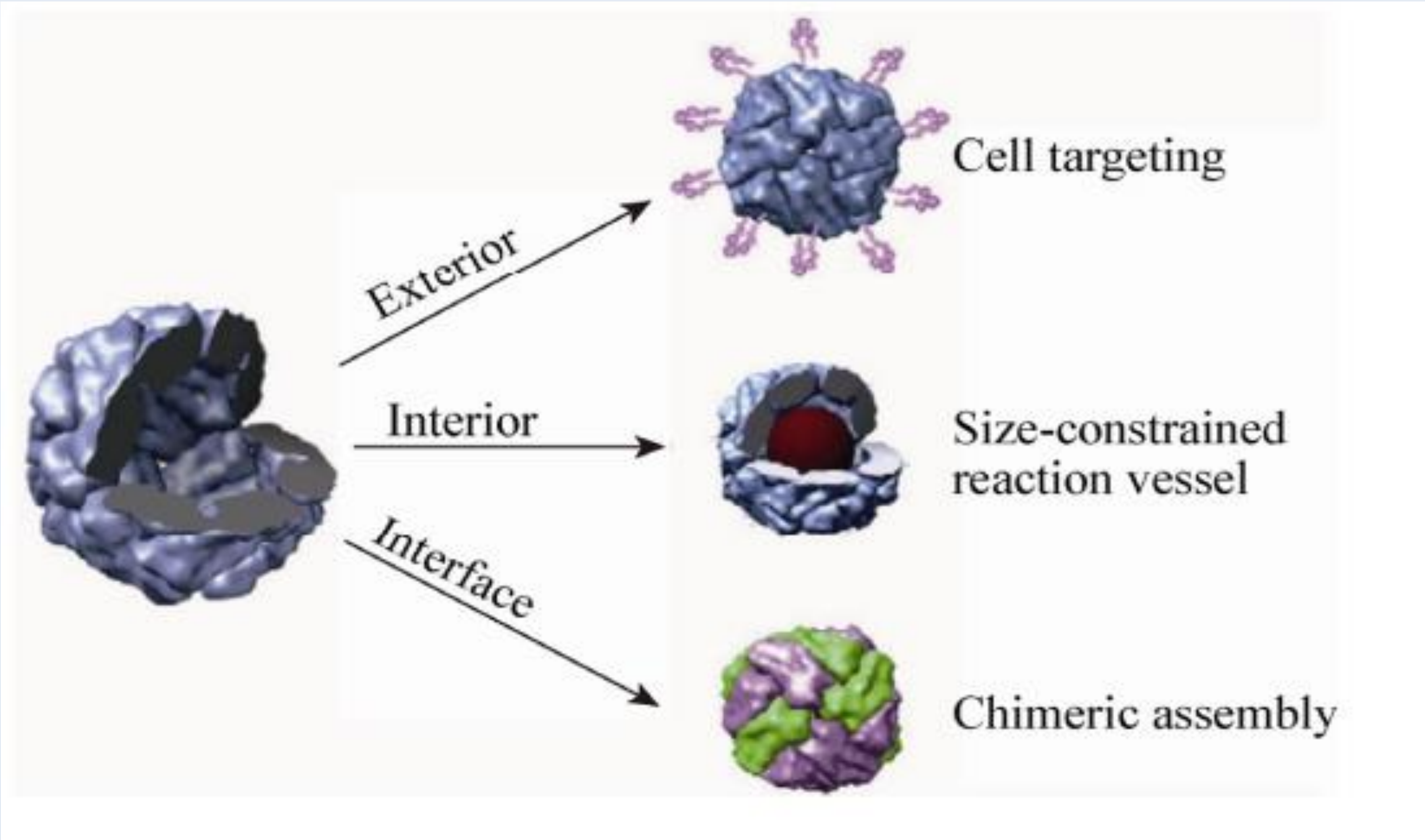
Artemin, crystallization



Diffracted to 2.3A
@ SSRF

Artemin, a new model for ferritin



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Halophilic bacteria as food for *Artemia*

Ruy Lopes-dos-Santos
Laboratory of Aquaculture and Artemia Reference Center,
Ghent University, Ghent, Belgium

Introduction

Artemia Production:

Natural salt lakes

Integrated in solar saltworks

Artemia cysts are produced in solar salt ponds by boosting primary production (phytoplankton) and supplementation with agricultural by-products, as food for *Artemia*.

Introduction

Lowering these production costs contributes to the sustainability of the cultures, and to the security of the *Artemia* supply.

Increased carbon and nitrogen supplementation in *Artemia* culture ponds results in higher cyst yields

L. Y. Sui · J. Wang · V. H. Nguyen · P. Sorgeloos · P. Bossier · G. Van Stappen

Unfortunately our lack of knowledge on interactions between ***Artemia*** and the **associated microbiota** hinders the widespread application of these techniques.

Research Specific Objectives

To investigate for the first time *Artemia* nauplii's **ability to survive and grow** on diets consisting exclusively of **halophilic bacteria biomass**, typical for the hypersaline environment where *Artemia* occurs.

Research Ultimate Objectives

To understand the relative importance of different halophilic bacterial genera and species for the *Artemia* life cycle as part of the **hypersaline food web**, and to shed light on the potential of this microorganisms to **maximize *Artemia* production in salt ponds**.

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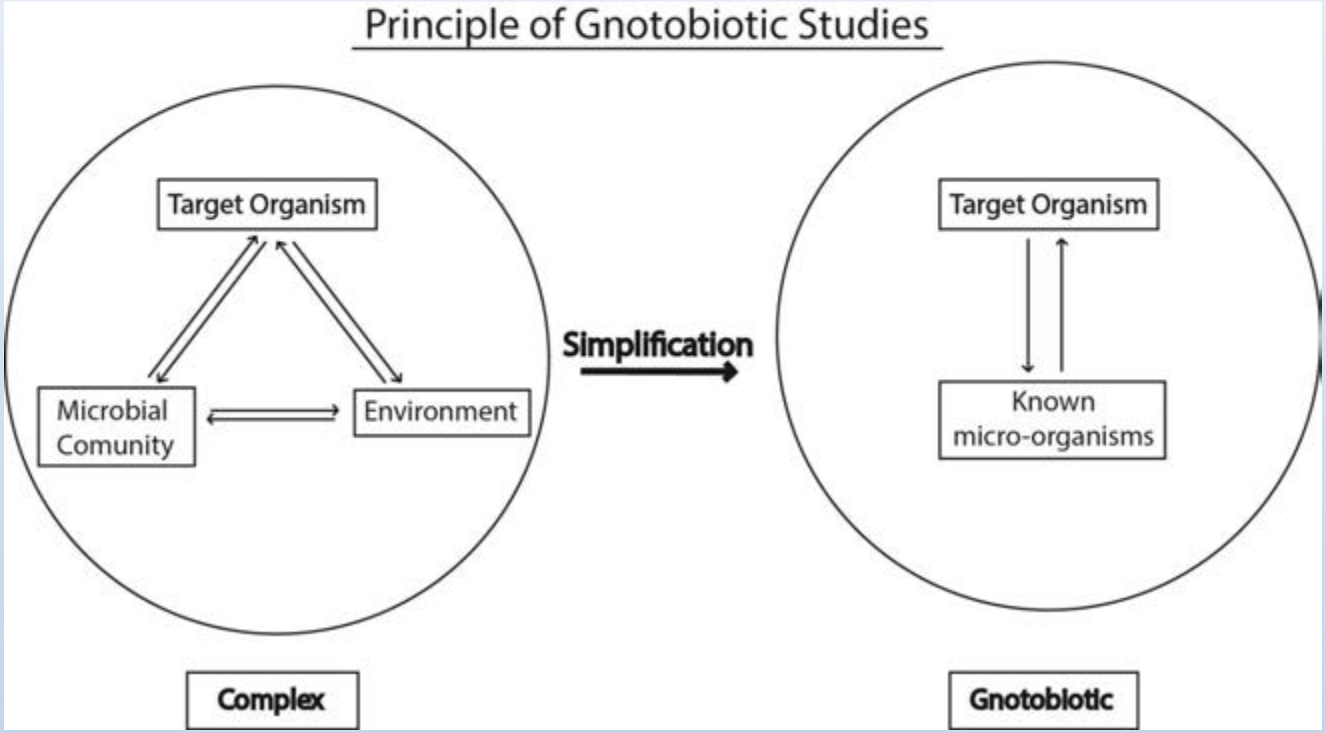
Materials and methods



Experimental design

Tested diets

- Two Halophilic bacterial strains belonging to genera associated with *Artemia* in natural ecosystems were evaluated as **mono-diets** for *Artemia* culture when offered as **live** or **dead** biomass.
- Two **controls** were used:
  - Negative Control (**NC**) → **Starvation**
  - Positive Control (**PC**) → Marine bacterial strain **LVS3 (*Aeromonas hydrophila*)**

Tested culture salinities

- Tests were conducted with culture water at **100 g/L** and at **35 g/L** salinity

Experimental design

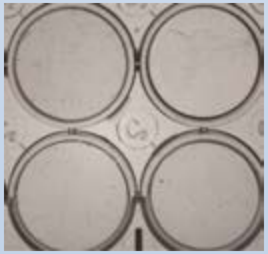
Experiment 1: 5 days culture experiments to assess **survival** and **growth** of *Artemia* nauplii when fed mono-diets of halophilic bacteria biomass.

- 20 axenic *Artemia* nauplii were cultured in screw cap tubes, containing 30 ml of filtered and autoclaved saline water (FASW). There were 4 replicates per culture.
- *Artemia* received daily an estabilished concentration of halophilic bacterial cells;
- Survival was checked daily for 5 days and at day 5 after hatching surviving *Artemia* were preserved in 1% lugol and length was measured under the microscope.

Experimental design

Experiment 2: 36 hours culture experiments to assess **swimming speed** of *Artemia* nauplii when fed mono-diets of halophilic bacteria biomass.

- 20 axenic *Artemia* nauplii were cultured in 48 well plates, with 1 nauplii per well containing 1 ml FASW. There were 3 well plates (replicates) for each culture.
- *Artemia* received the same concentration of halophilic bacterial cells
- Swimming speed was checked at 36 hours after hatching by following them for 2 minutes using video tracking software.

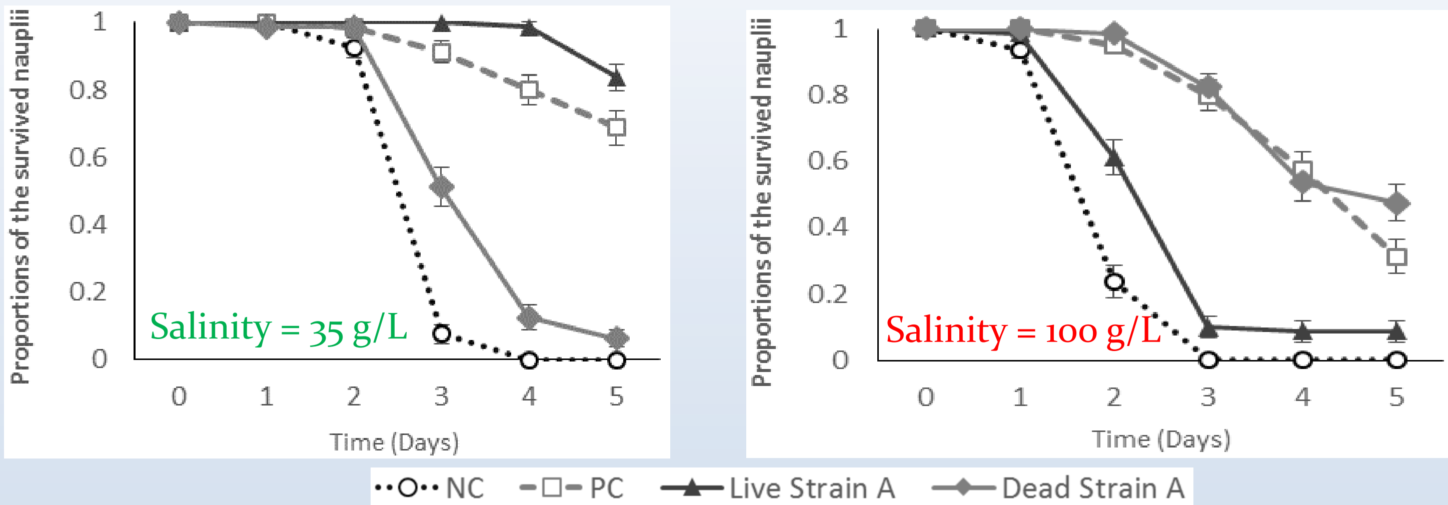


Results

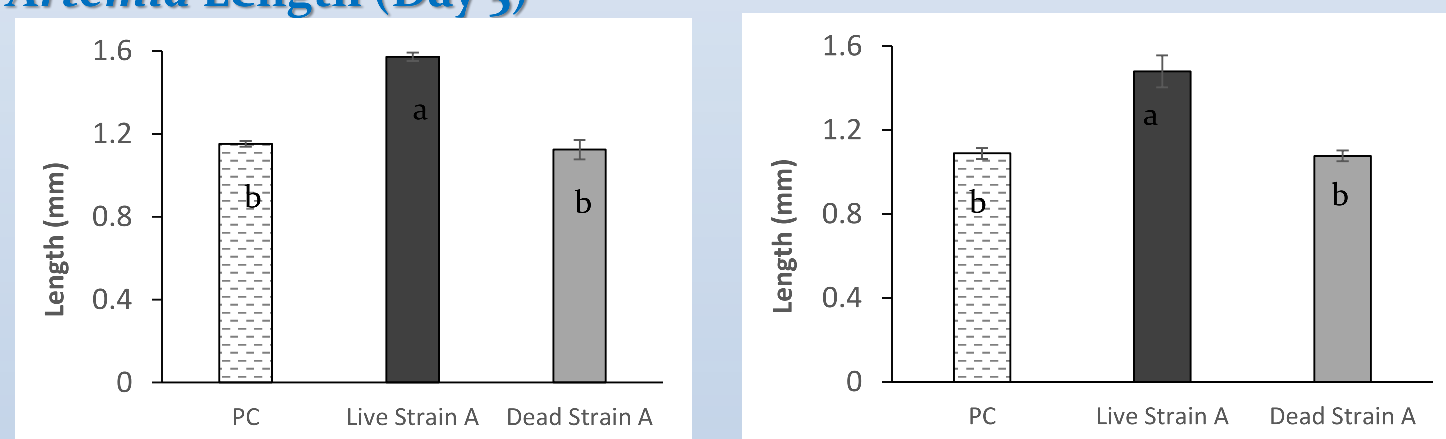
Experiment 1: 5 days culture experiments to assess **survival** and **growth** of *Artemia* nauplii when fed mono-diets of halophilic bacteria biomass

Halophilic bacteria: Strain A

Artemia Survival



Artemia Length (Day 5)



Different superscripts represent significant difference ($p<0.05$) between the tested diets at the same salinity

