Biometrical characters of *Artemia* from four Iranian regions

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Abstract

In order to introduce the best strain of Iranian *Artemia* cyst to larvicultural feeding of aquatic animals, biometrical characteristics of *Artemia* cysts and newly hatched nauplii of four different geographical regions of Iran (Maharloo Lake, Fars province, Meyqan Lake, Central province Urmia Lake and Fesendooze pond West Azarbaijan province) were determined. Whole cysts and decapsulated cyst diameters, chorion thickness, dry weight and total length of newly hatched nauplii were measured under stereomicroscope equipped with Motic 2000 software MLC – 150C, attached to a monitor. The obtained data were statistically analyzed by one way ANOVA and tested with multivariate Duncan test. The results showed that *Artemia urmiana* cyst has a significantly larger diameter and nauplius total length than the other cysts ($P<0.05$) (285.4 ± 0.53 µm and 511.8± 1.27 µm, respectively). Although the chorion thickness of the Meyqan cyst is higher than the others there are not any significant differences between them ($P>0.05$). The smallest cyst diameter (276.8± 0.58 µm), nauplii total length (491.2±1.17 µm) and consequently the largest number of cysts per gram were obtained from Fesendooz. Due to the suitable size of *Artemia* samples for shrimp post larvae, ornamental and marine fish larval stage feeding it could be a good candidate for replacing imported expensive cysts.

Keywords: *Artemia*, Cyst, Decapsulated cyst, Chorion thickness

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Introduction

Due to aquaculture development (especially shrimp culture and ornamental fish rearing), there is an increasing world wide demand for *Artemia* cyst consumption. Recently, there is an increasing demand for *Artemia* cyst that leads to its huge supply from the natural habitats and its culture in pools, ponds and salt water lakes. Since there are different species and strains of *Artemia*, biometrical studies of the existence strain can be help aquaculturists to provide economic live food for their fish culture. *Artemia* annual consumption of the world is more than 2000 tons and in Iran it is near to 50 tons of dry cysts (Lavens and Sorgeloos, 1996; Agh, 2004).

The populations of *Artemia*, exist over all temperate and tropical areas of the world covering 600 *Artemia* sites and have a great potential for systematic, taxonomic evolutionary, fishery studies and aquaculture (Hontoria and Amat, 1992; Lavens and Sorgeloos, 1996; Gajardo et al., 2002; Van Stappen, 2002). Hafezieh (2003) showed that there are 14 *Artemia* natural habitats in Iran 13 of which are parthenogenesis strains and one is *Artemia* bisexual in Urmia Lake. Abatzopoulos et al. (2006), illustrated 17 *Artemia* sites in Iran (Fig. 1); but after draining years during the previous years, these numbers decreased (Ahmady, 2002; Agh, 2004). Vanhaecke and Sorgeloos (1980) showed that the whole *Artemia* cyst, the decapsulated cyst and the chorion thickness diameter vary in 24 geographical regions.

Pilla and Beardmord (1994) measured the cyst diameter of *A. sinica*, *A. urmiana* and *A. sp.* and the results showed a significant difference among samples. Mohammad Yari (2002) measured the *Artemia* cyst and chorion thickness of several partenogenetic populations from Urmia lake, Qom salt lake and Injhe basin and showed that there was a significant difference of these parameters between Injhe basin and the two other populations. Asem (2005) measured the *Artemia* cyst, decapsulated cyst diameter and chorion thickness of *A. urmiana* from 26 stations of Urmia Lake and mentioned that the minimum and maximum of chorion thickness was 1.31 and 9.37, respectively.

The most harvestable *Artemia* cysts in Iran exist in Urmia Lake, Maharloo Lake, Arak Meyqan and Fesendooz Region. The objective of this study was to determine some biometrical characters of 4 strains of *Artemia* from four Iranian regions.

Materials and methods

The geographical position and other specifications of study regions have been tabulated in Table 1 and Figure1. Water salinities were measured in all sampling areas by refractometer ATAGO-Japan (Table 1). All cysts were collected by a 100µ mesh size sampler net from Urmia Lake, Maharloo Lake, Arak Meyqan and Fesendooz Region. After collection, cysts were separated and purified from mud, algae and Artemia carcass.

