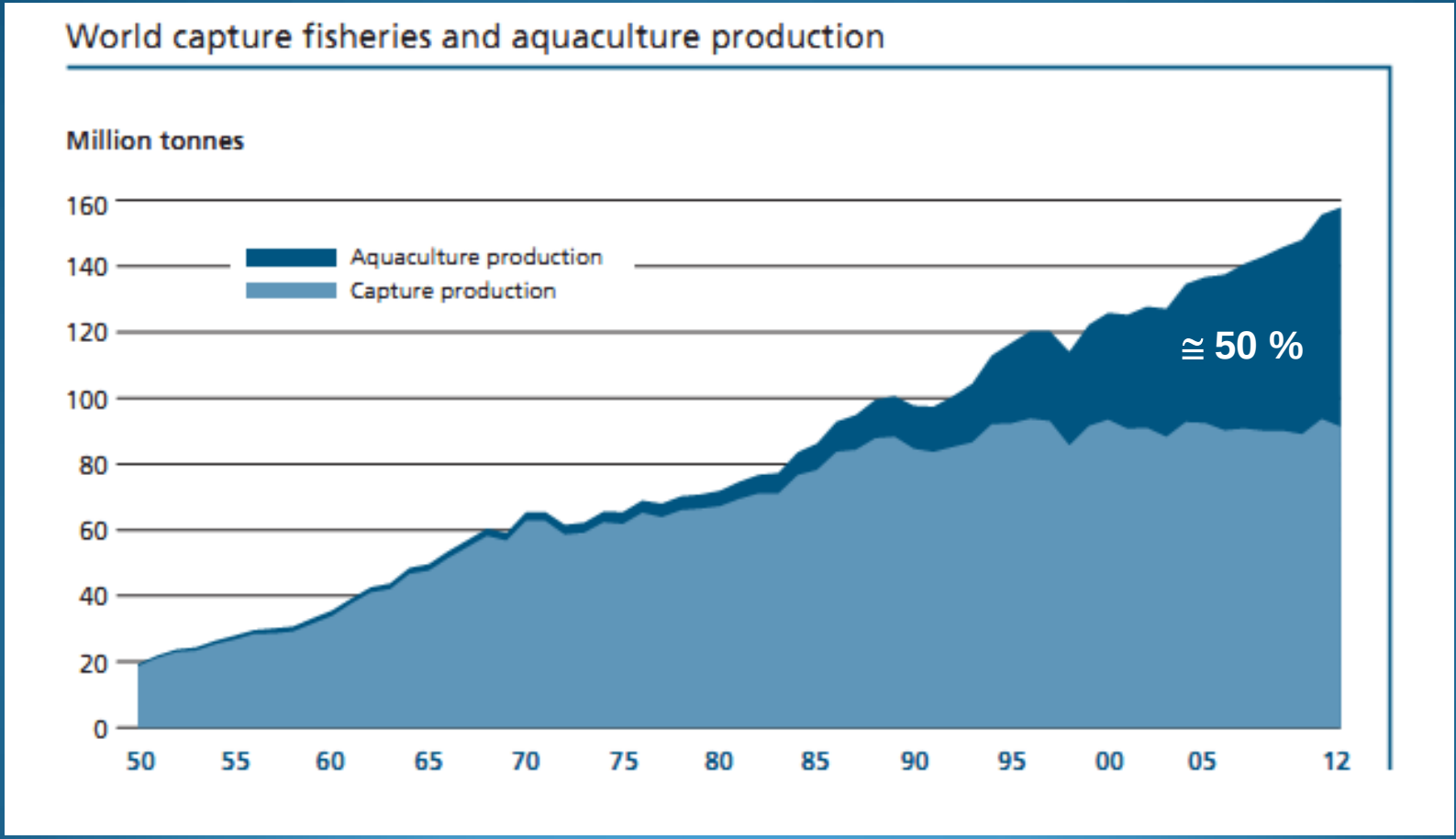
International workshop on "Sustainable use of marine and coastal resources in Kenya: from research to societal benefits"

# SUSTAINABLE ARTEMIA POND PRODUCTION IN COASTAL SALTWORKS AS A TOOL TO SOLVE AQUACULTURE CHALLENGES

By Nguyen Van Hoa , Nguyen Thi Ngoc Anh, Nguyen Thi Hong Van, Tran Huu Le, Patrick Sorgeloos and Gilbert van Stappen

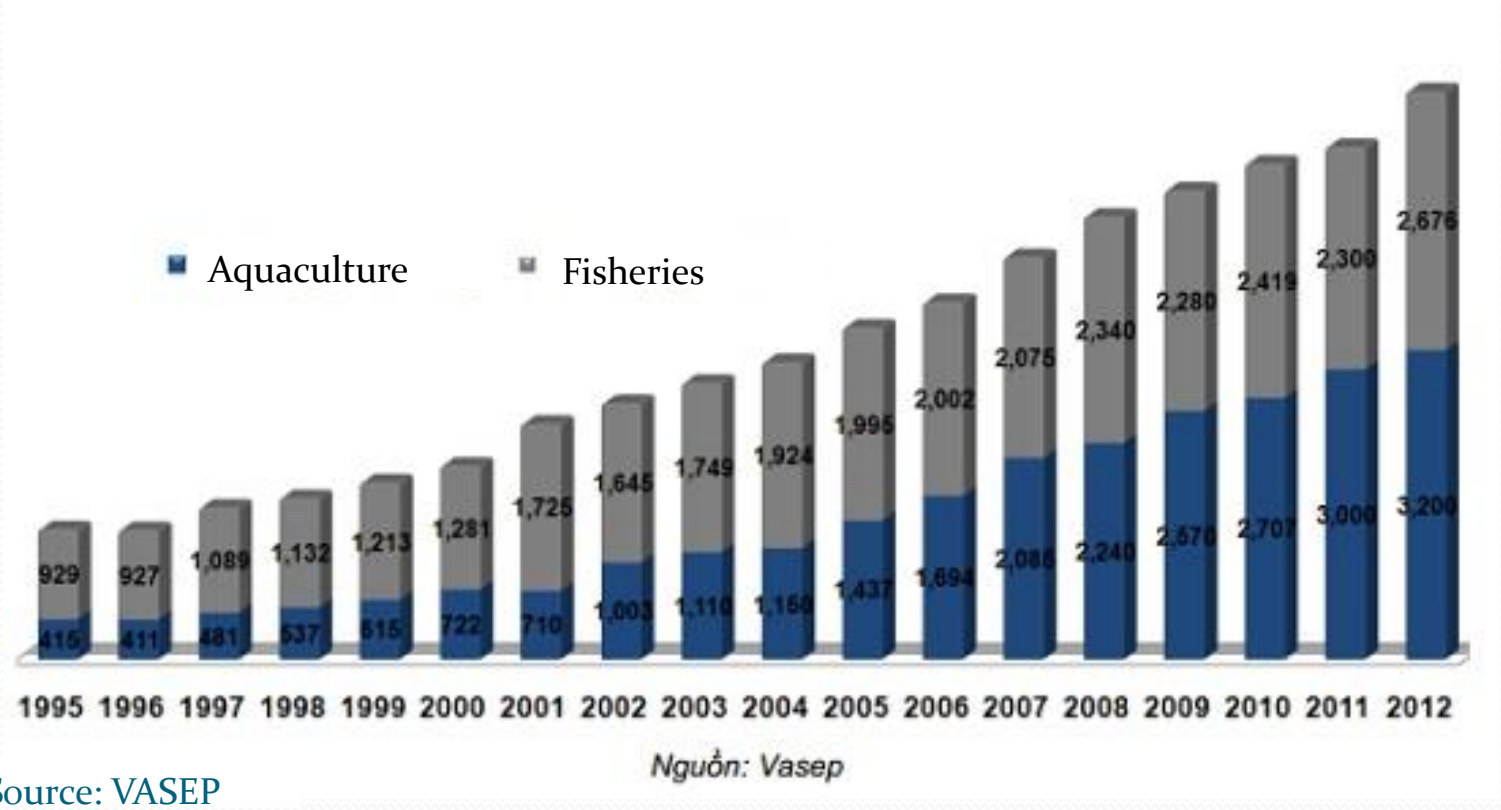
**Kenya, 27 – 29 October 2014**

# World Fisheries and Aquaculture production




(FAO, 2014)

# Vietnam Fisheries and Aquaculture production




≅ 70 %

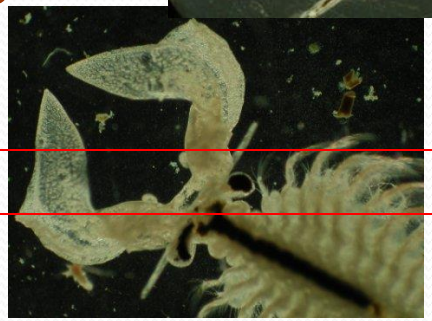
Source: VASEP

Total production in Vietnam is **6.05** mill tons in 2013

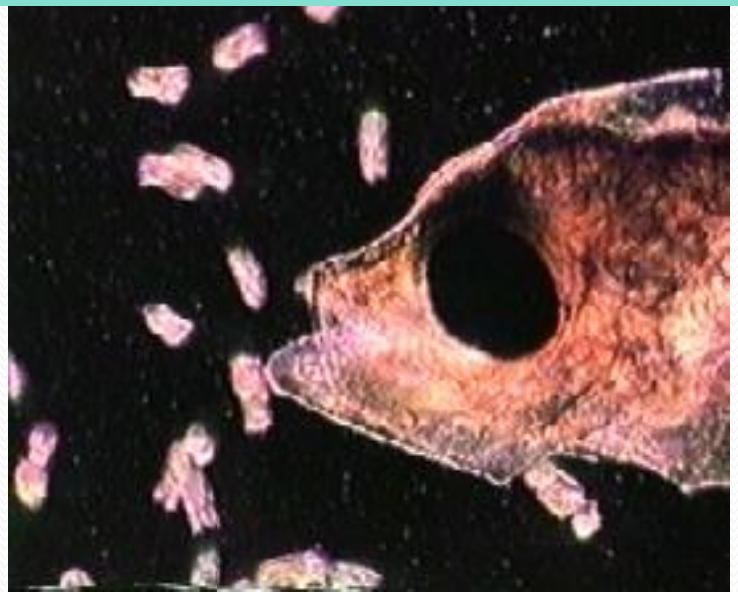
# Why Artemia?

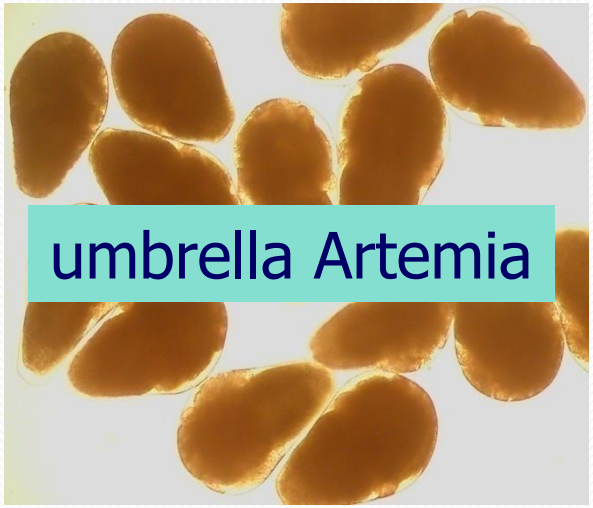
(Search in Google, 11/2011, 9/2014)

1. Environment: 224,000 ; **257.000 sites**
2. Flocculation: 14,100; **514.000**
3. Water treatment: 56,000; **88.100**
4. Toxicology: 48,100; **68.000**
5. Genetic: 105,000; 162.000
6. Biological: 243,500; 203,000
7. Culture systems: 37,200; 40,000
8. Nutrient: 103,000; 292,000
9. **Application in aquaculture:** 66,000; **234.000**
10. Pet-fish: 25,900; 40,900
11. As feed for human: 113,000; 103,000
12. Biotechnology: 1,030,000; 1.660.000
13. Molecular: 162,000; 149,000
14. Bacteria: 248,000; 210,000
15. Production: 332,000; **3,140,000**
16. Cyst production: 2,250,000; 89.900
17. Biomass production: 17,300; 59,200