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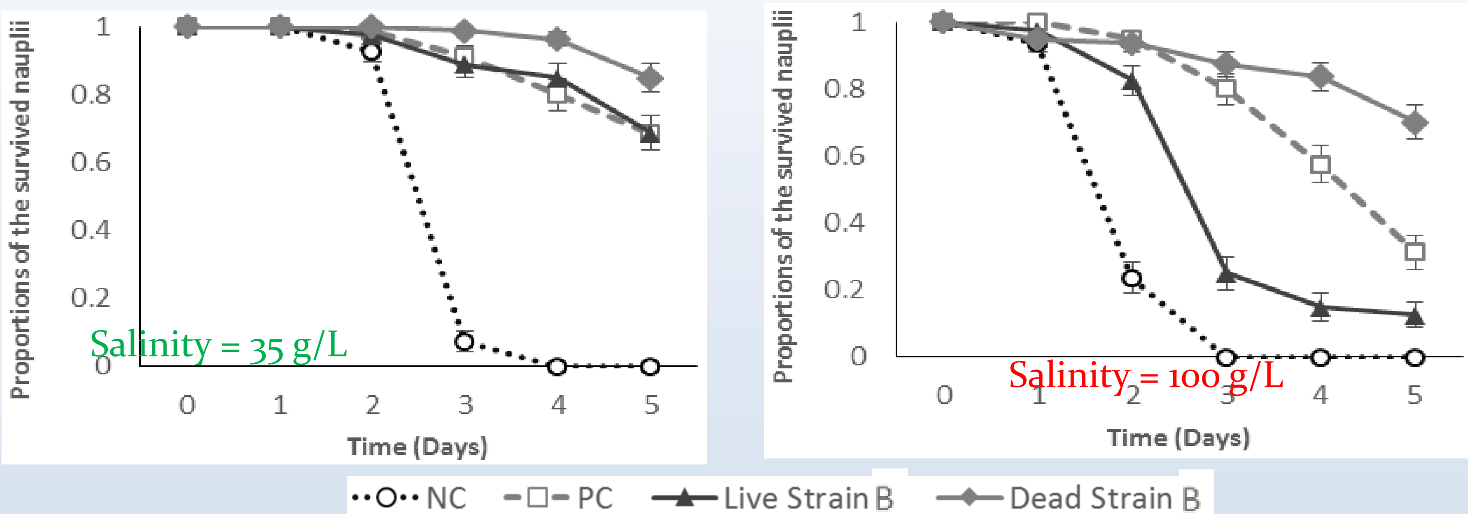
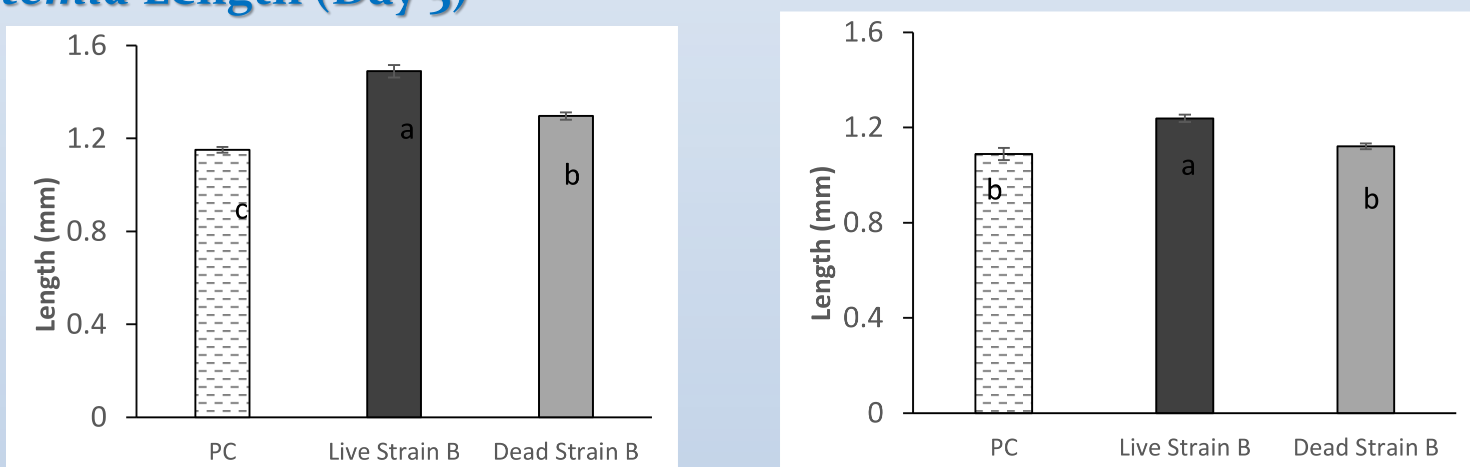
Halophilic bacteria: Strain B

Artemia Survival



Artemia Length (Day 5)



Different superscripts represent significant difference ($p<0.05$) between the tested diets at the same salinity

Artemia Development

Positive Control | **Live Strain A** | **Live Strain B**

35 g/L

100 g/L

Results

Experiment 2 : 36 hours culture experiments to assess **swimming speed** of *Artemia* nauplii when fed mono-diets of halophilic bacteria biomass

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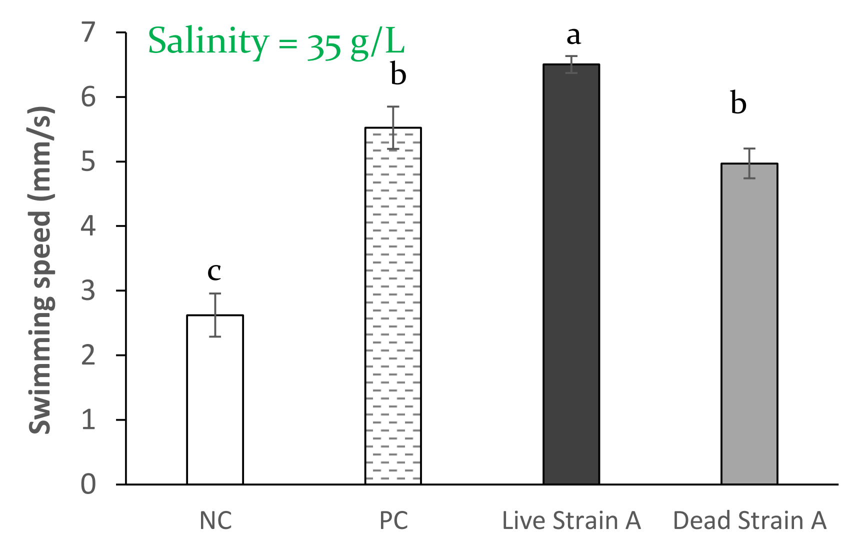
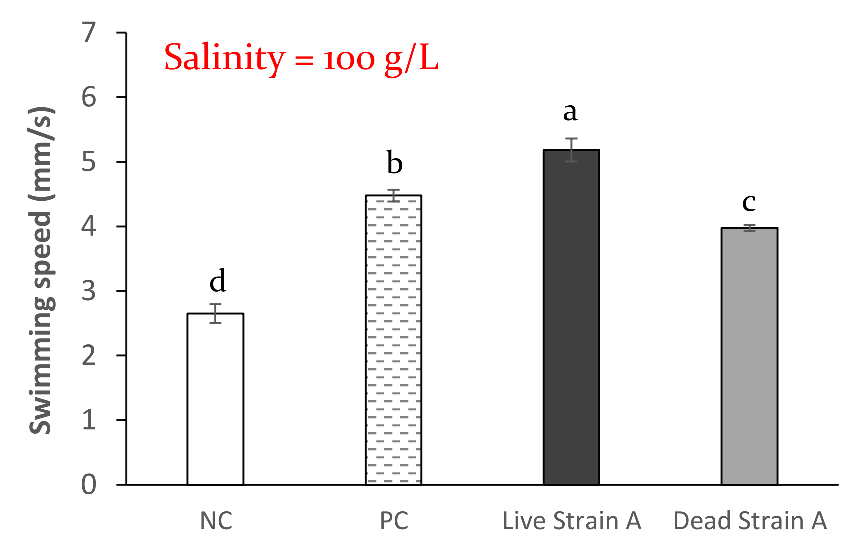
Halophilic bacteria: Strain A

Artemia Swimming Speed at 36 hours after hatching





Different superscripts represent significant difference ($p<0.05$) between the tested diets at the same salinity

Main findings

- *Artemia* nauplii have the ability to **survive** and **grow** on diets consisting of pure biomass of halophilic bacteria strains.
- The positive effects on **development** and **swimming speed** of the tested halophilic mono-diets compared to both controls in both salinities, clearly denotes their **value as food item** for Artemia culture.
- *Artemia* shows **better performance** when fed with its naturally associated halophilic microbiota than when fed with **marine bacteria** (Toi *et al*. 2014), or even with **axenic microalgae** (Van maele, 2015).

Toi, H. T., P. Boeckx, P. Sorgeloos, P. Bossier, and G. Van Stappen. 2014. Co-feeding of microalgae and bacteria may result in increased N assimilation in Artemia as compared to mono-diets, as demonstrated by a N-15 isotope uptake laboratory study. Aquaculture 422:109-114.

S. Van maele. 2015. Morphological examination and immunostimulation of the gastrointestinal tract in an *Artemia* model. PhD thesis, Ghent University, Ghent, Belgium.

Conclusion

- The acquired knowledge is a crucial contribution to understand the role of these bacteria in the hypersaline food webs, illustrating that they can be an **integral part of the *Artemia* diet.**

- The strategy to stimulate the formation of halophilic biofloc and bacterial aggregates in ponds should indeed provide a **valuable extra source of nutrients** for *Artemia*.

Thank You

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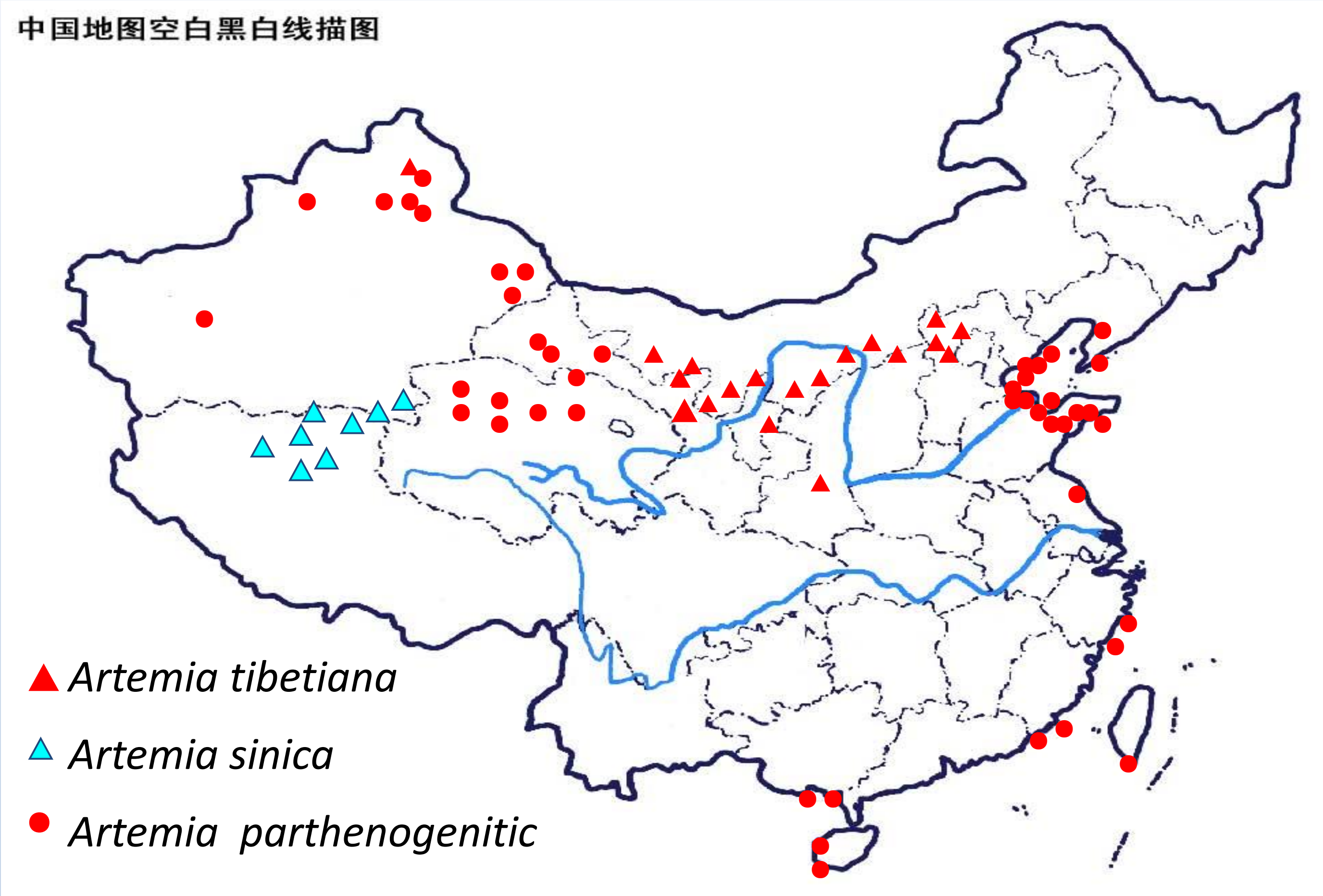
Artemia market situations in China

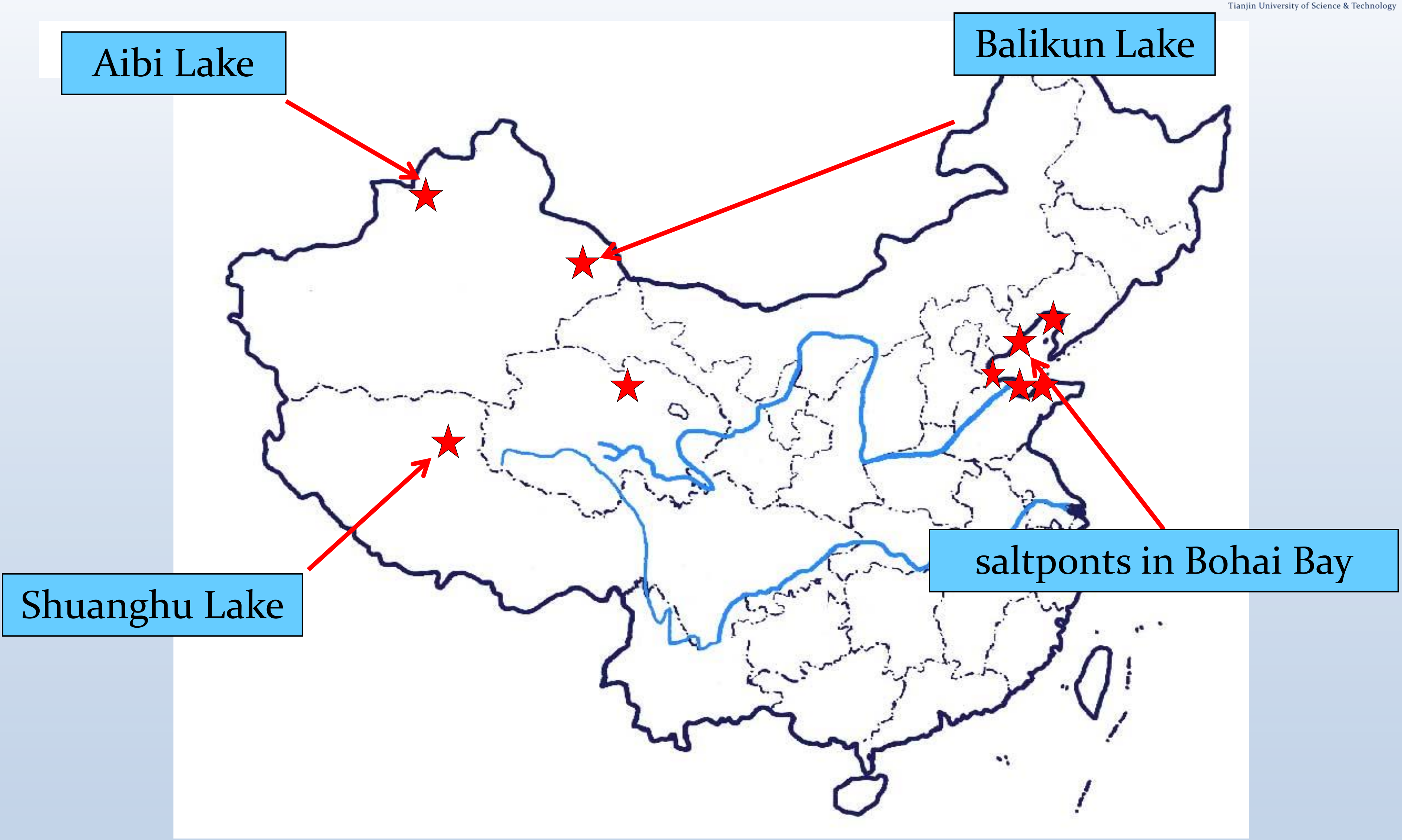
Bo Zhang
College of Marine and Environmental Sciences, Tianjin University of Science and Technology, Tianjin, China

Artemia distribution in China



Artemia can be found in more than 100 nature habitats in China.
Including 68 inland salt lakes and 41 coastal saltworks in 18 provinces of China.