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Table 1: The specifications of sampling area

region	Position	Altitude from Sea level (m)	Situation	Area (Km^2)	Salinity (g/l)
Urmia Lake	37 ° 20' N 45° 40' E	1278	17 km from East of Urmia	5750-6000	320
Maharloo Lake	29 ° 32' N 52° 42'E	1455- 2990	27 km from South-East of Shiraz	216	250
Arak Meyqan	34 ° 9'N 49° 55'E	1660	17 km from North-East of Arak	545.3	96
Fesendooz region	37 ° 15' N 45° 53' E	1278	35 km from Miandoab	20	100

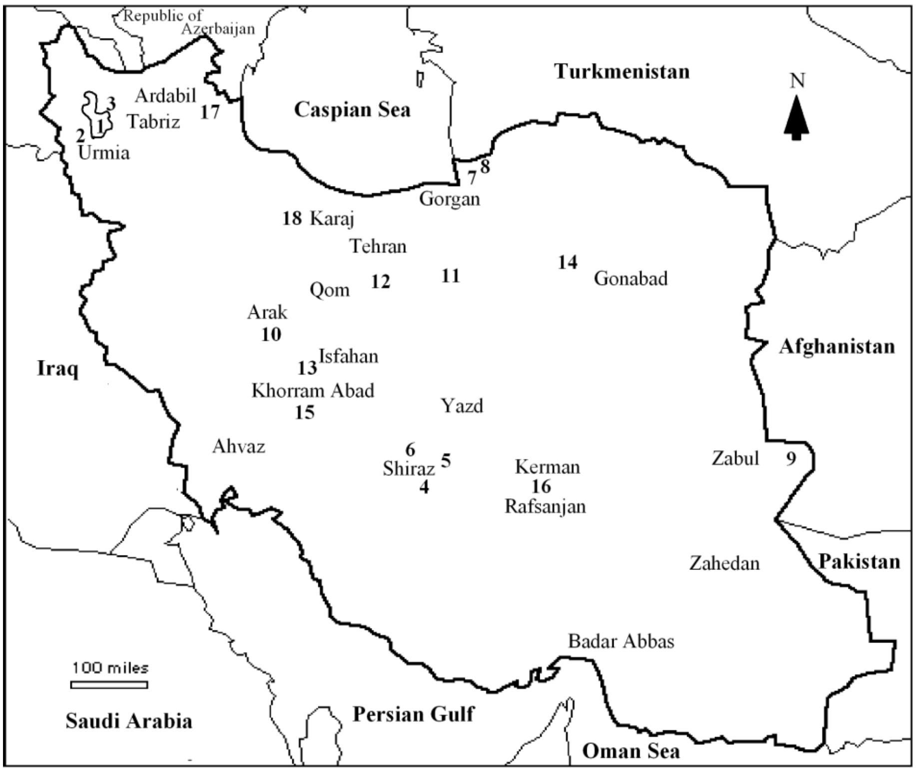

Fig 1: Distribution of Artemia sites in Iran. The names on the map refer to main cities while the numbers mentioned below indicate the map of studied geographical positions of sampling areas; 1, Urmia Lake; 2, Fesendooz Region; 4, Maharlou Lake; 10, Miqan Lake(Adapted from Abatzopoulos et al, 2006)

Measurement of cyst diameters and chorion thickness

A six gram cyst sample was provided from 4 different lakes for biometrical measurements. These cysts were hydrated in 4 small conical tubes with a capacity of 500 ^{cc} for 10 minutes. 0.5 ml of logul 1% (1 g in each tube) was separately added with gentle aeration. Cyst incubation was done according to Vanhaecke *et al*. (1980), with filtered water using 45µ mesh size net and Dietrich & Kalle medium. 0.5 ml logul was added to each tube, after 3 hours incubation. After 12 hours incubation in

