

Use of Seasalt for Artemia Biomass Culture In Corporation With Biofloc Technology

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Abstract: In this study, *Artemia* were cultured at three substitution rates of seawater by sea-salt water involving 0% (control; seawater); 50 and 100% incorporated with two ways of culture method known as normal and biofloc application. The two-factorial experiment produced 8 treatments and there were 3 replicates for each. *Artemia* were reared in 5L plastic bottles, contained 3L culture medium with a density of 500 nauplii/L, salinity 30‰ and lasted for 4 weeks. In the first two days after stock, *Artemia* was fed with fresh *Chaetoceros* algae and after that till the end of experiment, *Artemia* formulated feed was used as daily food. In biofloc treatments, the C/N ratios were regulated by adding molasses into the *Artemia* culture medium based on TAN concentration from days 5th. Results from experiment showed that most of followed parameters such as survival, growth, reproduction as well as biomass production were more or less similarity between treatments ($p > 0,05$) except reproduction rate. These results indicated that the sea-salt water can be used for *Artemia* biomass culture and bioflocs did not make sense when applied in this case, although the biomass production in biofloc treatments were a bit higher than in normal culture. However, it was calculated that the feed utilization in biofloc treatments was lesser 13.3% compared to normal culture for getting the same amount of live biomass at harvest. Besides that, biofloc application also saves water exchanges about 30% and labour cost for this work.

Index term: *Artemia*, substitution rate, biomass production, bioflocs

I. INTRODUCTION

Artemia is an ideal live food item that has widely used in aquaculture, especially in fish and crustacean larvae production due to their nutritional values such as high protein content (45-55%), essential fatty acids, pigments and vitamins ... (Léger *et al.*, 1986.; Nguyen Thi Ngoc Anh, 2009). Beside nutritious worth, the difference in sizes of biomass from nauplii (500 µm) till adults (8-10mm) easily to fit the larval mouth sizes during larval culture to nursery stages of shrimps, fishes. Former researchers announced that newly hatched *Artemia* nauplii can be feed directly to most of shrimp, fish larvae and resulting in higher survival rates, better performance compared to other live feed or artificial feed (Bengtson *et al.*, 1991). Moreover, *Artemia* biomass including pre-adults and adults when used as food source in nurseries most of aquaculture species gave better results in term both of utilization and economic efficiency (Olsen *et al.*, 1999). Additionally, *Artemia* had been documented that it contained rather large amount of vitamins, pigments as well as reproductive hormones which is very beneficially in pet fish culture and shrimp, fish and crab brood-stocks (Sorgeloos *et al.*, 1997; Lim *et al.*, 2003).

Although *Artemia* biomass has been cultured worldwide a long time ago but it is always incorporation with either salt lakes or salt-field for outdoor culture (Van Stappen., 2002). The indoor culture was not favorite because of high operation costs such as seawater transportations, electrical power, feed and labors...despite of many advantages known as serving in demand, disease control and no seasonal depending like outdoor culture (Hoa., 2002; Anh., 2009). Recently, aquaculture is fast developed in Vietnam, requiring many billions shrimp, fishes, crabs... babies a year and that leading to a big amount of *Artemia* biomass has been used as feed source for both nurseries and grow-out activities in the Mekong Delta (100-150 thousand hundred tons, data from our survey 2016-2017, not published yet). However, this biomass can only provide during the dry season (from February to June) by outdoor culture in salt-pans, out of this time it became shortage or had to storage under frozen form which is not attractive to many predators liked shrimp babies, pet fishes who is a hunter of live prey. For this reason, an indoor culture has been tried in a series of experiments based on *Artemia* biological characteristics known as easy to adapt with diversified habitats including differential salt compositions (Van Stappen, 2002) resulting in our trials of using sea-salt water; a cheap and always available instead of seawater (high transportation cost). Besides that, bio-floc technology which is well documented during recent years in aquaculture as a new

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way to save food, exchange water and environmental support, especially in *Artemia*, it has also been noticed by Sui *et al.*, 2013; Ronald *et al.*, 2013. These advantages should be tested in combination with the sea-salt water culture medium in order to produce not only actively fresh *Artemia* biomass year-round but also reasonable, clean and disease controlled live food.