Several aquatic organisms require rotifers as starter food





# Demand of Artemia cyst for 1 million of PL's

**30 kg**
mud-crab

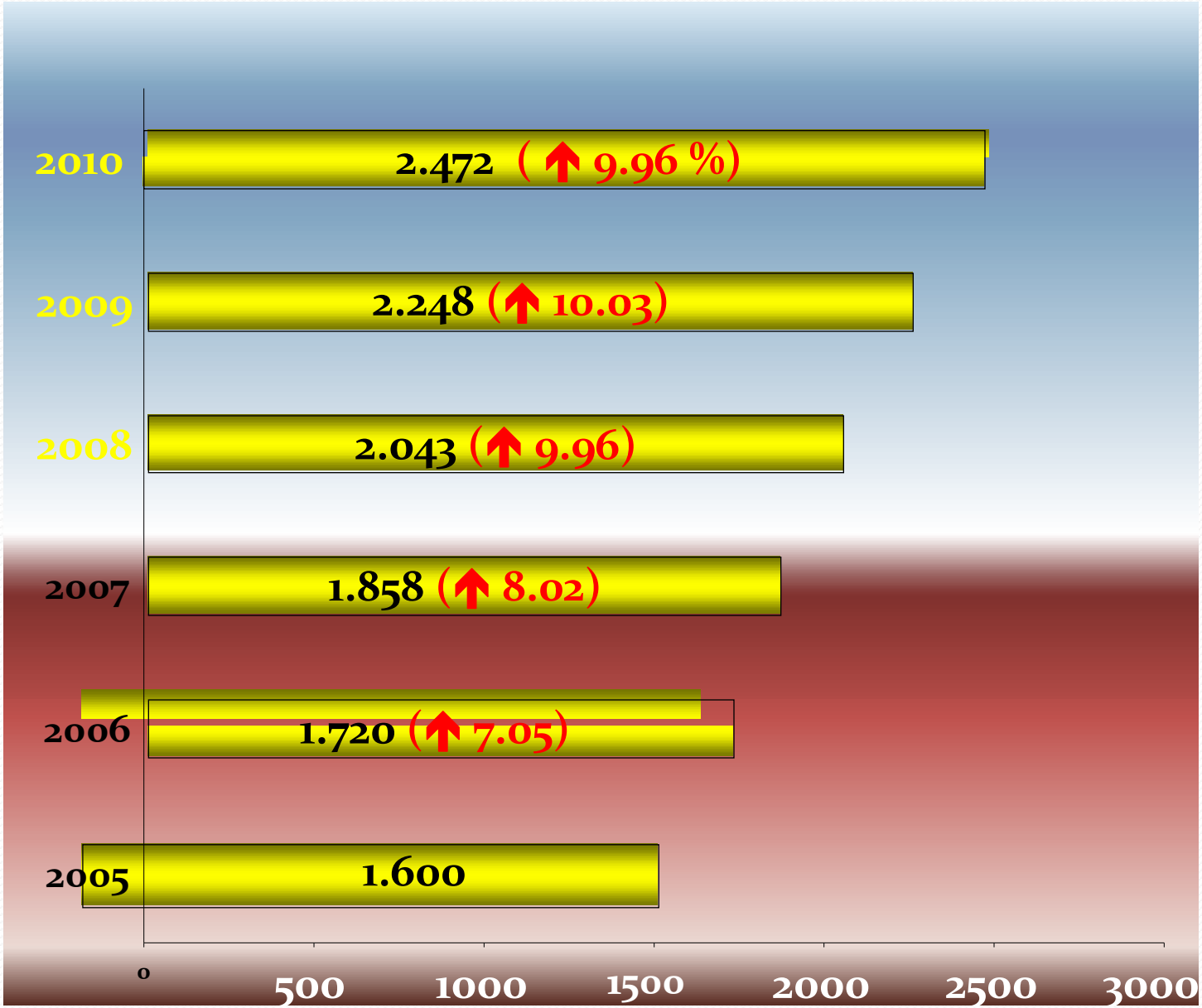
**10-13 kg**
prawn

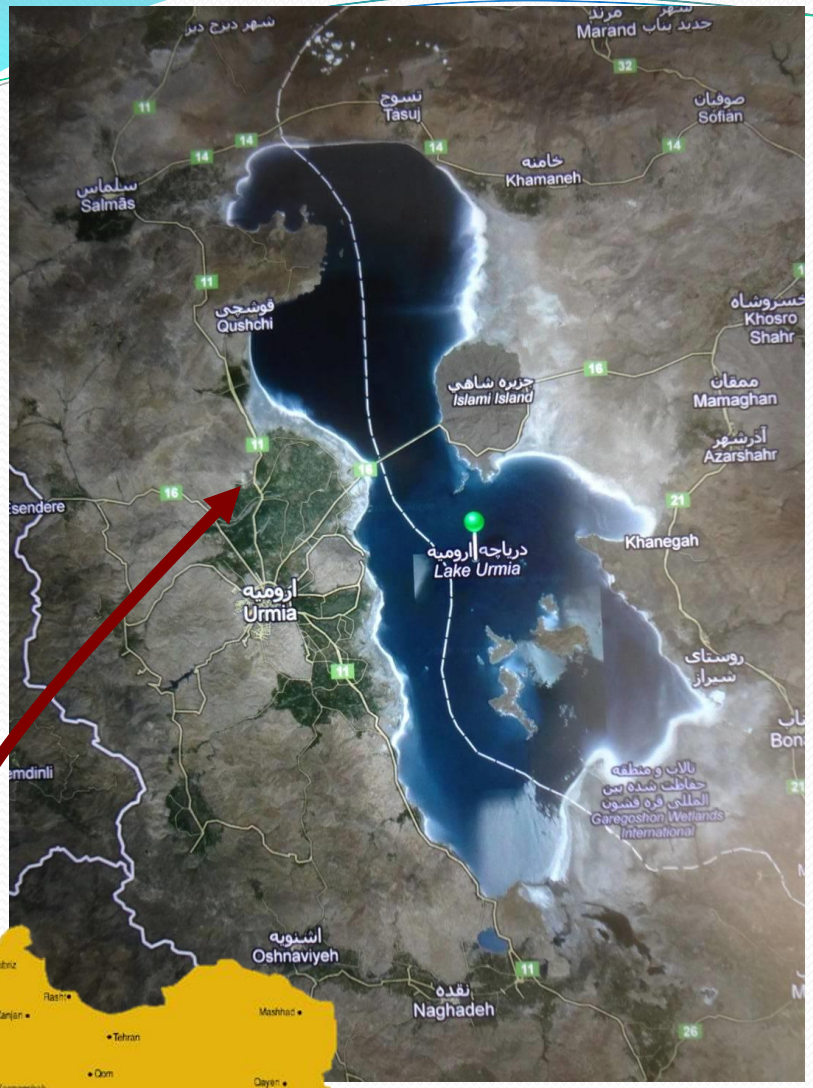
**3 kg**
tiger shrimp

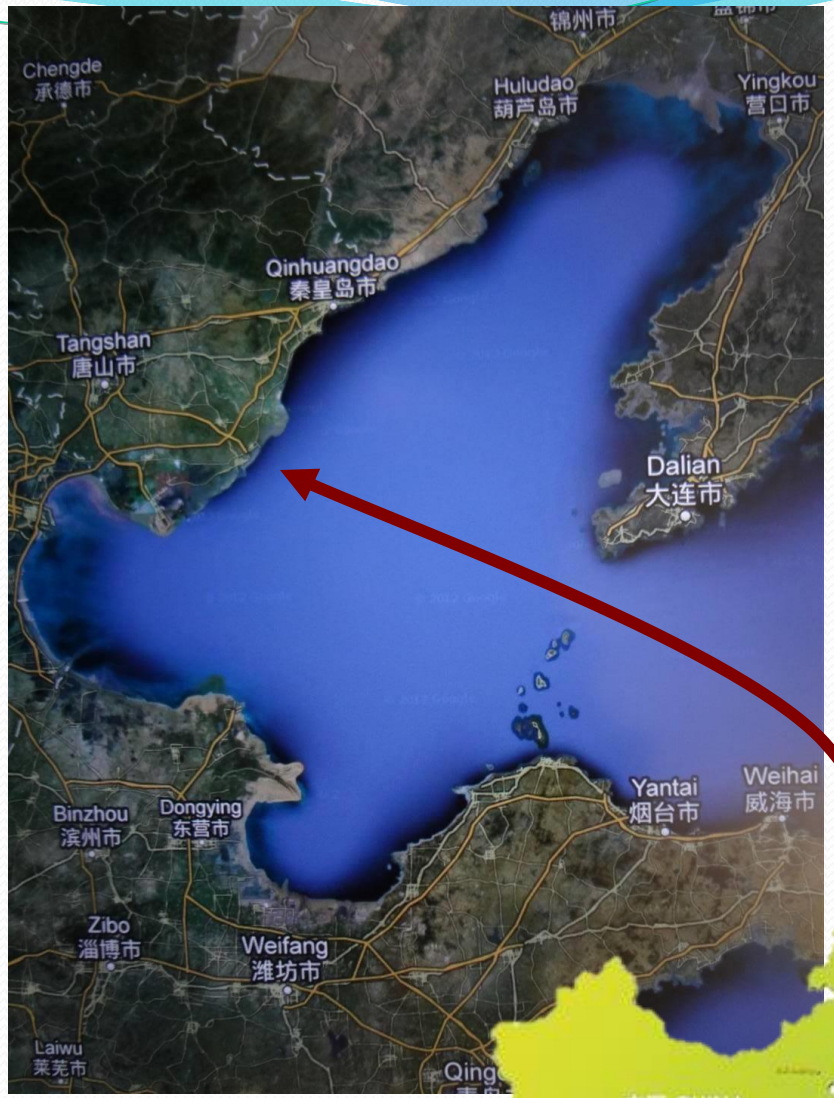
**3 kg**
white shrimp

**3 kg**
for **5000 cobia fry**
50-days old

# Artemia cyst demand forecast



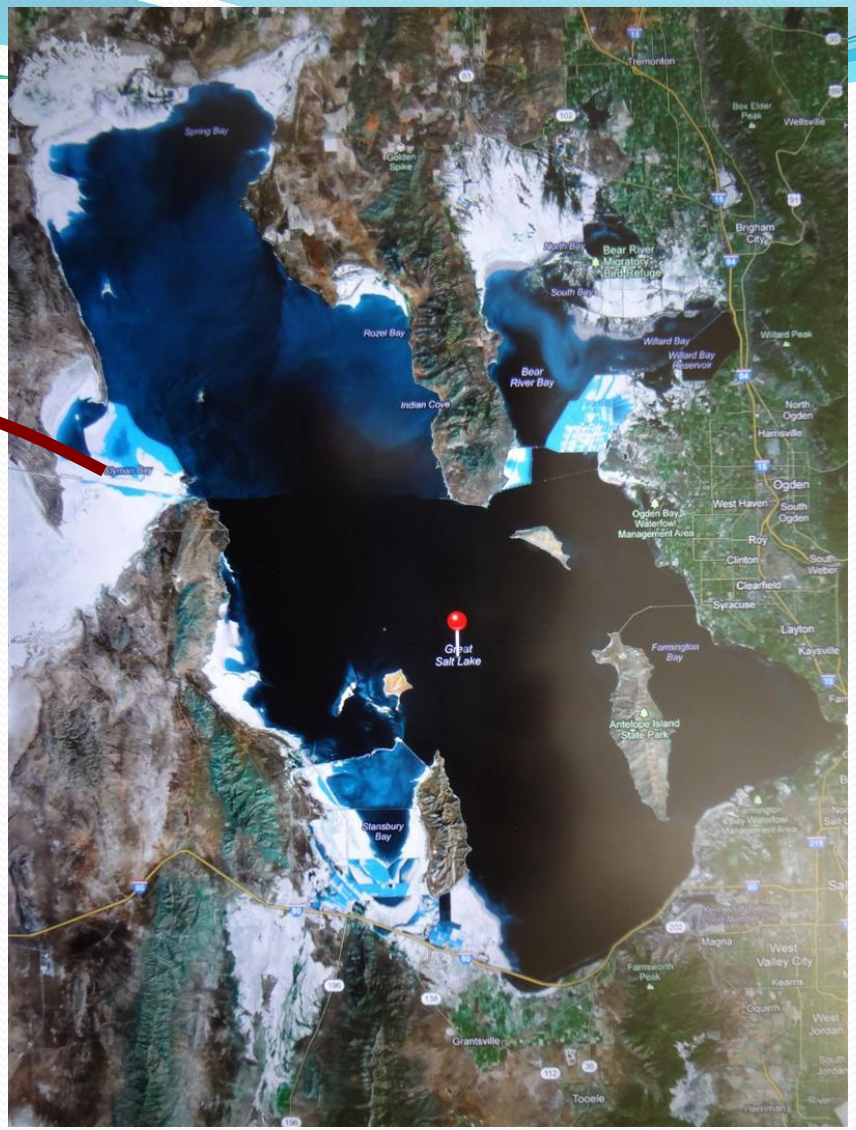


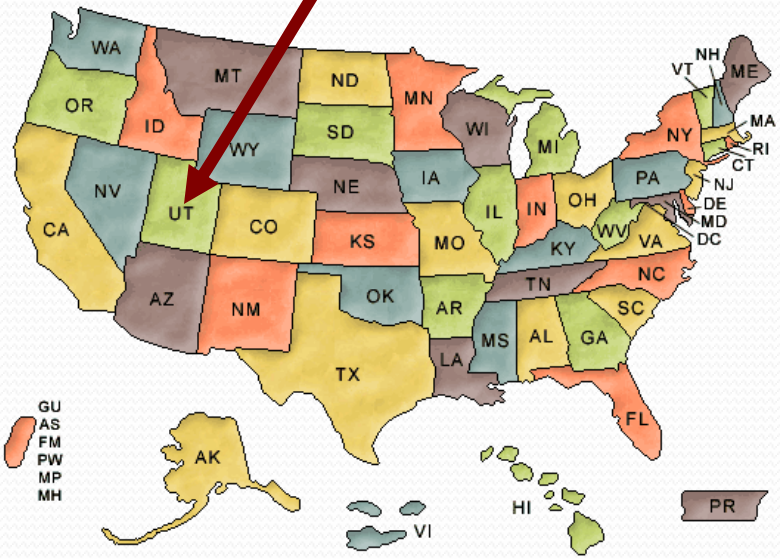





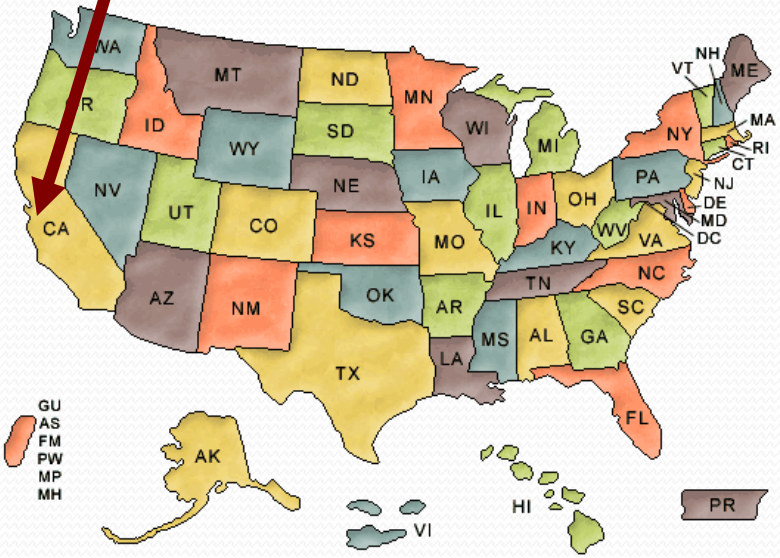
**Urmia Lake, Iran**


**Bohai Bay, China**



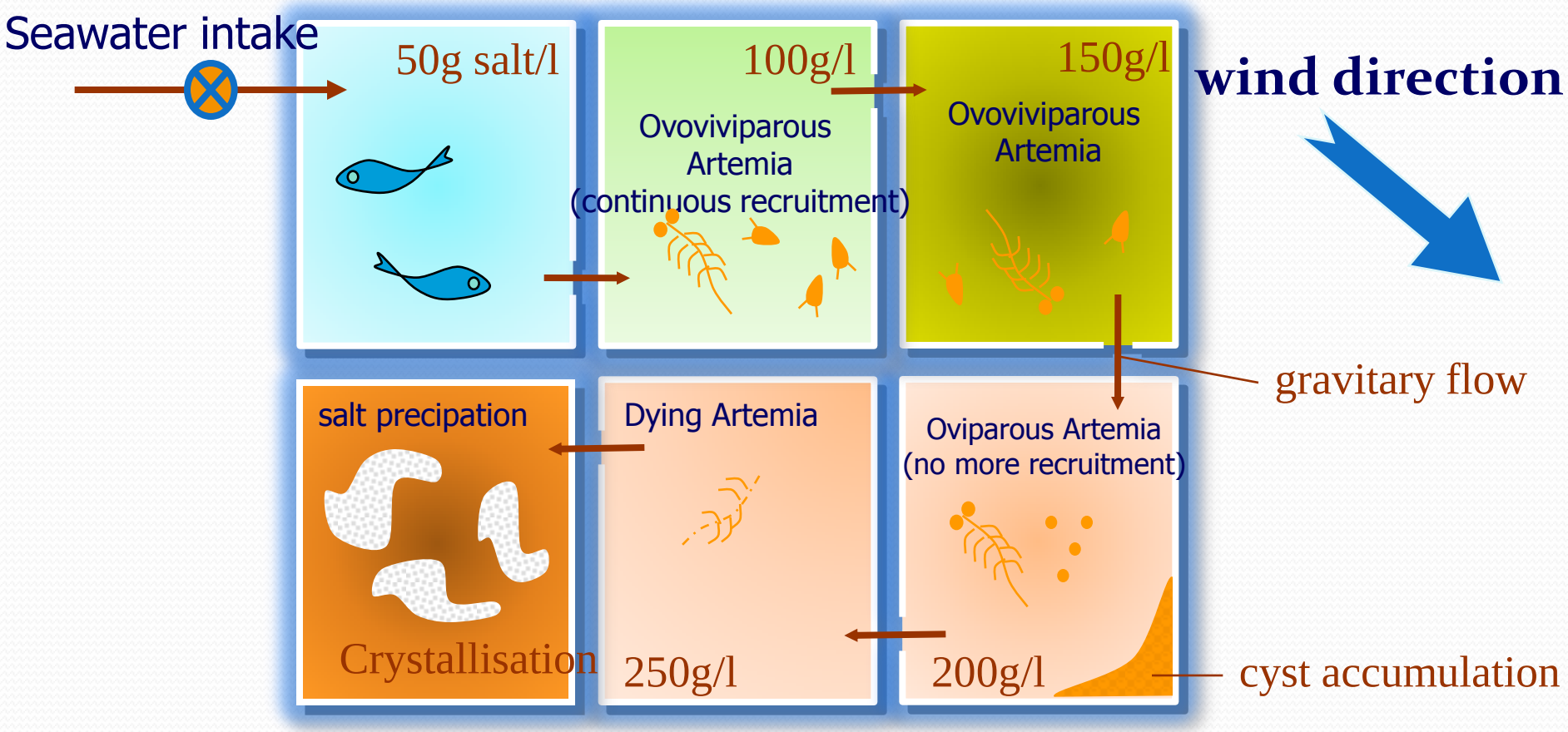




# Great Salt Lake, USA




# San Francisco Bay, USA

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



# LIFE CYCLE OF ARTEMIA

Oviparous

DRY CYSTS
= eggs

SW 35 ppt

HYDRATED CYSTS

BREAKING STAGE

UMBRELLA STAGE

Hatcheries

NEWLY HATCHED NAUPLII

SUB-ADULT

RIDING COUPLE

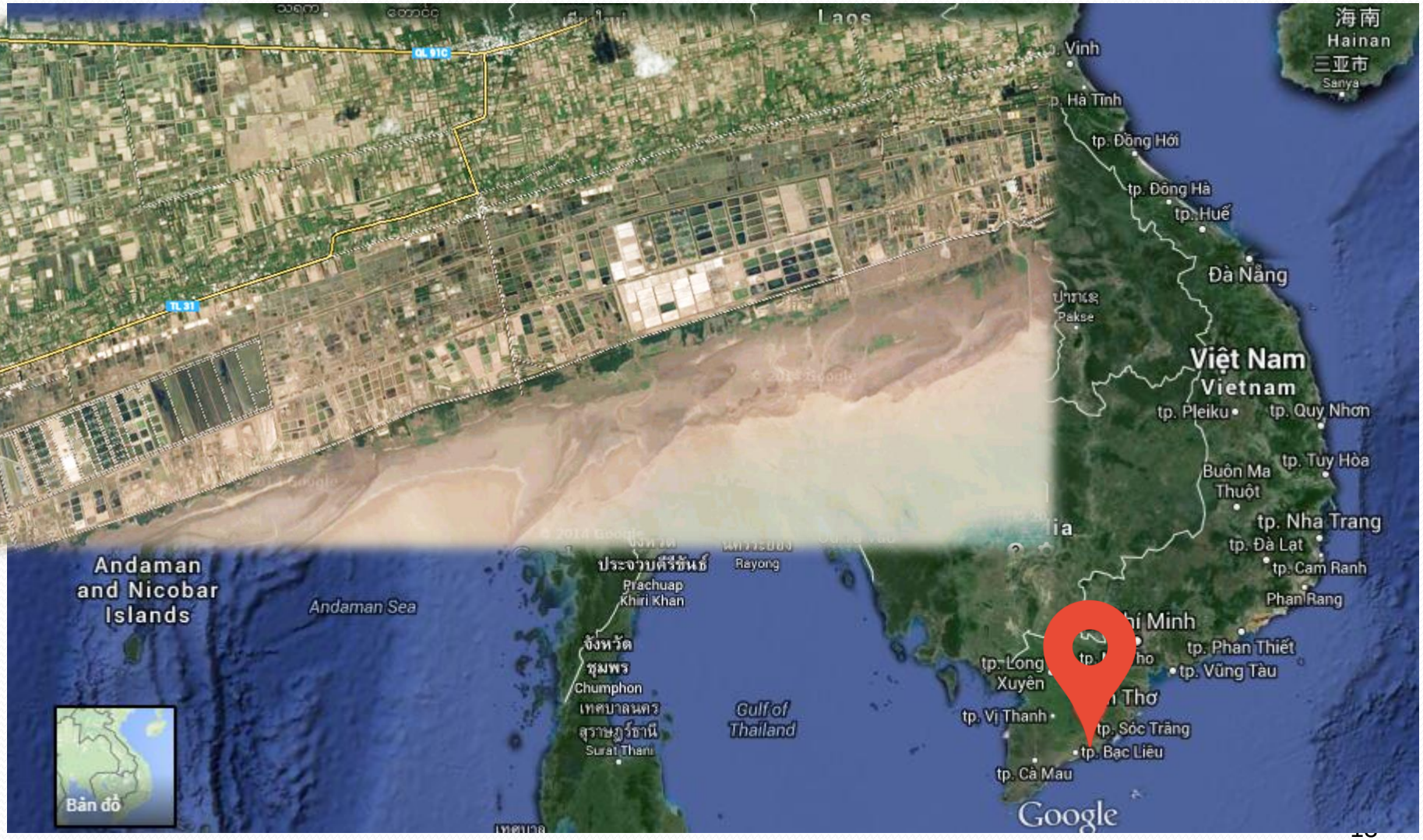
ADULTS