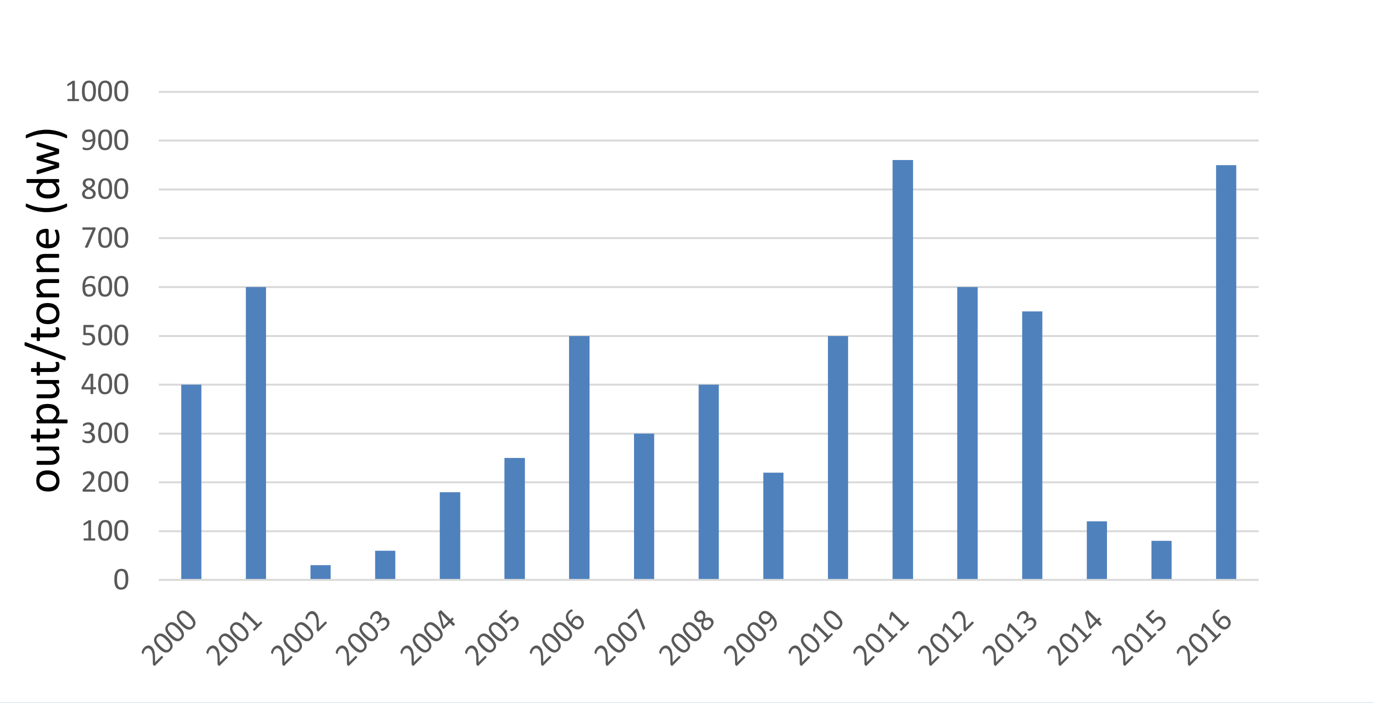
Artemia cysts resource in China



Aibi Lake

- Aibi lake=Ebinur lake
- The area is 500 km^2， average deep is 1.5 metre， altitude is 189 metre.
- Harvest: 300-600 t(dw)
- The right of harvesting: government to private person to company.
- The quality increased year by year.

The harvest of *Artemia* cysts from Aibi Lake over the years



Balikun Lake

- Balikun lake=Barkol lake
- 112 km^2， 0.8 m， 1 585 m
- harvest: 100 tonnes, 30-50 tonnes, 150-250 tonnes
- there are not management for harvesting, the quality is lower than others.

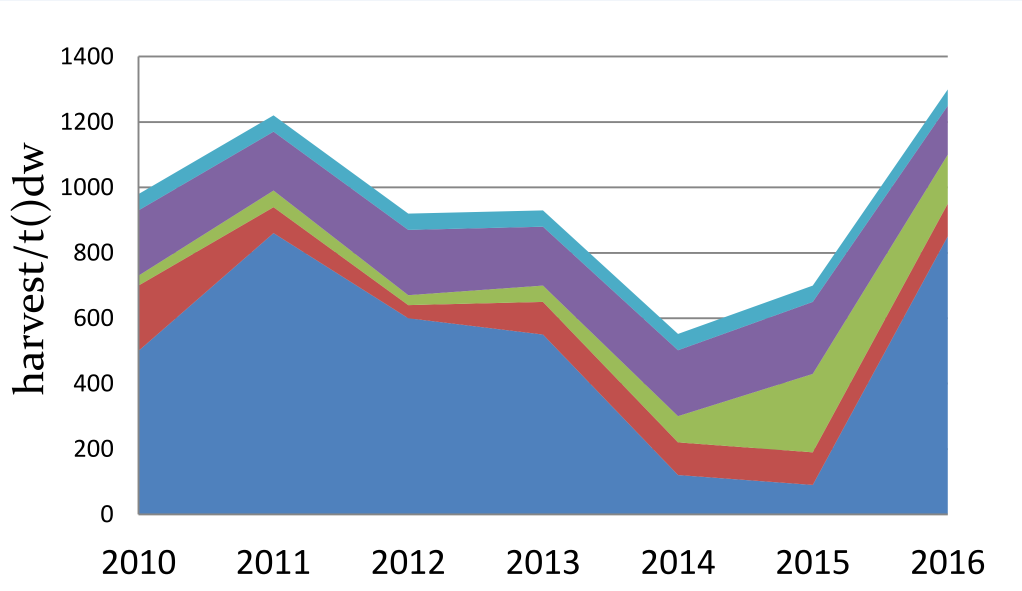
Shuanghu Lake

- the altitute is more than 5000 m.
- Only one company can get the permission these years.
- Harvest: 50-150 tonnes (dw)
- *Artemia* cysts have special characteristics different with others.

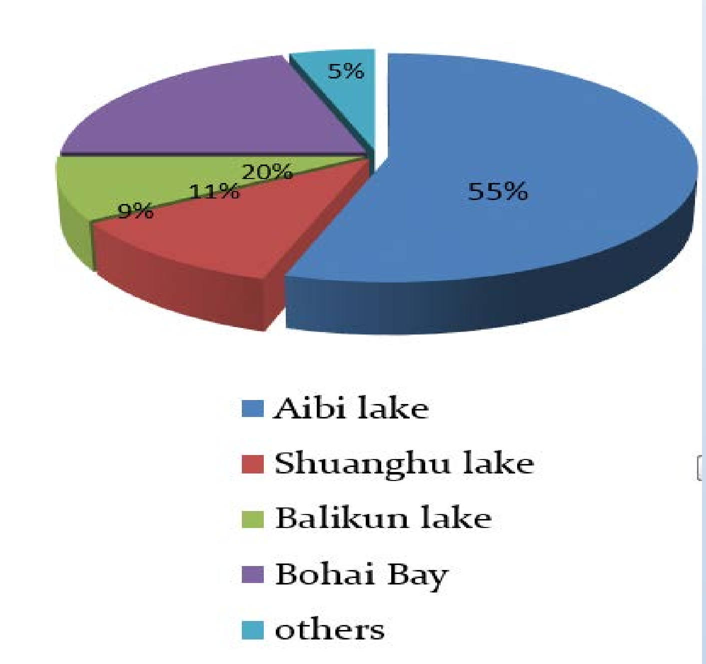
saltworks in Bohai Bay

- Fuzhouwan, Nanpu, Hangu, Tanggu, Huanghua, Lubei, Shouguang, Yangkou,……
- 500 tonnes, 300 tonnes, 150-250 tonnes.
- Harvest: 50-150 tonnes(dw)
- The price is higher than others.

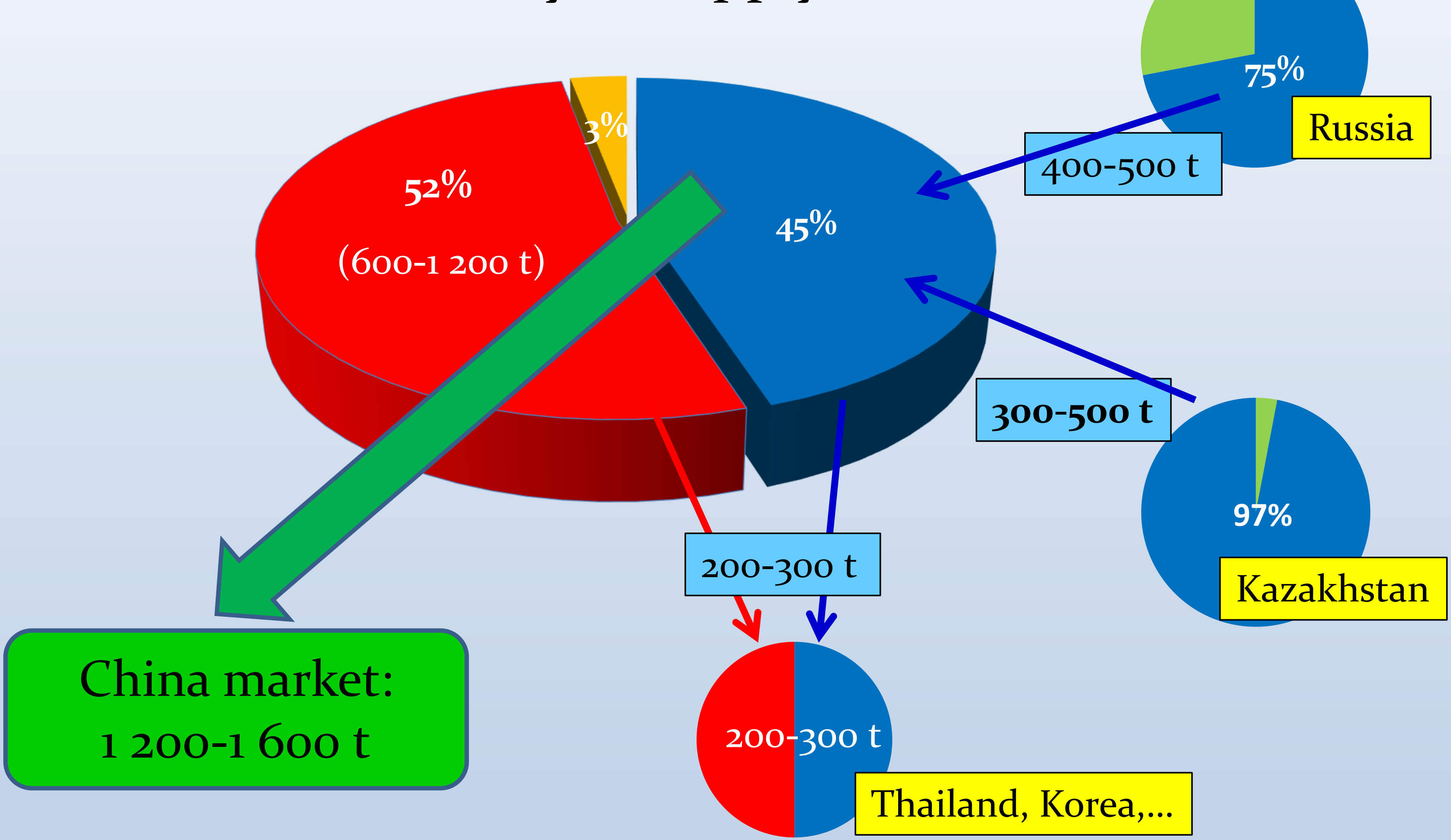
Artemia cysts resource in China



the harvesting volume of *Artemia* cysts in China



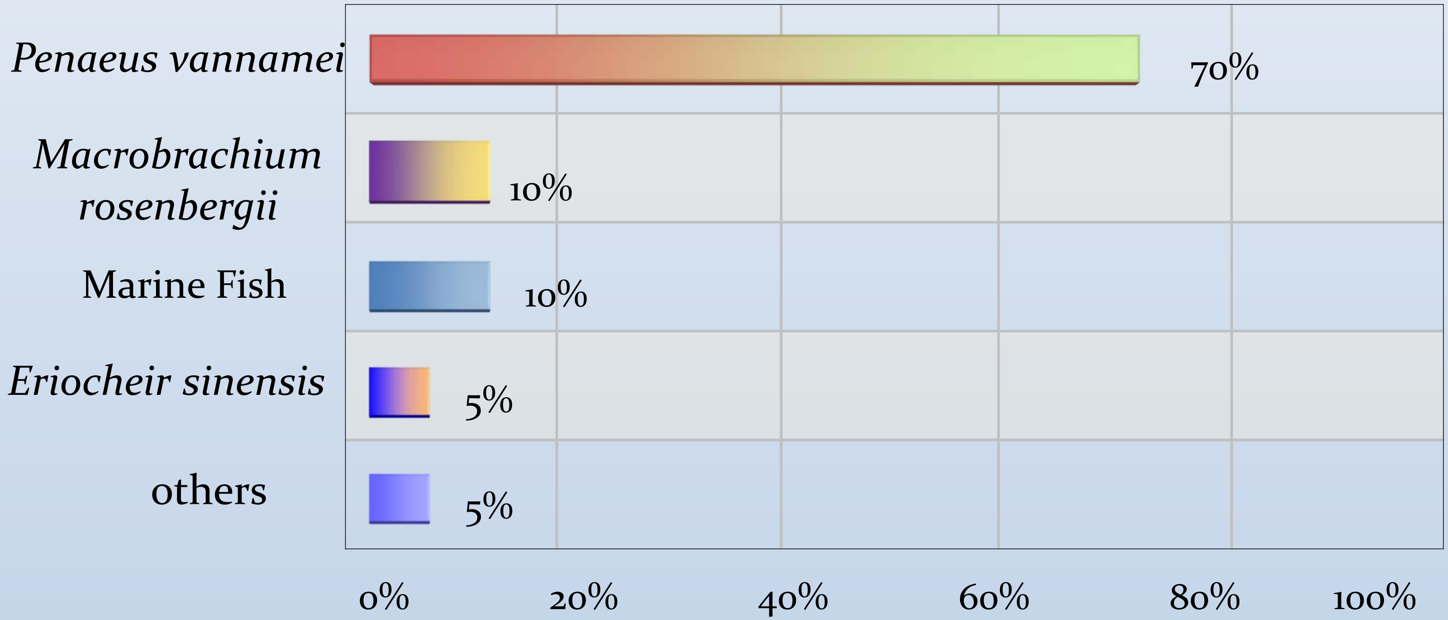
Artemia cysts supply of China



The strain characteristics in market of different *Artemia* cysts

strain	HO	size	colour	separation	nutrition	diapause	enrichment
GSL	4.0	S	+++	+++	+	+++	Ok
Bohai Bay	3.6	M	++	++	++	++	Ok
Aibi Lake	2.6	L	+	++	++	++	Ok
Balikun Lake	3.5	M	++	++	++	+/++	Ok
Shuanghu Lake	2.8	XL	++	+++	++++	+++	No
Big Yarovoe	3.2	M	+++	++++	?	+	?
Small Yarovoe	2.6	M	++	++	?	++	?
Kunlundinskoe	3.0	M	++	+++	?	+++	?
Borli	3.2	S	+++	++++	?	++	?
Marindy	2.5	M	+	+	?	+	?