II. MATERIALS AND METHODS

This study was carried out at the *Artemia* laboratory, Department of Coastal Aquaculture, College of Aquaculture and Fisheries, Can Tho University and *Artemia franciscana* Vinh Chau strain was used for experimental test.

Experimental setup

A two factorial experiment was set up in which the first factor were substitution rates of seawater medium (SM) by sea-salt medium (SSM) in turn of 0%, 50%, 75%, 100% and the second factor was with biofloc (BF) and without biofloc (NBF) application in culture medium. The combination of two factors resulted in 8 treatments as described in Table 1, there were three replicates for each treatment

Table 1: Describe of treatments in experiment

Substitution rates of SM by SSM	Biofloc application	
	None (NBF)	Bioflocs (BF)
0% (SM)	NBF_0	BF_0
50%	NBF_50	BF_50
75%	NBF_75	BF_75
100% (SSM)	NBF_100	BF_100

Preparation of culture medium: the seawater culture medium (SM) was prepared by diluting high saline water (80‰; was collected and transported from solar salt pans) in tap water to a salinity of 30‰; while the sea-salt water culture medium (SSM) was prepared by dissolving 3kg sea salt in 100L tap water. Both mediums were then treated with 30 mg/L chlorine and strong aeration for at least 48 hours before used. The mix culture medium (50% SM+50%SSW and 25% SM+75%SSW) was done with exact ration from both prepared medium.

Preparation of Artemia nauplii: One gram of *Artemia franciscana* Vinh Chau was added into the 1.5 L conical plastic bottle containing 1 L of seawater at 30‰, the optimal conditions for hatching were maintained at pH about 7.5-8.6, temperature at 28°C, light 1000 lux and continuously aeration (Sorgeloos *et al.*, 1986). After 24hours of incubation, newly hatched *Artemia* nauplii were harvested for stocking into the different culture medium (experimental treatments).

Artemia stock and culture maintaining: *Artemia* newly hatched were cultured according to treatments (Table 1) in 5L plastic bottles, contained 3L culture medium with the density was 500 nauplii/L, light aeration. All treatments were kept in room condition with air-conditioner during 28 days of culture period. *Artemia* were offered fresh microalgae *Chaetoceros* sp. (2 million cells/mL), during the first two days of culturing and from the 3rd onwards to the end of experiment, *Artemia* were fed with *Artemia* feed containing 30% of protein and 9% of lipid following feeding regime by Hoa (1993). *Artemia* feed was soaked in seawater (salinity 30‰) for 15 minutes, and then the solution was filtered through the 50 µm net before feeding. The total amount of daily feed was divided in four parts, and distributed to *Artemia* in four times per day (at 8:00, 11:00, 14:00 and 17:00). The amount of feed was adjusted by day based on the demand of *Artemia* through the observation of feed in water (water color) and in digestive track of *Artemia*.

Water exchange and C:N regulation: The NBF treatments was renewed 30% at day 7th and day 14th, after that water exchange was done depending on each culture bottle status, i.e. water quality and *Artemia* activities. The C:N=5 in biofloc treatments were regulated by adding molasses from day 5th of culture and every 3 days after that until end of experiment based on TAN concentration in culture medium.

Data collection and analysis

Water quality: pH and temperature were daily recorded at 8 AM and 2 PM by pH meter and thermometer, respectively. NH₄⁺/NH₃ (TAN) concentration was recorded every 3 days and nitrite (NO₂⁻) concentration was recorded weekly by Sera-test (Germany).

Survival at day after hatching 7th (DAH7) and at DAH14 was counted and calculated following the below formula:

Survival rate (%) = final number of Artemia at DAH7 or DAH14/Initial number of Artemia at stock x 100.

Individual length of *Artemia* at DAH7 and at DAH14 was recorded by randomly measuring the individual length of 30 *Artemia* in each treatment, *Artemia* was measured from the head to the telson of *Artemia* under specific binocular (Olympus SZ51, Japan)

Fecundity: Number of cysts or nauplii per female were recorded from 30 randomize females in each the brook sac of each *Artemia* female was opened and counted all the number of cysts (yellow-brown in color) or nauplii (orange in color) under microscope (10X).

Reproductive pattern: percentage of oviparity (cyst bearing) or ovoviviparity (naupliar bearing) females per total observed females; were recorded from 30 randomize females.

