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SHORT PAPER

Combined Effect of Salinity and Ferric Citrate on the Biometric Characterization of *Artemia* Cysts under Laboratory Condition

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Abstract

The quality of *Artemia* cysts size can be determined by biometrical characterization. In this study, the combined effects of salinity and ferric citrate were studied on the biometric characterization of *Artemia* by single clonal reproduction under the laboratory condition. Results of control treatment showed increasing of the salinity correlates non-significant with diameter of decapsulated cyst and negatively correlates with the size of untreated cyst and chorion thickness. The effect of low concentrations of ferric citrate (5 and 10 mg/L) led to release the largest cysts in low salinity (35 ppt), while in high concentrations of ferric (20 and 40 mg/L) the largest cyst was released in medium salinity (70 ppt).

Introduction

The brine shrimp *Artemia* is a commercial aquatic organism which has wider applications in aquaculture (Bengtson, Léger, & Sorgeloos, 1991). The annual demands for *Artemia* cysts has already increased on the world from a few tons in the mid 1970s to over 2,000 tons in the early 2000s (Sorgeloos, Dhert, & Candreva, 2001). Although the population consumption has been increasing rapidly, but the supply of *Artemia* cysts are not sufficient because the harvesting procedures are mainly performed gotten from natural salt lakes and solar salt-ponds (Sui, Wang, Nguyen, Sorgeloos, Bossier, & Stappen, 2013). Nevertheless, pond culture of *Artemia* has been developed and adopted by artisanal salt farmers over the past decades to produce the high quality cysts (Sui *et al.*, 2013). In *Artemia* production manuals, many efforts have made to help to choose suitable species/populations to be applied in aquaculture industry (Tackaert & Sorgeloos, 1991 ; Lavens & Sorgeloos, 1991; Barata, Hontoria, & Amat, 1996; Thi, Van, & Sorgeloos, 2013). Additionally, the effect of environmental parameters was studied on potential of cyst production (Barata *et al.*, 1996; Thi *et al.*, 2013 ; Gao, Wang, Ma, Stappen, & Sui, 2017; Nguyen, 2015; Thi, Pachecovega, Cadenaroa, Ascencio, Rangeldávalos, & Rojascontreras, 2015).

Biometrical characterizations of *Artemia* cysts can reflect the quality of production (Asem, Rastegar-Pouyani, & Agh, 2007). For example, high hatching efficiency is observed in the batches with small diameter of cysts (Sorgeloos, Persoone, Baeza-Mesa, Bossuyt, & Bruggeman, 1978, Asem *et al.*, 2007). Early research had indicated the existence of iron in the culture medium can cause an increased hemoglobin synthesis and cyst production (Baker, 1966). Female *Artemia* uses hemoglobin as a basic element in cyst shell formation (Versichele & Sorgeloos, 1980).

It has been indicated that the size of *Artemia urmiana* cyst significantly decreased with increasing the salinity in Urmia Lake (Asem, Rastegar-Pouyani, De Los Rios, Manaffar, & Mohebbi, 2010); but there is lack of information to evidence the relationships between biometrical changes and effective factors under experimental conditions. The aim of this study was to examine the correlation effects between biometric parameters and combined effect of salinity and ferric in the laboratory condition. We used single clonal reproduction mode of diploid parthenogenetic population.

Materials and Methods

A diploid clone (BRK-C53) was established from the parthenogenetic *Artemia* population of Barkol Lake, Xinjiang, China. Stock cultures of BRK-C53 were maintained at temperature of 25˚C, photoperiod of L(light): D(dark) = 18:6, and salinity of 70 ppt (for more information see Wang, Asem, & Sun, 2017).

For all stock and experimental cultures, different salinity levels (35, 70, 140 g/L) and iron (Fe) media (0, 5, 10, 20, 40 mg/L) were prepared by adding artificial sea salt and ferric citrate ($C_6H_5FeO_7 \cdot 5H_2O$) to sea water. Temperatures (25˚C) and photoperiods (L:D=12:12) were controlled by lighting incubators (GZX-300, Ningbo Jiangnan Instrument Factory, Ningbo, China). *Artemia* were fed with an 1:1 mixture of *Spirulina* sp. powder (Tianjian Biology Technology Co., Ltd, China) and LANSY-Shrimp ZM powder (INVE Asia Services Ltd, Thailand).

For each treatment, 10 individual's larva were selected as test animals, with each of them was reared separately in a 50 ml falcon tube containing ~40 ml culture medium. During the lifespan of animals under the experimental condition, the cysts reproduction was potentially collected, daily.

