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Culture of Vinhchau strain of *Artemia franciscana* Kellogg, 1906 (Crustacea: Anostraca) in Pakistan

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Abstract

The paper presents comprehensive information on the *Artemia* culture under the local climatic conditions. The work has been carried out in three 1-acre ponds during 2007-2008 in the coastal area of Ghorabari, Pakistan. Two strains of *Artemia*, native parthenogenetic and bisexual Vinhchau (VC) strain (Vietnam) were assessed under the laboratory conditions for inoculation on the basis of their reproductive performance, life span characteristics and the cyst production capability. VC strain showed an overall better reproductive performance and cyst production than the native parthenogenetic strain, hence was selected for inoculation. The inoculation density of 50 nauplii / liter in three semi-intensive ponds (1-acre size) yielded average 12.789 ± 1.31 kg wet cysts with no significant variation among three replicate ponds. The average survival was 63 ± 5. *Artemia* reached to adult stage in 10-11 days. Average size of male and female was 6.5 – 7 mm (n=10) and 8-9 mm, respectively. The nauplii production occurred during approximately first three days which followed by cyst production. The number of cysts varied from 6 to 257 in the brood pouch of a single female. The maximum number of cysts found in 3rd and 4th week of culture, which declined progressively from fifth week onwards. Individual observation on brood size revealed that the number of cysts varied from 15-75 per brood in the initial four broods; maximum cyst production of >200 cyst/brood occurred from 5th to 8th brood, i.e., the number increased progressively from 1st to 8th brood which declined gradually in the later broods. This study revealed that the suitable period for *Artemia* production may be from September-October to April-May (7-8 months) based on the annual temperature regime.

Keywords: *Artemia* inoculation, culture, cysts production

Introduction

Artemia are found in large saline water bodies naturally and the cysts with a worth of millions of dollars are harvested each year in considerable quantities from many countries like USA, Australia, China, Canada, India, and Iran. On the other hand in Vietnam, Thailand, Philippines and other Southeast Asian countries, natural populations of *Artemia* are not found and it is being cultured in man made coastal salt ponds. These countries have been able to produce hundreds of metric tons of superior quality *Artemia* cysts and biomass (Brazil, Vietnam, and Thailand). In Vietnam, major stimulus for *Artemia* inoculation was the larviculture of *Macrobrachium rosenbergii*; whereas, in Thailand it was the use of cyst and biomass in larval and nursery phases of penaeid—shrimp, *Penaeus monodon*.

The Vinhchau strain has been originated from *Artemia franciscana* in Vietnam and after almost 20 years has developed a temperature tolerance better than the original SFB strain (Hoa, 2002-2003). The developed characteristics were thought to be helpful in colonization of VC strain in other localities. The cyst of VC strain has been considered of excellent quality for its smaller size, hatching and nutritional qualities, hence, was selected for inoculation in Pakistan based on initial assessment. The present work is a part of studies to produce live feeds (*Artemia* and microalgae) for newly emerging shrimp farming activities in the country.

The VC strain was assessed in comparison to the native parthenogenetic strain for its suitability under the local climatic conditions prior to the inoculation. The native strain was recorded from the coastal salt works of Korangi Creek in 1991 (Sultana et al, 1991). *Artemia* found seasonally in the saltworks, appeared in October and disappeared in May (Sultana et al, 2000) each year till 1997. The population failed to appear naturally in salt works after 1997 due to a number of manmade factors, though was present in the laboratory. The two strains, mentioned above, were assayed for following ten reproductive and lifespan character-ristics under laboratory conditions: i) Number of total offspring per female, ii) offspring per brood per female, iii) number of broods per female, iv) time interval per brood (number of days) v) number of offspring per reproductive day, vi) offspring encysted (%), vii) age at first brood, viii) age at last brood, xi) reproductive period, and x) lifespan.

