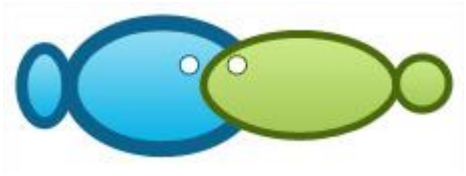

Study on *Artemia* culture in low salinity water in earthen ponds: how to avoid poor cysts production due to erratic rains and prolonged high temperatures resulting from the climate change

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Abstract. *Artemia* were reared in earthen ponds at a salinity lower than the recommended (80‰), which aimed to shorten the highly saline water preparation (scheduled yearly) and to avoid unseasonal rainfall, due to impacts of climate change, that affect *Artemia*. The experiment was carried out with three different salinities, by comparing 40‰ and 60‰ with the control treatment (80‰), at the inoculation day. Then, the salinity of the culture water was naturally increased by evaporation during the culture period, which aimed to assess the effect of an initially low salinity, at the inoculation day, on the survival and reproduction of *Artemia*. Results showed that the cyst production in the control treatment was the highest (108.8 kg ha^{-1}), which was not significantly higher than at a salinity of 60‰, but significantly higher than for a salinity of 40‰. In general, *Artemia* cultured at 60‰ salinity did not differ in survival, growth and yield, compared to 80‰, but shortened the time of the salt water preparation. Therefore, a 60‰ salinity can be applied to the *Artemia* culture, in order to reduce the costs by cutting the time of highly saline water preparation.
Key Words: *Artemia*, salinity, survival, growth, cysts production.

Introduction. The aquaculture industry is growing and becoming one of the key economic sectors of many countries, including Viet Nam. The aquaculture area is increasingly expanding in terms of farming area, resulting in an increasing demand for seeds (shrimp and fish larvae from hatcheries or from the wild for farming). In order to match this demand, the development of raising organisms to feed fish larvae in the production of seafood seeds also plays a very important role. Among the live foods (including phytoplankton and zooplankton), *Artemia* is an aquatic crustacean that can live in hypersaline lakes throughout the world and grow from nauplius to the adult stage in only 8 days. They are small, the nauplius has a body length of 400-450 µm and an adult has a body length of 80-100 mm. *Artemia* can reproduce in two ways. They can produce free-swimming *Artemia* nauplii if the living conditions are favorable. However, they have ability to produce dormant embryos of about 200-300 µm, known as cysts, when the living conditions deteriorate. Free swimming *Artemia* nauplii can be easily obtained when dry *Artemia* cysts are incubated in 30 g L^{-1} seawater for 24 h (Van Stappen 1996). *Artemia* can be found in low-medium salinity environments.

Artemia (nauplii and adults) have an important role in the rearing of aquatic larvae, being a highly nutritious species, and it is also used as an intermediary of essential nutrients through the path of enrichment (Lavens & Sorgeloos 1996). The demand for *Artemia* cysts for aquatic hatcheries is increasing year by year and sometimes the demand is higher than the available supply, therefore *Artemia* rearing has been developed in earthen ponds (constructed with soil materials used to keep a big volume of water for farming of aquatic animals) in many countries around the world (Toi 2014). In *Artemia* earthen pond culture, water salinity is one of the essential factors. *Artemia* is often raised in high salinity water bodies where salt production is practiced,

especially in South-East Asia, during the monsoon season. The expansion of the farming areas and the seasonality are among the constraints of the aquaculture development. The salinity of the culture water for *Artemia* cysts production is recommended to be kept at 80‰ and upwards to avoid some predators for *Artemia* nauplii in the first day of inoculation (Anh 2009). At a high salinity concentration, as recommended, some predators on the newly inoculated *Artemia* nauplii, such as copepods (zooplankton or small planktonic crustacean) and tilapia, cannot survive (Van Stappen 1996; Beart 1997). In practice, these predators living in lower salinity ponds are easily eliminated by concentrated derris roots extracts or rotenone (Hamilton 1941; Anderson 1970; Anh 2009).