hooked graspers appear at 10th stage

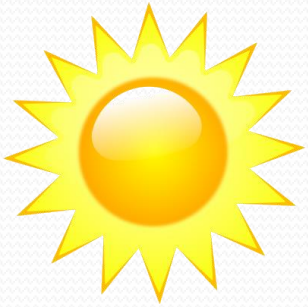
NAUPLII/CYST-BEARING FEMALE

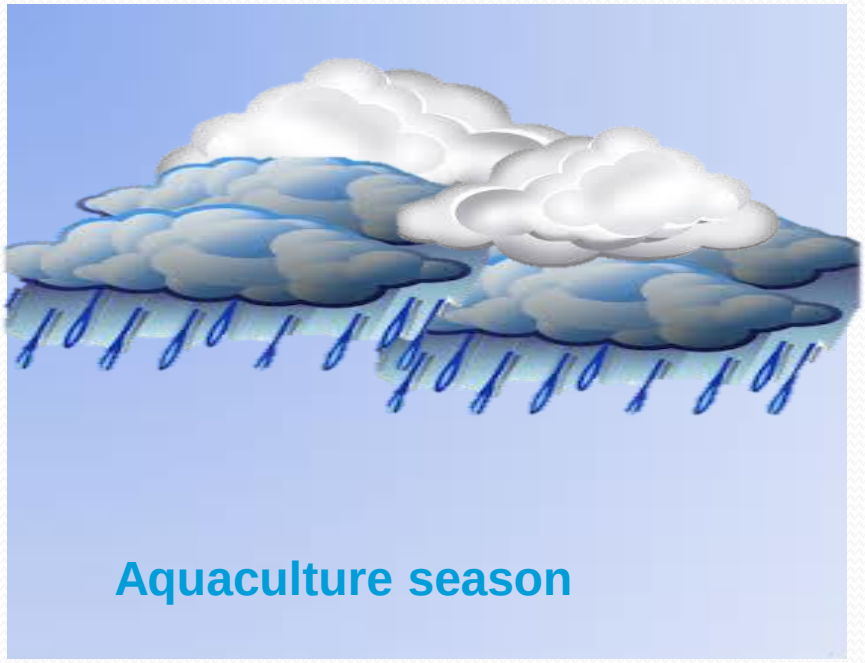
Ovoviviparous

# Vinh chau solar saltworks



# Artemia culture season in Vinh Chau and Bac Lieu

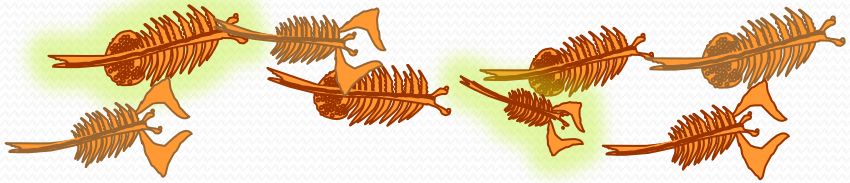




**Salt production season**

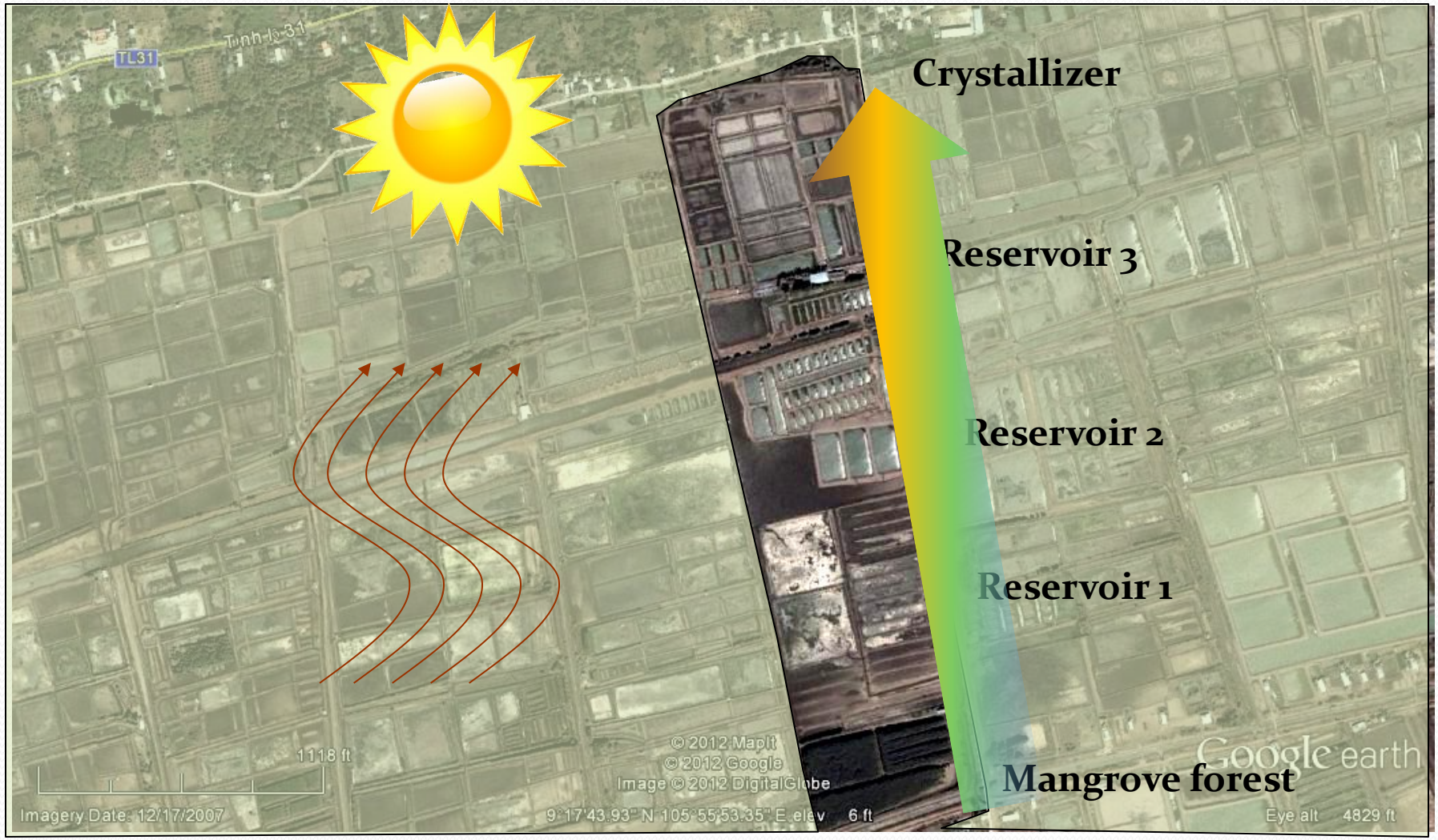
**Aquaculture season**

nov dec jan feb mar apr may jun jul aug sep oct

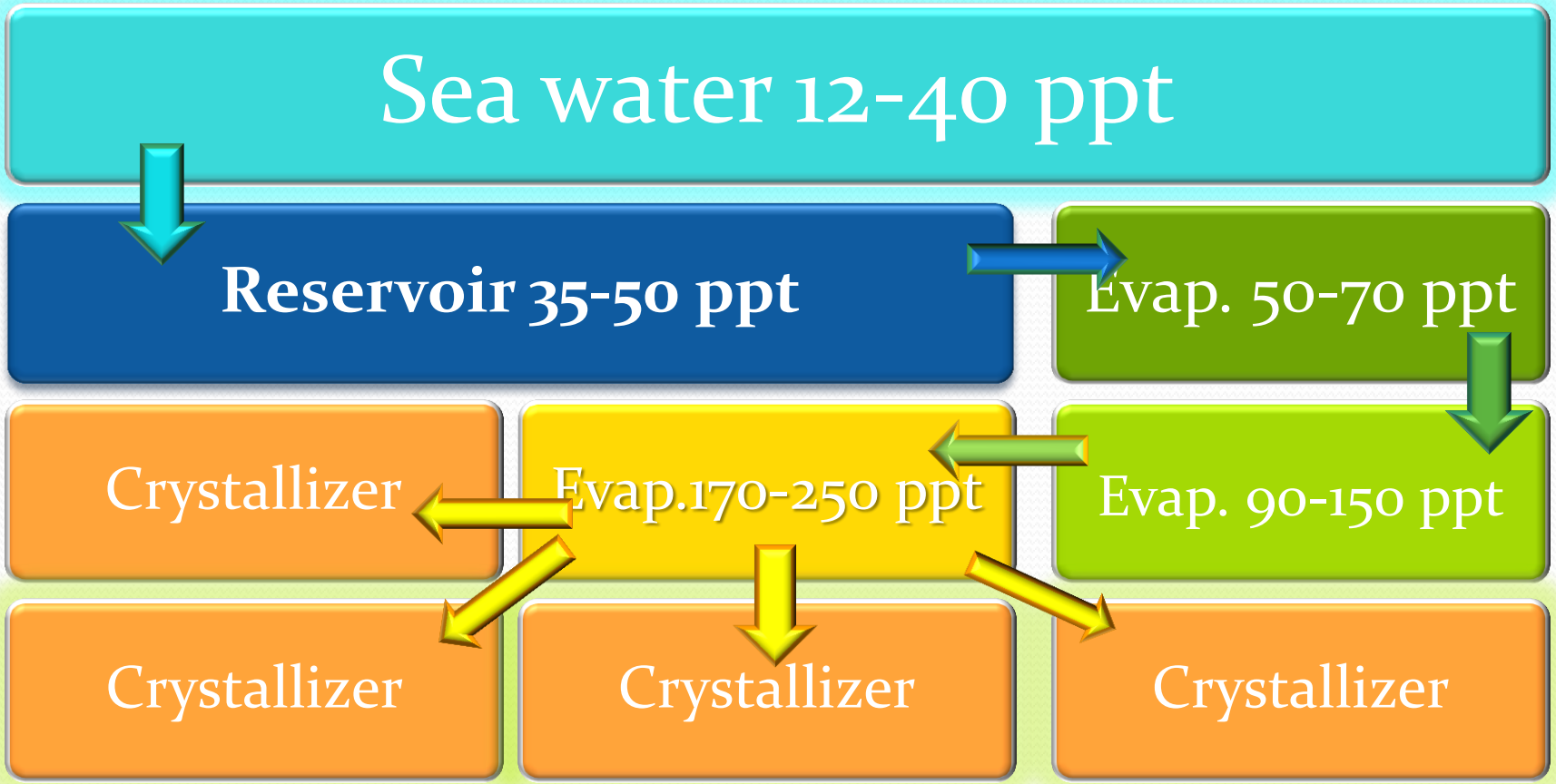


**Artemia production season**

# Experimental station in Vinhchau solar saltworks

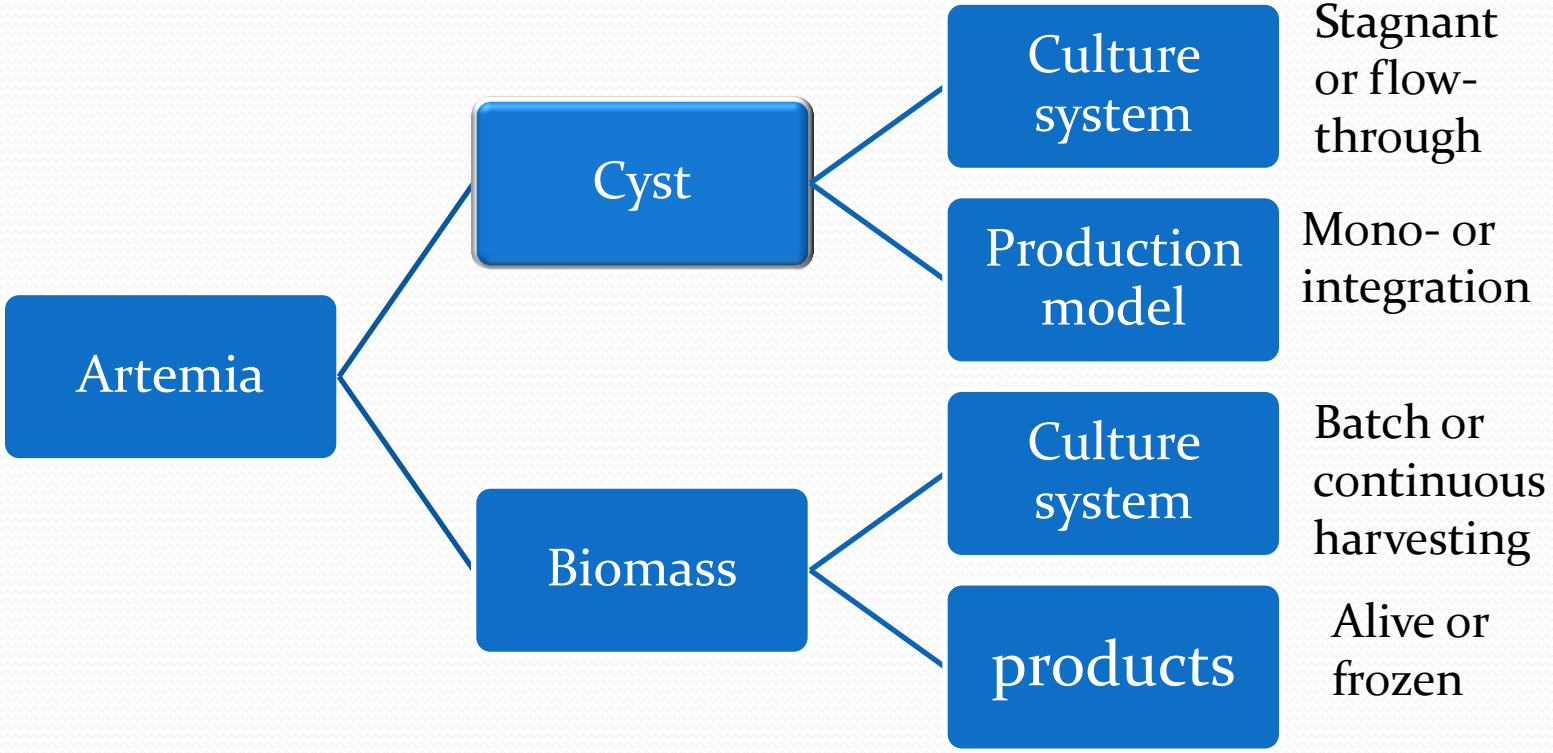


# Artemia culture in Vinh Chau and Bac Lieu salt-fields, Vietnam

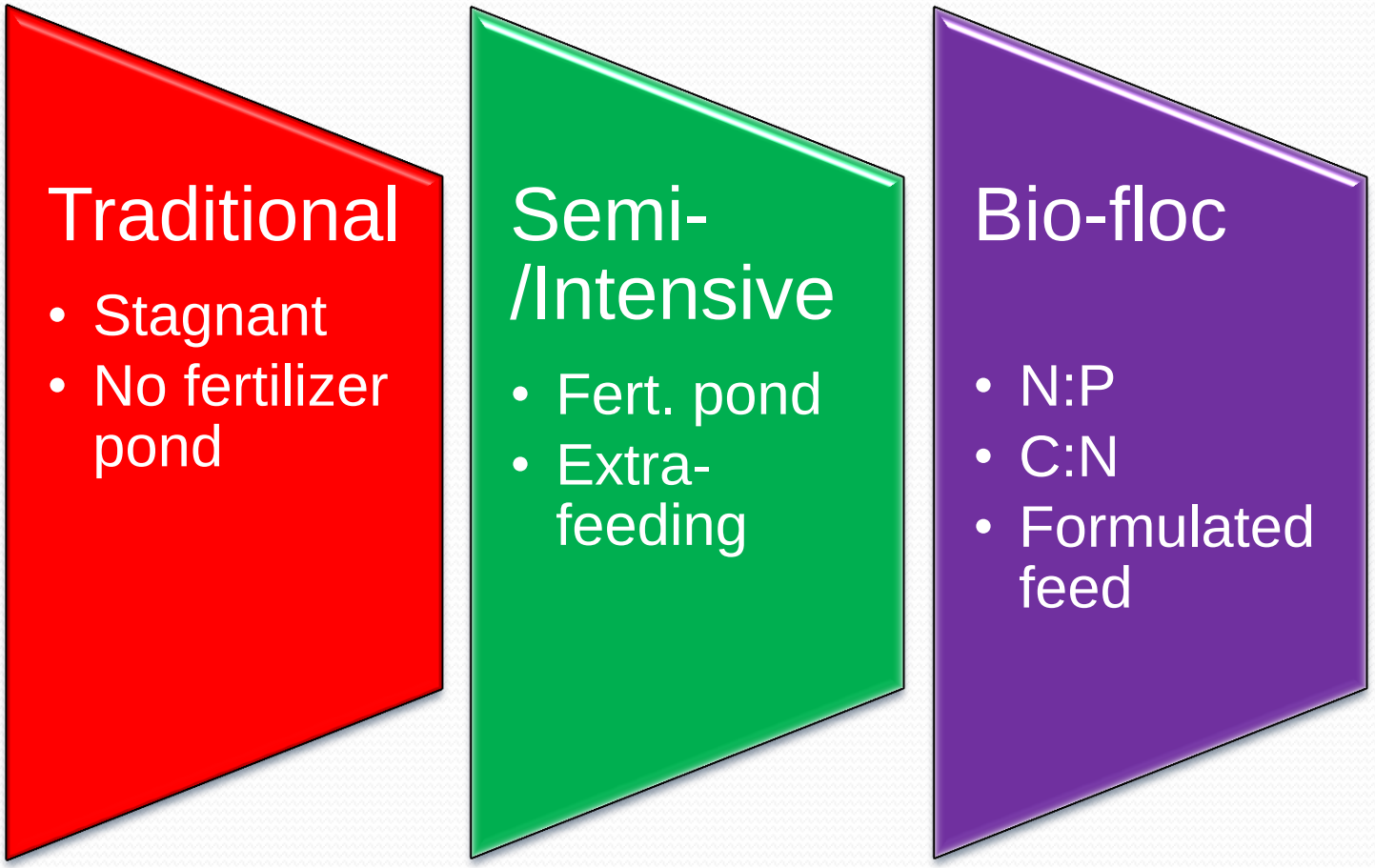



**A saltstreets in Vinhchau saltworks (not to scale)**

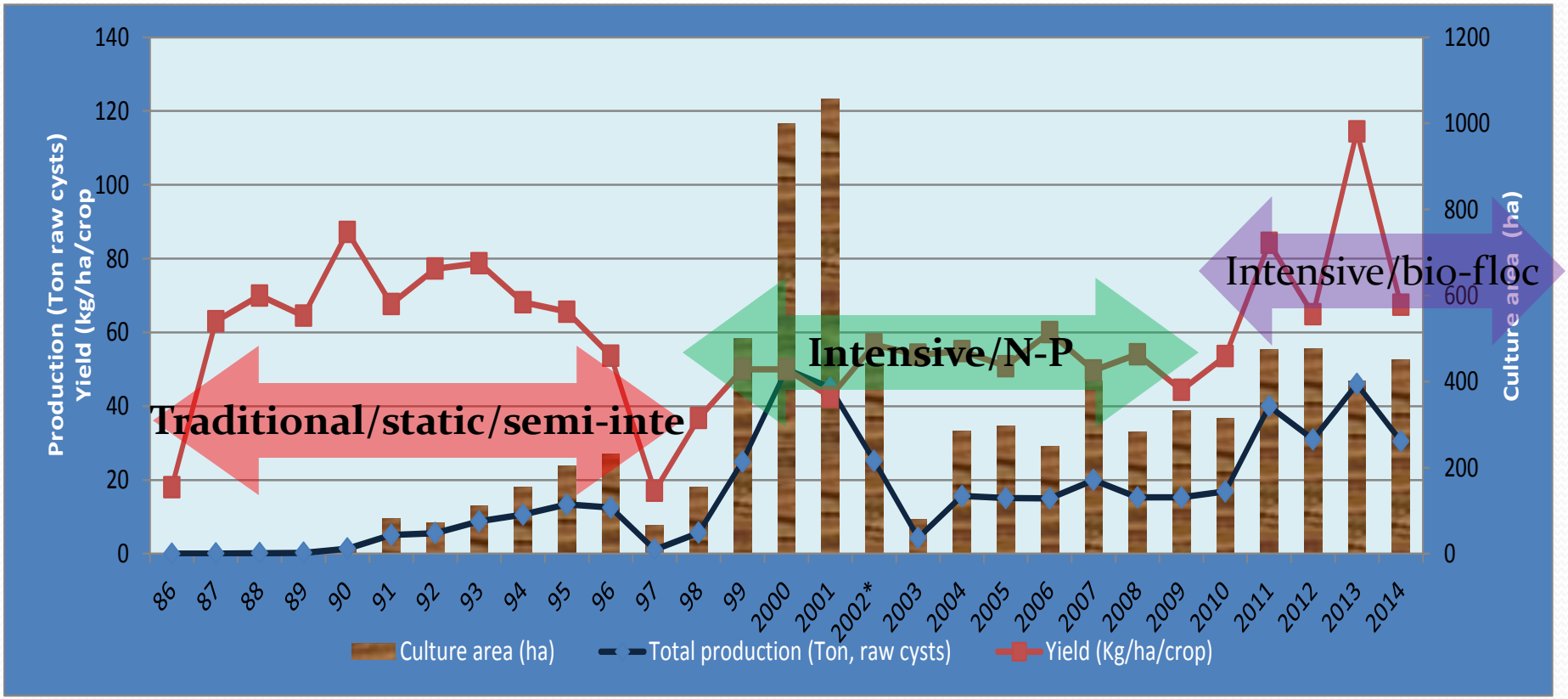
# Production


Artemia
Cyst
Biomass
Culture system
Production model
Culture system
products
Stagnant or flow-through
Mono- or integration
Batch or continuous harvesting
Alive or frozen

# Production


Traditional
• Stagnant
• No fertilizer pond
Semi-/Intensive
• Fert. pond
• Extra-feeding
Bio-floc
• N:P
• C:N
• Formulated feed

# Artemia cyst production in Vinh chau and Bac lieu



# Glance at Artemia production in Vinhchau

- **Artemia pond culture**
- **Solar saltworks**
- **Season: dry period**
- **Earthen pond**
- **Salinity 80-100 ppt**
- **Feeding: GW, RB**
- **Current culture area: 500-700 ha**
- **Cyst yields: 40-200 kg ww/ha/season**
- **Total cyst production: 30-50 Tons/season (year)**

# Cyst yield related to:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7$$

In which,

$\beta_0$ : Intercept

$\beta_1, \beta_2, \ldots\ldots, \beta_7$ : Parameters of respectively independent variables

$X_1$ :(**experiential years**),

$X_2$ :(**training**),

$X_3$ :( risk of **weather/materials quality**),

$X_4$ :(cost for variety per hectare),

$X_5$ :(cost for **feed and fertilizers** per hectare).