Artemia VC strain was inoculated in semi-intensive coastal ponds of one acre size (sq ft) at Ghorabari. The salinity was maintained from 130-132 ppt and was chosen exclude the predatory species of mysid, *Indomysis annandallei* from *Artemia* ponds. *I. annandallei* is an extremely eurythermal, euryhaline and carnivorous species, which feed on *Artemia* and proved to be a menace in the first two attempts of inoculation despite careful screening of intake water. *I. annandallei* was naturally found almost throughout the year in Ambro creek and other coastal areas of Sind and Baluchistan Coasts in salinities from 4 ppt to 41 ppt. Adequate experiments were done to determine the upper limit of salinity tolerance of the species, which was found to be 130 ppt (Sultana and Qazmi, 2008). It was therefore almost unavoidable to culture *Artemia* at salinity less than 130 ppt, particularly when *I. annandallei* was present.

A separate system for culture of microalgae was employed for feeding *Artemia*. Inoculation was performed in March. The cycle was continued for 30 days in 1-acre ponds and was terminated because of high mortality due to the increase in temperature which reached to 41 ^{0}C.

Materials and Methods

To assess the reproductive and life span characteristics in native parthenogenetic and Vietnamese bisexual strains, cysts were weighed and incubated for hatching under the standard conditions described by Sorgeloos (1986). Three replicates of 100 instar I nauplii were transferred to 250 ml beakers containing water of 80 ppt salinity. Microalgae (*Tetraselmis chuii* and *Isochrysis galbana*) cultured in the laboratory were given as feed. The temperature was kept at 28±2 ºC and mild aeration was provided. Twenty pairs of adult *Artemia* (in case of bisexual) and twenty adult females (in case of parthenogenetic) were kept

separately in 50 ml falcon tubes for comparative studies on their reproductive and lifespan characteristics. The photoperiod of 12: 12 h light/ dark cycle was provided by fluorescent light tubes. 80 ppt salinity water was prepared by dissolving crude sea salt. The salinity was measured by a temperature-compensated ATAGO refractometer (calibrated by distilled water). The tubes were examined daily for offspring production or deaths. Reproductive and lifespan characteristics were determined according to Browne et al. (1984, 1988) and Triantaphyllidis et al. (1995).

Artemia production system involved a separate culture pond of microalgae, which was one fourth of the production capacity; the system consisted of a reservoir pond (2 acre), three *Artemia* ponds (1 acre each) and one microalgae pond (1 acre). Reservoir pond was used for storage and evaporation of sea water to increase the salinity to the desired level (80 ppt). For production of *Artemia*, three shallow ponds of one acre size were prepared in the coastal area. Ponds had a marginal ditch. The depth of water at the center of pond was kept at 6-7 inches, whereas, in marginal ditch, it was 12-14 inches. The fourth pond of the same size, without marginal ditch was used for the culture of microalgae. The hardness of water was 54 mg $CaCO_3.l^{-1}$, therefore liming of pond was not performed. Water of high salinity (130 ppt) was pumped into *Artemia* pond from the reservoir; salinity in the microalgae pond varied from 37-45 ppt, though the much higher salinity tolerance was found during the experiments with microalgae. Native halophillic microalgae (*Dunaliella, Nannochloris*, *Chaetoceros calcitrans*) were collected from low and intermediate salinity ponds of coastal salt works isolated as pure cultures in the laboratory and were used in production ponds after gradual scaling up through primary to secondary and tertiary cultures.

Primary and secondary cultures of micro-algae were prepared by using Guillard and Ryther's Modified F- Medium (Guillard and Ryther, 1962) and were maintained by continuous sub culturing in the laboratory. The microalgae is being cultured as primary culture (in 25 ml culture tubes under sterilized conditions) and secondary cultures in 250 ml, 500 ml,1000 ml, 5000 ml flasks, which were subsequently used to inoculate in the tanks and small ponds as tertiary cultures. For tertiary cultures, urea and DAP were used at 1-10mg/liter and 0.3 – 3 mg/ liter respectively, dissolved in water and sprinkled evenly on the surface of microalgae pond, at a sunny day.