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the darkness, whole cysts of each tube were collected by 100µ mesh size net. The dimensions of 1000 Non Decapsulated cysts were measured by a binocular microscope equipped with Motic 2000 loop software MLC – 150C, attached to a monitor. Mean ± SE of data were calculated. Then, some cysts were decapsulated according to Bruggman *et al*., (1980) using hypochlorite sodium containing 5% causative substance. Then 1000 decapsulated cysts (D) were separated and their chorion thicknesses were calculated according to the following formula:

$$ch = \frac{(ND - D)}{2}$$

Where:
Ch: Chorion thickness, ND: Non-Decapsulated cysts, D: Decapsulated cysts

Measurement of individual dry weight of cyst & Nauplious

To determine the dry weight of cysts, 3 replicates each containing nearly 50000 cysts in freshwater were incubated by gentle aeration for 10-15 minutes. Ten subsamples, each 250 µl, from each tube were obtained for hydration and isolation of full cysts from empty ones (30 samples). Then full cysts were dried in oven 60 ˚C for 24 hours and weighted with digital sensitive scale (sensitivity 0/0001 g) and individual weights of dry cysts were obtained by the following formula:

Individual dry weight (µg) = (dry cyst and dish weight (µg) – dish empty weight (µg))/ Number of measured cysts

Dry weights of nauplii were measured by the same technique, but cysts were cultured in 3 trials under standard conditions. After 24 hours, 10 subsamples from each replicate were separated and the number of nauplii were counted, dried in oven (60˚C and 24 hours) and weighted. Total and individual weights of samples as µg were achieved.

Measurement of Instar I nauplii length

Cysts were cultured under the standard conditions (Sorgeloos *et al*., 1986).
Nearly 2000 nauplii were taken randomly from each tray and fixed in logul 5% solution. Lengths of 800 nauplii were measured from proximal head to the end of the abdomen by Motic software installed on a binocular microscope.

Results

Comparison of cyst diameter & chorion thickness

This study revealed that the largest cyst diameter is from Urmia Lake and the smallest one is from Fesendooz region cysts. Also, chorion thickness of cysts from Fesendooz region and Arak Meyqan are the smallest and largest ones, respectively (Table 2).

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Table 2: Mean and SE of cyst diameter, decapsulated cyst diameter and chorion thickness of *Artemia* (µm) from 4 geographical regions of Iran

Region	Cyst diameter(µ)	decapsulated cyst diameter(µ)	Chorion Thickness (µ)
Urmia Lake	285.4± 0.53	270.8± 0.54	7.30± 0.06
Maharloo Lake	281.3± 0.58	266.3± 0.59	7.50± 0.07
Arak Meyqan	280.1± 0.58	263.4± 0.56	8.40± 0.08
Fesendooz region	276.8± 0.58	263.5± 0.59	6.63± 0.06

A Duncan test (P< 0.05) on cyst diameter of 4 *Artemia* strains from different regions of Iran (Fig. 2) shows that the cyst diameter of samples obtained from Maharloo Lake and Arak Meyqan regions do not have any significant difference but these samples have a significant difference with Urmia Lake and Fesendooz region.

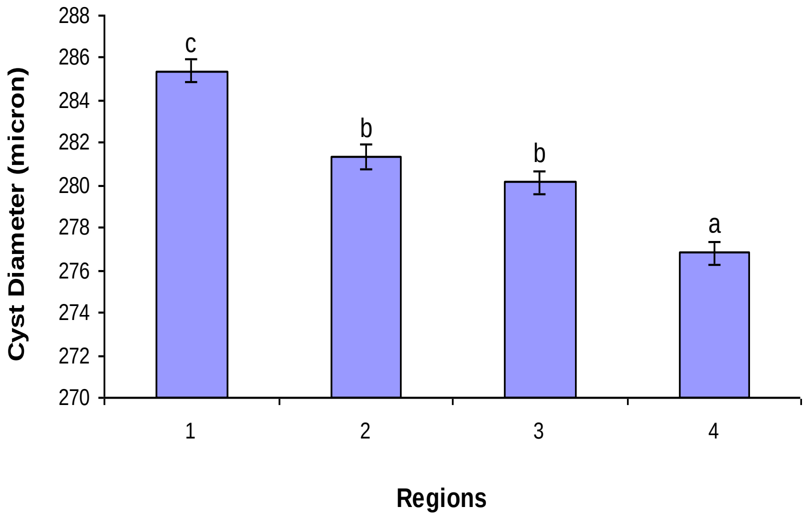

Figure 2: Comparison of Artemia cyst diameter (Mean ± SE) from 4 different regions (P< 0.05) (1; Urmia Lake , 2; Maharloo Lake , 3; Arak Meyqan 4; Fesendooz region).

