Optimization of hatching efficiency condition of *Artemia urmiana* cysts

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Abstract:
Nauplii in the Instar I –II stages are probably the form of Artemia most widely used in aquaculture. They are also the easiest and earliest live food obtained from the cysts. In order to optimize utilization of cysts for hatching into nauplii, it is useful to know as much as possible about the hatching characteristics of each batch. and in this regard, the critical factors are: temperature, salinity, and pH.
Three - way ANOVA with three factors each with three levels including, water salinity (28,30 and 33ppt), water temperature (25, 28 and 30°C) and pH (7, 8 and 9) has been analyzed on the hatching efficiency of *Artemia urmiana* cysts for determining the best points of these physical parameters in laboratory condition. There are significance differences between salinity, water temperature and interaction S* W.T, S*pH and W.T*pH (p<0.05) but there are not any differences between pH treatment and S*W.T*pH interactions The best points are 30ppt, 28°C and 7 for water salinity, water temperature and pH respectively.

Keywords: *Artemia urmiana*, Cysts, Hatching, Expression

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Introduction

Presently brine shrimp cysts are commercially available from natural sources in Argentina, Australia, Brazil, Canada, the People's Republic of China and the USA (Sorgeloos, 1980a).

Knowledge of hatching characteristics is important due to the variability among batches found by Vanhaecke and Sorgeloos, 1983. Percent hatch was found to vary from about 20 to 90% of the total cysts. This quality criterion for Artemia cysts obviously accounts for much of the price differences among Artemia batches. A batch with 80% hatch will clearly be worth more than a batch worth20% hatch. Number of nauplii hatching per gram of cysts can vary from<100,000 to >300,000

From the limited literature on this subject, it is known that the hatching rates of *Artemia* cysts vary from one strain to another (Sorgeloos and Persoone, 1975; Person-Le Ruyet and Salaun, 1977; Smith *et al.,* 1978) and depend on physical parameters such as salinity, Water temperature, pH and Oxygen dissolve (Van steppan, 1996).

According to the results obtained by Smith *et al.* (1978), even variation among batches from the same strain may be expected. This complicates the exact timing for the maximized harvest of instar I nauplii, which is critical both in fundamental research, e.g. in ecotoxicological studies (Sorgeloos *et al.,* 1979; Vanhaecke *et al.,* 1980), and in aquaculture (Benijts *et al.,* 1976; Sorgeloos, 1980b). Presently it is not clear whether the hatching rate is strain specific, as are several other cyst characteristics (Vanhaecke and Sorgeloos, 1980), or to what extent cyst processing and/or storage conditions are involved. Although hatching process is relatively simple but several conditions need to be considered if one wished to make optimal use of the cysts. Primary critical factors are: light, temperature, salinity, oxygen level, and pH and cyst density (Vanhaecke and Sorgeloos, 1983). A better knowledge of the parameters that influence the hatching efficiency might lead to improved techniques in the development of better *Arternia-cyst* products.

For obtaining the best physical condition for hatching of *Artemia urmiana* cyst it was focused on water salinity (W.S), water temperature (W.T) and pH and compared the Hatching efficiency (H.E) of cysts with Using Factorial ANOVA.

Materials and methods

The 3*3*3*3= 81 cylindro-conical tubes as experimental units were set up with each 2 gram disinfected cysts of *A.urmiana* from the same batch in 1litre preheated, filtered (e.g. with a filter bag) seawater (12 ppt) with adding chemical salt for increasing the salinity The hatching tubes were incubated at different water temperatures with using heater and pH, with adding dissolved sodium bicarbonate (up to $2g.l^{-1}$ technical grade $NaHCO_3$) or sodium carbonate solution drop by drop for changing the pH, with three replications. Other physical conditions such as light intensity (2000 lux) and gentle aeration were taken constant for all treatments

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After 24h, six 250µL sub samples were taken from the tubes with a micropipette and stained with lugol

-Count nauplii (n i) under a dissection microscope and calculate the mean value of the six sub-samples (N)

-Hatching efficiency (HE) = (N*4*1000): (2) $^{-1}$ (Sorgeloos *et al*., 1978).

