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CHARACTERIZATION OF BRINE SHRIMP *ARTEMIA* FROM SRI LANKA

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Abstract: Biometrical characteristics of *Artemia*, such as naupliar size, determine its acceptability as a 'live feed' by specific-sized larvae in aquaculture hatcheries. In Sri Lanka, a parthenogenetic strain of the brine shrimp, *Artemia parthenogenetica,* occurs naturally in salterns. Biometrics of *Artemia* from different sources were characterized and compared using natural populations from salterns in Palavi (PAL) and Mahalewaya (ML) as well as pond-cultured populations of *Artemia parthenogenetica* (PAL89 and PAL90) and San Francisco Bay *Artemia* (SFB). The hydrated cyst diameter varied from 248.7 ± 0.82µm in PAL90 cysts to 266.4 ± 0.94µm in PAL89 cysts. The decapsulated cyst diameter varied from 236.5 ± 0.76µm in PAL89 cysts to 245.3 ± 0.90µm in PAL cysts. The naupliar length ranged from 423.9 ± 3.01µm in PAL90 nauplii to 437.4 ± 1.84µm in PAL nauplii. Chorion thickness of cysts and the dry weight of nauplius ranged from 3.0 to 14.95µm and from 2.21 to 2.52µg, respectively. Hydrated and decapsulated cyst diameters and the naupliar length of SFB *Artemia* were significantly smaller ($p < 0.05$) than those of Sri Lankan *Artemia* originating from the two different sources. Cyst diameters (both hydrated and decapsulated) and naupliar lengths of natural populations from Palavi were significantly smaller than those from Mahalewaya populations.

The differences in biometrical characteristics of *Artemia* cysts and nauplii reported here for natural and pond-cultured populations in Sri Lanka provide the necessary data to meet the live-feed size requirements of specific fish and larval stages in commercial hatcheries.

Key Words: *Artemia*, biometrics, characterization, chorion thickness, cyst diameter, hatching, naupliar length.

INTRODUCTION

The demand for brine shrimp *Artemia* as a larval feed in aquaculture has led to the exploitation of *Artemia* populations throughout the world for commercial production. Studies on *Artemia* populations by several authors[1-7] led to the realization that the genus *Artemia* consists of several sibling species which are reproductively isolated.

The size of the freshly hatched *Artemia* nauplius (instar I) is of importance in aquaculture hatcheries especially for predatory larval stages of marine fish and shrimps[8] where prey size becomes a limiting factor in ingestion. If the size of *Artemia* as food does not cause ingestion problems, then according to the Optimal Foraging Theory[9,10] it would be beneficial to use larger sized nauplii as the predator would spend less energy in capturing a lesser number of larger nauplii to fulfill its nutritional needs.[7]

In Sri Lanka, a parthenogenetic strain of the brine shrimp, designated *Artemia parthenogenetica*[8] occurs naturally in solar salterns in Palavi (7°59' N, 79°50' E), Hambantota (6°08'N; 81°07'E) and Elephant Pass (9°44'N, 80°19'E)[11] Even though commercial culture of *Artemia* for cyst production offers high potential in Sri Lanka in view of the large quantities of *Artemia* that are imported into the country for use in commercial hatcheries (1993 imports totalled a value of US $ 20,000)[a], commercial cyst production is still not practised. The lack of data relating to biometrics of *Artemia* populations in Sri Lanka has made it difficult for hatchery managers to evaluate how Sri Lankan *Artemia* would perform relative to the accustomed imported *Artemia*, thus making them reluctant to initiate switching over to the local alternative. In turn, potential *Artemia* culturists are prevented from calculating projected market demand for a locally cultured product over a defined time course thereby making them unwilling to invest in commercial culture operations.

The present study presents data to characterise Sri Lankan *Artemia parthenogenetica* using cyst sources both from accessible natural populations and from earthen pond cultured populations. We also present the biometrical characteristics of imported *Artemia (A. franciscana)* arising from their culture in earthen ponds under Sri Lankan field conditions. Further, the biometrical characteristics of the different *Artemia* are compared in the hope that commercial hatcheries would use this data and become more receptive to import substitution and attract potential investors to their commercial culture in our country.

