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Artemia franciscana Kellogg, 1906 (Crustacea: Anostraca) production in earthen pond: Improved culture Techniques

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Abstract

Artemia culture in Vinhchau (Mekong Delta, Vietnam) solar-saltworks has been started since late 1980's with the main aim to produce cysts. Culture techniques have been improved and culture area has been enlarged year by year and thus the area could produce as high as 50 tons of raw cysts in the early of 1990's. However, cysts yield remained around 50 kg of raw cyst per hectare in average to imply other factors (e.g. soil, water quality, nutrients etc.) should be considered. In fact, present studies dealing with these allow enhancing not only cyst production but also cyst quality [high highly unsaturated fatty acid (HUFA) contents]. Moreover, these concerns are site/location related, therefore appropriated pond culture techniques/procedures should be adapted to the site where optimal culture condition for *Artemia* to be well considered.

Keywords: *Artemia*, pond culture, solar-saltworks, soil profile, poor vs. rich nutrient, *Chaetoceros*, biomass

Introduction

Artemia culture begun in solar-saltworks of Vinhchau in late 1980's. In early 1990's *Artemia* production and culture were quickly increased as they could reach around 50 tons (raw cyst material) in approximately 1200 ha. Nowaday, Vinhchau has become a well-known area where it can produce cysts of *Artemia* to fulfill the need for local aquaculture and partly for exportation. Statistically, total production increased sharply as a function of culture area extension rather than its productivity per ha (cyst yield) as indicated in Fig. 1.

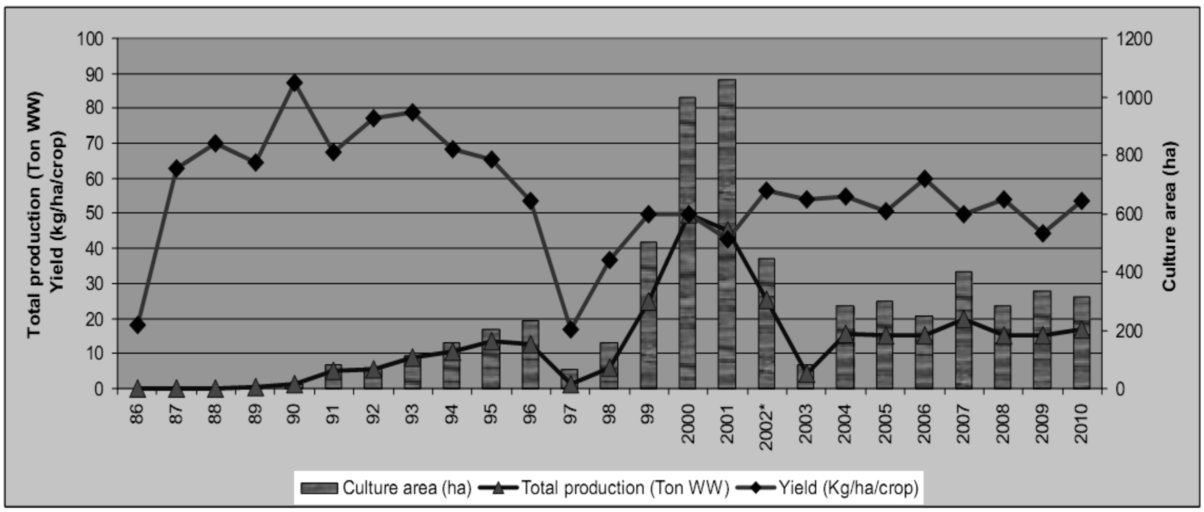

Fig. 1: *Artemia* production in Vinhchau solar saltworks during 1986-2010 (source: College of Aquaculture and Fisheries, Cantho University)

Therefore, trials have been made to study the soil, water and algal composition. Algal species in the sites, through soil profiles in Vinhchau solar saltworks has been characterized as rich- vs. poor-organic matter show its fluctuation by location accordingly.

Materials and Methods

<u>Soil and water quality influence on *Artemia* pond production</u>

Soil quality

A survey has been performed in two different pond groups, i.e. "trouble –ponds" vs. "untroubled-ponds" in term of their nutrient contents, water quality…to lead to negative impact on *Artemia* pond production. Soils samples have been taken on the depth of 0-5 cm from the pond surfaces. These samples were taken just in the moment farmers have started their pond preparation (at beginning season). Soil samples were air-dried and screened through the 0.5 mm mesh-size net for determination of physico-chemical parameters such as pH, EC, organic matter, total nitrogen, available nitrogen, total phosphorus, available phosphorus.

Water quality

Water samples were collected weekly through out the culture period, i.e. 1, 2, 3 and 4 weeks. Parameters to be monitored were pH, EC, total alkalinity, COD, BOD, DO, NO_3, NO_2, TN, TAN, DRP and TP (Clesceri *et al*., 1998). Besides, algal samples were collected for qualitative (Shirota, 1966), and quantitative analyzing by Sedgewick-Rafter (S-R) and were counted and calculated according to Clesceri et al., 1998.

Effect of different ratios of N:P on primary productivity: its combination with feeding strategies for *Artemia* biomass production in salt ponds

Fertilizer pond: Four earthen ponds with 90 m^2 each were made, and to start 10 kg $CaCO_3$ m^{-2} were provided and to keep drying for 7 days, then pig manure were added at the rate of 1.2 and 0.6 ton dry weight per ha at the beginning and every two weeks, respectively. After three days, the ponds were pumped with seawater at a level of 0.5 m, to the end water level was up to 0.8 m. Then, inorganic fertilizers (combination of Urea (46.3 %) and Supper phosphate (16.5 % P_2O_5) with the rate of N:P=5 (GW5) and N:P=10 (GW10)) were applied at the rate of 7 g m^{-3} initially and 2-5 g m^{-3} for the next 1-2 week. Green water appeared after a few day since fertilization, then was used as feed to *Artemia* ponds. As a rule of thumb, 30-40% of green water was kept as stock for the next green water cycle.