The biometrical characterizations of the cysts (n= 100) were measured and statistical analysis followed

Table 1. Mean (SD) of biometrical characters (n=100) of cyst under different treatments (UN: untreated cyst, DE: decapsulated cyst, CH: chorion thickness)

[Fe]	Salinity								
	35 ppt			70 ppt			140 ppt		
	UN	DE	CH	UN	DE	CH	UN	DE	CH
0	253.20 (12.20)$^{A, a}$	221.16 (9.70)$^{A, a}$	16.02	248.06 (5.09)$^{B, a}$	223.11 (5.08)$^{AB, a}$	12.475	243.40 (9.63)$^{C, a}$	224.47 (6.36)$^{B, a}$	9.465
5	245.42 (16.72)$^{A, ab}$	234.22 (13.08)$^{A, b}$	5.6	234.39 (15.65)$^{B, b}$	224.17 (12.52)$^{B, a}$	5.11	233.12 (11.54)$^{B, bc}$	218.57 (11.06)$^{B, b}$	7.275
10	242.02 (12.41)$^{A, b}$	220.95 (15.30)$^{A, a}$	10.535	240.58 (8.18)$^{A, c}$	217.89 (9.01)$^{AB, b}$	11.345	236.41 (7.11)$^{B, c}$	215.64 (7.40)$^{B, b}$	10.385
20	237.44 (14.31)$^{A, b}$	216.34 (9.17)$^{A, ac}$	10.55	248.29 (6.32)$^{B, a}$	221.54 (8.32)$^{B, ab}$	13.375	230.34 (7.38)$^{C, b}$	217.98 (7.13)$^{AB, b}$	6.18
40	237.14 (16.67)$^{A, b}$	211.86 (5.73)$^{A, c}$	12.64	248.99 (8.88)$^{B, a}$	218.68 (6.35)$^{B, b}$	15.155	234.17 (6.95)$^{A, c}$	217.43 (6.37)$^{B, b}$	8.37

Same upper cases in each row show non-significant difference among each biometric characters.
Same lower cases in each column show non-significant difference among each biometric characters.

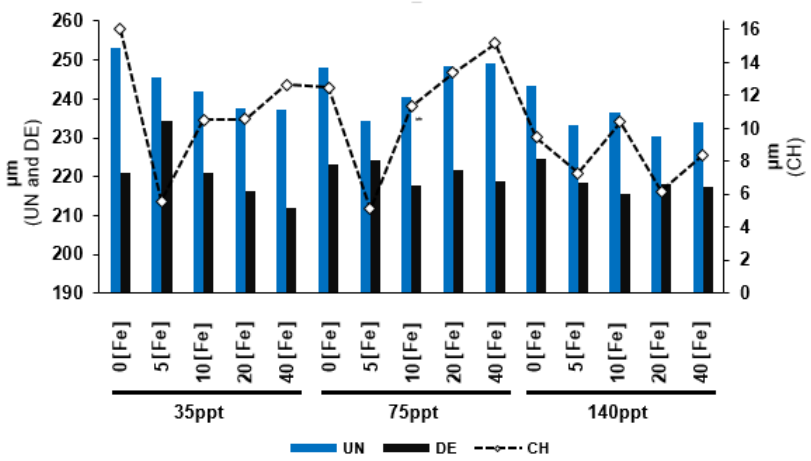

Figure 1: Mean of biometrical characters of cyst under different treatments (UN: untreated cyst, DE: decapsulated cyst, CH: chorion thickness, [Fe]: concentration of ferric citrate).

with Asem *et al.* (2007). Significant differences between means were determined by two-way ANOVA (Tukey, P<0.05). The computer program SPSS v.22 was performed for statistical analysis.

Results and Discussion

The mean values of biometrical characters of *Artemia* cysts were represented in Table 1 and Figure 1. The largest diameter of untreated (253.20 ± 12.20 µm) and decapsulated (234.22 ± 13.08 µm) cysts belong to 35 ppt-0 mg/L [Fe] and 35 ppt-5 mg/L [Fe], respectively. The treatments of 140 ppt-20 [Fe] and 140 ppt-10 mg/L [Fe] yielded the smallest untreated (230.34 ± 7.38 µm) and decapsulated (215.64 ± 7.40 µm) cysts, respectively. The thickest chorion was observed in 35 ppt-0 mg/L [Fe] (16.02 µm) and the thinnest chorion were found in 70 ppt-5 mg/L [Fe] (5.11 µm), followed by in 35 ppt-5 mg/L [Fe] (5.6 µm). The result of the two-way ANOVA documented the salinity level, concentration of ferric and interaction of them (salinity × [Fe]) have significant effects on untreated and decapsulated cysts size (Table 2).

With regard to the results of control treatment ([Fe] = 0 mg/L), an increasing of salinity level caused significant decreasing in diameter of untreated cyst and chorion thickness, by contrast the decapsulated cysts revealed an increasing size. There is a negative correlation between increasing salinity and cysts size in treatment of 5 and 10 mg/L of Fe. The largest cysts and thinker chorion were observed in 70 ppt of both treatments of 20 and 40 mg/L of Fe.