$X_6$ :(cost for **labor**) and

$X_7$ :(cost for **pond modification**)

Number of households: 28

# Pond management

- **Pond monitoring**
- **Pop observation**
- **Applying GW**
- **Raking**
- **Feeding**

# Traditional/static/semi-inte

- Stocking density > 20 naupli litre $^{-1}$
- Ponds are managed intensively (*i.e.* inoculation of selected strains, manipulation of primary and secondary production, predator control *etc*.)
- evaporation ponds (0.5-0.7 ha), *i.e.* where salinity varies from 90 ppt to 150 ppt
- a "kitchen pond" to produce green water as feed for *Artemia*

Vu Do Quynh anh Nguyen, 1987

Tackaert and Sorgeloos, 1991

# Traditional/static/semi-inte

- "kitchen pond" to produce green water as feed for *Artemia, in which*
  - *basic fert.:*
    - Organic manure: 0.5-1.25 Ton/ha
    - Inorg fert: 50-100 kg/ha
  - Additional fert.:
    - Organic manure: 0.5 Ton/ha/wk
    - Inorg fert: 30-50 kg/ha/wk
  - Total:
    - org manure app. 7 Ton/ha/crop
    - Inorg fert app.: 0.5-0.7 Ton/ha/crop

Vu Do Quynh anh Nguyen, 1987
Tackaert and Sorgeloos, 1991

# Traditional/static/semi-inte

- Recently, rice-bran or formulated feed are used as supplementary feeds to enhance the cyst production.
- In average, cyst production in Vinh Chau varies from less than **5 to 40 kg/ha/month**, depending on the culture system (extensive vs. semi-intensive and intensive, respectively), and the climatic conditions.

Vu Do Quynh anh Nguyen, 1987
Tackaert and Sorgeloos, 1991; Nguyen Van Hoa et al., 2012

# Intensive/N-P

## R11-SUB-PROJECT

**KEY ELEMENTS**

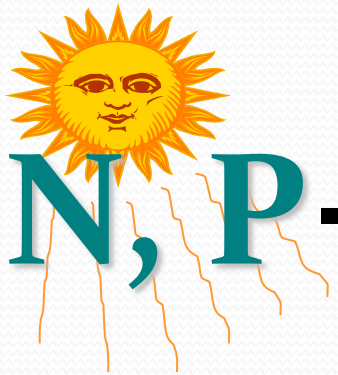
**CONTROL**

Nutrient management in *Artemia* pond culture

**AIM**

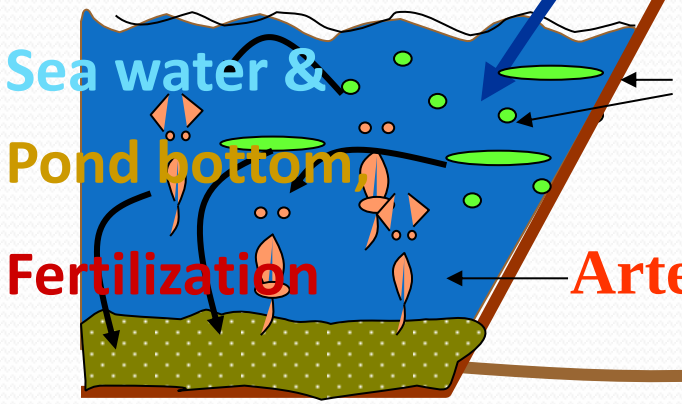
Enhancement

**Cyst** **uction**




**Controlled primary production**

Nutrients (*i.e.* N,P...)




- **Stocking density,**
- **Culture systems and pond management procedures.**

Control algae bloom, selected suitable algal species and mass culture

# Intensive/N-P

## Experimental set up

Stocking density ≥ 100 naupli liter -1, Fertilization, food: rice-bran, pig manure

- GW5+RB: green water (N:P=5:1) + rice bran;
- GW5+PM: green water (N:P=5:1) + pig manure;
- GW10+RB: green water (N:P=10:1) + rice bran;
- GW10+PM: green water (N:P=10:1) + pig manure;

⇒day 5 after inoculation. Pig manure was applied at 200-300 kg DW $ha^{-1}$ $week^{-1}$ and rice-bran was added at a rate of 20-30 kg $ha^{-1}$ $day^{-1}$

| Total: | |
|---|---|
| org manure app. | 2-3 Ton/ha/crop |
| Inorg fert app.: | **0.2-0.3 ???Ton/ha/crop** |
| Rice-bran: | 2.4 Ton/ha/crop |

Nguyen Thi Ngoc Anh, 2009

# **Table 7.** Estimates of conversion ratio of rice bran, pig manure and chlorophyll *a* to *Artemia* biomass (300m$^2$ pond$^{-1}$).

| | Treatment | | GW5+RB | GW10+RB | GW5+PM | GW10+PM |
|---|---|---|---|---|---|---|
| Total feed applied in *Artemia* pond | Rice bran (DW) | (kg pond$^{-1}$) | 40.66 | 40.66 | - | - |
| | | (kg ha$^{-1}$) | 1355.27 | 1355.27 | - | - |
| | Pig manure (DW) | (kg pond$^{-1}$) | - | - | 76.50 | 76.50 |
| | | (kg ha$^{-1}$) | - | - | 2550.00 | 2550.00 |
| | Chl *a* | (mg/L? pond$^{-1}$) | 52.71 | 60.72 | 52.71 | 60.72 |
| | | (g ha$^{-1}$) | 1.76 | 2.02 | 1.76 | 2.02 |
| Total *Artemia* biomass yield | kg WW pond$^{-1}$ | | 67.5±12.3 | 63.7±11.4 | 69.6±19.3 | 54.6±9.8 |
| | kg ha$^{-1}$ crop$^{-1}$ | | 2251±410 | 2123±380 | 2321±643 | 1821±327 |
| Conversion ratio (kg **rice bran** kg$^{-1}$ biomass) | | | **0.62 ± 0.12** | **0.65 ± 0.13** | - | - |
| Conversion ratio (kg **pig manure** kg$^{-1}$ biomass) | | | - | - | **1.17±0.38** | **1.43±0.26** |
| Conversion ratio (mg/L **Chl *a*** kg$^{-1}$ biomass) | | | **0.80±0.16** | **0.98±0.19** | **0.81±0.27** | **1.14±0.21** |

Nguyen Thi Ngoc Anh, 2009

# Conclusions

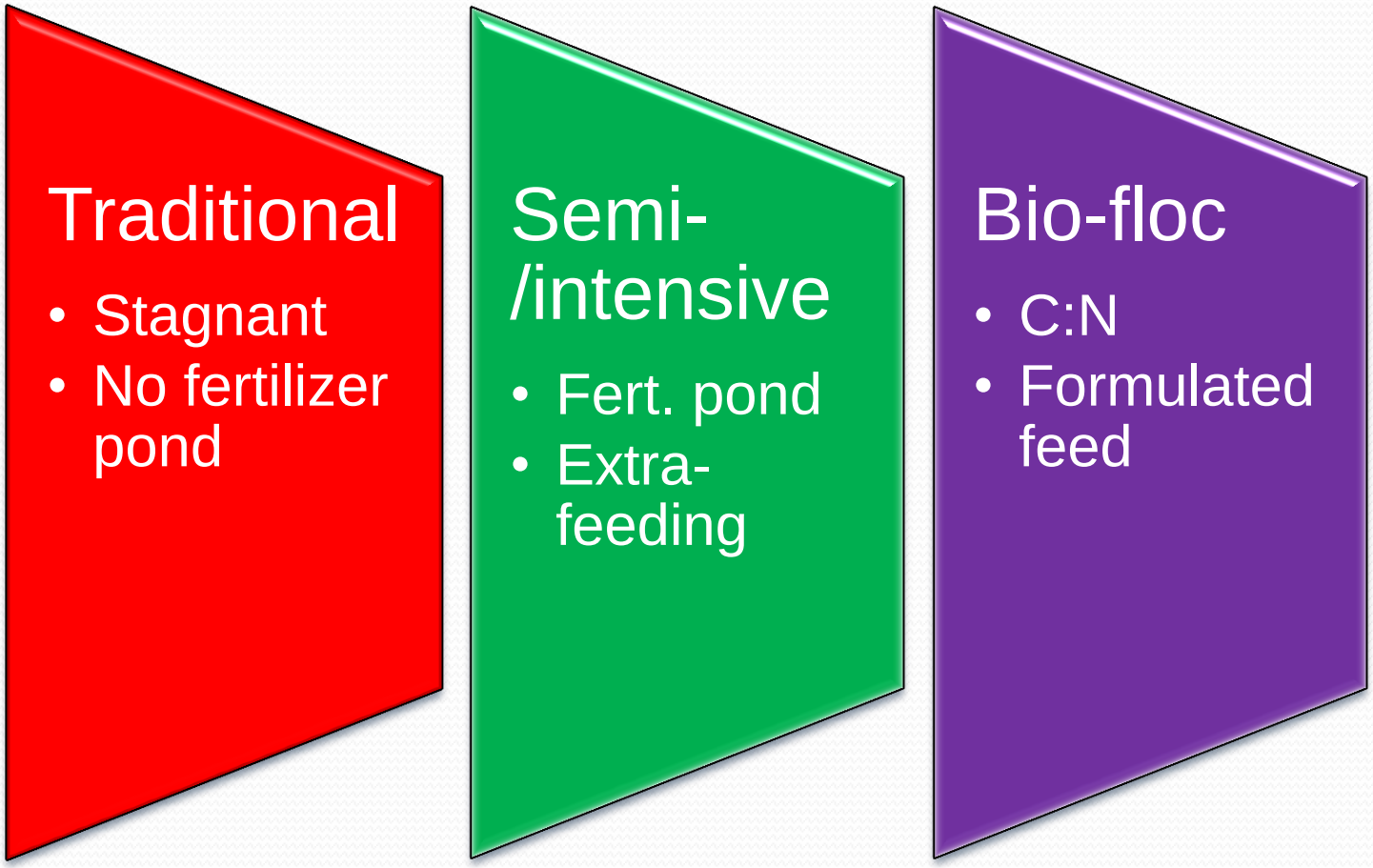
•applied N:P=5 and 10 in the fertilization pond; No differences in chlorophyll *a* concentration and algal composition. Bacillariophyta (diatoms) were the dominant group over the sampling period (*Nitzschia longissima, N. longissima var reversa and N. acicularis)*.

•using N:P=5 combined with rice bran or pig manure ⇒ *Artemia* growth, fecundity and total yields were enhanced >> N:P=10. However, biomass yield was not sig diff. ($p > 0.05$).

•N:P=5 gave a slightly higher income, net profit and economic return than the N:P=10 treatments.

- N:P=5 + RB ⇒ **2251±410** kg $ha^{-1}$ $crop^{-1}$
- N:P=5 + PM ⇒ **2321±643** kg $ha^{-1}$ $crop^{-1}$
- N:P=10 + RB ⇒ 2123±380 kg $ha^{-1}$ $crop^{-1}$
- N:P=10 + PM ⇒ 1821±327 kg $ha^{-1}$ $crop^{-1}$

# Environment



# Intensive/bio-floc

## Floc in aquaculture

If carbohydrate was added to the water column to enhance heterotrophic bacterial protein production, the protein level in the diet could be reduced from 40% to 25%, without compromising shrimp production.

Carbohydrate addition in combination with a decreased dietary protein level **improved the sustainability of shrimp farming** in extensive shrimp.

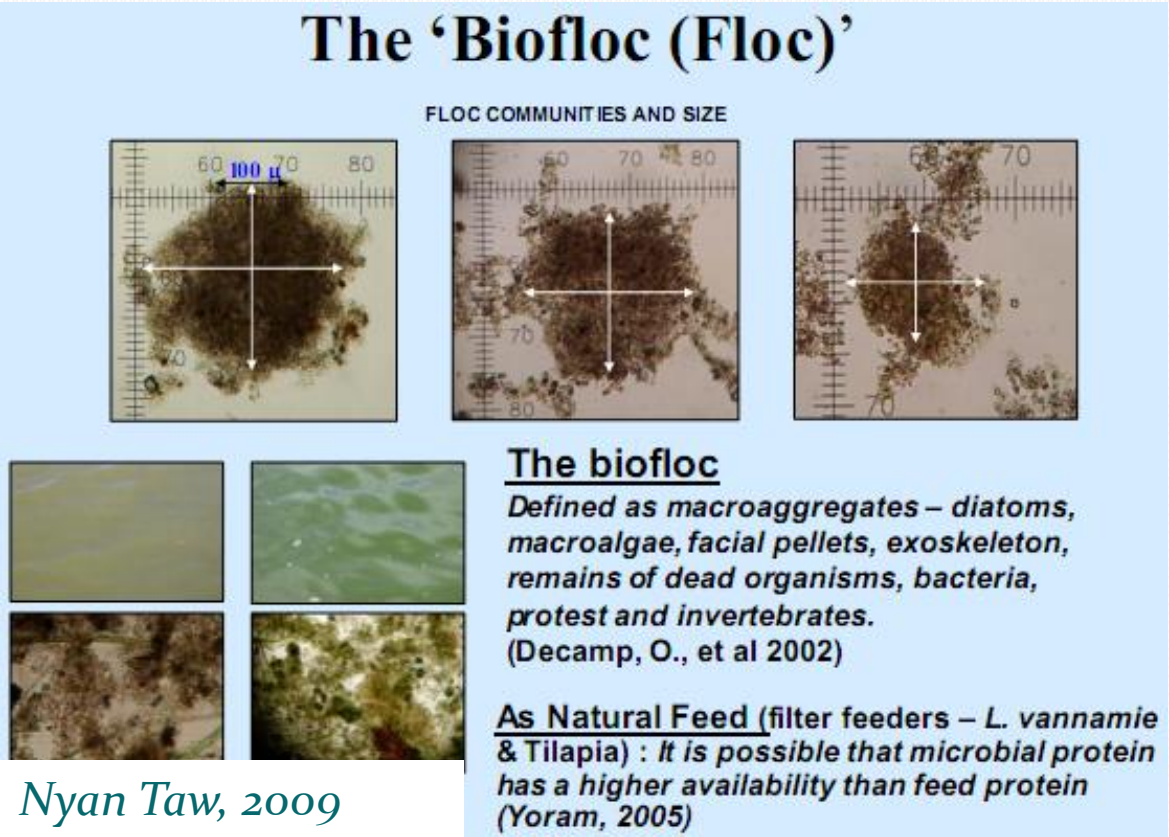
*Hari et al. (2006)*

# Intensive/bio-floc

## Floc in aquaculture

**Bio-flocs technology (BFT)** offers the possibility to: (1) maintain a good water quality within aquaculture systems and (2) produce additional food for the aquaculture organisms