Experimental set-up: A two factor experiment with three replicates for each treatment as follows was implemented:

GW5+RB (supplemented with rice-bran)
GW5+PM (supplemented with pig-manure)
GW10+RB (supplemented with rice-bran)
GW10+PM (supplemented with pig-manure)

Artemia pond: Pond preparation, inoculation, management were performed according to Nguyen Thi Ngoc Anh (2009)., The feeding procedure was managed either as: 1) 200-300 kg DW ha^{-1} $week^{-1}$ pig manure or 2) 20-30 kg DW ha^{-1} $week^{-1}$ rice-bran (fermented with 2-3 % alcohol yeast over 24 h prior to feeding).

Data collection: Water temperature (ºC), pH, salinity, turbidity, $N\text{-}NH_4^+$, $N\text{-}NO_3$, TN, PO_4^{3-}, TP and Chlorophyll-a were measured for fertilizer ponds, while water temperature (ºC), salinity, turbidity were recorded only for *Artemia* ponds (see Nguyen Thi Ngoc Anh, 2009).

Chaetoceros as the main food to enhance *Artemia* biomass quality

Artemia biomass quality was evaluated when fed with *Chaetoceros sp*. Compared to wild algae from fertilizer ponds, study was set-up with two treatments and four replicates each.

Cultures were carried out in two stages: the first phase was batch culture, in which *Artemia* were cultured in a 30 L composite tank , salinity 80 ‰, density 200 nauplii/L. Culture period was 40 days. Animals were fed ad-libitum four times a day, and were aerated continuously. The second phase was carried out in order to follow their reproductive characteristics, couples were separated when riding couples were observed; couples were randomly selected and each couple was cultured in a Falcon tube (125 ml), water exchange followed each reproductive cycle (i.e. after recording the number of cysts or nauplii releasing).

Data were recorded including of survival, length while HUFA contents of biomass were determined in ARC (*Artemia* Reference Center, Ghent University, Belgium).

Result and Discussion

Soil and water quality influence on *Artemia* pond production

Soil quality

Results of soil analysis was indicated in Table 1, there were difference between two pond groups in mainly % TP, available N, N-labile and available P.

Table 1: Chemical parameters of soil samples in *Artemia* ponds (early season)

Parameters	Pond characteristics		T-value	P
	Group1	Group 2		
pH $_{(1:5)}$	7.47	7.51	-0.38	0.72^{ns}
EC $_{(1:5)}$ (dS/m)	27.73	24.88	1.511	0.18^{ns}
%C	2.28	2.35	-0.57	0.59^{ns}
% TN	0.015	0.020	-1.90	0.11^{ns}
% TP	0.055	0.045	4.89	0.0027^{**}
P available (mg P/kg)	20.49	17.18	5.56	0.0014^{**}
N available (mg NH_4-N+NO_3-N/kg)	18.15	13.57	4.36	0.0047^{**}
N labile (mg/kg)	24.51	17.31	4.77	0.0031^{**}

Note: Group 1: "trouble pond" and Group 2: "untroubled-pond" in term of population development and cyst production

%TP in both groups 1 and group 2 was classified as rich in terms of P concentration (Tran Van Chinh, 2006) and this may lead to contamination (Sharpley, 1995). According to Olsen (1954) soil characteristic in group1 was high, while in group 2 was in average, the variation was considered because of soil profile and pond culture procedure. In fact, in order to enhance algal development in *Artemia* ponds there were a number of organic manures e.g. chicken manure, cow manure etc to be applied through out the culture. which were used. For nitrogen there were significant difference in N available and N labile for both groups; N labile is considered as main indicator for soil quality, and this varies according to the organic matters applied rather than the total organic matter. This element is normally absorbed efficiently by algae and bacteria.

Water quality

Water quality parameters was displayed in Table 2a, b, c, d, and e, and figures 2a, 2b, 2c, 2d, 2e 2nd 2f, in which pH varied closely to 8 throughout the culture period and had no effect on *Artemia* growth and development (Treece, 2000). Salinity had no strong effect on *Artemia*, as they could survive in the range of 3-300 ‰ (Wear and Haslett, 1986), however at salinity lower than 80 ‰ they have the tendency to turn into cyst reproduction (Vos and De la Rosa, 1980). Moreover, at low salinity it is more favor to nutrient release and thus higher algae density and composition (Gardolinski et al., 2004; Khoi et al, 2006); oxygen concentration was different between two groups in week 1 and 4, however most of them were higher than 5 ppm, which was suitable for *Artemia* growth and development (Lavens and Sorgeloos, 1996); COD was different in week 1 and week 3 between two groups and varied around 9.41-18.83 ppm, which was generally acceptable for aquaculture system (TCVN 5943-1995) (optimal COD range was 15-30 ppm and optimal concentration was 35 ppm). According to TCVN 5943-1995 BOD_5 below 10 ppm shows a poor nutrient content in water body versus to higher than 35 ppm which indicates a rich nutrient water. Therefore, both water samples had low nutrients although group 1 showed higher BOD_5 than group 2 except for week 4. Total alkalinity was significantly higher in group 1 compared to group 2 during week 1, 2 and 3, except week 4; higher alkalinity is a good buffering for pH of water medium and thus to sustain water quality. Total dissolved nitrogen and dissolved inorganic nitrogen were highly significant difference between group 1 and group 2 (see Fig. 2b); high nitrogen content in *Artemia* ponds came from both organic manure applied (i.e. chicken manure, pig-manure…) and soil profiles and explained by Boyd (1998) as NH_4^+ higher than 2 ppm to indicate ponds with high nutrient but when it was higher than 5 ppm the pond became contaminated. Total phosphorus and dissolved reactive phosphorus (DRP) in group 1 was three times higher than that of group 2, in which total phosphorus was 5.43 ppm which was too high in a water body (Van Dolah *et al*., (2002. DRP was 1.4 ppm in average again this level may induce the water-bloom (Seroka, 2004), too high phosphorus as a whole in water column may cause excess development of algae which is not suitable for *Artemia* culture (Fatimah et al., 2001). Rich in nutrients, especially N and P results in over development of algae which may have negative impact on *Artemia* culture as shortage of oxygen during the night time. Moreover, poor N but rich in P stimulate development of *Oscillatoria sp*. (cyano-bacteria) and they mainly cause water-bloom. Observation in pond condition indicated that algae density in groups 1 always was higher than group 2 (see Fig. 2.) and this concentration was indicated as water-bloom (Smith, 1996).

