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Biometrical, morphological and biochemical characterization of three *Artemia* (Crustacea: Anostraca) populations from South India

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Abstract

The aim of the present study is to enumerate variations in terms of size, growth, morphology and biochemistry among South Indian *Artemia* populations namely Puthalam, Thamaraikulam and Tuticorin. Cyst biometrics and hatching performances of the three *Artemia* populations were studied to enumerate the possible differentiation among them. A detailed post-embryonic development from cysts to adults, and morphological study with adults of two parthenogenetic (Puthalam, Thamaraikulam) and one bisexual (Tuticorin) populations of the brine shrimp *Artemia* was performed. Multivariate Discriminant Analysis was carried out to separate and/or group together populations which exhibit great similarities. The quantitative estimation of the biochemical constituents such as protein, lipid and sugars, of the populations were studied following standard procedures. Biometrical and hatching characteristics revealed distinct variation among the three populations which could be taken as a tool to discriminate the populations. Post embryonic development was observed to complete by 13 stages in Tuticorin population, 17 stages in Puthalam population and Thamaraikulam population, showing morphological and functional changes. Morphometric analysis showed that *Artemia* population from Tuticorin is morphologically divergent whilst the other two parthenogenetic populations partially overlap each other. The three populations of *Artemia* remain separated by the Discriminant Function, which has the total length, number of setae in a furca, eye diameter, head width and length of the furca as the morphological characteristics contributing most to the observed differences and contributing to the separation of parthenogenetic and the bisexual populations. Significant variations were observed in the relative concentrations of total proteins, total lipids, free cholesterol, glycoproteins and total free sugars among the populations with the Tuticorin population having higher concentration of all the biochemical constituents. This multi-trait approach for *Artemia* characterization is stressed as a way to obtain better descriptions and interpretations of the biological diversity in the genus.

Key Words: Biometry, post-embryonic development, morphometry, Discriminant Analysis, biochemical characterization

Introduction

The brine shrimp *Artemia* is a cosmopolitan Anostracan. The genus is a complex of species and superspecies defined by the criterion of reproductive isolation (Browne and Bowen, 1991). Salt lakes and brine ponds with *Artemia* populations are found all over the world (Vanhaecke *et al*., 1987; Triantaphyllidis *et al*., 1998) with exception of Antarctica (Claus *et al*., 1977). *Artemia* could thrive in saline and hypersaline environments from 30 to 350 $g.L^{-1}$; these water bodies are mainly located in very dry areas worldwide (Vanhaecke *et al*., 1987; Triantaphyllidis *et al*., 1998). The high salinities of these biotopes, together with other abiotic characteristics, make them extremely rigorous environments. These scattered and relatively isolated biotopes offer suitable conditions for maximal inter-population differentiation and ecological isolation (Abreu-Grobois, 1987; Bowen *et al*., 1988). The brine shrimp *Artemia* is one of the very few invertebrates that have the striking ability to adapt, live and reproduce in such extreme habitats, regulation the osmotic and ionic concentration of their tissues. Favored by the absence of predators and food competitors, it mostly develops into very dense populations. This anostracan branchiopod has a striking ability to live and reproduce in hypersaline waters which are mainly located in very dry areas worldwide (Vanhaecke *et al*., 1987; Triantaphyllidis *et al*., 1998). These scattered and relatively isolated biotopes offer suitable conditions for maximal inter-population differentiation and ecological isolation (Abreu-Grobois, 1987; Bowen *et al*., 1988). Except for Amat's unpublished data on the presence of the exotic *A. franciscana* in India, all the records in this country belong to parthenogenetic *Artemia* (Van Stappen, 2002). Nevertheless, relatively few systematic attempts have been performed to characterize the pattern of variation on *Artemia* populations in South India.

Artemia is a suitable organism to study the evolutionary processes and the modes of speciation in particular (Browne and Bowen, 1991), i.e. the populations exhibit a pronounced tendency for the development of local adaptations. The fact on the presence of *Artemia franciscana* in Tuticorin salterns as per previous reports is of great importance, since the invasion of this species as exotic invader in different areas of the world has provoked the complete replacement of the native *Artemia* forms. Quantitative analysis of reproductive and life span characteristics of *Artemia* has given insight into these features that have been difficult to test with other animals not amenable to cultivation.

Abatzopoulos *et al*. (1989; 2003; 2006) have studied the cyst characteristics of *Artemia* from Northern Greece and Iran and also studied the effect of salinity and temperature on reproductive and life span characteristics of clonal *Artemia*. The effect of salinity on maturation and life span characteristics has been well documented in four Egyptian *Artemia* populations (Baxevanis *et al*., 2004; El-Bermawi *et al*., 2004). Camargo *et al*. (2003; 2004; 2005) have determined the influence of external factors on biomass, cyst production and morphometry of *A. franciscana* from Colombian Caribbean. Recently, Agh *et al*. (2009) have studied the morphometric and genetic characteristics of *Artemia* from Iran. From post-diapause cysts of *Artemia franciscana*, Wu *et al*. (2011) have defined fourteen LEA (late embryogenesis abundant) and LEA-like genes, including four novel members (Afrlea1-5, Afrlea3-5, Afrlea3-like1 and Afrlea3-like2), which were classified into four groups: G1, G3, G3-like (LEA group3-like), and SMP-like (seed-maturation-protein-like), based on their conserved and diversified sequence motifs and amino acid compositions among bacteria, plants, and animals.

The decapsulated cysts of *Artemia* combine the physical properties of a dry artificial feed and the nutritional value of live *Artemia* nauplii (Lim *et al*., 2002). Subsequent studies demonstrated that decapsulated cysts are a good feed similar to freshly hatched *Artemia* nauplii for the larvae of marine shrimps, freshwater prawns and lobsters (Bruggeman *et al*., 1980). Sandoval *et al*. (1993) have studied the biochemical composition of whole cysts of *Artemia franciscana* from San Francisco (USA), San Francisco Bay Brand (USA), Baja California (Mexico) and Sonora (Mexico) to describe

their nutritional values. Garcia *et al*. (2011) have studied the nutritional efficacy of different preparations of decapsulated *Artemia* cysts for *Tinca tinca* larvae.

The nutritional value of decapsulated cysts might be sensitive to the quality of the decapsulation. During the decapsulation process the cysts are hydrated: as a consequence, the interrupted metabolism of the *Artemia* embryo resumes (Garcia-Ortega *et al*., 1998). This might result in a food with a biochemical and nutritional composition that diverges from both cysts and nauplii. Hines *et al*. (1980) followed the biochemical composition during the development of both cysts and nauplii. Moraiti-Ioannidou *et al*. (2009) have assessed the biochemical composition during naupliar development of *Artemia* sp. from Greece. Nguyen Thi *et al*. (2009 a,b) have studied the effect of different supplemental feeds and assessed the role of *Artemia* biomass as a fishmeal replacement in terms of their protein composition for the freshwater prawn, *Macrobrachium rosenbergii*. Using the smaller-sized Vietnam *Artemia franciscana* (AF) population instead of the Great Salt Lake *A. franciscana* population, Nhu *et al*. (2009) have shown that the rotifer-feeding period could be shortened with 3 days, resulting in significant improvements in larval survival and growth. This study verified the possibility to feed umbrella-stage *Artemia* for further shortening and eventually completely substituting rotifer start feeding. The possibility to use umbrella-stage *Artemia* has opened an opportunity to simplify the rearing protocol and to reduce production costs of cobia larviculture.

Rasawo and Radull (1986) have reported *Artemia* as a source of high protein content (40%). Dietary amino acids are of primary importance for successful growth. Bellini (1960) measured total free amino acids from hydration through several hours after hatching in *Artemia*. Dutrieu (1960) did a semi-quantitative survey of free amino acids in encysted embryos, nauplii and adults. In both studies, free amino acids increased steadily until emergence. Carbohydrate is used as a primary energy source, such it is recognized in nutritional studies of aquaculture (Whyte *et al*., 1989). Aquatic organisms are good sources for unsaturated fats that are essential for growth and survival (Bransdena *et al*., 2005). In the present study, geoeographically isolated and ecologically-different (coastal vs. inland) populations of the brine shrimp *Artemia* from Puthalam, Thamaraikulam and Tuticorin have been used to compare developmental, morphological differentiation and biochemical constituents among populations in order to evaluate variability among them.