Reproduction rate: percentage of females that released the offsprings per total oserved females; were calculated from 30 randomize females

Final *Artemia* biomass production is a lump sum of biomass harvesting in wet weight at the end of experiment according to treatments.

III. RESULT AND DISCUSSION

Culture condition

The experiment was set up in laboratory, equipped air-conditioner, therefore the temperature was rather stable and did not differ between treatments, average temperature at 7 AM and 2 PM was $27.2 \pm 1.3^{\circ}\text{C}$ and $28.0 \pm 1.2^{\circ}\text{C}$, respectively. In the other hand, the pH of culture water in this study was in the range of 8.1 – 8.7. Previous studies has confirmed that this temperature and pH levels were in suitable range for the performance of *Artemia* (Dhont et al., 1996; Hoa et al., 2007).

The water quality parameters including $\text{NH}_3/\text{NH}_4^+$ (TAN) and NO_2^- during experimental period varied from 0.1 – 2.5 mg/L; 0.1 – 2.0 mg/L, respectively with similar trend; low at beginning, then gradually increase by cultured timing and rather high concentration at the end of experiment due to accumulation of organic matters i.e. feces, moulted shell, extra feed...; especially TAN at biofloc treatments was highest because of no water exchange. Another notification was at those treatments using seawater; TAN và NO_2^- was stable while at treatments with different substituton rates presented a larger fluctuation. However, the values of these factors always reached higher with biofloc treatments (BFT) than in none biofloc treatments (NBFT) except the NO_2^- content at NBF_0 treatment (Fig.1), that might be related to the growth of bacteria when molasses were daily added. Stability of TAN và NO_2^- at SM despite of adding molasses or not (BF vs. NBF) was a proof of bacteria activity in BF application and enhancing water quality due to there was none water exchange in BF but it was almost 30% for NBF. Hari et al. (2006) had confirmed that adding carbohydrate into shrimp culture system resulted in reducing TAN và NO_2^- accumulation in water column and this seems to fit with the results of SM treatments.

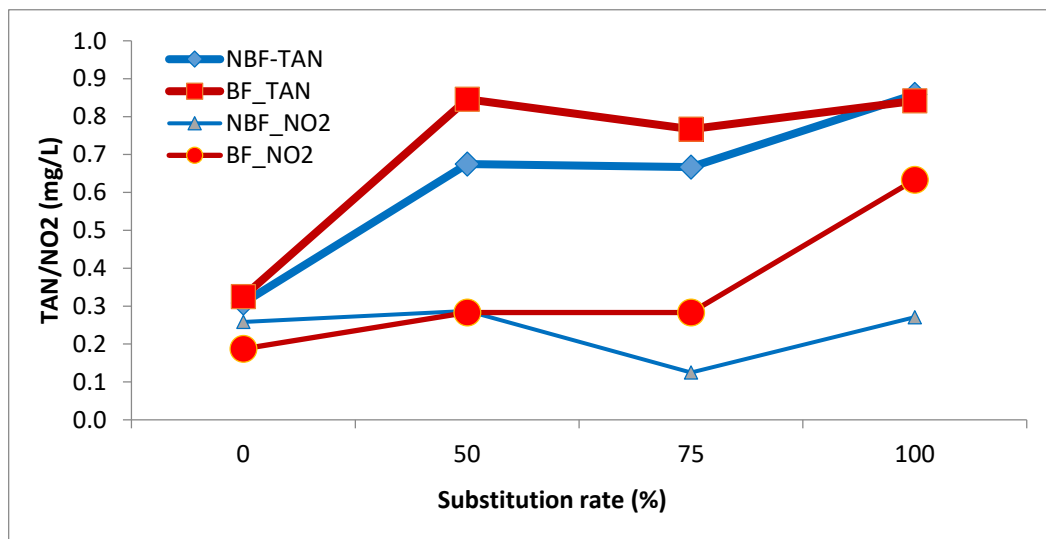


Fig.1. Average TAN and NO_2^- concentration in NBF and BF treatments

However, when replacing 50% SM by SSM upwards, TAN and NO_2^- concentration showed a larger variation than in SM, might concern to the growth and diversity of bacteria community because according to Shadia et al., (2016) there is a relationship between limiting nutrients and microbial growth, development and diversity in which varied with environmental factors. Regarding to this