In recent years, the weather of South-East Asia has been greatly affected by the climate change, causing erratic rains and prolonged high temperatures weather, deviating from the natural pattern, thereby affecting the *Artemia* farming. Although *Artemia* is a fairly widespread saltwater species, prolonged heavy rains have directly affected the saltwater preparation process for the crop and caused difficulties in the management of the pond. The early onset of heat has also caused a great impact on the crop, because the normal peak of *Artemia* cyst collection is from early January to the end of March. With the early onset of heat (above 35°C) from the end of March to the beginning of the rainy season, few cysts are collected during this time (Anh 2009). Therefore, in order to cope with the climate changes, such as the off-season rains during the preparation of saline waters, and to avoid production of *Artemia* cysts falling into the hot and dry months, in the middle of the drought season, it is suggested to rear *Artemia* earlier than the traditional *Artemia* farming season, in waters with a salinity lower than the recommended level for *Artemia* cyst production (80‰).

Material and Method

Pond and highly saline water preparation. Nine earthen ponds, of 1,000 m^2 each and 1 m in depth, from the artisanal salt work system, were used for the culture of *Artemia*. Before inoculating, the sludge was removed from the ponds, the dikes were compacted to prevent the water leakage and the ponds were left for drying during two weeks. Afterwards, highly saline water was poured into the pond system in a thin layer (seawater covering the pond bottom had a depth of around 5 cm). Before inoculating, predators of *Artemia*, such as copepods and tilapia, were eliminated by applying derris root extracts at 2 kg for 1,000 m^2 for 2 days.

Experimental design. Newly hatched *Artemia franciscana* Vinh Chau were inoculated at 150 nauplii L^{-1} in nine earthen ponds (1,000 m^2 $pond^{-1}$), with three salinities at the inoculating point, including 80‰ (acting as control; the treatment code was T3), 60‰ (the treatment code was T2) and 40‰ (the treatment code was T1). Each treatment was conducted by three replicates. The salinity of each treatment was naturally increased during the culture period by evaporation. The study was conducted in 6 weeks.

Artemia nauplii preparation. Dry *Artemia franciscana* cysts were incubated at standard conditions (30‰ seawater at 28℃, in 24 h).

Management. The fertilization pond occupied 30% of the *Artemia* culture area, being separated from the *Artemia* culture ponds. In the fertilization pond (green water), with a salinity managed around 30‰, phytoplankton was used as main food for *Artemia*. Then the green water from the fertilization pond was provided to the *Artemia* ponds. Phytoplankton were produced by adding between 0.2 and 0.5 ton WW (wet weight) ha^{-1} $week^{-1}$ of chicken of manure, urea and di-ammonium phosphate (DAP), at an amount of 3-7 g m^{-3} $week^{-1}$, with a ratio of N:P=5 (Anh 2009). The green water was provided to all *Artemia* culture ponds at the same volume (2 cm depth or column height), every two days, through a pumping system. Chicken manure was added to the *Artemia* pond at 45 kg WW 1,000 m^{-2}, to promote the microalgae growth.

Sample collection and analysis

Physical parameters. Water temperature (°C) and pH in the nine *Artemia* culture ponds was measured two times per day at 7:00 am and 14:00 pm. Salinity was measured by refractometer once per day at 7:00 am.

Biological parameters. The microalgae composition in the fertilization pond was determined every week during the experimental period. Samples for determination of microalgal concentration and microalgal composition in the fertilization pond were collected in a 50 mL Falcon tube, then the samples were preserved with formaline 5%. The concentration of microalgae in each sample was determined under an Olympus microscope at 40X magnification, by a Bürker chamber. Each microalgal species was identified based on their morphology.

The total length (TL) of *Artemia* was determined from 30 *Artemia* specimens, from the head to the telson, under a microscope specially equipped with a calibrated eyepiece, at days 7 and 14 of culture. *Artemia* were preserved in Lugol's solution before measuring. The actual total length of *Artemia* was then calculated using the formula below (Hoa et al 2021):

$$TL\ (mm) = A/10 \times 1/\gamma$$

Where:
A - the length of the sample was measured by the calibrated eyepiece;
γ - the magnification.

