New concepts for hatching quality and evaluation of brine shrimp *Artemia* (Crustacea: Anostraca) cyst

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The cysts of brine shrimp *Artemia* are widely used in the aquaculture industry (Sorgeloos 1980, Sorgeloos *et al*. 1998, Sorgeloos *et al.* 2001). They are produced commercially by culturists in many places or harvested from their natural habitat, such as the Great Salt Lake (Van Stappen 2003). Irrespective of the fact that each species or population has its own unique characteristics, the processing of *Artemia* cysts is an important factor in price determination. Thus, different definitions have been developed for the evaluation of the hatching quality of *Artemia* cysts. Those hatching percentage, hatching efficiency, hatching rate, hatching synchrony and hatching output (Sorgeloos *et al.* 1978, Van Stappen 1996).

- Hatching Percentage: The number of nauplii that can be produced under standard hatching conditions from 100 full cysts; this criterion does not take into account cyst impurities, such as cracked shells, sand or salt, and refers only to the hatching capacity of the full cysts.
- Hatching Efficiency: Number of nauplii that can be produced from 1 g of dry cyst product under standard hatching conditions.
- Hatching Rate: The time period for full hatching from the start of incubation (hydration of cysts) until nauplius release (hatching), at a number of time intervals.
- Hatching Synchrony: Time lapse during which most nauplii hatch, *i.e.* Ts = T90-T10.
- A high hatching synchrony ensures a maximal number of instar I nauplii available within a short time span; in case of poor synchrony the same hatching tank needs to be harvested several times in order to avoid a mixed instar I-II-III population when harvesting at T90.
- Hatching Output: Dry weight biomass of nauplii that can be produced from 1 gram dry cyst product incubated under standard hatching conditions; best products yield about 600 mg nauplii/g of cysts. The calculation is made as follows: hatching efficiency x individual dry weight of an instar I nauplius.

These concepts are used in *Artemia* based on research (Vanhaecke *et al.* 1981,Vanhaecke and Sorgeloos 1982, Drinkwater and Crowe 1991, Triantaphyllidis *et al.* 1993, Van Stappen *et al.* 2003, Saygi 2004, Kara *et al.* 2004, Abatzopoulos *et al.* 2006) and as quality determining factors for economic purposes (Vanhaecke and Sorgeloos 1983).

All five definitions, but in particular hatching percentage and hatching efficiency, have major roles in cyst quality determination. The major issue is that these definitions can provide a real role in cyst quality only where there is a suitable processing of cysts. Sometimes, cysts have a high percentage of shells and are cracked. Although freshwater-saturated brine technology is used for purification of *Artemia* (Baert *et al.* 1996, Treece 2000), in some cases the method is not efficient in separating shells and cracked cysts of from full *Artemia*[2] cysts. Present definitions and calculations are unable to determine the percentages of shell content and cracked cysts in samples.

In determining hatching percentage after decapsulation of cysts, the shells and cysts with undeveloped embryos are deleted. Most of the cracked cysts disintegrate during the first 3-4 hours after hydration, but they are included as part of the cyst batch. Even hatching efficiency is unable to determine quality of cysts, because it depends on their diameter (Asem *et al.* 2007). Moreover, because shells and cracked cysts are eliminated at the end of determining the hatching percentage, it doesn't seem unexpected for the cyst mass containing shells and cracked cysts to show a high percentage of hatching.

This article delineates six new concepts and definitions for examining the quality and evaluation brine shrimp cysts that can accurately show the hatching percentage, cyst quality and purification effectiveness.

New Concepts and Their Formulas

Since hatching percentage can't clearly provide cyst quality because cracked cysts are disintegrated in the first 2-3 hours after hydration and the shells are finally removed by sodium hypochlorite. Therefore, it is suggested the title percentage of false hatching (FH) should be substituted for hatching percentage.