Material and Methods

Samples studied

Artemia cysts were collected from the salt pans located at Puthalam (09^{o} 83'N 79^{o} 30'E), Thamaraikulam (09^{o} 39'N 78^{o} 30E) and Tuticorin (08^{o} 48'N 78^{o} 11'E), South India. Though these populations experience similar temperature range (25-30^{o}C), Puthalam and Thamaraikulam are inland salterns characterized by salinity range of 30-50 ppt, while Tuticorin salt pan, referred as a coastal site, presents a salinity range of 100-130 ppt. Cysts of the different populations were collected, washed with salt water, brought to the laboratory and subjected to washing with tap water to separate viable cysts. The viable cysts that settled at the bottom were flushed out through a fine sieve of 200 µm mesh. The cysts were then dried at 28 ± 2^{o}C and stored in airtight vials for further studies.

Biometry and hatching

For cyst biometric studies, about 200 *Artemia* dry cysts collected from Puthalam, Thamaraikulam and Tuticorin were measured under light microscope, Labex, India. About 200 *Artemia* cysts of each populations were counted and subjected to hypochlorite (10%) decapsulation treatment and kept for hatching in hatching medium (natural sea water) at a salinity of 60 ppt. The cysts were then observed for hatching and the hatching percentage, and efficiency were calculated by the following formulae and results tabulated.The time (in hours) spent from the beginning of incubation until the appearance of the first nauplii is given as T_0, and time taken for the hatching of 10% cysts is given as T_{10} and that of 90% cysts as T_{90}.

$$\text{Hatching percentage} = \frac{\text{Total number of cysts hatched}}{\text{Total number of cysts}} \times 100$$

Hatching efficiency = Total number of nauplii / gram of cyst

Hatching synchrony (Ts) = T_{90}- T_0

Laboratory culture experiments

After 24 hrs of hatching, nauplii were transferred to one liter cylindroconical glass tubes containing filtered sea water (60 ppt) for post-embryonic developmental and morphological studies. Initial density was one animal per 2 ml while from day 8 onwards it was reduced to one animal per 4 ml. The animals were kept under mild aeration, 25±1°C with 12 hr cool white fluorescent lighting daily. Each population had three replicates and the animals were fed with *Chlorella.*

Post-embryonic development

For post-embryonic development, a staging scheme based on the appearance of appendage rudiments in the thoracic segments was established that allows a fast and reliable method to classify the larvae (Benesch, 1969). In the present study, Instars are defined on the basis of thoracic and abdominal segmentation, where the development of the genital organs such as gonopods and brood pouch occurs together with the development and maturation of the thoracic limb buds. The post embryonic development was studied, and the observations were recorded using a CCD Microimaging Systems (Korea), at varying magnifications.

Morphometrical analysis

Cysts of each population were collected from the collection sites, subjected to hatching, reared in the laboratory and cultured until maturity, under standardized conditions following the procedure of Gajardo *et al.* (1998). For morphological measurements, as many as 60 adult animals of different populations were fixed in acetic acid (50%) to avoid the possible shrinkage. The morphometrical parameters such as abdominal length, abdominal width, distance between the compound eyes, eye diameter, head width, length of furca, number of setae per furca, total length, and abdominal to total length ratio were measured under a dissection microscope equipped with a camera lucida, Leica, Germany.

Biochemical characterization of cysts and nauplii

Artemia cysts (100 mg) were decapsulated with a solution of Sodium hypochlorite (10%) according to Sorgeloos *et al*. (1986). For biochemical characterization, cysts were incubated in natural sea water at 30 ppt salinity. Six different incubation times were used: 1, 6, 11, 16, 21 and 25 hrs which encompasses early embryo development, cyst hatching to early nauplii. Nauplii were allowed to attain adult stage and the nutritional composition of the females was also assessed.

Estimation of total proteins

Estimation of total proteins was done by the methodology of Bradford (1976). The colour developed was measured spectrophotometrically at 595 nm using a UV-Visible Shimadzu UV-160A Spectrophotometer, Japan.

Estimation of free amino acid nitrogen

Estimation of free amino acids was done according to Yemm and Cocking (1955). The colour developed was measured spectrophotometrically at 570nm.

Estimation of glycogen

Estimation of glycogen was done according to Carroll *et al.* (1956). The resultant colour complex was measured spectrophotometrically at 620 nm.

Estimation of total free sugars

Estimation of total free sugars and glycoproteins were done according to Roe (1955). The resultant colour complex was measured spectrophotometrically at 620 nm.

Estimation of total lipids

Estimation of total lipids was done according to Barnes and Blackstock (1973). Extraction of lipids from each sample was done following the procedure of Folch *et al.* (1957). The mixture was allowed to stand for 30min. and the colour developed was read with spectrophotometer at 520 nm.

Estimation of free cholesterol

Estimation of free cholesterol was carried out following Kabara (1957). The colour complex developed was read spectrophotometrically at 620 nm.

Estimation of phospholipids

Estimation of phospholipids was carried out according to Rouser *et al.* (1970). The colour complex developed was read spectrophotometrically at 700 nm.

Statistical analysis

Morphometrical data were processed with a Discriminant Function Analysis with the statistical package SPSS (Version 10) (Sokal and Rohlf, 1981; Hontoria and Amat, 1992 a,b); this multivariate procedure computes a series of new variable (Z1, Z2, ……..) which are linear functions of the morphometrical parameters considered in the form of Zn = l1X1 + l2X2 + …… (where ln are the calculated Discriminant coefficients and Xs the variables being considered). Discriminant Analysis was carried out separately on females, while the origin of each population was used as separation criterion. A stepwise variable selection method was applied based on the criterion of minimizing Wilks' lambda. Thus, at each step the variable that minimizes the overall Wilks' lambda is entered. Each entry or removal of a variable is considered a step. The rationale was to identify relationships between the qualitative criterion variable (population) and the quantitative predictive variable (morphometrical character) and to determine the boundaries between the three studied populations (Kachigan, 1986).

The results of biochemical analysis were subjected to Analysis of Variance (ANOVA) to reveal any significant difference in the biochemical constituents of the different *Artemia* populations.

Results

Biometry and hatching

Dry cysts collected from Tuticorin showed the higher cyst diameter among the three populations (236.39 ± 8.51 µm), followed by Puthalam and Thamaraikulam (Tab. 1). Also, Tuticorin cysts had a higher hatching percentage of 96.84 ± 2.12% and hatching efficiency of 187,500 nauplii/gm. The appearance of the first nauplii (hatching rate) fluctuates from a minimum of 16 hr after incubation for Tuticorin cysts, up to a maximum of 19 hr from Puthalam and Thamaraikulam (Tab. 1).

Post-embryonic development

The observed pattern of segmental and appendage development of *Artemia* illustrates clear variations among different populations of the present study (Tab. 2a-2d). Excystment of the embryo takes place by shell breaking in two phases. The embryo gradually emerges; head protrude first and still enclosed inside the transparent hatching membrane after 18-24 hrs of cyst hydration. After 6-8 hrs, the hatching membrane ruptured at the region near to the head and the embryo hatched out, leaving membrane and shell behind. In a fully emerged embryo, the body was not yet divided into head and trunk. However, the first three pairs of head appendages are present in rudimentary form. In subsequent stages of development, trunk, genital and abdominal segments begin to form.

Instar I (400-500mm in length) (Stage 0) had a brownish-orange colour and a red naupliar eye in the head region and three pairs of appendages, i.e., the antennule, the antennae and mandibles. The Instar I larva does not take up feed and hence its digestive system is non functional; it relies completely on its yolk reserve. After about 8 hrs from hatching, Instar II is formed, which feeds on small food particles, ingested into the digestive tract which is now functional. The larva grows further and body segment and appendages gets differentiated.