The applications of *Artemia* cysts in larvaeculture



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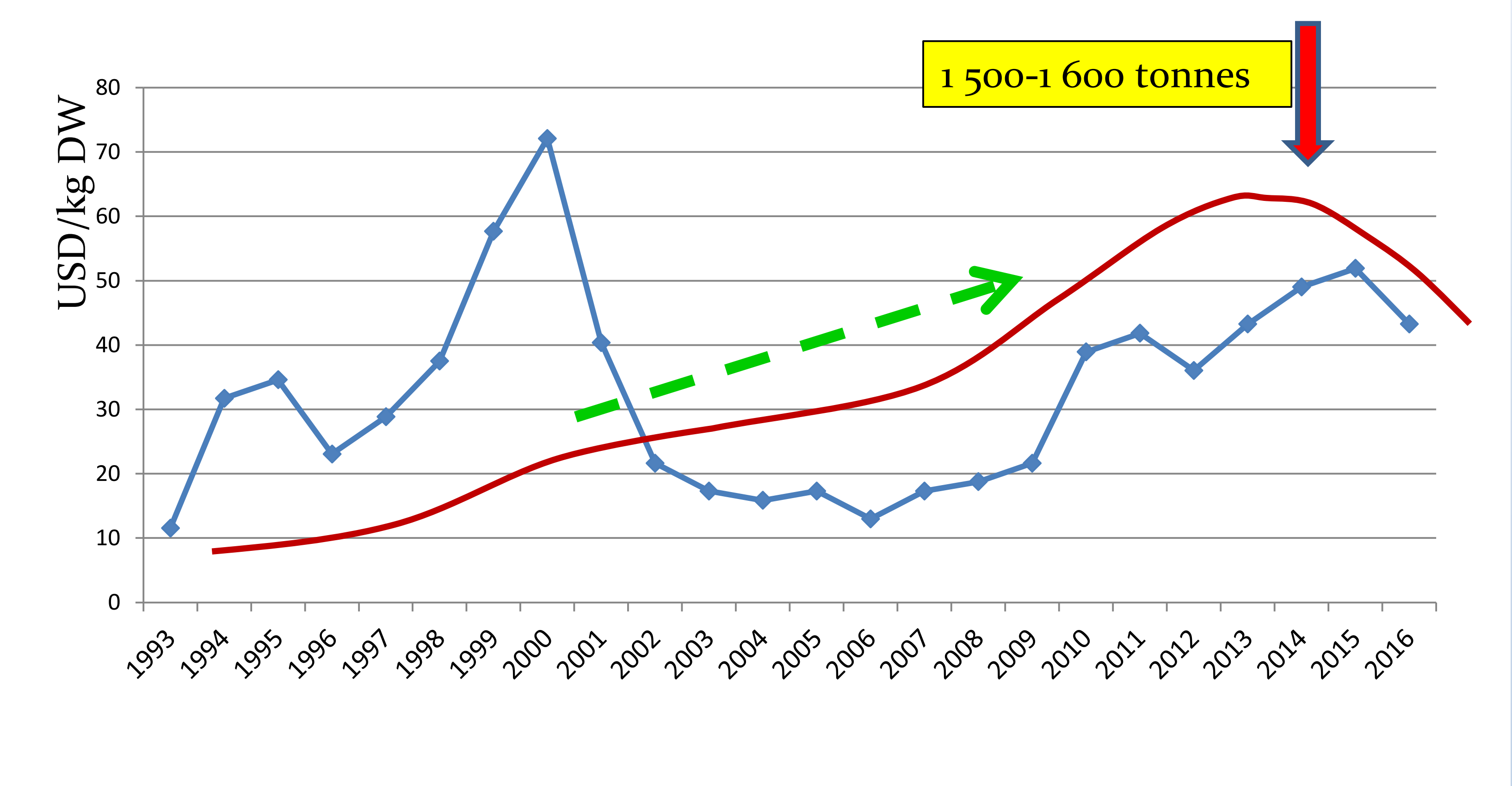
Evolution of *Artemia* cysts wholesale purchase prices in China



The reason of decrease on the demands of *Artemia* cysts in China

- Because of the price of *Artemia* cysts has been increased year by year, the cost of feed in hatcheries increased a lot. To reduce the cost, hatcheries use other artificial feeds and other alive feed (copepod) to replace *Artemia* nauplii.
- Improvement on the harvesting, processing, storage technology of *Artemia* cysts, enhanced the hatching quality. Farmers can get more *Artemia* nauplii from better quality of *Artemia* cysts.
- Farmers installed better hatching facilities in the hatcheries, and the *Artemia* nauplii centers are more professional, both improved the hatching efficiency of *Artemia* cysts in the application.
- Total production of shrimp/fish larvae reduced recently.

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Use of *Artemia* in the larviculture of commercially important crab species in China

Xugan Wu
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Contents

I. Background

- Crab culture are the big industry in Chinese aquaculture, valued more than **10 billions** US $ per year;
- **Three major culture species:** Chinese mitten crab (*Eriocheir sinensis*), swimming crab (*Portunus trituberculatus*) and mud crab (*Scylla Paramamosain*)

823 000 tonnes

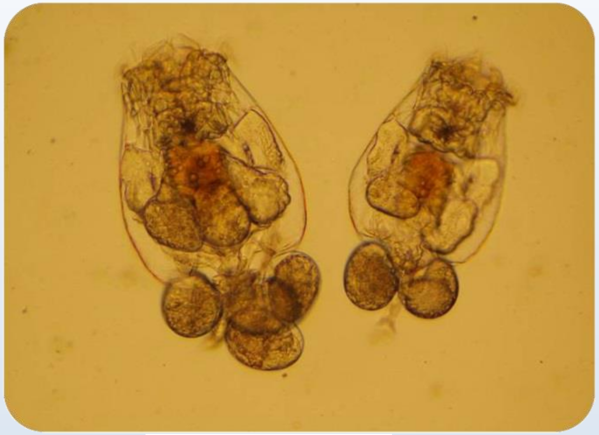
148 000 tonnes

124 000 tonnes

Seed sources – hatchery or wild collection?

- Chinese mitten crab: successful artificial hatchery, in earth ponds and indoor tanks.
- Swimming crab: a certian successful artificial hatchery for indoor tank scale;
- Mud crab: not successful, depending on wild collection.

Outdoor larval breeding of *E. sinensis*



Rotifer

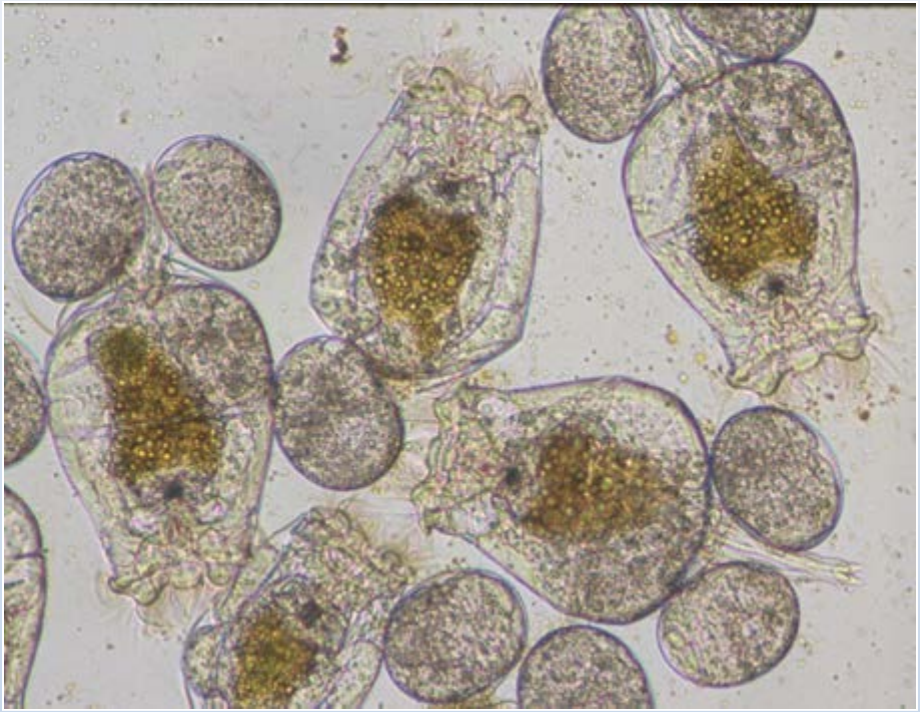
Artemia

Daphnia

Copepod

Mysis

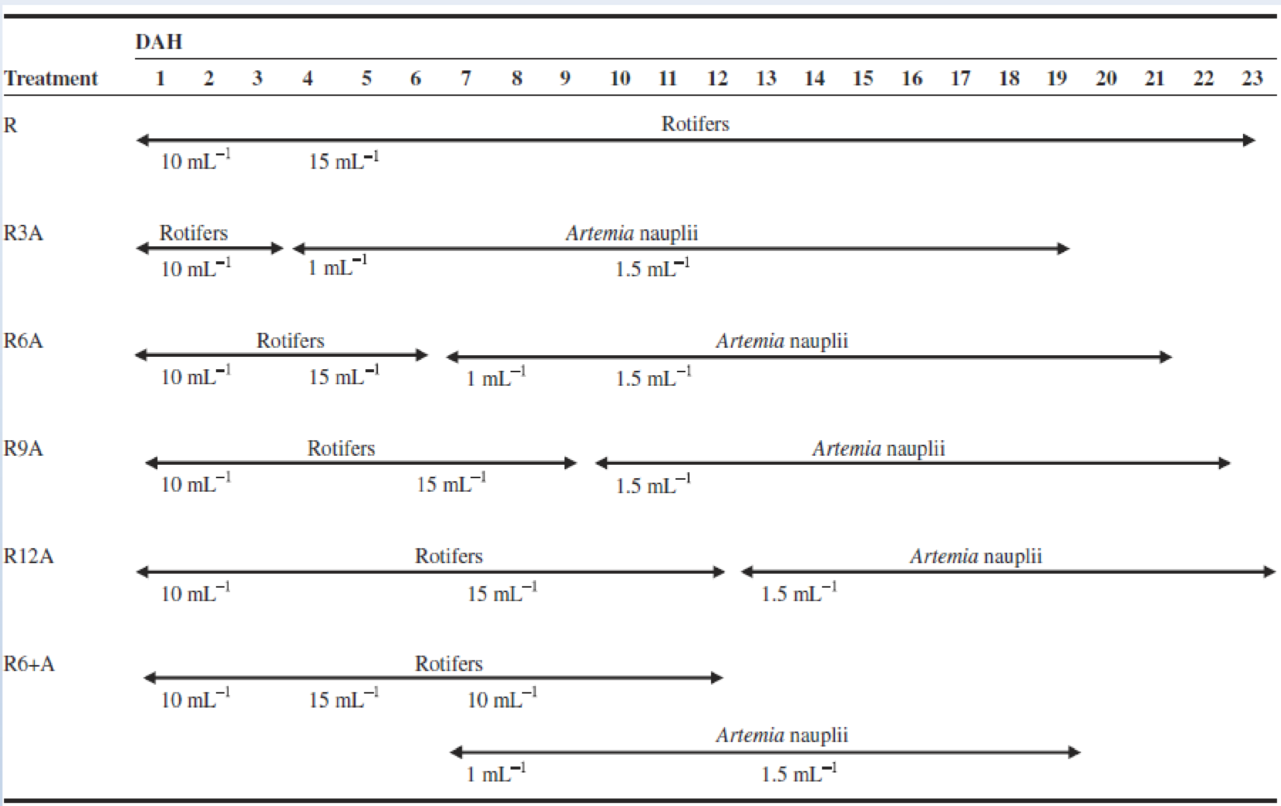
II. Feeding scheme and density- from Prof. Sui



B. rotundiformis

Artemia nauplii

Feeding scheme for Chinese mitten crab larvae



Survival, individual dry weight and duration of the zoeal stage of different treatments of larval *E. sinensis*

	Survival percentage (%)					Individual megalopa dry weight (μg)	Duration of zoeal stage (days)
Treatment	Z2	Z3	Z4	Z5	Megalopa		
R	90.7 ± 5.7^{a}	73.6 ± 2.4^{a}	52.1 ± 7.9^{a}	13.3 ± 3.9^{c}	6.2 ± 1.8^{c}	425.0 ± 5.0^{b}	22.2 ± 1.2^{c}
R3A	92.7 ± 2.8^{a}	73.2 ± 1.9^{a}	68.9 ± 4.9^{a}	28.6 ± 6.3^{ab}	19.0 ± 3.3^{ab}	465.0 ± 33.8^{ab}	18.2 ± 0.4^{a}
R6A	92.1 ± 4.2^{a}	73.7 ± 6.2^{a}	58.3 ± 4.6^{a}	37.8 ± 8.4^{a}	28.1 ± 2.9^{a}	563.6 ± 63.9^{a}	18.2 ± 0.4^{a}
R9A	88.9 ± 9.0^{a}	73.8 ± 9.1^{a}	58.9 ± 12.6^{a}	38.7 ± 2.4^{a}	27.7 ± 3.9^{a}	535.2 ± 54.9^{a}	18.8 ± 0.4^{a}
R12A	88.4 ± 4.8^{a}	79.7 ± 8.1^{a}	63.1 ± 14.1^{a}	21.7 ± 2.5^{bc}	16.7 ± 3.4^{b}	491.4 ± 38.6^{ab}	20.8 ± 0.3^{b}
R6+A	89.1 ± 4.2^{a}	70.7 ± 7.7^{a}	64.2 ± 7.1^{a}	37.6 ± 7.7^{a}	28.0 ± 5.5^{a}	548.9 ± 48.4^{a}	18.2 ± 0.4^{a}

Conclusion of part II

- Optimal rotifer density: Z1 and Z2 were 15 and 20 ml/l, respectively;
- *Artemia* density: Z3, Z4 and Z5 were 3, 5 and 8 ml/l, respectively.