The survival of *Artemia* at the 1st, 7th and 14th day was determined by sampling the *Artemia* population at five points, in the ponds. The samples at each point were collected by a zooplankton net (50 x 50 cm) through the vertical water column from the bottom to the water surface. Afterwards, the *Artemia* population was fixed in 4% formaline, the number of *Artemia* samples was counted, and the average density of *Artemia* was calculated from the five samples. Finally, the survival rate of each pond was estimated by the formula (Van & Toi 2017):

$$S\ (\%) = T/No \times 100$$

Where:
T- the number of *Artemia* at sampling;
No i - the initial number of *Artemia*.

The fecundity in term of embryos of *Artemia* per week was examined in 30 female *Artemia* specimens, from the 3rd week to the end of the culture period. First, the brood sac of each *Artemia* female was opened and then the number of embryos in the brood sac was counted by microscope (10X).

Cyst production: total cyst production was calculated as the sum of the daily cyst production (from the first day of cysts releasing to the last day of the study; cysts harvesting took around 22 days).

Statistical analysis. The dataset of each treatment was processed to get the mean and standard deviation, by the Microsoft Excel software, and the statistics performed were the one-way ANOVA and the Tukey-HSD tests, by Statistica 7.0.

Results. The temperature was around 25.0ºC in the morning and 31.8-33.2ºC in the afternoon. The pH ranged from 7.6 to 7.8 in the morning and from 7.6 to 8.3 in the afternoon (Table 1). The results of the tests showed that the salinity of the water culture tended to gradually increase during the culture period, due to the evaporation (Table 1). In the first week, the salinity was significantly different between the treatments ($p<0.05$). In the second and third week, the salinity of T1 reached 72.4 and 79.6‰, respectively, which is significantly lower than the salinity in the treatments T2 and T3, the control ($p<0.05$). However, the salinity of the culture water of the nine ponds continuously increased to finally reach very close values. There was no significant

difference between the treatments in salinity ($p>0.05$) in the end weeks of the culture period.

Table 1

Physical parameters of *Artemia* culture ponds

Week	*Treatment*	*Temperature (ºC)*		*pH*		*Salinity*
		7:00	*14:00*	*7:00*	*14:00*	
1	T1 (40‰)	25.0±0.5	32.6±1.9	7.8±0.1	8.3±0.1	50.4±7.1[a]
	T2 (60‰)	25.0±0.4	33.0±2.2	7.7±0.2	8.3±0.1	70.8±7.7[b]
	T3 (80‰)	24.9±0.4	33.1±2.1	7.6±0.1	8.2±0.1	84.5±2.5[c]
2	T1 (40‰)	24.5±0.9	32.7±2.0	7.7±0.2	8.3±0.1	72.4±5.9[a]
	T2 (60‰)	24.5±0.9	33.1±2.2	7.7±0.1	8.2±0.1	87.2±2.3[b]
	T3 (80‰)	24.4±0.9	33.2±2.0	7.6±0.1	8.2±0.1	86.9±2.1[b]
3	T1 (40‰)	24.8±0.5	31.8±1.0	7.6±0.2	7.7±2.3	79.6±2.1[a]
	T2 (60‰)	24.9±0.5	32.3±1.3	7.7±0.2	7.7±2.3	88.0±2.6[b]
	T3 (80‰)	24.8±0.5	32.2±1.1	7.6±0.2	7.6±2.3	86.5±1.9[b]
4	T1 (40‰)	25.5±0.9	31.8±1.7	7.5±0.1	8.2±0.1	79.9±2.1
	T2 (60‰)	25.5±0.8	32.1±1.8	7.4±0.1	8.2±0.1	85.0±2.8
	T3 (80‰)	25.7±0.9	31.6±2.8	7.5±0.1	8.2±0.1	86.0±2.6
5	T1 (40‰)	25.1±0.8	31.3±0.7	7.5±0.1	8.2±0.2	75.1±4.1
	T2 (60‰)	25.2±0.7	31.4±1.1	7.6±0.2	8.3±0.2	85.7±4.1
	T3 (80‰)	25.1±0.6	31.3±1.0	7.7±0.1	8.2±0.2	85.7±2.8
6	T1 (40‰)	25.1±0.3	31.5±1.2	7.5±0.1	8.1±0.1	80.1±3.1
	T2 (60‰)	25.3±0.6	31.6±1.1	7.5±0.1	8.1±0.1	89.3±3.7
	T3 (80‰)	25.2±0.5	31.2±1.1	7.7±0.1	8.2±0.1	91.8±3.2