For mass culture the water with a salinity of 35-37 ppt was directly pumped into the micro-algae culture pond after screening through a 100 µm mesh siz net. The inorganic fertilizers were added at 21 kg Urea and 7 kg DAP / acre (Urea: DAP ratio of 3:1). The fertilizers were dissolved in fresh water, and sprinkled over the entire pond, or if wind was blowing at fair speed, the pre-dissolved fertilizers were dispensed in wind direction to quickly spread in the pond. The cultures of *Dunaliella, Nannochloris and Chaetoceros calcitrans* (addition of a specific algae depends on water temperature, since *Nannochloris* grow well at low temperature (<30), whereas, *Dunaliella* and *Chaetoceros* which tolerate temperature >35 ^{0}C were added to pond and left until bloom occured, which was visible thorough its increased transpancy and green color. When algae was at exponential growth phase, up to 50 - 70% water can be supplied to *Artemia* ponds and the volume of water was replaced and fertilizers were added. In the next 2-3 days algae were increased and ready to use again. In microalgae pond water depth should be at least 50 cm, for maintaining the algal culture. The salinity increased to 55 ppt (occasionally 60 ppt) in micro-algae ponds having no effects on any of the three species.

Pond preparation

High salinity water (120-130 ppt) from the reservoir pond was pumped into the *Artemia* ponds. The pond was considered as ready when water salinity and depth reached to 132 ppt and 40 cm in deeper parts i.e., peripheral ditches respectively. Each pond was provided with a label and measuring scale/stick to measure the water depth.

Artemia cysts were hatched according to Sorgeloos (1986). The freshly hatched nauplii were harvested after separating the empty shells (to avoid the mixing of empty shell with the cysts

produced during the culture operation) and inoculated without any delay after sunset. The cysts were kept for hatching accordingly to get the hatched nauplii in the evening. The hatched nauplii were transferred into a polythene bag inflated with oxygen in a density of half of the hatching density. The nauplii were acclimatized well by dripping in the pond water and released in the wind direction to facilitate the dispersion in the ponds.

Pond Management

Pond raking was started from the next day after the inoculation, it was performed once a day in the morning; and twice a day, if there was no wind; no pond raking was done, when it was too gusty. Culture of micro-algae at an approximate density of $1x10^4$ cells/ml was pumped into the *Artemia* pond in sufficient quantity, which could be visually observed as the pond water imparts coloration.

General condition of *Artemia* (animal gut fullness, pellets, swimming behavior, animal color (normal/red/green), color of the brood pouch (white, brown), and animal appearance (appendages for any bacterial or fungal diseases or abnormalities) were visually examined every morning before the pond raking. Weekly samples were collected for population dynamics (i.e. the number of nauplii, juveniles, sub adults and adults) and brood size (number of the cysts or nauplii in brood pouch).

Cyst harvesters made of plastic netting mounted on the bamboo poles to make a screen, were used for harvesting. The cyst processing was done according to the methods described by Sorgeloos (1986). The air and seawater temperatures were measured twice a day at 9.00 a.m. and 2.00 p.m. from February 2006 to July 2007. DO and pH were monitored occasionally.

Results

Reproductive and lifespan characteristics of two *Artemia* strains

Six reproductive and four lifespan characteristics of the two strains cultured at same salinity have been summarized in Table 1. Vietnamese strain showed an overall better reproductive performance than the native parthenogenetic strain for having more offspring/cyst per female, broods per female and having smaller interval between the broods. The length of pre and post reproductive periods were not significantly ($p < 0.05$) different in both strains, while Vietnamese strain had a significantly ($p < 0.05$) shorter reproductive period and lifespan than the parthenogenetic strain. On the other hand, the former strain had a better reproductive performance in terms of total number of offspring per female. The parthenogenetic strain was suitable for the production of biomass since produced more nauplii than cysts, whereas the bisexual strain was a better cyst producer.