Table 2a: *Artemia* pond water pH

Sampling time (week)	pH *Artemia* pond water		T-value	P
	Group 1	Group 2		
Week 1	8.13	8.43	-4.36	0.0048**
Week 2	7.97	8.36	-11.86	0.0000**
Week 3	8.16	8.29	-2.055	0.086ns
Week 4	8.15	8.28	-2.66	0.038*

Table 2b: variation of salinity by time

Sampling time (week)	Salinity (‰)		T-value	P
	Group 1	Group 2		
Week 1	84.09	78.73	3.14	0.0200*
Week 2	84.76	82.75	0.44	0.6800ns
Week 3	84.76	82.75	0.44	0.6800ns
Week 4	77.49	86.31	-2.58	0.0400*

Table 2c: Variation of dissolved oxygen (DO) in *Artemia* ponds

Sampling time (week)	DO (O_2 mg/L)		T-value	P
	Group 1	Group 2		
Week 1	6.93	8.54	-8.03	0.0002**
Week 2	8.25	8.42	-0.94	0.3900ns
Week 3	5.71	5.52	0.40	0.7040ns
Week 4	5.02	6.10	-2.05	0.0860*

Table 2d: Variation of COD in *Artemia* ponds

Sampling time (week)	COD (O_2 mg/L)		T-value	P
	Group 1	Group 2		
Week 1	17.89	18.83	-3.15	0.0200*
Week 2	18.33	18.67	-0.86	0.4230ns
Week 3	9.41	12.54	-4.33	0.0050**
Week 4	14.40	14.00	1.44	0.2010ns

Table 2e: Variation of BOD_5 in *Artemia* ponds

Sampling time (week)	BOD_5 (O_2 mg/L)		T-value	P
	Group 1	Group 2		
Week 1	3.79	2.72	5.15	0.0021**
Week 2	5.64	4.72	2.81	0.0310*
Week 3	3.67	2.76	2.22	0.0690ns
Week 4	5.23	6.52	-2.86	0.0290*

