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ARTEMIA FROM MADAGASCAR REVEALS AN EXCEPTIONALLY HIGH n-3 HIGHLY UNSATURATED FATTY ACID CONTENT

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Introduction

Freshly-hatched nauplii of *Artemia* can be classified into two general groups, freshwater and marine type, depending on the amounts of 18:3n-3 (linolenic acid) and 20:5n-3 (eicosapentaenoic acid, EPA) (Watanabe et al., 1978). Most of the studied *Artemia* populations contain either 18:3n-3 levels higher than 20% of total fatty acids and EPA <5% or 18:3n-3 levels <10% and EPA levels between 5 and 13% (Bengtson et al., 1991). Only the latter *Artemia* batches are a suitable food for larvae of marine fish and crustaceans which require EPA levels >5%, whereas both types can be fed to freshwater organisms. Size of *Artemia* nauplii, although not so critical for crustacean larvae, is very critical for most marine fish larvae that require prey in the 50-100µm range during first feeding and are switched to small size *Artemia* in the first 1-2 weeks thereafter (*Artemia* instar-I nauplii range between 428-517µm, Vanhaecke and Sorgeloos, 1980).

In view of past and present *Artemia* cyst shortages, it is essential to explore and study *Artemia* sources other than the Great Salt Lake (Utah, USA). This paper reports on the exceptionally high levels of EPA found in a population from Madagascar and compares these values with other strains, both freshly hatched and enriched. We also discuss the possible reasons that contributed to such a high HUFA content.

Material and methods

Cysts have been collected from the Ankiembe saltworks, 5km south of Toliara, Madagascar. The population was found to be parthenogenetic and triploid (Triantaphyllidis et al., submitted). Cysts were incubated in seawater under optimal conditions (Sorgeloos et al., 1986). Instar-I nauplii were collected and their fatty acid methyl esters (FAME) were prepared through direct transesterification following the

method of Lepage and Roy (1984). FAME were separated on a Carlo Erba Mega Series 5160 HRGC gas chromatograph equipped with a 50m very polar capillary column BPX70, hydrogen as a carrier and the injection mode was on-column.

Results and discussion

Table I. Fatty acid methylesters (FAME) of instar-I *Artemia* nauplii hatched from cysts collected in Toliara (Madagascar) (the results are means from two analyses)

FAME	Area %	$mg.g^{-1}$ dry weight
14:1n-5	1.15	1.65
14:0	2.35	3.30
15:0	0.75	1.05
15:1n-5	0.45	0.60
16:0	13.15	18.55
16:1n-7	14.35	20.20
17:0	0.80	1.15
17:1n-7	1.80	2.55
18:0	5.20	7.35
18:1n-9	15.00	21.15
18:1n-7	12.80	18.15
18:2n-6-c	5.25	7.40
18:3n-6	0.40	0.55
18:3n-3	4.45	6.25
18:4n-3	0.60	0.90
19:1n-9	nd	nd
20:1n-9	0.20	0.20
20:4n-6	1.15	1.6
20:3n-3	nd	nd
20:4n-3	0.10	0.20
20:5n-3	17.30	24.45
$\sum$n-3:$\geq$20:3n-3	17.45	24.65
Total FAME		141.40

nd: not detected; *Content as a percentage of total fatty acids.

Table I shows the FAME composition of the population from Madagascar. Although there is no docosahexaenoic acid (DHA), a typical phenomenon for *Artemia*, EPA levels are very high (the highest levels ever reported for freshly-hatched *Artemia*). The levels of linolenic acid, 18:3n-3, are quite low and thus this strain is falling in the category of marine type *Artemia* (Watanabe et al., 1978). Since the fatty acid composition of *Artemia* is environmentally and not genetically determined (Lavens et al., 1989) one should seek the reasons of high HUFA content in the habitat conditions. In Madagascar, cyst production takes place in relatively low salinity ponds i.e. from 80-100ppt. Under these conditions it is possible that microalgal species such us diatoms, some haptophyceae (prymnesiophytes) and most cryptophytes that contain significant amounts of EPA and DHA (Volkman et al., 1989) could be present and

enhance the nutritional value of *Artemia*. Moreover, it has been shown that the total lipid content of the diatom *Navicula* sp. increased with increasing salinity of the medium from 30 to 100ppt but declined at 146ppt (Al-Hasan et al., 1990) while the total fatty acid content of the marine alga *Porphyridium cruentum* (Rhodophyceae) is increasing from 26 to 88ppt (Lee et al., 1989). In addition, the previous studies showed that the poly-unsaturated fatty acids either remained constant or slightly increased as the salinity elevated. In salinities above 120ppt halophyte green microalgae prevail, such us *Dunaliella* sp., with low HUFA content. Further study of the microalgal composition of the Madagascar saltworks is needed to isolate the species that results in high EPA levels in *Artemia*.