Table 1 Cyst biometrics and hatching characteristics of three *Artemia* populations from South India

	Puthalam	Thamaraikulam	Tuticorin
Mean cyst diameter (N= 200)	225.14 ± 8.17 µm*	219.94 ± 8.78 µm *	236.39 ± 8.51 µm*
Hatching percentage (%) (6 replicates)	48.50 ±3.01*	62.42 ± 4.98*	96.84 ± 2.12*
Hatching efficiency (nauplii/gm) (3 or 6 replicates)	97,920 ± 102*	134,540 ± 52*	187,500 ± 74*
Hatching rate (hrs)***			
T_0	19	19	16
T_{10}	22	20.5	17
T_{90}	33.5	29	23.5
Ts	11.5	8.5	6.5

* (mean ± SD)

**Values refer to the time (in hours) spent from the beginning of incubation until the appearance of the first nauplii (T_0), until the hatching of 10% cysts (T_{10}) and of 90% (T_{90}) of hatching efficiency of three replicates. Ts = T_{90} – T_0, is a measure of hatching synchrony.

Table 2a. Chart showing the post embryonic development of *Artemia* strain from Tuticorin

21	42	67	87	103	117	137	155	180	211	234	274	309	331	347	381	Hours of development
1/2	3/4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Instars
0	0+	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Stages
	◘	¤						♦								T1
	◘		¤						♦							T2
	◘			¤						♦						T3
	◘				¤						♦					T4
		◘				¤					♦					T5
			◘				¤					♦				T6
				◘				¤				♦				T7
					◘				¤				♦			T8
						◘				¤			♦			T9
							◘				¤			♦		T10
								◘				¤			♦	T11
									◘						▼	G1
										◘					▼	G2
											◘				●	A1
											◘				●	A2
												◘			●	A3
												◘			●	A4
													◘		●	A5
													◘		●	A6

◘ Segment formation
¤ Thoracic limb bud formation
♦ Completion of thoracopods
▼ Completion of gonopods and brood pouch
● Completion of abdominal segments
T1 – T11 (First 11 thoracic segments)
G1 & G2 (Genital segments)
A1 – A6 (Abdominal segments)

Table 2b. Chart showing the post embryonic development of *Artemia* strain from Puthalam

22	46	68	91	110	136	165	191	214	235	251	263	291	324	349	373	397	431	451	Hours of development
1/2	3/4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Instars
0	0+	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Stages
	◘	¤						♦											T1
	◘		¤						♦										T2
	◘			¤						♦									T3
	◘				¤						♦								T4
		◘				¤						♦							T5
			◘				¤						♦						T6
				◘				¤						♦					T7
					◘				¤						♦				T8
						◘				¤						♦			T9
							◘				¤						♦		T10
								◘				¤						♦	T11
									◘									▼	G1
										◘								▼	G2
											◘							●	A1
												◘						●	A2
												◘						●	A3
													◘					●	A4
													◘					●	A5
														◘				●	A6

◘ Segment formation
¤ Thoracic limb bud formation
♦ Completion of thoracopods
▼ Completion of gonopods and brood pouch
● Completion of abdominal segments
T1 – T11 (Thoracic segments)
G1 & G2 (Genital segments)
A1 – A6 (Abdominal segments)

The body segments are considered established by externally visible transverse constriction separates it off from the posterior non-segmented budding zone. Prior to being established, the presumptive thoracic appendages and genital organs go through a definite number of rudiment stages. While thoracic rudiments are formed at a rate of one per stage, the rudiments for segments 12 to 19 appear twice as fast. Processes of genital and abdominal segmentation differ from those in the thorax. In the genital phase of development while thoracic growth is interrupted, the 12th segment when formed is larger than thoracic segments. All abdominal segments at any given time have the same size, but their segmental stages differ serially by one stage. During abdominal phase of development, the thoracic segments undergo development.

Segment formation and limb bud development in thorax

Thoracic segments 1st, 2nd, 3rd and 4th are externally visible in stage 0+ and 5th thoracic segment appear in stage 1 (Instar 5), 6th in stage 2 (Instar 6), etc., and 11th in stage 7 (Instar 11). The observed pattern of segments and appendages development of the three *Artemia* populations considered in the present study revealed minor

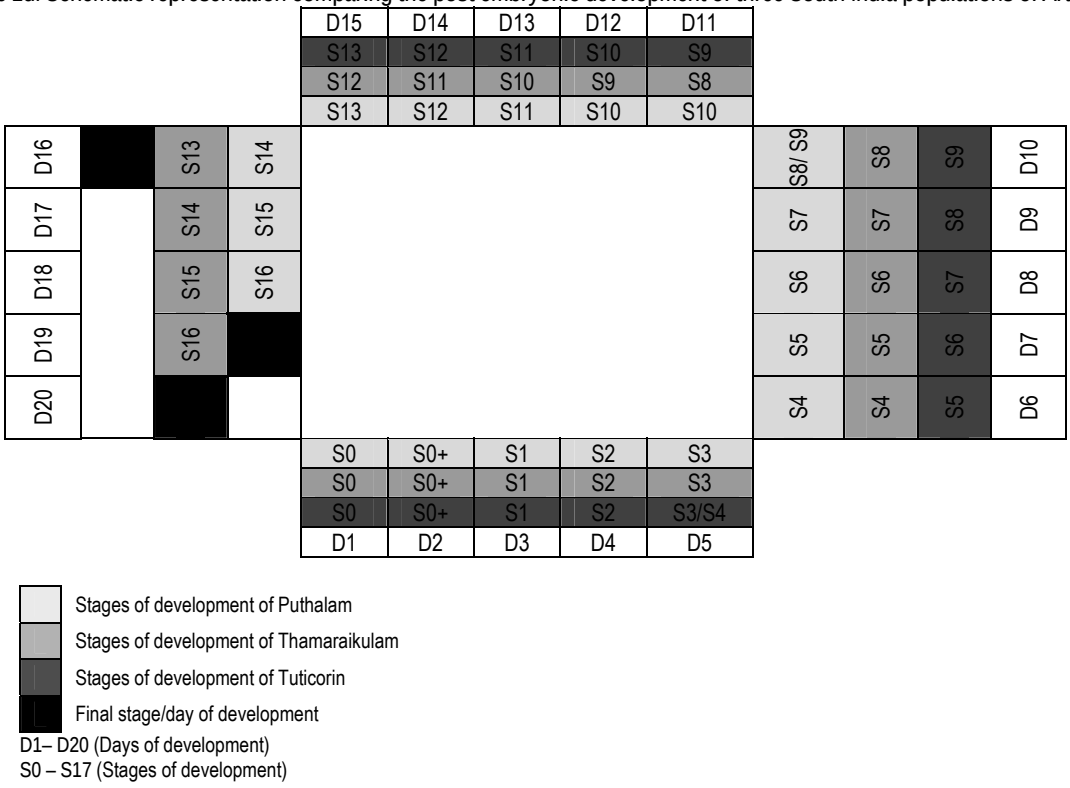
variations. The time spent to complete the formation of the 11 thoracic segments and the 2 pregenital thoracic segments was named the thoracic phase of larval development. At this phase, all the thoracic segments are very similar, and in subsequent stages thoracic limb bud growth are observed. In each stage, the developing thoracic limb attains a characteristic external and internal development anterio-posteriorly. For instance, the principal feature of the first 2 ensuing stages of the 1st thoracic limb bud is growth and elongation of appendage bud. In stage 3 a definite posterior curvature appears and in the next stage, smooth outline of the bud is lost, slight elevations indicating the position of future endites and exites appear, the rudimentary limb bud bears a few bristles. The leg at this stage may execute occasionally jerky and uncoordinated movements. Rhythmic motion is initiated only stage 5 just as the segment matures and gnathobase bears bristles. Further development of the mature appendage takes place till stage 7 consisting principally of a general increase in size. The exites enlarge considerably and endites also grow relatively fast and the proximal part of the gnathobase assumes a horizontal position. Thus the 1st thoracic limb bud formed in stage 1 attain full complements as a motile appendage by stage 7, 2nd formed in stage 2 matures in stage 8, etc. The thoracic segments are said to be mature only by the formation of thoracic limb buds and their independent motility as appendages. Mature thoracopods after this stage of development can be differentiated into three functional parts namely the telopodites and endopodites and the membranous exopodites.