1. Percentage of Potential hatching (%PH): the number of larvae harvested from 100 shells, perfect and cracked cysts.

$$\%\mathrm{PH} = \frac{Nauplii}{Total\ \ Cysts} \times 100$$

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2. Percentages of Absolute Hatching (%AH): is the number of larvae harvested from 100 embryo-bearing cysts whether perfect or cracked.

$$\%AH = \frac{Nauplii}{Total\ \ Decapsulated\ \ Cysts} \times 100$$

3. Percentage of Cyst Purity (%Cyst Purity): percentage of perfect and perfect embryo-bearing cysts out the total embryo-bearing cysts whether perfect or cracked

$$\%Cyst\ \ Purity = \frac{Nauplii + Umbrella + Embryo}{Total\ \ Decapsulated\ \ Cysts} \times 100$$

4. Shell Percentage (%Shell): the percentage of shells and imperfect embryo-bearing cysts

$$\%Shell = \frac{Total\ \ Cyst - Total\ \ Decapsulated}{Total\ \ Cysts} \times 100$$

5. Percentage of Cracked Cyst (%Cracked Cyst): the percentage of cracked embryo bearing cysts out of total embryo bearing cysts

$$\%Cracked\ \ Cysts = \frac{Total\ \ Decapsulated - (Nauplii + Umbrella + Embryo)}{Total\ \ Decapsulated\ \ Cysts} \times 100$$

6. Percentage of Hatching Potential Loss (%HPL): the difference between the %FH and %PH that indicates the potential loss due to existence of cracked cysts and sells. The lower the loss percentage and the higher the potential hatching, the better the quality of the cysts.

$$\%HPL = \%FH - \%PH$$

The abbreviations FH, PH, AH and HPL are used in Figure 1 and Table 2.

Methods and Materials

1. One gram of dry cysts are added to of 1,000 cc of 35 ppt salinity water and aerated.
2. After one hour, six samples are taken from replicates.
3. The total number of cysts in each the six samples are counted (including those cracked, fully cyst: hydrated or dehydrated cysts). The mean is calculated for the six samples so the total number of cysts can be calculated.
4. Immediately, the cysts are decapsulated with sodium hypochloride. At this stage shells and the cysts with imperfect embryos are eliminated but the cracked and full cysts remain and are decapsulated. Then, the decapsulated cysts are counted and mean from the replicates is obtained to determine the total number of decapsulated cysts.
5. After 24 hours (hatching time), the percentage of false hatching (%PFH) is calculated by the method of Van Stappen (1996).

$$\%FH = \frac{Nauplii}{Nauplii + Umbrella + Embryos} \times 100$$

Summing the Nauplii + Umbrella + Embryos shows the number of full cysts containing embryos (Van Stappen 1996).

6. Other conditions required for hatching must be observed.
7. All experiments should be repeated three times.

Finally, the value of each defined parameter is computed.

Hatching quality of cysts from three species of *Artemia* (*A. urmiana*, *A. franciscana* and a parthenogenetic population) were evaluated following the new concepts.

Table 1. Raw data from the study.

Sample	Repetition	Total Cyst	Total decapsulated	Nauplii	Umbrella	Embryo
	1	98.32	57.9	22.83	7.66	13.33
Parthenogenetic population	2	-	-	-	-	-
	3	87.5	57.07	25.66	6.83	16
	1	98.32	54.15	20.5	7.16	14.16
A. urmiana	2	102	55.4	19	6	13.33
	3	80	51.65	20	4.16	13
	1	88.75	46.87	33	7.5	5
A. franciscana	2	91.25	56.87	37.25	7	7.5
	3	91.25	56.25	39.5	9.25	7

Table 2. Mean (S.D) of the results of cysts quality (same letters in each column show significant difference, ANOVA; Tukey, $p<0.05$)