Comparison of individual dry cyst weight and number of cysts per gram

Table 3 shows that *Artemia urmiana*'s cysts encompass the highest dry weight and consequently it has the least number of cysts per gram but Fesendooz region's cysts are the smallest individual cysts and the most individual cyst per gram, also see Figure 3. The result shows that there are significant differences amongst all regions (P< 0.05).

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Table 3: Mean ± SE individual dry weight of *Artemia* Cysts from four geographical regions of Iran

Region	cysts dry weight individual (µg)	number of cyst per gram
Urmia Lake	3.66± 0.68	283000± 56000
Maharloo Lake	3.36± 0.45	303000± 43000
Arak Meyqan	3.52± 0.49	290000± 42000
Fesendooz region	3.22± 0.35	314000± 37000

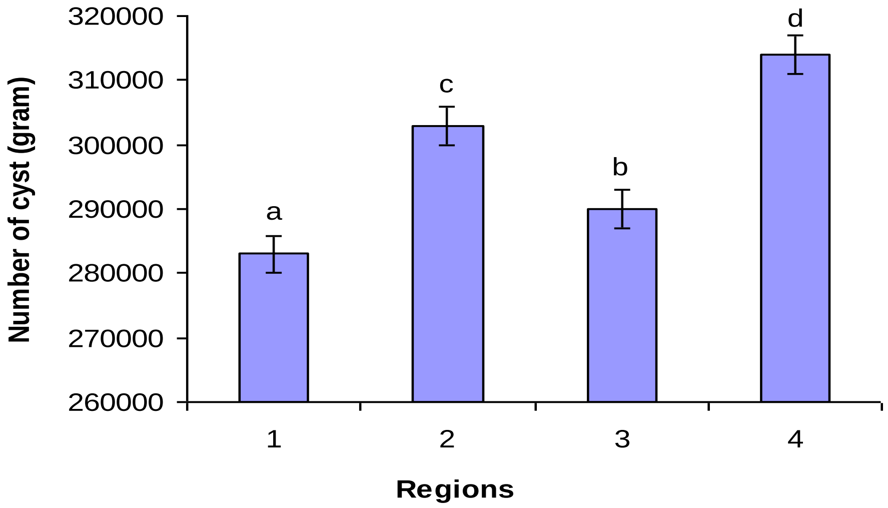

Fig. 3: Comparison of the cyst number per gram (Mean ± SE) for 4 study areas based on Duncan test (P< 0.05) (1; Urmia Lake, 2; Maharloo Lake, 3; Arak Meyqan 4; Fesendooz region).

Individual dry weight & Instar I nauplii length

The collected data indicate that the greatest individual dry weight of *Artemia* nauplii was found from Urmia Lake and the least one belongs to Fesendooz *Artemia* nauplii (Table 4). One Way ANOVA results indicate that there are significant differences in nauplious individual dry weight and length (P≤ 0.05) of 4 different regions (Table 4). The largest nauplii (511.8± 1.27 µ) belonged to *Artemia* nauplii of Urmia Lake and the smallest one (491.3± 1.17) to Fesendooz region.

Table 4: Mean ± SE individual dry weight and length of *Artemia* nauplii from 4 different regions

Regions	Instar I Nauplii individual dry weight (µg)	length of Instar I Nauplii length of *Artemia* (µm)
Urmia Lake	3.75 ± 0.03	511.7 ± 1.27
Maharloo Lake	3.04 ± 0.03	509.4 ± 1.25
Arak Meyqan	3.57 ± 0.04	504.9 ± 1.28
Fesendooz region	3.74 ± 0.03	491.3 ± 1.17

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Discussion

Although *Artemia* cyst structure is the same in all strains but they have quantitative differences that have a great impact on their use in aquaculture. Vanhaecke & Sorgeloos (1980) studied the whole cyst, decapsulated cyst and chorion thickness diameter varies in 24 geographical regions. The results showed significant differences of mentioned parameters in various populations. Also, they suggested that the cyst diameter was related to genetic characteristics.