Table 2c. Chart showing the post embryonic development of *Artemia* strain from Thamaraikulam

23	48	71	95	119	143	159	182	206	232	269	298	320	344	378	396	420	449	472	Hours of development
1/2	3/4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Instars
0	0+	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Stages
	◘	¤						♦											T1
	◘		¤						♦										T2
	◘			¤						♦									T3
	◘				¤						♦								T4
		◘				¤						♦							T5
			◘				¤						♦						T6
				◘				¤						♦					T7
					◘				¤						♦				T8
						◘				¤						♦			T9
							◘				¤						♦		T10
								◘				¤						♦	T11
									◘									▼	G1
										◘								▼	G2
											◘							●	A1
												◘						●	A2
												◘						●	A3
													◘					●	A4
													◘					●	A5
														◘				●	A6

◘ Segment formation
¤ Thoracic limb bud formation
♦ Completion of thoracopods
▼ Completion of gonopods and brood pouch
● Completion of abdominal segments
T1 – T11 (Thoracic segments)
G1 & G2 (Genital segments)
A1 – A6 (Abdominal segments)

Table 2d. Schematic representation comparing the post embryonic development of three South India populations of *Artemia*



Genital organs attain maturity, 5-6 stages after being laid down, viz., in stages 13-14 in Tuticorin and after 9 stages viz., in stages 16-17 in Puthalam and Thamaraikulam respectively. By the time, segmental maturity is attained, the segments show enlarged and fully developed gonads which extend through several segments and visible through the transparent body wall. In female, the median ends of the rudimentary oviducts fuse across the midline and at the point of juncture continuity is established with the distal portion of the reproductive duct and forms a median uterus, formed medially from the 13th segment which lies within a ventrally projecting sac, the rudimentary brood pouch. At the caudal tip of the gradually enlarging brood pouch a fairly large opening connects the developing uterine cavity to the outside. In the later stages, the yolk glands are formed and in the final stages, the sacs of the yolk gland fill up most of the free space in brood pouch and eggs are present. In male, the medial end of the duct on each side links up into a short tube in 13th segment and together they constitute the rudimentary vas deferens on that side. The most caudal portion of the latter has a ventrolateral finger-like outgrowth, the penis. At the tip of each penis the vas deferens opens to the outside. At this stage, it is proper to regard the animal as adult.

Appendage formation in head

Antennules: In Instars 1 and 2, the antennules

are short finger-like rods, freely projecting forward form the antero-lateral head surface. They become motile in stage 1 and increase in size in subsequent stages.

Antennae: The antennae, attaining their great relative size during the early larval stages of larval development, are motile in Instars 1 and 2. Set ventro-laterally into the head, they acquire a broad base by the stage of 5-6. The exopodites develop into the larger and functional more significant branch.

Mandibles: Mandibular development was well underway in Instars 1 and 2. The protopodite is directed ventrally and connects distally with mandibular palp. The gnathobase is small and conical. By stage 4 and in the following stages distinct toothing was apparent which increased in complexity with further development.

Maxillae: Both pairs of maxillae are well developed in Instars 1 and 2. By stage 4, the more anteriorly situated buds of the first maxillae have developed into short, sac-like outgrowths. In the following stages, the appendages enlarge assuming a stubby, finger-like shape. Maxillary motion is limited externally and the appendage, as a whole is slightly considered vestigial. Second maxillae (maxillulae) develop into short, triangular appendages and seen situated posterior-medially to the first pair of maxillae. A number of bristles are present at the tip. In stage 5, the final form of maxillulae is attained; the maxillulae always stay small and are completely vestigial.

Comparative studies on post embryonic development indicated that stage 13 of Tuticorin population, stage 17 of Puthalam population and Thamaraikulam population, showed important morphological and functional changes, i.e. the antennae lost their locomotory function and underwent sexual differentiation by the complete transformation of the male antennae 2 into hooked graspers (claspers), accessory structures for the process of mating in males, and the reduction of the female antennae 2 into unisegmented sensorial appendages.

Segment formation in abdomen

Rudiments of abdominal segments 13, 14, 15, 16, 17, 18 and 19 (A1, A2, A3, A4, A5 and A6) are laid down from stage 10 onwards. In Tuticorin population, rudiments of segments 13th and 14th occurs in stage 10, 16th and 17th in stage 11 and 18 and 18th and 19th in stage 12. Whereas in Puthalam and Thamaraikulam populations, segment 14th is laid down in stage 10, 15th and 16th in stage 11 and 17th and 18th in stage 12 and 19th in stage 13. The last abdominal segment develops furca at its posterior end. Complete formation and growth of abdominal segments is accomplished in the final stage of development. Anterior abdominal segments are larger than more posterior ones. Since no appendages develop, abdominal segments can be considered mature on complete growth.

Clear differences were observed in the furcal morphology of three *Artemia* populations. After complete development each population had its own characteristic shape as verified with 25 specimens from each population (Fig.1). The furcal groove between both furcae is narrower in Puthalam population than in Thamaraikulam and Tuticorin. This gradual post-embryonic development is completed at a faster rate in stage 14 (day 16) for Tuticorin population whereas it takes a bit longer time of 19 and 20 days at stage 17 in Puthalam and Thamaraikulam, respectively.

Morphological analysis

Discriminant Analysis was used in a number of studies based on *Artemia* morphological characteristics with very promising results. The mean values of the adult morphometric characters of the three populations are presented in Tab. 3. Wilks' Lambda resulted in F ratios, the significance of which revealed highly statistical differences ($P<0.0001$). The variables that present the larger F ratios are the total length, head width, distance between eyes, abdominal length to total length ratio, abdominal length, abdominal width and eye diameter.

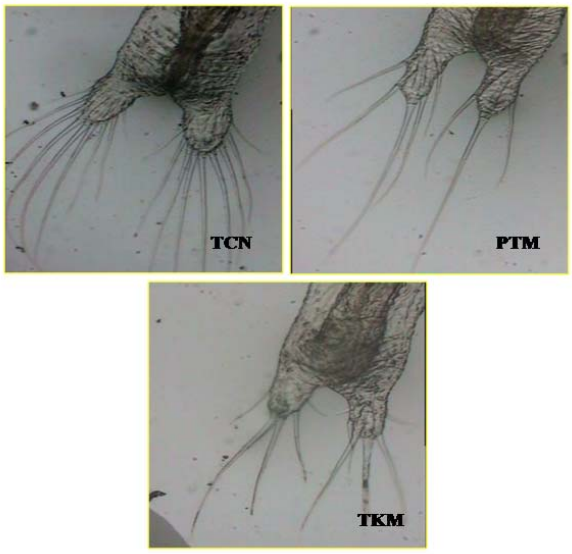


Fig. 1: Furcal morphology of different populations of *Artemia*
(TCN –Tuticorin, PTM – Puthalam, TKM – Thamaraikulam)

Table 3: Mean values (mm) of morphological characters of three South Indian parthenogenetic and bisexual *Artemia* populations

Characters	Puthalam	Thamaraikulam	Tuticorin	F ratio	Wilks' ambda	F probability
AL	6.32±0.20	6.37±0.29	5.96±0.31	11.195	0.547	<0.001
AW	0.49±0.04	0.51±0.06	0.58±0.03	10.325	0.567	<0.001
DE	1.83±0.95	1.82±0.89	1.72±0.84	20.665	0.395	<0.0001
ED	0.34±0.11	0.35±0.08	0.40±0.07	8.504	0.614	<0.01
HW	0.96±0.44	0.94±0.38	0.83±0.32	27.743	0.327	<0.0001
LF	0.25±0.08	0.25±0.04	0.21±0.03	4.039	0.770	<0.05
SF	8.59±1.02	8.70±0.11	8.67±0.12	2.136	0.863	n.s.
TL	11.70±4.80	11.84±4.71	11.99±5.01	60.971	0.181	<0.01
AL/TL	53.98±1.7	53.86±2.43	49.74±0.67	18.169	0.426	<0.001

Mean ± SD of 10 observations
Abbreviations:
AL: Abdominal length, AW: Abdominal width, DE: Distance between the compound eyes, ED: Eye diameter, HW: Head width, LF: Length of furca, SF: Number of setae per furca, TL:Total length, AL/TL: ratio abdominal and total length (%).
n.s. - not significant

Discriminant Analysis based on the origin of each population as a separate criterion resulted in 2 canonical Discriminant Functions. The two functions that appear in Tab. 4 resulted in 95.4% and 100% of variance, respectively. In Tab. 5, the standardized canonical Discriminant Function coefficients are also presented. The three populations of *Artemia* remain separated by the first Discriminant Functions, which have total length, number of setae in the both furca, eye diameter, head width and length of the furca as the morphological characteristics contributing most to the observed differences. On the other hand, the second Discriminant Function, with abdominal length, distance between the compound eyes, abdominal width and abdominal/total length ratio as relevant variables, contributes to the separation of parthenogenetic Thamaraikulam population and the bisexual Tuticorin population (Tab. 3). Using

morphometric characters each population can be classified correctly in one of three groups with an overall accuracy of 90% (Tab. 6). Predictability for the populations was 100% and the respective values for the two inland parthenogenetic populations were 80% and 90% respectively, showing their closeness (Tab. 6). Fig. 2 displays scatter plot depicting morphological relationships and degrees of separation between populations (Centroids provided for each population).