METHODS AND MATERIALS

Cyst material of the Sri Lankan *Artemia* strain were obtained from natural populations in Palavi Saltern (PAL) and from field culture experiments conducted in earthen ponds in Palavi in 1989 (PAL89) and in 1990 (PAL90). Characteristics of cysts from the natural populations at Mahalewaya Saltern[12] (ML) are also included for comparison. Cysts of the bisexual strain (SFB) consisted of those produced in earthen pond culture experiments at Palavi that were inoculated with San Francisco Bay Brand *Artemia* (SFB/SFBB 1157). Cysts from salterns in Elephant Pass are not included since this location is inaccessible due to the security situation in the north of the country.

Cyst preparation: Cysts were cleaned using the density separation technique[13] and air-dried under shade. For measuring the untreated hydrated cyst diameter, cysts were hydrated for two hours at a density of 250 mg cysts/100 ml of 35 ppt salinity sea water and 29 ± 1°C temperature with continuous aeration. The cysts were observed microscopically during the 2 hour period to ensure maximum hydration. After two hours the floating material with broken shells was drained out and the remaining cysts were fixed overnight with a few drops of Lugol solution.[7]

[a]External Trade Statistics, 1993, Sri Lanka Customs.

For measuring length of nauplii, dried *Artemia* cysts were incubated in natural sea water of 35 ppt salinity and 29 ± 1°C temperature under continuous illumination and gentle aeration provided from the bottom. The instar I nauplii were harvested and fixed in Lugol solution.

Decapsulation of cysts was carried out according to the standard procedure described.[8] The decapsulation solution consisted of 35.5 g $Ca(OCl)_2$ (bleaching powder) and 32.5 g Na_2CO_3 in 700 ml sea water.

Microscopic measurements: The diameter of 100 cysts (both hydrated and decapsulated) and the total body length of 100 nauplii were measured microscopically using a calibrated micrometer eyepiece.

Dry weight of nauplii: Dry weight of nauplii was determined in triplicate samples according to described methods.[7] Cysts were decapsulated and hatched as described earlier and rinsed well in distilled water. Five samples of approximately 50,000 hatched nauplii (around 0.25 g wet weight) were introduced to 100 ml distilled water and the number of nauplii in 10 subsamples of 250µl was counted. The nauplii were dried to constant weight at 60°C and the final weight noted as the dry weight of nauplii present in 100 ml volume. The individual weight of a nauplius was calculated as the mean of the number of individuals present in the five samples each in 100 ml distilled water.

Data analysis: Analysis of Variance was used to determine if differences existed among the means of hydrated cyst diameter, decapsulated cyst diameter and naupliar length of the *Artemia* samples, using the ANOVA procedure of SAS (Statistical Analysis Systems, 1985). The Least Significant Difference (LSD) values obtained from t tests were used for separation of means. Correlation coefficients (r) were calculated between hydrated cyst diameter, decapsulated cyst diameter and naupliar length of the *Artemia* and significance was tested at 5% level.

RESULTS

The biometrical characteristics of the *Artemia* populations are presented in Table 1.

In Sri Lankan *Artemia* from different sources, the hydrated cyst diameter (mean ± s.e.) varied from 248.7 ± 0.82µm in PAL90 to 267.9 ± 0.82µm in ML. PAL and PAL90 cyst diameter values were significantly smaller than that of ML. The decapsulated cyst diameter values of Sri Lankan cyst sources were significantly different from each other with ML cysts having the highest value (256.2 ± 0.59µm) and PAL89 having the least diameter (236.5 ± 0.76µm). PAL90 which had the smallest hydrated cyst diameter (248.7 ± 0.82 µm) from among the Sri Lankan cysts, possessed a decapsulated cyst diameter of 242.7 ± 0.65µm which was significantly different and larger than that of the PAL89 cysts.