Comparison results of increasing the concentration of ferric in each group salinity documented heterogeneity relationship with biometrical characters, nonetheless the largest untreated cysts generally belong to control treatments of each group.

Generally, the biometrical characterization of *Artemia* cysts had been referred to reproductive mode of each species (bisexual or parthenogenetic) (Vanhaecke & Sorgeloos, 1980; Abatzopoulos, Agh, Van Stappen, Razavi-Rouhani, & Sorgeloos, 2006a), ploidy levels of parthenogenetic populations (Triantaphyllidis, Abatzopoulos, Miasa, & Sorgeloos, 1996), habitat altitude (Asem & Sun, 2014), and physico-chemical parameters or food availability (Abatzopoulos *et al.*, 2006b).

Literature reviews of biometrical characterizations of *Artemia* cysts have revealed high intra-population variability (Asem *et al.*, 2010; Asem & Sun, 2014). Due to that majority of studies used naturally-collected cyst samples, so this might be difficult to discuss about the contributing parameters affecting *Artemia* cysts biometrical characterization, but the result of the present study indicated that cysts produced from the next generations of single diploid clone of parthenogenetic *Artemia,* could skip the effect of intra-specialty.

Asem *et al.*, (2010) documented the size of untreated and decapsulated of wild cyst form Urmia Lake decreased with increasing the salinity from 173.8 ppt in 1994 to 280.8 ppt in 2003/4 contrary to increasing chorion thickness. With regard to results of this study increasing of salinity in control treatment reveals non-significant correlation with diameter of decapsulated cyst but negative correlation with size of untreated cysts and chorion thickness. This contradiction results in the effect of increasing salinity on the size decapsulated cyst and chorion thickness between natural and laboratory conditions can be attributed to food availability and/or interaction of physico-chemical parameters.

The low concentrations of ferric (5 and 10 mg/L) produced the largest diameter of untreated and decapsulated cysts in the low salinity (35 ppt), while the high concentrations of ferric (20 and 40 mg/L) produced the biggest cyst and thicker chorion in medium salinity (70 ppt).

In conclusion, comparative studies on biometrical characterization of bisexuals and polyploids *Artemia* cyst populations are suggested to understand the role of ionic composition in determining the biometrical variation of *Artemia* cyst.

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Table 2. Statistical results of combined effect of salinity and [Fe] by two-way ANOVA

	UN			DE		
	df	F	Sig.	df	F	Sig.
Salinity	2	79.57	.000	2	6.05	.002
[Fe]	4	35.61	.000	4	31.96	.000
Salinity × [Fe]	8	16.68	.000	8	13.20	.000

References

Abatzopoulos, T.J., Agh, N., Van Stappen, G., Razavi-Rouhani, S.M., & Sorgeloos, P. (2006a). *Artemia* sites in Iran. Journal of the Marine Biological Association of the United Kingdom, *86*(2), 299-307. https://doi.org/10.1017/S0025315406013154

Abatzopoulos, T.J., Baxevanis, A.D., Triantaphyllidis, G.V., Criel, G., Pador, E.L., Van Stappen, G., Razavi Rouhani, S.M., & Sorgeloos, P. (2006b). Quality evaluation of *Artemia urmiana* Gunther (Urmia Lake, Iran) with special emphasis on its particular cyst characteristics (International Study on *Artemia* LXIX). Aquaculture, *254*(1-4), 442-454. http://dx.doi.org/10.1016/j.aquaculture.2005.11.007

Asem, A., Rastegar-Pouyani, N., De Los Rios, P., Manaffar, R., & Mohebbi, F. (2010). Biometrical comparison of *Artemia urmiana* Günther, 1899 (Crustacea: Anostraca) cysts between rainy and drought years (1994-2003/4) from Urmia Lake, International Journal of Biological and Life Sciences, *6*(2), 100-106.

Asem, A., & Sun, S. (2014). Biometric characterization of Chinese parthenogenetic *Artemia* (crustacea: anostraca) cysts, focusing on its relationship with ploidy and habitat altitude. North-Western Journal of Zoology, *10*(1), 149-157.

Asem, A., Rastegar-Pouyani, N., & Agh, N. (2007). Biometrical study of *Artemia urmiana* (Anostraca: Artemiidae) cysts harvested from Lake Urmia (West Azerbaijan, Iran). Turkish Journal of Zoology, *31*(2), 171-180.

Baker, M.J. (1966). *Autoecology of Artemia: Factors influencing haemoglobin, synthesis and cyst production* (ScM Thesis), San Francisco State College, California, USA.