study, the SSM eventhough have similar macronutrients with SM but lesser amounts of many trace elements found in natural seawater (Kolev et al., 2013) might cause lesser diverse of bacteria, especially chemochophic group who required chemical compoumd for they growth. However, Dhont *et al.* (1996) reported that *Artemia* have ability to resist high level of $\text{NH}_4^+/\text{NH}_3$ and NO_2^- content and LC50 (lethal concentration of 50%) of *Artemia* on $\text{NH}_4^+/\text{NH}_3$ and NO_2^- concentration 24 hours is 1000 mg/L and 320 mg/L, respectively. Therefore, $\text{NH}_4^+/\text{NH}_3$ and NO_2^- concentration in this study was in suitable range for the growth of *Artemia*.

Artemia performance

Survival rates

The survival rates of *Artemia* are showed in Table 2. A week from stock, the survival was recorded in range 85% - 97.7% and then slowed down around 7-10% more at day 14th. However, there was no statistical different between treatment on survival at both sampling time (DAH7 and DAH14; $p>0.05$)

Table 2: Survival and length of *Artemia* in different treatments at DAH7 and DAH14

Treatm ent	Survival rates (%)		Length (mm)	
	DAH7	DAH14	DAH7	DAH14
NBF_0	97, 7±1, 5 ^a	87, 3±8, 5 ^a	3, 87±0, 58 ^a	5, 53±1, 01 ^a
NBF_50	87, 3±3, 5 ^a	83, 3±4, 5 ^a	4, 40±0, 87 ^a	5, 27±1, 12 ^a
NBF_75	86, 0±8, 7 ^a	79, 3±9, 7 ^a	4, 23±0, 21 ^a	6, 70±0, 60 ^a
NBF_100	89, 7±6, 1 ^a	80, 0±5, 1 ^a	4, 37±0, 25 ^a	5, 80±0, 56 ^a
BF_0	97, 3±2, 9 ^a	89, 7±2, 1 ^a	3, 83±0, 21 ^a	6, 07±0, 25 ^a
BF_50	81, 3±15, 3 ^a	78, 3±15, 9 ^a	4, 63±0, 23 ^a	6, 97±0, 72 ^a
BF_75	86, 7±7, 0 ^a	79, 6±2, 8 ^a	4, 27±0, 25 ^a	6, 43±0, 38 ^a
BF_100	85, 0±12, 8 ^a	82, 7±13, 0 ^a	4, 50±0, 17 ^a	6, 00±0, 53 ^a

(Values shared the same character in the same column presented no statistic difference ($p>0.05$; Two-way ANOVA)

Results from Table 2 also revealed that the culture medium (with and without BF) had no effect on survival, the difference was very tiny (highest only 3% between BF and NBF). The explaining for this similarity could come the fact that heterobacteria was not developed yet in BF treatments since molasses just was added after day 5th of the culture. In another hand, *Artemia* just overcame two weeks; there was not much organic matter accumulation that migh influence to the water quality and in turn survival rates. This was demonstrated in water quality parameters of both type of culture medium; in which TAN and NO_2^- in BF and NBF was 0.28 Vs. 0.31; 0.3 Vs. 0.4mg/L, respectively.

According to the results in Table 2, survival was not enhanced when bioflocs was applied, while Toi *et al.* (2013; 2014) reported that *Artemia* culture in biofloc system with C/N=10 and using *Tetraselmis* sp as food resulted in better survival. In our study, C/N=5 was applied and this lower rate may be a reason for slow grow of heterobacteria and as a sequence giving a little amount of supplemental food by bacteria, moreover fresh *Tetraselmis* was always better food in term of biology and nutritional value compared to artificial food that was used in present study. Those reasons could be an explanation for none improved survival when BF application in our study compared to previous studies.

Regarding to the ability of using sea-salt instead of seawater through substitution rates, results from Table 2 illustrated that when replacing SM by SSM despite of BF and NBF; survival always was 8-16% lower than none replace (using seawater) but no statistic difference was found ($p>0.05$). The poorer survival in treatments that seasalt was used apart of full (100%) could be the result of lacking of micronutrients compared to seawater and this is not good for *Artemia* at young age but after that those can adapt with new environment, they became more flexible with it; i.e. at 7th survival between SM and SSM was 16% lower but it was only 7-10% at day 14th.