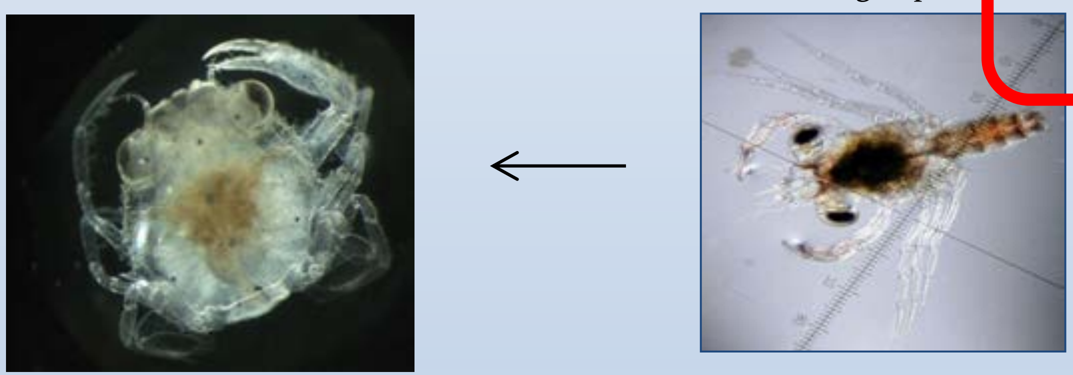
Sui *et al.* (2008) Aquaculture Research, 39, 568-576

III. Nutritional enrichment

Zoea I | Zoea II | Zoea III | Zoea IV | Zoea V

Larvae development of mitten crab

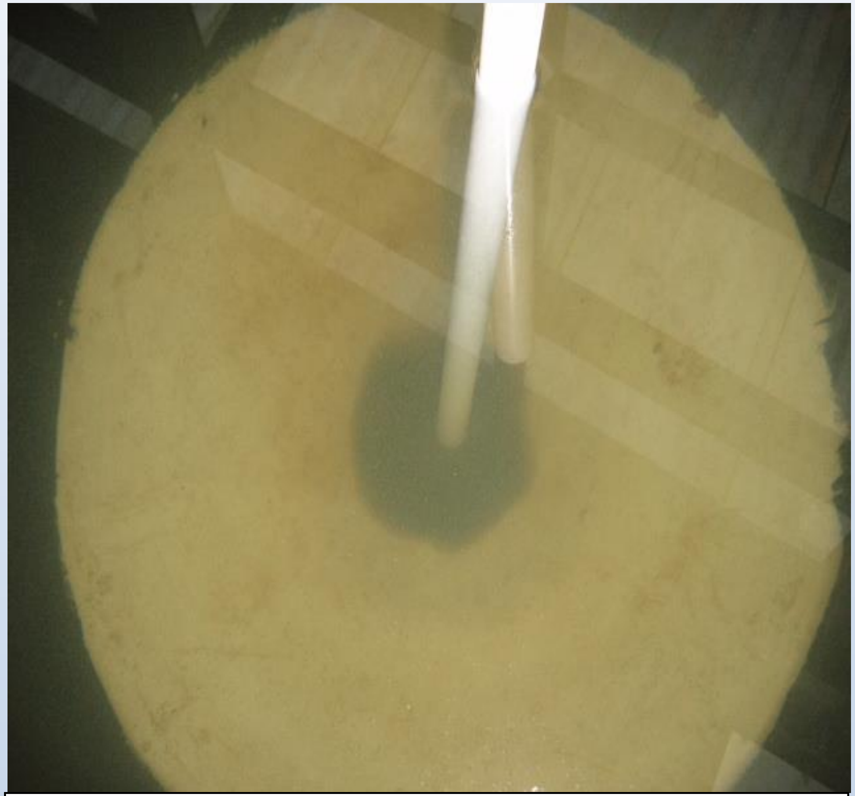
First crab | Megalopa



Development time approx. 20-35 days

Larvae with different quality

Active newly hatched larvae



Sinking and dead larvae

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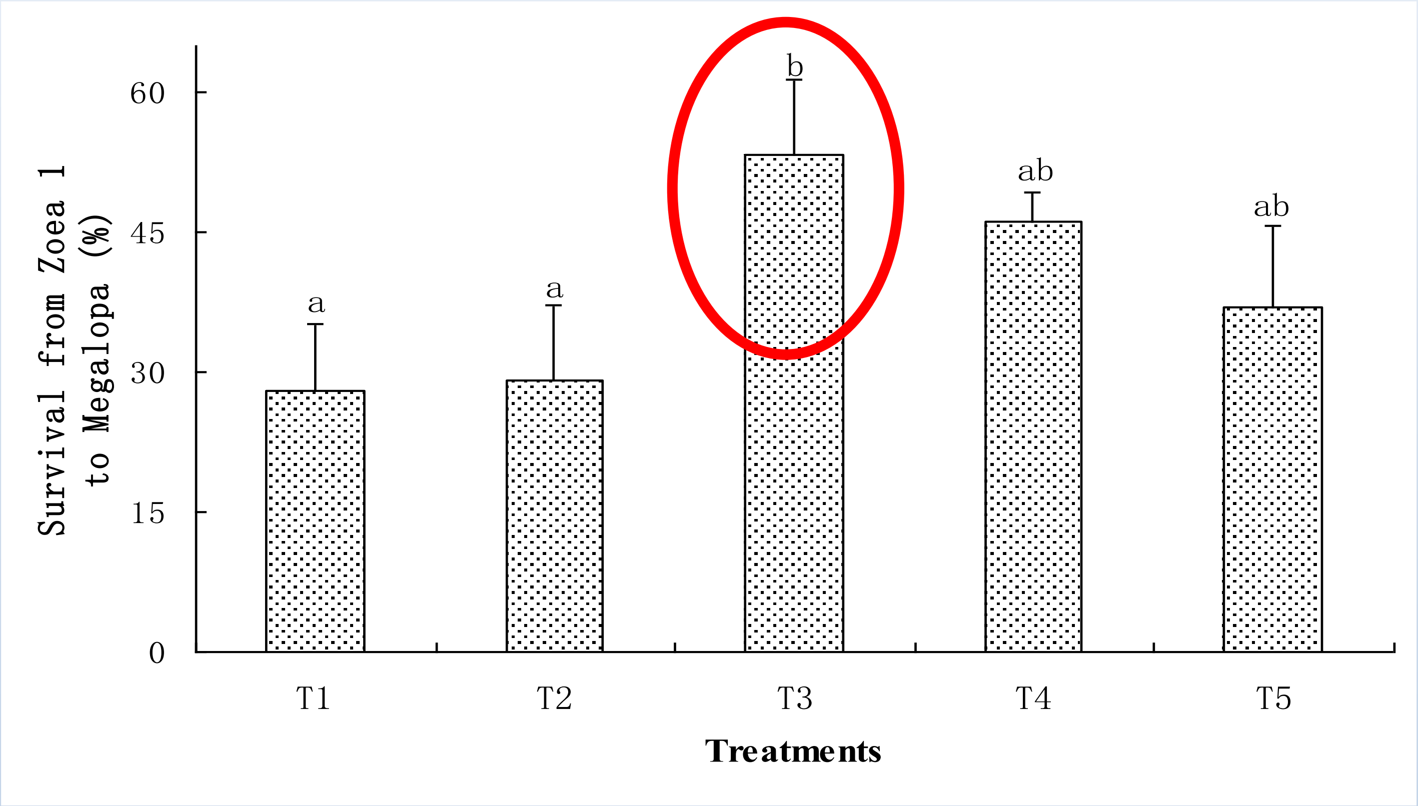
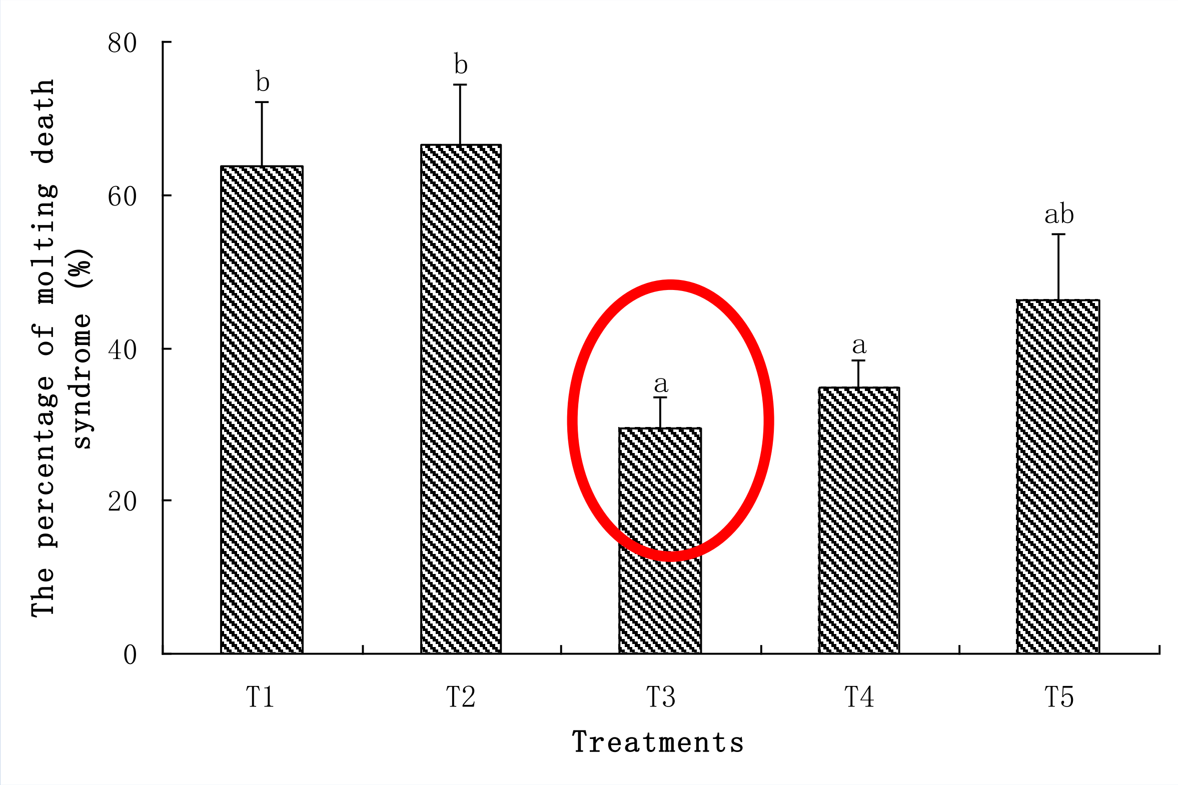
Artemia enrichment

Survival - crab larvae from zoea 1 to megalopa



Percentage of MDS of larvae fed enriched *Artemia* contained different DHA/EPA ratio during the period of zoea 4 to megalopa



Final carapace length and individual dry weight - crab larvae

Treatments	Carapace length (mm)	Dry weight (μg.ind.-1)
T1	2.18±0.03a	292.26±3.53ab
T2	2.23±0.03a	260.89±3.30a
T3	**2.34±0.05c**	**354.02±18.63c**
T4	2.30±0.07bc	322.87±22.31bc
T5	2.25±0.06ab	295.99±9.93ab

Conclusion of Part III

- For the crab larvae, the optimal DHA/EPA ratio of enriched *Artemia* around 0.50;
- For the crab larvae, the optimal ARA level in the enriched *Artemia* was around 3.0% TFA;
- For the crab larvae, the optimal HUFA level in the enriched *Artemia* was around 15-20% TFA;

IV. Replacement

- Early stage: rotifer;
- Later stage: daphnia, copepod and mysis;
- Formulated diets.

