STUDIES ON THE REPRODUCTIVE BIOLOGY OF TWO SPECIES OF BRINE SHRIMPS UNDER DIFFERENT ECO-PHYSIOLOGICAL CONDITIONS

THESIS SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN
FISH AND FISHERIES SCIENCE
(MARICULTURE)

OF THE
CENTRAL INSTITUTE OF FISHERIES EDUCATION
(DEEMED UNIVERSITY)
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Certified that the thesis entitled **"STUDIES ON THE REPRODUCTIVE BIOLOGY OF TWO SPECIES OF BRINE SHRIMPS UNDER DIFFERENT ECOPHYSIOLOGICAL CONDITIONS "** is a record of independent bonafide research work carried out by **Mr.Soniraj N** during the period of study from October 1997 to August 2001 under our supervision and guidance for the degree of Doctor of Philosophy in **Fish and Fisheries Science (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I here by declare that the thesis entitled **"STUDIES ON THE REPRODUCTIVE BIOLOGY OF TWO SPECIES OF BRINE SHRIMPS UNDER DIFFERENT ECO–PHYSIOLOGICAL CONDITIONS"** is an authentic record of work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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ACKNOWLEDGEMENTS

I wish to express my obligation, respect and gratitude to Dr. M. Rajamani, Senior Scientist, CMFRI under whose supervision this work has been conducted and also for his valuable guidance and critical suggestions, which helped me to complete the work successfully and prepare the thesis in the present form.

I avail this opportunity to express my gratefulness to Dr. Mohan Joseph Modayil, Director CMFRI, Kochi for providing the facilities of CMFRI and other necessary arrangements for my research work. My thanks are also due to Shri. D. C. V. Easterson, Senior Scientist and Officer-in-Charge TRC of CMFRI, Tuticorin for providing the working facilities and also for his valuable suggestions and advice for the research as a member of my advisory committee.

I am thankful to Dr. D.B James, Senior Scientist (Retd.), MRC of CMFRI, Dr. M. Srinath, HOD, FRA Division of CMFRI and Dr. H. Muhammad Kasim, Senior Scientist and Officer-in-Charge, Kakinada Centre of CMFRI for their contribution as the members of my advisory committee.

I am grateful to Dr. Peter Marian, CAS training Centre Nagercoil for his valuable suggestions regarding the experiments. Thanks are also due to Shri. N. K. Sanil, Scientist and Shri. Ayyappan Pillai, Technical Officer for their help in the successful completion of the Electron Microscopic work. I would also like to thank Dr. R. Paulraj, Officer-in-Charge, PGPM,

Dr. C. Suseelan, former Officer-in-Charge, PGPM, Dr. M. Devaraj, former Director CMFRI, Kochi for their timely help and encouragement. I also wish to thank Dr. A. A. Jayaprakash, Dr. J. P. George, Senior Scientists and Shri. S. Kaladharan Scientist (SS) for their constant encouragement. I am grateful to Shri. P. M. Aboobakar, Technical Officer of CMFRI and Mrs. Suvarna and other library staff for the help extended to me. Thanks are also due to the staff of PGPM, Administration, Audit and Establishment for their timely help.

I will be failing in my part if I am not thanking Dr. P. Muthiah, Shri. K. Ramadoss, Shri. A. Chellam, and Shri. M. Dharmaraj, Senior Scientists of the TRC of CMFRI for the help extended to me. Special thanks are due to Mrs. Asha, Mrs. Shoji Joseph, Miss Lakshmi Pillai , Mrs. Suja and Mrs. Jenny for the support they have given during my research work at Tuticorin. Thanks are also due to Shri.J.X. Rodrigo, Shri.D.Sundararajan, Shri. Vinod, Shri. Ramasamy, P.Jayaram and Soundrapandian for all their help. I will be always thankful to Shri. Joseph Sahayraj, Shri. Muthuvel and Shri. Gurusamy and other staff of main office and Karappadu field laboratory including the casual labourers Shri. Arumugam, Raj, Shakthivel, Prince and David for their help and service.

I have no words to acknowledge my love and gratitude towards Shri.T. N. P. Kurup and my senior colleague Dr. Rammohan and for their constant encouragement and support which helped me to overcome all the hardships during my stay at Tuticorin. Special thanks are due to my colleagues Anilkumar,P. K., Sunilkumar,P , Balu, S., Gireesh, Mrs. Suja Rammohan and Mary Asha Antony for their timely help and constant encouragement.

I express my sincere gratitude to Mr. Satish for his photographic assistance for the research work. Thanks are also due to my senior colleagues Manoj Nair, Najmudeen, Abraham, and juniors Binu, Anikuttan,

Samayakannan, Asok, Maharajan and Unnikrishnan for their support and love. I am also grateful to my batch mates Ansy, Prasad, Rupak, Raychal, Prameela and Sherly for their timely help.

I am grateful to CIFE for providing me financial assistance in the form of fellowship, which enabled me to complete the study successfully. I cannot forget the affection and support of my parents, relatives and friends during the study period.

Above all I am grateful to the Almighty for providing me the physical and mental strength for the successful completion of the Ph.D. work.

सारांश

टूटिकोरिन के लवण क्यारियों में अलैंगिक और लैंगिक *अर्टीमिया* जातियाँ मौजूद हैं और इन दोनों जातियों की प्रचुरता बता नहीं जा सकता. अनुमान है कि लवण क्यारियों में लैंगिक जातियों का आगमन स्थानीय स्फुटनशालाओं में उपयोग किये जानेवाले निर्यातित सिस्ट से विकसित हुआ होगा. इस अध्ययन में अर्टीमिया की लैंगिक व अलैंगिक जातियों के जीवविज्ञान पर पारिस्थितिक-शरीर क्रियात्मक स्थितियों जैसे लवणता, तापमान, अनशन, विभिन्न खाद्य और आहार के स्तर, दीप्तिकालिता (फोटोपीरियोडिसम) के प्रभाव पर अनुसंधान करने का प्रयास किया गया है. अध्ययन की गई अधिकांश स्थितियों में लैंगिक जातियों ने देशज अलैंगिक जातियों की अपेक्षा हावी दिखाई. नियंत्रित तापमान में अलैंगिक जाति के अंडे आकार में बडे होते हैं जबकि परिवेश ताप (एंबियन्ट टेम्परेचर) में लैंगिक जातियों के अंडे बडे आकार के होते हैं. उच्च लवणता में अलैंगिक जातियों की पूर्व-जननात्मक अवधि लैंगिक जातियों की अपेक्षा कम है बल्कि निम्न लवणता में लैंगिक जातियों की पूर्व-जननात्मक अवधि कम होती है. उच्च लवणता में अलैंगिक जातियों के अंडों का आकार लैंगिक जातियों की अपेक्षा बडा होता है बल्कि लैंगिक जातियों के अंडों का आकार निम्न लवणता में बडा होता है. अतः शीतकाल / वर्षा काल में लैंगिक जाति की और गर्मी के मौसम में अलैंगिक जाति की हावी होती है. अलैंगिक जातियों द्वारा निम्न तापमान में अंडज तरीके (ओवीपारस) का जनन होता है जो इस जाति की हावी स्थापित करता है बल्कि लैंगिक जाति निम्न तापमान में नोप्लियों का जन्म देती है. अर्टिमिया को प्रयोगशाला में पालन करते वक्त खाद्य के रूप में आइसोक्राइसिस जाति जैसे शैवाल खाद्य या धान चूर्ण देना उचित देख गया है क्योंकि इस से उच्च जननक्षमता दिखाई पडती है. दोनों लैंगिक और अलैंगिक जातियों में निम्न तापमान सिस्ट के उत्पादन के लिए अनुकूल देखा जाता है लेकिन अलैंगिक जाति अंडजरायुज तरीके का जनन नहीं करते हैं. इसलिए वे 25°C का निम्न तापमान पसंद करते हैं. शैवाल खाद्य पुटीभवन (एनसिस्टमेन्ट) केलिए प्रेरित करते है तो धान चूर्ण पुटीभवन को कम करता है. अतः तापमान और खाद्य पुटीभवन को प्रभावित करने वाले दो प्रमुख घटक हैं., अधिक लवणता और दीप्तिकालिता सिस्ट के उत्पादन की दर को प्रभावित कर सकती हैं.

ABSTARCT

There are asexual *Artemia parthenogenetica* (Barigozzi 1974) and sexual *Artemia franciscana* (Kellog 1906) species available at the saltpans of Tuticorin and the chances of dominance of either of these species can not be ruled out. Sexual species is believed to have entered the saltpans because of contamination from the local hatcheries where the imported cysts are being used. Here an attempt was made to study the effect of ecophysiological conditions such as salinity, temperature, starvation, different feeds and feeding levels, photoperiodism etc. on the biology of these two species of *Artemia*. Sexual species has the advantage of dominating the indigenous asexual species as they had performed better in most of the conditions studied. At fixed temperature the asexual species had a better brood size than the sexual species but at ambient temperature the sexual had larger brood size. The pre reproductive period of asexual species at higher salinities was less than the sexual species, while at lower salinities the sexual had shorter pre reproductive period. The brood sizes of asexual species were also large than the sexual species at higher salinities while at lower salinities sexual species had larger broods. Therefore rather than a complete dominance of either of these species the sexual species may dominate in the winter/rainy season and the asexual one in the summer season. The oviparous mode of reproduction by the asexual species at lower temperature also supports this view as the sexual species could produce nauplii offspring even at this temperature. In laboratory rearing of *Artemia* algal feeds like *Isochrisis* sp. or ricebran were found good for biomass production as the fecundity was high with these feeds. Low temperature could induce cyst production in both sexual as well as asexual species, while the asexual responded more positively to low temperature of 25 ^{0}C as they never turned back to ovoviviparous mode of reproduction. Algal feeds favoured the encystment rate, while ricebran fed animals could never produce cysted offspring. Temperature and feed were found to be the major factors, which influence the encystment while salinity increase and photoperiodism can only enhance the cyst production rate.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The brine shrimp *Artemia* is the most preferred over other live feeds as its nauplii, juveniles, adults and cysts could be used as feed for fishes and crustaceans particularly in the early larval stages. *Artemia* was first described by Schlosser in 1755 and later by Linnaeus in 1758 (Kueren and Baas-Becking 1938) under the binomen *Artemia salina*.

Artemia lives and reproduces in natural and artificial brine pools and lakes and is distributed widely in many parts of the world which are geographically isolated (Persoone and Sorgeloos, 1980; Vanhaecke *et al.*, 1987). In Africa, both sexual and asexual species have been recorded from Algeria, Egypt, Kenya, Libya, Madagascar, Morocco, Mozambique, Namibia, Niger, Senegal, South Africa and Tunisia. The *Artemia* finding sites in Australia and Newzealand are West Australia, South Australia, Newzealand, Queensland, and those in the American continents are Argentina, Bahamas, Bolivia, Brazil, British Virgin Islands, Carribean Islands, Canada, Chili, Colombia, Costarica, Dominican Republic, Ecuador, Mexico, Netherlands, Antilles, Peru, Puerto Rico, U.S.A. and Venezuela. In Asia *Artemia* are reported from the countries such as China, India, Iran, Iraq, Israel, Japan, Korea, Kuwait, Srilanka, Taiwan and Turkey and those of in Europe are, Bulgaria, Cyprus, France, Greece, Italy, Portugal, Rumania, Spain, USSR and Yugoslavia.

Natural population of *Artemia* in India was first reported in Vadala, Bombay, in 1953 by Kulkarni followed by Baid in 1968 from Sambar and Didwana Lake in Rajasthan. *Artemia* were also reported from the salt pans in Gulf of Kutch, Gujarat (Royan, 1979). There were reports of *Artemia*

occurrence also from the south east coast of India in the Karsewar islands of Tuticorin (Achari, 1971), Veppalodai salt pans of Tuticorin (Royan *et al.*, 1970), Kelambakkam near Madras and Vedaranyam near Muthupett lagoon (Kulasekarapandian *et. al.*, 1995)

The systematic position of *Artemia* in the animal kingdom is as follows

Phylum	:	Arthropoda
Class	:	Crustacea
Subclass	:	Branchiopoda
Order	:	Anostraca
Family	:	Artemidae
Genus	:	*Artemia* Leach -1819

Its speciation has always raised confusion, as the different geographically isolated sibling species had performed differently when studied in laboratory (Browne, 1980b; Browne *et al.*, 1988). Earlier taxonomists used to assign different species names to the populations with different morphological characteristics. Later on all the brine shrimps were collectively referred as *Artemia salina* Linnaeus 1758. Recently, different names are given to reproductively isolated populations or cluster of populations. Number of different *Artemia* population described today already exceeds 150 (Persoone and Sorgeloos,1980) and the genetic study of many of them revealed existence of atleast six non-interbreeding species (Bowen and Sterling, 1978).

Based on the mode of reproduction there are two types of *Artemia* populations, one which reproduces sexually and has both males and females in the population, whereas the other group reproduces parthenogenetically and has mostly females in it. It has been reported that the Old World strains of *Artemia* lack males in the population and

approximately 70% of the population studied so far reproduce only by parthenogenetic mode of reproduction (Browne and Halanych, 1989). On the other hand, *Artemia* in the Western hemisphere (New World strain) reproduce exclusively by sexual mode (Browne and MacDonald ,1982)

Among the sexual strains there are six known sibling species (Van Stappen, 1995)

Artemia salina Linnaeus 1758 : Lymingdon, England (now extinct), Meditteranian area.

Artemia tunisiana Bowen and Sterling 1978 synonym of *A.salina* : Europe,

Artemia sinica Yaneng 1989 : Central and eastern Asia

Artemia franciscana : America (North , Central and South). This include populations reproductively isolated in nature like *A.*(*franciscana*) *franciscana* Kellog 1906 and *A.* (*franciscana*) *monica* Verill 1869 (Monolake California)

Artemia persimilis Piccinelli and Prosdocimi 1968 : Argentina

Artemia urmiana Gunther 1900 :Iran

Among these *Artemia franciscana* is the most dominant species in the New World as the population is found throughout North and South America.

The asexual strains which are found mostly in Europe and Asia reproduce parthenogenetically, are genetically different with difference in ploidy level and isozyme pattern, which make their joint classification under *Artemia parthenogenetica* (Barigozzi, 1974; Bowen and Sterling, 1978) confusing. Therefore, only the genus name should be used until the

speciation is more clearly understood (Bowen and Sterling, 1978). Most of the Old World species of brine shrimp reproduce parthenogenetically (Cuellar, 1990,1991) which is not clearly reported among the New World strain. There are taxonomic and allosomic evidences (Abreu Grobois and Beardmore 1980, 1982; Browne *et al.*, 1984) that the Old World asexual populations have descended from the Old World sexual species *Artemia tunisiana*. A detailed biogeographical and ecological description of *Artemia* and its habitat is contained in Browne and MacDonald (1982), and Vanhaecke *et al.* (1987).

The *Artemia* was given the name brine shrimp, as it is an inhabitant of coastal waters where brine and salt are formed by evaporation of seawater in landlocked lagoons. Because of this geographical isolation, the brine shrimp population has diversified to more than 150 strains. The common feature of all *Artemia* habitats are their high salinity which is the predominant biotic factor determining its geographical distribution. Other parameters like temperature, light intensity, primary food production etc. have less impact and can only cause a temporary absence of *Artemia*. *Artemia* can tolerate extreme ranges of environmental conditions (salinity, pH, temperature etc.) although it is intolerant to certain substances like Potassium (Jennings and Whitaker, 1941). It can complete its life cycle even in sea water but requires environmental conditions like hypersalinity which exclude the predators as it is a defenceless creature. For this *Artemia* posses the best osmoregulatory mechanism known in the animal kingdom and hence it survive at higher salinity at which other aquatic animals cannot. In the extreme conditions its propagation is ensured by the formation of cyst which are dispersed to various places by wind and water birds. *Artemia* is a continuous nonselective filterfeeder consuming micro algae and inert materials of less than 50 micron in size available in the ecosystem.

Optimum temperature range for *Artemia* to perform all metabolic functions is 25 to 30 °C. But it can tolerate temperature as low as 6 °C and as

high as 35 °C and the tolerance limit varies from strain to strain. Dehydrated cysts can survive temperature up to many degrees below freezing and close to boiling. Most of the *Artemia* strains can also tolerate a wide range of salinities from 15ppt to 200ppt. Under extremely high saline conditions the animals barely manage to survive and most of their normal physiological and metabolic functions may not be accomplished. Its lower salinity level in which it can survive is a function of the presence of predating animals as it can thrive well in seawater and even in brackish water. As in the case of temperature there is no optimum for salinity range. *Artemia* is a typical euroxibiont since the animal can withstand a very low oxygen concentration in the medium, which is far below than the normal and a very low pH also.The nutritional quality of different *Artemia* strains differ, as they inhabit different habitats, with varying abiotic and biotic factors, which can influence the physiology of the animal. The environmental conditions also influence the type of food present in the *Artemia* habitat thus influencing the biochemical composition indirectly.

Artemia populations are either sexual or parthenogenetic and posses the unique adaptation, which may be rare in the animal kingdom, that it can reproduce oviparously and also by ovoviviparously. When the environmental conditions are conducive *Artemia* reproduce ovoviviparously, in which eggs are hatched with in the brood pouch of the female and nauplii are released, whereas when the conditions in the environment become unfavourable, *Artemia* switches over to oviparous mode of reproduction in which egg development is arrested at the gastrula stage and is coated with a thick chitinous layer of shell and are released as cyst, which remain viable in dormant condition for several years as long as they are kept dry under anaerobic conditions.

Dried cysts are approximately 240 microns in diameter and are deeply indented on one side which when placed in seawater or diluted

seawater regain its spherical shape by imbibing water (Jennings and Whitaker, 1941). If conditions, especially the environmental factors like temperature, pH and salinity are favourable for hatching, the nauplii are released from the cysts. Schrehardt (1987) has described all the developmental stages and their identifying features with the help of electromicroscopic photographs. According to him, the developmental phases can be grouped into a naupliar, metanaupliar, post - metanaupliar and post - larval period.

The adult animal matures within two weeks of time from their naupliiar stage .The reproductive system of female consists of two ovaries, two pouch like oviducts and a ventral median uterus or broodpouch. The eggs are generated in the ovary followed by a moult and are passed from the ovaries to the oviduct in less than two hours. Fautrez (1957) and Goldschmidt (1952) have reported that the eggs in the oviduct are in metaphase of the first meiotic division and remain there from 1 to 40 hours. Then it is being transferred to the uterus, which takes less than 30 minutes. Eggs remain there for 3 to 5 days. In case of sexual species normally fertilisation takes place when the eggs are in the brood pouch. Females generally do not store sperm from one generation to another. The female expels her eggs from the uterus as virgin eggs or nauplii or cysts depending on the environmental conditions and success of fertilisation. The birth process takes two to ten hours to complete. She molts in a few seconds and the next egg generation starts.

Matured sexual females produce active nauplii or viable cysts once they are fertilised with male, but the virgin females release transparent thin-shelled cysts, which sink to bottom and never hatchout. In the sexual females the eggs in the uterus develop, only after the occurrence of fertilisation, but in the asexual parthenogenetic strain the development starts as soon as the eggs reach the ovary.

Although *Artemia* was first described in the 17th century, and had been used extensively for biological studies, its value as a suitable food organism was discovered very recently. Seale (1933) and Rollefsen (1939) found freshly hatched *Artemia* nauplii as an excellent food source for fish larvae and from then on its application in larviculture increased significantly. Now *Artemia* is used as a source of live feed in the hatcheries of fresh water and marine fish and crustacean species (Bengtson *et al.*, 1991).

From the commercial point of view the oviparous reproduction has got more importance as the cysts fetch a very good price and demand in the aquaculture market. Handling of cysts is much easier as they can be easily collected, canned, shipped and later hydrated in seawater as and when required. Traditionally cysts have been collected from natural habitat or commercial salt works. But the increasing demand for cyst in the 1970's prompted the introduction of *Artemia franciscana* into coastal salterns (Camara and De Medeiros Rocha, 1987) in various countries including India (Persoone and Sorgeloos, 1980). The existing natural salterns and lakes have been already overexploited or mismanaged causing a decline in the cyst availability. The newly introduced population initially produce cyst in high rate and the production trend declines later (Berthelemy and HedgecocK, 1987). Possible reason for this may be the changed environmental conditions that shift the reproductive mode towards ovoviviparity or the continuous removal of cyst might have removed the genotype predisposed towards oviparity. The cyst production in these area can be restored either by reintroducing a highly oviparous strain or the environmental factors that effect cyst production should be identified and manipulated so as to enhance cyst formation. An understanding of the physiological basis underlining the oviparous mode in the female brine shrimp also may become a useful tool in the revival of cyst production rate.

Environmental stress particularly hypoxia is thought to induce the production of cyst in *Artemia*. Gilchrist (1954) and Gilchrist and Green (1960) showed that levels of haemoglobin and its metabolic end product haematin increase with hypoxic stress. Dutrieu (1960) suggested that under low oxygen concentration excretion of haematin via the shell gland situated in the ovisac induced dormancy of cyst contained in the uterus. Temperature and photoperiodism are also found to have some effect on the cyst production of *Artemia*.

There are reports showing the differences in the reproductive performances between the different geographical strains of same species and are thought to be because of the genetic dissimilarity between these strains. A similar kind of work can be done to study the reproductive performance of different strains available along the Indian subcontinent. The outcome of such a study can cast light in the selection of suitable strain for optimum cyst production in the natural ecosystem.

In the present study an attempt has been made to compare the two locally available species, one is indigenous and the other is exotic, which might have entered the local salt pans through the local hatcheries by contamination. The reproductive performance of both the exotic species *Artemia franciscana* and the local species *Artemia parthenogenetica* was studied in detail and results obtained are presented in this thesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Intensive research on *Artemia* started during 1940's when it was proved, that one of the best livefeed available for the larviculture was its freshly hatched nauplii (Rollefsen, 1939). Since then, most of the works were aimed towards an understanding of the mechanism involved in increasing its production under controlled environmental conditions. Improvement of cyst processing techniques and studies on the hatching efficiency had the priority in the research fields. As the quality of cyst collected from the wild highly varied due to various factors, attempts were made in several parts of the world to culture brine shrimp in solar saltpans in order to produce better quality cysts (Camara and De Medeiros Rocha, 1987; Persoone and Sorgeloos, 1980). Recent trend is to improve the nutritional quality of the nauplii of different *Artemia* strains, to suit the nutritional requirement of different larval forms by following different enrichment methods (Amat, 1980 b).

In India, *Artemia* was first reported in Bombay by Kulkarni (1953) and later by Baid in 1968 at Sambar lake of Rajasthan. At Tuticorin the presence of *Artemia* was recorded by Royan *et al.* (1970) and then at Karsewar Islands by Achari (1971). Royan in 1979 reported the presence of *Artemia* at the salt pans of Gulf of Kutch region of Gujarat. All the reported species were of parthenogenetic in nature. A lot of study has been conducted in India on *Artemia* so as to produce cyst using indigenous technologies both by inoculating the foreign species and also by using our own parthenogenetic strain. Continuous mass culture of the locally available *Artemia* species from Bombay was carried out by Dwivedi *et al.* in 1980. Availability of wild cyst from the salt condensation units of Gujarat is limited and is restricted to

premonsoon months. Inorder to get a steady supply of good quality cyst, more inoculation studies in the simulated salt ponds were conducted by Chayya *et al.* (1990). Biomass production of bisexual strain and parthenogenetic strain in air-water lift system with *Spirulina* as feed were tried by Royan *et al.* in 1990. Royan *et al.* (1991) had also conducted experiments to assess the cyst production potential of the natural Jamnagar *Artemia* population.

Population reproduction by either obligate parthenogenesis or by obligate sexual crossing as occurs in *Artemia* are found through out the animal kingdom, including beetles (Suomalainen, 1962), rotifers (Birky and Gilbert, 1971), ticks (Oliver, 1971), *Drosophila* (Carson, 1967) and reptiles (Maslin, 1971). Parthenogenetic *Artemia* produce only females with rare occurrence of males. At the same time bisexual *Artemia* strain produce both male and female at 1:1 ratio and both the species are reproductively isolated. Parthenogenetic females produce only females even if they are crossed with sexual males (Cai, 1986). Parthenogenetic shrimps have a slender abdomen and bisexual shrimp a thick and short one. Their brood pouches also have different shapes at opening of their protuberance. Jensen as early as 1918 followed by Relyea in 1937 had reported that brine shrimp from the Great Salt Lake in Utah could reproduce by parthenogenesis. Lochhead in 1941 stated that female *Artemia* had the ability to reproduce both by oviparous and ovoviviparous mode. Later Flowers and Evans (1966) also mentioned parthenogenesis in Utah shrimps. A recent study by Wirick (1972) on the ecology of shrimp from the Great Salt Lake revealed substantial seasonal variation in sex of the animal. In nature (Amat, 1980a) some salt ponds in southern Spain was found to have a mixture of parthenogenetic and sexual strains. Strain dominance varied seasonally as the sexual strain favoured low temperature and salinities (winter- early spring) while parthenogenetic strain dominate at warm temperature and high salinities (Browne and MacDonald, 1982; Amat, 1983). Number of investigations (Browne *et al.*, 1978; Browne

1980b; Abreu Grobois and Beardmore, 1980) had proved that the parthenogenetic *Artemia* are derived from Old World sexual populations. Life span and reproductive parameters of two population of brine shrimp, one raised under laboratory conditions for 25 years and the other naturally occurring group belonging to the same species were studied by Browne in 1983.

Various researchers have studied the effect of several factors on the mode of reproduction in Artemia. According to Barigozzi (1939) a slight correlation existed between high salinity and oviparity but later experiments conducted by Ballardin and Metalli (1963) could not find any correlation between oviparity and high salinities, temperature, photoperiodism, food type or densities in an Italian strain and they attributed the oviparity to stress in general. Algal feed was reported to have some inducing effect on the oviparity as reported by Dutrieu (1960) as the cultures with yeast predominantly resulted in ovoviviparous reproduction compared to the algal cultures. It may be due to the Iron component of the algal feeds, which is necessary for cyst formation (Bowen *et al.*, 1969). Studies on the sexual population of *Artemia* from the Great salt lake, Utah, USA and the parthenogenetic population from France showed the effect of photoperiodism on the mode of reproduction especially when applied 10 to 12 days before offspring deposition, while the eggs were still in the ovaries (Provasoli and Pintner, 1980). They also found that dimmer light favour oviparism. Versichele and Sorgeloos (1980) reported the importance of hypoxic stress in the cyst formation in intensive mass culture system. Large differences in percentage of encystment have been found between different strains raised under the same experimental conditions (Browne, 1980a) suggesting some genetic basis for encystment rates. Food shortage and oxygen stress have been found to be the influential factors controlling encystment in *Artemia* (Browne, 1983). Influence of chemicals like Fe EDTA on the encystment ability on different *Artemia* strains were studied by Versichele and Sorgeloos (1980)

and Balasundaram and Kumaraguru (1987). Geographical strains of the brine shrimps can be characterized by the diameter of their cyst, cyst volume and chorion thickness (Vanhaecke and Sorgeloos, 1980b). Growth and survival of larvae of different strain also differ but never among batches of the same strain (Vanhaecke and Sorgeloos, 1980a). Turki (1986) had conducted the qualitative and quantitative study of *Artemia salina* cyst occurrence in the Salinas of Megrine (Tunisiana) and the role of biotic and abiotic factors on cyst production. Cyst production techniques in Thailand salt ponds have been discussed by Tunsutapanich (1982) and that of *Artemia monica* cyst production in Mono lake by Dana *et al.* (1990). According to Berthelemy and Hedgecock (1987), maternal age, photoperiodism, temperature and salinity are found to be major factors controlling the mode of reproduction in San Francisco Bay strain. At low (16 °C) or medium (20 to 22 °C) temperature females are found to be 68 to 99% oviparous under photoperiod for 12 hours or less of light but are only 10% oviparous under long days or constant light. Maternal age was also found to have some influence on oviparity as the experimental animals produced 100% cysts from their third brood onwards. According to them no single environmental factor influences the oviparity but there exist an interaction of more than one factor.

Artemia live and reproduce in natural brine pools and other saline water bodies and can tolerate a wide range of salinity and other environmental factors (Jennings and Whitaker, 1941). Though *Artemia* is considered as euryhaline its salinity and temperature requirement vary from strain to strain (Sorgeloos *et al.*, 1976; Claus *et al.*, 1977). Brine shrimp nauplii are known to have the capacity to survive the sudden shift in the salinities compared to the juveniles and adults (Sorgeloos, 1980). In the natural environment *Artemia* can withstand higher salinities as high as 200ppt (Persoone and Sorgeloos, 1980) but in the laboratory there are problems in maintaining *Artemia* at this higher salinities (Wear and Haslett 1986; Wear *et al.*, 1986). Dana and Lenz (1986) reported low survivability of bisexual strain

of *Artemia* from Monolake, California at 159 and 179ppt salinities under laboratory conditions. VonHentig (1971) studied growth and mortality of Great salt lake (Utah) strain of *Artemia* in four salinities ranging from 5 to 70 ppt and Vanhaecke *et al.* (1984) studied survival of 13 geographical strains in 25 combinations of temperature (8 to 34 °C) and salinities ranging from 0 to 120ppt. Effect of salinity on the survival of Indian strain has been studied by Royan (1980) and Dwivedi *et al.* (1980). According to Triantaphyllidis *et al.* (1995) optimum salinity for parthenogenetic population is between 80 and 100ppt while *A. franciscana* appears to exhibit more euryhaline capacity with more survivability and better reproductive characteristics in higher salinities compared to the parthenogenetic species (Wear and Haslett, 1986; Wear *et al.*, 1986). Adult size, growth rate and maturation are found to have an inverse relation with the salinity as reported by Triantaphyllidis *et al.* (1995), Dana and Lenz (1986), Gilchrist (1960) and Bond (1933). Salinity is considered as one of the factors, which induces the cyst formation in *Artemia*. Berthelemy and Hedgecock (1987) had studied many of the possible factors including the salinity in order to find out their role in the encystment process of *Artemia* eggs. According to them salinities above 120ppt was found to have inhibitory effect on cyst formation. A comparative study between bisexual and local parthenogenetic population of Tangui salt pans of Peoples Republic of China were conducted by Triantaphyllidis *et al.* (1995) to examine the salinity effect on their survival, growth, biometrics, reproductive and lifespan characteristics.

Variation in temperature can have pronounced effect on the expression of fitness traits, more specifically on life history traits like the maturation period, length of reproductive period, total lifespan, brood size, brood number, inter brood period and total offspring production (Brown, 1929; Parsons, 1977; Murphy *et al.*, 1983). Browne et al. (1988) studied different life history traits at different temperatures for asexual and sexual population. Effect of different combinations of temperature and salinity on the

reproductive mode and growth of Srilankan strain of *Artemia* are detailed by Kuruppu & Ekratne (1995 a&b). Various studies show that temperature is the critical factor that determines the biogeographic distribution and competitive ability of sexual and parthenogenetic *Artemia* (Barata *et al.*, 1996a). Closely related species are often able to exclude each other, as the competition between them is intense. Distribution pattern of *Artemia* in local salt ponds depends more on species-specific response to abiotic factor as temperature rather than competitive exclusion. Usually in a mixed population of sexual and asexual animals, sexual strain appears first when temperatures are low, and asexual parthenogenetic diploid makes its presence when temperature increases and the triploid animals at low densities in summer during high temperature and saline conditions (Barata *et al.* 1992). Effect of diurnal variation and fixed temperature on survival of *Artemia* at different salinity levels were studied by Thoeye *et al.* (1987).

Artemia is a non-selective filter feeder that feeds on anything with size lesser than 50 micron. In nature they depend on the halophilic algae or other micro-organism present in the habitat but in the laboratory it is not economic to produce algal feed to maintain *Artemia* biomass, as it is a costly affair. There were various studies aimed to find out a substitute for algal feed for culturing *Artemia* and agricultural wastes like rice bran, wheat bran and other similar products were tried by many researchers. Biomass production is greatly dependent on the reproductive capacity of the animal, which may vary in accordance with the feed and also with strain and ecological factors. Cyst producing capacity is also known to vary in accordance with the feed. So it is important to study the reproductive characteristics of the strain with different feeds before it is selected for the inoculation or biomass production so as to find out its suitability.

There are different feeding experiments conducted to study the biology of *Artemia* under laboratory as well as wild conditions. Males are found to have significantly shorter lifespan than females when both are raised under conditions of low food in either a high-density environment (Browne, 1980b) or in isolation (Browne, 1982). In contrast, when food is not a limiting factor male and female have equal lifespan (Browne, 1982). *Artemia* can consume a large amount of food relative to body mass but the growth is found to have retarded by over feeding (Nimura, 1980) and it is important to know the optimum feeding range for the effective production of the animal.

Survival, growth and reproductive mode of the *Dunaliella* fed *Artemia parthenogenetica* strain of Srilanka were studied and they were raised from nauplii to reproductive maturity at different salinity and temperature combinations by Kuruppu and Ekratne (1995a). Its life cycle also has been studied in detail by them (Kuruppu and Ekratne, 1995b). *Artemia* from three different localities of Venezuela was studied to determine its biometric and reproductive features at different feed and salinity ranges (DeDonato and Graziani, 1993). Among the various algal feed tried in the mass culture system of *Artemia*, the diatom *Chaetoceros curvisetus* was proved to be the best and the minimum cell concentration at which *Artemia* can efficiently remove cells was found to be a constant factor throughout its lifecycle (Tobias *et al.*, 1979). Efficiency of nauplii and cyst production based on food taken by the shrimps have been studied by Nimura *et al.* (1994). Effect of different algal feeds on different strains of *Artemia* has been experimented by Dedonato and Graziani (1993). Coutteau *et al.* (1993) tried the algal substitutes like Yeast, Ricebran, Kaolin etc. to study its effectiveness as feed for *Artemia*.

Effect of specific ions and of ion antagonism on the excystment of *Artemia* has been studied by Boone and Becking (1931) and the effect of salinity on the same was studied by Jennings and Whitaker (1941). It has

strain of *Artemia* stand first among the Indian strain in terms of biomass production (Tobias *et al.*, 1980). The variation in the population structure of *Artemia* in the salt pans of Tuticorin over the year with respect to temperature, salinity and phytoplankton was described by Joslet Mathew (1990). Growth and reproduction rate of Tuticorin strain of *Artemia* with respect to different food type, salinity and the effect of EDTA on cyst production were studied by Balasundaram and Kumaraguru (1987). Basil and Pandian (1991) had tried to culture the Tuticorin strain of *Artemia* in organic and agricultural waste at different salinities as the complete dependence on microalgal feeds for the *Artemia* production are not economically viable. There were reports of occurrence of sexual population in the Karapadu salt pans of Tuticorin where usually only parthenogenetic population were reported to have occurred (Rajamani *et al.*, 1998) and the fecundity and inter spawning periodicity of these strain were also determined (Rajamani *et al.*, 1999). So it is important to study the reproductive biological aspects of this exotic population in comparison with the indigenous parthenogenetic population so as to find out the possibility of competitive exclusion of either of these population and also the viability of selecting either of these to culture in the salt pans of Tuticorin. Therefore, in the present investigation reproductive biology of the two locally available species were conducted under different ecophysiological conditions in order to understand the reproductive potential of both native and exotic species. The results of the present study may be helpful in the future inoculation trials that can be carried out with these strains in the salt ponds of Tuticorin region, where there is immense scope for *Artemia* cultivation due to the presence of vast areas of saltpans.

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

1. Collection of animals for the experiment

Two locally available species of brine shrimps were selected for the study. The native asexual strain *Artemia parthenogenetica* (Plate 2a) was collected from the Thirespuram (Plate 1a) salt pan areas which is located about 5 km north of Karappadu field laboratory at Tuticorin (Latitude 8 °48' N, Longitude 78°11' E), where the present study was carried out. Parthenogenetic species have mostly female individuals in its population, which have significantly longer antennae than the sexual females as shown in plate 3b and 3c (Triantaphyllidis *et al.*, 1995). The sexual species *Artemia franciscana* (plate 2b) was collected from the salt pans of Veppalodai (plate 1b) situated about 25 km north of Karappadu laboratory. Sexual species have both male and female individuals in its population. Males have well developed antennae in the form of claspers (Plate 4a) and paired penis (Plate 4b).

Animals were collected from the salt pans using a tea strainer and were transferred to a bucket containing brine collected from the same salt pan and then transported to the laboratory where they were maintained as stock. Both the species were maintained in a FRP tank of capacity 25 litres each, after diluting the habitat brine gradually to 100ppt. Aeration was provided for 24 hours. Healthy matured brooders were taken from this stock for salinity, temperature, photoperiodic and feeding experiments. Fresh collections were carried out before the commencement of each set of experiments. Stocking medium was replaced weekly and feeding was done with *Chlorella* sp. with a cell concentration of approximately 1.5 million

PLATE 1

a) Salt pans at Thirespuram from where asexual species of *Artemia* were collected

b) Salt pans at Vepalodai from where sexual species of *Artemia* were collected

Plate 1

PLATE 2

a) Asexual species of *Artemia* collected from Thirespuram salt pans of Tuticorin

b) Sexual *Artemia* species collected from Vepalodai salt pans of Tuticorin.