The results of survival and growth in length of *Artemia* was presented in Table 2. The total length of *Artemia* varied from 6.30 to 6.49 mm at day 7 and there was no significant difference ($p>0.05$) between treatments. The length of *Artemia* at 60‰ was bigger (8.66 mm) than in the control treatment at day 14, but there was no significant difference ($p>0.05$).

Survival of *Artemia* in the first two weeks of rearing is shown in Table 2. *Artemia* was affected by the salinity of the water culture at the inoculation day. The mortality of *Artemia* increased when the salinity of the culture pond was at a low concentration. In day 7, the highest survival reached 96% at 80‰ salinity, statistically significant ($p<0.05$) compared to the survival reached at 40‰whereas the lowest survival, of 72.4%, was obtained at 40‰. In day 14, the highest survival was also obtained at 80‰ and it was significantly higher ($p<0.05$) than the survival reached at 40‰, but there was no significant difference ($p>0.05$) when compared to the survival reached at 60‰.

Table 2

Individual length and survival of *Artemia*

Treatment	*Length (mm)*		*Survival (%)*	
	Week 1	*Week 2*	*Week 1*	*Week 2*
T1 (40‰)	6.41±0.52[a]	8.01±0.91[a]	72.4±10.8[a]	48.7±8.5[a]
T2 (60‰)	6.49±0.61[a]	8.66±0.39[a]	91.1±6.1[ab]	61.2±6.1[ab]
T3 (80‰)	6.30±0.52[a]	8.62±0.66[a]	96.0±2.9[b]	72.0±3.8[b]

The fecundity of *Artemia* (Table 3) tended to increase from the week 3 to the week 4 and the difference was not statistically significant ($p>0.05$) among the treatments. In the week 6, the fecundity of *Artemia* in the treatment at 80‰ was significantly lower ($p<0.05$) than in the other two treatments. The highest fecundity was obtained in the treatment with 40‰.

Table 3

Fecundity (embryos female^{-1}) of *Artemia*

Treatment	*Weekly observation*			
	Week 3	*Week 4*	*Week 5*	*Week 6*
T1 (40‰)	178±31^{a}	251±58^{a}	280±47^{b}	263±41^{b}
T2 (60‰)	177±35^{a}	252±51^{a}	221±44^{a}	275±50^{b}
T3 (80‰)	196±46^{a}	233±33^{a}	222±42^{a}	238±46^{a}

Cyst production. The highest cyst production reached 108.8 kg ha^{-1} for the 80‰ salinity, significantly higher than for 40‰, but there was no significantly higher figure than for 60‰ (Figure 1).