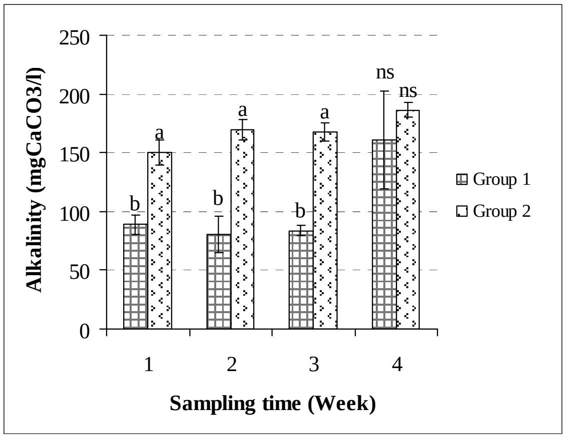

Fig. 2a: Variation of total alkalinity in *Artemia* pond by time

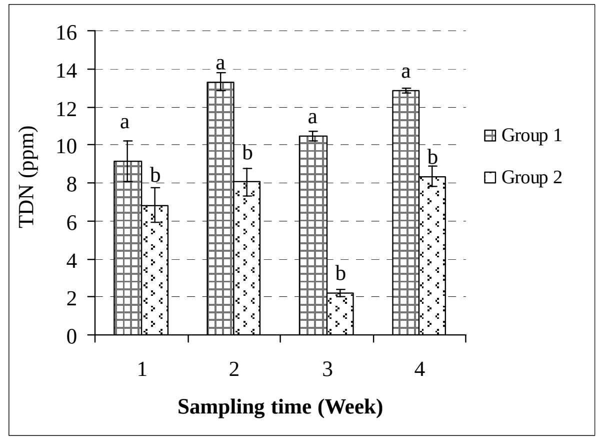

Fig. 2b: Variation of total dissolved nitrogen in *Artemia* pond by time

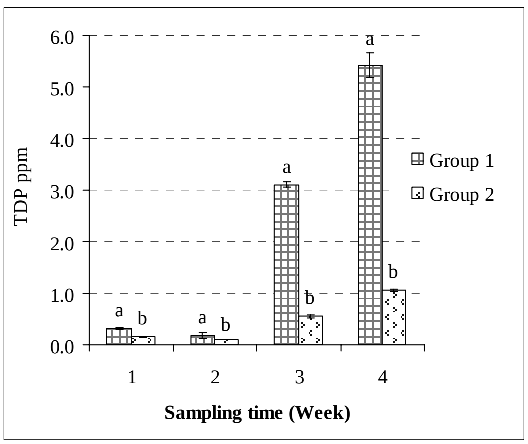

Fig. 2c: Variation of total dissolved phosphorus in *Artemia* pond by time

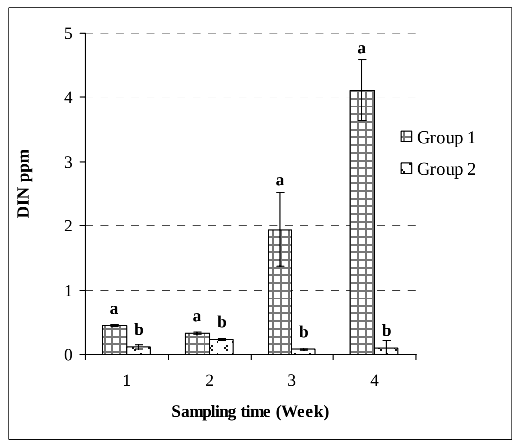

Fig. 2d: Variation of dissolved inorganic nitrogen in *Artemia* pond by time

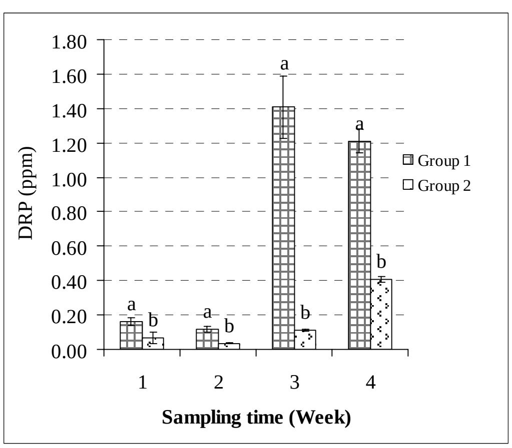

Fig. 2e: Variation of dissolved reactive phosphorus in *Artemia* pond by time

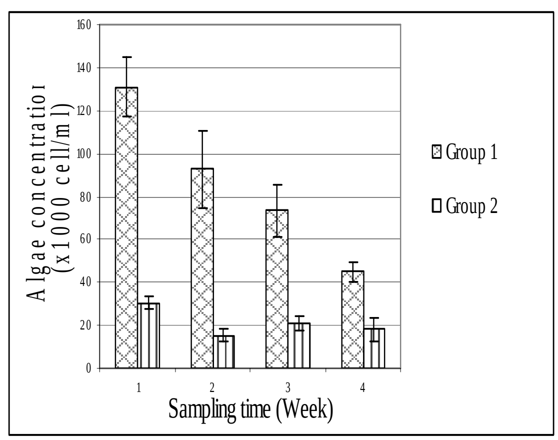

Fig. 2f: Variation of algae density in *Artemia* pond by time

Algae play an important role in *Artemia* growth and development (Mason, 1963) however, *Artemia* could not filter larger algae, especially filamentous algae (e.g. Ossilatoria) as they become like a trap to prevent *Artemia* movement and thus reduce their survival (Sorgeloos et al., 1986; Dhond and Lavens, 1996). Moreover, too dense algae make *Artemia* thoracopods become stuck, while overfeeding and slow down their digestion rate as algae passing quickly through the gut (Sevrin-Reyssac and Pletikosic, 1990) will reduce sharply *Artemia* survival. As a result (Table 3) after 7-10 days since inoculation *Artemia* from group 1 displayed its retarded growth, slow movement and finally low survival rate (15 %) versus to 80 % survival rate in group2, which almost double growth (i.e. 7.22 mm) after 7-10 days and all animal were healthy, active and brightly colored. Consequently, total cyst production of cysts (in wet weight) of group 2 varied in the range of 100-120 kg ww/ha/crop (3-4 months) compared to around 50 kg ww/ha/crop in group 1.

Table 3: Differences in development and cyst production of *Artemia* between group 1 and group 2

Observation	Group 1	Group 2
7-10 days after inoculation		
+ Length (mm) + Survival (%) +Animal behavior (swimming)	4.58 15 Swimming slowly, in-active	7.22 80 Active, brightly colored
Production		
+ Cysts Yield (kgWW/ha)	50	100-120

WW: wet weight

In summary, soil profile and water quality both play important roles in *Artemia* pond culture, and in Vinhchau saltfarm variations between rich- and poor-organic bottom are mainly relying in 1) percentage of total phosphorus, 2) P and N available and 3) and N labile. Then, together with mineralization occurs during the process of pond preparation and culture procedure available nutrients were released into water column resulted in water eutrophication by organic rich bottom which produced *Artemia* with poor growth, low survival rate, and thus low fecundity and cysts production.

Effect of different ratios of N:P on primary productivity: its combination with feeding strategies for *Artemia* biomass production in salt ponds

Fertilizer pond: Variation of abiotic conditions during 12 weeks are indicated in Figs. 3 and 4. As shown in the tables the water temperature had an increasing pattern from the beginning of the culture period to the end. pH increased from week 3 -8 then dropped closely to 8; turbidity showed its declining after 1 week and stabilized from week 3 afterwards (around 20 cm); salinity increased by time and maximal was closely to 43 ppt then down to 30 ppt. TN increased gradually by time with representative by $N\text{-}NH_4$, $N\text{-}NO_3$ increased sharply for the last two weeks. Except for week 1 and 7 PO_4 which was increased, during the rest of the period it was stable around 0.1-0.2 ppm. TP increased but TN was rather stable from week 4 afterwards, therefore TN: TP was 16.8 from beginning and was close to the Redfield ratio (Redfield *et al*., 1963) but decreased gradually until the end of the experiment that indicated that N is limited factor for the growth of algae (Howarth, 1988; Khoi et al., 2006). However, with Chlorophyll-a varied 78-274µg L-1 for GW5 and 60-253 µg L-1 for GW10, turbidity 10-30 cm, TN 1.2-11.6 mg L-1, TP 0.07-1.84 mg L^{-1} for GW5 and 0.07-1.19 mg L^{-1} for GW10, N:P varied from 6.4-13.6 and 7.4-20.6 for GW5 and GW10, respectively were suitable for maintenance algae development (Lin and Yi, 2002).