Plate 2

PLATE 3

a) Broodpouch of sexual species of *Artemia* with cysts – Oviparous mode of reproduction

1 - Cyst

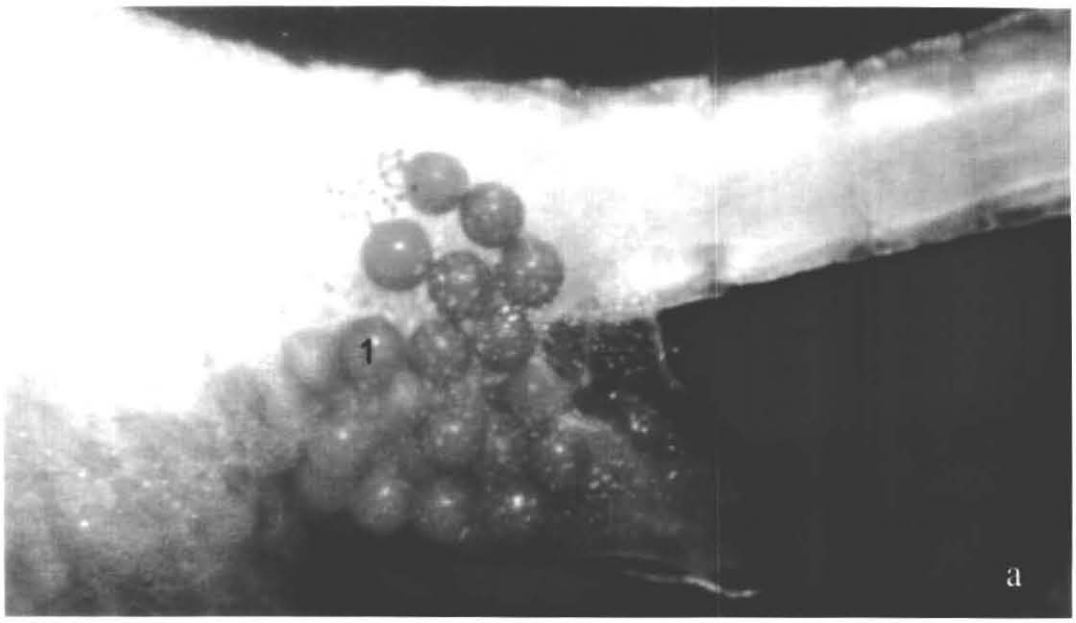
b) Head of female Artemia with short antennae, characteristic of sexual *Artemia* species.

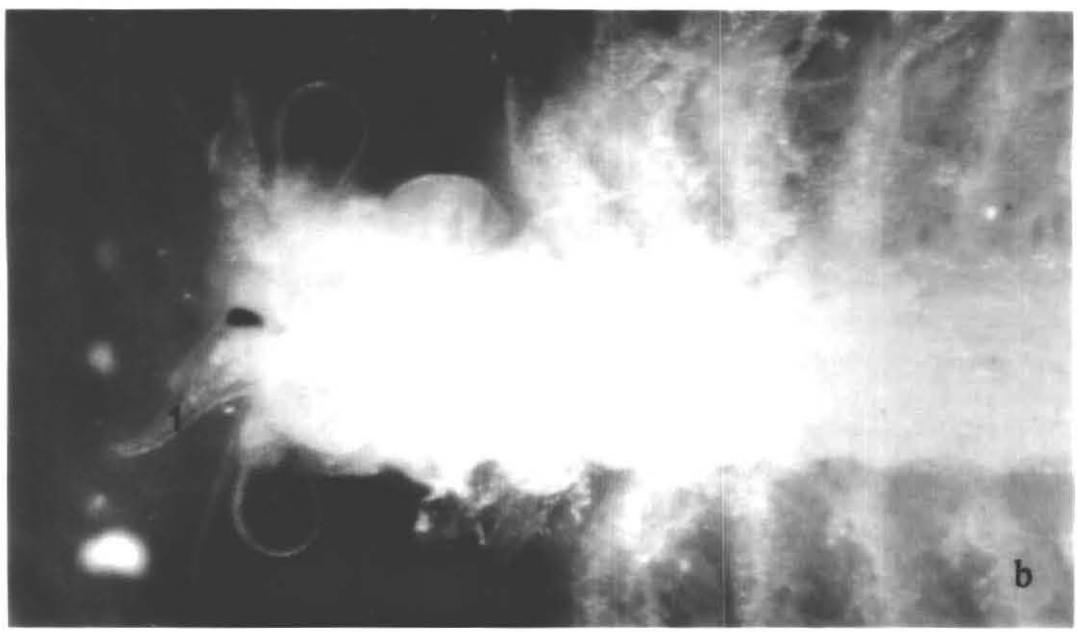
1 – Antennae

c) Head of female *Artemia* with long antennae, characteristic of asexual Artemia species

1 – Antennae

Plate 3





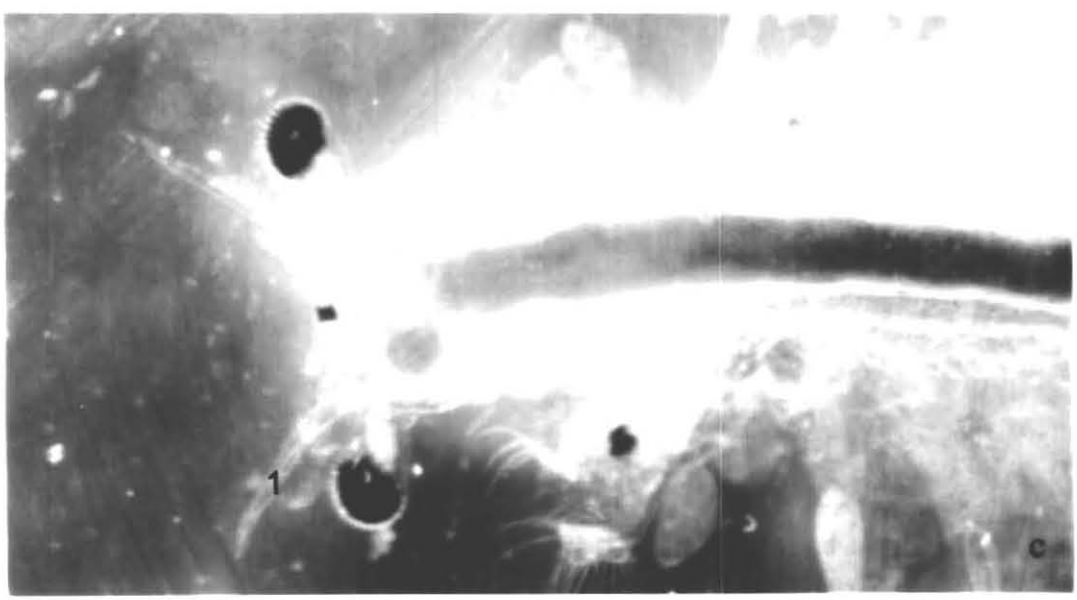


PLATE 4

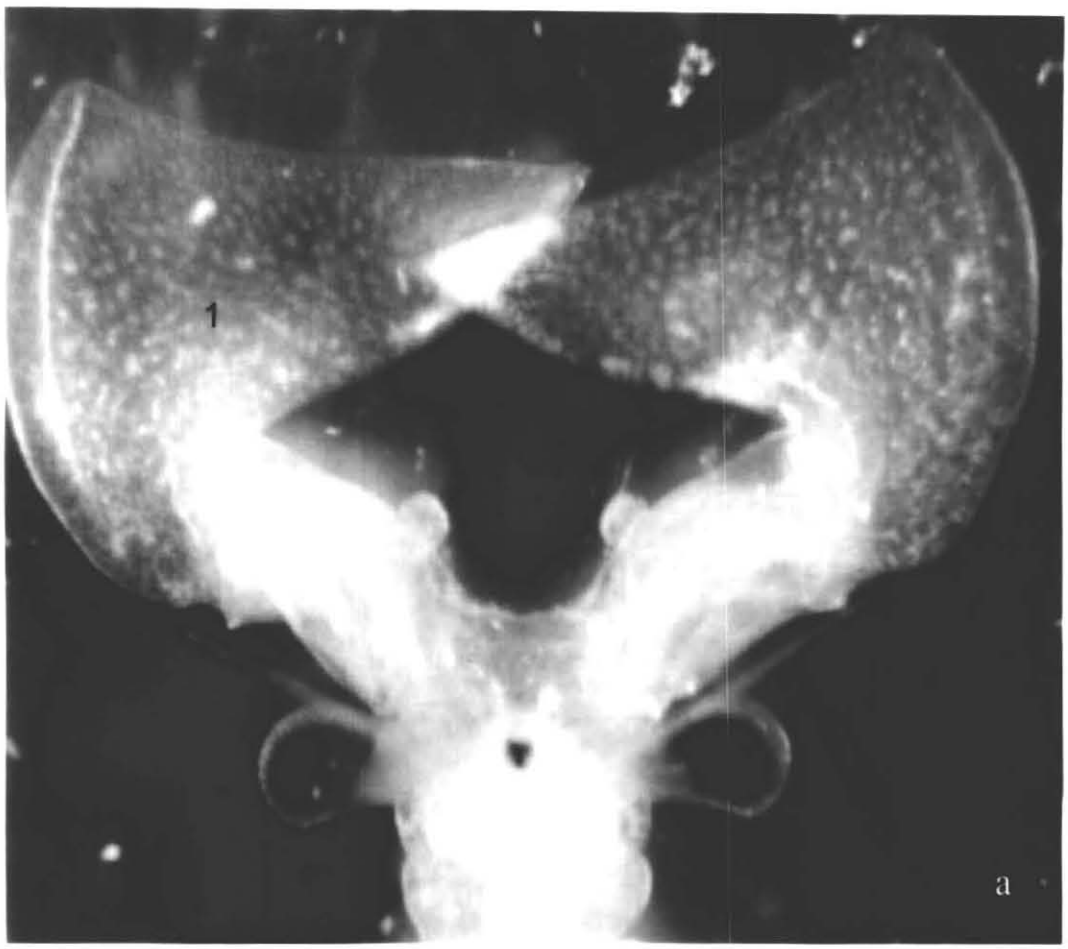
a) Head of male animal with claspers, belongs to the sexual *Artemia* species.

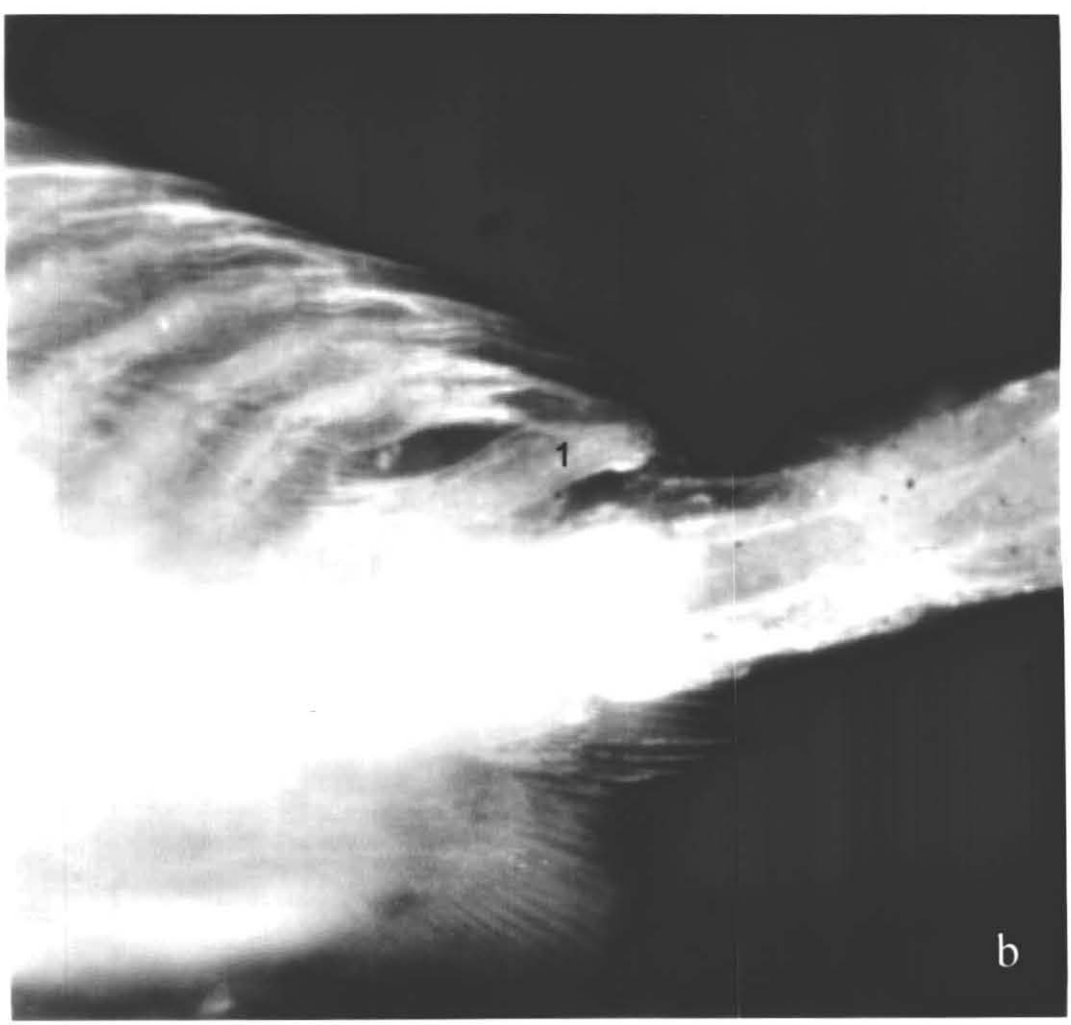
1 – Antennae

b) Genital organ of male of sexual species of *Artemia*.

1 – Male genital organ

Plate 4





cells/ml. Ecological parameters like temperature, salinity and pH were checked at regular intervals.

2. Hydrographic observations

Temperature was observed daily using a Celsius thermometer. pH was determined using digital pH meter. The pH meter was standardised by using buffer solutions of acidic (0.4) and alkaline (9.2) range. Daily salinity readings were taken by a refractometer and was confirmed by titration which was carried out twice a week following Mohr- Kundson method as given by Strickland and Parson (1972). In this method, 10 ml of sample was taken in 250-ml conical flask. Four drops of Potassium Chromate were added as indicator and sample was titrated against Silver nitrate solution, till the colour turned brick red. Algal count in the culture medium was noted using a standard Haemocytometer weekly twice. Faecal matter settled in the bottom of the container was removed daily, with the help of a dropper and the quantity of the culture medium lost was replaced with the fresh medium.

3. Experimental setup

Reproductive and lifespan characters such as Prereproductive period, Reproductive period, Postreproductive period, Total lifespan, Percentage reproductive period of total lifespan, Number of offspring per brood, Broods per female, Inter brood periods, Cysts per female, Nauplii per female, Percentage encystment, Cyst size, Nauplii size, Adult size at maturity etc. of the two *Artemia* species were studied under different ecophysiological conditions to assess the i) effect of temperature ii) effect of salinity iii) effect of quality and quantity of feeds and iv) effect of starvation. The nauplii for the experiments were obtained from the brooders from the respective stock, which were collected from the salt pans from two selected areas as mentioned above. The experiments were continued till the animals were found dead.

Prereproductive period was studied by keeping twenty nauplii each per one litre culture medium in 1.5-litre capacity plastic jars and the experiments were run in triplicates. A minimum number of three matured animals were transferred individually to 500 ml capacity transparent plastic jars containing 300 ml of culture medium from each 1.5 litre jar. Jars were checked daily for the presence of nauplii or cyst. When mortality was observed in the case of male in sexual strain, it was replaced by another mature male.

4. Salinity experiments

Experiments were run at eight different salinities viz. 20, 45, 80, 100, 120, 145, 170 and 195ppt, at ambient temperature of 31±3 °C. *Chlorella* sp. was given as feed and cell concentration in the rearing medium was maintained at 1.5 million cells per ml. All the experiments were carried out in natural light. The experiments were run in three series. Salinities 145,170 and 195 were tested in the first series. All the three salinities were obtained by diluting brine with seawater by regularly checking the level of salinity with a refractometer. In the second series 80,100 and 120ppt treatments were carried out. Culture medium was prepared as mentioned above. In the final series 20 and 45-ppt salinity effects were studied. The 20-ppt medium was prepared by diluting seawater with fresh water and 45 ppt was obtained by adding brine. Brine was prepared by adding salt crystals collected from the salt pan areas, to the seawater till saturation and was stored so as to prepare required range of salinity. This was done because the present experiment was carried out during December, January months when operation in the salt pans were suspended temporarily due to rain and there was difficulty in getting brine as the pumping of brine through the bore wells was stopped.

Flow chart showing the experimental set-up

Wild sexual and asexual species

↓

Acclimation in the laboratory

↓

Few healthy matured animals from each species, about-to release the offspring are Isolated to individual plastic containers (both male and female in case of sexual species)

↓

20 nauplii (40 in case of sexual species including male population) to each salinities with triplication

↓

3 matured females from each replicate till the death of atleast two females.

Initially the salinity experiments with eight salinities were designed with an interval of 25ppt salinity each. But later, when the experiment was conducted there was a slight variation in the salinity difference in the second series of experiment as the salinity difference decreased to 20 (80,100 and 120ppt salinities). But this was not considered as a major factor as the primary aim of the experiment was the comparative study of the sexual and asexual species and the treatments were given similarly to both the species.

The *Chlorella* sp. cultures for the experiment were kept out door in FRP tanks and was found contaminated with some filamentous algae. The cultures were then transferred to indoor transparent plastic buckets and were covered completely using polythene sheets.

5. Temperature experiments

For the present study, a temperature range of 25 to 34 °C were selected as Tuticorin being the tropical area. A factorial experiment was used involving nine combinations of three temperatures 25, 30, 33.5 °C and three salinities 80,100,120 ppt to study various aspects of the reproductive biology of the asexual and sexual *Artemia* species. Only the mid ranges of the eight salinities tested in the previous experiment were selected to study the temperature effect as these salinities were found most suitable in the last experiment.

Flow chart showing the experimental set-up

Wild sexual and asexual species

↓

Acclimation in the laboratory

↓

Few healthy matured animals from each species, about-to release the offsprings are Isolated to individual plastic containers (both male and female in case of sexual species)

↓

20 nauplii (40 in case of sexual species including male population) to each temperature salinity combinations with triplication

↓

3 matured females from each replicate till the death of atleast two females.

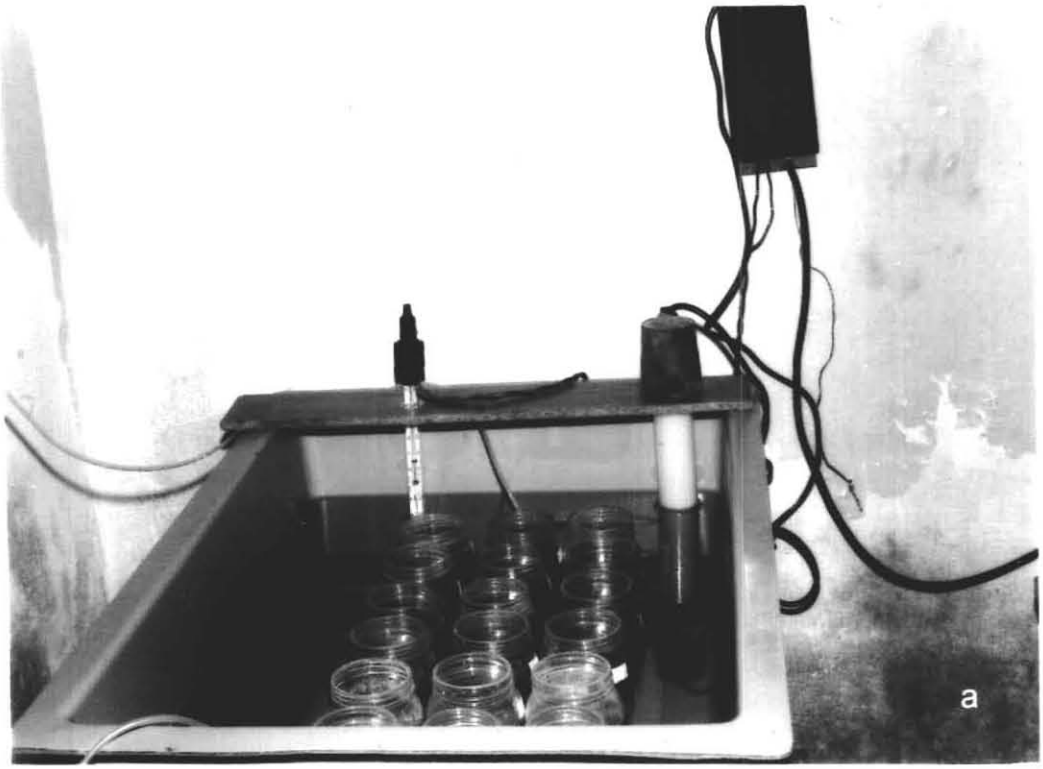
Different salinities were obtained by dissolving salt collected from salt pan areas into the seawater and a saturated brine solution was prepared as stock and the required salinities were prepared by diluting this

PLATE 5

a) Experiment set up for the temperature experiments with Jumo thermometer, matured females are reared to study the reproductive characteristics

b) Experiment set up for rearing nauplii till maturity , temperature experiment with Jumo thermometer.

Plate 5



with seawater. Salt was collected because brine was not available in salt pan as pumping of brine through the bore well was suspended due to rain. *Chlorella* sp. was given as feed for the experimental animal. Same feeding schedule was adopted for all the salinities and temperature.

Diurnal temperature variation was observed to vary between 25 to 35.5 °C, and therefore three temperatures of 25, 30 and 33.5 °C was selected for the present study. Temperature was maintained at fixed level with the help of a jumo contact thermometer, with an electric relay and immersion heater (Plate 5a and 5b). The experiment at lower temperature (25 °C) was carriedout in an air-conditioned room. The containers for the experiment were arranged on a platform kept in a Perspex tank of 50 litre capacity. The tank was then filled with fresh water and to get a uniform distribution of temperature, air circulation was arranged. Containers were arranged on the platform in such a way that a great part of them was immersed in the fresh water and were getting heated in accordance with the water filled in the tank, which in turn was regulated as depicted above.

6. Feeding experiments

The effect of two algal feeds, *Chlorella* sp. and *Isochrisis* sp. and an inert feed on the reproductive characters of the two locally obtained sexual and asexual *Artemia* species were studied. Salinity of each jar was maintained by adding either distilled water or brine. Medium was replaced with fresh medium on every fifth day. Algal concentration was checked twice a week using a standard Haemocytometer.

Flow chart showing the experimental set-up

Wild sexual and asexual species

↓

Acclimation in the laboratory

↓

Few healthy matured animals from each species, about-
to release the offsprings are isolated to individual plastic
containers (both male and female in case of sexual species)

↓

20 nauplii (40 in case of sexual
species including male population)
to each feed and salinity combinations
with triplication

↓

3 matured females from each
replicate till the death of atleast two females.

Chlorella sp. was cultured in door in plastic transparent buckets of 20 litre capacity. Commercial medium (of Urea, Potash, Phosphate) @ 1 gram / litre was added to filtered and sterilised seawater before the inoculam was added to the culture and aeration was provided. Bloom was obtained in a week's time and was maintained by regularly harvesting and adding fresh sterilised seawater. The count was maintained approximately at 3 million cells/ ml for high feeding sets and 1.5 million cells/ml for low feeding sets. Salinity was maintained by adding either distilled water or brine. Algal concentration was checked twice a week. *Isochrisis galbana* was obtained from the algal culture laboratory of TRC of CMFRI. *Isochrisis* sp. concentration was maintained at 0.1 million/ml for high feeding sets and 0.05 million/ml for the low (normal) feeding sets.

Rice bran was purchased from the local market. It was weighed and then ground using an electric mixer and was sieved through a common kitchen sieve and was weighed again. The sieved ricebran was then boiled in fresh water and was allowed to cool. This was again sieved through one 50 μ net, and was taken in a cylindrical container provided with vigorous aeration and was allowed to settle after 2 hours. Rahaman and Rathinasamy

(1997) have mentioned that the dry ricebran was sieved through 50 μ size micro filter and then was micronized with seawater before stored in refrigerator. As it was difficult to sieve dry powdered ricebran through 50 μ sieve here rice bran was first boiled in freshwater then was mixed with seawater before sieving through 50 μ filter. The settled rice bran was collected and dried in oven at 45 to 50 °C and was stored for further feeding of the experimental animals. Approximately 74 grams of the final product was obtained from one kilogram of raw ricebran purchased from the market.

For feeding the experimental animals, a solution of rice bran in seawater was prepared by powdering and mixing the ricebran with seawater with a mortar and pestle @ 1 gram /10 ml seawater. From this solution 0.5 ml and 1 ml were added to individual animals kept in 500 ml jars for low and high feeding animals respectively and proportionate feeding was done with the nauplii rearing experiments also. Before feeding the animal, the feed remnants and faecal matters settled at the bottom were removed using a pointed dropper every day from the jars and the medium was changed with fresh medium on every fifth day.

7. Photoperiodic experiments

An experimental setup was arranged to study the influence of light on brood encystment of offspring. One set of animals was kept exposed to natural variations of light 12:12, L: D and another set was covered using a black cloth so as to prevent light, 0:24, L:D. The salinity selected was 100 ppt and the feed was *Chlorella* sp.

8. Cyst - hatching

Hatchability tests could not be conducted on all the cysts obtained from different experiments as the cyst availability was very less in many of the conditions. Enough quantity of cysts was obtained from the temperature experiments of 25 °C, from both the species. Apart from that

some cysts were collected from the animal stock maintained at laboratory. Wild cysts were obtained only for *Artemia parthenogenetica* and their hatchability also was tested.

Hundred numbers of cysts from each batch was taken and was put into hatching medium having salinity of 15 ppt, which was obtained by mixing seawater and freshwater and was taken in a transparent plastic jar with curved bottom and vigorous aeration was provided. Experiments were run in triplicates. From the 12^{th} hour onwards regular observation at every one hour was made to record the appearance of any nauplii so as to find out the time taken for first hatching .The experiment was terminated after thirty hours.

9. Data analysis

Data were analysed using two factor (for salinity experiments and photoperiodism experiments) and three factor (for feeding experiments and temperature experiments) ANOVA to study the effect of different ecophysiological interventions (Sokal and Rohlf, 1995). Pair wise comparisons between treatments were made using the Fishers LSD test, wherever the interactive effects were not significant. For all statistical tests, values for percent were normalised by arcsine transformation.

Experiments conducted

Expmt / Salin (ppt)	Asexual species			Sexual species		
	Temp (°C)	Feed	Cell conc. (mill/ml)	Temp (°C)	Feed	Cell conc. (mill/ml)
20	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5
45	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5
80	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5
100	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5
120	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5
145	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5
170	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5
195	31±3	*Chlorella* sp.	1.5	31±3	*Chlorella* sp.	1.5

Expmt / Temp. (°C)	Asexual species			Sexual species		
	Salin (ppt)	Feed	Cell conc. (mill/ml)	Salin (ppt)	Feed	Cell conc. (mill/ml)
25	80	*Chlorella* sp.	1.5	80	*Chlorella* sp.	1.5
	100	*Chlorella* sp.	1.5	100	*Chlorella* sp.	1.5
	120	*Chlorella* sp.	1.5	120	*Chlorella* sp.	1.5
30	80	*Chlorella* sp.	1.5	80	*Chlorella* sp.	1.5
	100	*Chlorella* sp.	1.5	100	*Chlorella* sp.	1.5
	120	*Chlorella* sp.	1.5	120	*Chlorella* sp.	1.5
33.5	80	*Chlorella* sp.	1.5	80	*Chlorella* sp.	1.5
	100	*Chlorella* sp.	1.5	100	*Chlorella* sp.	1.5
	120	*Chlorella* sp.	1.5	120	*Chlorella* sp.	1.5

Expmt Feed	Asexual species			Sexual species		
	Salin. (ppt)	Temp (°C)	Cell conc. (mill/ml)	Salin. (ppt)	Temp (°C)	Cell conc. (mill/ml)
Isochrisis sp. (Normal)	80	31±3	.05	80	31±3	.05
	100	31±3	.05	100	31±3	.05
	120	31±3	.05	120	31±3	.05
Isochrisis sp. (High)	80	31±3	0.1	80	31±3	0.1
	100	31±3	0.1	100	31±3	0.1
	120	31±3	0.1	120	31±3	0.1
Chlorella sp. (Normal)	80	31±3	1.5	80	31±3	1.5
	100	31±3	1.5	100	31±3	1.5
	120	31±3	1.5	120	31±3	1.5
Chlorella sp. (High)	80	31±3	3.0	80	31±3	3.0
	100	31±3	3.0	100	31±3	3.0
	120	31±3	3.0	120	31±3	3.0
Ricebran (Normal)	80	31±3	.05g/ 300ml	80	31±3	.05 g/ 300ml
	100	31±3	.05g/ 300ml	100	31±3	.05 g/ 300ml
	120	31±3	.05g/ 300ml	120	31±3	.05 g/ 300ml
Ricebran (High)	80	31±3	0.1g/300ml	80	31±3	0.1g/300ml
	100	31±3	0.1g/300ml	100	31±3	0.1g/300ml
	120	31±3	0.1g/300ml	120	31±3	0.1g/300ml
Starvation	80	31±3		80	31±3	
	100	31±3		100	31±3	
	120	31±3		120	31±3	

Expmt	Asexual species				Sexual species			
Photo. D:L	Temp (°C)	Salin (ppt)	Feed	Cell conc. (mill/ml)	Temp (°C)	Salin. (ppt)	Feed	Cell conc. (mill/ml)
12:12	31±3	100	*Chlorella* sp.	1.5	31±3	100	*Chlorella* sp.	1.5
24:0		100	*Chlorella* sp.	1.5		100	*Chlorella* sp.	1.5
12:12	25	100	*Chlorella* sp.	1.5	25	100	*Chlorella* sp.	1.5
24:0		100	*Chlorella* sp.	1.5		100	*Chlorella* sp.	1.5

Abbreviations used

Temp - Temperature
Salin - Salinity
Cell con - Cell Concentration
Mill/ml - Million cells per millilitre
Photo - Photoperiod
Expmt - Experiment

CHAPTER 1

CHAPTER 1.

Effect of ecophysiological conditions on the life span characteristics

1.1. Introduction

Variation in habitat ecological conditions can have major effect on life history and reproductive characteristics of Artemia population belonging to a particular species existing at different geographical areas (Browne *et al.*, 1988). There are reports of co-occurrence of both sexual and asexual population in some Spanish salina (Amat, 1980a, 1983) where one population dominates the other in accordance with the climatic changes. In India, the presence of both the native parthenogenetic species and exotic sexual species are reported from the salt pans at Tuticorin and the possibility of finding both the species in the same pans cannot be ruled out and either of these species may dominate the other in due course of time. Detailed study of lifespan characteristics of both the population were conducted as these are important in determining the competitive interaction of both the species and the possible exclusion of either of it from the mixed population.

Entire life span of the females are divided into three components like prereproductive period, reproductive period and post reproductive period, so as to avoid confusion regarding the actual reproductive period of the animal throughout its life span. Among these pre reproductive period and the reproductive period have more affect on the fitness of the animal (Cole, 1954).

1. 2. Materials and methods

Experimental set up and other methods used for this study are detailed in the General Materials and method and the Life span characteristics observed are detailed below.

1. 2.1. Female life span and life span components

Total female life span is composed of pre reproductive, reproductive and post reproductive period. Percentage reproductive period of total life span is also calculated so as to find out the actual reproductive period spent by the female in the Total life span.

1. 2.1.1. Female prereproductive period

Average time in days taken by 50 % of the nauplii (20 numbers in the present experiment) in the 1st instar to mature and release the first batch of the nauplii or cysts (f1 generation) (Browne *et al.*, 1988).The sexual strains of *Artemia* have both males and females in the population and the males are identified with their modified antennal claspers and females with their uterus. In the asexual strain only females are found in the population.

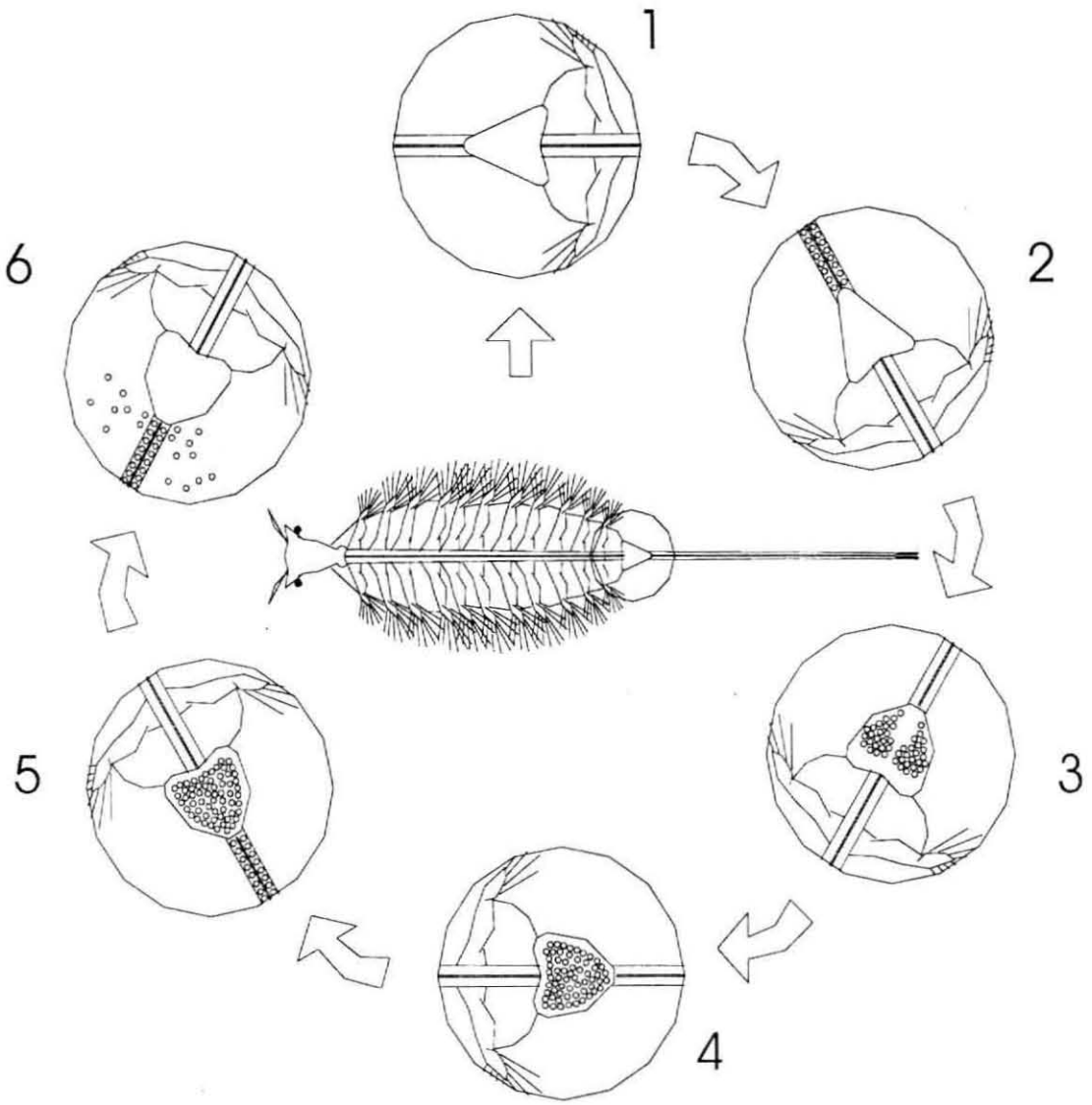
Females were considered mature when the uterus or ovisac was first visible to the naked eyes as a swelling behind the last pair of thoracopods. The developments in the ovary during maturation have been described in detail by Bowen (1962). In the present study the entire maturation cycle was further divided into six stages(Figure a) and the photographs were taken using microscope fitted with Pentax camera.

1. 2.1.1.1. Stage 1

This stage marks the beginning of the gonadal developments and do have a prominent uterus devoid of ova, just behind the last pair of thoracopods(plate 6a).

Figure a

MATURITY STAGES OF *ARTEMIA*



1. 2.1.1. 2. Stage 2

At this stage ovary can be seen on the either sides of the uterus, extending towards the tail end, as two yellowish white stripes(Plate 6b, Plate 6c). The colours of the developing eggs were found to differ depending on the feed. Rice bran gave a whitish appearance where as the *Chlorella* fed animals gave a light green appearance to the ovaries.

1. 2.1.1. 3. Stage 3

Here eggs are translocated to the uterus from the ovaries and can be seen as two bundles on its either side as shown in Plate 7a.

1. 2.1.1.4. Stage 4

The two bundles are united to form a single mass of eggs and remains in the uterus, till they are matured and are ready to get released (Plate 7b).

1. 2.1.1. 5. Stage 5

At this stage the next set of ova appears in the ovary as two white stripes and the previous set are still present in the uterus in their last stage of development (Plate 7c).

1. 2.1.1. 6. Stage 6

The fully developed eggs are released to the medium either as cysts or nauplii depending on the prevailing ecological conditions. The uterus remains empty to receive the fresh set of matured eggs from the ovary.