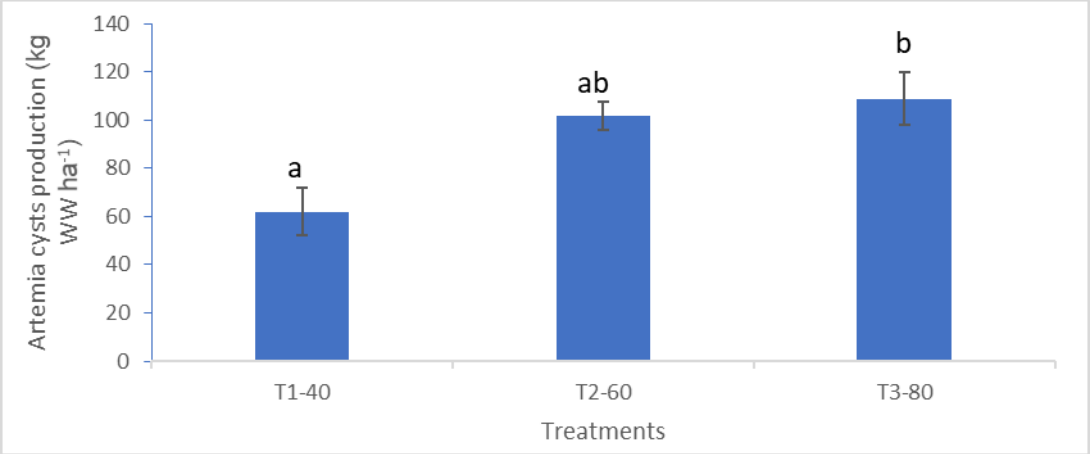

Figure 1. *Artemia* cyst production (kg WW ha^{-1}).

Microalgae composition in fertilization pond. A total of 16 microalgal genus in five classes were observed. Chlorophyta (*Dunaliella*, *Tetraselmis*) and Bacillariophyta (*Chaetoceros*) were found in abundance in the fertilization water, during the experimental period (Table 4).

Table 4

Common microalgae weekly identified in the fertilization pond sampling

Division	*Genus*	*Weekly microalgae community identification*					
		W1	*W2*	*W3*	*W4*	*W5*	*W6*
Chlorophyta	*Dunaliella*	++	+	++	+	++	+
	Tetraselmis	++	++	++	+	++	+
	Chlamydomonas	+	+	+	+	+	+
	Chlorococcum	-	-	++	-	-	-
Bacillariophyta	*Pleurosigma*	-	+	-	-	-	-
	Surirella	+	-	-	+	+	-
	Nitzschia	-	+	-	-	-	+
	Grammatophora	-	-	-	-	-	-
	Cymbella	-	-	-	+	+	-
	Navicula	+	-	-	-	-	+
	Coscinodiscus	-	-	-	-	-	-
	Chaetoceros	++	+	++	+	+	+
Ochrophyta	*Nannochloropsis*	++	+	+	-	+	+
	Amphora	-	-	+	-	-	-
Euglenophyta	*Euglena*	-	+	-	+	-	-
Dinophyta	*Gymnodium*	+	-	+	-	-	-

++: high abundance; +: less abundance; -: non-detected; W: week.

The density of phytoplankton in the fertilization pond was nearly 1.3 million cells mL^{-1} at the first week, increasing to 2.4 and 2.7 million cells mL^{-1} for the second and third weeks, respectively. Then, the density of phytoplankton slightly decreased in the fourth week but increased to 2.4 million cells mL^{-1} in the last two weeks of the experimental period (Figure 2).

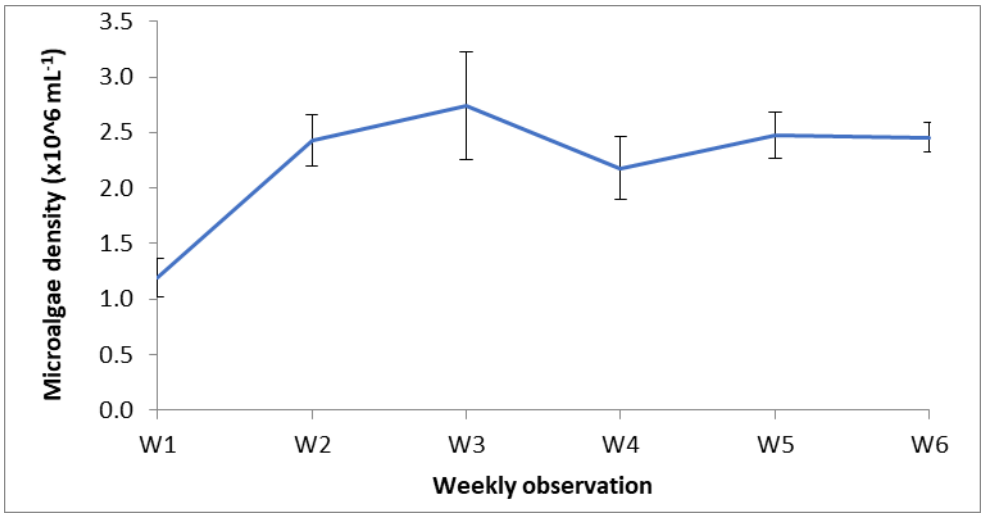

Figure 2. Phytoplankton density during the experimental period.