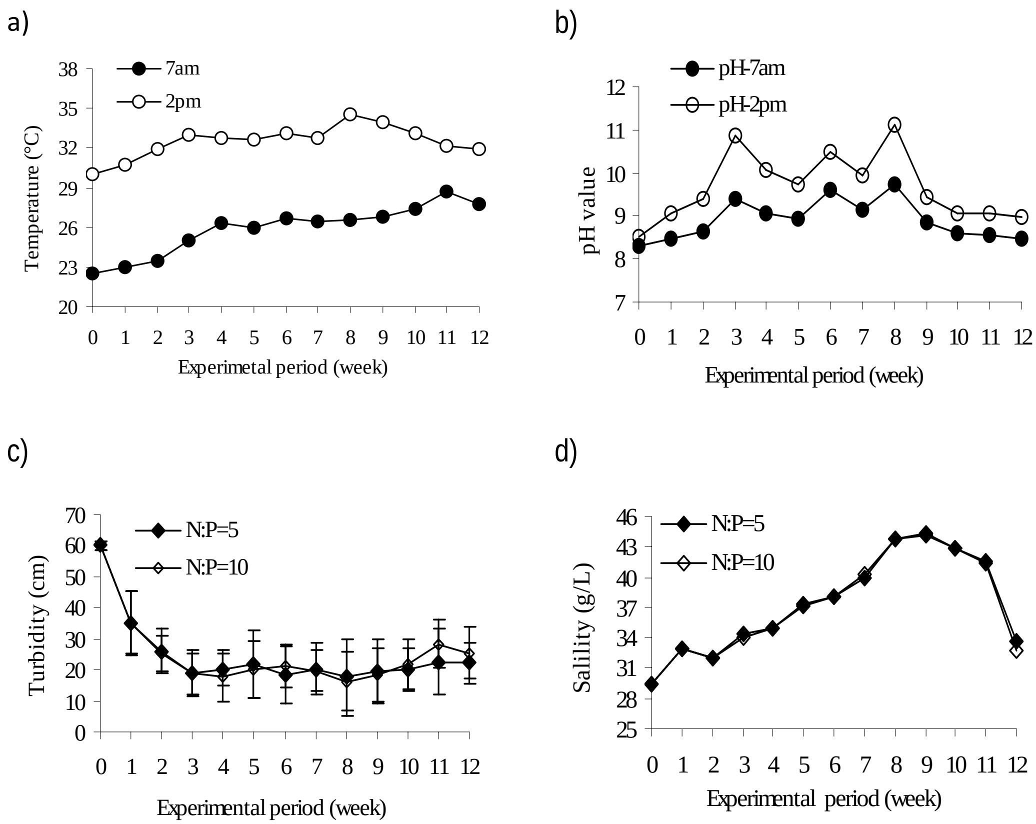

Fig. 3. Fluctuation of abiotic water parameters in the fertilization ponds.
(a) Temperature, (b) pH, (c) Salinity, (d) Turbidity

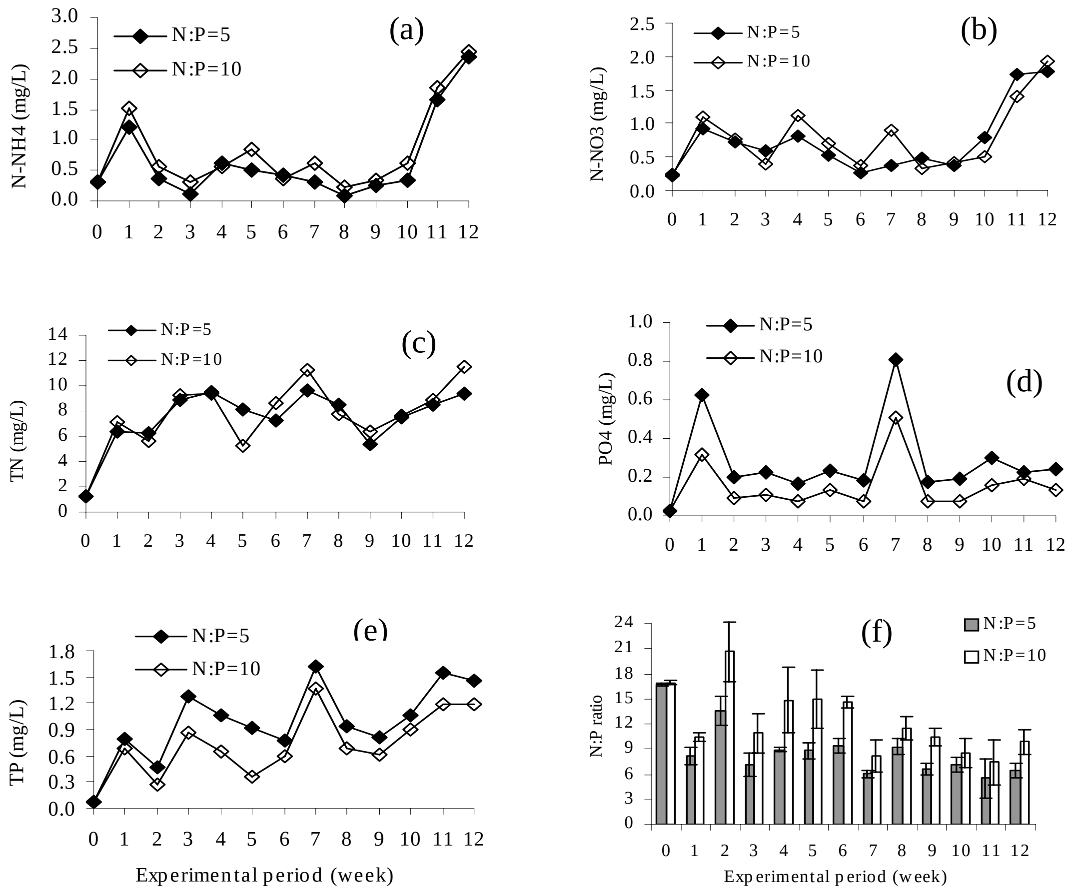

Fig. 4. Fluctuation of nutrient parameters in the fertilization ponds.
(a) $N\text{-}NH_4^+$; (b) $N\text{-}NO_3^-$; (c) TN; (d) $P\text{-}PO_4^{3-}$; (e) TP; (f) TN:TP ratio

In the site the phylum Bacillariophyta seems to be promoted when applied GW5 and GW10 (see Fig. 5). Chlorophyta, Cyanobacteria and Dinophyta were observed in both treatments from the beginning of the culture period, but then disappeared during weeks 1-5. Variations of chlorophyll-a explained in Fig. 6, in which there were observed two cycles of algae development the first of which was during weeks 1-5 and the second during weeks 7-12.