Barata, C., Hontoria, F., & Amat, F. (1996). Estimation of the biomass production of *Artemia* with regard to its use in aquaculture: temperature and strain effects. Aquaculture, *142*(3-4), 171-189. https://doi.org/10.1016/0044-8486(95)01250-8

Bengtson, D.A., Léger, P., & Sorgeloos, P. (1991). Use of *Artemia* as a food source for aquaculture. In R.A. Browne, Sorgeloos P., Trotman C.A.N. (Eds.), *Artemia* Biology(pp. 225–285). Boca Raton, Florida, USA: CRC Press., 374 pp.

Gao, M., Wang, J., Ma, G., Van Stappen, G., & Sui, L. (2017). Carbon supplementation and microbial management to stimulate *Artemia* biomass production in hypersaline culture conditions. Aquaculture Research, *48*(3): 1240–1250. https://doi.org/10.1111/are.12965

Nguyen, N.T.N. (2015). Yield and economic efficiency of green water in combination with supplemental feeds for *Artemia* production in Vietnamese salt ponds. Journal of Applied Aquaculture, *27*(1), 72-86. https://doi.org/10.1080/10454438.2015.1006496

Lavens, P., & Sorgeloos, P. (1991). Production of *Artemia* in culture tanks. In R.A. Browne, Sorgeloos P., Trotman C.A.N. (Eds.), *Artemia* Biology (pp. 317-350). Boca Raton, Florida, USA: CRC Press., 374 pp.

Sorgeloos, P., Dhert, P., & Candreva, P. (2001). Use of the brine shrimp, *Artemia*, spp. in marine fish larviculture. Aquaculture, *200*(1-2), 147-159. http://dx.doi.org/10.1016/S0044-8486(01)00698-6

Sorgeloos, P., Persoone, G., Baeza-Mesa, M., Bossuyt, E., & Bruggeman, E. (1978). The use of *Artemia* cysts in aquaculture: the concept of "hatching efficiency" and description of a new method for cyst processing. Proceedings of the annual meeting - World Mariculture Society, *9*, 715–721. http://dx.doi.org/10.1111/j.1749-7345.1978.tb00285.x

Sui, L.Y., Wang, J., Nguyen, V.H., Sorgeloos, P., Bossier, P., & Stappen, G.V. (2013). Increased carbon and nitrogen supplementation in *Artemia* culture ponds results in higher cyst yields. Aquaculture International, *21*(6), 1343-1354. http://dx.doi.org/10.1007/s10499-013-9637-6

Tackaert, W., & Sorgeloos, P. (1991). Semi-intensive culturing in fertilized ponds. In R.A. Browne, Sorgeloos P., Trotman C.A.N. (Eds.), *Artemia* Biology (pp. 287-315). Boca Raton, Florida, USA: CRC Press, 374 pp.

Thi, N.A.N., Van, H.N., & Sorgeloos, P. (2013). *Artemia* biomass production and its use in aquaculture in Vietnam. Balti, Moldova: Scholars Press. 200 pp.

Thi, N.A., Pachecovega, J.M., Cadenaroa, M.A., Ascencio, F., Rangeldávalos, C., & Rojascontreras, M. (2015). Assessment of endemic microalgae as potential food for *Artemia franciscana* culture. Latin American Journal of Aquatic Research, *43*(1), 23-32. http://dx.doi.org/10.3856/vol43-issue1-fulltext-3

Triantaphyllidis, G.V., Abatzopoulos, T.J., Miasa, E., & Sorgeloos, P. (1996). International study on *Artemia*. LVI. Characterization of two *Artemia* populations from Namibia and Madagascar; cytogenetics, biometry, hatching characteristics and fatty acid profiles. Hydrobiologia *335*(2), 97-106. http://dx.doi.org/10.1007/BF00015271

Vanhaecke, P., & Sorgeloos, P. (1980). International study on *Artemia* IV, The biometrics of *Artemia* strains from different geographical origin. In G. Persoone, Sorgeloos P., Roels O., Jaspers E., (Eds.), The Brine Shrimp *Artemia* (pp. 393-405). Ecology, Culturing, Use in Aquaculture. Wettern, Belgium: Universa press, 636 pp.

Versichele, D., & Sorgeloos, P. (1980). Controlled production of *Artemia* cysts in batch cultures. In G. Persoone, Sorgeloos P., Roels O., Jaspers E., (Eds.), The Brine Shrimp *Artemia* (pp. 231-246.). Ecology, Culturing, Use in Aquaculture. Wettern, Belgium: Universa press, 636 pp.

Wang, Z.C., Asem, A., & Sun, S.C. (2017). Coupled effects of photoperiod, temperature and salinity on diapause induction of the parthenogenetic *Artemia* (Crustacea: Anostraca) from Barkol Lake, China. North-Western Journal of Zoology, *13*(1), 12-17.