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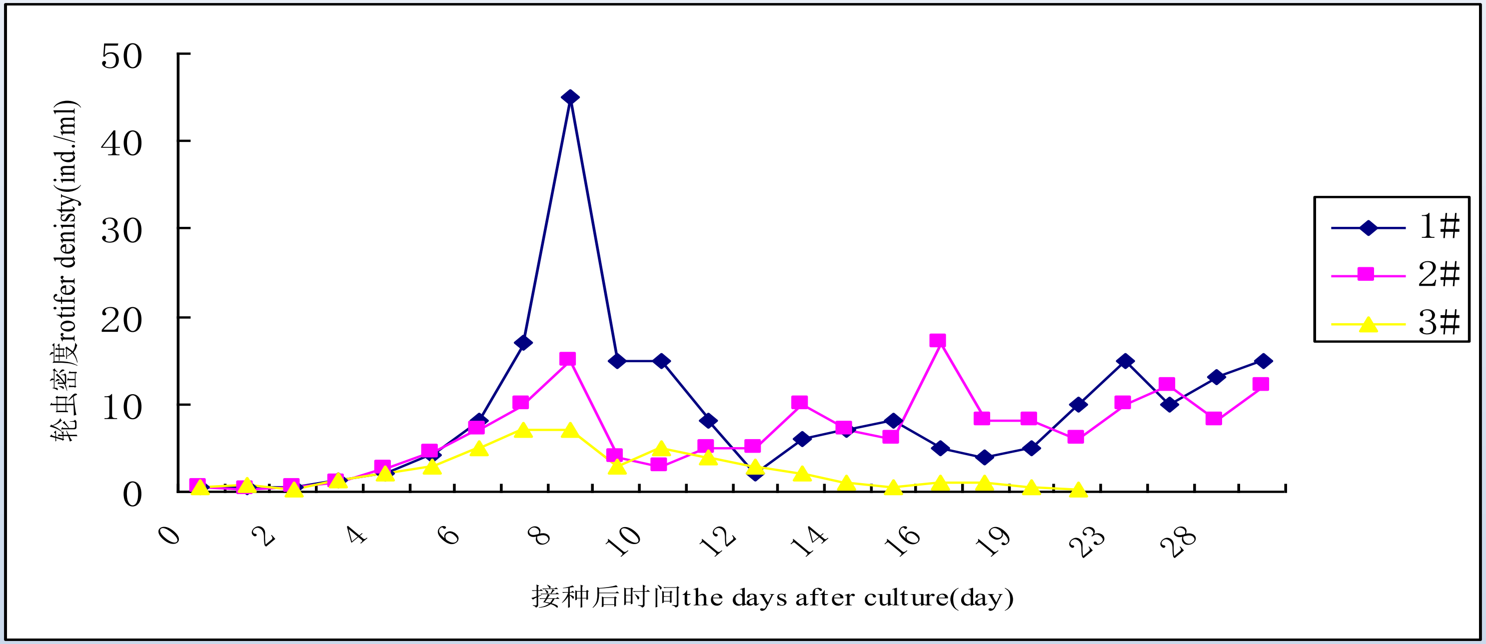
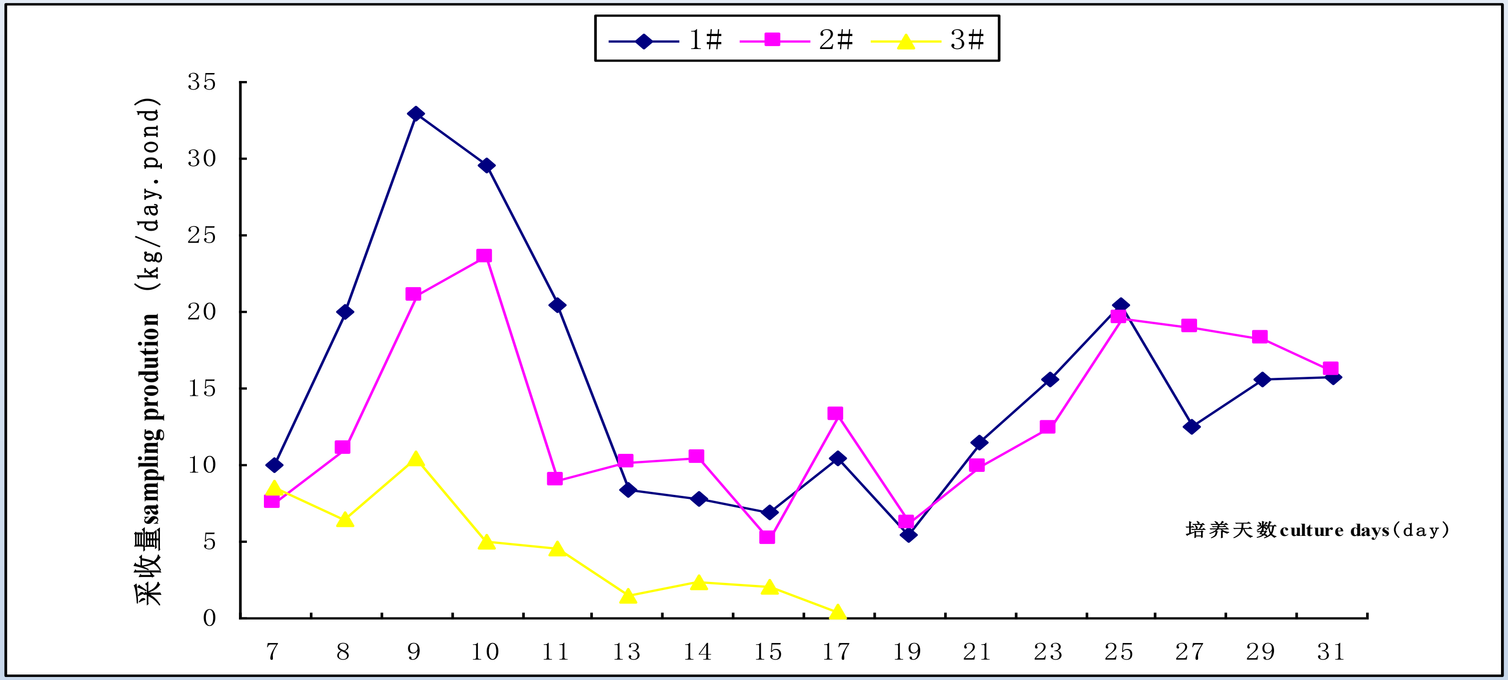
Changes of rotifer density in open earth ponds



The changes of rotifer density during the breeding period

The changes of rotifer production（wet weight/pond/day) in three breeding ponds



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Using copepod and mysis in larval culture of Chinese mitten crab

上海水產大學
Shanghai Fisheries University

Treatments

- Treatment 1: Nauplius of *Artemia sp*.
- Treatment 2: Enriched nauplius of *Artemia* sp.
- Treatment 3: *Centropages dorsispinatus*
- Treatment 4: *Centropages dorsispinatus +Neomysis joponica*

Results

The survival rate, period from Z_4 to Megalopa and Carapace length of megalopa of larvae of *E. sinensis*

Item	T1	T2	T3	T4
Survival rate (%)	55.82±12.71	56.26±11.10	66.81±7.18	70.48±6.27
Days Z4 to Megalopa	12-13	11-12	10-11	10-11
Carapace length of megalopa (mm)	2.18 ± 0.08^a	2.25 ± 0.09^a	2.40 ± 0.12^b	2.36 ± 0.09^b

Summary

1. More efforts should be invested in nutritional enhancement of *Artemia* (e.g. vitamin and mineral) depending on crab species;

2. There is long way to minimize the *Artemia* requirement by the cost effective replacement;

3. It is necessary and very important to setup a *Artemia* Reference Centre in Tianjin, then we standardize the research methods, do more cooperation, and efficiently support the aquaculture industry.

Thank You

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INVE's experiences in the exploration of new commercial *Artemia* resources for the aquaculture industry

Eddy Naessens
INVE Aquaculture, Dendermonde, Belgium

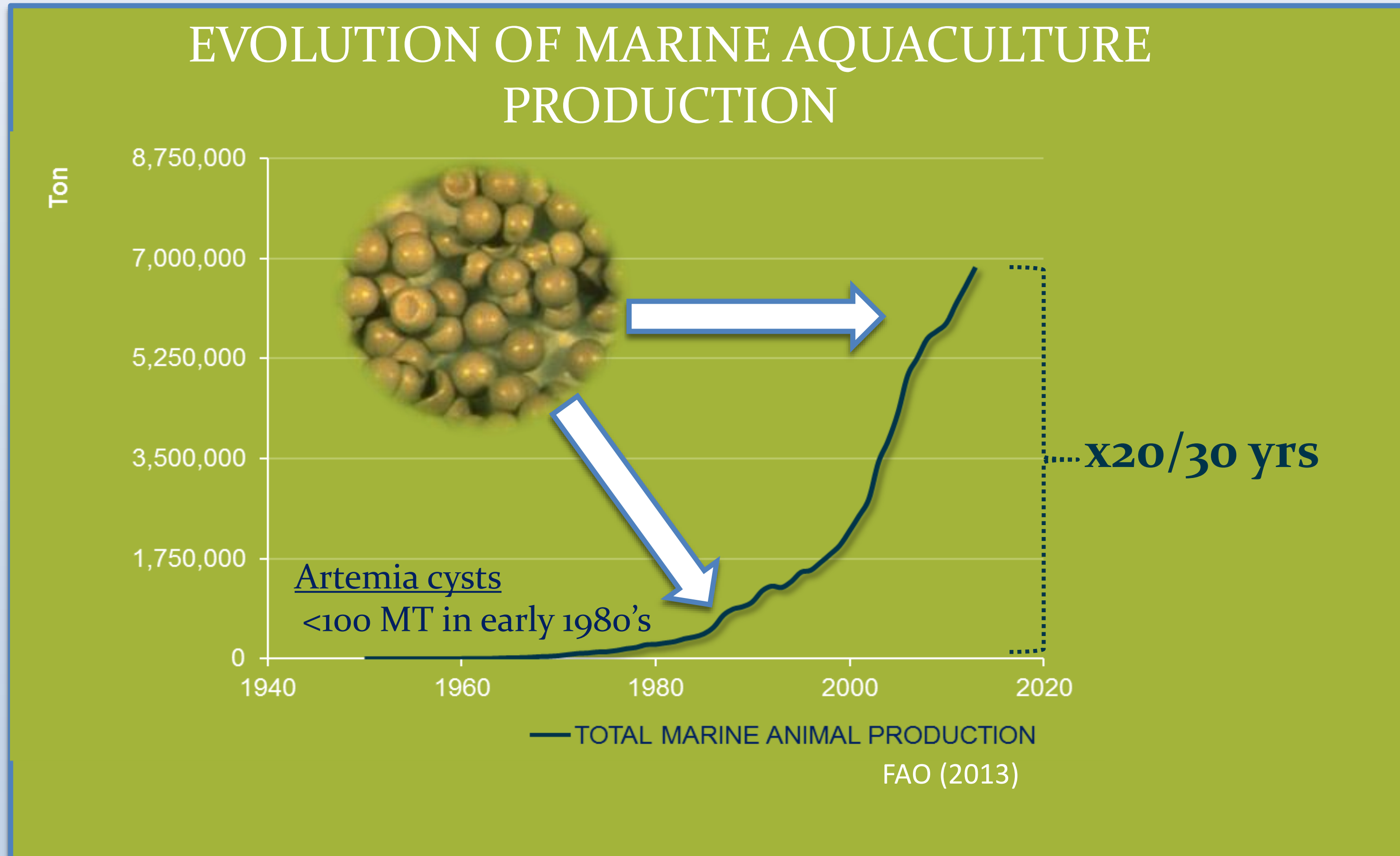
Food and Agriculture Organization of the United Nations

Tianjin University of Science & Technology

BACKGROUND

- Rapid **expansion of aquaculture** in 1980's-1990's
  - Larger production volumes
  - More species cultured

MORE *ARTEMIA* NEEDED (OR REDUCTION OF *ARTEMIA* IN THE FEEDING SCHEDULES)



BACKGROUND

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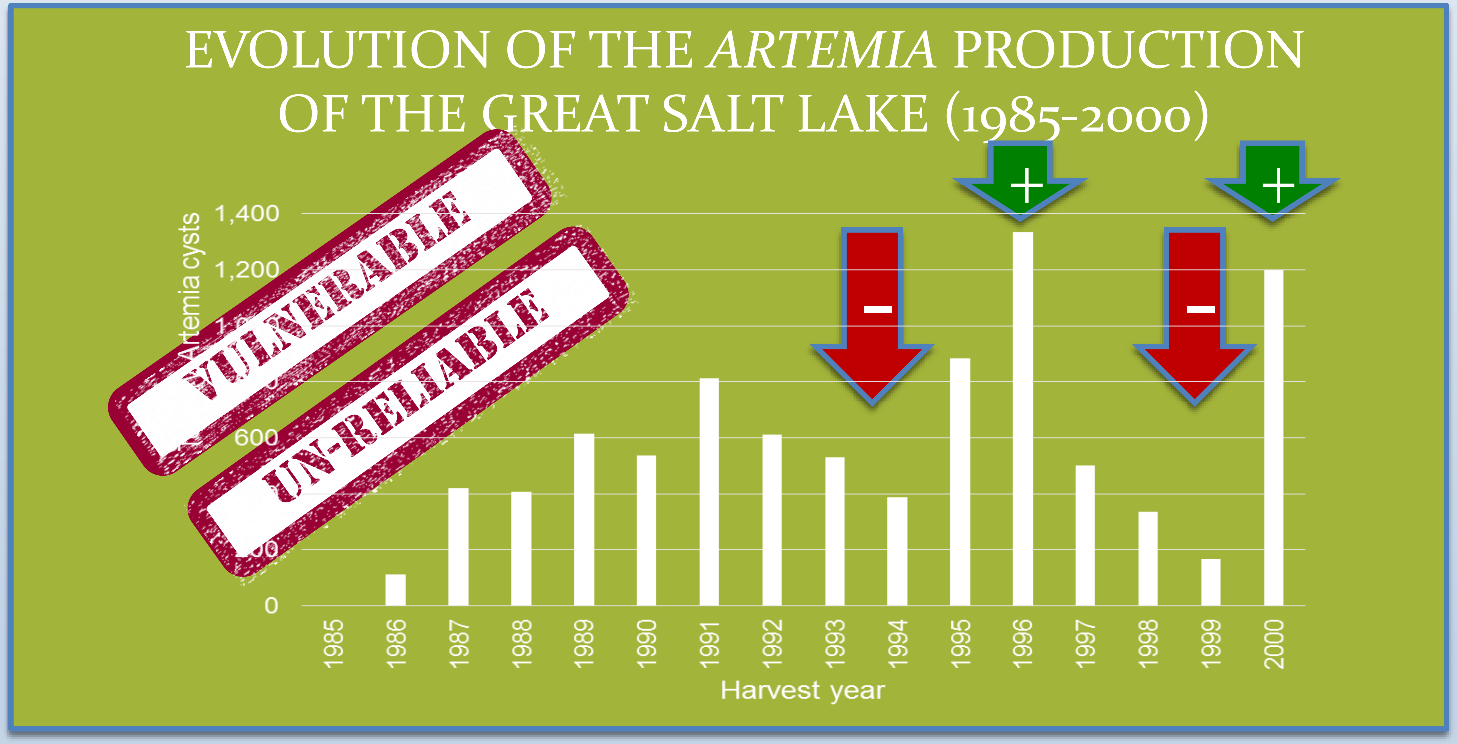
MORE *ARTEMIA* NEEDED (OR REDUCTION OF *ARTEMIA* IN THE FEEDING SCHEDULES)

- Worrying ***Artemia* supply**
  - One main source: Great Salt Lake; variable output
  - Some small suppliers e.g. San Francisco Bay



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- Worrying ***Artemia* supply**
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  - Some small suppliers e.g. San Francisco Bay

- Worrying ***Artemia* quality**
  - Unsufficient understanding of:
    - conditions for dormancy breaking
    - temperature tolerance during processing
    - moisture content of processed product
    - storage conditions

- Worrying ***Artemia* use**
  - Hatching conditions for optimal nauplius yield
    e.g. - influence of light, temperature, salinity, etc
  - Nutritional quality
    e.g. - nutritional profiling
    - instar I / II differences
    - nutritional enhancement

BACKGROUND

- Rapid **expansion of aquaculture** in 1980's-1990's
  - Larger production volumes, more species cultured
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INVE
Artemia Task Force

- Diversify & stabilize the global *ARTEMIA* SUPPLY
- Develop local PRODUCTION, QC & STORAGE METHODS
- Establish & implement QUALITY CRITERIA
- Establish optimal *Artemia* APPLICATION PROTOCOLS

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INVE Artemia Task Force

Objectives

- Diversify & stabilize the global *ARTEMIA* SUPPLY
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multi-disciplinary approach

Implementation

In-house expertise

Collaboration with specialized partners

- universities, laboratories
- local partners (public and private institutions, companies)

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- Develop local PRODUCTION, QC & STORAGE METHODS
- Establish & implement QUALITY CRITERIA
- Establish optimal *Artemia* APPLICATION PROTOCOLS

multi-disciplinary approach

Tasks

Site surveys & explorations

- ACCESSIBILITY of the site/resource
- ECOLOGICAL STUDIES (population dynamics, sustainable production level)
- evaluation of ECONOMICAL RELEVANCE

Establishment of local representation

- defining LOCAL PARTNERS
- creation of LOCAL INVE COMPANIES
- installation of necessary INFRASTRUCTURE (**production** and **QC-lab facilities**)
- TRAINING LOCAL STAFF (incl. training at international institutions (e.g. ARC) and INVE companies (e.g. ITECH, UTAH *Artemia* production plant)

Exploitation of the resource

- HARVESTING
- PROCESSING and QUALITY CONTROL
- COMMERCIALIZATION (local and export)

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INVE Artemia Task Force

Diversify & stabilize the global Artemia supply

Russia, 1991 - ...

Exploration of various lakes

In collaboration with:

- Federal State Centre for Fisheries (Rosrybkhoz)
- ARC

Inland salt lakes (> 10 lakes)

- Altai area
- Kurgan area
- Omsk area

Creation of INVE companies

In collaboration with local companies (e.g. Barrom, Arsal)

INVE Altai
INVE Kurgan

INVE Artemia Task Force

Diversify & stabilize the global Artemia supply

China, 1994 - ...