Table 1: Biometrical characteristics of *Artemia parthenogenetica* from Sri Lanka and *A. franciscana* cultured in Sri Lanka. Measurements are presented in μm ± s.e.

Source	Hydrated cyst diameter (μm)	Decapsulated cyst diameter (μm)	Naupliar length (μm)	Chorion thickness (μm)	Dry weight of nauplius (μg)	Ratio HCD/NL
ML	267.9 ± 0.82[a]	256.2 ± 0.59[a]	475.4 ± 1.62[a]	5.85	2.77 ± 0.05	0.5
PAL	254.9 ± 1.30[b]	245.3 ± 0.90[b]	437.4 ± 1.84[b]	4.80	2.52 ± 0.03	0.58
PAL 89	266.4 ± 0.94[a]	236.5 ± 0.76[c]	434.6 ± 1.65[b]	14.95	2.31 ± 0.03	0.6
PAL 90	248.7 ± 0.82[c]	242.7 ± 0.65[d]	423.9 ± 3.01[c]	3.0	2.21 ± 0.02	0.59
SFB 91	218.4 ± 1.10[d]	206.3 ± 0.89[c]	379.4 ± 2.01[d]	6.0	1.61 ± 0.02	0.57

ML and PAL = natural populations in Mahalewaya and Palavi salterns, respectively. PAL89 and PAL90 = Sri Lankan *Artemia* cultured in Palavi experimental ponds in 1989 and 1990, respectively. SFB = *A. franciscana* cultured in Palavi in 1991. Values with the same superscript are not significantly different ($p > 0.05$). HCD = Hydrated cyst diameter, NL = Naupliar length.

Mean naupliar length in ML (475.4 ± 1.62 μm) was significantly larger than that of all other sources. PAL 90 possessed the smallest naupliar length ($p < 0.05$) from among the Sri Lankan *Artemia*. There was no significant difference between naupliar lengths of PAL89 and PAL. The hydrated cyst diameter (218.4 ± 1.10μm), decapsulated cyst diameter (206.3 ± 0.89μm) and the naupliar length (379.4 ± 2.01μm) of the cultured SFB *Artemia* were significantly smaller than corresponding sizes of Sri Lankan *Artemia*.

The chorion thickness was greatest in PAL89 (14.95μm) while in PAL 90, the chorion was only 3.0μm thick. The chorion thickness of the SFB cysts was 6.0μm while the dry weight of SFB nauplius was 1.61μg.

The correlations between the naupliar length and selected biometrical characteristics of the Sri Lankan Artemia are presented in Table 2. The r values reveal that there exists a highly significant correlation between the naupliar length and other characteristics such as the hydrated cyst diameter, decapsulated cyst diameter and the individual naupliar dry weight of the Sri Lankan *Artemia* from the different sources. The highly significant correlation between hydrated cyst diameter and naupliar length resulted in the ratio between them maintaining a constancy (Table 1, last column).

Table 2: Correlation between biometrical characteristics of Sri Lankan *Artemia*.

Correlation	r value
Hydrated cyst diameter - naupliar length	0.916
Decapsulated cyst diameter - naupliar length	0.946
Naupliar length - naupliar dry weight	0.941

DISCUSSION

The two natural populations of Sri Lankan *Artemia* from Palavi and Mahalewaya salterns showed significant differences with respect to the reported biometrical characteristics. Differences between characteristics of naturally occurring populations are explained in terms of geographic isolation.[7] The Mahalewaya salterns are located 335 km southeast (as measured along the main coastal roadway) of Palavi Salterns. The differences in the biometric data indicate that the *Artemia* populations of these two salterns are geographically isolated from each other.

Although cyst sizes were similar in ML and PAL89, we found the naupliar length to be significantly different. This can be explained by differences in their chorion thickness which was almost thrice in PAL89 when compared to that of ML. Removal of this thick chorion in PAL89 resulted in a reduced decapsulated cyst diameter which was significantly correlated (Table 2) to a reduced naupliar length.