An analysis of variance- factorial analysis and Duncan's test on these data revealed the two best physical conditions the hatching efficiency.

Results

The results including measurements of H.E in different salinity, water temperature and pH were gathered in Table 1 and the analysis were brought in Table 2.

Table1: Average of three replications of H.E (*1000) in different salinities, water temperature and PH treatments.

	H.E 1	H.E 2	H.E 3	Average
S33,T30,pH9	98	102	100	100ab
S33,T30,pH8	111	109	110	110bc
S33T30,pH7	107	105	103	105bc
S33,T28,pH9	110	111	112	111bc
S33,T28,pH8	120	120	117	119bc
S33,T28,pH7	112	112	112	112bc
S33,T25,pH9	97	99	98	98ab
S33,T25,pH8	100	100	100	100ab
S33,T25,pH7	93	95	91	93ab
S30,T30,pH9	123	125	127	125cd
S30T30,pH8	129	129	129	129cd
S30,T30,pH7	127	130	130	129cd
S30,T28,pH9	125	125	131	127cd
S30,T28,pH8	158	152	155	155e
S30,T28,pH7	151	151	151	151e
S30,T25,pH9	98	94	96	96ab
S30,T25,pH8	90	92	88	90ab
S30,T25,pH7	70	72	68	70a
S28,T30,pH9	78	72	78	76a
S28,T30,pH8	92	75	90	89ab
S28,T30,pH7	81	75	84	80a
S28,T28,pH9	82	77	78	79a
S28,T28,pH8	98	99	97	98ab
S28,T28,pH7	100	100	100	100ab
S28,T25,pH9	70	69	71	70a
S28,T25,pH8	76	76	76	76a
S28,T25,pH7	78	77	73	76a

S=Salinity(ppt), T=Water Temperature (°C), H.E=Hatching efficiency

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Table 2: Factorial ANOVA.

Source	df	SS	MS	F
Salinity	2	1868	934	650**
Water Temp.	2	1353	676	471**
pH	2	50	25	17ns
Salinity * W.T	4	582	145	101
Salinity* pH	4	173	44	30*
W.T* pH	4	121	30	21
8	8	24	3	2ns
Error	54	79	1.43	
Total	**80**	**4245**		

Discussion and conclusions

Results and statistical analysis show, not only each parameter has effect on H.E separately, but also, the combinations or interactions of them are so. Then the best point of critical physical parameters for hatching of *Artemia urmiana* cysts are 30 ppt, 28°C and 7-8 for Water salinity, Water temperature and pH respectively. Van sttapen (1996) suggested 33 ppt. salinity for hatching of *A.fransiscana.* this difference perhaps is caused, by the differences in outer thickness of cyst layer. The size of *A. urmiana* cyst is ticker than *A.fransiscana* cyst because of alveolar layer. This layer is porous and in *A.urmiana* is ticker than *A.fransiscana*.(Hajirostamloo, 2001) It seems the *A. urmiana*'s embryo can break this layer in lower salinity. On the other hand, physiological condition of *A.urmiana*'s embryo for hatching are stimulated at lower salinity than *A.fransiscana* embryo (Clegg, 2001).

According to these results, during the projects these points of parameters for hatching of *Artemia urmiana* cysts were used and it is recommended all sectors who works with *A.urmiana* cysts, used these conditions for increasing the hatching efficiency.

1. The hatching efficiency is an important criterion in the evaluation of the overall hatching quality *of Artemia* cyst samples.
2. The hatching efficiency is not only a function of the geographical origin of the cyst material but is to a very large extent masked by the hatching physical conditions.
3. In order to ensure the optimal use of *Artemia* in aquaculture hatcheries, the hatching efficiency of a new batch of cysts should be determined prior to use.
4. The optimal hatching quality of *Artemia urmiana* cysts can be ensured by preparing 30ppt, 28°C and 7-8 salinity, water temperature and pH respectively.

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