Table 4: Results of the Discriminant Analysis on three South India *Artemia* populations showing the percentage of the variance accounted for the two Discriminant Functions generated

	Eigen value	Percentage of variance	Cumulative percentage	Canonical correlation	Wilks' lambda	Chi-square	df	P
Function 1	7.596	95.4	95.4	0.940	0.085	64.023	6	<0.0001
Function 2	0.365	4.6	100	0.517	0.733	8.089	2	<0.05

Table 5: Standardized coefficients for the two Discriminant Functions in nine traits used for the morphological analysis of three South India *Artemia* populations

Characters	Function 1	Function 2
Abdominal length	0.300	0.632*
Abdominal width	-0.005	-0.256*
Distance between the compound eyes	0.438	0.448*
Eye diameter	-0.351*	-0.198
Head width	0.324*	0.000
Length of furca	-0.230*	-0.147
Number of setae per furca	0.360*	-0.158
Total length	-0.758*	0.637
Ratio abdominal/ total length	0.406	0.542*

* Largest absolute correlation between each variable and any Discriminant Function

Table 6: Classification results of Discriminant Analysis showing the percentage of individuals of three South Indian populations, classified in each group

Actual group	Number of cases	Predicted Group Membership (%)		
		Puthalam	Thamaraikulam	Tuticorin
Puthalam	10	80.0	20.0	0
Thamaraikulam	10	10.0	90.0	0
Tuticorin	10	0	0	100.0

(The diagonal elements are the number of cases classified correctly into the groups and serve as an indicator of the effectiveness of the Discriminant Analysis. The percent of 'grouped' cases correctly classified is 90%)

Biochemical composition

The protein, lipid and carbohydrate compositions of different times of incubation of the embryos until hatching are given in Tab. 7. The relative compositions of glycoproteins, total lipids and cholesterol were significantly higher in decapsulated cysts than in any other stage of postembryonic development (P<0.0001).

Total protein

Results showed a gradual decrease in the total protein content ($P<0.0001$) from decapsulated cysts to 25 hrs incubated cysts and adults (Fig. 3a). In Puthalam population, the total protein decreased from 54.33 ± 3.15 mg/g dry weight in decapsulated cysts (0 hr) to 52.61 ± 3.02 mg/g dry weight after 6 hr of incubation. Gradual decrease was observed from 51.77 ± 3.24 mg/g dry weight at 11 hr (umbrella stage) to 49.12 ± 3.14 mg/g dry weight at 25 hr (I Instar nauplii). Similar trend was noticed in the Thamaraikulam and Tuticorin populations decreasing from 55.27 ± 2.02 and 59.33 ± 2.99 mg/g dry weight in decapsulated cysts to 50.63 ± 2.07 and 55.50 ± 3.15 mg/g dry weight in Instar I nauplii.

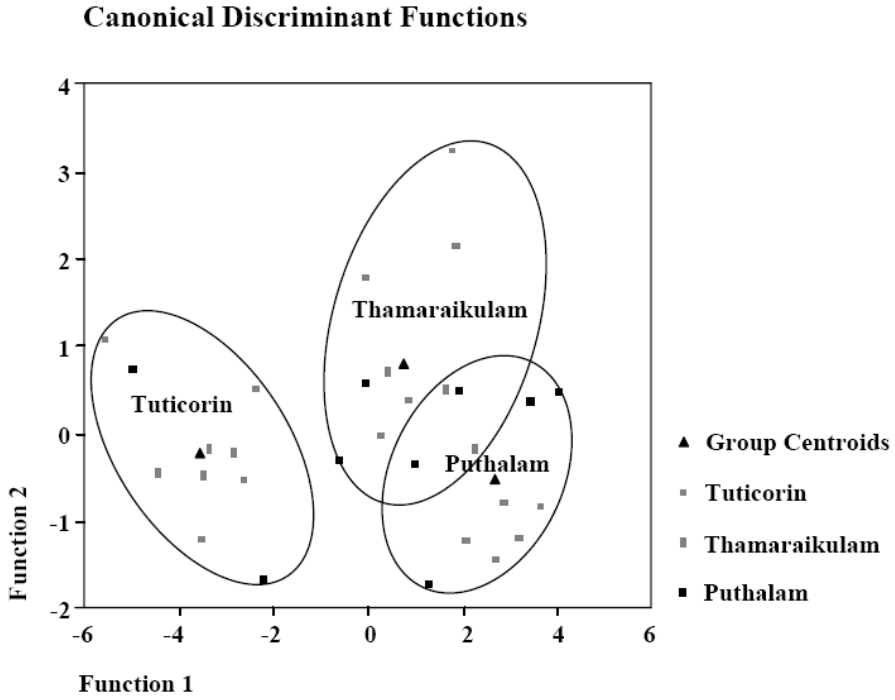


Fig. 2: Scatterplot of individual Function scores in *Artemia* samples from South India

Total free amino acid nitrogen

A steady increase in free amino acid nitrogen levels was noticed after 1 hr incubation from 0.51 ± 0.03, 0.60 ± 0.02 and 1.02 ± 0.06 µg/mg dry weight to 0.68 ± 0.03, 0.79 ± 0.04 and 1.48 ± 0.03 µg/mg dry weight, till 21 hr in Puthalam, Thamaraikulam and Tuticorin populations, respectively (Fig. 3b). The total free amino acid nitrogen levels began to decrease significantly thereafter to Instar I nauplii (25 hr) and in adults ($P<0.001$).

Glycogen

Glycogen, the storage form of sugar, amounted to 0.080 ± 0.02, 0.166 ± 0.01 and 0.250 ± 0.03 mg/g dry weight in decapsulated cysts of Puthalam, Thamaraikulam and Tuticorin populations (Fig. 3c). In the first hour of incubation, the glycogen content increased marked to 4.27 ± 0.11, 4.52 ± 0.28 and 6.27 ± 0.45 mg/g dry weight respectively and then decreased gradually ($P<0.05$).

Total free sugars

Total free sugars showed a pattern similar to the glycogen profile in all the populations (Fig. 3d). An upsurge was noticed from 4.53 ± 0.17, 5.00 ± 0.29 and 6.46 ± 0.45 mg/g dry weight after 1 hr incubation to 28.57 ± 2.25, 29.62 ± 2.45 and 38.14 ± 3.12 mg/g dry weight. Dramatic decrease was noticed in cysts after 6 hr incubation to Instar I nauplii and also in adults (P<0.0001).

Glycoprotein

A gradual decline in the glycoprotein levels was observed in all the populations studied (Fig. 3e). The decapsulated cysts stand ahead in the glycoprotein profiles i.e., 29.27 ± 2.37, 30.16 ± 2.57 and 39.16 ± 3.11 mg/g dry weight in Puthalam, Thamaraikulam and Tuticorin populations respectively. Statistically significant results show a decreasing trend upto the Instar I nauplii, while higher glycoprotein levels were recorded in the adults (P<0.0001).

Total lipids

The total lipid profile during development showed a gradual decrease during post embryonic development in all the populations studied (Fig. 3f). Significant decrease in the lipid profile was observed from 5.384 ± 0.17, 5.946 ± 0.22, 7.121 ± 0.36 mg/g dry weight in decapsulated cysts to 0.48 ± 0.06, 0.98 ± 0.02 and 0.84 ± 0.06 mg/g dry weight in Instar I nauplii of all the populations respectively (P<0.0001). Adults had a greater proportion when compared to the different hours of incubation of cysts and decapsulated cysts (5.606 ± 0.13, 6.370 ± 0.28 and 7.359 ± 0.41 mg/g dry weight).