Sample	%PH	%AH	%FH	%Cyst Purity	HE	%shell	%cracked	%HPL
Parthenogenetic *Artemia*	26.27 (4.32)a	42.20 (3.91)a	52.51 (0.58)a	80.32 (6.56)a	96980(8004)a	37.94(4.48)	19.68(6.56)a	10.31(3.33)a
A. urmiana	21.49 (3.23)b	36.96 (2.35)b	50.80 (2.63)b	72.79 (4.09)b	79333(3055)b	42.02(5.71)	27.21(4.09)b	13.84(2.33)b
A. franciscana	40.43 (3.07)ab	68.71 (2.78)ab	71.79 (0.85)ab	95.73 (4.22)ab	146333(13203)ab	41.07(5.31)	4.27(4.22)ab	3.08 (3.04)ab

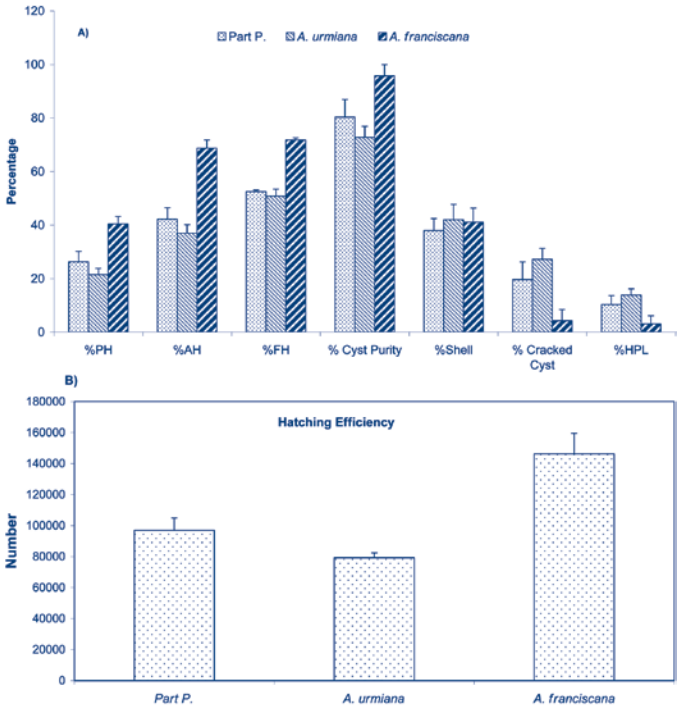

Fig. 1. Cyst quality.

Results

Before examination, all cyst samples were purified using freshwater-saturated brine method. Tables 1 and 2 show the raw data and summarize the results of cysts quality. The results are shown in Figure 1.

In Table 2 the percentages of potential hatching (PH), absolutely hatching (AH), false hatching (FH), hatching efficiency (HE) and cyst purity of the *A. franciscana* were higher than those of *A. urmiana* and the parthenogenetic population. The lowest percentage of cracked cyst were associated with *A. franciscana*. Results shown in Table 2 indicate the shell percentage in the three samples have weree not significantly different but the percentage of cracked cysts in the *A. franciscana* sample was the lowest. This difference can be attributed to the method of processing the cysts and not to the purification method.

Conclusion

Although the three cyst samples were purified by the freshwater-saturated brine method, the results show even if this method separates a considerable amount of the cyst waste, it isn't perfect. It was concluded that because of the low percentage of hatching potential loss and high hatching efficiency, *A. franciscana* cysts are of higher quality than the other two types evaluated. Finally, it seems that the new concepts can provide another approach to studies to assess the economics brine shrimp cysts.

Notes

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References

Abatzopoulos, T.J., A.D. Baxevanis, G.V. Triantaphyllidis, G. Criel, E.L. Pador, G. Van Stappen and P. Sorgeloos. 2006. Quality evaluation of *Artemia urmiana* Günther (Urmia Lake, Iran) with special emphasis on its particular cyst characteristics (International Study on *Artemia* LXIX). Aquaculture 254:442-454.