*P. De Schryver, R. Crab, T. Defoirdt, N. Boon, W. Verstraete (2008)*



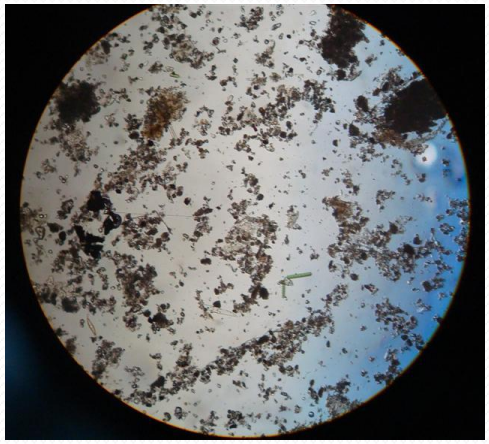

*Nyan Taw, 2009*

**Biochemical** Composition of the Floc:

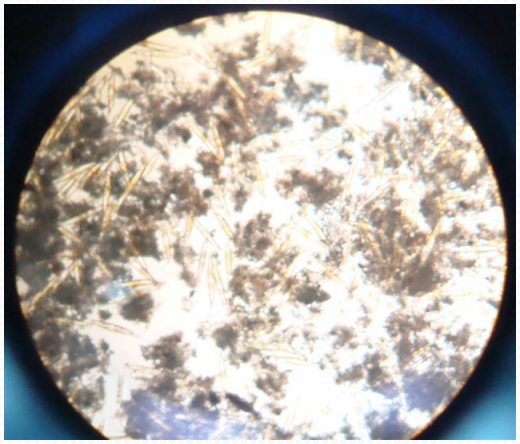
- –Crude protein: 35-50 %
- –Crude lipid: 0.6-12%
- –High ash: 21-32%

# Intensive/bio-floc

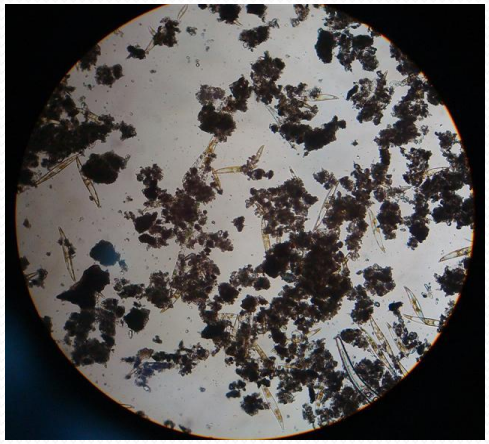
**Recycling of nutrients in eutrophicated coastal waters of the Mekong delta, Vietnam, by Artemia production** (Bilateral Project (2009-2010) and RIP project (2012-2016)



Treat 1



Treat 2



Treat 3

## Experiment 1: Formation of biofloc in fertilizer pond at different salinities (35, 60, 80 and 100 ‰)

- Experimental set-up (3 replicate each).
  - Treatment 1: 35 ‰ (control)
  - Treatment 2: 60 ‰
  - Treatment 3: 80 ‰
  - Treatment 4: 100 ‰

**Monitoring**

Temp(°C), pH: twice a day (7 am and 2 pm)

Oxy, salinity, hardness, turbidity, water level, water color: daily (7 am):

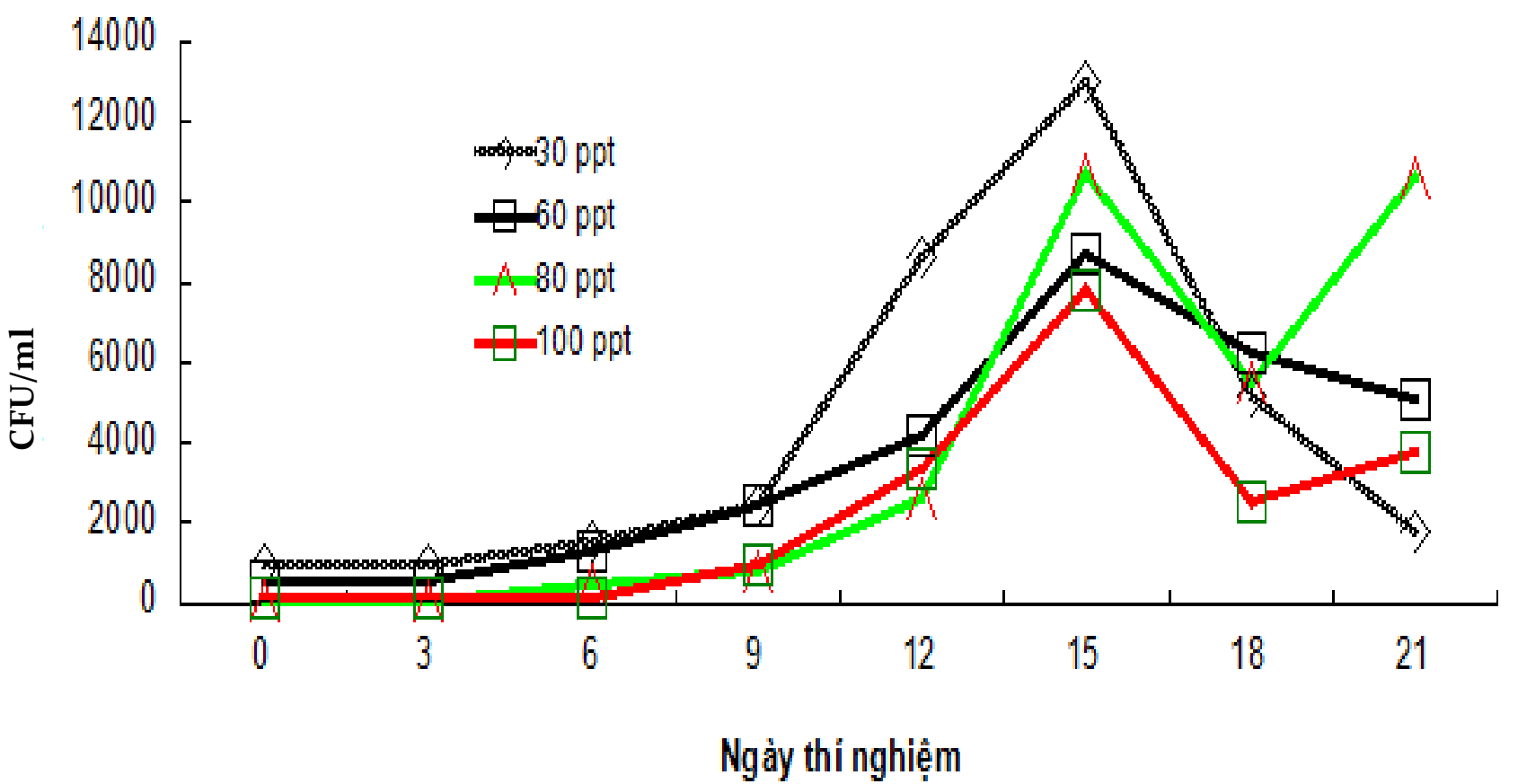
TN, $NH_4^+$, $NO_2^-$ , TP, $PO_4^{3-}$ , TOC,TSS, VSS, volume bio-floc: every 3 day.

Bacteria.

Alage (composition, density)

# Experiment 1: Formation of biofloc in fertilizer pond at different salinities (35, 60, 80 and 100 ‰)

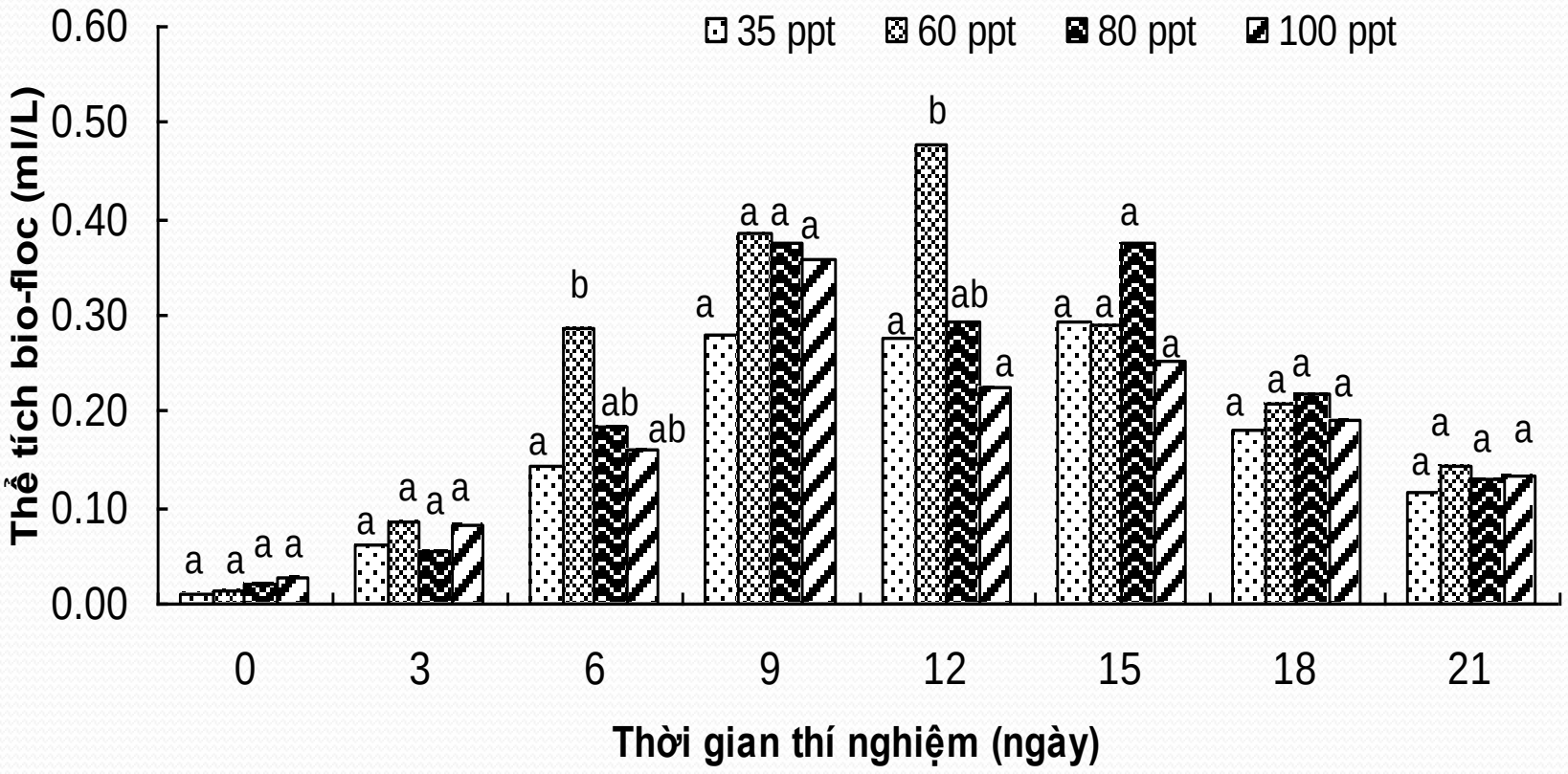
## Total count bacteria




Vibrio, Baccillus, Nitrosomonas and Nitrobacter, Bacillus is dominant group

# Experiment 1: Formation of biofloc in fertilizer pond at different salinities (35, 60, 80 and 100 ‰)

## Volume of bio-floc



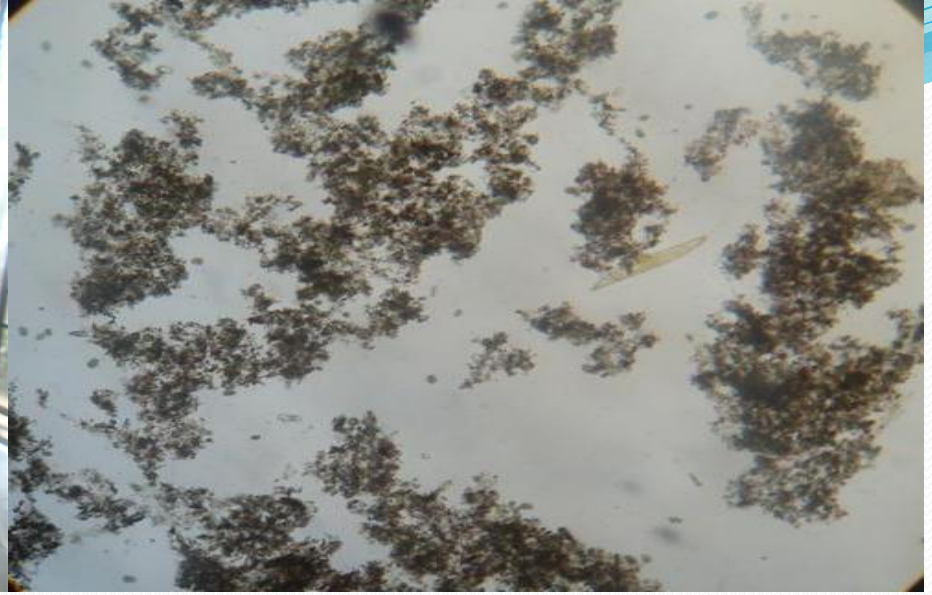

Shrimp pond varied 2 – 40 ml/L, fish pond goes up to 100 ml/L (Avnimelech, 2009)