1. 2.1. 2. Female reproductive period

The potential reproductive days of the animals are counted from the first brood which release the first nauplii or cysts to the last brood.

PLATE 6

a) Stage 1. *Artemia* (asexual species) at early stages of maturity where brood pouch is in the developing stage

1 – Brood pouch

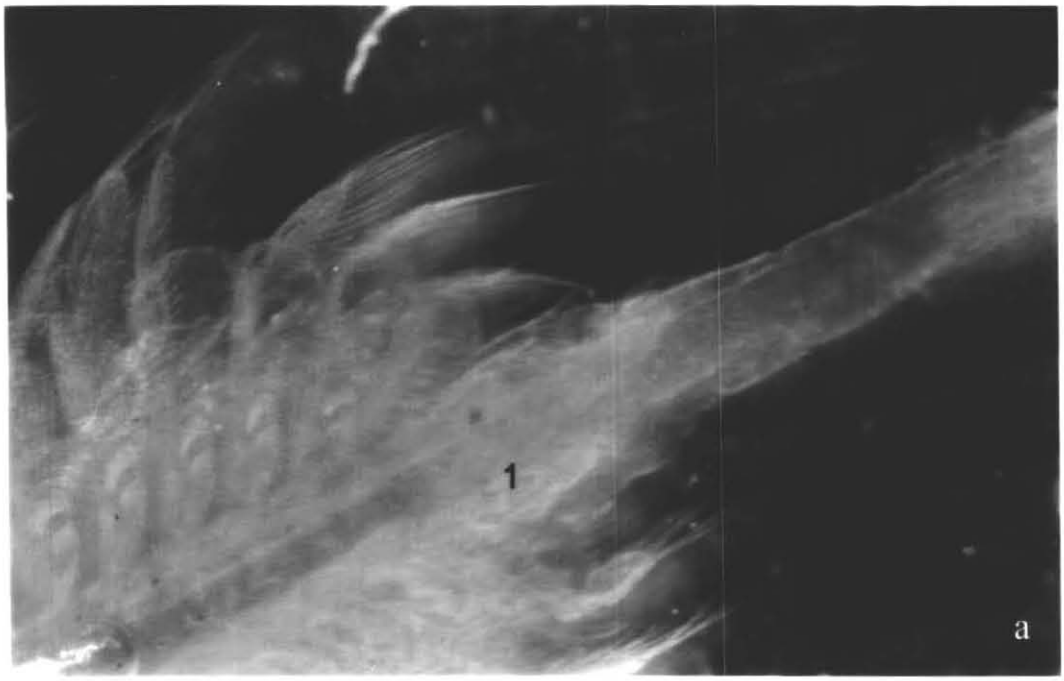
b) Stage 2. *Artemia* (asexual species), dorsal view of the ovary carrying matured eggs.

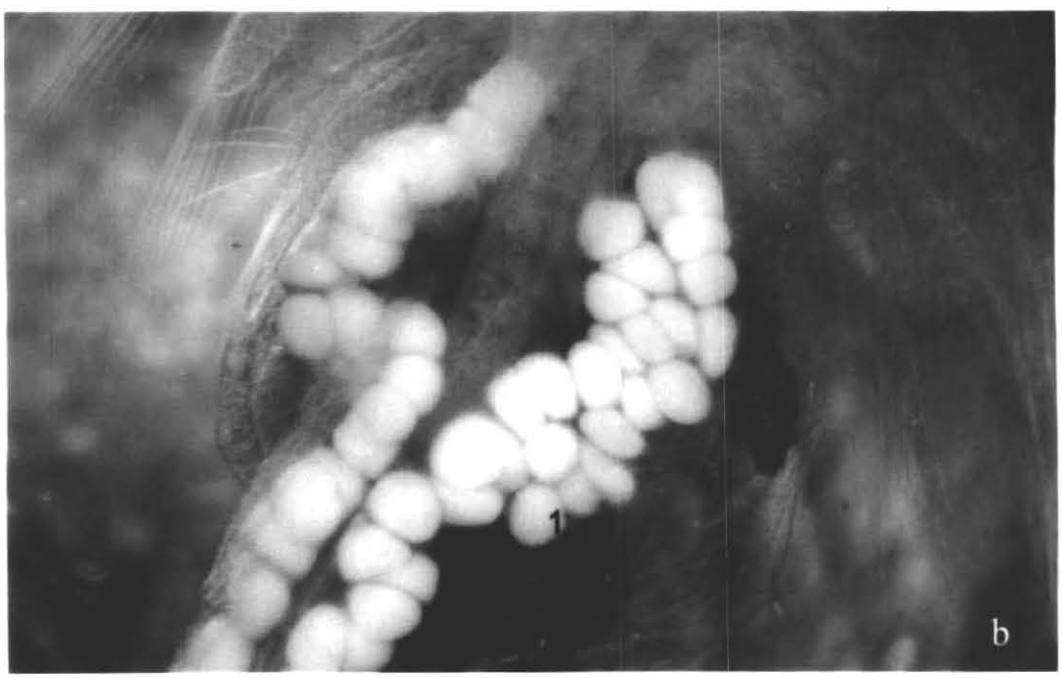
1 – Matured egg
2 – Brood pouch

c) Stage 2. Artemia (asexual species), ventral view of ovary carrying matured eggs.

1 – Matured egg
2 – Brood pouch

Plate 6





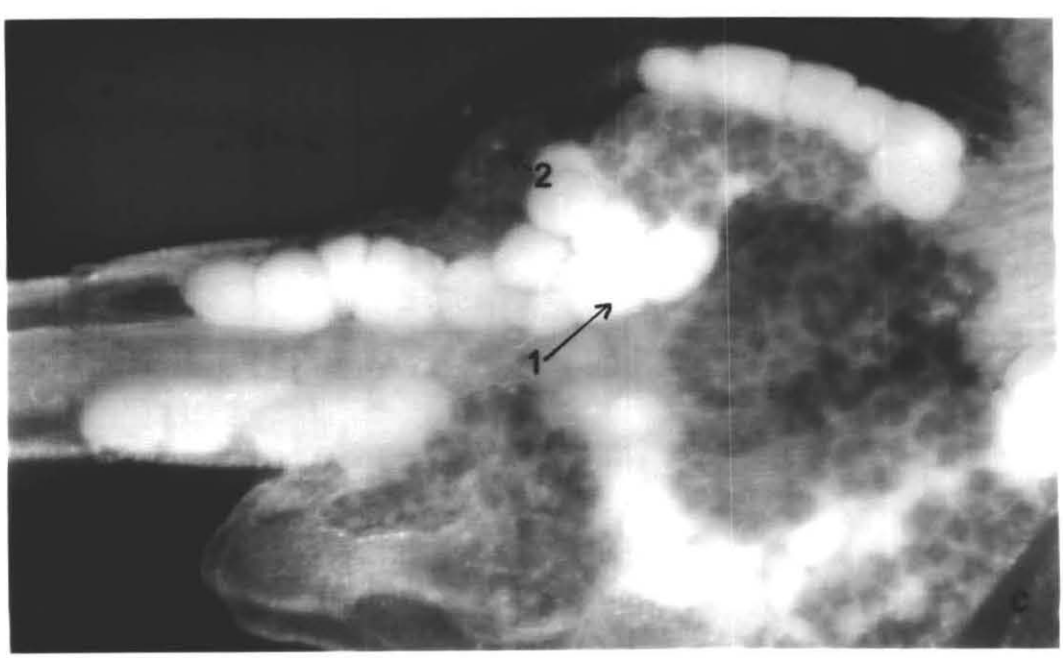


PLATE 7

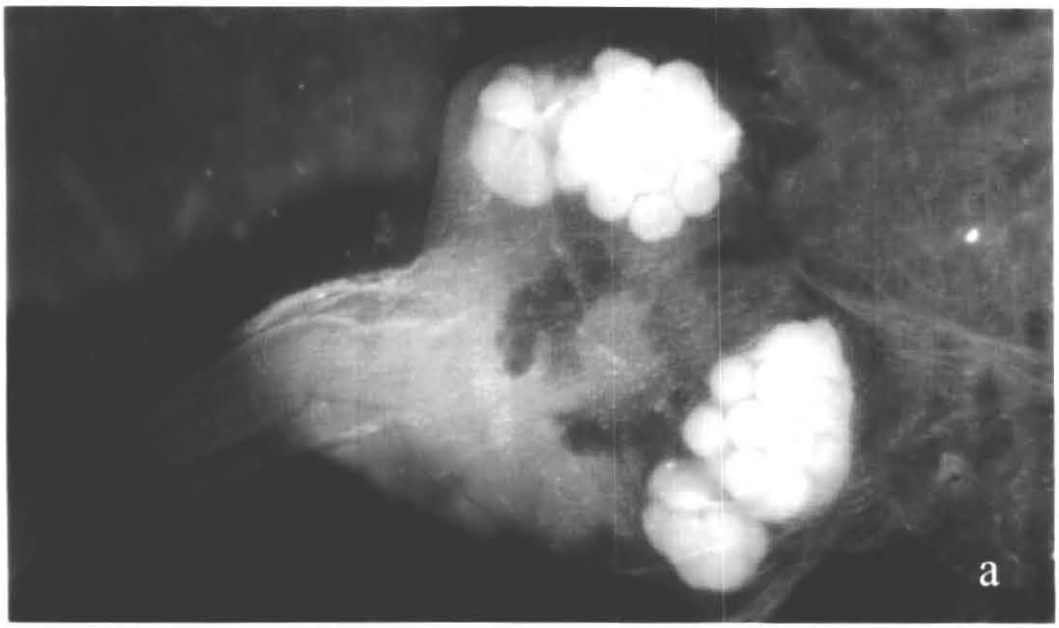
a) Stage 3. *Artemia* (asexual species), eggs are passed on to the brood pouch from the two ovaries.

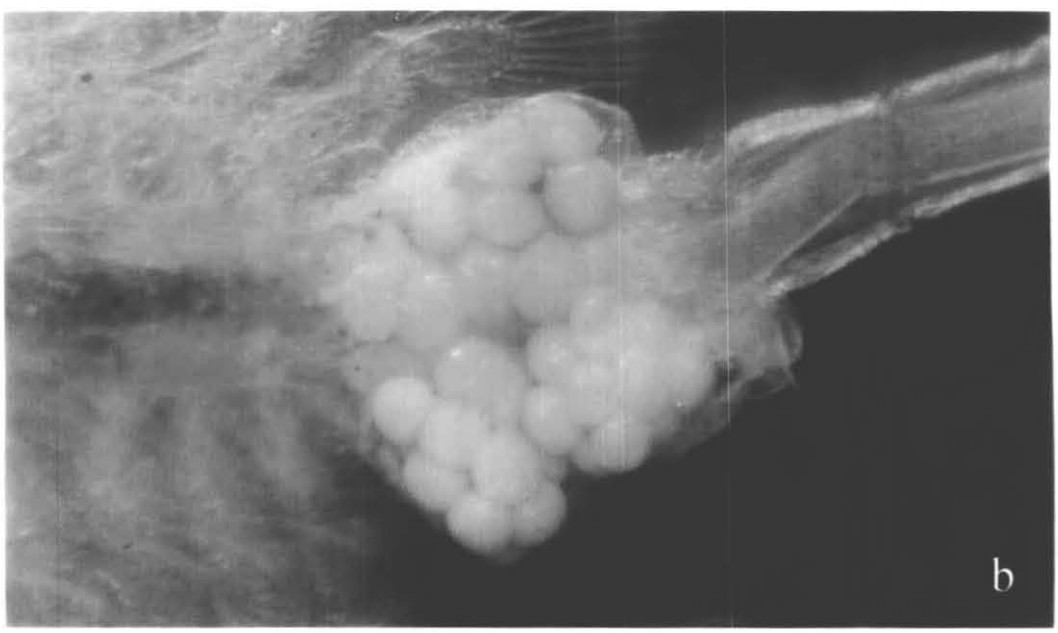
b) Stage 4. *Artemia* (asexual species), eggs from both the ovaries are in united form

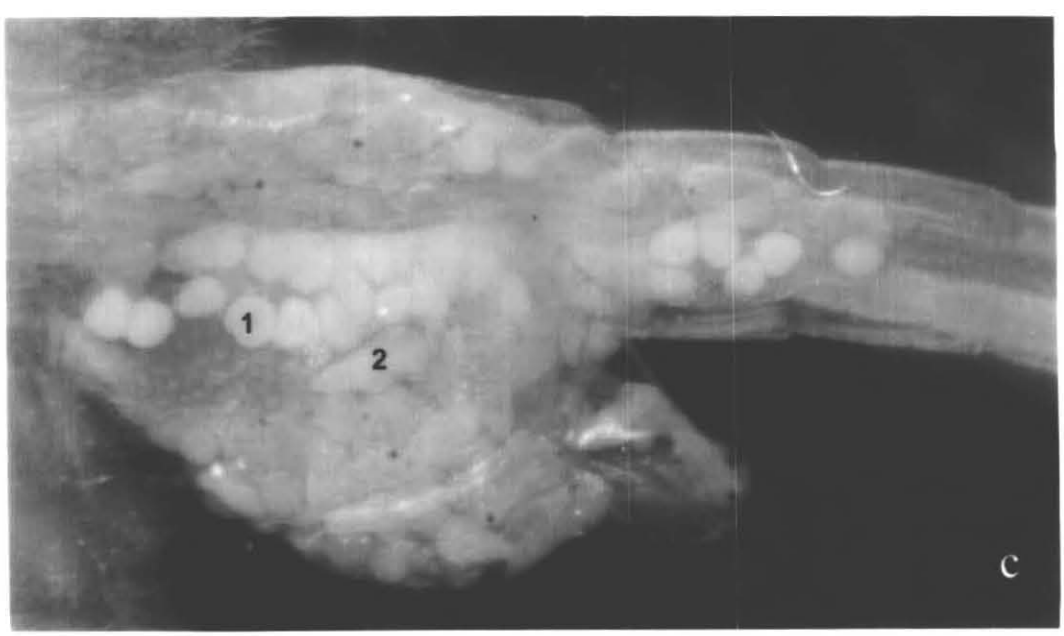
c) Stage 5. *Artemia* (asexual species), ovary with maturing eggs, brood pouch hold both the matured eggs and hatched nauplii indicating the ovoviviparous mode of reproduction.

1 – Matured egg
2 - Nauplii

Plate 7







1. 2.1. 3. Female postreproductive period

Number of days counted from the last brood till the death of the animal

1. 2.1.4. Total female lifespan

Total number of days taken by the nauplii to mature, reproduce and die. This is obtained by adding prereproductive period, reproductive period, and postreproductive period.

1. 2.1. 5. Percentage of reproductive period of total lifespan

Calculated as the percentage of reproductive period to the total lifespan of the respective female.

1. 3. Results

1. 3.1. Prereproductive period

Results of the Effect of ecophysiological condition on the pre-reproductive period of both the sexual and asexual species of *Artemia* are listed here.

1. 3.1.1. Effect of salinity.

Results on the length of the prereproductive periods of both the species of *Artemia*, at different salinities are presented in Figure 1a and Table 1a. The values of prereproductive period in Figure 1a are shown in the Appendix 1a. As both the species did not reproduce at 170 and 195ppt salinities, only the results obtained at six salinities from 20 to 147ppt could be used for statistical analysis. Salinity was found to have significant effect on the prereproductive period of the animals ($P<0.01$) and its interactive effect with species also were highly significant. Maturation was delayed in both the cases at higher (145 ppt) as well as lower (20ppt) salinities. The asexual females took only 27 days whereas the sexual females needed 32 days to attain maturity at 145ppt. Above 145 ppt animals could not complete their pre-

reproductive period as there was mortality before they attained maturity. Maturation was faster for sexual species at 20, 45, 80 and 100ppt salinities whereas the asexual species had a faster maturation at 120 and 145ppt salinities. There was an increase in the maturation period as the salinity increased from 80 ppt for both the species. Similar increase was recorded with decrease in salinity also indicating 80ppt as the optimum salinity range for faster maturation of both the species. Higher prereproductive period recorded in both the species at 20ppt decreased gradually as salinities increased upto 80ppt and it increased again at higher salinities.

1. 3.1. 2. Effect of temperature.

Effect of temperature on the length of prereproductive period at three different salinities 80, 100 and 120ppt for sexual and asexual species of *Artemia* are given in Table 1b and Figure 1b . The values of prereproductive periods in Figure 1b are shown in the Appendix 1b. The prereproductive period of asexual females was higher than the sexual females at all the combinations of salinities and temperature except in combinations of 31°C and 120ppt, where the sexual females took more time to release their first batch of offspring than the asexual females. The length of prereproductive period increased with the increase in salinity. At all the three salinities the pre-reproductive period declined from 25 °C to 30 °C and then increased in 33.5 °C indicating the temperature nearer to 30 °C is favourable at all salinities.

At lower temperature, 25 °C, the maturation process was slower, which may be due to a lesser metabolic rate. Increase in salinity also hindered the maturation process, as there was an increase in prereproductive days alongwith increase in salinity for both the species at all the salinity temperature combinations.

As asexual females could not mature at 33.5 °C only results from 25 and 30 °C were taken for statistical analysis. Data from ambient temperature also were not considered for the analysis. At 25 and 30°C interactive effect of temperature and salinity on both the species had significant effect on the prereproductive period, $P<0.05$. Interaction of temperature and salinity, temperature and species and salinity and species also had significant effect. Sexual species could reproduce at all the three temperatures and the temperature alone and also in interaction with salinity had significant effect on the prereproductive period of the species.

1. 3. 1. 3. Effect of Quality and quantity of feeding.

Results on the effect of quality and quantity of the three types of feed on the length of prereproductive period of the two *Artemia* species are listed in the Table 1c, Figures 1ca and 1cb. The values of prereproductive periods in Figure 1ca and 1cb are shown in the Appendix 1c. Among the three types of feeds tested *Isochrisis* is found to be the best suited feed for both sexual and asexual species of *Artemia* at all the three salinities, as the prereproductive period of all the animals fed with *Isochrisis* sp. was lower than the animals fed with other two types of feed at all salinities except in 120ppt where the sexual *Artemia* fed with low (normal) concentration of ricebran had lower prereproductive period. Further, the sexual species fed with all the three types of feed at 100ppt appear to have lower pre-reproductive period than at 80 and 120ppt. Where as there seems to be very little variation in the prereproductive period of asexual species fed with all the three types of feed. In general, among the three types of feed *Isochrisis* sp. is the best suited feed followed by *Chlorella* sp. and ricebran (Figure 1ca,1cb) for faster maturation.

Comparing the two species the sexual species always had less

Table 1a

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the pre reproductive period of the female individuals

Treatments	Degree of freedom	Sum-of - Squares	Mean-square	F-ratio	P
Salinity	5	960.5	192.1	115.3	0.00**
Species	1	53.8	53.8	32.3	0.00**
Salinity & Species	5	259.6	51.91	31.1	0.00**
Error	24	40.0	1.7		

Table 1b

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the prereproductive period of the female individuals

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	484.0	484	300.4	0.00**
Temperature & Salinity	2	15.2	7.6	4.7	0.02*
Temperature & Species	1	215.1	215	133.5	0.00**
Temperature, Salinity & Species	2	52.7	26.4	16.3	0.00**
Error	24	38.7	1.6		

* Significant
** Highly significant

Figure 1a

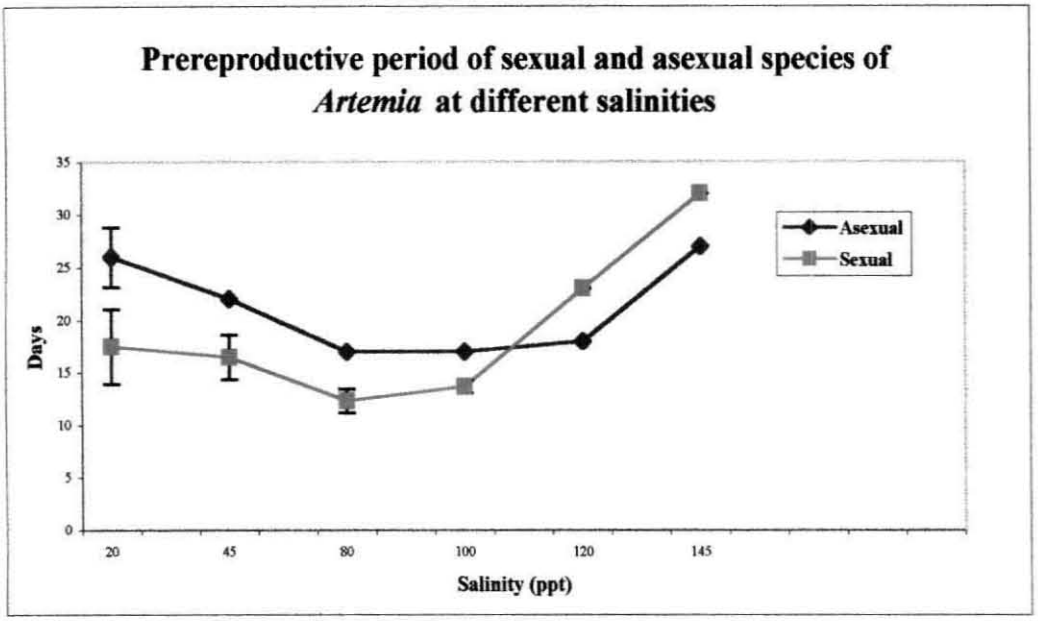
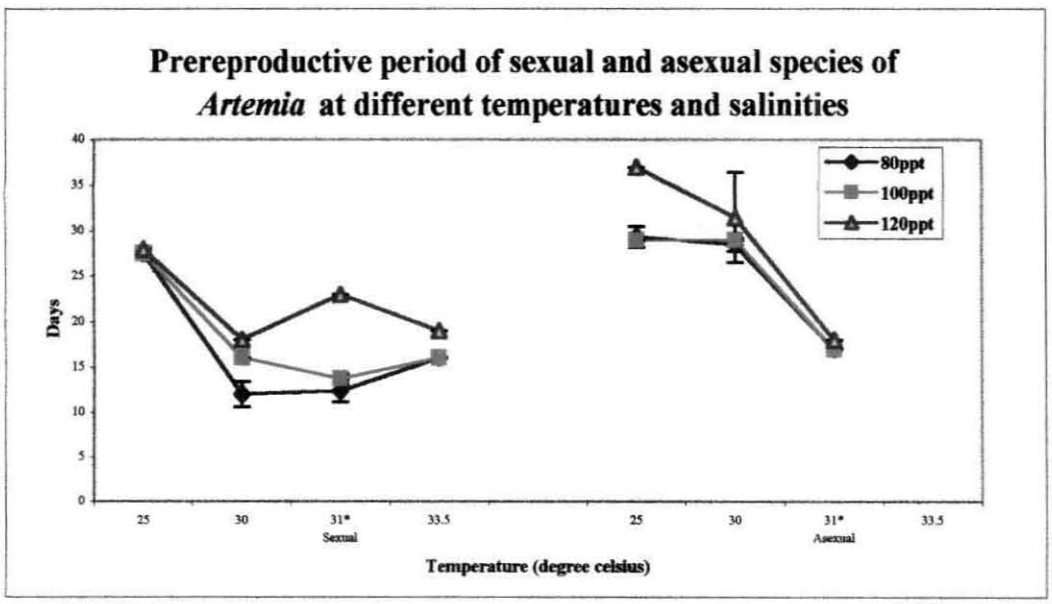


Figure 1b



* Ambient tempertaure

prereproductive period than that of asexual strain, except at 80ppt with rice bran as feed and at 120ppt with *Chlorella* as feed. There was significant difference between the prereproductive periods of the two species, which changed with feed type, feed quality and salinity ($P<0.01$). Individual effect of feed and salinity and also their interactive effect on prereproductive period of both the species were highly significant ($P<0.01$) (Table 1c). Interactive effect of feed quantity, type, salinity and species were also highly significant ($P<0.01$).

1. 3. 2. Reproductive period.

Reproductive period is the time span in days between the first and last brood production of the females and represents the reproductively viable period of total lifespan. Effect of ecophysiological parameters on this characteristic of both the sexual and asexual species is described here under.

1. 3. 2. 1. Effect of salinity.

The results on the length of reproductive period of both the species of *Artemia* in different salinities are given in Table 1d and Figure 1d. The values of reproductive periods in Figure 1d are shown in the Appendix 1d. The sexual species of *Artemia* have relatively longer reproductive period than the asexual counter part. The sexual animals at 45ppt salinity were reproductively active for the maximum number of days (46.67 ± 8.5) among the tested salinity ranges. In the sexual strain reproductive period was relatively longer in the lower saline ranges (20, 45, 80 and 100ppt) and was short in the higher salinity ranges (120,145, 170 and 195ppt). For the asexual animals also at 45ppt salinity the reproductive period was maximum (27 ± 1.41). In the asexual species reproductive period increased towards higher saline ranges (145,170 and 195 ppt). Salinities between 45 and 100ppt are the most advisable as it gave maximum reproductive period for both the species.

Table 1c

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the prereproductive period of the female individuals.

Treatments	Degree of freedom	Sum-of-quares	Mean-square	F-ratio	P
Feeding quantity	1	31.1	31.1	46.7	0.00**
Feed type	2	356.4	178.2	267.3	0.00**
Feed quantity & Feed type	2	16.1	8.1	12.1	0.00**
Feed quantity & Salinity	2	10.9	5.5	8.2	0.00**
Feed quantity & Species	1	59.3	59.3	88.9	0.00**
Feed type & Salinity	4	409.3	102.3	153.5	0.00**
Feed type & Species	2	28.4	14.2	21.3	0.00**
Feed quantity, Feed type & Salinity	4	111.5	27.9	41.8	0.00**
Feed quantity, Feed type & Species	2	1.7	0.8	1.3	0.29
Feed quantity, Salinity & Species	2	15.7	7.8	11.8	0.00**
Feed type, Salinity & Species	4	284.6	71.1	106.7	0.00**
Feed quantity , Feed type, Salinity & Species	4	96.7	24.2	36.3	0.00**
Error	72	48.0	0.7		

* Significant
** Highly significant

Figure 1ca

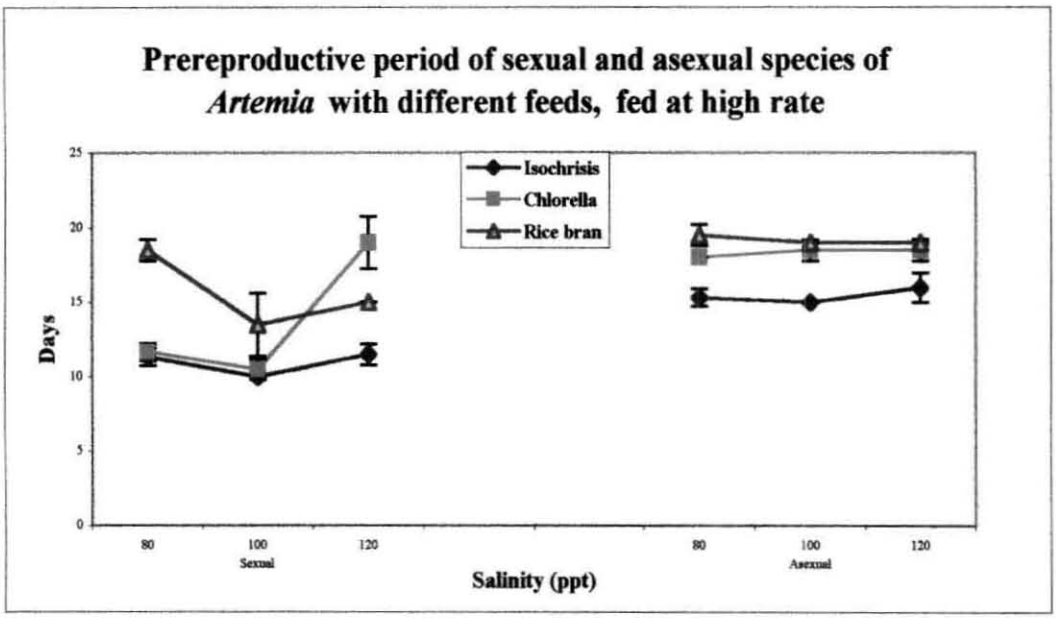
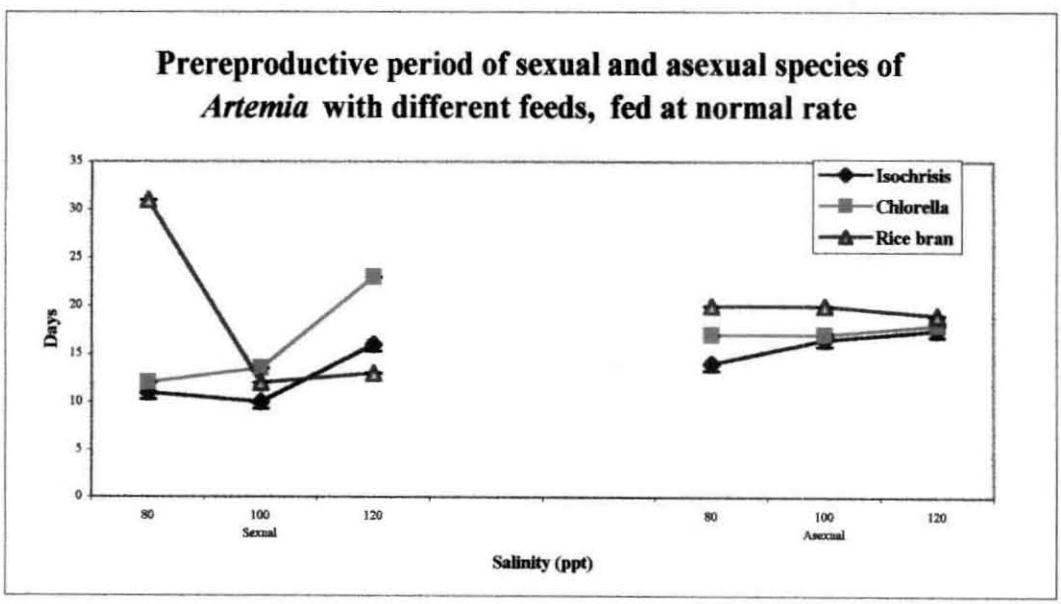


Figure 1cb



1. 3. 2. 2. Effect of temperature.

Effect of temperature with change in salinities on the length of reproductive period of two *Artemia* species is detailed in the table 1e and Figure 1e. The values of reproductive periods in Figure 1e are shown the Appendix 1e. Among the two species sexual females had longer reproductive period than the asexual females. Temperature is the master character which influenced the reproductive duration of both the species profoundly as there was a decrease in the reproductive period with increase of temperature. Asexual females had a continuous decrease with increase in temperature and at 33.5°C there was no reproduction at all. The sexual females had a slight increase in the reproductive period at 30 °C except at 100ppt salinity, and then decreased steeply as the temperature increased to 33.5°C. At 25°C an increase was observed in reproductive period along with increased salinity both in case of sexual as well as asexual species. At 30°C there was a decrease in the reproductive period as the salinity increased from 80 to 100ppt and then increased as the salinity increased to 120ppt and had same effect for both the species. At the upper most temperature, 33.5°c, reproductive period decreased along with increased salinity in case of sexual species and the asexual females never reproduced at this temperature. For both the species maximum reproduction was seen at 120ppt for 25 and 30 °c but at 33.5°c, 80ppt was found to be the best salinity range for sexual females and for asexual females there was no reproduction at this temperature. There was no significant difference on reproductive periods of the two species at different temperature in interaction with salinity. At the same time temperature ($P<0.01$) and salinity ($P<0.05$) individually had significant effect on the length of reproductive period of sexual and asexual species. There was significant difference between the reproductive periods of the two species. At 80 and 120ppt salinities relation between temperature and reproductive period of sexual species was parabolic as the mid temperature 30°C had longer whereas 25 and 33.5°C had shorter reproductive period,

Table 1d

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia,* on the reproductive period of the female individuals

Treatments	Degree of freedom	Sum-of - Squares	Mean-square	F-ratio	P
Salinity	7	3191.3	455.9	19.4	0.00**
Species	1	2080.3	2080.3	88.7	0.00**
Salinity & Species	7	330.7	47.2	2.01	0.08
Error	32	750.7	23.5		

Table 1e

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the reproductive period of the female individuals.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	1201.8	1201.8	13.8	0.00**
Temperature & Salinity	2	205.4	102.7	1.2	0.32
Temperature & Species	1	498.8	498.8	5.7	0.03*
Temperature, Salinity & Species	2	499.5	249.5	2.9	0.08
Error	24	2084.0	86.8		

* Significant
** Highly significant

Figure 1d

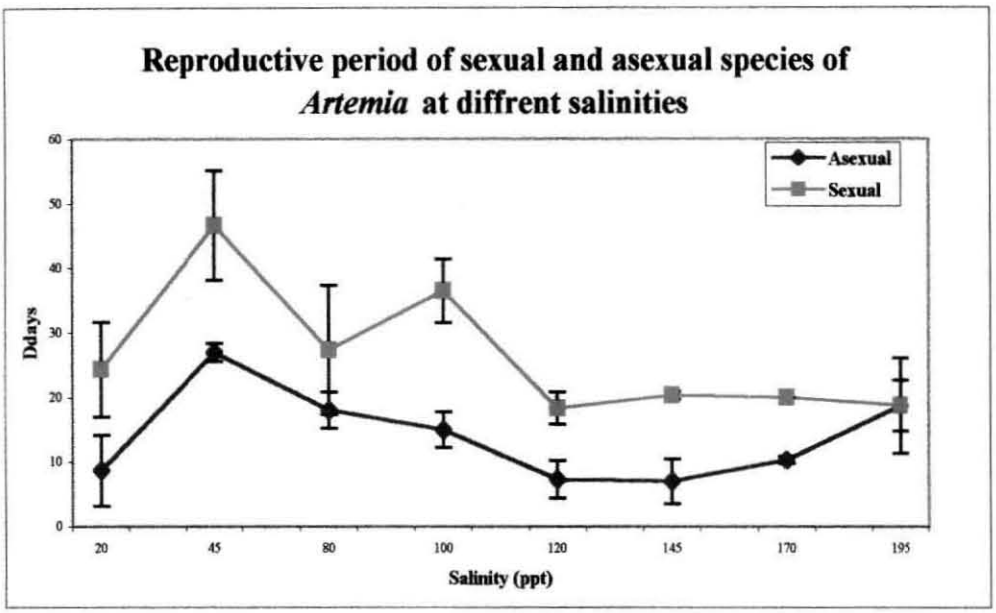
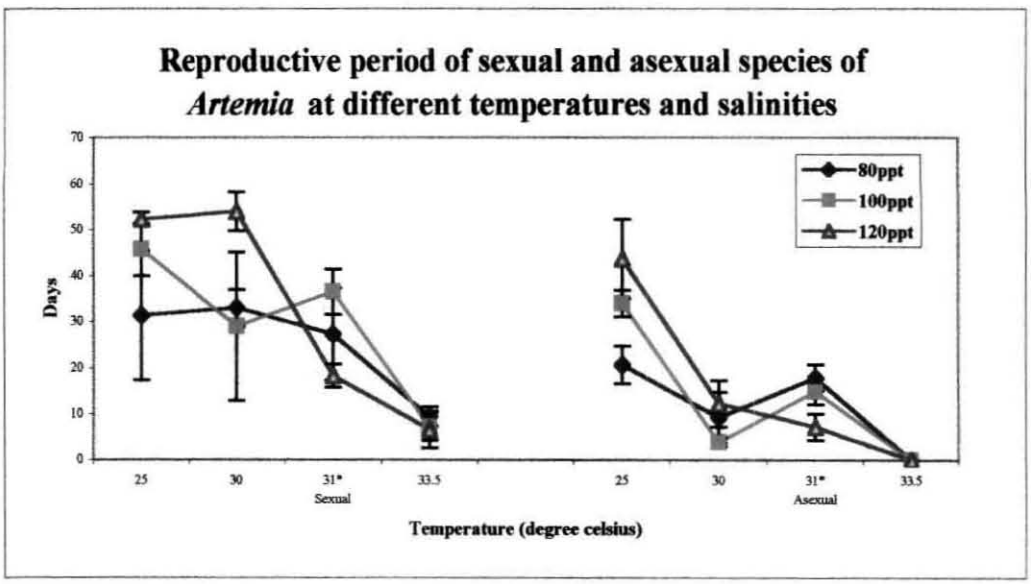


Figure 1e



* Ambient tempertaure

while at 100ppt the relation was linear (excluding the data at ambient temperature), Figure 1e. Reproductive period of asexual species had a linear relation with temperature as the reproductive period decreased with increased temperature (Figure 1e).

1. 3. 2. 3. Effect of quality and quantity of feeds.