Apart from variations among geographical strains of *Artemia*, variations may also be expected among batches of the same strain.[7] Such a variation was demonstrated between PAL89 and PAL90 cysts, even though the inoculum for the 1990 culture consisted of PAL89 cysts produced in these same ponds in the previous year. The differences related to culture conditions and period may be the responsible factors leading to biometrical differences among the Palavi strains. This possibility, however, was not examined in Mahalewaya, since culture experiments using different culture conditions were conducted only in Palavi. PAL89 was cultured from September to October 1989 (prior to the onset of the rainy season) and PAL90 from April to July 1990 (during the dry season). PAL89 culture was carried out using inorganic fertilizer while organic fertilizer was used for PAL90 culture. High salinity conditions (168ppt) at commencement of cyst production in PAL89 culture may have caused the Artemia to produce extremely resistant cysts carrying a thick chorion. As opposed to this, cyst production commenced at a low salinity of 124ppt in PAL90 culture and the *Artemia* produced cysts possessing a thinner chorion of 3.0μm.

Reports on the biometrics of various *Artemia* strains reveal that the differences within strains are very small in comparison to the large variations among strains.[7] For example, two batches of *Artemia* (TUT 1 and TUT 2) from India-Tuticorin had hydrated cyst diameters of 283.8μm and 282.9μm, respectively. This was also true for some biometrical characteristics among the Palavi strain such as the absence of a significant difference between the naupliar length of PAL and PAL89 (Table 1).

The present findings indicate that the cysts of Sri Lankan *Artemia* are much smaller than the cysts of the Indian strain but the China *Artemia* had a cyst diameter of 267.0μm[7] which is similar to that of the ML cysts.[12] Though a large cyst size is associated with parthenogentic strains (e.g. Italy, France and India[7]; Milos-Greece[2]; Portuguese strains[5]), the Sri Lankan strain possesses cysts that are towards the lower end of the parthenogenetic cyst size range.

With regard to the biometrics of the inoculated SFB strain, observations are in keeping with those of other authors who found no significant difference between the parental SFB strain and those originating from inoculations in either Brazil or the Philippines.[7]

The naupliar lengths of Sri Lankan *Artemia* in the present study were found to be smaller than values for parthenogenetic strains of China (493 ± 37.2μm, 1SD) and Margherita de Savoia, Italy (517 ± 29.5μm, 1SD).[7] The length of 434.6μm of PAL89 freshly-hatched nauplii was smaller than most values for bisexual Portuguese strains[5] but was comparable to that of San Pablo Bay (USA) *Artemia*.[7]

A highly significant correlation has been found between the naupliar length and both hydrated and decapsulated cyst diameters and the individual naupliar dry weight of the *Artemia* in the present study. Similar correlations have been found between untreated hydrated cyst diameter and naupliar length ($r = 0.864$),

between decapsulated cyst diameter and naupliar length (r = 0.906) and between naupliar length and individual naupliar dry weight (r = 0.945).[7] The individual naupliar dry weights observed in this study are in conformity with those reported by the above authors for other parthenogenetic strains.

The Sri Lankan *Artemia* was completely parthenogenetic with no males found in the samples analyzed throughout this study. From the present study, the parthenogenetic *Artemia* existing in Palavi and Mahalewaya can be said to belong to two geographically isolated strains while the differences observed in the cultured Sri Lankan *Artemia* at a given location can be attributed to varying environmental conditions in ponds that existed during the culture periods.

The size of the freshly hatched nauplius of *Artemia* is important when larval stages of marine fish and shrimps are fed in commercial hatcheries.[8] The existence of significantly different sizes of *Artemia* nauplii among the Sri Lankan brine shrimp, as reported here, should serve as an impetus for the development of the brine shrimp industry in Sri Lanka, because such findings could be developed further to culture commercially selected Sri Lankan strains having naupliar sizes appropriate for feeding specific life history stages of fish and shrimp species.

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