Free cholesterol

The cholesterol level increased significantly from 2.50 ± 0.15, 2.30 ± 0.28 and 3.74 ± 0.34 mg/g dry weight in decapsulated cysts to 0.025 ± 0.01, 0.096 ± 0.01 and 0.159 ± 0.02 mg/g dry weight in the Instar I nauplii (P<0.0001) in Puthalam, Thamaraikulam and Tuticorin populations respectively (Fig. 3g). This shows a similar trend to the decreasing levels of total lipids among populations. Adults of Tuticorin populations were found to have a higher cholesterol level of 02.76 ± 0.21 mg/g dry weight followed by Puthalam (02.12 ± 0.14 mg/g dry weight) and Thamaraikulam (01.81 ± 0.06 mg/g dry weight) populations (P<0.0001).

Phospholipids

Significant increase in the levels of phospholipids were observed from 0.176 ± 0.01, 0.246 ± 0.01 and 0.323 ± 0.02 mg/g dry weight in decapsulated cysts, through hatching to 0.259 ± 0.01, 0.302 ± 0.02 and 0.371 ± 0.01 mg/g dry weight in Instar I nauplii was observed in Puthalam, Thamaraikulam and Tuticorin populations respectively (P<0.05) (Fig. 3h).

Variations in the biochemical constituents among populations:

Analysis of variance studies revealed significant variations in the biochemical constituents among the different populations of *Artemia*. Amongst the populations, Tuticorin population was observed to have higher concentration of all the biochemical constituents than Puthalam and Thamaraikulam populations (Tab. 7). Variations in the concentrations of the biochemical constituents between the populations were highly significant (P<0.0001).

To summarize, the relative concentrations of total proteins, total lipids, free cholesterol, glycoprotein and total free sugars showed highly significant variations (P<0.0001) during the different hours of incubation among populations, than that of free amino acid nitrogen (P<0.001), phospholipids and glycogen (P<0.05). A steady decline was observed in the biochemical constituents viz., total proteins, glycogen, free sugars, lipids and cholesterol, from 1 hr to 25 hrs of development followed by gradual raise in adults.

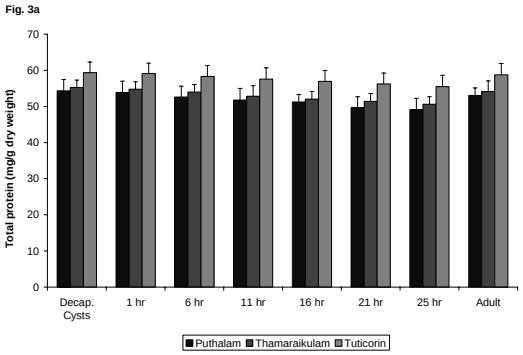


Fig. 3a: Total protein content in cysts (different hours of development) and adults of different *Artemia* populations

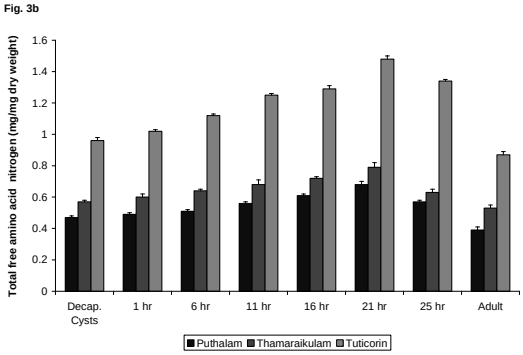


Fig. 3b: Total free amino acid nitrogen content in cysts (different hours of development) and adults of different *Artemia* populations

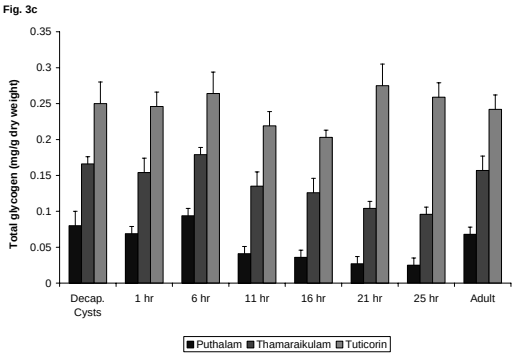


Fig. 3c: Total glycogen content in cysts (different hours of development) and adults of different *Artemia* populations

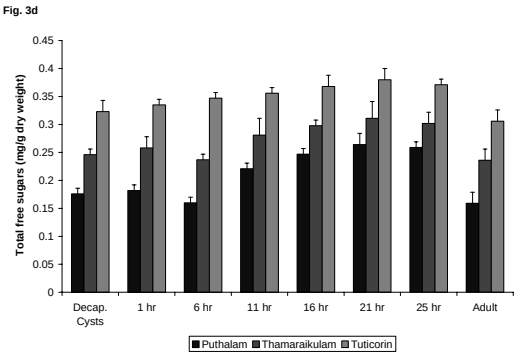


Fig. 3d: Total free sugar content in cysts (different hours of development) and adults of different *Artemia* populations

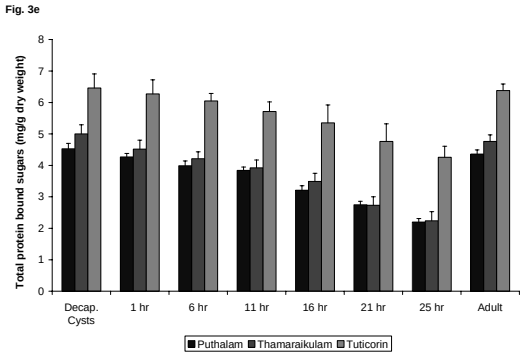


Fig. 3e: Total protein bound sugar content in cysts (different hours of development) and adults of different *Artemia* populations

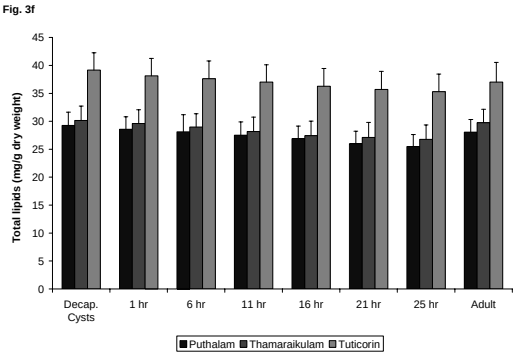


Fig. 3f: Total lipid content in cysts (different hours of development) and adults of different *Artemia* populations

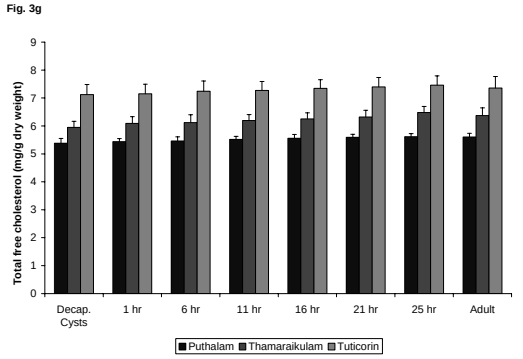


Fig. 3g: Total free cholesterol content in cysts (different hours of development) and adults of different *Artemia* populations

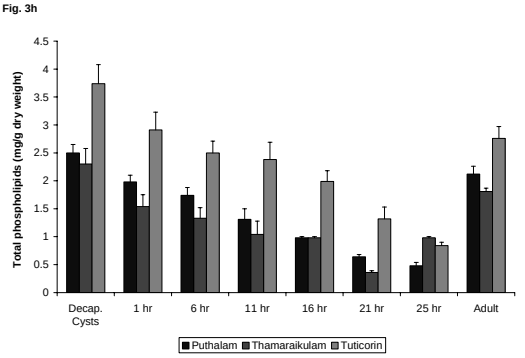


Fig. 3h: Total phospholipid content in cysts (different hours of development) and adults of different *Artemia* populations

Table 7. Biochemical composition during different hours of development and adults of several South India *Artemia* populations