The length of reproductive period of two Artemia species with different feeds at different salinities are shown in Figures 1fa and 1fb. The values of reproductive periods in Figure 1fa and 1fb are shown in the Appendix 1f. There was significant difference between the reproductive period of the two species fed with normal as well as high feeding ($P<0.01$) which are shown in Table 1f. The sexual females had longer reproductive period than asexual females at almost all the tested conditions except at 100ppt with *Isochrisis* and ricebran as feed when feeding was normal and with *Isochrisis* as feed at 80ppt with high feeding. With normal feeding rate, *Isochrisis* gave better reproductive period, 30days, at 80ppt in case of sexual females and 21.3 days at 100ppt for asexual females. With high feeding the *Isochrisis* fed animals gave the maximum reproductive days at 120ppt (43 days) for sexual females and at 80ppt (29 days) for asexual females. With *Chlorella* as feed at normal concentration, maximum reproductive period obtained for sexual females was 36.5 days at 100ppt and for asexual females 18 days at 80ppt . No significant difference on the reproductive periods between normal and high feeding individuals was noticed but was found differed with species as asexual species ($P<0.01$) had longer reproductive period with normal feeding rate while sexual species had longer reproductive period with high feeding at 80 and 120ppt salinities. With rice bran maximum reproductive period of 20 days was obtained at 80ppt, whereas it was 22 days for asexual females at 100ppt with normal feeding.

Figure 1fa

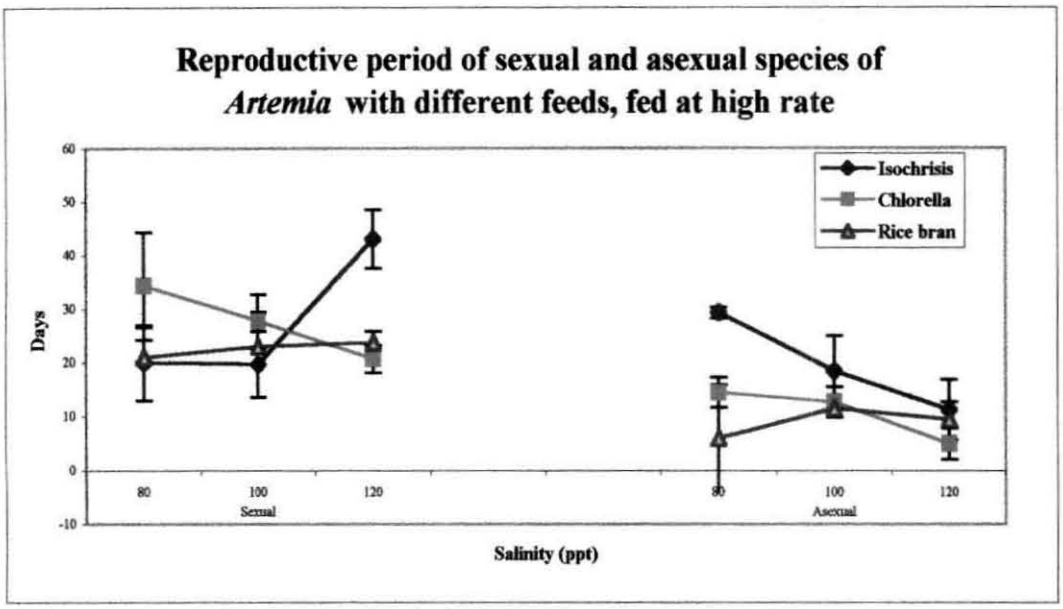
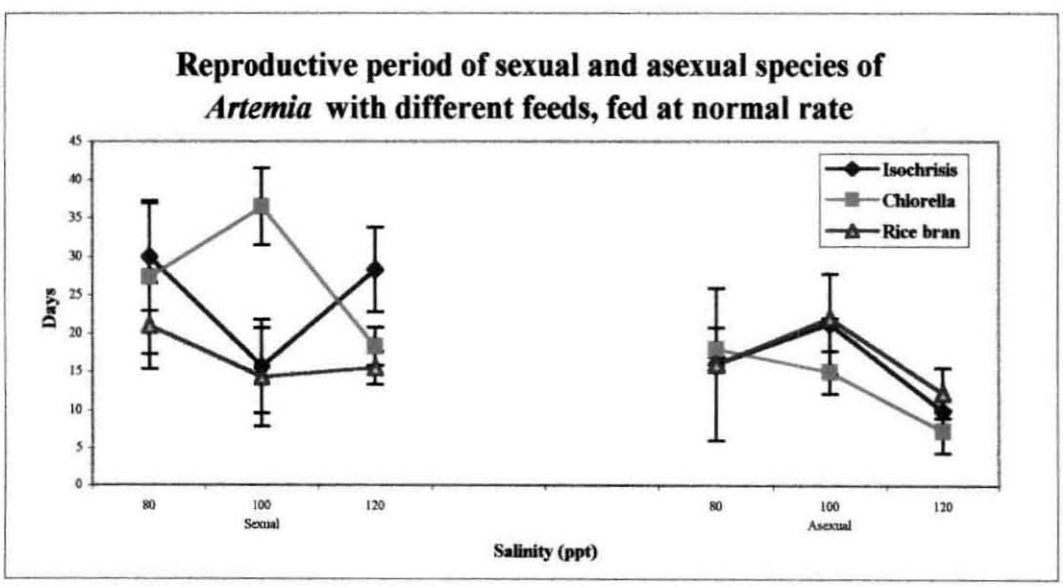


Figure 1fb



1. 3. 3. Postreproductive period.

This factor is not a determining factor, which influence the reproductive performance of the animal but is important as it affect the total lifespan of the animal. It is calculated as the days survived after the last brood.

1. 3. 3. 1. Effect of salinity

The length of postreproductive period of the two *Artemia* species with different salinities are listed in Figure 1g and Table 1g. The values of postreproductive periods in Figure 1g are shown in the Appendix 1g. In the case of sexual strain postreproductive period showed an increasing trend alongwith increase in salinity (Figure 1g), but in asexual species there was no clear trend on the postreproductive period at different salinities. Effect of salinity on this characteristic was found to be significant ($P<0.01$) (Table 1g). But there was no significant difference between the species. At higher salinities the asexual animals were alive for a longer period even after the last brood when compared to those at lower salinities (except 80ppt), and same was the case also for sexual strain.

1. 3. 3. 2. Effect of temperature

Temperature effect on the length of postreproductive period of both the species are shown in Figure 1h. The values of postreproductive periods in Figure 1h are shown in the Appendix 1h. Asexual species had more postreproductive days i.e. Reproductively sterile period at 25 °C and with 80ppt. Ambient temperature at 80ppt also had longer postreproductive days (9 days). The sexual species had relatively longer postreproductive days at 33.5 °C. Asexual species had relatively longer postreproductive period compared to the sexual species. Statistically, temperature had no effect on postreproductive period of any of the species (Table 1h).

Table 1g

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the post reproductive period of the female individuals

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	233	33.3	10.7	0.00**
Species	1	0.5	0.5	0.2	0.67
Salinity & Species	7	149	21.3	6.8	0.00**
Error	32	100	3.1		

Table 1h

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the postreproductive period of the female individuals

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	0.1	0.1	0.0	0.88
Temperature & Salinity	2	2.1	1.0	0.2	0.8
Temperature & Species	1	1.8	1.8	0.4	0.53
Temperature, Salinity & Species	2	2.4	1.2	0.3	0.76
Error	24	104.7	4.4		

* Significant
** Highly significant

Figure 1g

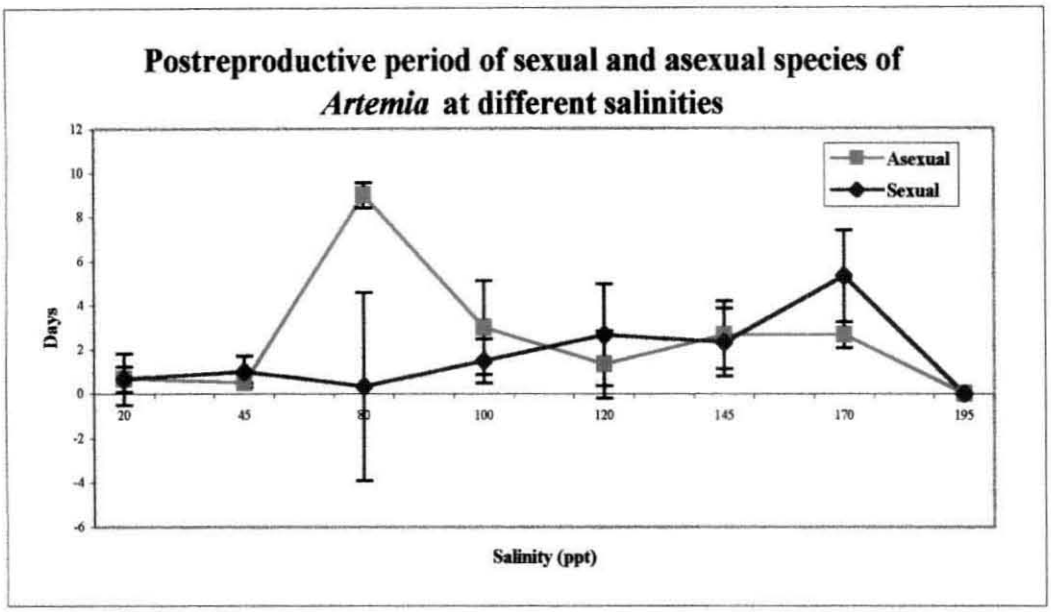
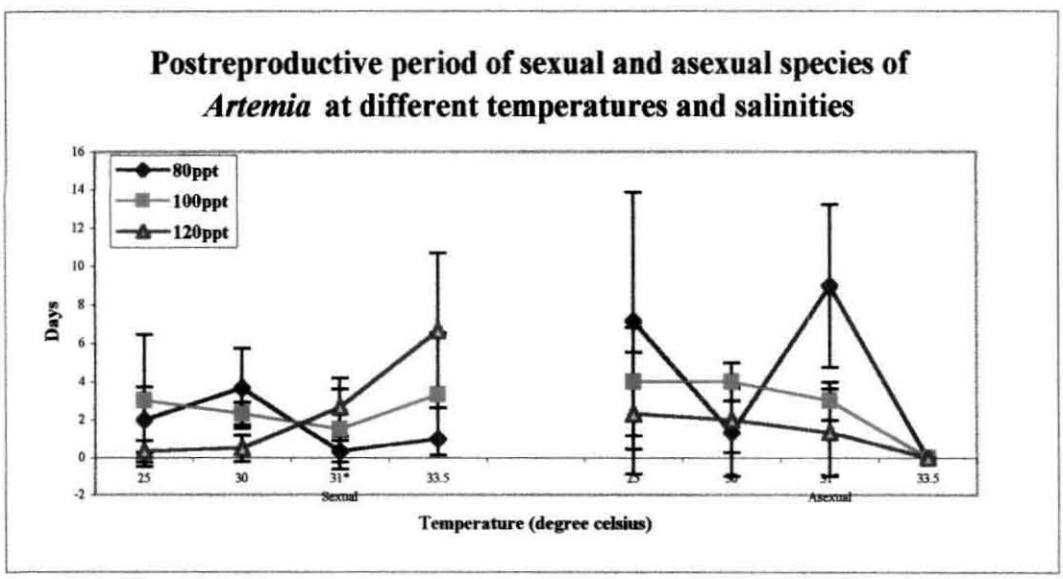


Figure 1h



* Ambient temperature

1. 3. 3. 3. Effect of quality and quantity of feed

The length of postreproductive period under different feeding regimes are shown in Table 1i, Figures 1ia and 1ib. The values of postreproductive periods in Figure 1ia and 1ib are shown in the Appendix 1i. Interactive effect of feed quantity and salinity on the length of postreproductive period are significant ($P<0.05$) while feed type had no affect on the postreproductive period. Interactive effect of feed quantity, type, salinity and species were also highly significant (Table1i).

1. 3. 4. Lifespan.

Lifespan is the sum of prereproductive, reproductive and post reproductive period of the animal. This factor is mainly influenced by the pre reproductive and reproductive period.

1. 3. 4. 1. Effect of salinity.

The average lifespan of both the studied species under different salinity treatments are shown in Figure 1j. The values of lifespan in Figure 1j are shown in the Appendix 1j. Life span of the animals reared at 170 and 195ppt could not be calculated as it was not possible to study the pre reproductive period due to the mortality of the animals before attaining maturity. Among the other salinities 45 ppt gave the longest lifespan for both the groups as they lived for 63.3 days and 49.5 days for sexual and asexual species respectively. The next maximum was obtained at 145ppt for the sexual species and 80ppt for asexual females. At 120 ppt the asexual females experienced the least lifespan. Salinity effect on life span of both the species was highly significant ($P<0.01$) (Table 1j). Both the species had an increase in the average lifespan when salinity increased to 45 from 20ppt

Table 1i

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the postreproductive period of the female individuals.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	0.5	0.5	0.2	0.67
Feed type	2	1.1	0.6	0.2	0.8
Feed quantity & Feed type	2	14.7	7.3	2.9	0.06
Feed quantity & Salinity	2	18.5	9.2	3.7	0.03*
Feed quantity & Species	1	6.7	6.8	2.7	0.1
Feed type & Salinity	4	20.4	5.1	2.0	0.1
Feed type & Species	2	9.7	4.9	2.0	0.15
Feed quantity, Feed type & Salinity	4	32.1	8.0	3.2	0.02*
Feed quantity, Feed type & Species	2	9.5	4.8	1.9	0.16
Feed quantity , Salinity & Species	2	58.4	29.2	11.7	0.00**
Feed type, Salinity & Species	4	39.9	10.0	4.0	0.01**
Feed quantity , Feed type , Salinity & Species	4	46.8	11.7	4.7	0.00**
Error	72	179.3	2.5		

* Significant
** Highly significant

Figure 1ia

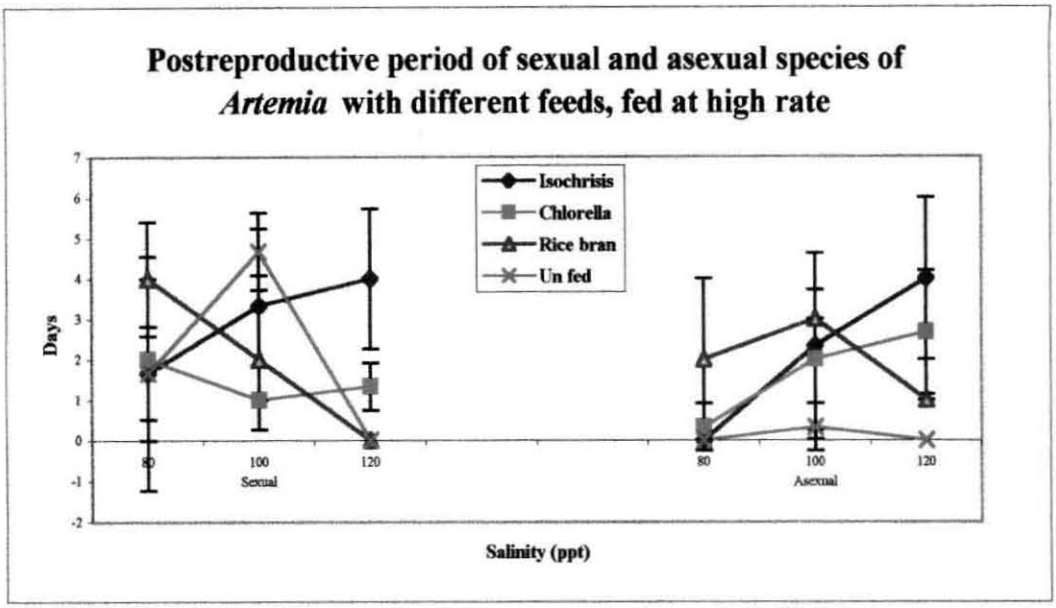
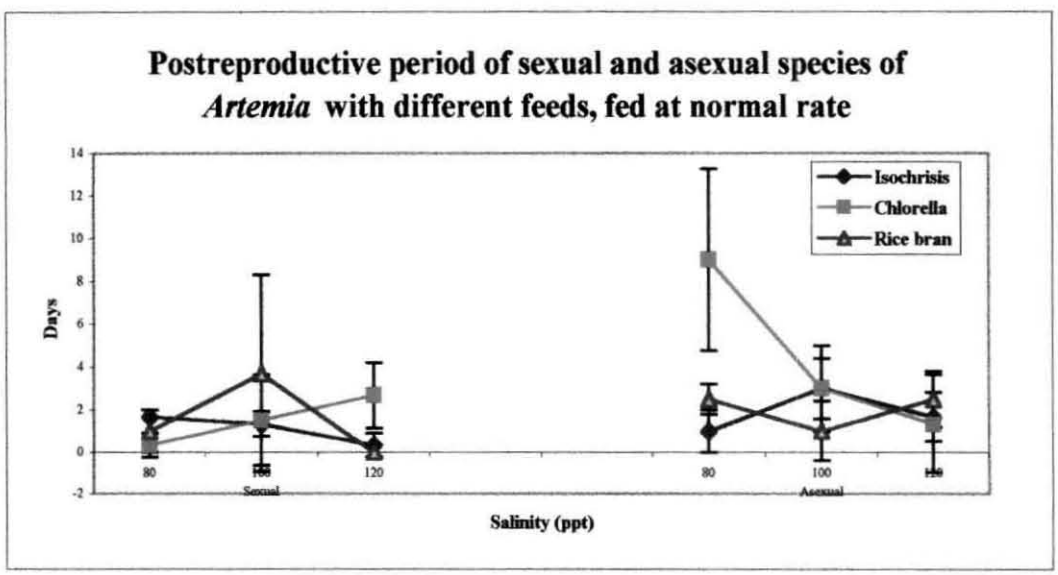


Figure 1ib



and had a decrease with further increase in salinity with a final increase at the extreme salinity (145ppt).

1. 3. 4. 2. Effect of temperature.

Temperature effect on the average lifespan of two species of *Artemia* are shown in Figure 1k. The values of lifespan in Figure 1k are shown in the Appendix 1k. Sexual females had a better lifespan in all the experimental units except at 120ppt with 25 °C temperature, and 100ppt with 30 °C temperature. At ambient temperature when the salinity was 80ppt also asexual females had a longer lifespan. With temperature the lifespan related linearly as it decreased with increase in temperature, including the ambient temperature, at all the three salinities in both the species. At elevated salinities sexual females had longer lifespan relatively at all the temperatures while asexual females had increase in their lifespan with increased salinity only at 25 °C. Total female lifespan of both the species had a linear relation
with temperature (Figure 1k). Temperatures individually had significant effect on the lifespan of the two species ($P<0.01$) (Table 1k).

1. 3. 4. 3. Effect of quality and quantity of feed.

Effect of feed type and quantity on the lifespan of the two species are shown in, Figures 1la, 1lb and Table 1l. The values of lifespan in Figure 1la and 1lb are shown in the Appendix 1l. Asexual females had a life span of 38.5 and 43 days respectively at 80 and 100 ppt salinities with rice bran as feed. Asexual females fed with algal feeds showed maximum longevity when feeding rate was high. High feeding with rice bran effected the lifespan of both the species negatively as they had shorter lifespan at this condition than that of with normal feeding. High feeding with rice bran and *Chlorella* affected the longevity of asexual females as their longevity decreased at all salinities with increase

Table 1j

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the life span of the female individuals

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Salinity	5	1850.3	370.1	13.4	0.00**
Species	1	1056.3	1056.3	38.4	0.00**
Salinity & Species	5	465.6	93.1	3.4	0.02*
Error	24	660.7	27.5		

Table 1k

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the life span of the female individuals

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	2916.0	2916.0	32.4	0.00**
Temperature & Salinity	2	288.7	144.3	1.6	0.22
Temperature & Species	1	121.0	121.0	1.3	0.26
Temperature, Salinity & Species	2	788.7	394.3	4.4	0.24
Error	24	2156.0	89.8		

* Significant
** Highly significant

Figure 1j

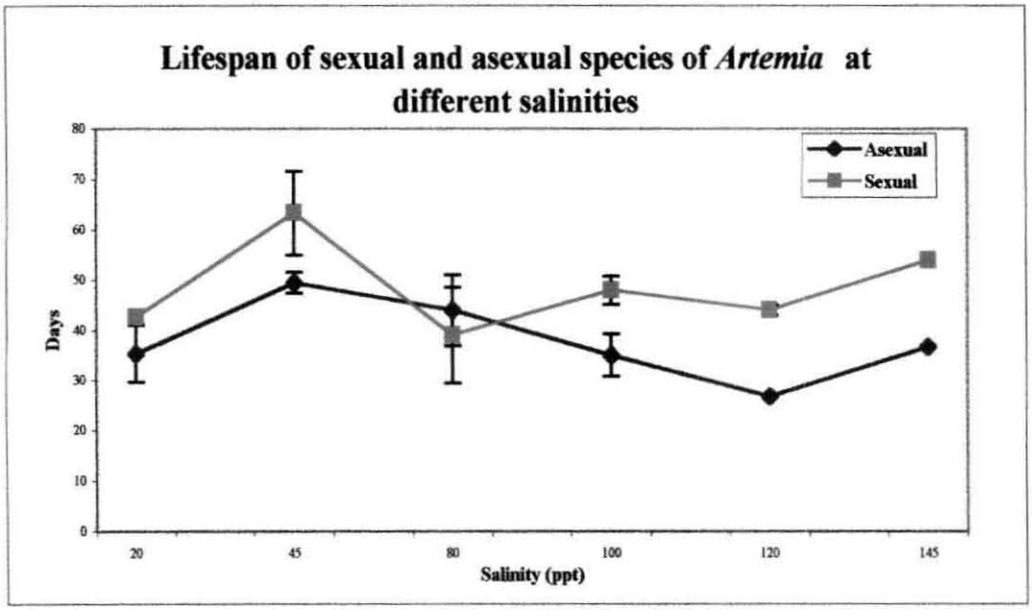
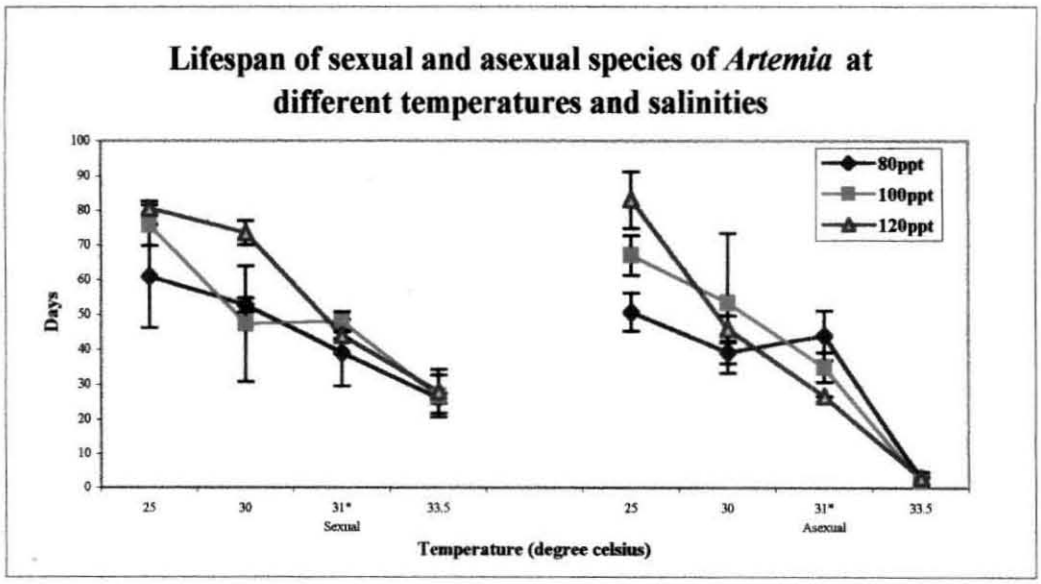


Figure 1k



* Ambient temperature

Table 1l

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the Total life span of the female individuals.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	46.7	46.7	1.1	0.3
Feed type	2	408.8	204.4	4.8	0.01*
Feed quantity & Feed type	2	569.0	284.5	6.7	0.00**
Feed quantity & Salinity	2	269.6	134.8	3.1	0.05*
Feed quantity & Species	1	277.1	277.12	6.5	0.01**
Feed type & Salinity	4	410.7	102.7	2.4	0.05*
Feed type & Species	2	445.8	222.9	5.2	0.01**
Feed quantity, Feed type & Salinity	4	302.6	75.6	1.8	0.14
Feed quantity , Feed type & Species	2	76.4	38.2	0.9	0.41
Feed quantity, Salinity & Species	2	319.1	159.6	3.8	0.03*
Feed type, Salinity & Species	4	1309.6	327.4	7.7	0.00**
Feed quantity, Feed type , Salinity & Species	4	1293.5	323.4	7.6	0.00**
Error	72	3060.0	42.5		

* Significant
** Highly significant

Figure 1Ia

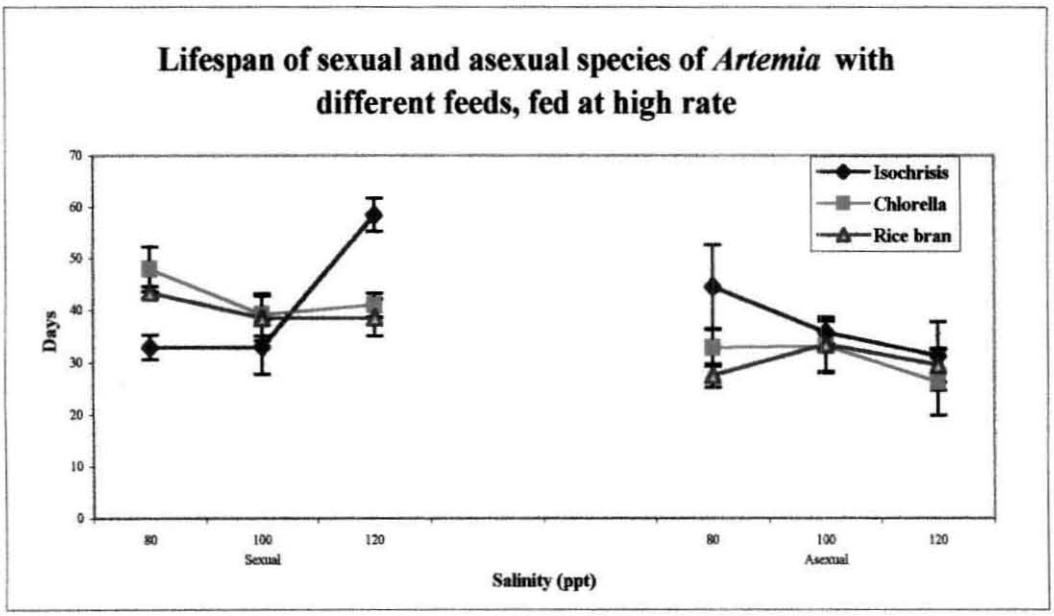
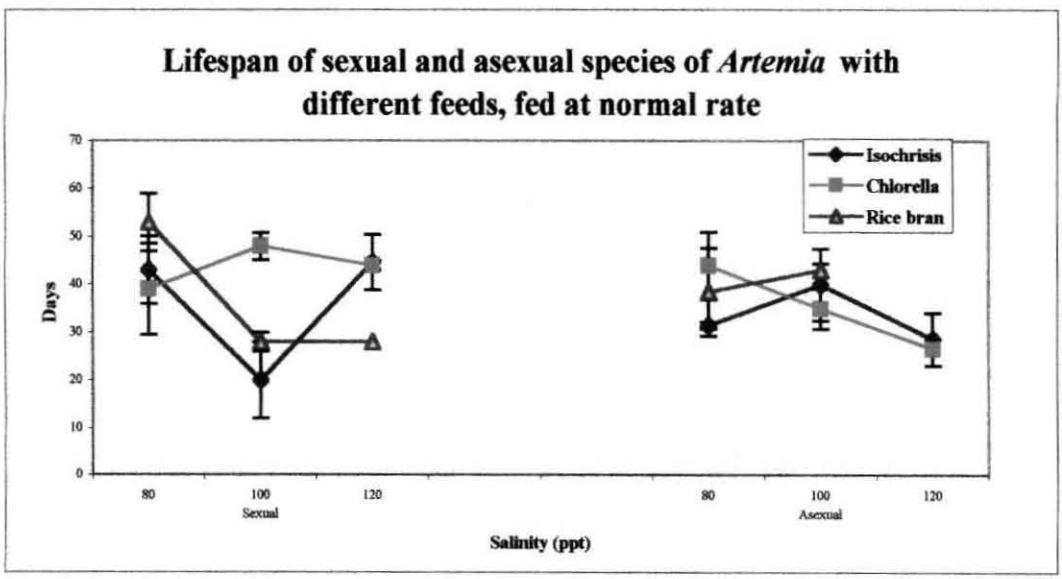


Figure 1Ib



in feeding rate while the *Isochrisis* fed animals had an increase in their life span with high feeding at all the three salinities. At normal feeding asexual females fed with *Isochrisis* had the longest lifespan (40 days) at 100ppt and with *Chlorella* as feed at 80ppt (44 days). With rice bran the asexual females had maximum longevity (43 days) at 100ppt.

For sexual species, high feeding with rice bran and *Isochrisis* at 100 and 120ppt (Figure Ia) increased their total lifespan compared to those of normally fed females (Figure Ib), while at 80ppt there was a reduction in the lifespan for the sexual females when the concentration of these feeds were increased. Sexual females fed with *Isochrisis* at normal level gave maximum lifespan(44 days) at 120ppt. With *Chlorella* as feed at normal feeding rate sexual females had the longest life span at 100ppt (48 days) and with rice bran they had the longest lifespan (53 days) at 80ppt. Between normal and high feeding there was significant difference only between those females fed with rice bran ($P<0.01$). Feed quantity and type had interactive effect on the average lifespan of both the species.

1. 3. 5. Percentage reproductive period of lifespan.

Percentage reproductive period of both sexual and asexual species at different ecophysiological conditions were calculated so as to determine the actual potentially reproductive period of the total female lifespan.

1. 3. 5. 1. Effect of salinity

Reproductive period of the two species of *Artemia* as a percentage of its total lifespan at different salinities are detailed in Figure 1m and Table 1m. The values of percentage reproductive period to the lifespan in Figure 1m are shown in the Appendix 1m. Sexual species had greater percentage of reproductive period of their total lifespan than the asexual species at all the tested salinities ($P<0.01$). Maximum percentage of reproductive period was obtained at 45, 80 and 100ppt salinities. For sexual species lowest percentage reproductive period was obtained at higher salinities (120 and

145ppt) while highest of 77% at 100ppt salinity. In case of asexual species the lowest percentage reproductive period was noticed at higher (145ppt) as well as lower salinity (20ppt) and maximum (54%) at 45ppt. Effect of salinity on percentage reproductive life of both the species are highly significant ($P<0.05$). In short, females of both the species at the mid range of salinities had maximum percentage reproductive period.

1. 3. 5. 2. Effect of temperature

Percentage reproductive period of the total lifespan of both the *Artemia* species studied are listed in Figure 1n. The values of percentage reproductive period to the lifespan, in Figure 1n are shown in the Appendix 1n. The difference between percentage reproductive period of the two species at all the tested temperature are highly significant ($P<0.01$) (Table 1n). The reproductive period, in percentage to the total lifespan of sexual species marginally increased from 25 °C to 30 °C at all the salinities and then drastically declined at 33.5 °C in all the three salinities. Further, it increased with the increase in salinity from 80 to 120ppt at 25 and 30 °C, where as at 33.5 °C it continued to decrease from 80 to 120ppt indicating that the optimum temperature 30 °C is favourable for reproduction and higher salinity still favoured the reproductive performance. The sexual species had the maximum reproductive period in percentage to the lifespan at 30 °C (73.4%).

Among asexual species, the reproductive period declined from 25 to 30 °C at all the three salinities. At 25 °C the reproductive period continued to increase with increase in salinity. Whereas, at 30 °C initially it declined in 100ppt and then increased in 120ppt. This shows that 25 °C and higher salinities are favourable for better reproduction by asexual *Artemia*

Table 1m

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the percentage reproductive period to the total life span of the female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	5	7278.5	1455.7	28.4	0.00**
Species	1	4606.6	4606.6	89.8	0.00**
Salinity & Species	5	911	182.2	3.6	0.02*
Error	24	1230.8	51.3		

Table 1n

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the percentage reproductive period to the total life span of the female individual

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	169.3	169.3	4.2	0.05*
Temperature & Salinity	2	27	13.5	0.3	0.72
Temperature & Species	1	608	608	15.1	0.00**
Temperature, Salinity & Species	2	51.5	25.7	0.6	0.54
Error	24	965.4	40.2		

* Significant
** Highly significant

Figure 1m

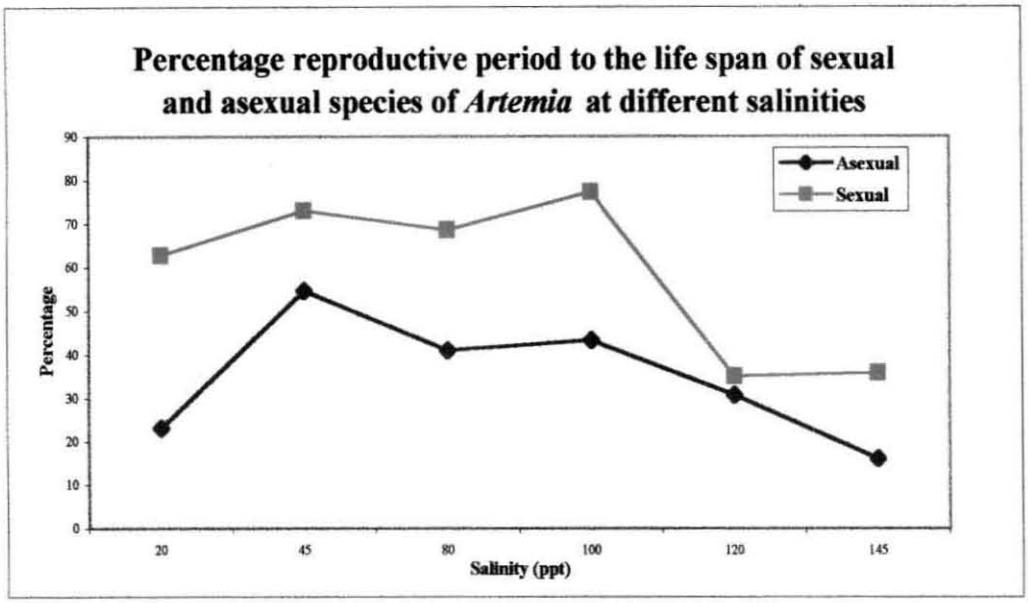
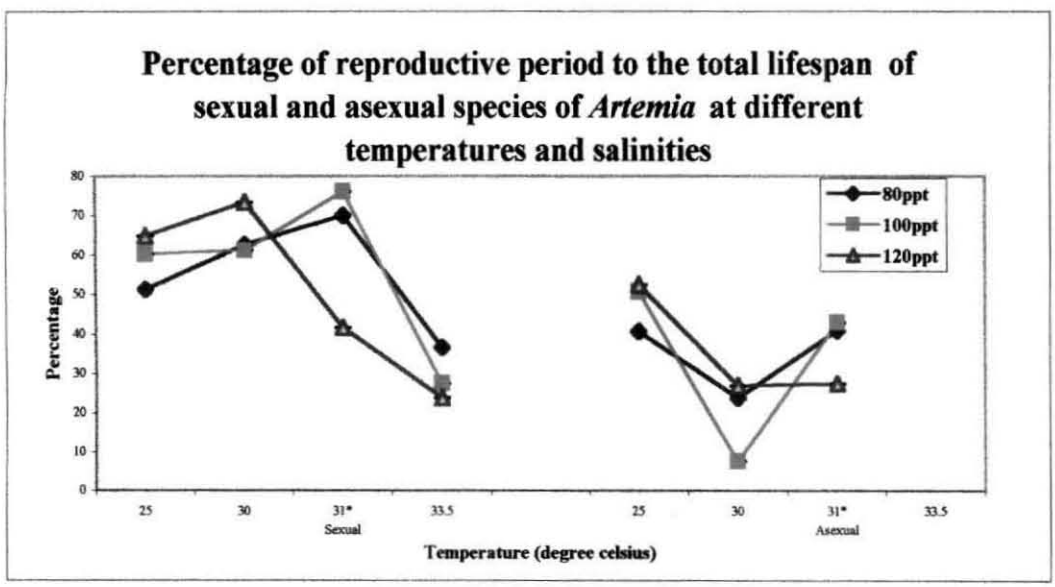


Figure 1n



* Ambient temperature

species. There was no reproduction at 33.5 °c for asexual species and it has the maximum reproductive period at 25 °C (52.6%).

At the ambient temperature (31±3), with 100ppt there was maximum percentage reproduction for both the species. While at 120ppt lowest percentage reproductive period was recorded. Percentage reproductive period of sexual species had a parabolic relation with increase in temperature while asexual species responded linearly (excluding the readings at ambient temperature).

1. 3. 5. 3. Effect of quality and quantity of feed.

Reproductive period of two *Artemia* species as a percentage of total lifespan with different feeds and concentration are shown in Figures 1oa and 1ob, Table 1o. The values of percentage reproductive period to the lifespan, in Figure 1oa and 1ob are shown in the Appendix 1o. With normal as well as high feeding the sexual species had significantly longer percentage reproductive period than that of asexual species (Table 1o).

With increased feeding sexual females fed with *Chlorella* sp. gave maximum percentage of reproductive period at 80 and 100ppt while at 120ppt *Isochrisis* and rice bran gave maximum percentage. For asexual species *Isochrisis* was the best at all the salinities. Generally at low salinities algal feed and at high salinities rice bran were found to be the best for both the species (Figure 1oa, 1ob). Normal feeding with *Isochrisis* gave maximum percentage reproductive period at 100ppt for sexual (78%) as well as asexual (53%) females. *Chlorella* fed females of both species had the maximum percentage of reproductive period of their lifespan at 100 ppt.