Exploration of various lakes and solar saltworks

In collaboration with:
- Salt Research Institute (SRI)
- ARC

Inland salt lakes
- Aibi lake (Ebi Nur)
- Balikun lake
- Qinghai area
- Lakes on the Tibet plateau
- Inner Mongolia

Solar salt works
- Bohai Bay area

Creation of INVE company

In collaboration with SRI **TIAC**

INVE Artemia Task Force

Diversify & stabilize the global Artemia supply

Turkmenistan, 1995 - 2001

Exploration of Karabogaz Gol

In collaboration with:

- Ministery of Natural Resources,
- Ministery of Oil and Gas,
- Turkmenistan Academy of Sciences
- ARC

Inland salt lake

- Karabogaz Gol

Creation of INVE company

In collaboration with Ministery of Natural Resources

Turkmenistan *Artemia*

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Diversify & stabilize the global Artemia supply

Kazakhstan, Uzbekistan, 2003 - …

Exploration of various inland lakes

In collaboration with:

- Institute of Bioecology of Karakalpak
- Laboratory of Ichthyology and Hydrobiology (Uzbek Academy of Sciences)
- ARC

Inland salt lakes

- Sor Kaidak (Western Kazakhstan)
- Lakes in the Pavlodar Area
- Aral lake (KAZ & UZ territories)

Creation of INVE company (KAZ)

In collaboration with:

- Ministery of Natural Resources (Kazakhstan)
- Local company

INVE Mangistau Bio-Resources (IMBR)

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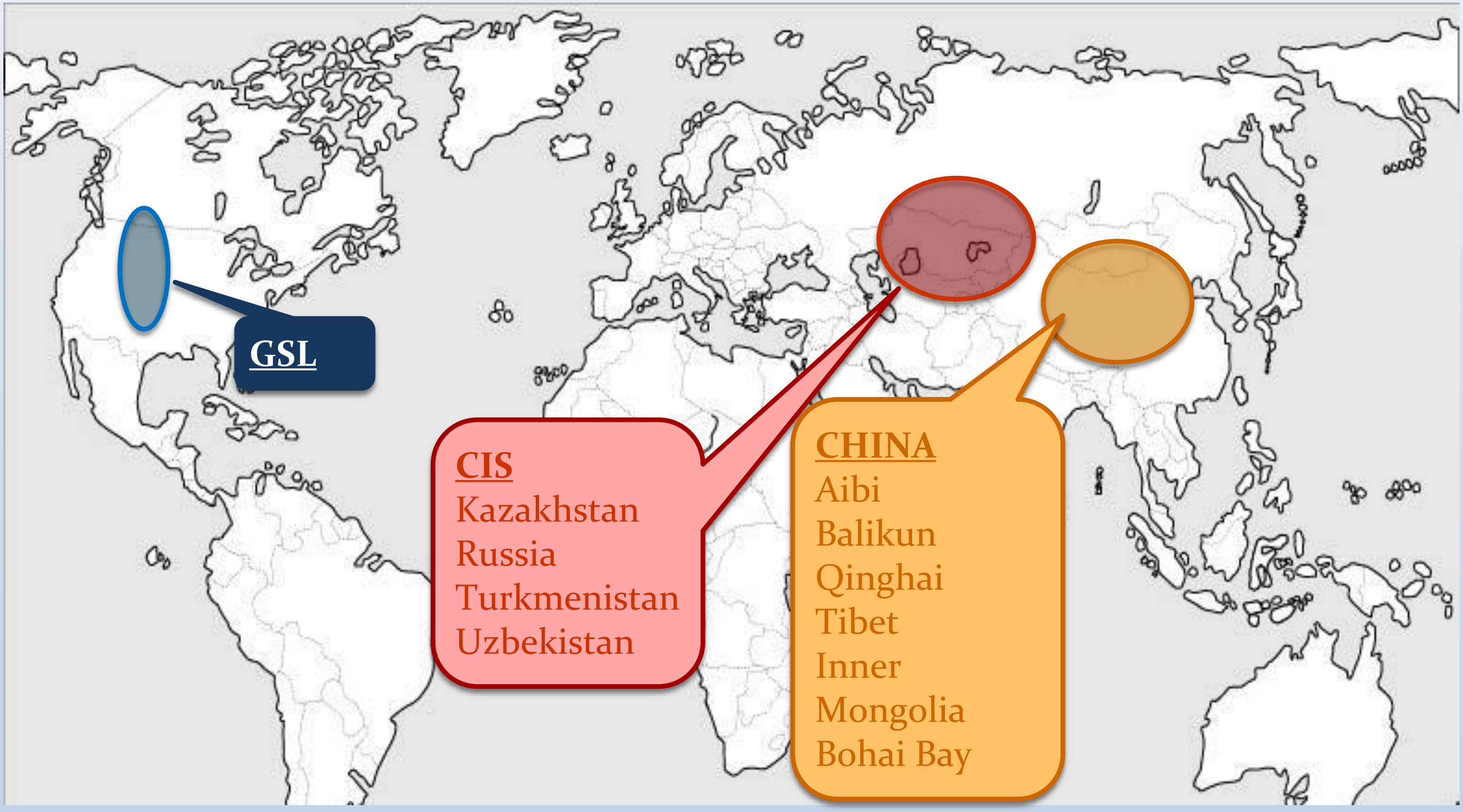
Diversify & stabilize the global Artemia supply



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INVE Artemia Task Force

Diversify and stabilize the global *Artemia* supply

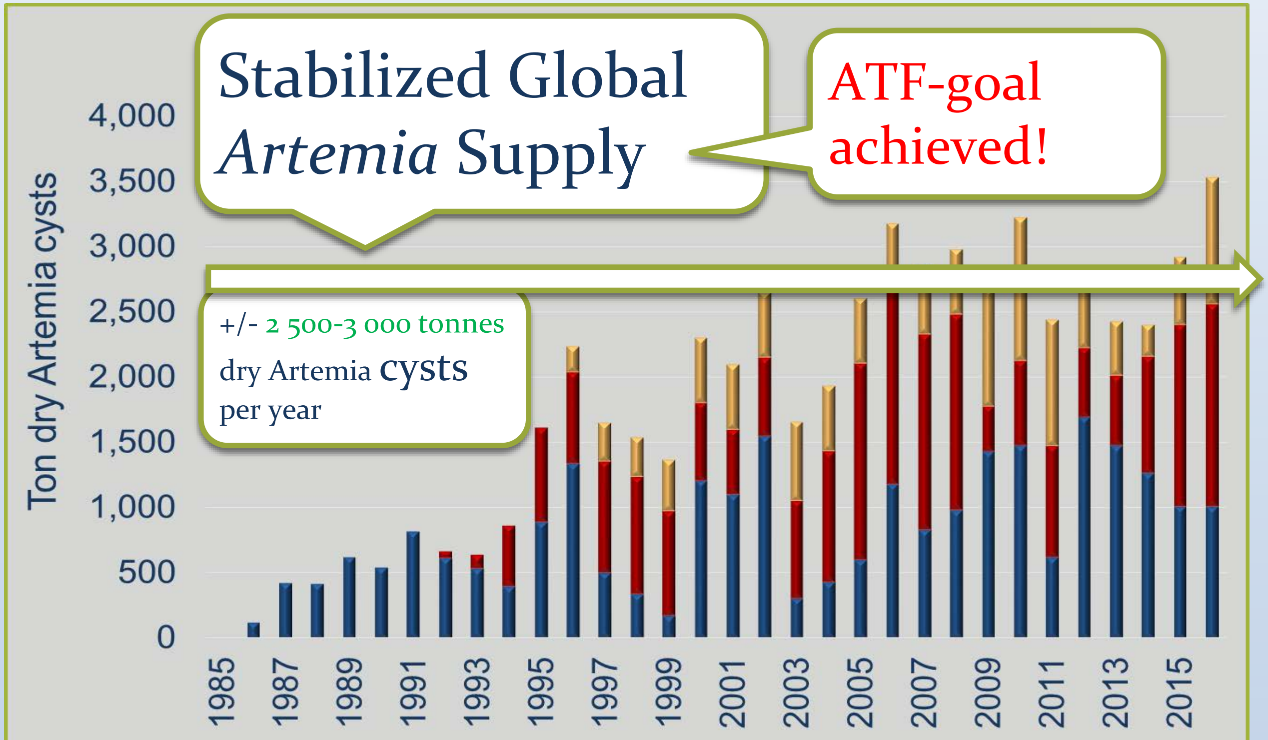
INVE "Classic"

GSL

INVE – ATF

CIS
Kazakhstan
Russia
Turkmenistan
Uzbekistan

CHINA
Aibi
Balikun
Qinghai
Tibet
Inner Mongolia
Bohai Bay



Evolution of global Artemia supply

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Objectives

- Diversify & stabilize the global *ARTEMIA* SUPPLY
- Develop local PRODUCTION, QC & STORAGE METHODS
- Establish & implement QUALITY CRITERIA
- Establish optimal *Artemia* APPLICATION PROTOCOLS

multi-disciplinary approach

Tasks

Harvest methods

- Open water harvests
- Shore harvests

Processing methods

- Wet processing / brine washing
- Dry processing

Quality assessment and quality control

- Up-to-date laboratory facilities
- Trained local personnel

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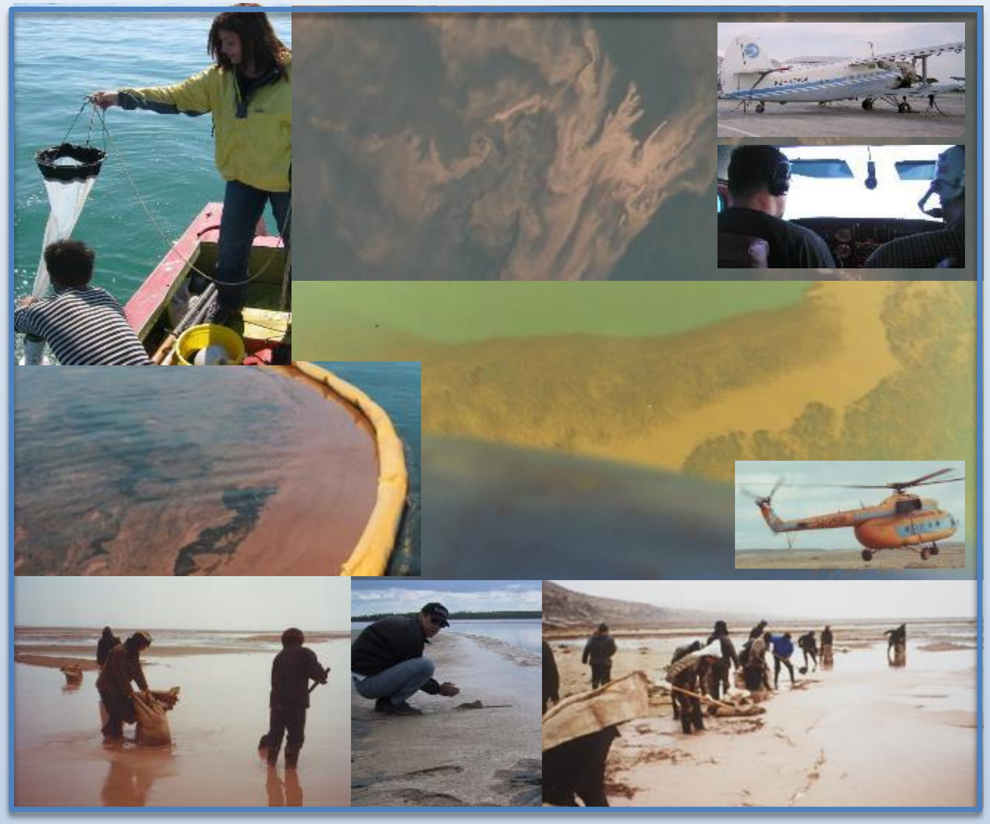
INVE Artemia Task Force

Develop optimal production & storage conditions

Harvest methods



- Sampling campaigns to estimate the lake's sustainable production
- Advanced methods of *Artemia* location (air-spotting)
- Traceability protocols
  - "from harvest location to can (to customer)"
- Efficient harvest methods
  - introduce open water harvest technique
  - apply best local alternatives

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INVE Artemia Task Force

Develop optimal production & storage conditions

Processing & QC methods

- Elaborate brine/freshwater cleaning protocols
  - maximal removal of impurities
- Automated drying processes allowing in-line monitoring & control of cyst temperature and moisture content
  - optimize the economics of the drying process
  - maximal safeguarding of the hatching potential
  - guarantee optimal shelf life of the cysts
- Strict QC of final product
  - intercalibration between all INVE labs and international research centres (ARC)

INVE Artemia Task Force

Objectives

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multi-disciplinary approach

Tasks

ARC criteria for hatching quality determination

- Hatching percentage
- Hatching efficiency
- Hatching kinetics
- Global implementation

Strain characterization

- Biometrics of cysts/nauplii
- Nutritional value

Product upgrading

- Hatching enhancing

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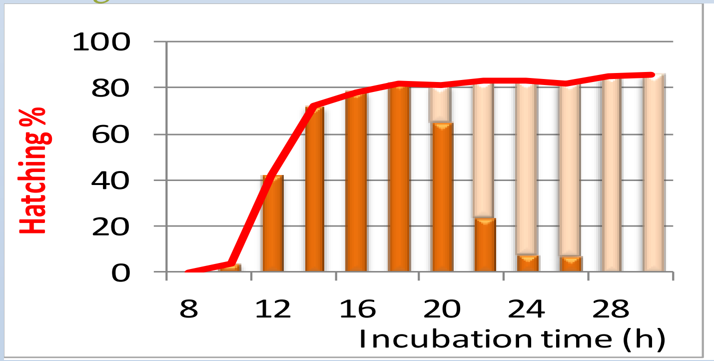
Establish & implement quality criteria

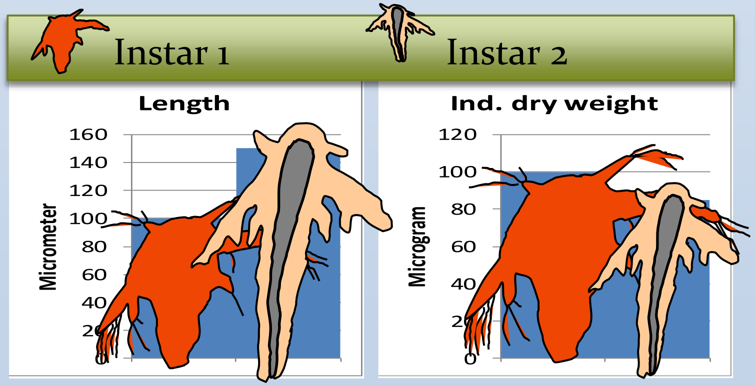
ARC criteria for hatching quality determination

Hatching percentage
number of nauplii hatched out of 100 full cysts)
→ Cyst GRADE: e.g 75%, 80%

Hatching efficiency
number of nauplii / gram of cysts
→ Cyst GRADE: e.g. 210,000 nauplii/gram