They concluded that the largest cyst diameter was due to parthenogenesis population of Margarita Di Savia Italy (284.9+14.6 µ) whereas *Artemia franciscana* from Sanferansisco Bay had the smallest cyst diameter (223.9±11.7 µ).The largest and smallest decapsulated cyst diameters were recorded from *Artemia sp.* from Toticorin- India (262.7±11.5µ) and *A. franciscana* from Sanfrancisco Bay (207.7±11.1 µ), respectively (Vanhaecke & Sorgeloos 1980). Pilla and Beardmore (1994) reported that the whole cyst diameter for *A. sinica, A. urmiana* and *Artemia sp.* was 232.75±11.22 µ, 265.85±15.85µ and 232.75±11.22 µ, respectively; which shows a significant difference among specimens.

Mayer (2002) showed that *Artemia sp.* populations from Portorico & Dominican have a significant difference on whole cyst diameters. Asem et al., (2007) did a survey in Urmia Lake and carried out sampling from 26 stations and consequently found out that cyst & decapsulated cyst diameters and chorion thickness of collected cysts were different; namely the largest cyst diameter was (259.34±11.36µ) in $N_{(3-1)}$ inhabitant (Asem et al. 2007, Peikaran Mana, 2007). In this study, the Mean ± SE of *Artemia* cyst & decapsulated cyst diameters and chorion thickness parameters were measured from 4 different geographical regions of Iran including: Urmia, Maharloo, Arak and Fesendooz. The results indicate a high variation of 280.1 ± 0.58-285.4 ± 0.53, 263.4 ± 0.56-270.8 ± 0.54 and 6.63 ± 0.06- 8.4 ± 0.08 µ, respectively. *Artemia* cysts' diameters of Urmia Lake (265.85 ± 15.85µ) have been reported by Pilla and Beardmore (1994) and Asem et al., (2007), is different from obtained results. The reason may be due to salinity changes, food availability, environmental changes especially nutritional and other physico-chemical factors, precision of measuring instruments. Triantaphyllidis *et al*. (1996) showed that the diameters of untreated cysts from Namibia and Madagascar were 247.7 ±11 µ and 285.9± 11.6 µ; also for decapsulated cysts were 233.1±9.8 µ and 246.2 ±11.7 µ, respectively. Their study indicated that the cysts from Namibia were smaller than Madagascar ones. Abatzopoulos et al. (1998) reported that *A.tibetiana* is the biggest recorded in size for *Artemia* species *(323*± 11.2 µ and 230±14.6 µ). Cohen et al. (1999) found diameter ranges between 246.1± 21 µ and 230.3 ± 1 µ for *Artemia* populations from Argentina.

Comparing the results of this survey with other researches on other species of *Artemia*, it can be concluded that in spite of the existing variety in *Artemia* cyst diameter in 4 geographical regions of Iran, their size are in the range

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of other species or strains of *Artemia* cysts or a little larger. The world average of nauplii dry weights have been reported as: 1.63-3.09µg, whereas, dry weight of nauplii studied in the 4 Iranian regions were in the range of 3.04 – 3.75µg, that shows a little difference, therefore they have more advantages comparing other nauplii achievement from other parts of the world. It is concluded that cysts of Fesendooz region have smaller sizes and greater cyst numbers per gram than the others. On the other hand, nauplii from cysts of Fesendooz, regarding to their small and suitable size may have a great potential for use in larviculture of various aquatic animals especially for shrimp, and have a great potential to compete with cysts from other parts of the world, especially after processing, drying and packaging.

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References

Abatzopoulos, T. J., Agh, N., Van Stappen, G., Razavi Rouhani, S. M. and Sorgeloos, P., 2006. *Artemia* sites in Iran.In: *Journal of the marine biological association of the United Kingdom*, 86, 299-307.

Abatzopoulos, T. J., Zhang, B .and Sorgeloos, P., 1998. International study on *Artemia*: 59. *Artemia tibetiana*: preliminary characterization of a new *Artemia* species found in Tibet (people' s Republic of China).*International Journal of Salt Lake Research*, 7, 41-44.