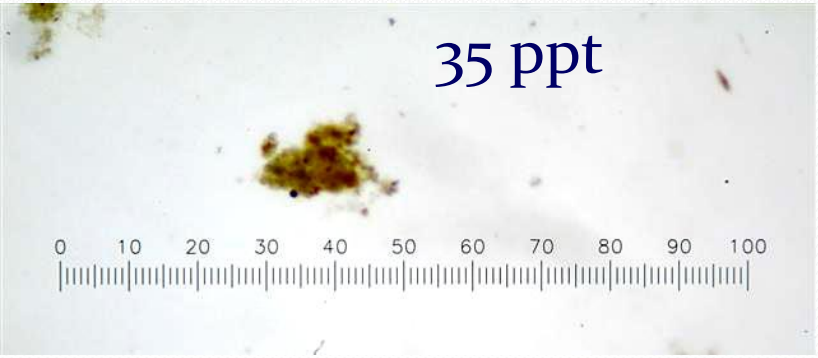
# Experiment 1: Formation of biofloc in fertilizer pond at different salinities (35, 60, 80 and 100 ‰)

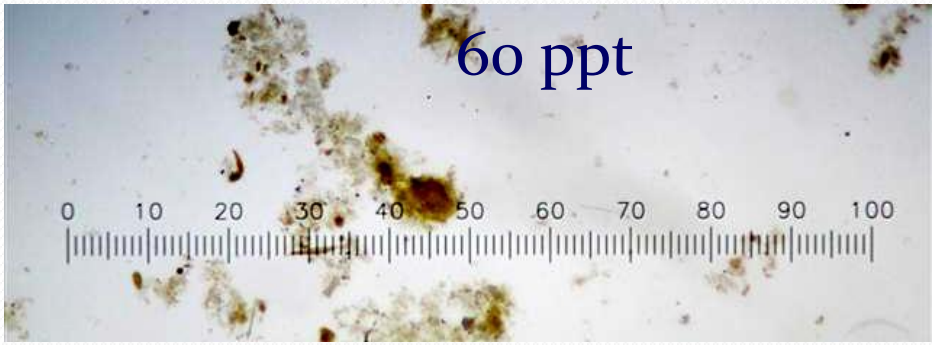
## Dimension of bio-floc (x±std, µm; n= 30)

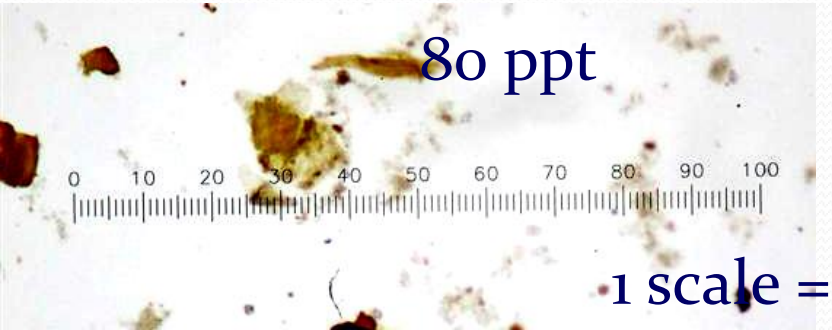
| Day | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 35 ppt | | 60 ppt | | 80 ppt | | 100 ppt | |
| | Width | Length | Width | Length | Width | Length | Width | Length |
| 7 | 62,6±30,2 | 93,6±30,4 | 59,8±25,9 | 76,7±27,4 | 51,6±28,8 | 81,4±46,6 | 39,7±11,4 | 74,5±30,5 |
| 14 | 71,0±20,4 | 105,3±30,9 | 57,5±22,3 | 102,6±36,4 | 61,5±30,5 | 97,9±51,4 | 37,1±26,1 | 61,3±36,8 |
| 21 | 52,3±18,7 | 98,2±55,7 | 55,3±19,4 | 80,6±41,6 | 32,5±19,6 | 66,3±28,3 | 33,8±15,8 | 49,7±22,2 |

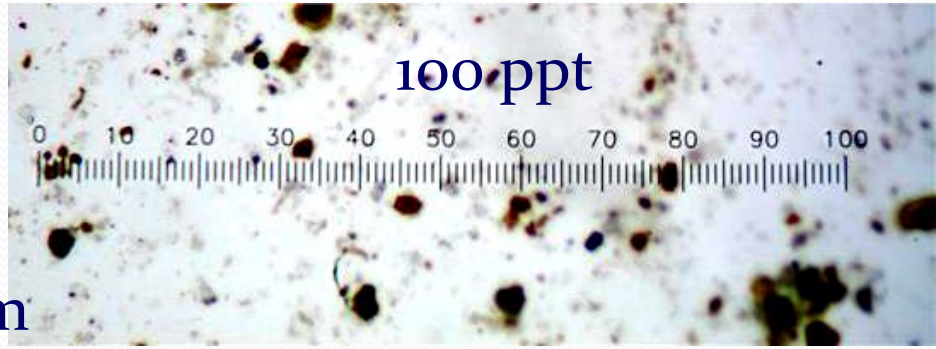
Dimension of bio-floc increased slightly at day 14




35 ppt
0 10 20 30 40 50 60 70 80 90 100


60 ppt
0 10 20 30 40 50 60 70 80 90 100


80 ppt
0 10 20 30 40 50 60 70 80 90 100


100 ppt
0 10 20 30 40 50 60 70 80 90 100

1 scale = 25 µm

# Experiment 1: Formation of biofloc in fertilizer pond at different salinities (35, 60, 80 and 100 ‰)

## Basic biochemical composition of bio-floc

| Treatment | Experimental duration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 7 | | | Day 14 | | | Day 21 | | |
| | Protein | Lipid | Tro* | Protein | Lipid | Tro | Protein | Lipid | Tro |
| 35 ppt | 12,2 $^{b}$ | 0,80 $^{a}$ | 69,9 $^{b}$ | 17,4 $^{a}$ | 1,05 $^{a}$ | 67,1 $^{a}$ | 9,3 $^{a}$ | 0,65$^{b}$ | 86,4 $^{a}$ |
| 60 ppt | 10,5$^{ab}$ | 0,89 $^{a}$ | 76,2 $^{ab}$ | 16,4 $^{a}$ | 0,92 $^{a}$ | 70,4 $^{a}$ | 12,4 $^{b}$ | 1,02 $^{a}$ | 74,8 $^{a}$ |
| 80 ppt | 9,8 $^{a}$ | 0,77 $^{a}$ | 80,6 $^{a}$ | 15,6 $^{a}$ | 1,08 $^{a}$ | 69,9 $^{a}$ | 10,4 $^{a}$ | 0,83 $^{ab}$ | 81,0 $^{a}$ |
| 100 ppt | 8,5 $^{a}$ | 0,67 $^{a}$ | 82,2 $^{a}$ | 15,9 $^{a}$ | 0,94 $^{a}$ | 71,7 $^{a}$ | 9,6 $^{a}$ | 0,91 $^{a}$ | 78,9 $^{a}$ |

* $Na^{+}$ (Sodium), $K^{+}$ (Potassium), and $Ca^{2+}$ (Calcium

## Experiment 1: Formation of biofloc in fertilizer pond at different salinities (35, 60, 80 and 100 ‰)

# Conclusion

- Bacteria were classified into: Baccillus/Nitrosomonas/Nitrobacter; vibrio was negligible and no observation at 100 ppt. However, more than 50% in the sample were not yet identified.
- Dimension of biofloc at salinity of 35 and 60 ppt > 80 and 100 ppt. Bio-floc at salinity of 80 and 100 ppt (Length x Width) ≅ 50 um, are suitable for Artemia.
- Biochemical of bio-floc: protein content is a bit low (the higher the salinity the lower the protein content be indicated)

# Experiment 2: Nutritional level of bio-floc and used as feed to *Artemia*

***Bio-floc as feed to Artemia***

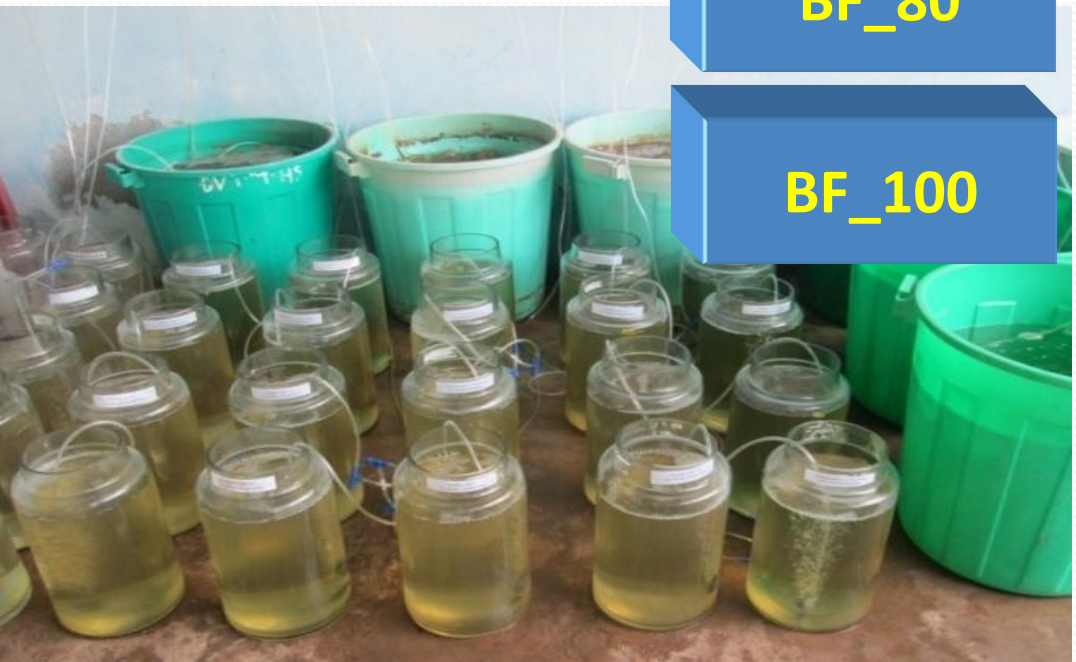
**(1) Batch culture to adult: survival and growth**