Table II summarizes literature data for several *Artemia* strains in terms of their EPA content, total n-3 content and their size. The population from Madagascar exhibits the highest EPA and sum of n-3 HUFA content compared to most commercially available strains as well as from several other parthenogenetic and bisexual populations. The size ofinstar-I nauplii from Madagascar is 490µm and is similar to the GSL nauplii which is the main source of cysts for aquaculture purposes. GSL nauplii need to be enriched for 24h with an emulsified product to obtain the same levels of EPA as the strain from Madagascar. This also means that the size of the nauplii will be much larger (~800µm) after the enrichment and thus of limited value when size is important. The only limitation in using the Madagascar strain is the lack of DHA which plays an important role during the early development of fish larvae (Watanabe, 1993).

Table II. Content of EPA, total (n-3)HUFA and size of several strains of *Artemia* (freshly-hatched instar-I nauplii, unless stated otherwise)

Artemia strain	EPA ($mg.g^{-1}DW$)	Σ(n-3)HUFA ≥20:3n-3	Size (in µm)	Reference
Toliara, Madagascar	24.5	24.7	490	1
San Francisco Bay, USA	11.8	14.6	428-431	2
Great Salt Lake (GSL), USA	0.5-4.3	1.9-6.0	486-489	2
Citros, Greece	5.3	9.8	483	3
Megalon Embolon, Greece	3	7.9	479	3
Kalloni, Greece	11.8	14.4	521	4
Inner Mongolia, P.R. China	1.4-4.1	1.8-5	NA	5
GSL enriched with SELCO®[a] for 24h	21.3	37.4	~800	6

1: This study; 2: Léger et al., 1986; 3: Abatzopoulos et al., 1989; 4: Triantaphyllidis et al., 1993; 5: Dhert et al., 1993; 6: Sorgeloos and Léger, 1992.
NA: data not available.
[a]Self emulsifying (n-3)HUFA enrichment concentrate (INVE Aquaculture NV, Belgium).

Conclusions

The high EPA content of the population from Madagascar together with the relatively small size of nauplii makes this population very attractive for use in aquaculture. The very high EPA content can be explained from the fact that cyst production occurs under relatively low salinities (not higher than 100ppt) that allow growth of microalgal species with high HUFA content.

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References

Abatzopoulos Th., G. Karamanlidis, P. Léger, and P. Sorgeloos. 1989. Further characterization of two *Artemia* populations from Northern Greece: biometry, hatching characteristics, caloric content and fatty acid profiles. Hydrobiologia 179:211-222.

Al-Hasan R.H., A.M. Ali, H.H. Ka'wash, and S.S. Radwan. 1990. Effect of salinity on the lipid and fatty acid composition of the halophyte *Navicula* sp.: potential in mariculture. Journal of Applied Phycology 2:215-222.

Bengtson D., P. Léger, and P. Sorgeloos, 1991. Use of *Artemia* as food source. p.255-285. In: Browne R., P. Sorgeloos, and C.N.A. Trotman (Eds). *Artemia* Biology. CRC, Boca Raton.

Dhert P., P. Sorgeloos, and B. Devresse. 1993. Contributions towards a specific DHA enrichment in the live food *Brachionus plicatilis* and *Artemia* sp. p.109-115. In: Fish Farming Technology. Reinertsen H., L.A. Dahle, L. Jorgensen, and K. Tvinnereim (Eds). A.A.Balkema, Rotterdam, Brookfield.482p.

Lavens P., P. Léger, and P. Sorgeloos. 1989. Manipulation of the fatty acid profile in *Artemia* offspring using a controlled production unit. p.731-739. In: Aquaculture - a biotechnology in progress. De Pauw N., E. Jaspers, H. Ackefors, and N. Wilkins (Eds). European Aquaculture Society, Bredene, Belgium. 1222p.

Lee Y.K., H.M. Tan, and C.S. Low. 1989. Effect of salinity of medium on cellular fatty acid composition of marine alga *Porphyrium cruentum* (Rhodophyceae). Journal of Applied Phycology 1:19-23.

Léger P., D.A. Bengtson, K.L. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. Oceanography and Marine Biology: An Annual Review 24:521-623.

Lepage G. and C.C. Roy. 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. Journal of Lipid Research 25:1391-1396.

Sorgeloos P. and P. Léger. 1992. Improved larviculture outputs of marine fish, shrimp and prawn. Journal of the World Aquaculture Society 23:251-264.

Sorgeloos P., P. Lavens, P. Léger, W. Tackaert, and D. Versichele. 1986. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. Laboratory of Mariculture, State University of Ghent.319p.

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(1):59-68.

Vanhaecke P. and P. Sorgeloos. 1980. International study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. p.393-405. In: The brine shrimp *Artemia*.Vol.3. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren. Belgium. 556p.

Volkman J.K., S.W. Jeffrey, P.D. Nichols, G.I. Rogers, and C.D. Garland. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology 128:219-240.

Watanabe T. 1993. Importance of docosahexaenoic acid in marine larval fish. Journal of the World Aquaculture Society 24:152-161.

Watanabe, T., F. Oowa, C. Kitajima, and S. Fujita. 1978. Nutritional quality of brine shrimp, *Artemia salina*, as a living feed from the viewpoint of essential fatty acids for fish. Bulletin of the Japanese Society of Scientific Fisheries. 44(10):1115-1121.