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Original Paper

BIOMETRY OF *Artemia franciscana* FROM THREE DIFFERENT BATCHES

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ABSTRACT

Biometry of Artemia franciscana from three different batches were characterized under laboratory conditions. The results from diameter measurement of cyst from PR, SI and AS batches were 240 µm, 238 µm and 245 µm respectively. The diameter of decapsulated cyst from the same batches were 223 µm (PR), 221 µm (SI) and 220 µm (AS). The length of Instar I nauplii from PR, SI and AS batches were 476 µm, 497 µm and 498 µm. Hatching efficiency after 48 hours of incubation were 2.76 x 10^5 nauplii/g cyst, 2.80 x 10^5 nauplii/g cyst and 2.90 x 10^5 nauplii/g cyst from PR, SI and AS batches. The statistical analysis of the results indicated that there were no significant differences (P=0.1) in diameter of cyst, length of Instar I nauplii and hatching efficiency of cyst. The hatching percentage of cyst from AS batch at 24 hours (89%), and respectively 48 hours (91%), was significantly higher (P=0.1) than those of SI (83% and 88%) and PR (74% and 80%).The hatching percentage of cyst from PR batch was significantly lower (P=0.1) than SI.

Keyword : Biometry, cyst, *Artemia franciscana*

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INTRODUCTION

Most marine fishes and crustaceans in intensive culture require live food during early stages. Collecting or cultural natural food for fish and shrimp larvae is either commercially unfeasible or technically hard to obtain.

Artemia, also known as brine shrimp, is one of live food used widely in larviculture of fish and shrimp. Freshly hatched *Artemia* nauplii is an excellent food source for fish larvae. Normally brine shrimp is used as freshly hatched nauplii, because *Artemia* cysts can be stored for a long period of times in cans, and used as an off-the-self food. To produce *Artemia* nauplii, a very simple procedure can be carried out by incubating the cysts in seawater. Since the brine shrimp *Artemia* has high economic value it is important to analyze the commercial characteristics with regard to their potential for application in aquaculture.

The nutritional effectiveness of a food organism is determined by its ingestability, and as consequence by its size and configuration.

The aim of this study was to compare biometry of *Artemia franciscana* produced from three different lake locations with different water salinities.

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MATERIALS AND METHODS

Source of cysts

In this study, *Artemia franciscana* was collected from three different batches in Great Salt Lake (Utah, USA). The first batch was taken from Promontery Point Bear River (PR) with salinity of about 90 ppt; the second batch from Stansburry Island East Side (SI) with salinity of about 140 ppt; and the last batch from Arm Salt Pump # 1 (AS) with salinity of about 200 ppt.

Biometry of cyst
Diameter of cysts

The diameter of cyst was measured for hydrated untreated- and decapsulated cyst. For each sample, the sum of 500 cysts were examined under microscope equipped with scale.

Hatching percentage and hatching efficiency

For each cyst sample, three cylindroconical glass tubes were set up, each with 1.8 g cysts in 900 ml D&K solution (Sorgeloos, 1980). The glass tubes were incubated at temperature 25 ± 1 ^{o}C and exposed to a light intensity of at least 1000 lux. The cysts were kept in suspension and gently aerated.
After 24 and 48 hours of incubation, six subsamples containing each 250 µl were taken from the cylindroconical glass tubes with an automatic micropipette. Subsamples were fixed with one drop of Lugol solution. After number of nauplii were counted, few drops of hypochlorite solution were added to subsamples to distinguish nauplii to the unhatched embryo's. Two parameters were determined in the following formulas:

Hatching percentage (%) = Number of nauplii x 100 /number of nauplii + number of unhatched embryo's
Hatching efficiency = number of nauplii per gram of cyst

Length of Instar I Nauplii

To measure the length of naupliar size, each sample of cyst from different batch culture were incubated in seawater (35 ppt) at a temperature 25 ± 1 ^{o}C and exposed to a light intensity of at least 1000 lux. After 18 hour-incubation, the number of 300 Instar I larvae were counted using microscope equipped with scale. The larvae were anesthetized with chloroform saturated-seawater in order to prevent naupliar movement.

Reproductive and Lifespan Characteristics

The reproductive performance of *Artemia franciscana* was tested at salinity 100 ppt D&K solution (Sorgeloos, 1980) and at temperature of 25 ± 1 ^{o}C. When males started clasping females at the age of about 15 days, twenty pairs of *Artemia franciscana* from each batches were separated and placed into 50 ml plastic cylindroconical tube containing 50 ml of 0.45 µm filtered D&K medium. The animals were fed once daily with a mixed diet of algal culture *Dunaliella tertiolecta* Butch and yeast cells based formulated feed (Lanzy PZ) in the ratio of 1 to 3. The medium was renewed every 5 days. The tubes were examined daily for survival and off-spring production. The off-spring was harvested from medium by paper filtration and the number was counted. During a period incubation, when one of the male from each pair was death, the new males substituted for the dead male.