Length

Artemia performance in term of individual length was presented in Table 2. At DAH7, the length of *Artemia* was in range 3.8-4.4 mm and there was no difference found between treatments ($p>0.05$). The length of *Artemia* in BFT varied from 3.83 – 4.63mm, a bit longer than those in NBFT (3.87 – 4.40mm) and the same tendency was recorded at DAH14 with BFT was in range of 6-7mm, while NBFT reached around 5,2- 6,7mm. Although, the difference in length of *Artemia* from BFT and NBFT did not differ from each other ($p>0.05$; Table 2) but it was observed that in BFT, they grew better and this result suited with Toi *et al.* (2013) who confirmed in his

study that the higher growth performance in term of length was obtained in the carbon added treatment, which may result from bacteria grown in the culture medium when they used added carbon as nutrition source. As a consequence, *Artemia* benefitted from these bacteria because they can use as direct food source (Intriago et al., 1993; Hoa et al., 2007).

Table 3: p-value of experimental factors on *Artemia* performance

p-value	Source of variation		
	Biofloc application (BA)	Substitution rate (SR)	BA x SR
Survival at DAH7	0,4693	0,0703	0,8831
Survival at DAH14	0,9822	0,3442	0,8862
Length at DAH7	0,5962	0,0583	0,9480
Length at DAH14	0,0772	0,2762	0,1352

(***:p<0,001; **: p<0,01 và *: p<0,05)

In general, results from Table 2 and detail statistics (Table 3) demonstrated the substitution rates of SM by SSM as well as the biofloc application did not have neither independent effect nor combine effect on *Artemia* performance in term of survival and leng growth (p>0.05)

Reproduction characteristics

Browne et al. (1984) stated that *Artemia* displays two of their reproductive partterns including oviparity (release cysts) and ovoviviparity (release nauplii) and which of these happen depended on a lot of environmental factors such as food available, stress, salinity and temperature changes.... Some authors assumed that like animals who can born the dormant cysts when their living condition was out of their stand, *Artemia* also tends to release cysts to remain their population when they have to challenge with rigorous change in their living condition (Sorgeloos et al., 1980; Hoa et al., 2007). In this study, *Artemia* got mature at DAH12 and the fecundity in NBFT varied from 61-75 offspring/female, a bit higher than what recorded in BFT (51-72 offspring/female); however, these differences was not statistically significant (Table 4). This result was rather similarity as found in previous studies on (Anh, 2009; Van anh Toi, 2018) demonstrating *Artemia* population was in normal development despite of using apart of full SSM to culture them.

Table 4 : Reproduction characteristics in different treatments

Treatment	Fecundity (offspring/female)	Ovoviviparity (%)	Reproduction rate (%)
NBF_0	75 ± 14 ^a	58,3 ± 2,9 ^a	100,0 ± 0,0 ^b
NBF_50	61 ± 4 ^a	49,3 ± 12, ^a	80,0 ± 10,0 ^b
NBF_75	62 ± 8 ^a	46,1 ± 6,0 ^a	80,0 ± 8,7 ^b
NBF_100	61 ± 7 ^a	64,6 ± 25,3 ^a	65,0 ± 18,0 ^{ab}
BF_0	51 ± 3 ^a	63,3 ± 32,1 ^a	38,3 ± 17,6 ^a
BF_50	72 ± 12 ^a	49,3 ± 12,9 ^a	83,3 ± 10,4 ^b
BF_75	58 ± 13 ^a	35,8 ± 10,4 ^a	68,3 ± 24,6 ^{ab}
BF_100	58 ± 19 ^a	30,9 ± 4,3 ^a	65,0 ± 8,7 ^{ab}

(Values shared the same character in the same column presented no statistic difference (p>0.05; meanwhile had different character showed a statistic difference (p<0.05); Two-way ANOVA)