Table 1o

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the Percentage reproductive period to the total life span of the female individual

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	7317.6	7317.6	216.1	0.00
Feed type	2	399.4	199.7	5.9	0.00
Feed quantity & Feed type	2	200.5	100.2	3.0	0.06
Feed quantity & Salinity	2	107.9	53.4	1.6	0.21
Feed quantity & Species	1	4620.0	4620.0	136.5	0.00
Feed type & Salinity	4	535.1	133.8	4.0	0.01
Feed type & Species	2	186.5	93.2	2.8	0.07
Feed quantity, Feed type & Salinity	4	419.0	104.8	3.1	0.02
Feed quantity, Feed type & Species	2	375.6	187.8	5.5	0.01
Feed quantity , Salinity & Species	2	39.2	19.6	0.6	0.56
Feed type , Salinity & Species	4	232.1	58.0	1.7	0.16
Feed quantity , Feed type, Salinity & Species	4	344.5	86.1	2.5	0.05
Error	72	2437.6	33.9		

* Significant
** Highly significant

Figure 1oa

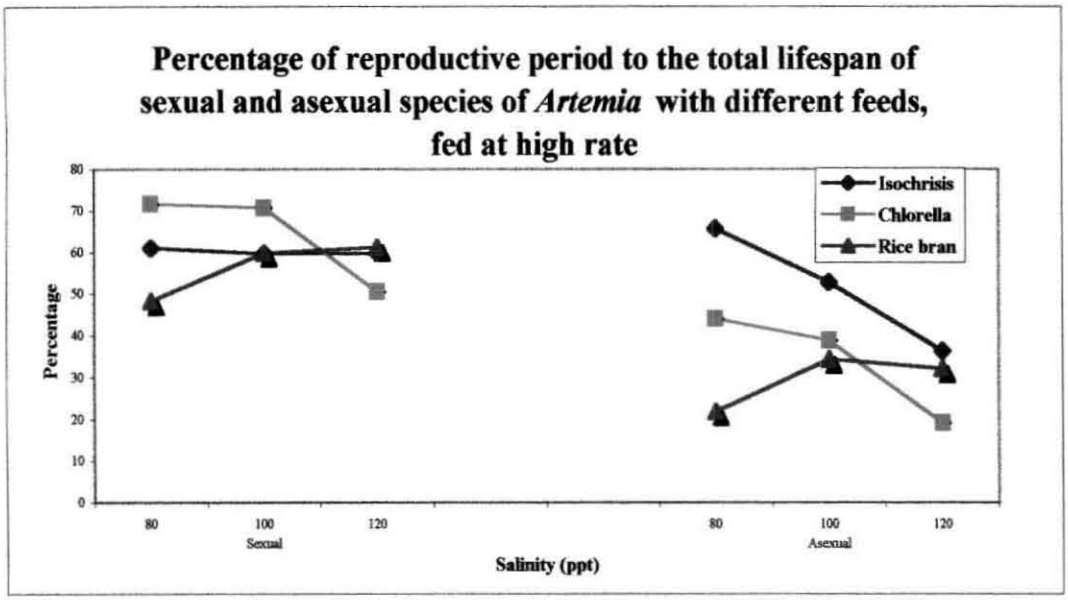
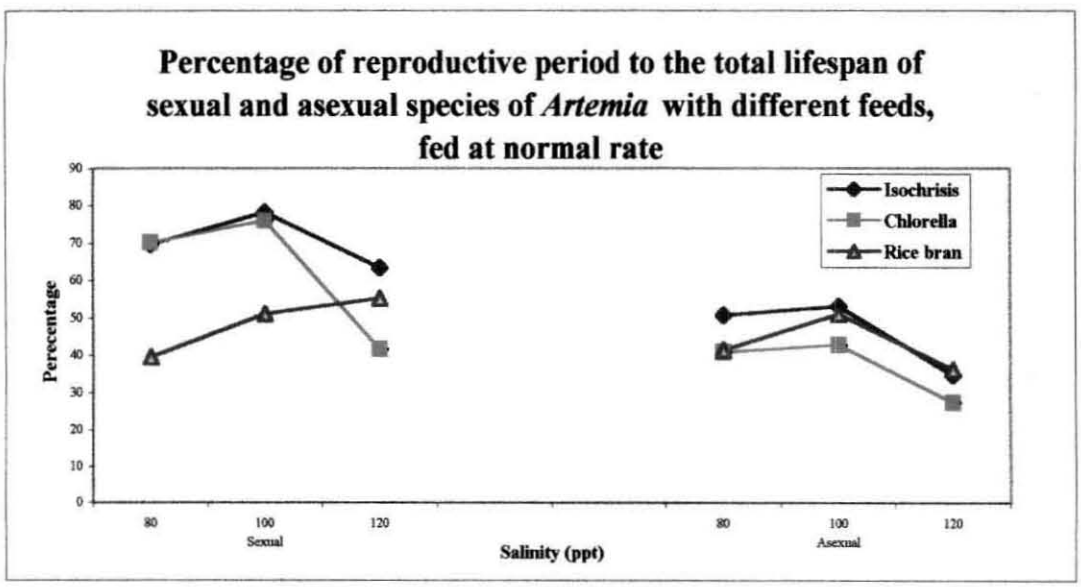


Figure 1ob



Rice bran fed sexual females had maximum percentage reproductive period at 120ppt, while asexual females at 100ppt. Interactive effect of feed quantity, feed type and salinity on the reproductive period of sexual as well as asexual species as a percentage to the total lifespan are highly significant.

1. 4. Discussion

Information on the effect of salinity on various lifespan characteristics of brine shrimp from tropical environments are rather limited. On the otherhand, several workers have observed the effect of salinity on the lifespan characteristics of brine shrimp in temperate environments. The results of the investigation on the lifespan characteristics of both *A. parthenogenetica* and *A. franciscana* under different salinities clearly indicate that the range of 80 to 100ppt is the best as both the lower and higher salinities affected the prereproductive period of both the species (Figure 1a). At high salinities 170 and 195ppt, females of both the species died before attaining maturity. In a previous work conducted by Browne and Hoopes (1990) in a temperate climatic region total mortality was noticed only at 230ppt while in the present study animals stopped maturing beyond 170ppt. This may be due to the high temperature prevailing at Tuticorin region where the present experiment was conducted. According to Wear *et al.* (1986) , decreasing growth efficiency which in turn affect the process of maturation in higher as well as lower salinities may be due to increased proportion of assimilated food being appropriated for osmotic work. Rearing *Artemia* at salinities above 200ppt has been found to be difficult by various workers (Wear *et al., 1*986; Dana and Lenz, 1986; Brown and Hoopes, 1990). In the present study salinities between 45 and 120ppt are found to be favourable for the asexual species and between 20 and 100ppt for the sexual species for longer percentage reproductive life of their total lifespan as shown in Figure 1m. And it was between these salinities that the animals had their shortest prereproductive period also. As the salinity increased reproductive period of lifespan (Figure 1m) also was found to decline.

Maturation period of *Artemia franciscana* strain and a parthenogenetic strain from China, at higher salinities were found to be longer than those at lower salinities by Triantaphyllidis *et al.* (1995). Sexual strain from San Francisco Bay had generally longer reproductive period than the parthenogenetic strain from China in their experiments and maturation rate was found inversely proportional to salinity especially above 140ppt. Total life span was also found to be longer for sexual species than that of asexual species in this experiment. Postreproductive period was not found to be significantly different and was also seen true in the experiments of Browne *et al.* (1984).

Experiments carried out at three different temperatures viz., 25, 30 and 33.5 °C at three salinities have shown that 30 °C was the optimum for asexual as well as sexual species. Tolerance limit for temperature by asexual species was less compared to the sexual species as the asexual females failed to mature at 33.5 °C while the sexual species showed comparatively faster maturation at this temperature than at 25 °C (Figure 1b). Similar results were obtained by Browne *et al.* (1988) as they observed shortest prereproductive period for *Artemia franciscana* at 33.5 °C and the asexual species at 30 °C were not reproducing. But in the present study asexual females could reproduce at 30 °C because they are used to the tropical climate of Tuticorin.

According to Vanhaecke *et al.* (1984) the parthenogenetic strain of Tuticorin is a triploid organism and is resistant to high temperature and to certain extent low salinities. Polyploid populations are genetically buffered against extreme environmental conditions than diploid animal (Artom,1931; Chapman 1968; Metalli and Ballardin, 1972). In the experiments of Barata *et al.*(1996b) at 30 °C death occur prior to maturity in sexual species . According to Zang and Lefcort (1991) the polyploidy had increased survival compared to diploids at extreme temperature of 0 and 37.5 °C but Zang and King (1993)

suggested that life span differences can also be due to the different geographical origin of population and localised adaptation and are not directly related to ploidy level. According to Royan (1980) the optimum temperature for parthenogenetic strain was 27°c at 30 to 40ppt salinity. Salinity tolerances of Sanfrancisco bay strain was between 15 to 120ppt and found that 50% mortality occur at29 to 30°c. Great Salt Lake strain was found highly tolerant to temperature up to 35°C (Vanhaecke *et al.* 1984). Maturation time of *Artemia* found to be decreased with temperature increase from 15 to 30°c(Browne *et al.*,1988). Browne *et al.*(1984,1988) found sexual *Artemia tunisiana* had longer pre reproductive period than parthenogenetic strain and Indian tetraploid strain had short pre reproductive period than French and Spanish parthenogenetic diploid strain. Majority of parthenogenetic polyploid strain have longer maturation period (Zang and King(1993). Findings of Wear *et al.*(1986) shows that pre reproductive period and the reproductive period were more heavily influenced by temperature than by salinity and the interactive effect was also found significant. At almost all salinities pre reproductive period decreased with increase in temperature up to 26°c but extended at 32°c and referred 24°c as the most favorable.

According to Vonhentig (1971) GSL strain of *Artemia franciscana* matured more rapidly at 30°C than those at 20°C at all three salinities 15,32 and 70ppt. It was also found that time to attain maturity in 70ppt at four temperatures between 10 and 30°c are similar to those obtained in 32ppt at same temperature and reinforces the statement that temperature is the most important factor that determines maturation rate. In the present study also prereproductive period responded uniformly to the tested temperatures at all the salinities (Figure 1b) asserting the role of temperature as the primary factor controlling maturation process at tropical condition as reported at temperate conditions. Prolonged prereproductive period of both the species at 25°C ,which is the lowest temperature tried, could be due to the physiological mechanism getting affected as at lower temperature swimming rate which in turn control the feeding rate may be getting lower and all the energy assimilated may be

directed to somatic growth and maintenance with very little remain for reproduction.

At ambient temperature salinity affected reproductive period negatively (Figure 1e and 1n) but at temperature 25 and 30°c maximum reproductive period was obtained with 120ppt salinity (Figure 1e and 1n) indicating that the temperature is the most determinant factor on reproduction. For sexual species *Chlorella* and *Isochrisis* at high feeding favored faster maturation (Figure 1ca) while ricebran fed at normal level gave good results at high salinity (Figure 1cb). *Chlorella* and *Isochrisis* at high feeding favored faster maturation for asexual species. At the same time *Chlorella* fed females had faster maturation at normal feeding (Figure 1cb). Percentage of reproductive period of total life span of sexual species was maximum at 30°c than 25 and 33.5°c irrespective of salinities while for asexual species the reproductive period was maximum at 25°c while at 33.5°c there was no reproduction at all (Figure 1n). In both the species difference between pre reproductive periods at different salinities were meager compared to those at different temperatures. Time taken to 50%maturity, was found more highly influenced by temperature rather than salinity as reported by Wear *et al.* (1986). According to them time taken to 50%maturity decreased with increasing temperature up to 26°c but was extended at 32°c. According to Vonhentig (1971) Great Salt Lake strain of *Artemia franciscana* matured more rapidly at 30°c than those at 20°c at 15,32 and 70ppt salinities. He could not find much variation in the maturation time of *Artemia* at 32 and 70ppt salinities. Increase in temperature could increase the maturation rates of the animals at all the tested salinities. According to some studies conducted by Weisz (1946) and Baid (1963) decreased salinity also had a positive effect on increased maturation rate of *Artemia*.

Algal feeds fed at normal rate were found to be the best at lower salinities where as at higher salinities rice bran gave fastest maturation for sexual species while asexual species had almost same pre reproductive period with rice bran (19 days)at all tested salinities. Starved

animals managed to survive and reproduce at 100ppt, but not with 80 and 120ppt. At this salinity animals of both the species could survive with what is available in the medium, with out any supplementary feeding.

Time taken to attain sexual maturity for Tuticorin strain has been reported as 14 days by Tobias *et al.* (1980) but according to Balasundaram and Kumaraguru (1987) with a compound feed of algae, rice bran, decomposed cabbage and yeast it was still less. Survival rate of *Artemia* was always found to be best with algae as feed (Rahaman, 1993) but its practical applicability was less due to high cost of production and Rahaman and Rathinasamy (1997) has tried steamed rice bran as an alternative feed because of its nutritional value and found it as good as algal feeds. Parthenogenetic strain had their fastest maturation (14 days) with *Isochrisis* as feed with normal as well as high feeding rate, but found to increase with increase in salinity (Figure 1ca,1cb). The sexual species also had their fastest maturation with *Isochrisis* as feed (10 days). With ricebran females of sexual species delayed maturation at 80 ppt salinity while at 120ppt ricebran fed sexual females matured faster than that of *Chlorella*, while asexual females had no effect. Early maturity (12 days) was found to be one of the characteristic of Indian strain according to Royan *et al.* (1978) while at all the experimental conditions of the present study sexual species bettered in the prereproductive period than that of asexual Indian strain. *Chlorella* was not advised as a good feed for *Artemia salina* because of its low assimilation efficiency (Sick,1976). In the present experiment *Chlorella* fed females under performed to *Isochrisis* fed females of both the species with normal as well as high feeding, but matured faster than those fed with rice bran at lower salinities.

Reeve (1963) observed high growth efficiency in lower food concentration and explained that this is because food remains in gut progressively for longer period as there is less pressure on it from the incoming food to move through the gut. Longer stay in gut permits digestive efficiency. Beyond a certain limit of algal concentration the growth efficiency was found stagnant for *Artemia* (Manson, 1963). For

asexual species with *Chlorella* as feed, females fed at normal rate were found to have more reproductive days than those at high feeding.

Reproductive period is dependent on the feed selected as at 120ppt *Isochrisis* sp. was found to be most appropriate feed with high feeding rate (for both sexual as well as asexual strain) followed by rice bran and *Chlorella* sp. While at 80ppt, *Isochrisis* and *Chlorella* gave maximum percentage of reproductive period than rice bran. Generally at lower salinities algal feeds are preferred as they very well thrive in that salinities but rice bran get decayed very soon in salinities lower than 100ppt. But at 100ppt and above rice bran is a stable feed and gave good results.

The percentage reproductive period of asexual females varied with change of quantity and quality of feed. With rice bran and *Chlorella* sp. high feeding retarded the percentage reproductive period at 100 and 120ppt salinities. But with *Isochrisis* sp., high feeding favored longer percentage reproductive period for the majority of experiments. Irrespective of asexual females sexual females had more percentage reproductive period with high rice bran feeding as well as with *Chlorella* with majority of salinities. *Isochrisis* at normal level gave good results. It is assumed that effect of quantity of feed on life span characteristics varies with feed quality and with species.

Post reproductive period is not a determining factor to measure the fitness of the animal during its entire life span. Post reproductive period of the animals at any of the condition have not shown any relation with any of the factors studied. Still post reproductive period of asexual species was longer at

lower temperature as indicated by Browne *et al.* (1988) whereas sexual species had no such relation with temperature.

Total female lifespan of both the species has a linear relation with temperature (Figure 1k) and a similar relation was also mentioned by Browne *et al.* (1988). The lifespan components of the animal like pre reproductive (Figure 1b) period and reproductive period (Figure 1e) of both the species was not linearly related to temperature but their total lifespan is linearly related supporting the findings of Browne *et al.* (1988). For sexual species total lifespan was maximum at 25 °C while percentage reproductive period was found maximum at 30 °C. With asexual species lifespan as well as percentage reproductive period of lifespan are found to be maximum at 25 °C.

Parthenogenetic and sexual population clearly differ with regard to their reproductive and lifespan characteristics (Browne *et al.*, 1984). Competitive ability of the population is also related to their life history traits (Browne, 1980a; Browne *et al.*, 1984). Cole (1954) and many other subsequent investigators emphasise that prereproductive period which can also be called as generation time, is a key factor in determining the population growth rate and subsequent competitive exclusion among the population in a resource limited environment as the initial batch of offspring have the advantage of utilising the available resource. In the inoculation experiments it is always important to compare the generation times of the selected species before they are inoculated. In wild if there is a co-occurrence of two species there is every chance of competition between them and the most adapted to the prevailing environmental conditions will emerge as the winner. Here also the generation time plays an important role in the selection. Intra and inter strain correlation between the variables prereproductive, reproductive and postreproductive were considered for a fixed temperature salinity regime by Browne (1980a) and Browne *et al.* (1984). Effect of changing values for temperature and salinity on these variables were discussed by Wear *et al.* (1986). Lenz (1980) estimated two generations per

year for *Artemia monica*, Gillespie and Stephens (1977) suggested four or five generations for Utah strain of *Artemia franciscana*. But there may be eight generations annually in San Francisco Bay strain (Carpelan, 1957). With adequate food and in absence of possible crowding factor, the temperature range 20 to 28 °C in salinities of 100 to 120ppt are optimal conditions for growth, survival, maturation, fecundity and generation time for *Artemia* (Wear *et al.*, 1986).

Competition, which is regarded as a means of limiting species diversity (Mac Arthur, 1972) also leads to evolutionary changes. Possibilities of competition in *Artemia* population were studied by Browne and Halanych (1989). In the majority of the studies conducted, in many of the species competitive exclusion requires on the order of 10 to 100 generation, but for *Artemia* where the generation time is approximately 20 days exclusion occurs in 2 to 3 generation , may be due to complete niche overlap between *Artemia* populations. Parthenogens were always out competed by the sexual at high as well as low food levels in the experiments of Browne *et al.* (1984). But according to Browne and Hyanych (1989) sexual population from Old World were always outcompeted by parthenogenetic species indicating that the outcome of sexual and parthenogenetic competition linked to whether sexual population is of New or Old World origin. The biogeography of *Artemia* support this view as the New World population are exclusively sexual (*Artemia franciscana*) while the areas where the Old World sexual and parthenogenetic population are both found, parthenogens dominate (Browne and MacDonald, 1982). But the possibility of competitive exclusion of the local *Artemia* species in areas where the inoculation of exotic strains are done is an important factor which is to be studied. At Tuticorin sexual strains are assumed to have come from San Francisco Bay population. Considering the lifecharacteristics of the two available species of Tuticorin salt pans, in the present study, there is every possibility that the exotic sexual population can dominate over the local parthenogenetic species.

In the present investigation there was no co-occurrence of the exotic sexual and indigenous parthenogenetic species in the wild, but its possibility in the near future can not be ignored as they are seen in the adjacent salt pans of the Tuticorin region. In almost all the experimental conditions sexual ones had the dominance over the asexual parthenogenetic species regarding their life history characteristics. The two species were reared in separate containers and were never put together while giving the treatments. Sexual females had lesser generation time than the asexual females. Sexual females had longer reproductive days than the parthenogenetic females. According to the reports of AbreuGrobois and Beardmore (1980, 1982) and Browne *et al.* (1984), New World sexual, Old World sexual and Old World parthenogens are quite distinct both genotypically and phenotypically (Browne *et al.*, 1984) which can cause the competitive exclusion of either of these species possible.

But there are some other logic factors, which are also to be considered before reaching to a conclusion. The male lifespan is important factor in determining the population growth of sexual population, as the sexual females need to be reproduced with their males so as to have a successful reproduction. Male sexual span was comparatively less compared to the females as at a larger number of occasion the males had to be replaced during the experiments. This was assumed due to increased male swimming rate and lower energy stores than females (Browne, 1982). In a mixed population there is a possibility of sperm robbing (Browne, 1980b; Lynch, 1984) and it can play a role in the elimination of sexual species as they cannot reproduce without sperms from male while the parthenogenetic females can reproduce in the absence of male. Males do not discriminate between mating with either type of females (Browne,

1980b) and as they pair with parthenogenetic females the available male number for the sexual females to fertilise them is reduced and they have to be fertilised after each brood as they cannot store sperm. Fertilisation has no effect in parthenogenetic females except for very rare occurrence of polyploidy (Browne and Halanych, 1989). Hence a complete exclusion of native asexual population by sexual exotic population may not be possible due to the above discussed reasons.

1. 4. 1. The negative aspects of the population mixing

As a result of the various experiments, which are carried out at Tuticorin salt pan areas there are every possibility that the purity of the native population will be lost, not due to the genotypic variations of the local strain, which happens rarely in *Artemia*, but due to the mixing of the cysts produced by both the population which in turn can effect the marketability of the cysts. The hatchability and the required hatching conditions of both the species vary, mixing of the same will lower the quality of the cyst harvested from the mixed population. Sorting the cysts produced by the two separate species are also quite impossible. So measures are to be taken to ensure that the cultured population are not contaminated.

If we opt sexual strain for culturing in the local salt ponds there is every possibility of getting mixed cyst as the females of asexual strain can also thrive in the ponds and can reproduce under the same conditions. On the other hand, if we go for asexual strain chances of getting sexual cysts from the population is very much limited. This is because we can confirm the purity of population by checking the presence of male as the male occurrence is rare in the asexual population. Even if the sexual females are present in the cultured ponds along with the asexual females they do need males to reproduce and

they will not produce offspring neither as cysts nor as nauplii. Hence only the parthenogenetic culture alone can give pure cyst harvest once they are cultured.

CHAPTER 2

CHAPTER 2.

Effect of ecophysiological conditions on the reproductive characteristics

2. 1. Introduction

In the previous chapter effect of environmental changes on sexual and asexual species of Tuticorin were discussed and found that the lifespan characteristics of both the species manifested differently in different conditions. In the entire lifespan studies, the reproductive period of the animal has more importance as it determines the chances of the survivability of that particular animal in the habitat. In this chapter the effect of ecophysiological conditions on the fitness of two locally available *Artemia* species were studied. Reproductive characters are important to determine the reproductive success of a population in a particular habitat. The long reproductive period need not necessarily help an animal group to successfully inhabit a particular environment but is actually depended on the total reproductive out put, which in the case of *Artemia* depends on offspring production, interbrood period and broods per female. Among the two species one was the indigenous parthenogenetic and the other exotic sexual species, which appeared later as a result of contamination from the shrimp hatcheries or by a purposeful inoculation of the same by a farmer to culture them in the local salt ponds. As the local environmental conditions can change, the exotic species as they try to acclimatise to the new conditions there may be changes affecting their genetic as well as phenotypic expressions such as the life span and reproductive characters. According to Browne *et al.* (1988), there are significant differences not only among species but among populations

within a species. This can be due to the geographical isolation of the individuals among the same species. So this can happen when a new species is inoculated to a different country, as the new one will be affected by the existing environmental conditions.

2. 2. Materials and methods

Experimental set up is the same for the entire experiment as described in the General Materials and Methods and the reproductive characters studied in this Chapter are determined as detailed here. Since there are two species available from the same locality dominance of either of these species in terms of its reproductive performance finally succeed in replacing the other in the habitat. Following reproductive variables of the two species were studied in detail, so as to have a complete knowledge of the two. They are, 1) Broods per female, 2) Interbrood period, 3) Offspring per brood, 4) Total offspring per female and 5) Percentage of offspring production per each brood.

2. 2. 1. Broods per female

Number of broods obtained from each female in its entire lifespan.

2. 2. 2. Interbrood period

Days taken by the female to mature and release offspring from one brood to the next.

2. 2. 3. Offspring per brood

Total number of nauplii or cysts obtained from a single brood.

2. 2. 4. Total offspring per female

Total offspring produced by individual females in its reproductive lifespan.

2. 2. 5. Percentage of offspring production per each brood

Percentage of offspring production per each brood to the total fecundity was calculated for each female.

2. 3. Results

2. 3.1. Broods per female

Effect of different ecophysiological conditions on the number of broods produced by females of both the sexual and asexual species of *Artemia* are listed here.

2. 3.1.1. Effect of salinity.

Results on the number of broods per female of both the species of *Artemia* in different salinities are detailed in Table 2a and Figure 2a. Values of brood per female in the Figure 2a are shown in Appendix 2a. Maximum number of broods (12.5±0.7) for the sexual strain was obtained at 100ppt and that for the asexual strain (7±0) at 45ppt. The lowest number of broods were produced at 120ppt both for sexual and asexual strains. The number of broods per female increases with the increase in salinity from 20ppt to attain a peak of 12.5 broods per female at 100ppt among sexual species and 7 broods per female at 45ppt among asexual species. Then it tends to decrease and reach a minimum of 4.67 broods at 120ppt among sexual species and 2 broods per female at 145ppt among asexual species. Sexual species had more number of broods in all the tested salinities than asexual species (Figure 2a). Difference between the salinity treatments were highly significant (Table 2n). Interactive effect was significant indicating the dependence of salinity and species.

2. 3.1. 2. Effect of temperature

Effect of temperature on number of broods per female of sexual and asexual species of *Artemia* are shown in Figure 2b. Values of brood per

Table 2a

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the broods per female

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	195	27.9	14.7	0.00**
Species	1	212.5	212.5	112.1	0.00**
Salinity & Species	7	54.6	7.8	4.1	0.00**
Error	32	60.7	1.9		

Table 2b

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the broods per female.

Treatments	Degree of freedom	Sum-of Squares	Mean-square	F-ratio	P
Temperature	1	0.3	0.3	0.1	0.81
Temperature & Salinity	2	3.5	1.8	0.4	0.68
Temperature & Species	1	61.4	61.4	13.9	0.00**
Temperature, Salinity & Species	2	22.4	11.2	2.5	0.1
Error	24	106	4.4		

* Significant
** Highly significant

Figure 2a

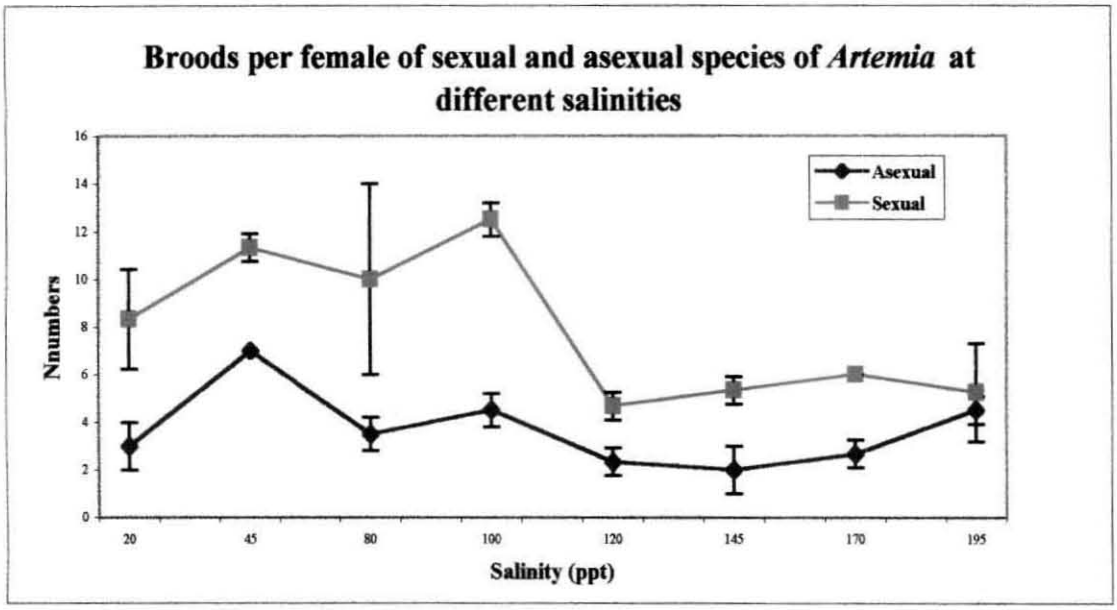
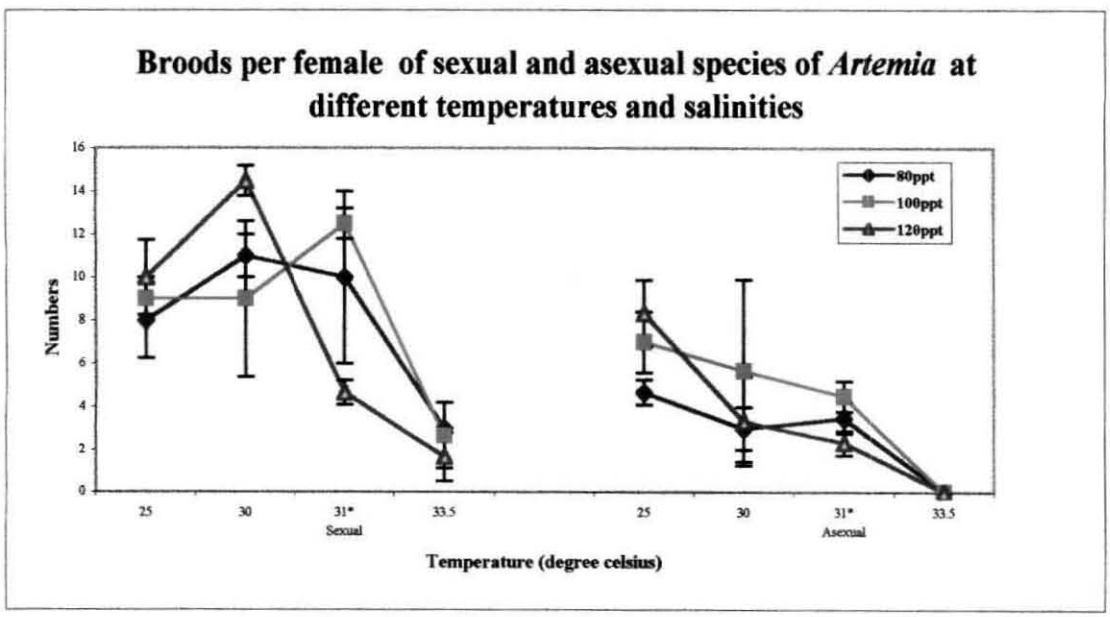


Figure 2b



* Ambient temperature

female in the Figure 2b are shown in Appendix 2b. At higher temperature only sexual females could reproduce and brood numbers were 3, 2.67 and 1.67 at 80, 100 and 120ppt salinities respectively at 33.5 °C and was found less compared to 25 and 30 °C. For sexual strain at all the tested salinities (excluding those at ambient temperature) the brood numbers increased at mid temperature (30 °C) and decreased at both low and higher temperature conditions parabolically. In case of asexual females, the brood number decreased linearly , Figure 2b, with increase in temperature. Broods per female of sexual as well as asexual species increased with increase in salinity at 25 °C. At ambient temperature the brood number of both the species increased as salinity increased to 100 from 80 and decreased at 120ppt.

Temperature individually had no significant effect on the brood number of either of the species but in interaction with species ($P<0.01$) there was significant difference (Table 2b).

2. 3.1. 3. Effect of quality and quantity of feed

Results on the effect of quality and quantity of feed on broods per female of sexual and asexual species of *Artemia* are shown in Table 2c and Figures 2ca, 2cb. . Values of brood per female in the Figure 2ca and 2cb are shown in Appendix 2c. Sexual females at 80 and 100ppt salinity had maximum number of broods with *Chlorella* sp. as feed both at high and normal feeding but at 120ppt the *Isochrisis* sp. fed animals were found to have had more number of broods than those fed with *Chlorella* sp. and rice bran. The asexual females had more brood number with *Isochrisis* sp. with all the three salinities with high and normal feeding. Asexual females had good number of broods also with ricebran when fed at normal concentration. Sexual species was found to have produced more broods when fed with *Chlorella* sp. especially at 80 and 100ppt salinities. *Isochrisis* sp. and rice bran were found good for asexual species in terms of brood numbers. It was proved statistically that there was significant difference in brood numbers

Table 2c

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the broods per female

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	0.1	0.1	0.0	0.88
Feed type	2	59.5	29.7	8.1	0.00**
Feed quantity & Feed type	2	18.1	9.0	2.5	0.09
Feed quantity & Salinity	2	22.2	11.1	3.0	0.06
Feed quantity & Species	1	8.9	8.9	2.4	0.13
Feed type & Salinity	4	92.8	23.2	6.3	0.00**
Feed type & Species	2	62.4	31.2	8.5	0.00**
Feed quantity, Feed type & Salinity	4	10.1	2.5	0.7	0.6
Feed quantity, Feed type & Species	2	1.7	0.8	0.2	0.8
Feed quantity, Salinity & Species	2	17.8	8.9	2.4	0.1
Feed type, Salinity & Species	4	173.5	43.4	11.8	0.00**
Feed quantity, Feed type, Salinity & Species	4	37.4	9.3	2.5	0.05*
Error	72	265.3	3.7		

* Significant
** Highly significant

Figure 2ca

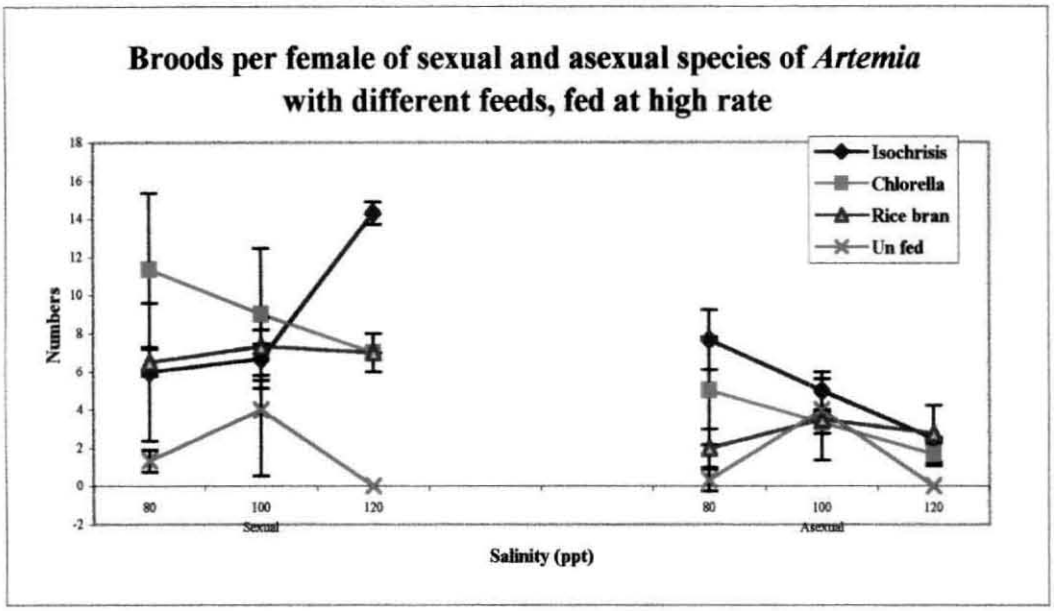
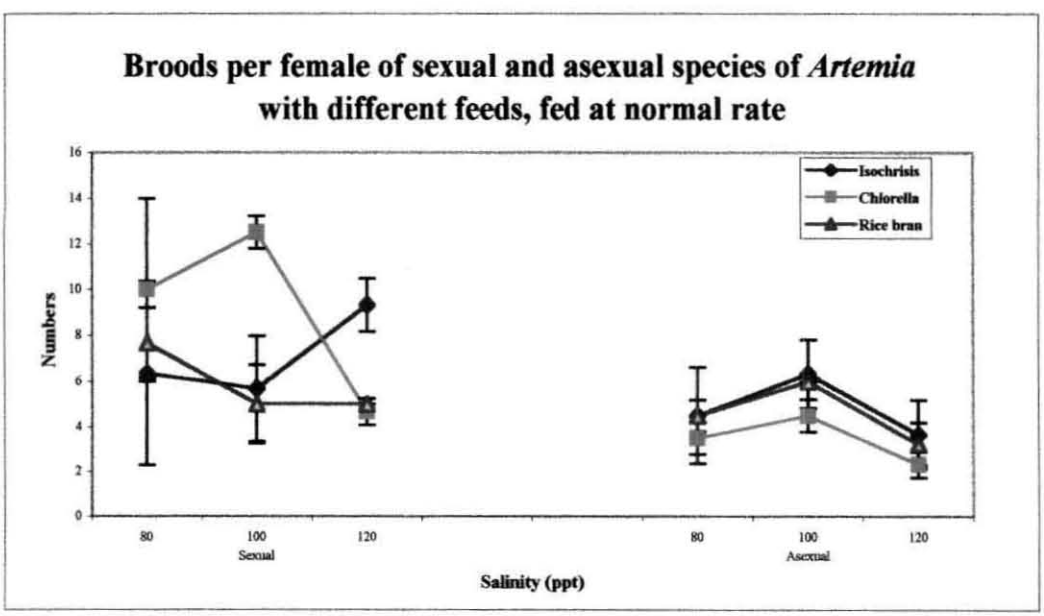


Figure 2cb



between animals fed with different feed type ($P<0.01$), while increased feeding had no effect (Table 2c).