Agh, N., 2004. Final project report on stock assessment of Urmia Lake *Artemia*, *Artemia* and Aquatic Animals Research Center, Urmia University, pp. 90.

Ahmady, R., 2002. Final project report on Effects of environmental factors on cyst formation of *Artemia urmiana* in cultural ponds. *Iranian Fisheries Research Organization*, pp.53.

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

Asem, A., 2005. *Artemia urmiana* systematic in Urmia lake: A morphological study, M.Sc. thesis , Kerman shah Razi University , pp. 107

Bruggman, E., Sorgeloos, P. and Vanhaecke, P., 1980. Improvements in the decapsulation technique of *Artemia* cysts. In: The brine shrimp *Artemia*. Vol.3. Ecology, culturing and use in Aquaculture.persoone G., P.Sorgeloos, O.Roels and E. Jaspers (eds), Universa press, Wetteren, Belgium. Pp.357-372 and pp: 260-268.

Cohen, R. G., Amat, F., Hontoria, F., and Navarro, J. C., 1999. Preliminary characterization of some Argentinean *Artemia* population from la pama and Buenos Aires provinces. *International Journal of Salt Lake Research*, 8, 329-340.

Gajardo, G., Abatzopoulos, T. J., Kappas.I. And Beardmore, J. A., 2002. Evolution and speciation .In: *Artemia* Basic and Applied Biology. (eds.T.J.Abatzopoulos, J.a.Beardmore, J.S.Clegg, P.Sorgeloos), Kluwer Academic Publisher, Dordrecht, pp.225-250.

Hafezieh, M., 2003. Natural resources of Artemia in Iran. Asian –Pacific aquaculture 2003, Bangkok, Thailand. Abstract Book.

Hontoria, F.and Amat, F., 1992. Morphological characterization of adult *Artemia* (Crustacea, Branchiopoda) from different geographical regions. American populations, Journal of Plankton Research, 14, 1461-1471

Lavens, P. and Sorgeloos, P., 1996. Manual on the production and use of live food for aquaculture, (eds), Food and Agriculture Organization of the United Nations, pp.375.

Mayer, R. J., 2002. Morphology and biometry of three populations of *Artemia* (Branchioopoda: Anostraca) from the Dominican Republic and Puerto Rico, Hydrobiology, 486, 29-38.

Mohammad Yari, A., 2002. The biometric, morphologic and life cycle of three populations of Iran Artemia. MSc. Thesis, Tehran University.

Peikaran Mana, N., 2007. Quantitative and Qualitative assessment of *Artemia* Cysts, Decapsulated cysts and Nauplii from 3 Geographical regions of Iran, MSc thesis, Lahijan Islamic Azad University, advised by Dr. H.vahabzadeh pp.144.

Pilla, E. J. S. and Beardmore, J. A. 1994. Genetic and morphometric differentiation in old World bisexual species of *Artemia* (the brine shrimp), 73, pp: 47-56.

Sorgeloos, P.; Lavens, P. and Legera, Ph. 1986. Manual for the culture and use of Brine Shrimp Artemia in aquaculture, pp: 45-49.

Triantaphyllidis, G. V.,Abatzopoulos ,T. J.,Miasa,E. and Sorgeloos, P., 1996. International study on *Artemia* population from Namibia and Madagascar; cytogenetics, biometry, hatching characteristics and fatty acid profiles .*Hydrobiologia*, 335, 97-106.

Van Stappen, G., 2002. Zoogeography. In: *Artemia* Basic and Applied Biology (eds.T.J.Abatzopoulos,J. A. Beardmore,J.S.Clegg,P.Sorgeloos) Kluwer Academic Publishers,Dortrecht,pp.171-224.

Vanhaecke, P. and Sorgeloos, P., 1980. International study on *Artemia*, IV. The biometrics of *Artemia* strains from different geographical origin, In*:* The brine shrimp *Artemia*. Vol.3. Ecology, culturing and use in Aquaculture, Persoone G., P. Sorgeloos, O. Roels and E. Jaspers (Eds), Universa press, Wetteren, Belgium. pp 357-372.

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