		Total protein (mg/g dw)	Total free aminoacid N (µg/mg dw)	Total Glycogen (mg/g dw)	Total free sugars (mg/g dw)	Total glycoproteins (mg/g dw)	Total lipids (mg/g dw)	Total Free cholesterol (mg/g dw)	Total Phospholipids (mg/g dw)
Puthalam	Decapsulated cysts	54.33 ±3.15	0.47 ±0.02	0.080 ±0.02	4.53 ±0.17	29.27 ±2.37	5.384 ±0.17	2.50 ±0.15	0.176 ±0.01
	Time of development 1 hr	53.87 ±3.12	0.51 ±0.03	4.27 ±0.11	28.57 ±2.25	5.438 ±0.11	1.98 ±0.12	0.069 ±0.01	0.182 ±0.01
	6 hr	52.61 ±3.02	0.49 ±0.03	3.99 ±0.15	28.12 ±3.05	5.464 ±0.15	1.74 ±0.14	0.094 ±0.01	0.160 ±0.01
	11 hr	51.77 ±3.24	0.56 ±0.04	3.84 ±0.11	27.52 ±2.38	5.518 ±0.11	1.31 ±0.19	0.041 ±0.01	0.221 ±0.01
	16 hr	51.21 ±2.09	0.61 ±0.04	3.21 ±0.14	26.89 ±2.24	5.557 ±0.14	0.98 ±0.02	0.036 ±0.01	0.247 ±0.01
	21 hr	49.68 ±3.01	0.68 ±0.03	2.75 ±0.11	26.01 ±2.21	5.594 ±0.11	0.64 ±0.04	0.027 ±0.01	0.264 ±0.02
	25 hr	49.12 ±3.14	0.57 ±0.02	2.20 ±0.11	25.47 ±2.17	5.616 ±0.11	0.48 ±0.06	0.025 ±0.01	0.259 ±0.01
	Adult	53.02 ±2.12	0.39 ±0.02	0.068 ±0.01	4.36 ±0.13	28.05 ±2.28	5.606 ±0.13	2.12 ±0.14	0.159 ±0.02
Thamaraikulam	Decapsulated cysts	55.27 ±2.02	0.57 ±0.03	0.166 ±0.01	5.00 ±0.29	30.16 ±2.57	5.946 ±0.22	2.30 ±0.28	0.246 ±0.01
	Time of development 1 hr	54.77 ±2.04	0.60 ±0.02	4.52 ±0.28	29.62 ±2.45	6.090 ±0.24	1.54 ±0.21	0.154 ±0.02	0.258 ±0.02
	6 hr	53.98 ±2.09	0.64 ±0.04	4.21 ±0.22	28.97 ±2.38	6.121 ±0.28	1.33 ±0.19	0.179 ±0.01	0.237 ±0.01
	11 hr	52.81 ±2.94	0.68 ±0.03	3.92 ±0.25	28.16 ±2.58	6.197 ±0.21	1.04 ±0.24	0.135 ±0.02	0.281 ±0.03
	16 hr	52.02 ±2.14	0.72 ±0.03	3.49 ±0.26	27.42 ±2.61	6.252 ±0.22	0.98 ±0.02	0.126 ±0.02	0.298 ±0.01
	21 hr	51.41 ±2.15	0.79 ±0.04	2.73 ±0.27	27.11 ±2.69	6.324 ±0.24	0.36 ±0.03	0.104 ±0.01	0.311 ±0.03
	25 hr	50.63 ±2.07	0.63 ±0.02	2.24 ±0.29	26.77 ±2.57	6.480 ±0.22	0.98 ±0.02	0.096 ±0.01	0.302 ±0.02
	Adult	54.12 ±2.97	0.53 ±0.04	0.157 ±0.02	4.76 ±0.21	29.75 ±2.42	6.370 ±0.28	1.81 ±0.06	0.236 ±0.02
Tuticorin	Decapsulated cysts	59.33 ±2.99	0.96 ±0.07	0.250 ±0.03	6.46 ±0.45	39.16 ±3.11	7.121 ±0.36	3.74 ±0.34	0.323 ±0.02
	Time of development 1 hr	59.12 ±2.87	1.02 ±0.06	6.27 ±0.45	38.14 ±3.12	7.154 ±0.34	2.91 ±0.32	0.246 ±0.02	0.335 ±0.01
	6 hr	58.34 ±2.97	1.12 ±0.05	6.05 ±0.24	37.63 ±3.18	7.241 ±0.37	2.50 ±0.21	0.264 ±0.03	0.318 ±0.01
	11 hr	57.57 ±3.12	1.25 ±0.05	5.71 ±0.31	37.01 ±3.11	7.274 ±0.32	2.38 ±0.31	0.219 ±0.02	0.356 ±0.01
	16 hr	56.94 ±3.01	1.29 ±0.04	5.35 ±0.57	36.28 ±3.19	7.343 ±0.31	1.99 ±0.19	0.203 ±0.01	0.368 ±0.02
	21 hr	56.24 ±2.99	1.48 ±0.03	4.76 ±0.56	35.71 ±3.23	7.398 ±0.34	1.32 ±0.21	0.175 ±0.03	0.380 ±0.02
	25 hr	55.50 ±3.15	1.34 ±0.06	4.26 ±0.35	35.29 ±3.15	7.460 ±0.33	0.84 ±0.06	0.159 ±0.02	0.371 ±0.01
	Adult	58.75 ±3.14	0.87 ±0.07	0.242 ±0.02	6.38 ±0.21	37.02 ±3.51	7.359 ±0.41	2.76 ±0.21	0.306 ±0.02

(Mean ± SD of 6 observations)

Discussion

This study offered an opportunity to expand the knowledge on conditions that promote morphological and post-embryonic differences in the genus *Artemia* under laboratory conditions. Hatching percentage, hatching efficiency and hatching rate are important criteria in the evaluation of the overall hatching quality of *Artemia* cyst samples. These parameters are not only a function of the geographical origin of cyst material but are to a very large extent marked by harvesting, processing and storage and hatching conditions. In order to ensure optimal use of *Artemia* in aquaculture hatcheries, the hatching characteristics of a new batch of cysts should be determined prior to use (Vanhaecke and Sorgeloos, 1980). Differences between *Artemia* populations in diapause deactivation sensitivity might be related to variations in habitat conditions or related to the reproductive strategy of the population. The differences in hatching results of Puthalam, Thamaraikulam and Tuticorin populations support the idea of local adaptation. According to Brown and Carpelan (1971), temperature and oxygen levels are the most important variables in humid climates, while osmotic pressure and oxygen are more relevant in arid regions. The great intrapopulation variations among the different populations of *Artemia* in cyst diameter, observed in this study, may be caused by the effects that different physical and chemical conditions may have on ovigerous females. Vanhaecke and Sorgeloos (1980) have found that temporal variations in food conditions and salinity of the ponds could affect the diameter of the cysts.

Variations in period of growth have been found occur between the geographical populations studied. Compared to Puthalam and Thamaraikulam populations, Tuticorin population showed faster growth by completing its development by day 16. It was first suggested by Baid (1963) that the density of the medium in which *Artemia* lives is specific to a particular body form, i.e., stronger the brine, the relatively longer and thinner the abdomen. As soon as a variation takes place in the conditions of mass and time, together constituting the growth-rate pattern, a change in the differentiation pattern is also found to occur (Weisz, 1947). Vanhaecke and Sorgeloos (1980) have observed in their study that *Artemia* populations that grew significantly faster were bisexual. However, Gilchrist (1960) reported that a parthenogenetic population grew faster than a bisexual *Artemia* population. Gilchrist (1960) worked on *Artemia* of different localities and suggested that growth and form of *Artemia* is influenced by a number of intrinsic factors when animals are reared under standard conditions and also varies with size, sex and stock of animals. Amat (1980) also revealed that the increase in salinity reduced the furca length and the number of setae in the furca. Similar observations were recorded in the populations of the Dominican Republic (Tejeda, 1987).

Effective overall morphological discrimination based on adult body characteristics was observed between all the three *Artemia* populations studied by Discriminant Analysis. Characteristics such as total length, number of setae in a furca, eye diameter, head width and length of furca contributed much to the Discriminant Function between all the three populations studied. Populations of the present study could be correctly placed in one of the three groups with an overall accuracy of 90%. Multivariate analysis showed that the Tuticorin population appeared to be more 'isolated' from the other populations. Moreover, the statistical analyses on the morphometry of females revealed that Puthalam population is morphologically closer to the Thamaraikulam population. The two parthenogenetic populations reveal around 10-20 % overlapping in their morphological characters. Taking into account that they are geographically close there is good evidence that these populations might be of the same origin.