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RESULTS AND DISCUSSION

Results

Results of measurement of a mean diameter of cysts with chorion- and decapsulated cyst are shown in **Table 1**.

Table 1. Diameter of cyst with chorion- and decapsulated cyst

Batch	Cysts with chorion(µm)	Decapsulated(µm)
PR	239.7 ± 12.1 [a]	223.3 ± 9.0 [a]
SI	238.4 ± 10.0 [a]	221.3 ± 10.6 [a]
AS	244.8 ± 10.6 [a]	219.6 ± 10.6 [a]

In the same column, values followed by the same letter are not significantly different at P=0.1

The mean diameter of chorionic- and decapsulated cysts varied from 238 to 245 µm and 220 to 223 µm, respectively. The statistical analysis (P=0.1) among means indicated that the cysts produced from different batch (salinity) were not significantly different.

Table 2. shows the means values for the length of Instar I nauplii for the different batches.

The statistical analysis indicated that there were no significantly different (P=0.1) from different batches. The mean length of Instar I nauplii varied from 475 to 498 µm.

Table 2. Length of Instar I nauplii from different batches

Batch	Length of nauplii (µm)
PR	475.94 (41.10) [a]
SI	497.07 (45.7) [a]
AS	497.50 (29.51) [a]

In the same column, values followed by the same letter are not significantly different at P=0.1

The results of hatching efficiency and hatching percentage of cysts are presented in Table 3. The statistical analysis indicated that there were no significantly differences (P=0.1) in hatching efficiency. The hatching efficiency ranged from 2.76 x 10^5 to 2.90 x 10^5 N/g cysts. There were significant differences in hatching percentage among batches from the same strain. The values of hatching percentage ranged from 79% to 91%.

In the same column, values followed by the same letter are not significantly different at P=0.1

Table 3. Hatching efficiency and hatching percentage of from different batches

Batch	Hatching efficiency (N/g csyt)		Hatching percentage (%)	
	24 hours	48 hours	24 hours	48 hours
PR	$2.04x10^5(7.2x10^3)$ [a]	$2.04x10^5(7.2x10^3)$ [a]	74.12 (1.58) [a]	79.57 (0.61) [a]
SI	$2.04x10^5(7.2x10^3)$ [b]	$2.04x10^5(7.2x10^3)$ [a]	82.86 (0.88) [b]	88.26 (0.67) [b]
AS	$2.04x10^5(7.2x10^3)$ [b]	$2.04x10^5(7.2x10^3)$ [a]	88.95 (0.63) [c]	91.18 (0.77) [c]

The reproductive and lifespan characteristics of *Artemia franciscana* among batches are summarized in **Table 4.** The reproductive and lifespan characteristics of Artemia from different batches were not significantly different (P=0.1) except for offspring per female per day.

Discussion

The data obtained demonstrated that there are some differences in biometrical parameters among batches from the same strain (**Table 3 and table 4**).This study indicates that the mean size of cyst varies among batches from the same strain.

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Table 4. Reproductive and lifespan characteristics of Artemia among batches

	PR	SI	AS	LSD (0.1)
A	84.3 (6.8) a	86.5 (7.9) a	91.4 (6.4) a	16.7
B	12.6 (1.4) a	10.5 (1.7) a	12.5 (2.3) a	3.87
C	17.0 (1.4) a	26.5 (6.0) b	20.8 (3.0) a	9.33
D	3.8 (0.4) a	2.9 (0.4) a	3.7 (0.4) a	0.97
E	43.8 (6.2) a	40.5 (6.3) a	42.6 (4.5) a	13.5
F	1195 (148) a	1084 (178) a	1349 (182) a	402
G	20.5 (0.4) a	19.9 (0.5) a	20.2 (0.9) a	1.5
H	65.1 (8.0) a	51.3 (7.8) a	66.9 (8.0) a	18.8
I	6.8 (2.9) a	4.5 (2.8) a	2.5 (0.9) a	5.4
J	92.3 (8.7) a	75.7 (8.8) a	89.8 (8.5) a	20.4

In the same row, values followed by the same letter are not significantly different at P=0.1
A=offspring/brood; B=broods/females; C=offspring/female.day during the reproductive period; D=days between broods; E=percent offspring encysted; F=total offspring/female; G=prereproductive period (days); H= reproductive period (days); I= postreproductive period (days); J=total lifespan (days)