Hatching kinetics
hatching rate
→ Harvest the MOST NUTRITIOUS stage





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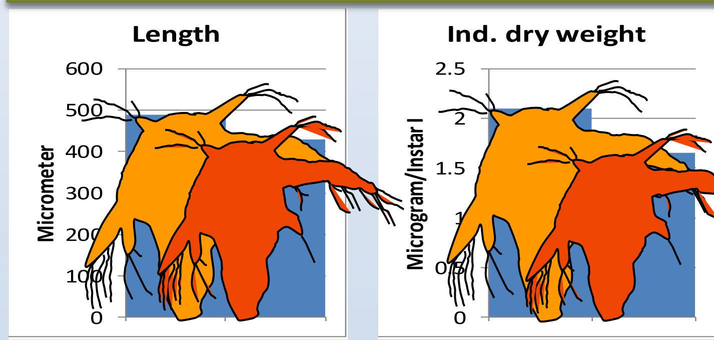
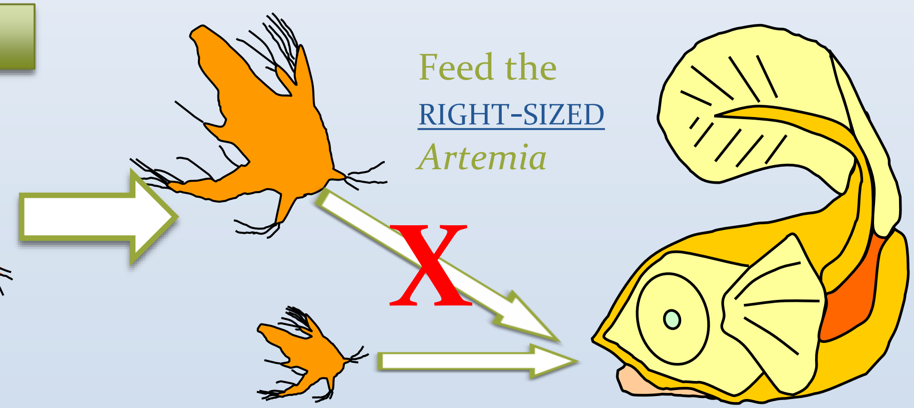
Establish & implement quality criteria

Strain characterization

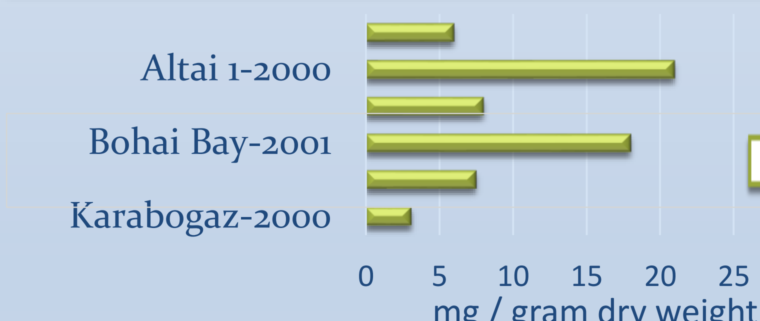
Biometrics SFB GSL





Feed the RIGHT-SIZED *Artemia*

Nutritional value HUFA profile (20:5n-3)



Observe the nutritional requirements of the predator

Feed the NUTRIONALLY APPROPRIATE *Artemia*

INVE Artemia Task Force

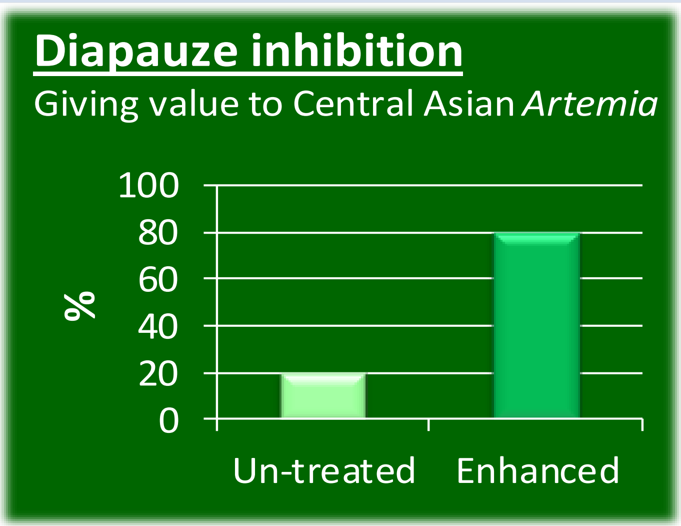
Establish & implement quality criteria

Product upgrading

Research

Diapauze inhibition
(hatching enhancing)

PROPRIETORY TECHNOLOGY
PATENT N°: WO02/28173



New Product Development

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INVE Artemia Task Force

Objectives

- Diversify & stabilize the global *ARTEMIA* SUPPLY
- Develop local PRODUCTION, QC & STORAGE METHODS
- Establish & implement QUALITY CRITERIA
- Establish optimal *Artemia* APPLICATION PROTOCOLS

multi-disciplinary approach

Tasks

Instructions for use

- Hatching
- Harvesting
- Cold storage
- etc.

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Establish optimal Artemia application protocols

Instructions for use

Research

ARC + INVE

- Establishing the hatching requirements/conditions for maximal nauplii output
- Establishing optimal harvesting methods
- Establishing methods for cold storage of *Artemia*

→ **Becoming the industry standards**

Practical guidelines

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DIVERSIFIED & STABILIZED *Artemia* supply

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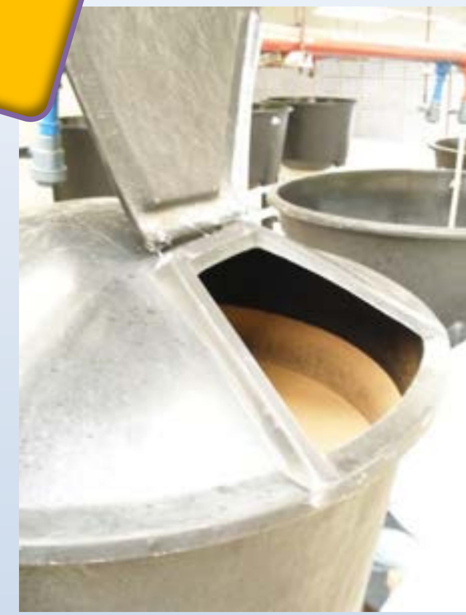
Certified Quality



PRODUCED, QUALIFIED & APPLIED according to top industry standards

INVE Artemia Task Force

Thank You

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Microbiome: criteria for the quality control of *Artemia* products

Zizhong Qi
Laboratory of Applied Microbial Technology, College of Marine Life Sciences, Ocean University of China, Qingdao, Shandong, China

The quality of *Artemia* products can be improved via

- Improve hatching rate
- Enrichment for increasing nutritional values
- Management of associated microbial community

Artemia inhabits diversified hypersaline environments associated with various of microbes

…Bacterial sequences most closely related to the genera *Halomonas* and *Vibrio* were commonly extracted from Great Salt Lake adult Artemia, while cysts yielded bacterial sequences from the genera *Idiomarina* and *Salinivibrio*, which were absent from adults and water…

- Riddle M R, Baxter B K, Avery B J. Molecular identification of microorganisms associated with the brine shrimp *Artemia franciscana* [J]. Aquatic Biosystems, 2013, 9(1):1-11.

Artemia cysts and adults are possible vectors for the delivery of harmful bacteria and virus

- Pathogenic Vibrios and WSSV are notorious for the causing severe infection disease.
- Result showed *Artemia franciscana* cultivated for 2 days had high numbers of presumptive vibrios and haemolytic bacteria.
- WSSV positive results both found in wild adult *Artemia* and filial generation, in an average rate of above 50%.
- Disinfection, although beneficial, may not prevent a re-colonization of the live food within a short time period

- Use of disinfected *Artemia* nauplii in combination with probiotics to ensure that beneficial rather than potentially pathogenic bacteria dominate the bacterial community

Number of *vibrio* bacteria in cultured water of *Artemia* after exposing to *Bacillus* mixture agent (*Bacillus subtilis*, *Bacillus licheniforms* and *Bacillus amyloliquefaciens*).

	Counts of Vibrio in cultured water (CFU/mL)	
	24h	48h
Control	1.1×10^6	3.0×10^6
Treatment	2.4×10^6	5.4×10^5

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Hatching rate of *Artemia* after exposing to Bacillus mixture agent (*Bacillus subtilis*, *Bacillus licheniforms* and *Bacillus amyloliquefaciens*).

	Hatching rate
	24h
Control	78.6±5.3
Treatment	85.2±3.7

Probiotics improved hatching rates!

Quantification of associated bacteria is an important factor in assessing the quality of *Artemia* cysts and nauplii

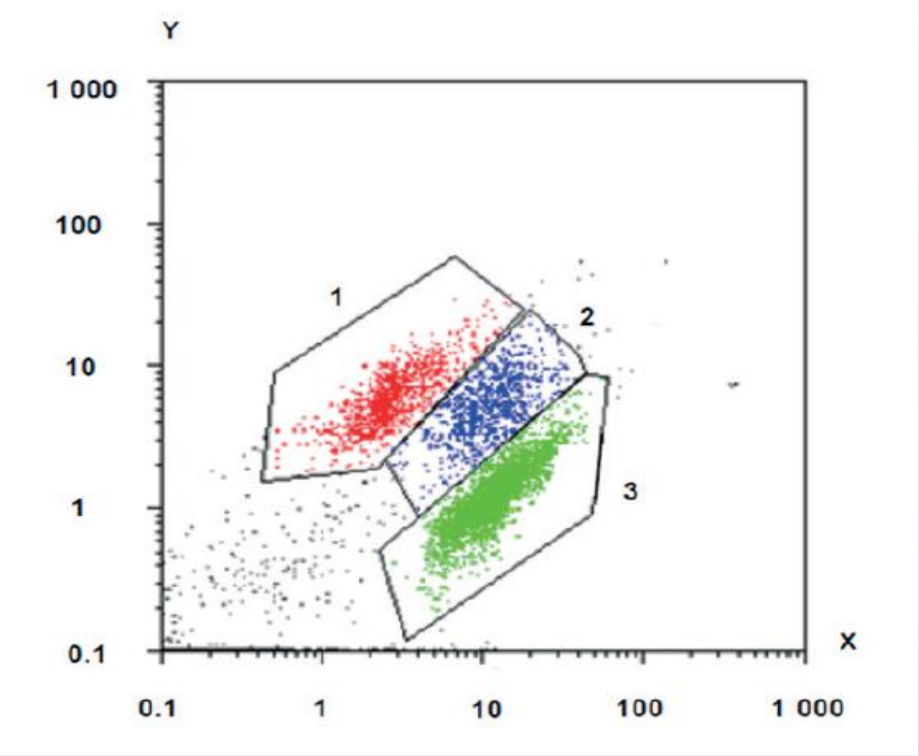
- Plate count techniques being the most traditional and widely used.
- Newer techniques include flow cytometry, metagenomics analysis which are able to determine cells as active and/or total units and species involved.

Advantages of the use of flow cytometry

- Low variation
- Differentiation between active, damaged and total cells
- High throughout

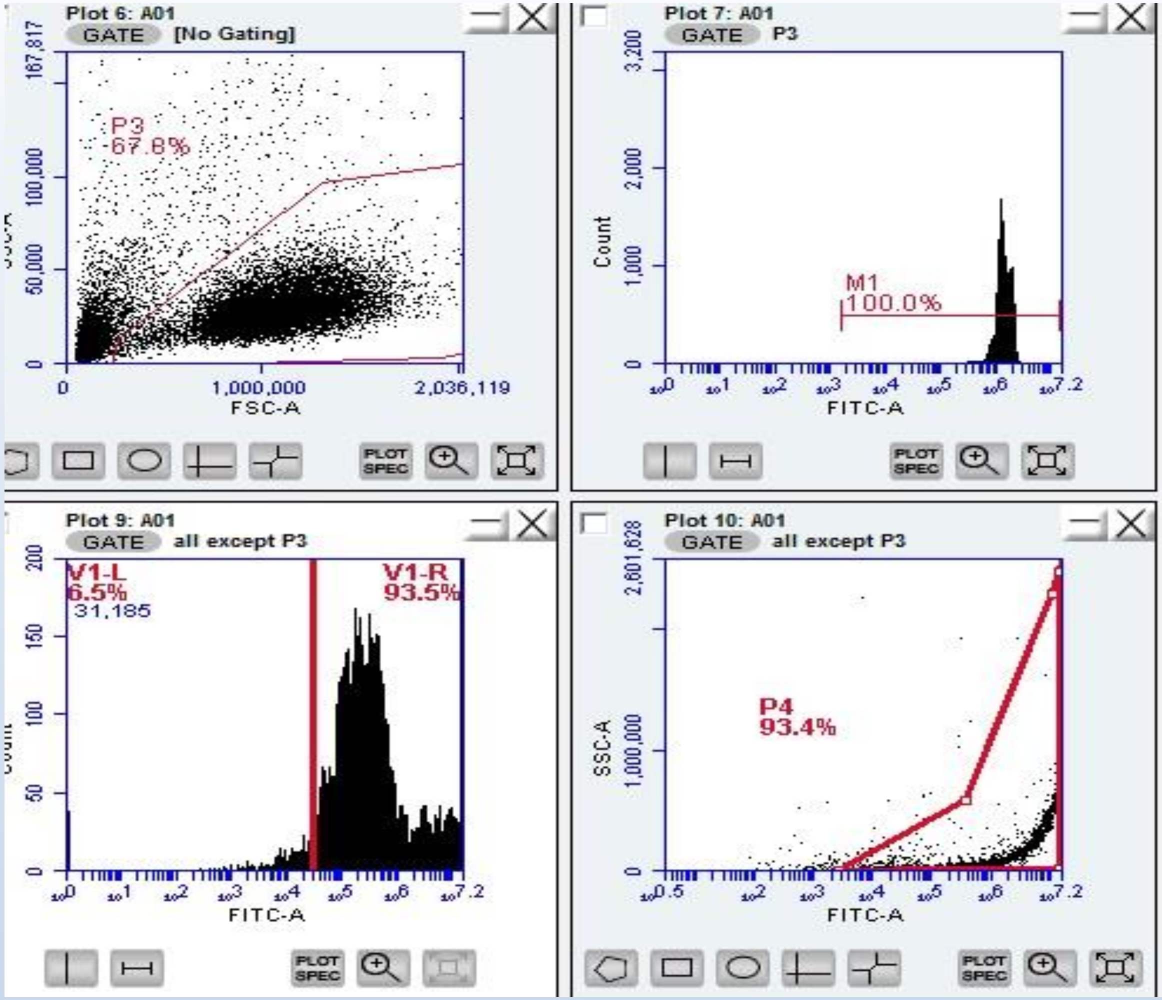
Advantages of the use of flow cytometry active cells vs. total cells

- The fitness of a given probiotic population
- Improved production process and shelf-life



1. Non-active; 2. Damaged; 3. active cells

(plots from BS ISO 19344:2015)



FCW analysis showed lactic acid bacteria fit the process of hatching *Artemia* and improved the quality of *Artemia* production

Further metagenomic study allow us to identify the potential pathogenic microbial populations, the beneficial compositions and activities of *Artemia* associated microbial communities, and understand their interactions with hosts and culture environment.

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