Sexual females had produced broods even without any feed at 80 and 100ppt and at lower salinities it can survive without much feed. But as salinity is increased female died before releasing the first batch of brood and may be because at salinities above 80ppt, microplanktons cannot survive and animals had to completely depend on supplementary feeding.

Increased feeding helped the sexual as well as asexual animals to have more broods at 120ppt in their reproductive life with *Isochrisis* sp. and rice bran. Generally low feed help to have more broods for asexual species at 100 and 120ppt. Unfed animals of both the species at 100ppt produced 4 broods each in their reproductive life.

2. 3. 2. Interbrood period.

2. 3. 2.1. Effect of salinity.

The results on the inter brood period of sexual and asexual females of *Artemia* for all the tested salinities are shown in Table 2d and Figure 2d. Values of interbrood period in the Figure 2d are shown in Appendix 2d. Interbrood period ranged from 2.88 days to 5.28 days for sexual strain and that for asexual strain varied from 3.88 to 4.5 days. Inter brood period showed minimum days at 80 and 100 ppt salinities for the sexual species (2.8 ± 0.1 and 3.0 ± 0.1) and 20 and 100ppt salinities for the asexual strain (3.94 and 3.88 days). Different salinity levels had significant effect on time between the broods ($P<0.05$), of both *Artemia* species (Table 2d). Interbrood period of both the species had a general trend to increase the inter brood period with increase in salinity.

2. 3. 2. 2. Effect of temperature

Results on temperature effect on interbrood period of sexual and asexual species of *Artemia* are shown in Table 2e and Figure 2e. Values of interbrood period in the Figure 2e are shown in Appendix 2e. Interbrood period of asexual females of almost all the experimental conditions were

longer than that of sexual females. Temperature was found to have a significant effect on this characteristic ($P<0.01$) (Table 2e). Alongwith increase in temperature interbrood period of both the species (excluding readings at ambient temperature) at all the salinities showed a decrease except at 80ppt where a marginal increase was noticed as the temperature increased from 30 to 33.5 °C in case of sexual species. Both the species had longer interbrood period at 25 °C at all the three tested salinities. At 25 °C there was a decrease in the interbrood period of sexual female along with an increase in the salinity from 80 to 100ppt and then had a increase with further increase in salinity to 120ppt. In case of asexual females it was 5.6 at 80ppt and increased to 6.2 days as the salinity increased to 100ppt and then decreased to 5.96 days as the salinity again increased to 120ppt. At 30 and 33.5 °C sexual inter brood period had a decrease in the mid salinity and then increased as the salinity increased to 120ppt. In case of asexual females interbrood period at 100ppt was longer compared to those at 80 and 120ppt. At ambient temperature the interbrood period was short at 80 and 100 ppt for asexual females, but at 120 ppt it was marginally higher. Temperature had highly significant effect on inter brood period of both the species ($P<0.01$) (Table 2e).

2. 3. 2. 3. Effect of quality and quantity of feed

Results on the effect of quality and quantity of feed on the length of interbrood period of sexual and asexual species of *Artemia* are shown in Figures 2fa, 2fb and Table 2f. Values of interbrood period in the Figure 2fa and 2fb are shown in Appendix 2f. Sexual species generally had significantly short interbrood period than the asexual species irrespective of the type and quantity of feed as shown in Figure 2fa, 2fb. In case of asexual species the shortest interbrood period was noticed with *Isochrisis* sp.

Table 2d

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on inter brood period of the female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	19.1	2.7	2.7	0.03*
Species	1	1.8	1.8	1.7	0.20
Salinity & Species	7	6	0.9	0.8	0.57
Error	32	32.8	1		

Table 2e

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the inter brood period of the female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	19.5	19.5	115.8	0.00**
Temperature & Salinity	2	0.7	0.4	2.2	0.14
Temperature & Species	1	0.0	0.0	0.0	0.83
Temperature, Salinity & Species	2	0.3	0.2	1	0.39
Error	24	4.0	0.2		

* Significant
** Highly significant

Figure 2d

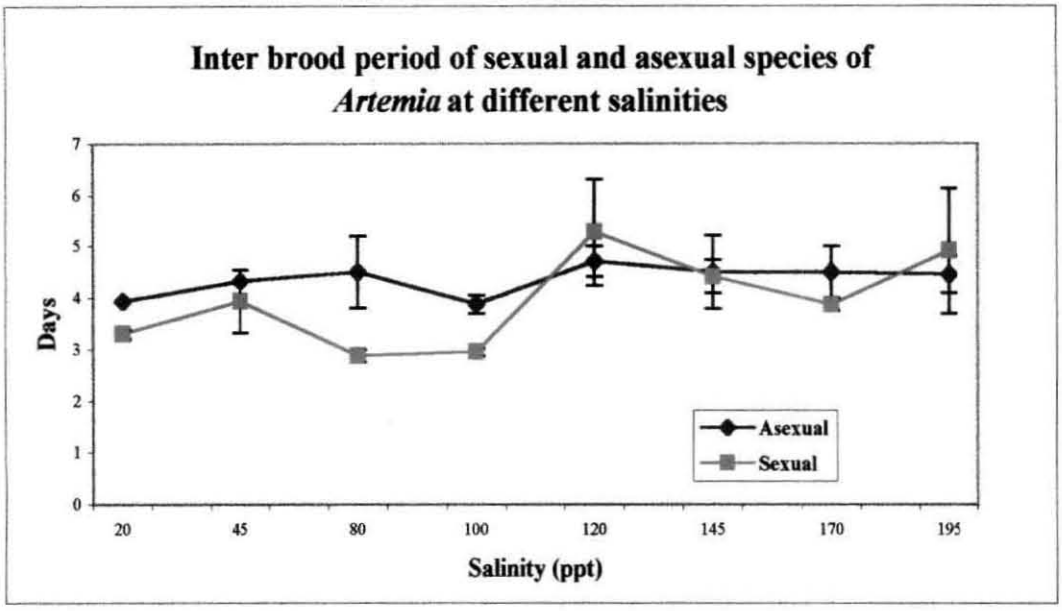
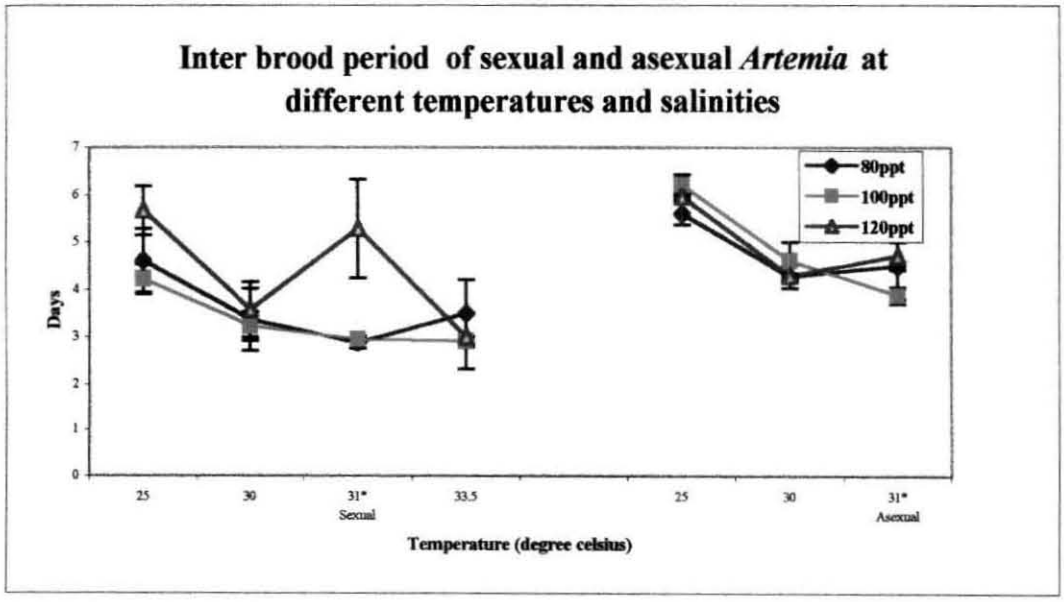


Figure 2e



* Ambient temperature

at 120ppt, when fed both at high and normal rate and the longest with rice bran at 80ppt, when fed at high rate. Asexual species with algae fed females had shorter interbrood period than those of rice bran fed animals except at 100ppt fed at high rate. In case of sexual species with normal feeding *Chlorella* sp. fed animals had shorter inter brood period at 80ppt (2.88 days) but at 120ppt the same had longest interbrood period (5.28 days) than those fed with rice bran and *Isochrisis* sp. There was significant difference in interbrood period with different feed type ($P<0.01$) (Table 2f). Sexual species fed with algae at high rate had short interbrood period than rice bran fed animals. Unfed females also had comparatively low interbrood period.

2. 3. 3. Offspring per brood

Effect of different ecophysiological conditions on the brood size of sexual as well as asexual species are listed here.

2. 3. 3.1. Effect of Salinity.

Results on the effect of salinity on the offspring per brood of sexual and asexual species of *Artemia* are presented in Table 2g and Figure 2g. Values of offspring per brood in the Figure 2g are shown in Appendix 2g. Broods of the sexual strain at 45ppt had produced maximum number of offspring (126.66 ± 3.95) among all the salinities and 170ppt gave least number of offspring per brood (15.59 ± 1.07). In the asexual strain those at 195ppt gave the maximum (81 ± 17.45) and those at 120ppt the minimum. For the sexual strain there was a trend to increase its brood productivity along with decrease in salinity. Asexual strain also had good production at lower (20, 45, 80 and 100ppt) as well as upper most (195ppt) salinities (Figure 2g). Salinity alone and also in interaction with species had significant effect on offspring per brood ($P<0.01$). At low salinities (20, 45, 80, 100 and 120ppt) sexual females had produced more offspring than asexual females, while at salinities 145,170 and 195 asexual females had produced more number of offspring.

Table 2f

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the Inter brood period of the female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	0.2	0.2	0.6	0.4
Feed type	2	3.0	1.5	5.1	0.01*
Feed quantity & Feed type	2	2.1	1.0	3.7	0.03*
Feed quantity & Salinity	2	1.0	0.5	1.8	0.17
Feed quantity & Species	1	1.0	1.0	3.5	0.07
Feed type & Salinity	4	5.6	1.4	4.8	0.00**
Feed type & Species	2	0.1	0.0	0.1	0.89
Feed quantity, Feed type & Salinity	4	3.7	0.9	3.2	0.02*
Feed quantity, Feed type & Species	2	0.9	0.4	1.5	0.24
Feed quantity , Salinity & Species	2	0.4	0.2	0.7	0.49
Feed type, Salinity & Species	4	1.4	0.3	1.2	0.33
Feed quantity, Feed type, Salinity & Species	4	0.7	0.2	0.6	0.69
Error	72	20.8	0.3		

* Significant
** Highly significant

Figure 2fa

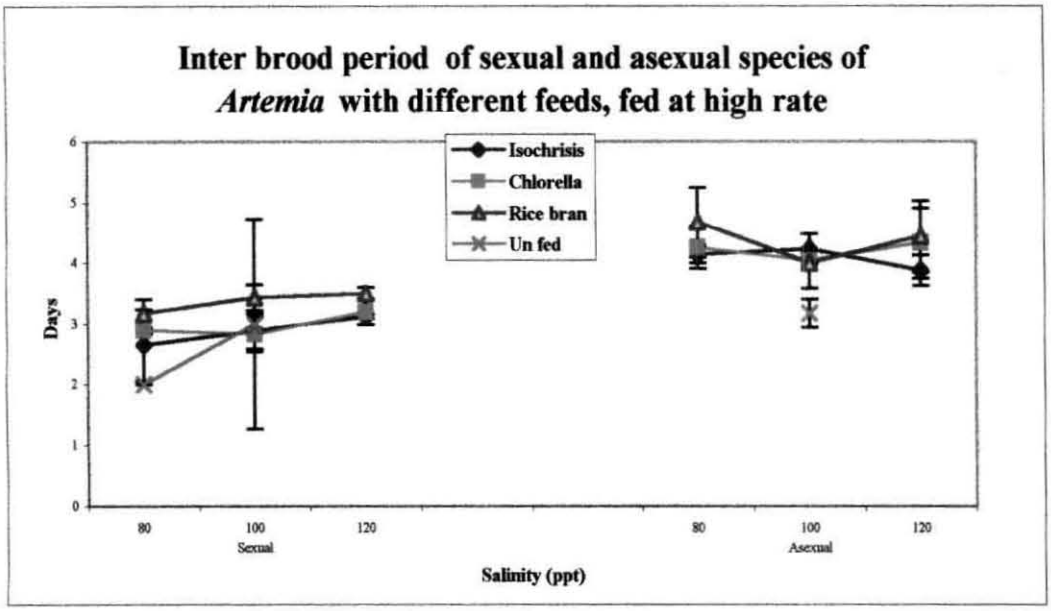
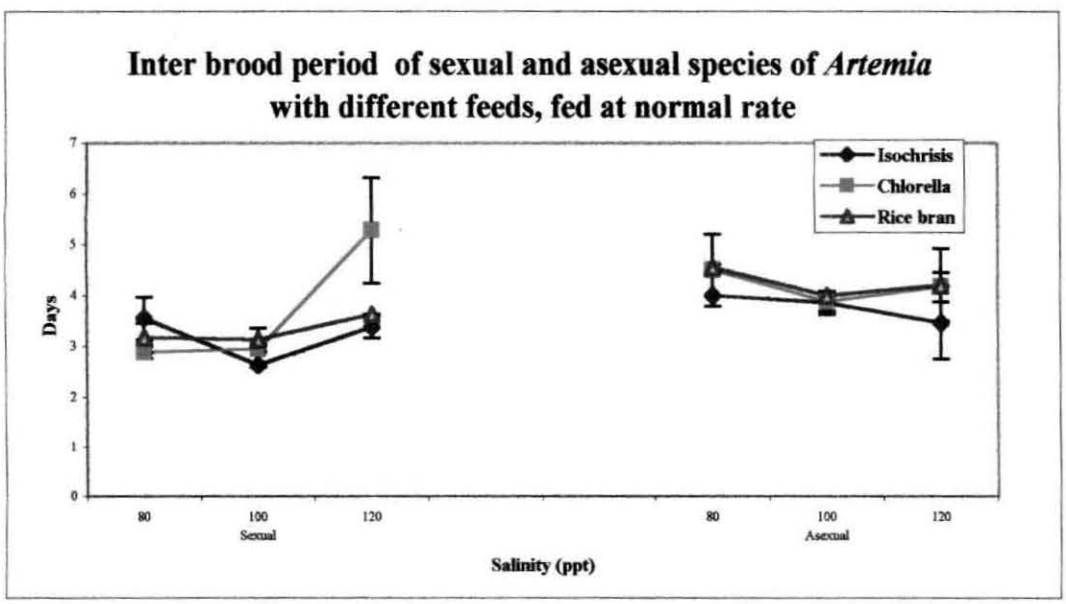


Figure 2fb



2. 3. 3. 2. Effect of Temperature

Results on the effect of temperature on offspring production of sexual and asexual species of *Artemia* are shown in Figure 2h and Table2h. Values of offspring per brood in the Figure 2h are shown in Appendix 2h. Asexual females were found to have produced significantly more offspring per brood than the sexual females (excluding the readings at ambient temperature). Temperature was found having no significant effect on the offspring production of broods of both the species (Table 2h). Only at 30 °C when the salinity was 100 ppt, asexual females had produced less number of offspring than the sexual females. Maximum number of offspring (55.67 numbers) per brood were produced at a temperature of 30 °C at 100ppt for sexual female. For asexual species it was (74.87 numbers) at 80ppt and 25 °C. At 30 °C and 33.5 °C the optimum salinities for sexual females were 100 ppt and 120ppt respectively. In case of the asexual females at 30 °C it was 120ppt which gave the maximum offspring and at 33.5 °C there was no reproduction at all. At 25 °C both the species had maximum offspring per brood at 80ppt. At ambient temperature sexual species had more offspring per brood than asexual species at all the tested salinities.

2. 3. 3. 3. Effect of quality and quantity of feed

Results on the effect of quality and quantity of feed on offspring per brood of sexual and asexual species of *Artemia* are listed in Figures 2ia, 2ib and Table 2i. Values of offspring per brood in the Figure 2ia and 2ib are shown in Appendix 2i. Sexual species in general had more number of offspring produced by each brood both with normal and high feeding. With high feeding *Isochrisis* sp. was found to be good at lower salinity of 80ppt than that of 100 and 120ppt as the offspring production per brood was more for both the species at this salinity. Asexual species with normal feeding also produced more offspring with *Isochrisis* sp. as feed than that of *Chlorella* sp. and rice bran at 80 and 100ppt, but the sexual species with normal feed gave maximum offspring per brood with *Isochrisis* sp. as feed at 80ppt and

Table 2g

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on offspring per brood of the female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	37559	5365.6	30	0.00**
Species	1	6031.2	6031.2	33.7	0.00**
Salinity & Species	7	11867	1695.3	9.5	0.00**
Error	32	5726.7	179		

Table 2h

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the offspring per brood of the female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	35	34.9	0.2	0.7
Temperature & Salinity	2	858.3	429.1	1.9	0.17
Temperature & Species	1	5.5	5.5	0.0	0.88
Temperature, Salinity & Species	2	1315.4	657.7	2.9	0.08
Error	24	5526.4	230.3		

* Significant
** Highly significant

Figure 2g

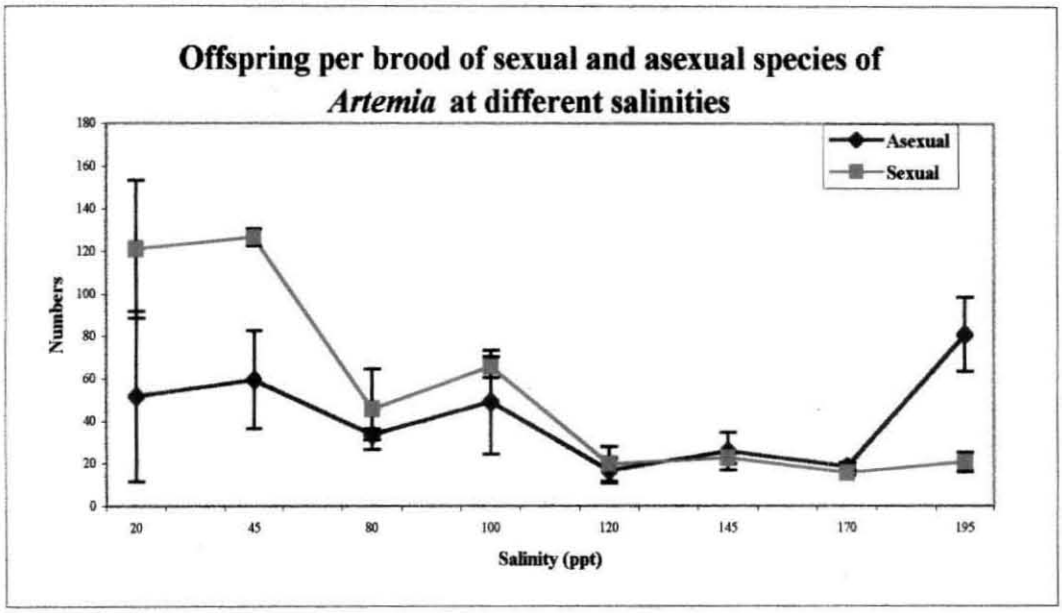
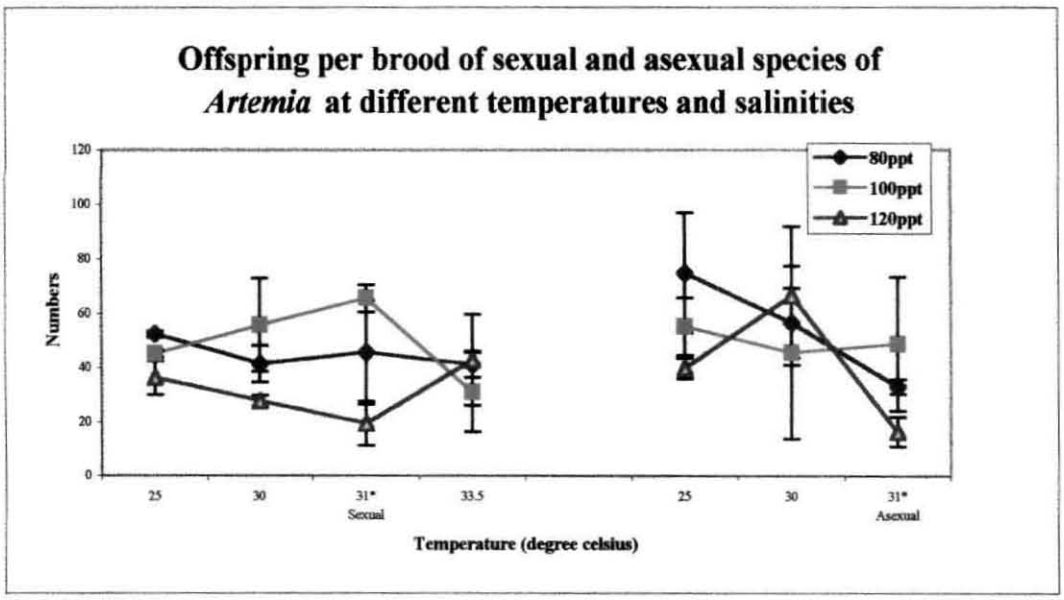


Figure 2h



* Ambient temperature

with *Chlorella* sp. as feed at 100pt and with rice bran as feed at 120ppt salinity respectively. The feed type and quantity individually and also interactively effected offspring production significantly ($P<0.01$) (Table 2l). High feeding with algae *Isochrisis* sp. and *Chlorella* sp. increased the offspring production almost at all the salinities for both the species, while with rice bran there was an increase in the offspring production rate at 80ppt but a decreased production at 100 and 120ppt salinities.

2. 3. 4. Total offspring per female

2. 3. 4.1. Effect of salinity.

Results on the effect of salinity on the offspring production potential of sexual and asexual *Artemia* species are shown in Figures 2j and Table 2j. Values of Total offspring per female in the Figure 2j are shown in Appendix 2j. There was an increase in offspring production per female of both the species with decrease in salinity. Total number of offspring produced by the sexual females at 20, 45, 80 and 100ppt salinities respectively were 1040.6±450.73, 1395.00±161.08, 508.33±371.12 and 820.00±12.73 and the offspring of asexual strain at these salinities were 126.66±94, 501.5±98.29, 118±32.5 and 211.5±76.66. Total offspring produced at 120, 145, 170 and 195ppt were lesser than hundred both for sexual and asexual strains (Figure 2J). There was significant effect of salinity and also in interaction with the species on the total offspring production ($P<0.01$) (Table 2j).

2. 3. 4. 2. Effect of temperature

Results on the effect of temperature on total offspring production per female of sexual as well as asexual species of *Artemia* are shown in Figure 2k and Table 2k. Values of Total offspring per female in the Figure 2k are shown in Appendix 2k. For sexual females 30 °C was the optimum (excluding ambient temperature)as the offspring number increased as the

Table 2i

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the offspring per brood of the female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	6059.4	6059.4	23.9	0.00**
Feed type	2	21348.6	10674.3	42.1	0.00**
Feed quantity & Feed type	2	7194.4	3597.2	14.2	0.00**
Feed quantity & Salinity	2	783.5	391.8	1.5	0.22
Feed quantity & Species	1	5522.1	5522.1	21.8	0.00**
Feed type & Salinity	4	8406.5	2101.6	8.3	0.00**
Feed type & Species	2	714.2	357.1	1.4	0.25
Feed quantity, Feed type & Salinity	4	125.1	31.3	0.1	0.97
Feed quantity, Feed type & Species	2	3503.3	1751.6	6.9	0.00**
Feed quantity, Salinity & Species	2	884.9	442.4	1.7	0.18
Feed type, Salinity & Species	4	797.6	199.4	0.8	0.54
Feed quantity, Feed type, Salinity & Species	4	624.7	156.2	0.6	0.65
Error	72	18248.4	253.5		

* Significant
** Highly significant

Figure 2ia

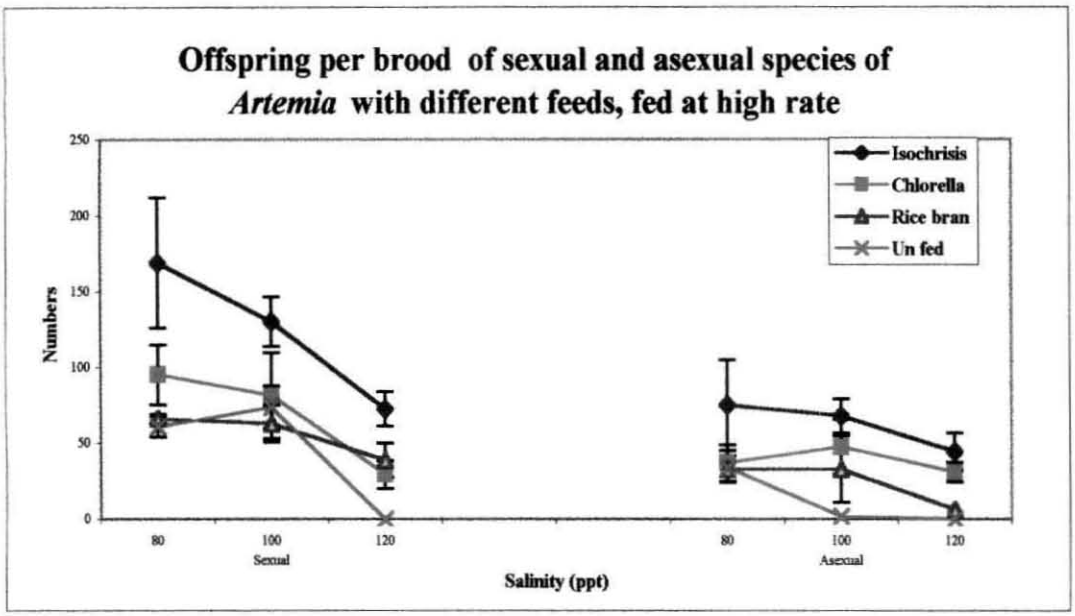
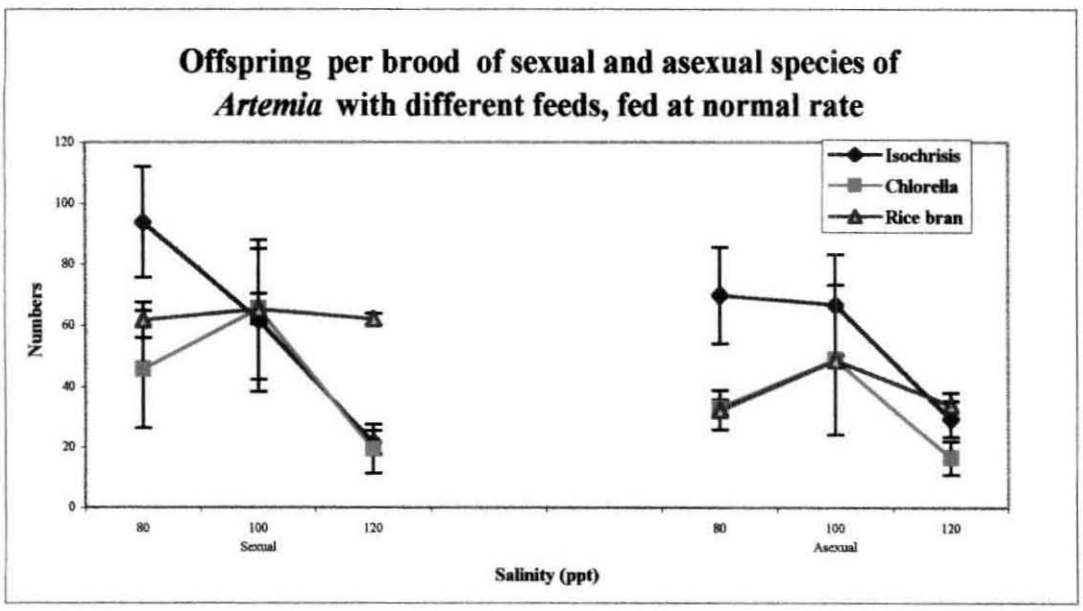


Figure 2ib



temperature increased to 30 from 25 °C and decreased at 33.5 °C irrespective of salinities and was found parabolically related with temperature. In case of asexual females number of offspring kept on decreasing linearly along with the increase in temperature. In sexual species at 80 and 100ppt salinities experiments at ambient temperature gave better results but at 120ppt the number came down. Asexual females had produced less offspring at ambient temperature than at 25 and 30 °C but was better than 33.5 °C. Temperature alone had no significant effect on total offspring production. Interactive effect of temperature and species had significant effect on offspring per female ($P<0.01$) (Table 2k).

2. 3. 4. 3. Effect of quality and quantity of feed

Results on the effect of quality and quantity of feed on total offspring production per female of sexual and asexual species of *Artemia* are shown in Figures 2la, 2lb and Table 2l. Values of offspring per female in the Figure 2la and 2lb are shown in Appendix 2l. Inter species variation on offspring production was highly significant as sexual species had more fecundity than the asexual species at almost all the conditions. With the normal feeding, sexual species had maximum fecundity with *Chlorella* sp. as feed at 100ppt salinity. Asexual females at normal feeding found to have had maximum fecundity with *Isochrisis* sp. as feed at all the three salinities. Rice bran fed animals had the second best fecundity followed by *Chlorella* sp. Sexual females had maximum fecundity with *Isochrisis* sp. and *Chlorella* sp. as feed at 80ppt with normal feeding. At 120ppt salinity rice bran fed females had the maximum fecundity with normal feeding but had a decrease with increase in quantity of feed. When the quantity of feed was increased the sexual species had their maximum fecundity with *Isochrisis* sp. as feed at 120ppt salinity. Asexual species at 80ppt with *Isochrisis* sp. had good fecundity. Rice bran when fed with high concentration had increased the fecundity along with increase in salinity from 80 to 100ppt for both the species

Table 2j

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the total offspring per female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	4326150	618021.4	25.2	0.00**
Species	1	1701027	1701027	69.4	0.00**
Salinity & Species	7	1631285	233040.8	9.5	0.00**
Error	32	784676	24521.1		

Table 2k

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the total offspring per female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	22650.3	22650.3	1.5	0.23
Temperature& Salinity	2	1140.5	570.3	0.0	1.00
Temperature& Species	1	163620.3	163620.3	10.7	0.00**
Temperature, Salinity & Species	2	14941.2	7470.6	0.5	0.62
Error	24	365315.3	15221.5		

* Significant
** Highly significant

Figure 2j

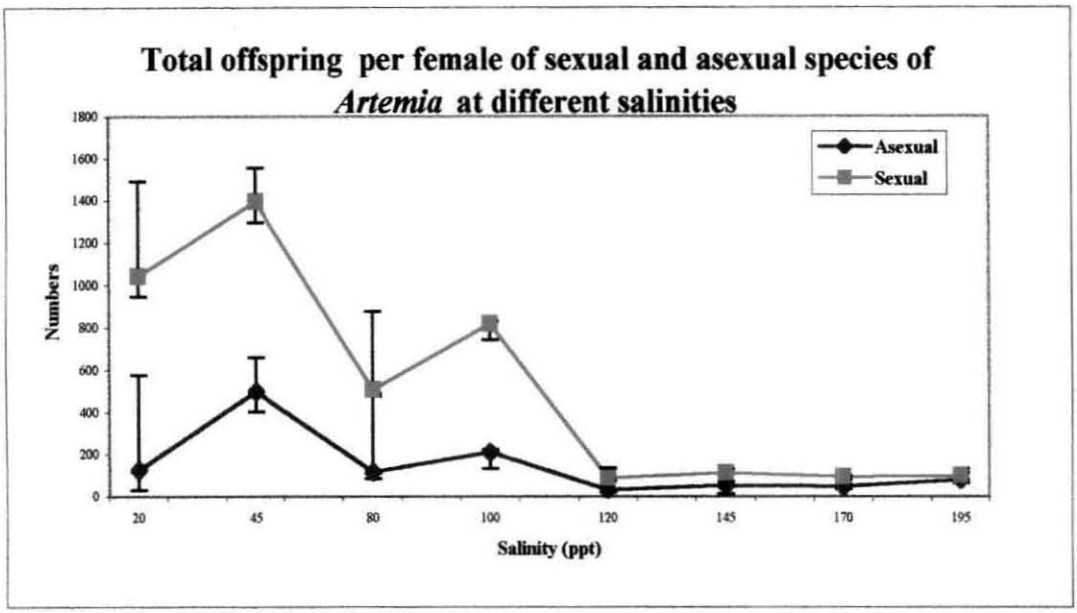
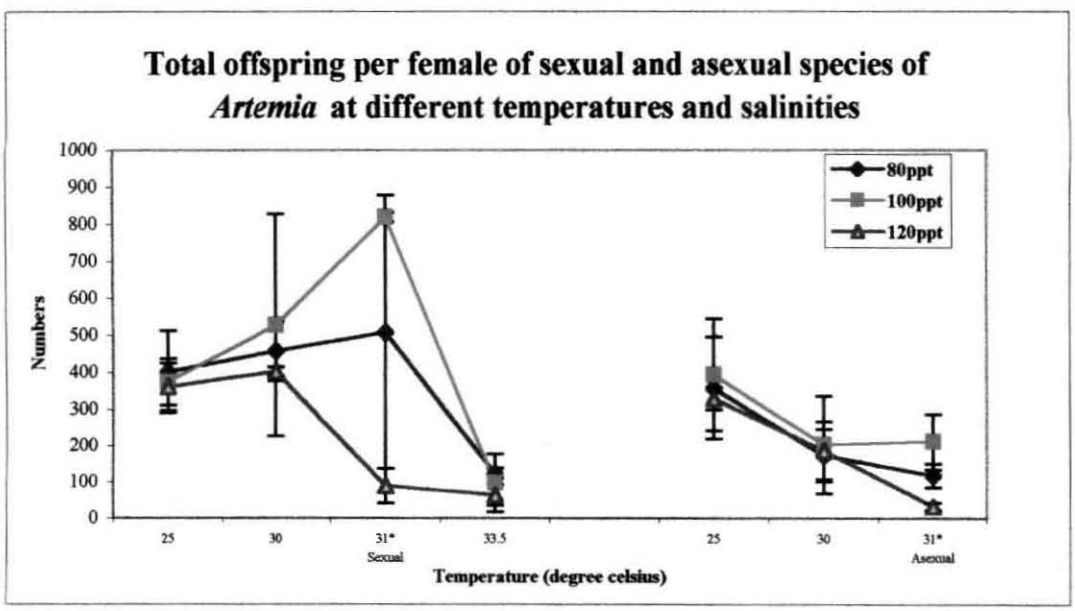


Figure 2k



* Ambient temperature

but decreased when salinities are increased to 120ppt. Total offspring production significantly differed with feed type and quantity (Table 2l).

2. 3. 5. Percentage offspring production per each brood

Percentage offspring production per each brood at different salinity, temperature and salinity combinations and feed and salinity combinations are shown in Figures 2ma, 2mb, 2mc, 2md, 2me, 2mf, 2mg, 2mh, 2na, 2nb, 2nc, 2oa, 2ob, 2oc, 2od, 2oe, 2of, 2og, 2oh and 2oi respectively. With different salinities sexual females had produced maximum of its offspring towards its penultimate broods in most of the cases (Figure, 2ma, 2mb, 2mc, 2md, 2me, 2mf, 2mg, 2mh). For asexual species at salinities of 20, 45, 80 and 100ppt maximum percentage of offspring was produced at the later reproductive period and at salinities above 100ppt the percentage offspring production reduced with the brood age of the female (Figure 2ma, 2mb, 2mc, 2md). At 25 °C the production of offspring of both the species were almost constant and regular through out reproductive period at all the tested salinities (Figure 2na, 2nb, 2nc). With 30 °C temperature the sexual females had constant offspring production through out the reproductive period but the asexual species had decreased number of offspring towards the later broods than that of the earlier broods. At 33.5 °C only sexual species had produced offspring and the production rate increased with the brood age. With different feed also there was an increased fecundity in mid ages brood and there was a dip towards the last brood (2oa, 2ob, 2oc, 2od, 2oe, 2of, 2og, 2oh and 2oi).