Table 1: Reproductive and lifespan characteristics of two *Artemia* strains. Mean of 35 replicates. Different alphabetic letters denote significant differences ($P < 0.05$)

Characteristics	Parthenogenetic (Native strain)	Bisexual (VinhChau strain)
Total offspring/female	983.73 ± 0.33^b	1449.75 ± 0.52^a
Offspring/brood/female	126.11 ± 1.83^b	138.07 ± 1.42^a
Broods/female	7.8 ± 0.62^b	10.5 ± 0.31^a
Days between broods	6.5 ± 1.60^a	3.97 ± 0.51^b
Offspring/reproductive days	16.78 ± 2.33^b	33.52 ± 1.98^a
% Of encysted embryos	64.0 ± 1.65^a	33.08 ± 1.21^b
Age at 1st brood (Pre-reproductive period)	20.1 ± 0.41^a	20.0 ± 0.22^a
Reproductive period	58.6 ± 2.38^a	43.25 ± 2.12^b
Post-reproductive period	3.0 ± 0.11^a	3.5 ± 0.02^a
Life span	81.5 ± 1.32^a	69.25 ± 1.55^b

The brood interval was significantly different in both strains. The parthenogenetic population showed 6.50 days between broods (Tabel 1) while bisexual one had 3.97 days. The parthenogenetic population had significantly longer lifespan (81.5 days) and longer total reproductive period (58.6 days) but had fewer offsprings while bisexual population showed shorter life span (69.25 days), shorter reproductive period (43.25 days) but had more number of total offspring (1450 vs 984) indicating significantly better reproductive performance than the local parthenogenetic population.

Inoculation studies

The first inoculation was carried out on March 3, 2007 in three one-acre ponds in coastal areas with an inoculation density of 50 nauplii/L. *Artemia* reached to adult stage in 10-11 days. Average size of females was 8-9 mm, whereas, size of male was 6.5 – 7 mm (n=10). The overall survival rate was 56-67 %. The sampling of population revealed nauplii production for about three days, whereas, cysts production initiated after about 14-15 days of inoculation in different ponds. The number of cysts is a highly variable parameter and varied from 6 to 257 in the brood pouch of a single female. The maximum number of cysts was observed in 3rd and 4th week of culture, which declined progressively from fifth week onwards. Individual observation on brood size revealed that the number of cysts varied from 15-75 per brood in the initial four broods; the cysts number increased progressively from 1st to 4th brood. Maximum cyst production occurred from 5th to 8th brood (>200 cyst/brood), which declined gradually in later broods.

Amount of cysts harvested from three ponds has been given in Table 2, which varied from 11.87-14.29 kg with an average of 12.79±1.31kg. Significantly higher cysts production in pond 3 as compared to ponds 1 and 2 may be attributed to the comparatively higher concentration of microalgae in pond 3.

Table 2: Cyst production in three 1-acre ponds. Different alphabet letters denote significant differences (P <0.05)

Date	Cyst Production (wet wt. in Kg)			Mean ± SD
	Pond 1	Pond 2	Pond 3	
17-Mar-07	1.68	1.76	2.08	1.840 ± 0.21
20-Mar-07	2.976	3.12	3.52	3.205 ± 0.28
26-Mar-07	4.00	3.984	5.104	4.363 ± 0.64
3-Apr-07	3.216	3.344	3.584	3.381 ± 0.19
Total	11.872^{b}	12.208^{b}	14.288^{a}	12.789 ± 1.31

Table 3: Quality evaluation (H%: hatching percentage, HE: hatching efficiency, HR: hatching rate, Ts: hatching synchrony) of *Artemia* cysts harvested from three ponds. Different alphabet letters denote significant differences (P <0.05)