Parthenogenetic populations have been discriminated from females of bisexual populations as demonstrated by Triantaphyllidis *et al*. (1995, 1997 a,b) for *A. franciscana* and a parthenogenetic population from Tanggu (People's Republic of China). By applying Discriminant Analysis, the morphological differences between parthenogenetic and bisexual females allowed allocation to the correct species with almost 100% accuracy and in salinities ranging from 35-180 ppt (Triantaphyllidis *et al*., 1995, Bernawi *et al*., 2004). Similar results were accounted with the present study, with the

parthenogenetic females being discriminated from the bisexual females of South India with 100% accuracy. Therefore, the comparison of this phenotypic information with any sort of genetic data should provide more biologically useful interpretations.

Comprehensive literature reviews on the use of *Artemia* as live food in fish and shellfish larviculture has been published by Leger *et al*. (1986) and Sorgeloos *et al*. (1998, 2001). Unlike algae or rotifers, different life stages of *Artemia* are used as larval food; from the embryonic form (as decapsulated cysts), through non-feeding nauplii and enriched nauplii, to adult biomass. Since *Artemia* is a non-selective filter-feeder with a relatively high ratio of gut content to body volume, its composition is highly dependent on its diet (Sorgeloos *et al*., 1986).

Decapsulated *Artemia* cysts have been tested successfully for larval rearing of African catfish (Verreth *et al*., 1987; Lim *et al*., 2002) and common carp (Vanhaecke *et al*., 1990). An important application of the decapsulated cysts is direct feeding to the predator, in which case even low-hatch or no-hatch cysts can be used for feeding. Studies have also demonstrated that decapsulated cysts are a good feed similar to freshly hatched *Artemia* nauplii for the larvae of marine shrimps and freshwater prawns such as *Metapenaeus monocerous, Penaeus monodon, Penaeus indicus, Macrobrachium rosenbergii* (Bruggeman *et al*., 1980). While, Garcia-Ortega *et al*. (1998) have enumerated a gradual decrease in the protein content due to the steady utilization of these essential ingredients in the metabolic resumption of the cysts to evolve into fully developed nauplii and adult, the results of the present study also demonstrate the clear utilization of protein for development. Increased level of proteins in adults could be contributed to those attained through feeding. Evjemo and Olsen (1999) have reported that the growth in terms of Individual dry weight and carbon content, ingestion rate, growth rate and production rate of different developmental stages of *A. franciscana* was strongly influenced by the food concentration.

Total free amino acid nitrogen as in the present study has been reported to increase gradually from decapsulated cysts to 21 hr of development which could be augmented to be the result of protein catabolism. Clegg and Lovallo (1977) reported an increase in the free amino acid pool during cyst hydration as the result of a proteolytic degradation of yolk platelets. Gulbrandsen *et al*. (2009) have studied the post mortem proteolysis of *Artemia franciscana* in terms of free amino acid (FAA) concentrations at several temperatures to indicate potential nutritive value. Results indicated that the most pronounced proteolysis took place at 20 °C which could be due to considerable autolytic proteolysis and represent a significant contribution to FAAs for energy supply and protein synthesis.

The importance of carbohydrate metabolism in the emergence process in *Artemia* has been reported by Clegg (1964). About 98% of the total carbohydrate in *Artemia* cysts consists of the dissacharide trehalose, the polyol - glycerol and polysaccharide glycogen besides glucose (Clegg and Conte, 1980). When the dormant embryo is rehydrated under appropriate conditions, its trehalose level immediately begins to fall, decreasing essentially to zero by the time the nauplius is fully formed as observed in *Artemia* (Dutrieu, 1960). It has also been reported that trehalose oxidation is the major and probably the only source of energy during the transition of the dormant embryo into the nauplius in *Artemia* (Clegg, 1964). Clegg (1964) reported that in *Artemia*, most of the trehalose gets converted into glycogen, which is then catabolism to glucose and energy. Decrease in glycogen content after 1 hr of incubation till hatching has been observed in the present study. Based on measurements of Alcohol soluble carbohydrates (ASC), Clegg and Campagna (2006) proposed that the disaccharide trehalose, critical for desiccation tolerance in many animal cells, has been maintained in the metabolic repertoire of *Parartemia* whose cysts have well developed tolerance to severe desiccation than *A. franciscana*.

From the results of the present study it would appear that the prime importance of lipids might essentially be that of a food reserve, providing energy for metabolic preparation at the induction of

anhydrobiosis, energy for ongoing metabolism during dessication or an available food source upon resumption of development (Womersley, 1981). The results also suggest the role of high lipid contents in cysts is essentially that of a food reserve and is gradually depleted during resumption of development. This explains the significant decrease of total lipid content of cysts during development. This together with the decrease in protein content reflects the need to feed the nauplii with microalgae/yeast when *Artemia* are being used as food for fish larvae (Garcia-Ortega *et al*., 1998).

The phospholipids provide energy whenever there is a need, besides their role in the formation of two messenger molecules like diacylglycerol, arachidonic acid etc. by hydrolysis. These second messenger molecules are essential for cell signaling during development (Barritt, 1992). Considering the importance of lipids, a steady decrease in the level of lipids during gradual rehydration and the accumulation of phopholipids during dormancy in the cysts of the different *Artemia* populations implies that they have an important role in maintaining the viability of encysted embryos of *Artemia* (Perona *et al*., 1987).

The differences in carbohydrate and lipid profile that are observed among the three populations here considered can reflect either genetic characteristics or the lipid profile of the food of the parental population (Triantaphyllidis *et al*., 1995; Han *et al*., 2000). However, it is considered to be environmentally rather than genetically determined, as several authors have demonstrated that the lipid profile of *Artemia* adults and their offspring clearly reflects the composition of the parental diet, regardless of the population (Lavens *et al*., 1989; Navarro and Amat, 1992). Aquaculturists have noticed highly significant differences when using different batches of *Artemia* from the same geographical origin (Leger and Sorgeloos, 1984). In particular, protein and lipid profiles seemed to differ widely from population to population (Leger *et al*., 1987).

The resumption of metabolism inside the cysts requires mobilization and utilization of these biochemical constituents for the growing embryo (Helland *et al*., 2000). Thus till the Instar I nauplii stage, there happens to be a steady decrease in biochemical constituents. Instar II and stages onwards develop their own feeding, their nutritional efficacy mainly rely on the feed provided. Hence adults have comparatively higher proportion of the biochemical constituents than developmental stages to repair, mate and reproduce. More important differences might be expected for other factors such as the structure and digestibility of proteins and the food intake stimulation. Additional information in this regard is required to define the factors that influence the better utilization of live food by the fish larvae when compared to artificial diets.

Conclusion

Biometrical and post embryonic development studies would provide the basic information on the size, hatching parameters and the period taken to attain sexual maturity and naturally those populations with greater biometry and faster development could be chosen for the day to day aquaculture industry. Our results verify the role of Discriminant Analysis to characterize a population and it can be used for preliminary population allocation, assignation and identification of the populations. This method also allows todiscriminate morphometrically, genetically different populations, in the present work, parthenogenetic ones from bisexual populations. Knowledge on the nutritional requirement of the *Artemia* populations would help to formulate a satisfactory diet for the initial feeding larvae of fishes and prawns after their transition from endogenous to exogenous feeding. Biochemical studies revealed higher level of all the biochemical constituents in Tuticorin cysts followed by Thamaraikulam and Puthalam. Higher lipid content in the cysts of South Indian populations would have a tremendous effect on the survival and stress resistance for the consumer organisms. Compared with the use of nauplii and adult, direct feeding of decapsulated cysts would be cheaper, alleviate the heavy work load in hatchery operations, as the labour intensive nauplii and adult production is no longer necessary. Furthermore, the coastal *Artemia* populations (Tuticorin) remain suitable to aquaculture needs both

in terms of size, growth and biochemical composition than the inland populations (Puthalam and Thamaraikulam).

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