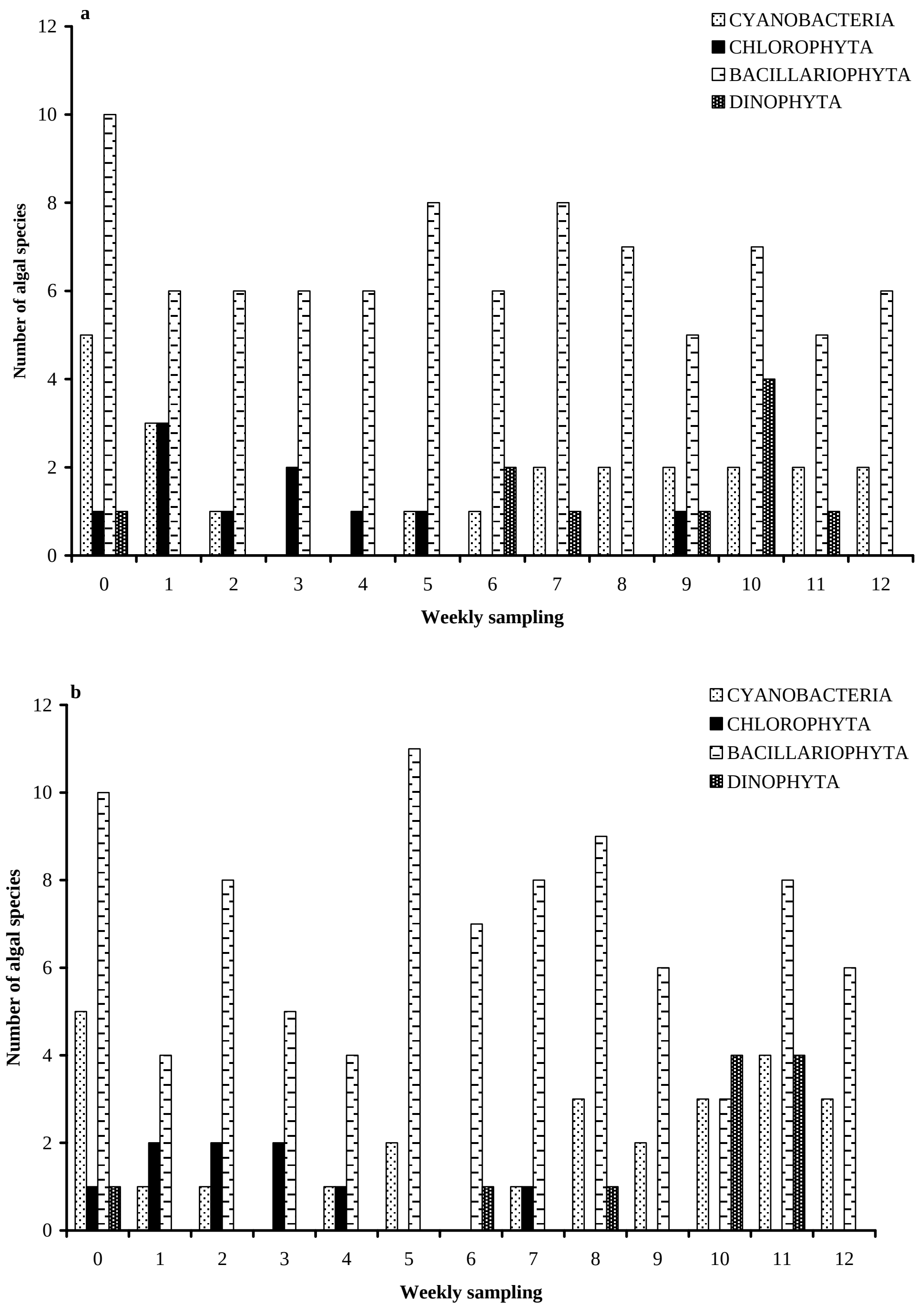

Fig. 5: Occurrence of main algal groups in fertilization ponds as a function of time.
(a) N: P=5 treatment, (b) N: P=10 treatment

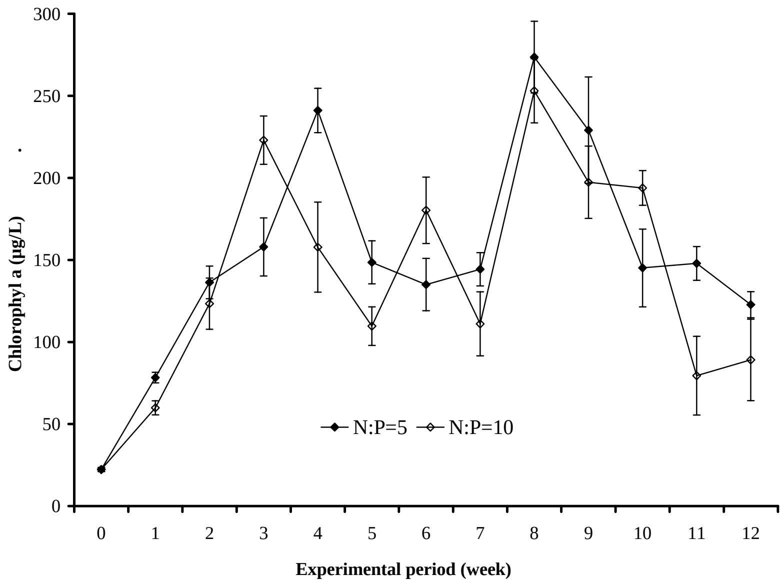

Fig. 6: Chlorophyll a levels in fertilizer ponds by time

The algal composition found in this study agreed to earlier studies (Ut et al., 2008; Oanh et al., 2008) however, they were somehow lower than what have been found by Nguyen and Ha, 2006 and probably because this study was subjected to high salinity (33-48 g L^{-1}), short duration of experiment (12 weeks) and restricted ecosystem (focused on fertilizer pond) (Nguyen Thi Ngoc Anh, 2009). Moreover, between GW5 and GW10 there was neither significant difference in Chlorophyll-a nor the algal composition and thus a further range of N:P needed to be evaluated.