Pond	Number of cysts/g*	H% (nauplii from 100 full cysts)	HE (nauplii/g of cysts)	HR (hrs)			
				T_0	T_{10}	T_{90}	T_s
1	266,770.3 ± 5,699^{a}	79.4 ± 14.6^{b}	125,555.6 ± 6.3^{b}	11	13	26.0	13.0
2	215,135.0 ± 8,453^{c}	80.5 ± 9.0.3^{b}	119,757.9 ± 10.9^{b}	11	12	25.5	13.5
3	235,543.1 ± 3,543^{b}	85.2 ± 5.3^{a}	146,155.3 ± 14.9^{a}	12	14	25.5	11.5

The water content of cysts was removed using a spinner and subsequently dried in a locally fabricated cyst dryer. The moisture content of the cysts was kept from 5-8 %. Approximately 2.7-2.9 kg of wet cysts yielded one kg of dry cyst. Dried cysts were packed under vacuum condition in plastic bags.

The quality evaluation of *Artemia* cysts collected is presented in Table 3. Hatching quality was, generally good in all the ponds and the hatching percentage varied from 79.4 - 87.4 % in all three ponds.

During the course of study (March 3, 2007-April 3, 2007) at Ghorabari, temperature ranged from 21.5 to 41 ^{0}C, salinity from 132 - 137 ppt, pH from 7.3 to 8.5, DO from 3.9 to 6.3 mg/ L.

Annual variations in air temperature

Figure 1 shows minimum, maximum and average values of air temperature during February 06-July 07 to give an idea of temperature changes in the inoculation area. Air temperature ranged from 10 to 42 ^{0}C during the study period. The minimum value for temperature was found in January 2007, whereas, maximum value occurred in April 2007. May and June are usually considered as the hottest months of the year but occasionally high temperature values (i.e. ≥ 40 ^{0}C) are found in April too. Air temperature varies mainly with the season and affected by the wind velocity and presence or absence of cloud cover. The large differences were found in minimum and maximum temperature values during winter months, which indicate the large temperature difference during day and night. According to the temperature regime (Fig 1) suitable period for *Artemia* production is from September-October to April-May (7-8 months) i.e when the day temperature varies from 21 to 35 ^{0}C. Two to three production cycles can be run during this period.

Discussion

Life history and reproductive characteristics of strains are important factors when *Artemia* introduction in a new habitat is considered, especially when competition with a local strain is to be expected. These competitive abilities are related to factors like the length of reproductive, pre- and post-reproductive periods, total lifespan, number of offspring per brood, broods per female, time in-between broods etc. In general, bisexual populations have a large number of offspring per brood, a large number of offspring/ day/ female and a fast development time to sexual maturity, which favors this group against parthenogenetic *Artemia*. Age at first spawning is a key factor determining the population growth rate.

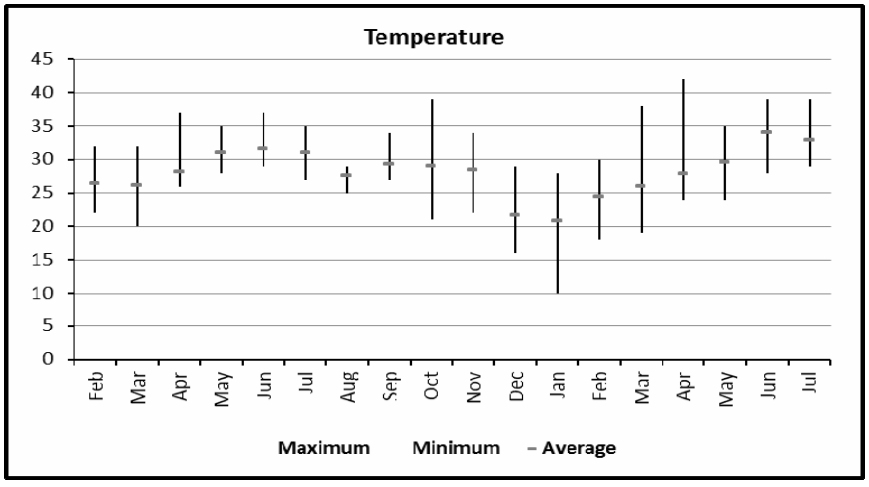

Fig.1: Annual variation in sea water temperature in coastal areas of Sindh during February 2006-July 2007