2. 4. Discussion

It is clear from the previous chapter that the reproductive period of the sexual species is longer than the asexual species at all the salinities and temperature combinations. With different feed also the sexual species had longer reproductive period except with *Isochrisis* sp. as feed at 80 and

Table 2l

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the total offspring per female individual.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	495455.8	495455.8	13.4	0.00**
Feed type	2	1052377.2	526188.6	14.3	0.00**
Feed quantity & Feed type	2	630334.5	315167.3	8.5	0.00**
Feed quantity & Salinity	2	100125.4	50062.7	1.4	0.26
Feed quantity & Species	1	524868.9	524868.9	14.2	0.00**
Feed type & Salinity	4	457638.8	114409.7	3.1	0.02*
Feed type & Species	2	210990.3	105495.1	2.9	0.06
Feed quantity, Feed type & Salinity	4	97044.7	24261.2	0.7	0.62
Feed quantity, Feed type & Species	2	230723.2	115361.6	3.1	0.05*
Feed quantity, Salinity & Species	2	20936.1	10468.0	0.3	0.75
Feed type, Salinity & Species	4	591433.5	147858.4	4.0	0.00**
Feed quantity, Feed type, Salinity & Species	4	330886.3	82721.6	2.2	0.07
Error	72	2656542.7	36896.4		

* Significant
** Highly significant

Figure 2Ia

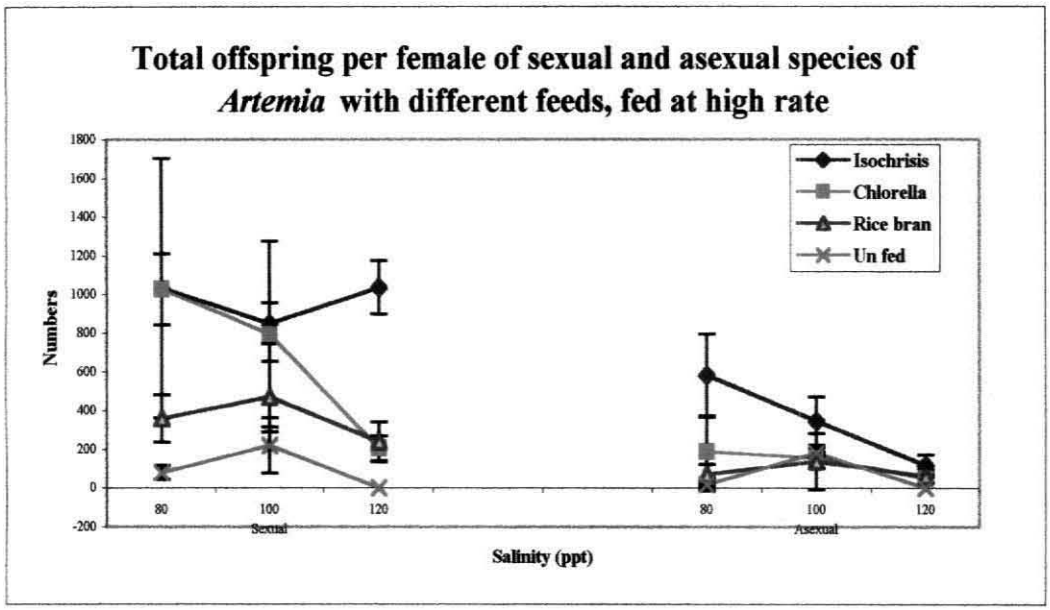
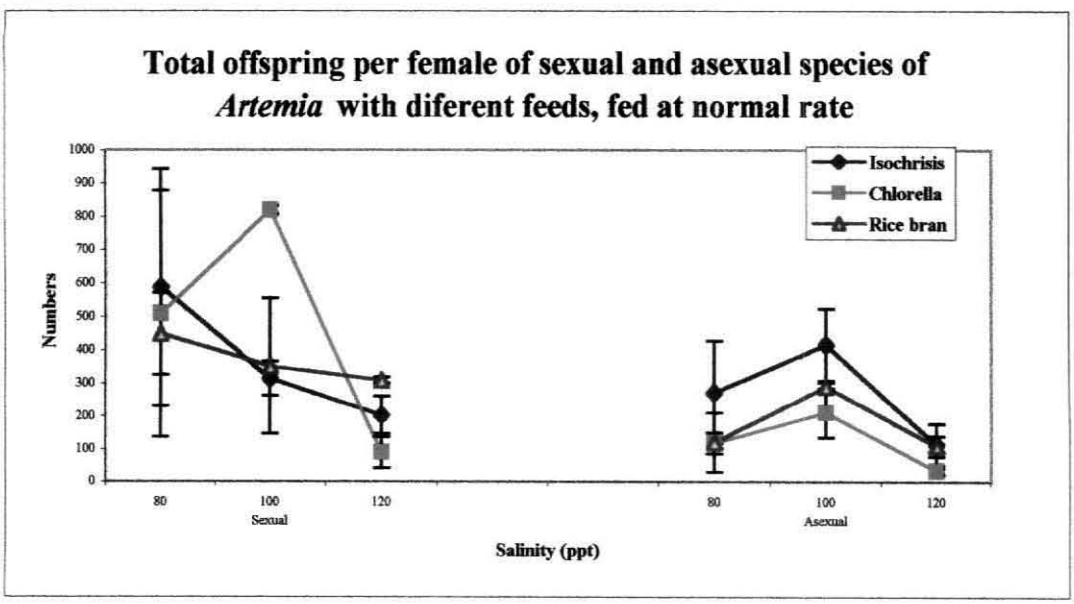


Figure 2Ib



100ppt salinities. Since the reproductive output of an *Artemia* species is controlled mainly by the differences in reproductive characteristics like brood numbers, inter brood period and offspring production, these characteristics of both the species under tropical conditions were studied in detail and are discussed in this chapter. The reproductive characters of the sexual and asexual species clearly differ as reported by many authors (Browne,1980b; Browne *et al.*, 1988; Wear *et al.*, 1986; Triantaphyllidis *et al.*, 1995) who conducted the study in temperate conditions and found that the reproductive characters are influenced with the prevailing ecological factors.

Artemia franciscana appears more euryhaline, exhibiting better reproductive characters in broader range of salinities (Wear and Haslett, 1986; Wear *et al.* 1986). Parthenogenetic and sexual species clearly differ with regard to their reproductive characteristics as reported by Browne *et al.* (1984). The results of the present investigation have proved that the reproductive characters of the studied sexual species were better than that of the local parthenogenetic species, and can be assumed that they belong to the New world sexual population. Browne (1980b) had also found significant difference between strains for a number of reproductive characters such as the number of offspring per brood, number of offspring per female over life time, broods per female, offspring per day and reproductive period.

The sexual species had higher fecundity than the asexual species at all the tested salinities (Figure 2j) with increased production at lower salinities. But the brood size of both the species was found to be good at both lower and higher salinities. Offspring per brood of sexual species ranged between 127 and 16 numbers being the maximum and the minimum at 45 and 170ppt respectively whereas in asexual species it ranged between 81 and 17 the maximum and the minimum at 195 and 120ppt respectively. Knight (1974) reported that in field females carry 45 to 55 eggs with little variation up to

Figure 2ma

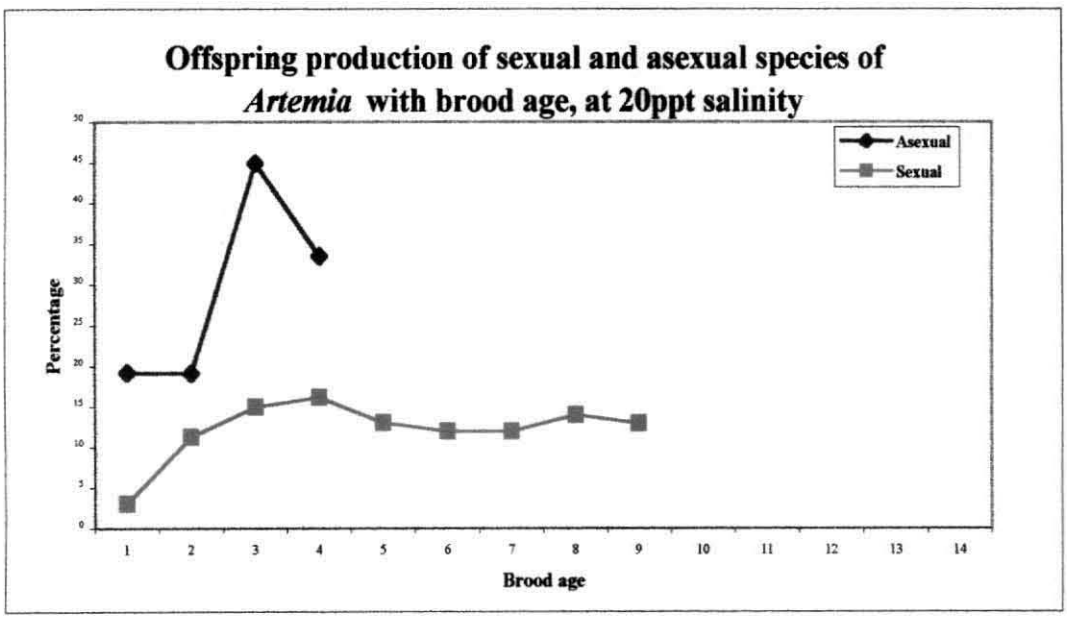


Figure 2mb

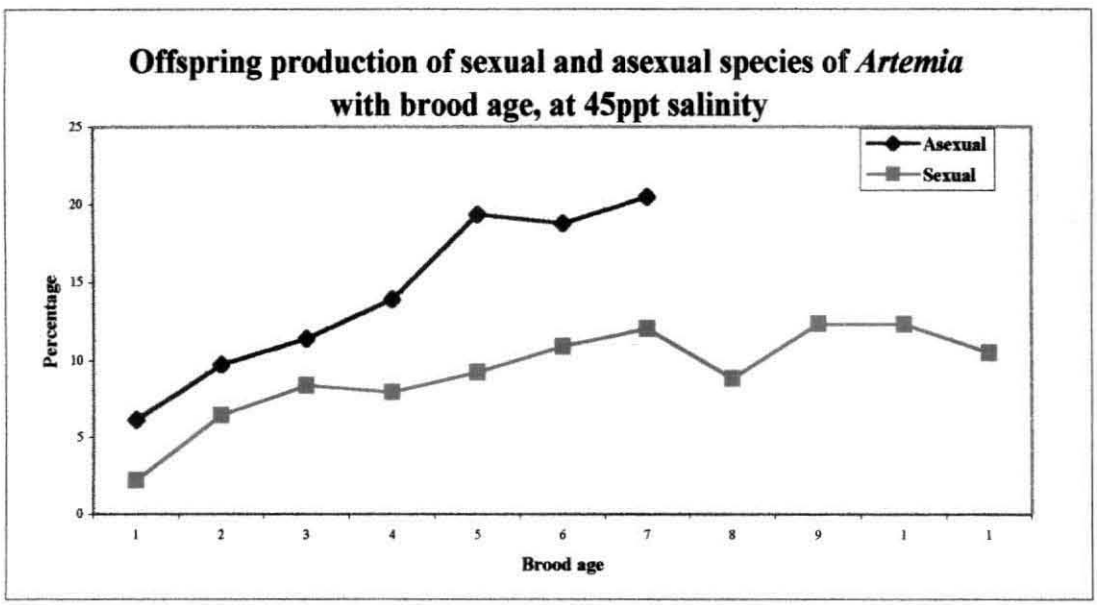


Figure 2mc

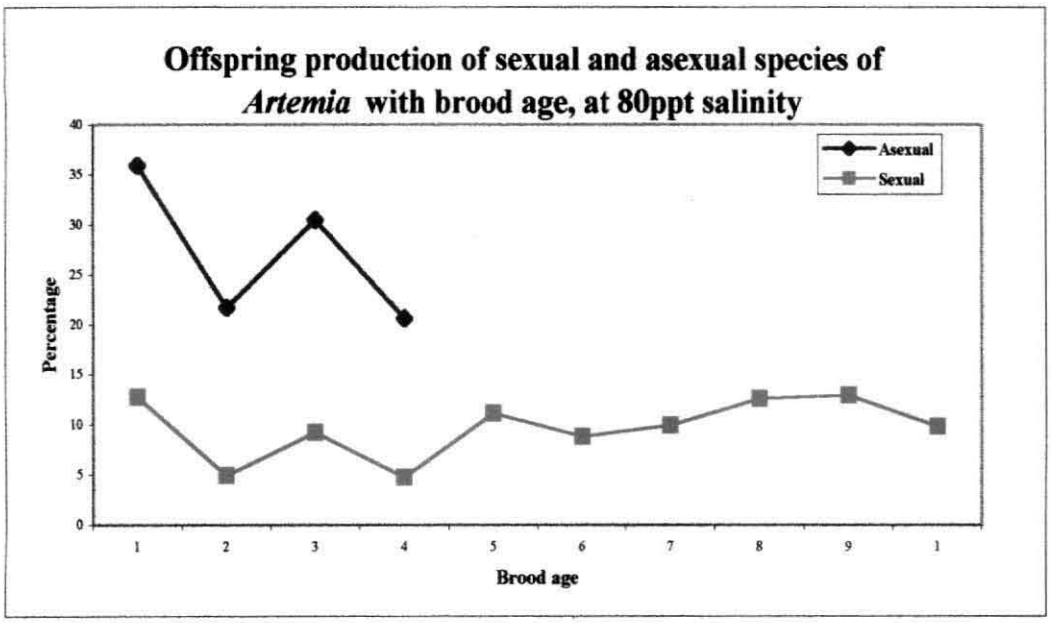


Figure 2md

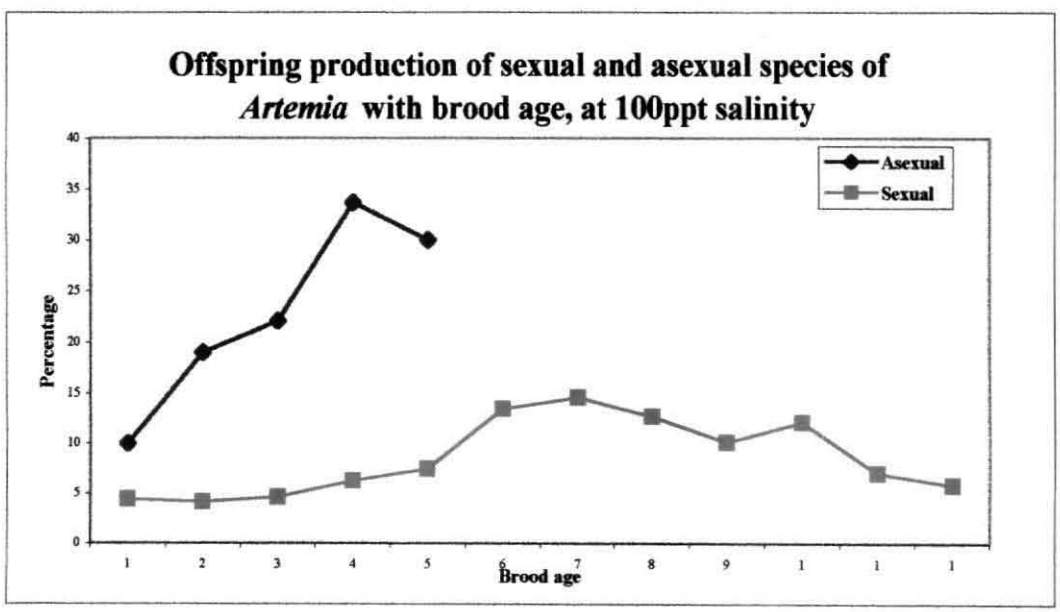


Figure 2me

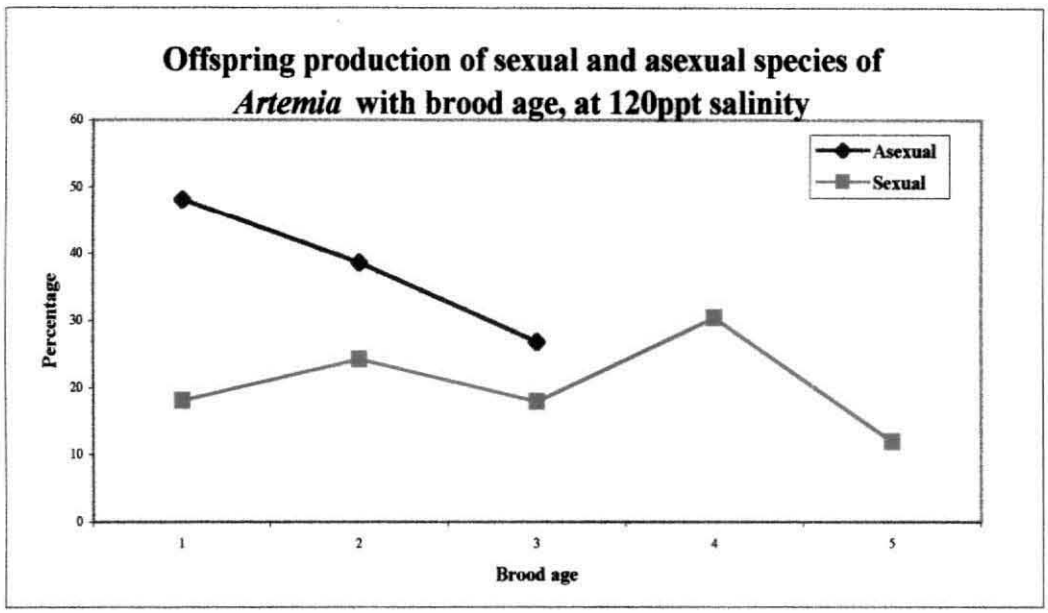


Figure 2mf

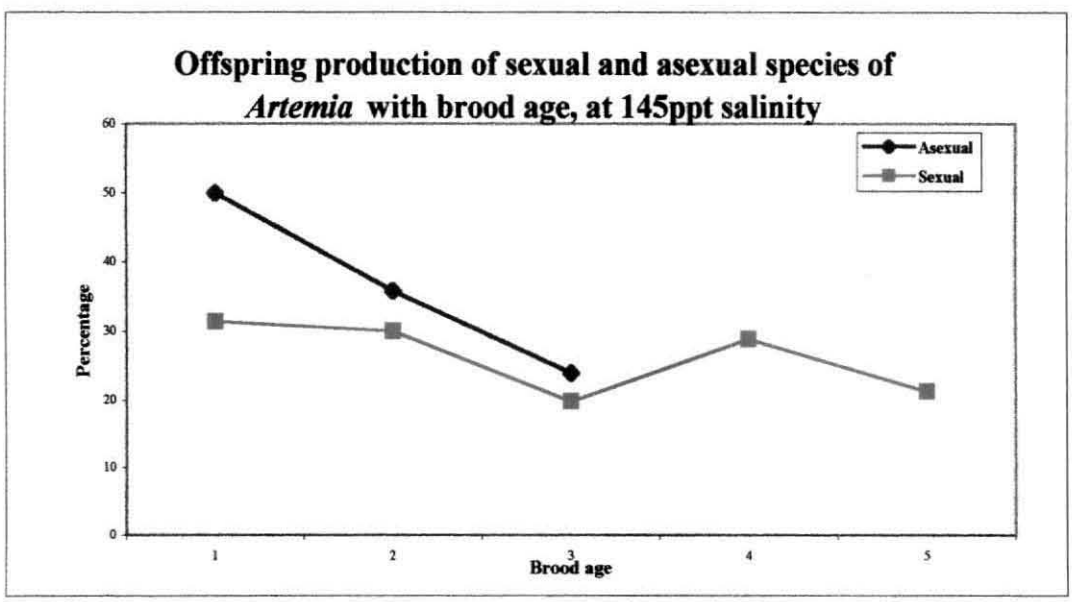


Figure 2mg

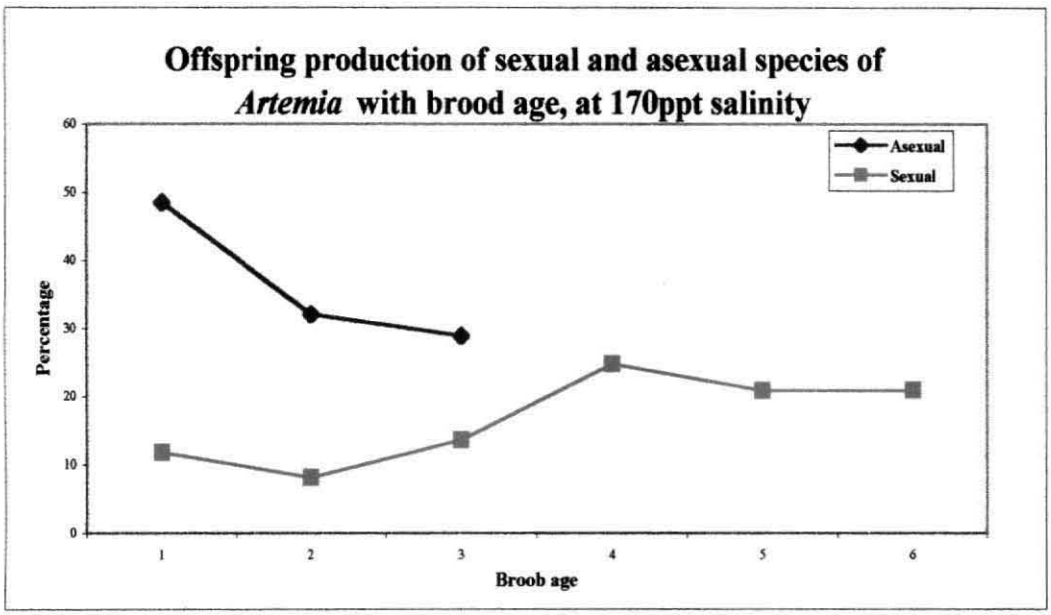


Figure 2mh

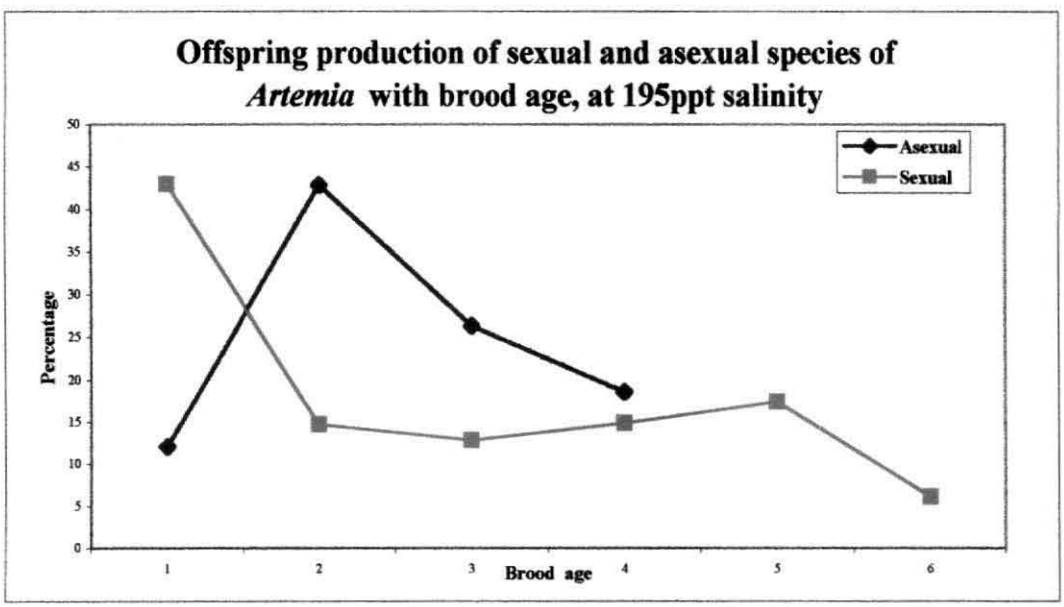


Figure 2na

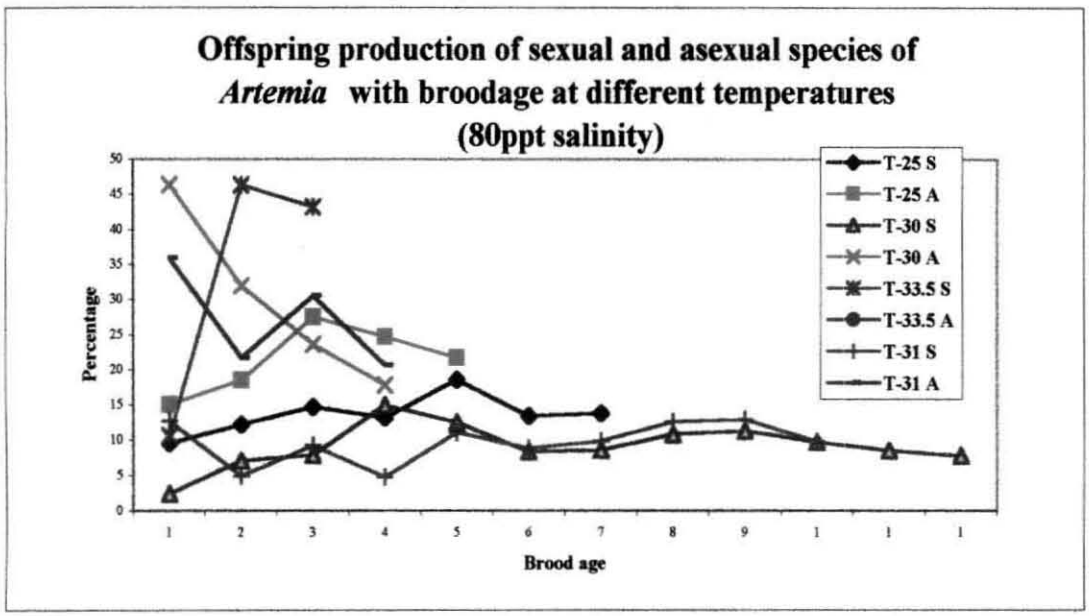
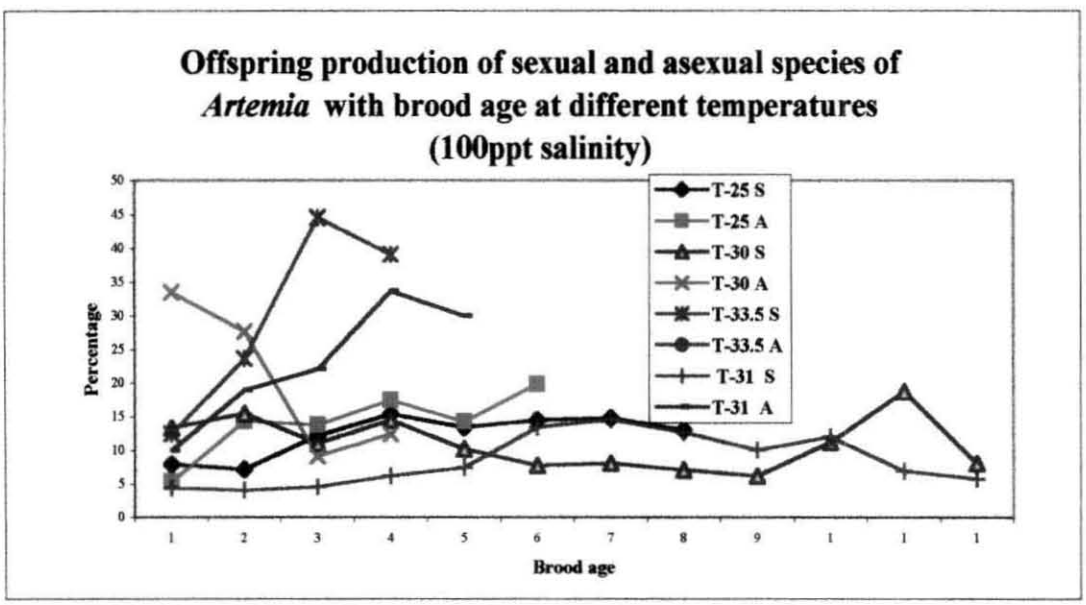


Figure 2nb



T-25S: Temperature 25 C with sexual species, T-25A: Temperature 25 C with asexual species
T-30S: Temperature 30 C with sexual species, T-30A: Temperature 30 C with asexual species
T-33.5S:Temperature 33.5 C with sexual species,T-33.5A:Temperature 33.5 C with asexual species
T-31S: Ambient temperature with sexual species,T-31A:Ambient temperature with asexual species

Figure 2nc

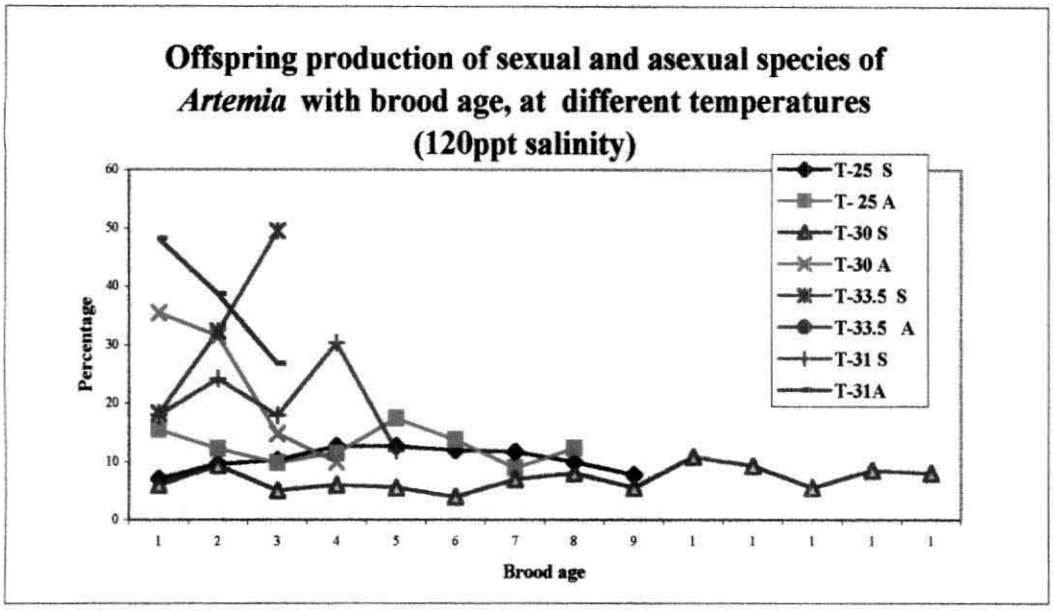
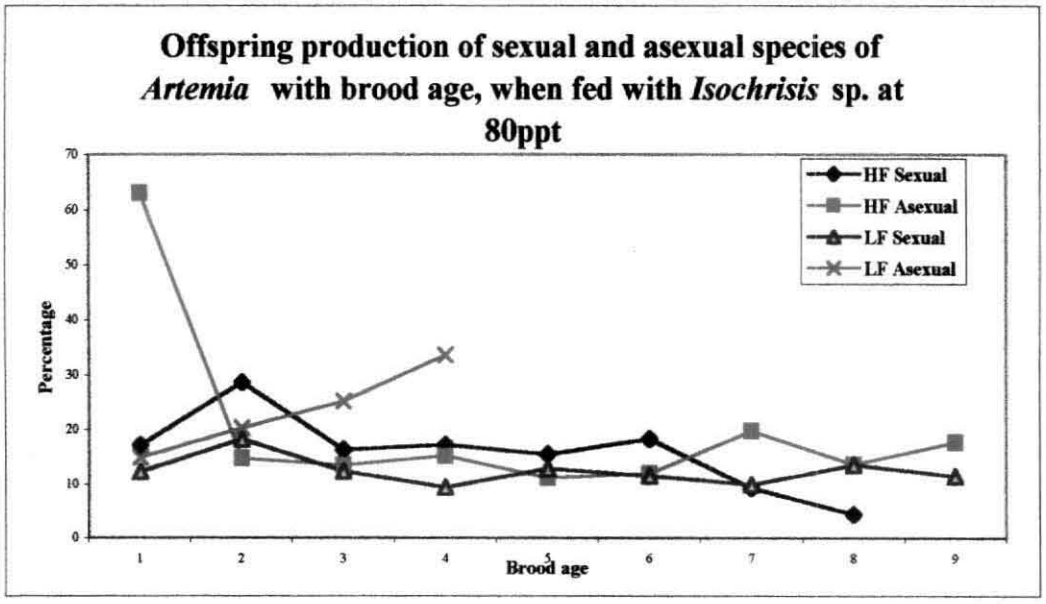
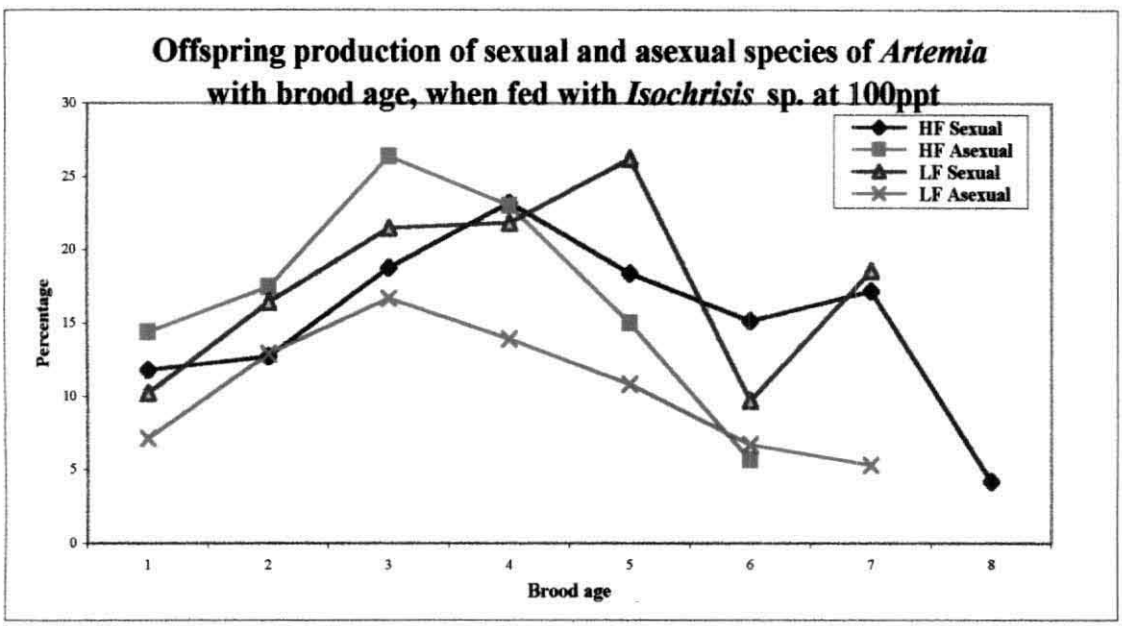


Figure 2oa



T-25S: Temperature 25 C with sexual species, T-25A:Temperature 25 C with asexual species
T-30S: Temperature 30 C with sexual species, T-30A:Temperature 30 C with asexual species
T-33.5S:Temperature 33.5 C with sexual species,T-33.5A:Temperature 33.5 C with asexual species
T-31S: Ambient temperature with sexual species,T-31A:Ambient temperature with asexual species
HF- Hgh feedng, LF- Low/ Normal feeding

Figure 2ob



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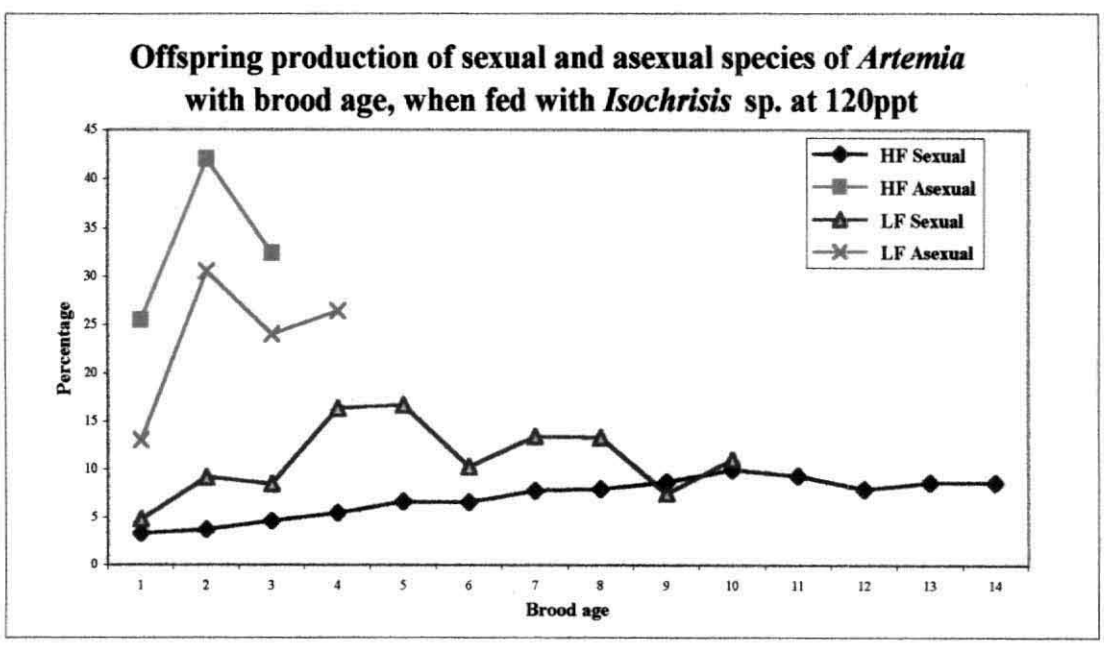