These variations may be due to differences in environmental conditions, especially related to different salinities in the salt ponds (Camargo *et al.*, 2005) It appeared from laboratory test that decreases of salinity from 180 to 90 ppt yielded increases of the diameter of ca.1.5 µm in both hydrated untreated and decapsulated cysts (Vanhaecke and Sorgeloos, 1980). Asem *et al.*, (2007) analyzed *Artemia urmiana* and found a correlation between cyst size and brine density in natural habitat. The present study demonstrated that there was variation in diameter of both hydrated untreated and decapsulated cysts from different batch (**Table 1**). Vanhaecke and Sorgeloos (1980) found that the cyst size was not affected by temperature within the experimental range. Other factors such as variation in food types for the adult or widely separated harvest sites- especially in very large salt ponds- might probably cause small differences in the cyst size among batches. The packaging techniques could also contribute to the small cyst size differences, for example segregation during cans filling or handling. Small differences exist in term of length of nauplii from different batches from the same strain (**Table 2**). Since the length of nauplii originated from AS and SI batches was bigger than 480 µm, it appeared that they could be used as food for fish and shrimp larvae at later stages (Vanhaecke *et al*., 1987) or freshwater larvae. From a practical value with regard to the selection of a food source for larval fishes or crustaceans in aquaculture hatcheries, one might expect that the use of large nauplii with a higher individual organic weight will be beneficial. The predator will spend less energy taking up a smaller number of larger nauplii to fulfill its food demand. This is especially the case for fish larvae which are not very efficient in prey hunting. The beneficial effect of feeding bigger Artemia is apparent from the experimental results of Beck *et al*., (1982): Menidia larvae grew significantly faster on a diet of large nauplii from Margherita di Savoia, Great Salt Lake and Shark Bay as compared to those silverside larvae fed with the smaller nauplii from San Francisco Bay and Macau. In the case the naupliar size is critical for the ingestion mechanism of the predator, better growth might be expected when using small nauplii. The use of a particular or specific Artemia strain may even result in a total failure in culturing a specific predator on brine shrimp

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because of the inability of the predator to ingest this specific Artemia strain.

In feeding trial with newly hatched Menidia larvae: there is a high mortality in the group of fish which were offered large size of nauplii, similar to the one noted for starved fish, was recorded during the first three days of the experiment. After this time, the mortality of fish larvae that fed on large nauplii did not exceed the mortality of those fed smaller size of nauplii.

The small differences in hatching efficiency of cysts (**Table 3**) among batches from the same strain may be due to the exposure of the cysts to suboptimal condition –before harvesting- which can result in mortality of some embryos (Sorgeloos *et al*., 1986; Vanhaecke and Sorgeloos, 1980). The significant differences (P=0.1) exist in hatching percentage (**Table 3**) among batches from the same strain. The hatching percentage can be slightly improved by incubation of cysts in a 5 ppt hatching medium, where the energy consumption is certainly lower. Combination of the highest hatching efficiency and the highest length of nauplii from AS batch can be beneficial for predator where Artemia size is not critical.

Table 4 indicates that different batches have no effect on the life cycle characteristics and length of life. In term of reproductive output (offspring/female per day) there are significant differences between PR batch and SI batch. Gajardo and Beardmore (1989) showed that there is correlation of heterozygosity with the reproductive and life span characteristics.

They mention that "more heterozygous females produces more zygotes, and tend to produce more broods and start to reproduce at a younger age than less heterozygous females". **Table 4** show that life span and reproductive characteristics (except for offspring/female per day) are similar among batches. It means that heterozygosity among batches are similar. Comparison of the results in this study with the results published by Browne *et al.*, (1984) are presented in **Table 5**, and indicate similarity.

Table 5. Comparison of reproductive and lifespan characteristics of *Artemia* from different batches from Great Salt Lake with *Artemia* from San Francisco Bay

	PR		SI		AS		SFB*	
A	84.3	(6.8)	86.5	(7.9)	91.4	(6.4)	111.4	(43.8)
B	12.6	(1.4)	10.5	(1.7)	12.5	(2.3)	13.6	(7.1)
C	17.0	(1.4)	26.5	(6.0)	20.8	(3.0)	27.8	(12.2)
D	3.8	(0.4)	2.9	(0.4)	3.7	(0.4)	4.1	(0.7)
E	43.8	(6.2)	40.5	(6.3)	42.6	(4.5)	28.1	(32.1)
F	1195	(148)	1084	(178)	1349	(182)	1619	(1051)
G	20.5	(0.4)	19.9	(0.5)	20.2	(0.9)	30.5	(9.5)
H	65.1	(8.0)	51.3	(7.8)	66.9	(8.0)	56.2	(29.5)
I	6.8	(2.9)	4.5	(2.8)	2.5	(0.9)	5.2	(5.2)
J	92.3	(8.7)	75.7	(8.8)	89.8	(8.5)	92.8	(23.6)

* data subtracted from Browne *et al.* (1984)
A=offspring/brood; B=broods/females; C=offspring/female day during the reproductive period; D=days between broods; E=percent offspring encysted; F=total offspring/female; G=pre-reproductive period (days); H= reproductive period (days); I= post-reproductive period (days); J=total lifespan (days)

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CONCLUSIONS

From the measurement and analysis, can be concluded:
Different batches have no influence to the diameter of cysts with chorion and decapsulated, the length of Instar I nauplii and hatching efficiency. The diameter of cysts with chorion and decapsulated ranged from 238 to 245 µm and from 220 to 223 µm, respectively. The length of the Instar I nauplii varied from 476 to 498 µm. The hatching efficiency and the hatching percentage of cysts from different batches ranged from 2.76 to 2.90 x 10^5 N/g cyst and from 79 to 91%, respectively.

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