The other important factors for inoculation are the environmental factors like salinity, temperature and annual rainfall. However, recent investigations point to temperature as a critical factor in determining the biogeographic distribution and competitive ability of sexual and parthenogenetic *Artemia* (Browne et al 1995).The suitable salinity level in most of *Artemia* production systems is >80-120 ppt and temperature 24-26 ^{0}C to support a good reproduction (Hoa, 2003).. However, in Pakistan, due to the presence of euryhaline and predatory species of mysid *I. annandallei*, suitable salinity is ≥132 ppt, in which all the predators die, though *Artemia* survived very well and reproduced; however, further experimentations are underway to compare the reproductive performance at 80- 120 ppt and 130 ppt. Abatzopoulos et al, (2002) reported the occurrence of first brood after 41 days at 120 ppt in clonal *Artemia*. In the present study, the effect of temperature, not the salinity, delayed the time reaching to sexual maturity and in turn the first brood.

Temperature in coastal areas of Sindh is generally high during summer and autumn and may reach to >40 ^{0}C. It followed a typical bimodal pattern of subtropics with a major peak in summer months (April-June) and a minor peak during autumn (September-October). The rainy season falls in July and August. The average rainfall in coastal areas of Sindh is low i.e only 150-180 mm. To minimize the effect of high temperature, the water depth was kept ≥ 40 cm in deeper areas of pond.

In addition to temperature and salinity, the most important and critical parameter for the cyst production was the quantity and quality of micro algal species which exert effect on the length of life cycle and number of cyst in the brood pouch which in turn determines the cyst yield. The number of cysts per brood pouch varied greatly and reached from 6 to a maximum of 257 cysts. The number of cysts in brood pouch also varied with the age of *Artemia*; initially number of cysts in brood is quite less and reached to the maximum level from 5th to 8th brood, and again decreased in the later broods. When *A. franciscana* was introduced to Vinhchau, high mortality of *Artemia* took place in the first two years because the maximum temperature in this region often exceeds 36°C, especially at the end of the season (April and May) whereas, the maximum water temperature recorded in San Francisco Bay never exceeds 33°C (Hoa, 2003). However, the production yield of *Artemia* in this area gradually improved during the later years, explained by an adaptation/selection of the newly introduced *Artemia* population to high temperature in the new habitat. Later, Hoa (1997) introduced the idea of a multicycle system rather than a single cycle in Vietnam. Vos and Tansutapanit (1979) showed that *Artemia* may adapt to higher temperatures with every following generation under the same circumstances. Adaptation of a newly inoculated strain may result in phonotypical and genotypical variations in the existing stocks, eventually yielding a new *Artemia* genotype (Vanhaecke and Sorgeloos, 1989). Hoa (2002) compared the potential of temperature adaptation between the SFB *Artemia* newly inoculated in the field of Vinhchau and the strain introduced to the salt field ten years earlier. The result showed that the cyst production of the newly inoculated *Artemia* was relatively low, explained by the high temperature in Vinhchau resulting in a lower survival rate. On the other hand, the *Artemia* strain which introduced in the area more than ten years before, showed a better heat resistance. SFB *Artemia* has acquired good reputation after previous inoculations in Brazil, Macau and Vietnam for its temperature adaptability, which require long term acclimatization to the environment; each successive generation exhibited a temperature tolerance better than the previous one.