**(2) Ind. Couple culture: reproductive characteristics and life-span**

# Experiment 2: Nutritional level of bio-floc and used as feed to *Artemia*

**ARTEMIA LAB CULTURE**

- Wild algae
- BF_35
- BF_60
- BF_80
- BF_100



***Culture volume: 7 L (x 4)***

***Aeration***

***salinty:*** **80 ppt**

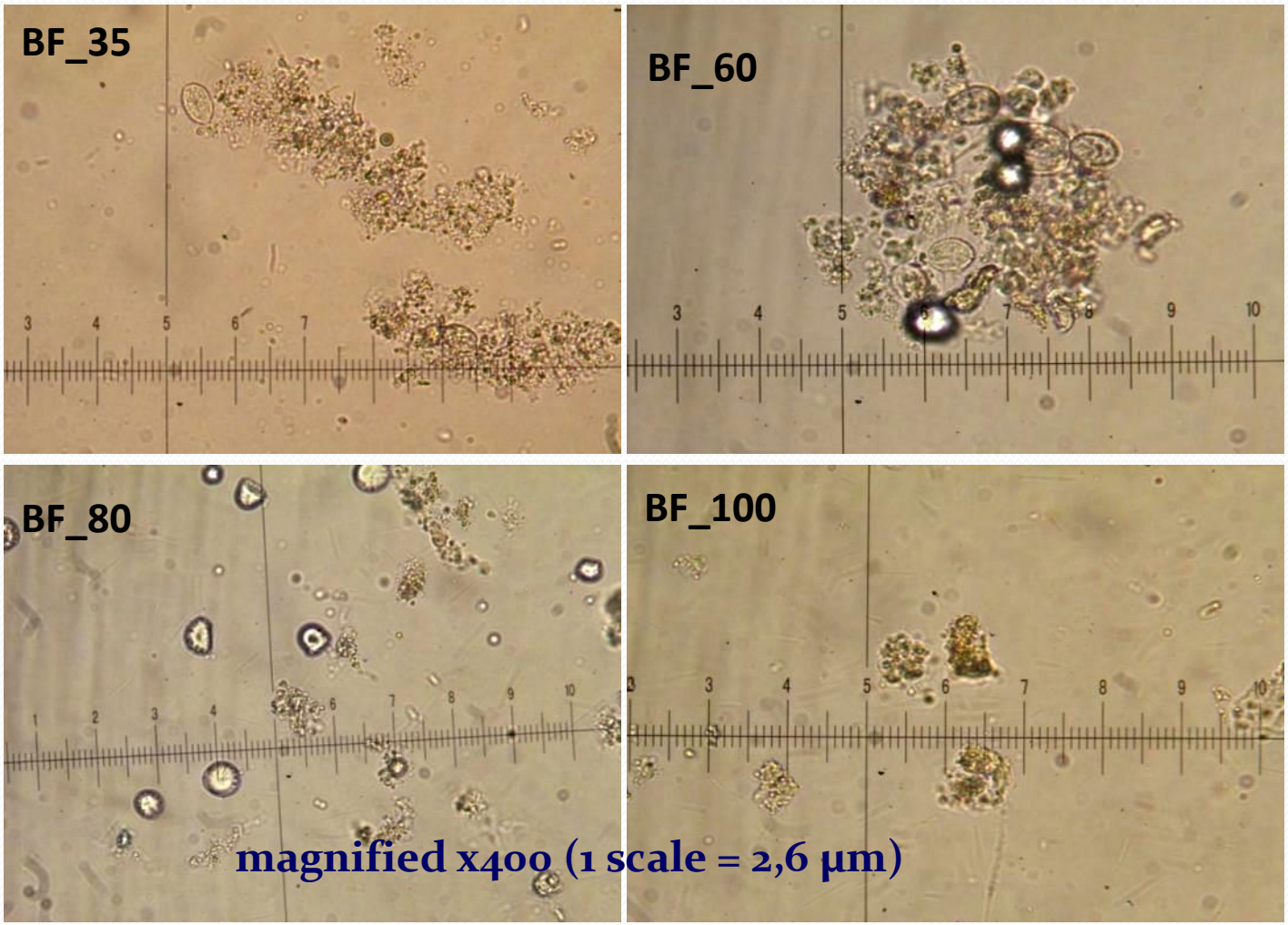
***density:*** **100 ind./L**

***feeding:*** **bio-floc was screened with 50 µm mesh-size prior feeding**

***duration:*** **13 day**

# Experiment 2: Nutritional level of bio-floc and used as feed to *Artemia*

## dimension of bio-floc

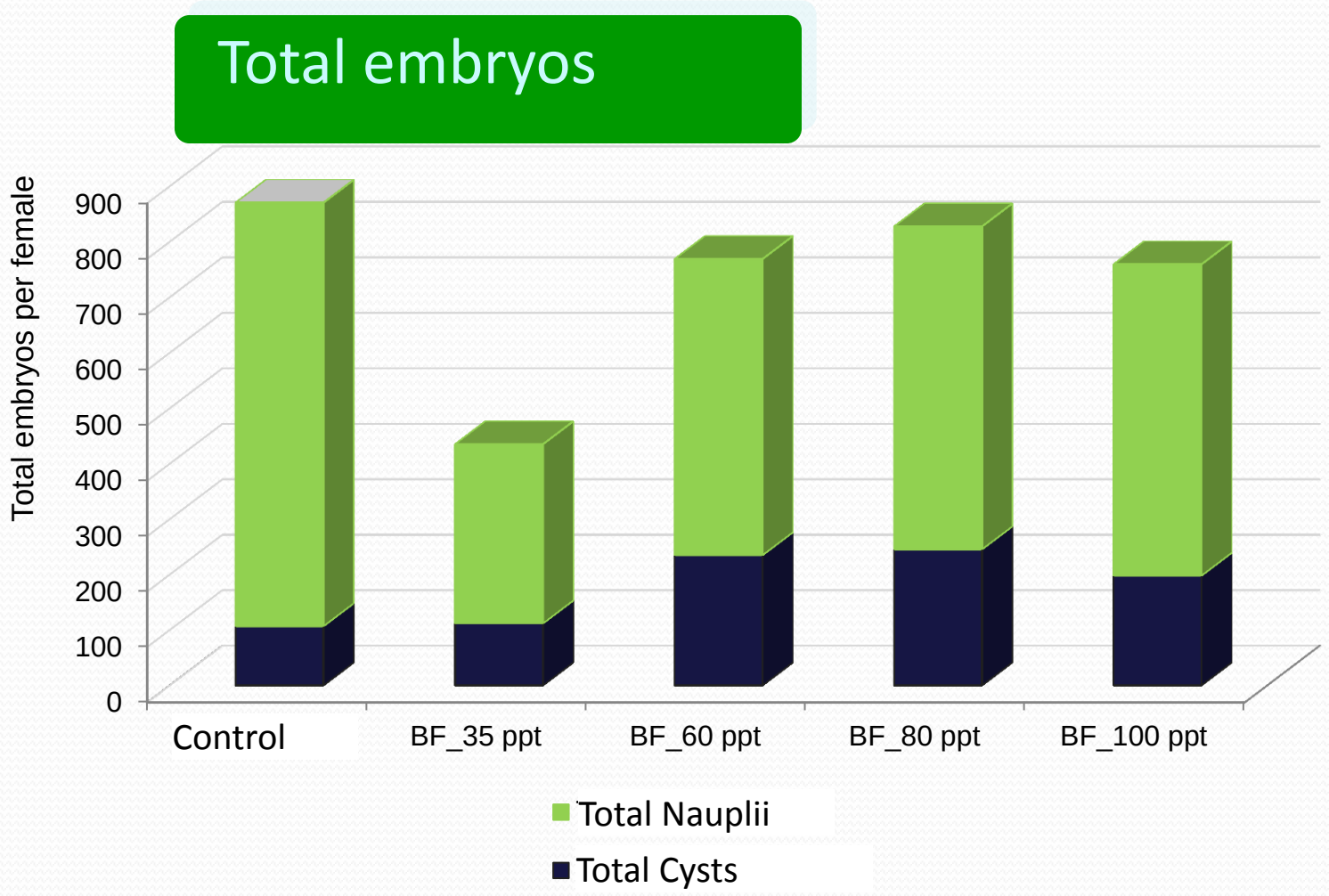


# Experiment 2: Nutritional level of bio-floc and used as feed to *Artemia*

## Reproductive characteristics of *Artemia*

| Parameters | Wild algae (control) | BF_35 | BF_60 | BF_80 | BF_100 |
|---|---|---|---|---|---|
| Total embryo/ female | 870,3±25,2$^{b}$ | 435,1±40,1$^{a}$ | 768,8±52,8$^{b}$ | 764,5±100,4$^{b}$ | 728,5±65,8$^{b}$ |
| Cyst ratio /life span (%) | 12,4±4,8$^{a}$ | 26,2±11,0$^{a}$ | 29,8±15,3$^{a}$ | 33,9±8,5$^{a}$ | 26,4±6,4$^{a}$ |
| No of brood | 7,6±0,3$^{b}$ | 5,3±0,4$^{a}$ | 7,2±0,4$^{b}$ | 7,2±0,5$^{b}$ | 7,4±0,6$^{b}$ |

# Experiment 2: Nutritional level of bio-floc and used as feed to *Artemia*



***Culture volume: 150 m2 (x3)***

***salinty:*** **80 and 100 ppt**

***density:*** **100 ind./L**

***feeding:*** **bio-floc Vs control**

***duration:*** **6 weeks**

# ARTEMIA POND CULTURE

- BF_80
- Control_80
- BF_100
- Control_100

# Experiment 3: formation and development of bio-floc in *Artemia pond*

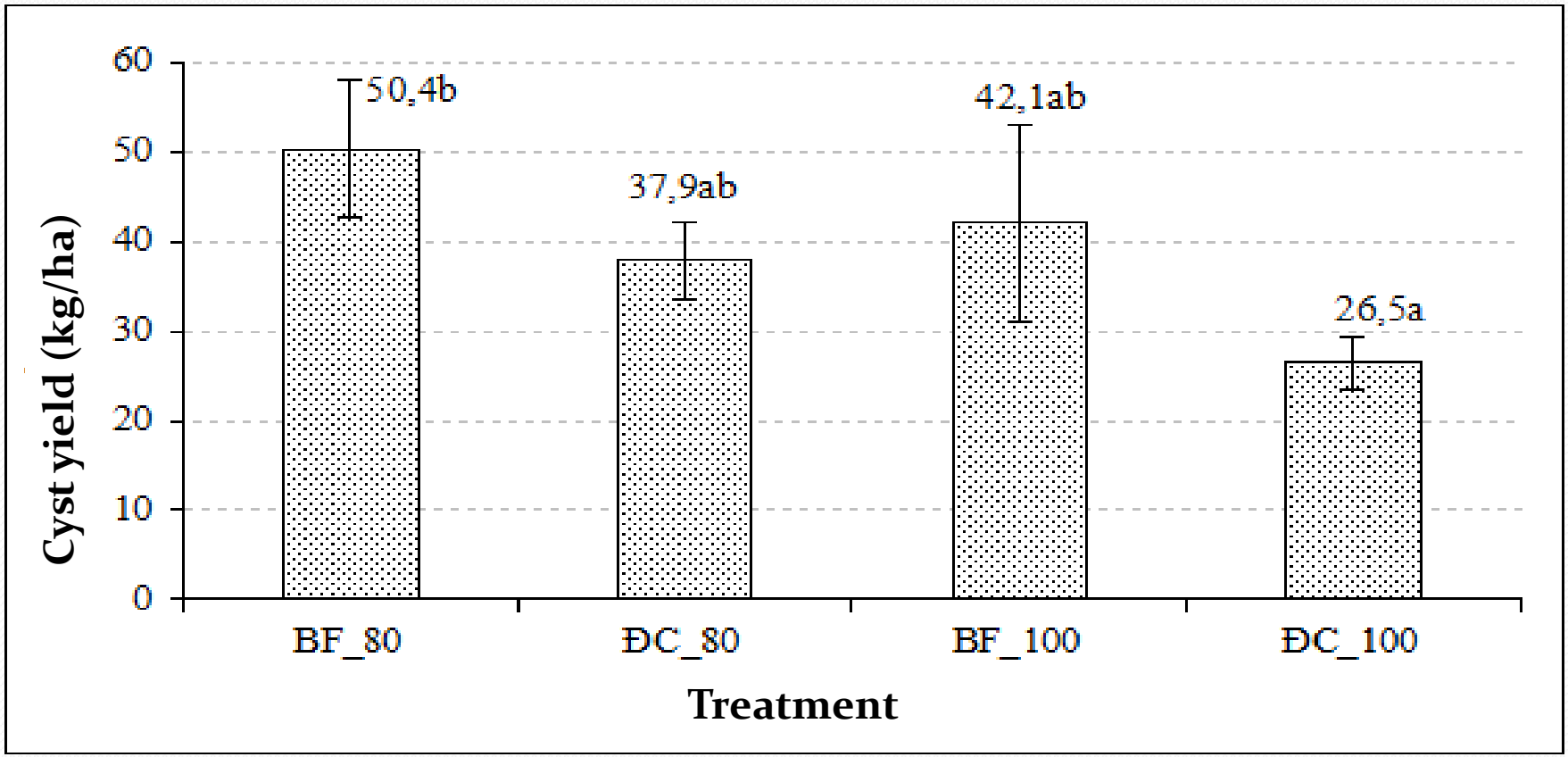
## Fecundity of Artemia

| | Treatment | Week 2 | Week 3 | Week 5 | Week 6 |
|---|---|---|---|---|---|
| Fecundity (No Cyst/ female) | ĐC_80 | 36,1±16,3$^{a}$ | 48,8±22,0$^{a}$ | 90,5±20,2$^{c}$ | 77,1±10,0$^{b}$ |
| | BF_80 | 58,9±57,9$^{b}$ | 60,8±28,2$^{b}$ | 73,8±16,9$^{b}$ | 92,6±15,1$^{c}$ |
| | ĐC_100 | 60,3±39,9$^{b}$ | 62,2±38,8$^{b}$ | 66,8±8,5$^{a}$ | 70,9±8,6$^{a}$ |
| | BF_100 | 112,7±40,5$^{c}$ | 104,3±27,0$^{c}$ | 75,9±11,2$^{b}$ | 78,1±9,8$^{b}$ |

ĐC : control

# Experiment 3: formation and development of bio-floc in *Artemia pond*

## Cyst yield (kg/ha)



## Experiment 3: formation and development of bio-floc in *Artemia pond*

# Conclusion

- Bio-floc developed well at 80 -100 ppt.
- Bio-floc formed at 80-100 ppt are suitable feed for Artemia.
- Bio-floc improved water quality and enhanced cyst production of Artemia (37,9 up to 50,4 kg/ha and 26,5 up to 42,1 kg/ha at 80 ppt and 100 ppt, respectively).

# Conclusion

**Extensive ➡ Semi-Int ➡ Intensive**

**Extensive**

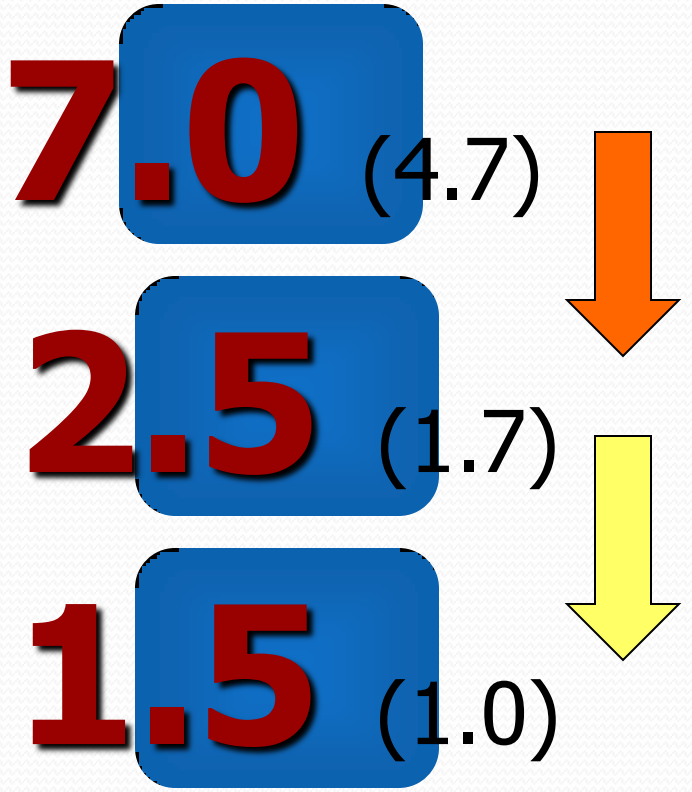
org manure app. 7 Ton/ha/crop
Inorg fert app.: **0.5-0.7** Ton/ha/crop

**Semi-Int**

org manure app. 2-3 Ton/ha/crop
Inorg fert app.: **0.2-0.3** **Ton/ha/crop**
Rice-bran: 2.4 Ton/ha/crop

**Intensive (BFT)**

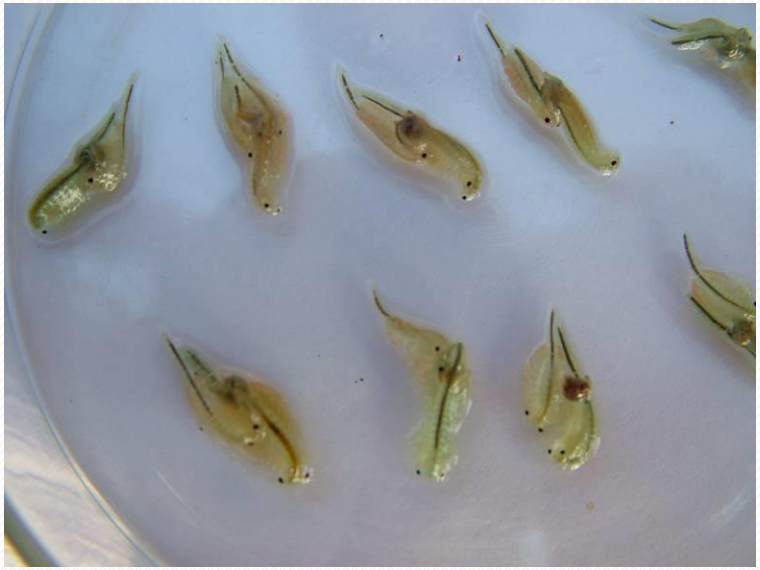
org manure app. 1.5 Ton/ha/crop
Inorg fert app.: **negligible**
Tapioca: 0.72 Ton/ha/crop



# Conclusion

| | Ext | Semi | Int |
|---|---|---|---|
| Manure (T) | 7.0 | 2.5 | 1.5 |
| Yield (kg/ha) | 30-50 | 60-120 | 150-200 |
| Profit (x 1000 usd) | 1.5-2.0 | 3.5-4.0 | 6.0-7.5 |

# Don’t forget …Artemia biomass is also an excellent food for human being!!!



# Please....

# Enjoy  with us…


thank you for your attention