The ovoviviparity percentage in population of this experiment displayed a big amplitude resulted in no difference seen between treatments, as had been explained above on the reproductive parttern, beside environment effect, some unknown reasons had been noticed (Sorgeloos et al., 1980; 1996; Hoa et al., 2007). Nevertheless, except the NBF-100 treatment, there was a tendency for substitution rate, the higher substitution rate, the lower of ovoviviparity percentage was observed in both BF and NBF application (Table 4). This is fit with statement from previous studies that vigorous living condition led to *Artemia* tends to release cysts. The lack of some micronutrients may be produced some effected on *Artemia* reproduction as well as reproductive mode because data showed that in BF and NBF treatment that used 100% SSM, the reproduction rates were lower in both of them compared to other

substitution rate treatments such as 50 and 75% (65% Versus 68-80%). An interesting observation was lowest reproduction rate found in BF_0 treatment (only 38%) while other treatments reached 65-100% (Table 3) and this was significant difference with most of NBF treatments. The difference in may be the result of the massive growth of bacteria in BF_0 treatment due to the medium (seawater) not only with better micronutrients but also was added molasses that stimulate the huge development of bacteria in the culture. According to Toi et al. (2013), bacteria with adequate amount would be a good food for *Artemia* but they become harmful when presented with high density and that would be the the explanation in this case.

Table 5: p-value indicated the effect of experimental factors on reproduction however characteristics and biomass of *Artemia*.

p-value	Source of variation		
	Biofloc application (BA)	Substitution rate (SR)	BA x SR
Fecundity	0.2742	0.7304	0.0938
Ovoviviparity	0.1643	0.2480	0.2194
Reproduction rate	0.0079**	0.2423	0.0036**
Biomass	0.4253	0.5137	0.9689

(***: p<0.001; **: p<0.01 and *: p<0.05)

Results from statistic analysis in Table 5 also confirmed that fecundity and reproduction pattern both were not effected by experimental factors (biofloc application and substitution rate) in term of either independent or combined effect (p>0.05); meanwhile there was an effected on reproduction rates by biofloc application and as a sequence the interation between the two factors was found (Table 4; p<0.05).

Biomass at harvest

After 28 days of culture period, the highest biomass weight was collected at BF_50 treatment (12,2g), lowest was at NBF_0 treatment (9.1g). Despite of culture medium in which bioflocs was applied or not; the substitution rates of SM by SSM from 50-75% always gave better biomass yield compared to other substitution rates, eventhough the difference was not statistically significant. Results from Fig 2 and Table 4 indicated that the average biomass in BFT was higher than in NBFT (11,4 ± 2.4g versus 10.5 ± 2.0g but this was not proved by statistic signification (p>0.05). However, the biomass weight at harvest of all treatments in present study was better than those was recorded in previous trial of Thong and Hoa (2018) when the culture was done at the same stocking density and salinity (average 3.3g/L versus 1.08-1.48g/L); or similar with those reported by Toi and Van (2017) but shorter in culture time (42 vs. 28 days). Base on the biomass at harvest and the amount of feed was used during experimental period, result from calculation showed that the feed for producing 1g *Artemia* biomass was 1.47g in BFT; while it was 1.7g for NBFT, saving about 13,3%. Moreover, those treatments with BF application was zero water exchange during the culture but for NBFT (normal culture) water exchange was started at day 7th and upwards by every 5-7 days depending on culture status; resulted in total water exchange was about 20%-30% for whole culture period plus the labour cost for doing this job.