Artemia ponds: Chemical composition of added foods for *Artemia* ponds as indicated in Table 4, in which N was almost the same but rice-bran had lower P, consequently it had higher C:N and N:P ratios compared to pig manure. Similar abiotic conditions in different treatments were recorded throughout the culture poeriod, in which salinity was in the range of 74-99 ppt, temperature varied in the range of 22-29ºC at 7 a.m. and 31-36ºC at 2 p.m., respectively. Turbidity varied in the range of 28-40 cm during the culture period. Survival rate was measured at day 11, while length and weight were recorded at day 14, together with final yield results have been presented in Table 5:

Table 4. Chemical composition of the two supplementation products (% dry matter). Values are average and standard deviation of three replicates.

Chemical composition	Rice bran	Pig manure
Dry matter	88.58 ± 1.28	50.02 ± 13.94
Carbon	42.31 ± 2.08	30.61 ± 4.38
Nitrogen	2.63 ± 0.36	2.98 ± 0.39
Phosphorus	1.39 ± 0.22	2.40 ± 0.32
C :N ratio	16.24 ± 1.43	10.27 ± 0.28
N:P ratio	1.95 ± 0.53	1.25 ± 0.26

Table 5. Mean survival at day 11, length and weight at day 14 and final yield of *Artemia* biomass cultured with different green water source (fertilizing with different N: P ratio) and supplementary feed. Values are average and standard deviation of three replicate ponds.

Main effect	Survival (%)	Length (mm)	Weight (mg)	Biomass yield (ton ha^{-1} $crop^{-1}$)
Green water				
GW5	71.84±3.56a	9.34±0.71a	9.39±0.37a	2.29±0.48a
GW10	71.98±3.60a	9.22±0.75a	9.19±0.44a	1.97±0.36a
Feed supplement				
RB	73.54±3.01a	9.24±0.77a	9.29±0.32a	2.19±0.36a
PM	70.28±3.20a	9.31±0.70a	9.29±0.49a	2.07±0.53a
P value				
Green water (1)	0.946	0.114	0.065	0.267
Feed supplement (2)	0.617	0.359	0.981	0.671

Values within a column with different letters were significantly different (P<0.05).

Considered the effect of green water there was indicated that most of survival, length and weight were the same (not significantly difference) and thus the final biomass yield remained also with no variations, although a slightly higher biomass yield was observed at GW5 compared to GW10 (2.29 vs. 1.97 ton ha^{-1} $crop^{-1}$), similarly adding RB gave a bit higher biomass yield compared to PM (2.19 vs. 2.07 ton ha^{-1} $crop^{-1}$). It was indicated that *Artemia* survival, growth could be affected as a function of salinity, temperature (Hoa, 2002), therefore similar results in this study may engage with the same culture condition, especially the food item and finally remaining in the biomass yield (Table 5). Productivity of females between treatments has been displayed in Table 6:

Table 6. Reproduction characteristics (percentage of ovoviviparity and brood size) at first spawning and average values of the whole culture period of *Artemia* biomass cultured with different green water source (fertilizing with different N: P ratio) and feed supplement.

Main effect	First spawning		Whole culture period	
	Ovoviviparity (%)	Brood size (No. nauplii $female^{-1}$)	Ovoviviparity (%)	Brood size (No. nauplii $female^{-1}$)
Green water				
GW5	13.06±3.86	44.5±5.4b	19.17±10.59	47.5±3.7b
GW10	11.94±1.95	30.2±1.7a	18.36±10.04	41.3±3.5a
Feed supplement				
RB	12.78±3.60	36.4±6.5	18.43±9.92	44.7±4.0
PM	12.22±2.51	38.4±10.5	19.09±10.71	44.0±5.8
P value				
Green water (1)	0.585	0.000	0.228	0.011
Feed supplement (2)	0.783	0.344	0.320	0.731

Values within a column with different letters were significantly different (P<0.05).

One way ANOVA indicated that the nauplii brood-size in GW5 was almost 1,47 times higher than GW10 for the first spawning, and 1,15 for the whole culture and those were significantly different. Surprisingly, both supplementary foods gave similar brood-size (p >0.05) throughout the culture. However, when applied GW5, there was significantly higher brood-size compared to GW20, and it seemed that Chlorophyll-a plays a main role. Besides, GW5 contained more suitable species (e.g. Nitzschia sp, *Chaetoceros sp*.) for *Artemia* (Thinh *et al*., 1999; Toi *et al*., 2006) than GW10.

Chaetoceros sp as main food to enhance *Artemia* biomass quality

Chaetoceros sp. was first isolated and scaling-up culture before feeding to *Artemia*, while wild algae were collected in fertilizer pond in Vinhchau station through out the culture, in which the species composition and frequencies has been recorded in Table 7:

Table 7: Algal composition collected in Vinhchau saltfarm

Species	Frequency
Chlorella sp.	+
Lyngbya sp.	+
Nanochloropsis sp.	+
Isochysis sp.	++
Cyclotella caspia	+
Navicula derecta	+
Nitzchia longissima	+

Note: + common species, ++ Dominant species

Survival of *Artemia* fed on *Chaetoceros sp*. and wild algae are displayed in Fig. 7, through which there were decline in survival for animals fed with *Chaetoceros sp* or wild-algae. Wild algae supported *Artemia* survival for the first three days then significant difference in survival appeared and lasted until day 15 of the batch culture.