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

Baert, P., T. Bosteels and P. Sorgeloos. 1996. *Artemia*: Pond production. Pages 196-251 *In* P. Lavens and P. Sorgeloos, editors. Manual on the production and use of live food for aquaculture. FAO, Rome, Italy.

Drinkwater, L.E. and J.H. Crowe. 1991. Hydration state, metabolism, and hatching of Mono Lake *Artemia* cysts. Biological Bulletin 180: 432-439.

Kara M.H., K.A. Bengraine, F. Derbal, L. Chaoui and M. Amarouayache. 2004. Quality evaluation of a new strain of *Artemia* from Chott Marouane (Northeast Algeria). Aquaculture 235:361-369.

Saygi, Y. 2004. Characterization of parthenogenetic *Artemia* populations from Camalti (Izmir, Turkey) and Kalloni (Lesbos, Greece): Survival, growth, maturation, biometrics, fatty acid profile and hatching characteristics. Hydrobiologia 527:227-239.

Sorgeloos P., G. Persoone, M. Baeza-Mesa, E. Bossuyt and E. Bruggeman. 1978. The use of *Artemia* cysts in aquaculture: The concept of "hatching efficiency" and description of a new method for cysts processing. Pages 715-721 *In* J.W. Avault, Jr., editor. Proceedings of the 9th Annual Meeting of the World Mariculture Society. Louisiana State University, Baton Rouge, Louisiana, USA.

Sorgeloos, P., P. Coutteau, P. Dhert, G. Merchie and P. Lavens. 1998. Use of the brine shrimp, *Artemia* spp. in larval crustacean nutrition: A review. Reviews in Fisheries Science 6 (1&2): 55-68.

Sorgeloos, P., P. Dhert and P. Candreva. 2001. Use of the brine shrimp, *Artemia* spp. in marine fish larviculture. Aquaculture 200:147-159.

Sorgeloos, P. 1980. The use of the brine shrimp *Artemia* in aquaculture. Pages 25-46 *In* G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers, editors. The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium.

Treece, G.D. 2000. *Artemia* production for marine larval fish culture. SRAC publication. 702:1-7.

(Continued on page 58)

(Continued from page 13)

Triantaphyllidis, G.V., T.J. Abatzopoulos, R.M. Sandaltzopoulos, G. Stamou and C.D. Kastritsis. 1993. Characterization of two new *Artemia* populations from two solar saltworks of Lesbos Island (Greece): Biometry, hatching characteristics and fatty acid profile. International Journal of Salt Lake Research 2:59–68.

Van Stappen, G. 1996. *Artemia*: Use of cysts. Pages 107-137 *In* P. Lavens and P. Sorgeloos, editors. Manual on the production and use of live food for aquaculture. FAO, Rome, Italy.

Van Stappen G. 2003. Production, harvest and processing of *Artemia* from natural lakes. Pages122-144 *In* J.G. Støttrup and L.A. McEvoy, editors. Live feeds in marine aquaculture. Wiley-Blackwell, Hoboken, New Jersey USA.

Van Stappen, G., L. Sul, N. Xin and P. Sorgeloos.2003. Characterisation of high-altitude *Artemia* populations from the Qinghai-Tibet Plateau, PR China. Hydrobiologia 500:179-192.

Vanhaecke, P. and P. Sorgeloos. 1982. International study on *Artemia*. XVIII. The hatching rate of *Artemia* cysts - a comparative study. Aquaculture Engineering 1:263-273.

Vanhaecke, P., A. Cooreman and P. Sorgeloos. 1981. International Study on *Artemia*. XV. Effects of light intensity on hatching rate of *Artemia* cysts from different geographical origin. Marine Ecology Progress Series 5:111-114.

Vanhaecke, P. and P. Sorgeloos. 1983. International Study on *Artemia*. XIX. Hatching data for ten commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept. Aquaculture 30:43-52.