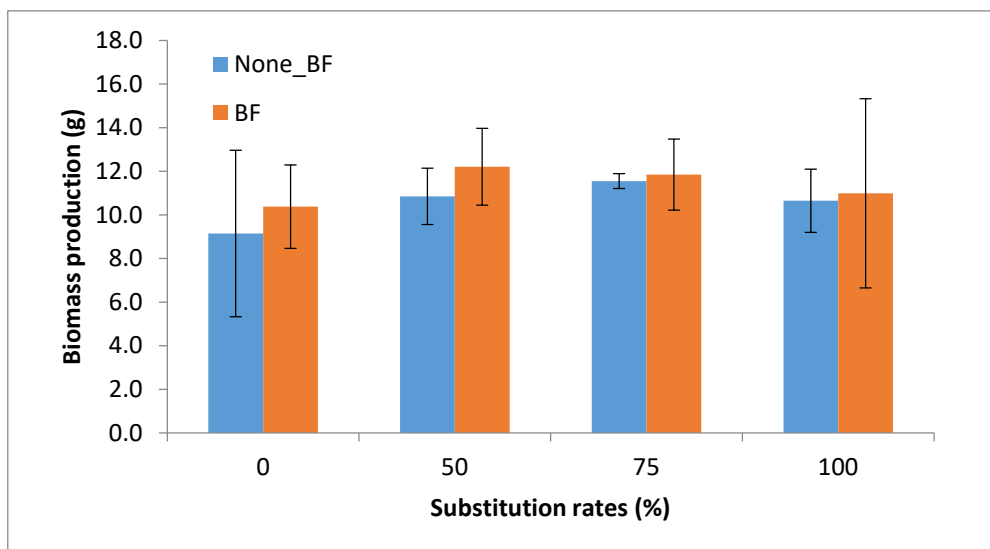


Fig 2: Biomass at harvest from experimental treatments

IV. CONCLUSION AND RECOMMENDATION

Conclusion

Survival and length of *Artemia* were not affected by neither biofloc application nor substitution rates of seawater by seasalt water and also no interaction between them.

There was difference in reproduction characteristics of *Artemia* between treatments but no statistical signification was found, except the reproduction rate. However, the higher percentage of salt water was used in culture medium, the more females had oviparity mode (cyst bearing) and the lesser reproduction rate in female.

Biofloc application in *Artemia* culture was effected on reproduction rate of female, especially at seawater medium (0% substitutions) showed the lowest reproduction rate (2-2.5 folds lesser than others)

The biomass weight in biofloc treatments was higher than those was collected in normal culture about 9%, and saving 13,3% feed use as well as 20-30% water exchange.

Recommendation

The sea-salt can be used for *Artemia* biomass culture incorporation with biofloc application in order to break down the seasonal depending of *Artemia* biomass and high saline water transportation for inland shrimp/fish hatcheries or nurseries. However, the trial in larger scale should be tested before mass production for evaluation the real cost-effect. Besides that, microbial flora during the culture, especially with biofloc system should take into account for further research for best microbial management to avoid the crash of culture batch due to bacteria growth in excess.

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Reference

1. Anh, N. T. N., 2009. "Optimisation of *Artemia* biomass production in salt ponds in Vietnam and use as feed ingredient in local aquaculture." PhD thesis, Ghent University, Belgium.
2. Bengtson DA, Leger P, Sorgeloos P., 1991. Use of *Artemia* as a food source for Aquaculture. In: *Artemia Biology* (ed. by Browne et al.), CRC Press. Boca Raton. FL. USA: 255–285.
3. Browne, RA., Sallee, SE., Grosch, D. S, Segerti, W. O. and Purser, S. M., 1984. Partitioning genetic and environmental components of reproduction and lifespan in *Artemia*. *Ecology*, 65(3):949-960.
4. Dhont, J. and Lavens, P., 1996. Tank production and use of on grown *Artemia*. In: Sorgeloos, P., Lavens, P. (Eds.), *Manual on the Production and Use of Live Food for Aquaculture*. Fisheries Technical Paper No. 361. Food and Agriculture Organization of the United Nations, Rome, pp.164–194.
5. Hari, B., Madhusoodana Kurup, B., Varghese, J. T., Schrama, J. W. and Verdegem, M. C. J., 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. *Aquaculture* 252(2-4): 248-263.
6. Hoa, N. V., 2002. Seasonal Farming of brine shrimp *Artemia franciscana* in artisanal salt-ponds in Vietnam: Effect of temperature and Salinity. Ph.D thesis, Academic year 2002-2003. Ghent University. 184pp.
7. Hoa, N. V., Van, N.T.H, Anh, N.T.N, Ngan, P.T.T, Toi, H.T and Le, T.H., 2007. Study on *Artemia* culture and its application in aquaculture. Can Tho University, Agriculture publishing house: 639.7-A100.
8. Intriago, P. and Jones, D. A., 1993. Bacteria as food for *Artemia*. *Aquaculture*. 113(1-2): 115-127.
9. Kolev H, G. Tyuliev, C. Christov, K. L. Kostov. 2013. Experimental study of the surface chemical composition of sea salt crystallized during evaporation of seawater under natural conditions. *Bulgarian Chemical Communications*, 45 (4): 584–591
10. Léger, Ph., Bengtson, D.A., Simpson, S.L and Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. *Oceanography Marine Boil. Ann. Rev.* 24: 521-623.
11. Lim, L.C., Dhert P., Sorgeloos P., 2003. Recent developments in the application of live feeds in the freshwater ornamental fish culture. *Aquaculture*, 227, 319–331
12. Nguyễn Thị Ngọc Anh, 2011. Sử dụng sinh khối *Artemia* làm thức ăn trong ương nuôi các loài thủy sản nước lợ. *Tạp chí Khoa học Đại học Cần Thơ* 19b, 168-178.
13. Nguyen Van Hoa, 1993. Effect of Environment Conditions on the Quantitative Feed Requirements of the Brine Shrimp *A. franciscana* (Kellogg). University of Ghent. Thesis Submitted in Partial Fulfilment of the Requirements for the Academic Degree of Master of Science in Aquaculture.
14. Olsen, Y., Evjemo, J.O., Olsen, A., 1999. Status of the cultivation technology for production of Atlantic halibut (*Hippoglossus hippoglossus*) juveniles in Norway, Europe. Flatfish culture. Flatfish Symposium, January 1998, Las Vegas, Nevada, USA. *Aquaculture* 176, 1–13.