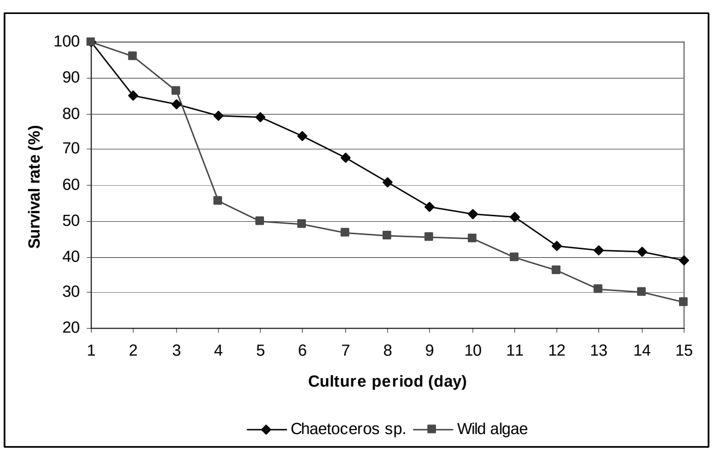

Fig. 7: Survival rate (%) of *Artemia* batch culture when fed either Chaetoceros or wild-algae

In the couple culture their survival were stable until day 15 then declined, those were fed with wild-algae maintained their survival until day 22 then the survival crashed sharply, and no survivors observed at day 28 afterwards. Females population fed on *Chaetoceros sp*. were slightly declined from day 15-20 then stabilized until the end of the culture period (closed to 78 %) (see Fig. 8).

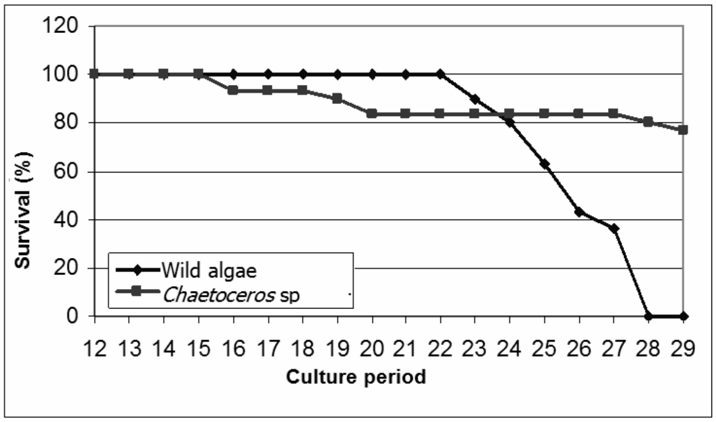

Fig. 8: Survival of *Artemia* females fed either *Chatoceros sp*. or wild algae

Reproductive parameters were recorded and are displayed in Table 8, in which embryos per brood, embryos per female, cysts per female and nauplii per female of females fed with Chaetoceros were almost two times more than those fed with wild algae ($p < 0.05$), however significant difference was not found for number of cysts per female ($p > 0,05$), the rest had not significant difference. Results from this study agreed with those of Quynh and Tho (1993) as females were fed with wild algae (dominated with *Chaetoceros* sp) gave always higher life characteristics.

Table 8: Reproductive characteristics as a function of feed applied

Parameters	Wild-algae	*Chaetoceros sp*.
Embryos per brood	66±16[a]	120±48[b]
Embryos per female	284±99[a]	661±406[b]
Cysts per female	59±72[a]	117±187[a]
Nauplii per female	226±98[a]	545±411[b]
Number of broods	4.23±1.04[a]	5.03±2.07[a]
Cyst-broods per female	0.87±0.94[a]	0.90±1.12[a]
Nauplii broods per female	3.67±1.81[a]	3.33±1.32[a]
Brood interval	3.22±0.61[a]	3.64±1.01[a]

Values within a row with different letters were significantly different ($P<0.05$).

Fatty acids in *Artemia* biomass when fed either on *Chatoceros sp*. or wild algae has been presented in Table 9 and clearly indicated that food quality has a strong effect on the biomass fatty acids. Fatty acids percentage in biomass fed with *Chaetoceros sp* was higher than those fed with wild-algae (i.e. 22.6 vs. 9.99 %); the same pattern when compared in dry weight HUFA was almost 2.6 times (26.63 vs.

7.22) for those were fed with *Chaetoceros sp* compare to those fed with wild algae. However, DHA content of *Artemia* when fed with *Chaetoceros* sp was only one-third in term of dry weight compared to wild algae and this was similar to observation of Thinh et al., (1999). It was stated that for marine fish larval culture the ration of EPA/DHA should be in the range of 1:1,5-1:8 (Copeman *et al*., 2002), while in other live feeds (e.g. marine algae, rotifer…) this ratio is in average of 1:2.5 (Lavens and Sorgeloos, 1996). Therefore, in order to meet the nutritional requirement of larval culture, especially to marine species DHA content should be level up via enrichment with DHA emulsion (e.g. SELCO from INVE, Belgium) of feeding with alga rich in DHA prior to larval feeding.

Table 9: Fatty acid composition in *Artemia* biomass

Fatty acids	Food			
	Chaetoceros sp.		Wild-algae	
	% Fatty acid	mg/g in dry weight	% Fatty acid	mg/g in dry weight
SFA	26.7	32.4	32.0	23.2
MUFA	40.0	48.5	38.9	28.2
PUFA	28.4	34.3	24.2	17.5
HUFA	22.06	26.63	9.99	7.22
DHA (Docosahexaenoic acid)	0.1	0.2	0.9	0.7
EPA (Eicosapentaenoic acid)	18.4	22.2	5.7	4.1

Conclusion

Under different soil profiles (i.e. rich vs. poor organic matter in term of N, P contents) and via mineralization, N, P were released in ponds water accordingly. When soil contained 20.49 mg/kg P available, 18.15 mg/kg N available and 24.51 mg/kg N labile and considered as rich organic soil profile, there were tremendous increase of N, P in water column which resulted in algal-bloom and caused negative effect on *Artemia* culture ("trouble pond").

At GW5 combined to rice-bran or pig-manure there was observed better results in terms of growth, fecundity, and biomass yield compared to GW10 but not statistically difference.

Chaetoceros sp. isolated from the site (Vinhchau) could promote *Artemia* survival and biomass quality in terms of fatty acids profile, however before applying to marine laval culture, *Artemia* should be enriched with rich HUFA ingredients.

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