The performance of Vinhchau (VC) strain has been found suitable under the local climatic/environmental conditions and has good potential for commercial production in coastal areas of Pakistan. Based on the temperature regime, suitable period for *Artemia* production in the region may be from September-October to April-May (7-8 months).

References

Abatzopoulos T.J., Bermawi N.E. and Vasdekis C. (2002) Effects of salinity and temperature on reproductive

and life span characteristics of clonal *Artemia*. Hydrobiologia 492, 191-199.

Browne R.A., Sallee S.E., Grosch D.S., Segreti W.O. and Purser S.M. (1984) Partitioning genetic and environmental components of reproduction and life span in *Artemia.* Ecology 65, 949-960.

Browne R.A., Davis L.E. and Sallee S.E. (1988) Temperature effects on life history traits and relative fitness of sexual and asexual *Artemia*. J. Exp. Mar. Biol. Ecol. 124,1-20.

Browne R.A. and Wanigasekera G. (2000) Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia.* J. Exp. Mar. Biol. Ecol.244, 29-44.

Browne R.A., Barata C., Hontoria F. and Amat F. (1995) Competition between sexual and parthenogenetic *Artemia*: temperature and strain effects. J. Exp. Mar. Biol. Ecol.196, 313-328.

Camara M. R. and Rocha R. M. (1987) *Artemia* culture in Brazil; an overview, In *Artemia* Research and its applications, III, Proceedings of the Second International Symposium.

Guillard R.R.L. and Ryther J. H. (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and Detonula Confervaces (Cleve) Gran Can. J. Microbiol. 8, 229-239.

Hoa N.V., Baert P. and Anh N.T.N. (1997) Increasing cyst yield in *Artemia* culture ponds in Vietnam: the multi-cycle system. Aquaculture Res.28, 809-814.

Hoa N.V. (2003) Seasonal farming of the Brine Shrimp *Artemia franciscana* in artisanal salt ponds in Vietnam: Effects of temperature and salinity, Ph.D thesis, Ghent University Belgium.

Sorgeloos P., Lavens P., Leger P., Tackaert W. and Versichele D. (1986) Manual for the culture and use of Brine shrimp *Artemia* in Aqua-culture.319pp.Ghent: Artemia Reference Center.Belgium.

Sultana R., Abbas Shah S.G. and Qadri R.B. (1991) Report on the occurrence of parthenogenetic brine shrimp, *Artemia* in solar saltworks of Karachi coast. Pak. J. Sci. Ind. Res. 34, p 499.

Sultana R., Abbas Shah S.G. and Qadri R.B. (2000) On the *Artemia* population from Korangi Creek Saltworks (Karachi, Pakistan). Pak. J.Sci.Ind. Res.43, 285-288.

Sultana R., Kazmi Q. B. Nasir M., Amir F., Ali W. and Shadrin N. (2011) Extremely eurythermal and euryhaline opossum shrimp, *Indomysis annandalei* W. Tattersall, 1914 (mysida: mysidae) from pakistan coastal waters. Mar.Ecol. J, Kovalevsky Institute of Biology of the Southern Seas National Academy of Sciences of Ukraine (Accepted).

Triantaphyllidis G.V., Poulopoulou K., Abatzopoulos T.G., Pinto Perez C.A. and Sorgeloos P. (1995) International study on *Artemia* XLIX.Salinity effects on survival, growth, biometrics, reproductive and life span characteristics of a bisexual and a parthenogenetic population of *Artemia.* Hydrobiologia 302, 215-227.

Vanhaecke P. and Sorgeloos P. (1989) International study on *Artemia* XXXII The effect of temperature on cyst hatching, larval survival and biomass production for different geographical strains of brine Shrimp *Artemia* sp. Annl. Soc.R. Zool. Belg., 119, 7-23.

Vos J. and Tansutapanit. (1979) Detailed report on *Artemia* cyst inoculation in Bangpakong Chach-oengsao Province. FAO/UNDP Field document. 54p.