Discussion. *Artemia* is known as a non-selective filter feeder, which can uptake all the particles including phytoplankton in a water column with a size of less than 50 µm. Phytoplankton was grown in the fertilization pond and provided to the *Artemia* ponds. The density of phytoplankton in the fertilization pond in the present study was always maintained at high levels (more than two million cells mL^{-1} from the second week) and it was the main food source for *Artemia*. Its composition was mainly *Dunaliella*, *Tetraselmmis* and *Chaetoceros* during the experimental period. These microalgae were repeatedly shown to be good food for *Artemia* (Fábregas et al 1996; Naegel 1999; Thinh et al 1999; Marques et al 2005). Therefore, the phytoplankton composition in the present study was suitable for feeding *Artemia*.

Besides the food, environmental conditions are really important for *Artemia*. The temperature and salinity of the culture water has been analyzed in previous studies. Temperature is one of the physical factors affecting the growth and survival of *Artemia franciscana*. The optimum temperature for *A. franciscana* ranges from 20 to 35ºC (Thoeye et al 1987). Under earthen pond conditions, the optimum temperature has been reported to range between 28 and 30ºC for *Artemia* and a high mortality occurred when the temperature reached 36-37ºC (Hoa 2002). The pH of the water affects the survival of *Artemia*, and the mortality is high in the nauplius stage when pH drops less than 6.5 (Doyle & McMahon 1995).

A. franciscana are an aquatic species that can live in a wide range of salinities; they can survive in salt concentrations of up to 250‰ (Van Stappen 1996). The tolerance of salt concentrations by *Artemia* depends on the temperature and a better survival has been recorded in a low salinity when the temperature of the culture water is maintained at 30ºC, whereas a low survival in high salinity is obtained when temperature is at 15ºC (Browne & Wanigasekera 2000). In earthen pond conditions, the salinity for *A. franciscana* was recommended to be maintained from 80 to 140‰ (Hoa 2002); in this range copepods spend more energy for osmoregulation, so it is not a suitable salinity for copepods (Chen et al 2006). However, the predators (copepods, tilapia) were eliminated in this present study, so the inoculated *Artemia* were not affected by this factor. The low salinity affects *Artemia*, so the survival was reduced by nearly 1.3 times when the culture was at 40‰ as compared to 80‰, but the survival of *Artemia* at 60‰ was not significantly lower than at 80‰. According to Van & Toi (2017), the reproductive capacity of *Artemia* is low when raised at a salinity of 10-50‰ compared to a salinity of 80‰, but in the current experiment the reproductive capacity of *Artemia* cultured at 40

and 60‰ was higher than at 80‰, and this may be related to the density of *Artemia* in the ponds. However, low salinity only affected the survival of *Artemia*; resulting in a low cyst production, but the fecundity and growth of *Artemia* were not affected by low salinity.

In addition, using brine water at a salinity lower than the recommended salinity for *Artemia* culture in earthen ponds can shorten the time to prepare and accumulate the saline water. The highly saline water in South-East Asia countries is obtained by evaporated seawater process over several days. It only took 12 days to prepare water at 40‰ and 16 days for 60‰, but it took 23 days to reach a salinity of 80‰. The shortened time to prepare a highly saline water is one of the positive factors which make the *Artemia* farming season occurring earlier in the year, due to the suitable temperatures for the *Artemia* cysts production in the early season and to the lower operation costs when time is saved during the highly saline water accumulation process.

Conclusions. The results of the present study indicated that *Artemia* cysts production can be performed at 60‰ without significant alterations, as compared to 80‰. This result shows that a 60‰ salinity of the culture water at can be used to replace the 80‰ salinity for *Artemia* cyst production. In addition, the use of a low salinity to culture *Artemia* reduced the time taken to prepare the culture water in the South East Asia region.

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Conflict of interest. The authors declare no conflict of interest.

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