15. Ronald, L., G. Van Stappen, N. Van Hoa and P. Sorgeloos, 2013. Effect of carbon/nitrogen ratio manipulation in feed supplements on *Artemia* production and water quality in solar salt ponds in the Mekong delta, Vietnam. *Aquaculture Research*: 1-4.
16. Shadia M. Abdel-Aziz, Vijai K. Gupta, D Sukmawati, and M. Fadel. 2016. Role of nutrient in microbial developments and microbial metabolic diversity: Recent Advancements and Future Developments. In book: *Microbial Applications*.
17. Sorgeloos, P., 1980. Life history of the brine shrimp *Artemia*. In Persoone, G., Sorgeloos, P., Roels, O. and Jaspers, E., (Eds), *The brine shrimp Artemia. Morphology, genetics, Radiobiology, toxicology*. Universa Press, Wetteren, Belgium 1: 19-22.
18. Sorgeloos, P., 1986. Life history of the brine shrimp *Artemia*. In: *the brine shrimp Artemia*, Proceeding of the International Symposium on the brine shrimp *Artemia salina*. Corpus Chritis, Texa, USA, August 20-23, 1979. Volume 1: Morphology, Genetics, Radiobiology, Toxicology, G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers, (Eds.), Universa Press, Wetteren, Belgium: 19-22.
19. Sorgeloos, P., Bossuyt, E., Lavina, E., Baeza Mesa, M., Persoone, G., 1977. Decapsulation of *Artemia* cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture* 12, 311–315.
20. Sui, L. Y., J. Wang, V. H. Nguyen, P. Sorgeloos, P. Bossier and G. Stappen, 2013. Increased carbon and nitrogen supplementation in *Artemia* culture ponds results in higher cyst yields. *Aquaculture International* 21(6): 1343-1354.
21. Thong, L. V. and Hoa, N. V., 2018. Influence of salinity, stocking density and partial harvesting to biomass production of *Artemia franciscana* (Vinh Chau strain) in hatchery. *Journal of Science, Can Tho University, special issue on Aquaculture*, 54: 129-141
22. Toi, H. T. and Van, N. T. H., 2017. Contribution of bacteria to *Artemia* nutrition in conditions of degressive algal feeding. *International Journal of Scientific and Research Publications*. 7 (12): 34-45
23. Toi, H. T., Boeckx, P., Sorgeloos, P., Bossier P. and Van Stappen, G., 2014. Co-feeding of microalgae and bacteria may result in increased N assimilation in *Artemia* as compared to monodiets as demonstrated by a ¹⁵N isotope uptake. *Aquaculture*. 422-423: 109-114
24. Toi, H. T., P. Boeckx, P. Sorgeloos, P. Bossier and G. Van Stappen, 2013. Bacteria contribute to *Artemia* nutrition in algae-limited conditions: A laboratory study. *Aquaculture* 388-391: 1-7.
25. Van Stappen, G., 2002. "Zoogeography," in *Artemia: Basic and Applied Biology*, eds Abatzopoulos Th. J., Beardmore J. A., Clegg J. S., Sorgeloos P., editors. (Dordrecht: Kluwer Academic Publishers) 171–224.
26. Van, N. T. H. and Toi, H. T., 2018. Effect of different C/N ratios on *Artemia* biomass culture. *International Journal of Scientific and Research Publications* 8(2): 17-21