HF- Hgh feedng, LF- Low/ Normal feeding

Figure 2od

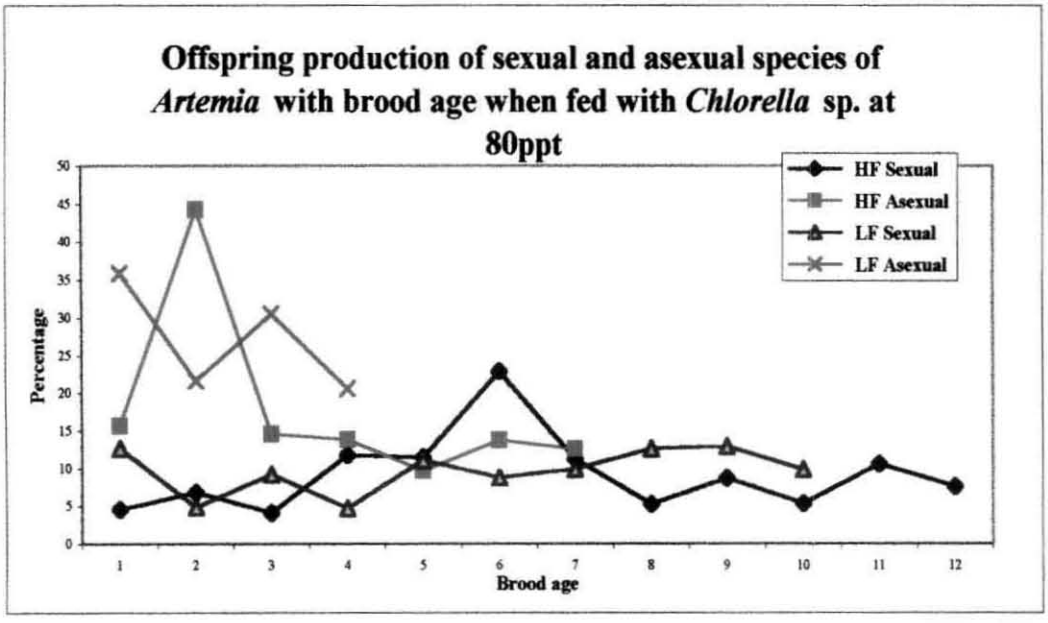
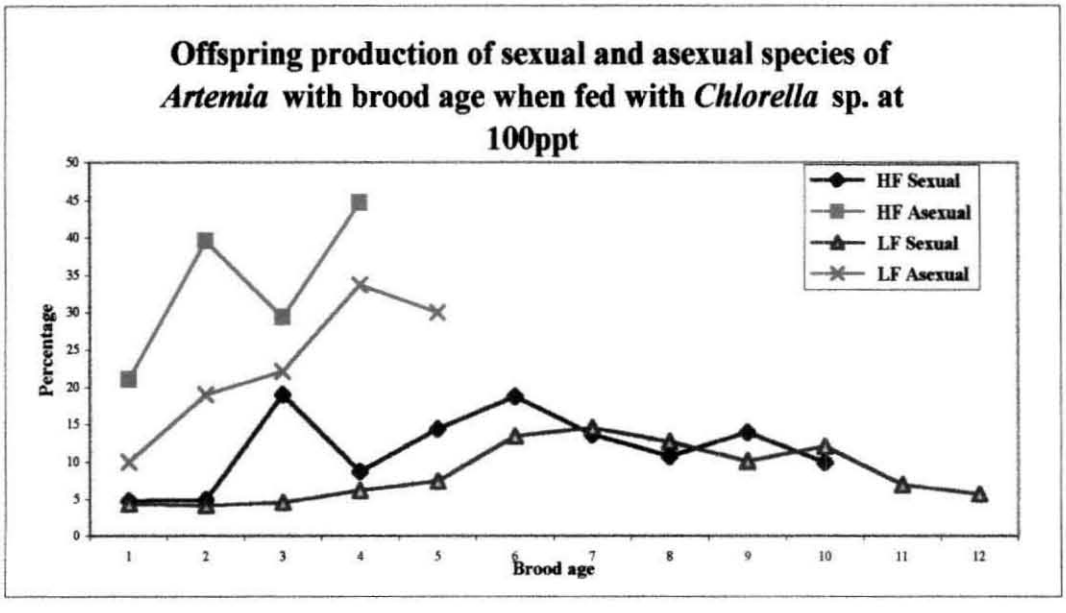
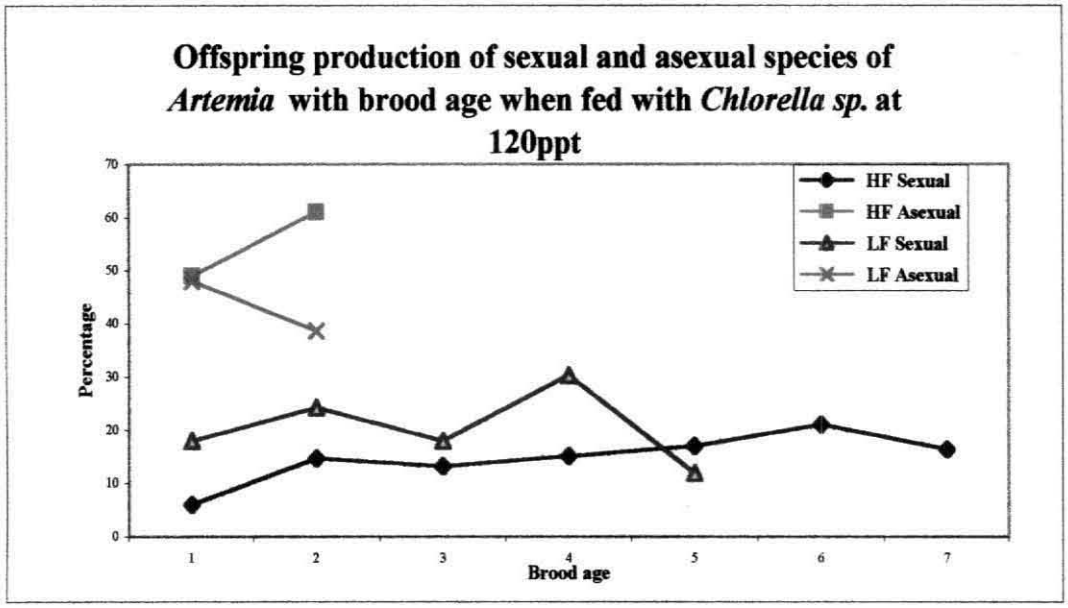


Figure 2oe



HF- Hgh feedng, LF- Low/ Normal feeding

Figure 2of



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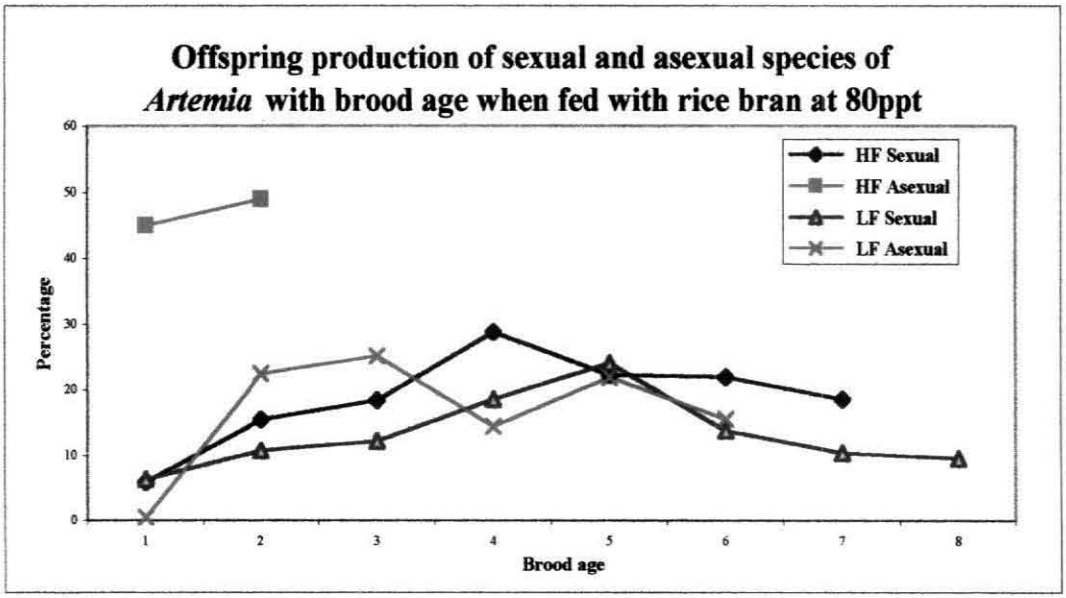


HF- Hgh feedng, LF- Low/ Normal feeding

Figure 2oh

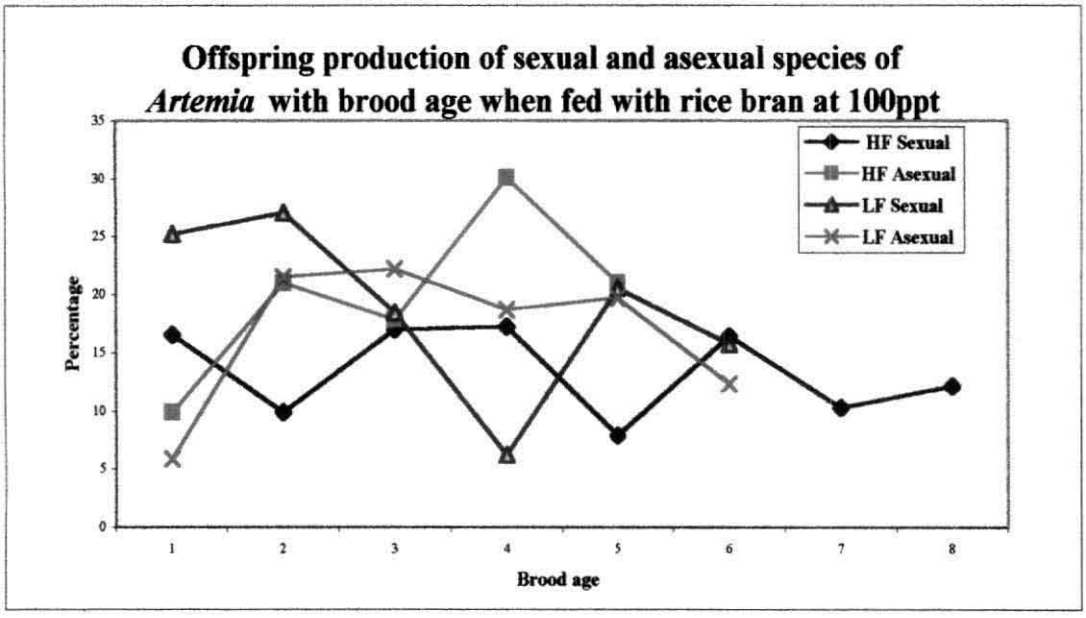
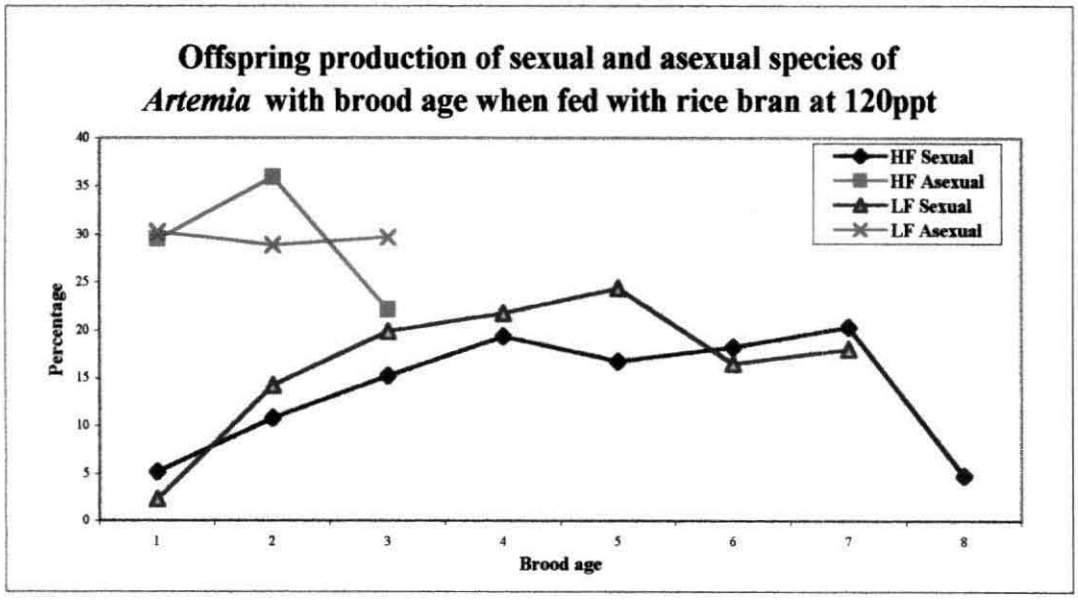


Figure 2oi



HF- Hgh feedng, LF- Low/ Normal feeding

215ppt salinity, then reducing the number steeply with increasing salinity to 20 eggs per female at 282ppt salinity in *Artemia franciscana*. Subsequent lab studies with unlimited food revealed similar trend, indicating dominant influence of salinity on fecundity and this was also supported by the findings of Wear *et al.* (1986). In the present study also sexual and asexual species had marginally less fecundity at high salinities but had improved fecundity at low salinities. Triantaphyllidis *et al.* (1995) reported that the reproductive characters of parthenogenetic population from China and sexual strain from San Francisco Bay are significantly different at 35, 60 and 100ppt with greater values for San Francisco Bay population while at 140 and 180ppt there was no significant difference for majority of characteristics studied as the salinities above 140ppt reduce the reproductive out put, especially that of San Francisco Bay strain and was found true in the tropical conditions of Tuticorin also as per the present study. Sexual species had better brood size at low salinities and asexual species had more offspring per brood than sexual species at high salinities (Figure 2a). Usually in field females carry eggs with little variation in their number upto 215ppt salinity but a further increase to 282ppt cause a steep reduction in egg number (Knight, 1974). In any given temperature regime fecundity of Lake Grassmere strain of *Artemia* (*A. franciscana*) measured with respect to intra uterine egg count or batch size is significantly and primarily controlled by salinity and this is also reflected in pond system biomass (Haslett and Wear, 1985).

Temperature was found to be one of the determining factor in brood size. At fixed temperatures of 25 and 30 °C, the asexual species had more offspring per brood than the sexual species (except at 30 °C and 100ppt) where as at ambient temperature of 31$\pm$3 °C sexual females had more brood size (Figure 2h). At high salinities of 145,170 and 195 ppt asexual females had more brood size as discussed before, while at rest of the tested salinities sexual females had more number of offspring per brood (Figure 2g). Considering the temperature effect on brood size, the lower temperature of

25 °C had the maximum brood size at 80ppt, where as at temperatures of 30 °C and 33.5 °C the sexual females produced maximum brood size at 100 and 120ppt salinities. The total fecundity of sexual females had a parabolic relation with increase in temperature as it increased when the temperature increased to 30 from 25 °C and then decreased along with a further increase of temperature. In the case of asexual species the maximum brood size was obtained at 80 and 100ppt with 25 °C temperature and as the temperature increased the brood size decreased linearly but at 120ppt there was an increase and at 33.5 °C there was no production at all. The total fecundity of asexual species also decreased linearly with increase of temperature (Figure 2k). These are in accordance with the findings of Wear *et al.* (1986), as he reported that high temperature of 32 °C can reduce the fecundity of *A. franciscana* strain. Offspring per brood of sexual species at different tested salinities ranged from 15 (120ppt) to 126 (45ppt) numbers and that of asexual species from 16 (120ppt) to 81 (195ppt) numbers at ambient temperature. The brood size of sexual species at different temperatures ranged between 27 (30 °C and 120ppt) to 55 numbers(30 °C and 100ppt) and that of asexual species between 40 (at 25 °C and 120ppt) to 75 numbers (at 25 °C and 80ppt). With change in feed also there was great variation in the brood size as the sexual species had 168 (*Isochrisis* sp. fed with high rate at 80ppt) to 29 numbers (*Chlorella* sp. fed with high rate at 120ppt) of offspring while asexual species had 75 (*Isochrisis* sp. with high rate at 80ppt) to 7 numbers (rice bran with high feeding at 120ppt) of offspring. The fecundity rate in experiments conducted by Rahaman and Rathinasamy (1997) ranged between 40 to 94 per brood for Kelambakkam strain and between 41 to 71 per brood for San Francisco Bay strain at ambient temperature. In the present experiment total offspring per female of sexual species at different salinities ranged between 89 (120ppt) to 1395 (45ppt) numbers and that of asexual species between 33 (120ppt) to 501(45ppt) numbers. Total fecundity of sexual species at different temperatures ranged between 528 (30 °C to 100ppt) to 63 numbers (33.5 °C and 120ppt) and that of asexual species

between 394 (25 °C and 100ppt) to 173 (30 °C and 80ppt) numbers. The total fecundity of sexual species with *Isochrisis* sp. had maximum fecundity of 1037 numbers from 14 broods with high feeding and the lowest at 89 numbers from 5 broods with *Chlorella* sp. as feed with low feeding. Asexual species had a maximum of 583 numbers from 8 broods with *Isochrisis* sp. as feed with high feeding and a lowest of 33 numbers from 2 broods with *Chlorella* sp. as feed with normal rate of feeding. In the experiment conducted by Browne *et al.* (1984), number of offspring per female ranged from 112 to 1619 from 4 to 19 broods. According to Wear *et al.* (1986) the offspring per brood varied from 1001 in 15 batches at 26 °C with 180ppt salinity and obtained a maximum of 1500 numbers in 12 batches at 26 °C with 140ppt salinity and reported that high temperature reduces the fecundity while low temperature increases it.

Parthenogenetic Kutch strain from India and the sexual San Francisco Bay strain appeared to be well adapted and tolerant to upper temperatures of 24 to 30 °C according to the experiments of Browne *et al.* (1988). They have also found that *Artemia franciscana* was phenotypically more plastic than the parthenogenetic population. According to Wear *et al.* (1986) and Vanhaecke *et al.* (1984) temperature is the major factor effecting the reproductive characters of *Artemia* than the salinity which have little effect only at extreme conditions where *Artemia* is less likely to occur in nature. However, apart from the environmental factors, the brood size in *Artemia* are under genetic control to a very great extent (Amat, 1983; Browne *et al.*, 1984). Browne *et al.* (1984) found that mean brood size of different *Artemia* population ranged from 21 to 111eggs per brood under identical laboratory conditions and the largest difference was between parthenogenetic and Old World bisexual strain.

Isochrisis sp. was found to be the best feed in terms of brood size for both the strain especially at a low salinity of 80ppt than the other two

tested salinities of 100 and 120ppt (Figure 2ia, 2ib). The total fecundity also was found to be maximum with *Isochrisis* sp. as feed especially at 80ppt. The *Isochrisis* sp. and *Chlorella* sp. concentrations present in the rearing medium were found to have influenced the brood size of both the species as the animals fed with high level had more brood size than those fed with low concentration which is in agreement with what has been reported by Browne (1982). High concentration of rice bran as feed in the medium retarded the fecundity at 100 and 120ppt salinities. *Chlorella* sp. was the second best feed as observed from the results but the ricebran was equally good or even better than the algal feeds at 120ppt as at higher salinities algae failed to survive and the inert feed like ricebran had high stability at this conditions and never got decayed as happened at 80 and 100ppt salinities. Salinity effect on brood size was also significant with *Isochrisis* as feed as the brood size of both the species decreased with increased salinity.

Considering the individual effect of salinity on Interbrood interval of *Artemia*, asexual species had longer inter-brood period than sexual species except at 120 and 195ppt salinities (Figure 2d). With an increase in salinity there was an increase in the interbrood period of both the species. Temperature also had linear relation to Inter-brood period as the inter-brood period of both the species decreased with increase in temperature except at 80ppt at which the sexual females had longer Interbrood period at 33.5 °C than at 30 °C. Inter brood interval has nearly linear response to temperature for almost all populations according to the experiments conducted by Browne *et al.* (1988) and Wear *et al.* (1986). The feed also was found to have some effect as the *Isochrisis* sp. fed sexual females had comparatively shorter interbrood period than those fed with other feeds.

In almost all the tested salinities and temperature salinity combinations and feed the sexual females had more number of broods than the asexual females (Figure 2a). The females of both the species had highest

number of broods at lower saline ranges than that at higher salinities. The temperature also affected the brood numbers significantly as the sexual females had a parabolic relation with increase of temperature while the asexual species related linearly with increase of temperature as the brood number decreased with increased temperature (Figure 2b). With different feed also the sexual females had more brood numbers except at 80 and 100ppt with *Isochrisis* sp. as feed (Figure 2ca, 2cb). Asexual females had good number of broods at 100 and 120ppt with *Isochrisis* sp. as well as rice bran as feed both with low and high feeding levels. Asexual females had the lowest number of broods with *Chlorella* sp. as feed. But the sexual species had more broods with *Chlorella* sp. as feed than the other feed except at 120ppt at which *Isochrisis* sp. fed females had more number of broods. Salinity alone and also in interaction with temperature had significant effect on brood number (Wear *et al.*, 1986). Brood number of asexual species with fixed temperatures were marginally less compared to sexual species. Therefore, high brood size of asexual species, as discussed before at these conditions literally have no effect on success of the species in the habitat as they have fewer brood numbers than sexual species. Apart from that the sexual species had small brood size than asexual species with fixed temperature while at ambient temperature sexual species had larger brood size. The prereproductive period of sexual species was shorter than that of sexual at low salinities while the asexual species had shorter pre reproductive period at higher salinities of 120 and 145ppt salinities while at salinities higher than this both the species could not mature in laboratory (see Chapter 1, Figure 1a). The brood size of the asexual species where also higher than that of sexual species at 145,170 and 195ppt salinities (Figure 2g). Therefore, instead of a total dominance of either of these species one of them may dominate, probably *A. parthenogenetica* in summer when the salinities in salt pans normally go higher while the other one in winter or rainy season when the salinities will be marginally lower. The findings discussed in the next chapter shows that the low temperature can induce parthenogenetic females completely to oviparous mode (Figure 3h) and strongly reinforce this

possibility as the sexual species can produce more nauplii offspring even at low temperature (reffer Chapter 3, Figure 3e) and can produce new recruits to the habitat while the asexual species will have to wait till their cysts are hatched.

Vanhaecke *et al.* (1984) conducted some survivability studies of 13 *Artemia* strains, at varied salinity and temperature (18 to 34 °C) combinations and found that *Artemia tunisiana* population was least tolerant to high temperatures, as they had high mortality at 30 °C which supported the findings of Browne *et al.* (1988). In the present experiment asexual species of Tuticorin showed least tolerance towards higher temperature of 33.5 °C but the exotic sexual species from the same region could survive and reproduce at this temperatures. Vanhaecke *et al.* (1984) had reported that with regard to temperature most strain appear to tolerate the lowest temperature very well with the exception of Tuticorin strain. According to them parthenogenetic strain are more tolerant to high temperature which is contradictory to the observations made in the present study as here the sexual species could withstand high temperature while the asexual ones could not survive at this temperature. Usually Polyploid populations (asexual populations are considered as the Polyploid individuals) are better adapted against the extreme environmental conditions than diploid animals (Artom,1931; Chapman, 1968; Metalli and Ballardin, 1972). Indian strain including the Tuticorin strain of *Artemia parthenogenetica* are assumed to be genetically triploid as per the reports of Browne *et al.* (1988) and Vanhaecke *et al.* (1984). It shows that many of the strain differ in performance as many of the characters are influenced by local adaptations.

Indian asexual species produced offspring relatively at constant rate over its reproductive period, while San Francisco Bay species produced majority of offspring relatively late in its reproductive life (Browne, 1980b). Changes in age specific fecundity rate are temperature and strain dependent (Barata *et al.*, 1996a, 1996b). From the present study it is understood that

the brood age and offspring production are not only strain dependent but are also related with environmental characters. Temperature had more influence (Figure 2na,2nb and 2nc) than the salinity, (Figure, 2ma, 2mb, 2mc, 2md, 2me, 2mf, 2mg and 2mh), and feed as at different temperatures the brood age and offspring production differed between two species and also within the species. According to Wear *et al.* (1986) in most of the temperature-salinity combinations number of nauplii per batch was found smaller at beginning and near end of reproductive life of each female but was large and more variable in mid stages. On the other hand, in the present study the offspring per batch was relatively constant throughout the reproductive period at 25 °C but had significant change with increased temperature especially for the asexual species (Figure 2na, 2nb, and 2nc).

Competitive interaction between sexual and asexual species of *Artemia* are effected by temperature (Browne, 1980b). In his studies *Artemia parthenogenetica* bettered the performance of *Artemia tunisiana*, but *Artemia franciscana* outcompeted both the population. As the asexual population does avoid the cost of meiosis (Williams, 1975), they should have performed better than the sexual population. Lower fecundity rates of asexual species as observed in the present study may not affect as almost all the nauplii produced by them can develop into females with potential reproductive capacity which again can contribute to the population. But the sexual females can produce only half number of females against their total fecundity considering a 1:1 sexual ratio. And these new females have to be fertilised by the males so as to perform a successful reproduction, which is not necessary in the case of asexual species. So the chances of competitive exclusion of local asexual population by the sexual *Artemia* population are very less. As the offspring production of both the species varies with salinity, as at higher salinities asexual females and at lower salinities sexual females had more fecundity, at some conditions one population may dominate the other in

relation to the existing ecological conditions. These findings may be helpful in predicting the population boom of both the locally available sexual and asexual population in wild and the possible competitive exclusion of either of them, using the expected temperature salinity fluctuations of the environment.

CHAPTER 3

CHAPTER 3

Effect of ecophysiological conditions on the mode of reproduction

3. 1. Introduction

The brine shrimp *Artemia* exhibits two modes of reproduction, viviparous and ovoviviparous. In the ovoviviparous mode live nauplii will be released from the uterus of the matured female while during the oviparous mode dormant cyst will be released by the females and these modes of reproduction are mutually exclusive with some exceptions. As the dormant cysts can be easily collected and more importantly stored for a long period without losing its viability, and later hydrated and used whenever required *Artemia* cysts became an important commodity in the aquaculture market. Traditionally cysts were collected from the natural habitat and its high demand initiated its introduction into the coastal salterns in various countries (Persoone and Sorgeloos, 1980; Camara and De Medeiros Rocha, 1987). Apart from the initial high yield the production of cysts in these inoculated ponds started declining after a few years. It is important to study the environmental factors of the site before inoculation which must be manipulated in order to have a profitable yield. Environmental stress particularly hypoxia was thought to be the prime factor which induce oviparity (Gilchrist, 1954; Gilchrist and Green, 1960; Dutrieu, 1960). Other factors like salinity (Barigozzi, 1939) algal feed (Dutrieu, 1960) and light and temperature (Provasoli and Pintner 1980; Berthelemy and Hedgecock, 1987) were found to be the other factors which are responsible for influencing the encystment of *Artemia* cysts.

In the present experiment effect of salinity, temperature, quality and quantity of feeds and photoperiodism on the zygotic encystment was

studied and the results are discussed in detail. Some of the cysts obtained from the laboratory conditions and also those obtained from the wild were studied to determine the hatching rate.

3. 2. Materials and methods

Experimental set-up for the investigation is the same as discussed in the General Materials and Methods. The details of the characteristics studied for the present experiment are discussed here.

3. 2.1. Cysts per female

Total number of cysts obtained from a single brood.

3. 2.2. Nauplii per female

Total number of nauplii produced by one female.

3. 2.3. Percentage of oviparous broods

Total numbers of encysted brood were calculated as percentage to the total broods produced by the female.

3. 2.4. Percentage of offspring encysted

Percentage of cyst production per female was calculated.

3. 3. Results

3. 3.1. Cyst per female

3. 3.1.1. Effect of salinity.

Results on the number of cysts produced by sexual and asexual species of *Artemia* at different salinities are described in the Table 3a and Figure 3a. Values of cyst per female in the Figure 3a are shown in Appendix 3a. Cysts were produced at almost all the tested salinities except at 20 ppt for the sexual strain. For the asexual species cysted offspring were produced only at 100 (9 ± 15.6) and 170 ppt (13 ± 22.52) salinities. For sexual strain number of cysts produced were maximum at 80ppt (178.67 ± 91.5) followed by 195ppt where it was 90.25 ± 29.7. From 120ppt onwards there was a regular increase in the cyst production up to 195ppt for the sexual strain.

Table 3a

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on cysts per female.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	37361.5	5337.4	6.5	0.00**
Species	1	41477.5	41477.5	50.4	0.00**
Salinity & Species	7	37233	5319	6.5	0.00**
Error	32	26316	822.4		

Table 3b

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on cysts per female

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	325090.0	325090.0	46.4	0.00**
Temperature & Salinity	2	11564.2	5782.1	0.8	0.45
Temperature & Species	1	47742.2	47742.3	6.8	0.02*
Temperature, Salinity & Species	2	7098.0	3549.0	0.5	0.61
Error	24	168222.0	7009.2		

* Significant
** Highly significant

Figure 3a

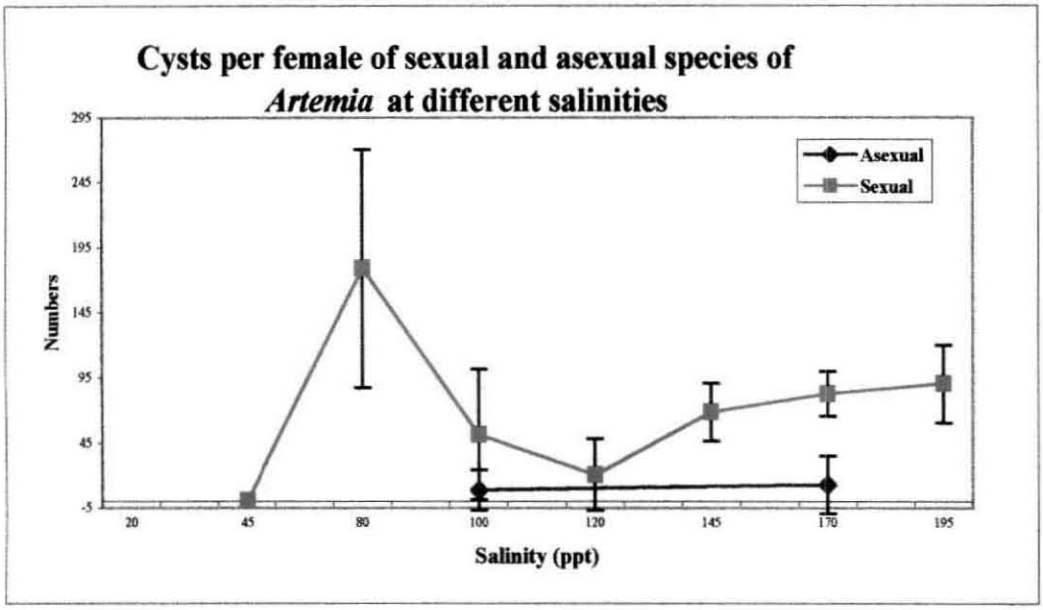
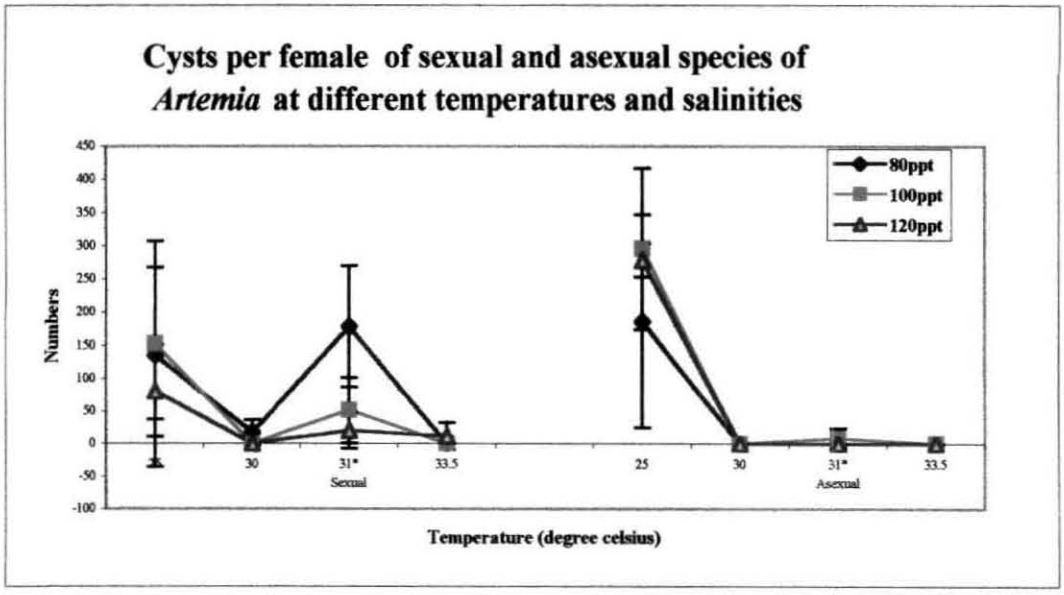


Figure 3b



* Ambient temperature

Cyst production differed significantly between the two species and also between the salinities ($P<0.01$) (Table 3a). Interactive effect of salinity and species on cyst production of female was also highly significant ($P<0.01$).

3. 3.1. 2. Effect of temperature.

Results on the effect of different temperatures and salinities on the cysts per female of sexual and asexual species of *Artemia* are detailed in Table 3b and Figure 3b. Values of cyst per female in the Figure 3b are shown in Appendix 3b. Only those females which were reared at 25 °C produced cysts both in the case sexual and asexual species. At 100ppt there was considerable increase in the encystment rate for both the species at 25 °C. For asexual species there was cyst production only at 25 °C while in case of sexual species there was cyst production not only at 25 but also at 30 and 33.5 °C but number of cyst produced were very high at 25 °C compared to other temperatures. Sexual species had produced cyst also at ambient temperature, but not the asexual species. Asexual species had produced more number of cysts than the sexual species at 25 °C (Figure 3b). The results support that there is significant effect of temperature for the cyst production for asexual and sexual ($P<0.01$) species (Table 3b).

3. 3.1.3. Effect of quality and quantity of feed.

Results on the effect of different quality and quantity of feed on the cysts per female of sexual and asexual species of *Artemia* are detailed in Table 3c and Figures 3ca and 3cb. Values of cyst per female in the Figure 3ca and 3cb are shown in Appendix 3c. At normal feeding sexual species had a tendency to produce cyst at all the three tested salinities with algal feeds while the asexual species could produce cyst only at 100ppt with algal feeds. Both the species could produce cysts with algal feed especially with *Chlorella* sp. as feed. *Chlorella* sp. fed (fed at normal rate) sexual *Artemia* had a decrease in the cyst production rate from 178 cysts per female

Table 3c

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the cysts per female.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	5348.1	5348.1	5.1	0.03*
Feed type	2	18335.6	9167.8	8.7	0.00**
Feed quantity & Feed type	2	2692.1	1346.0	1.3	0.29
Feed quantity & Salinity	2	10469.4	5234.7	5.0	0.01**
Feed quantity & Species	1	4961.3	4961.3	4.7	0.03*
Feed type & Salinity	4	12736.4	3184.1	3.0	0.02*
Feed type & Species	2	13509.9	6754.9	6.4	0.00**
Feed quantity , Feed type & Salinity	4	5989.0	1497.3	1.4	0.24
Feed quantity , Feed type & Species	2	2950.9	1475.4	1.4	0.25
Feed quantity, Salinity & Species	2	12976.2	6488.1	6.2	0.00**
Feed type, Salinity & Species	4	10369.0	2592.3	2.5	0.05*
Feed quantity, Feed type, Salinity & Species	4	7076.9	1769.2	1.7	0.16
Error	72	75785.3	1052.6		

* Significant
** Highly significant

Figure 3ca

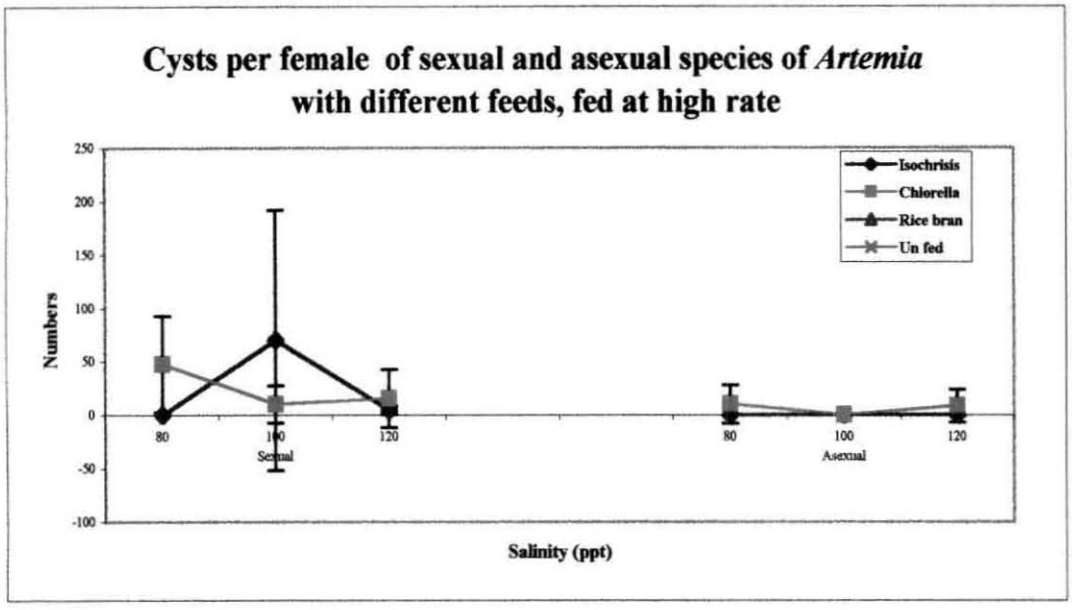
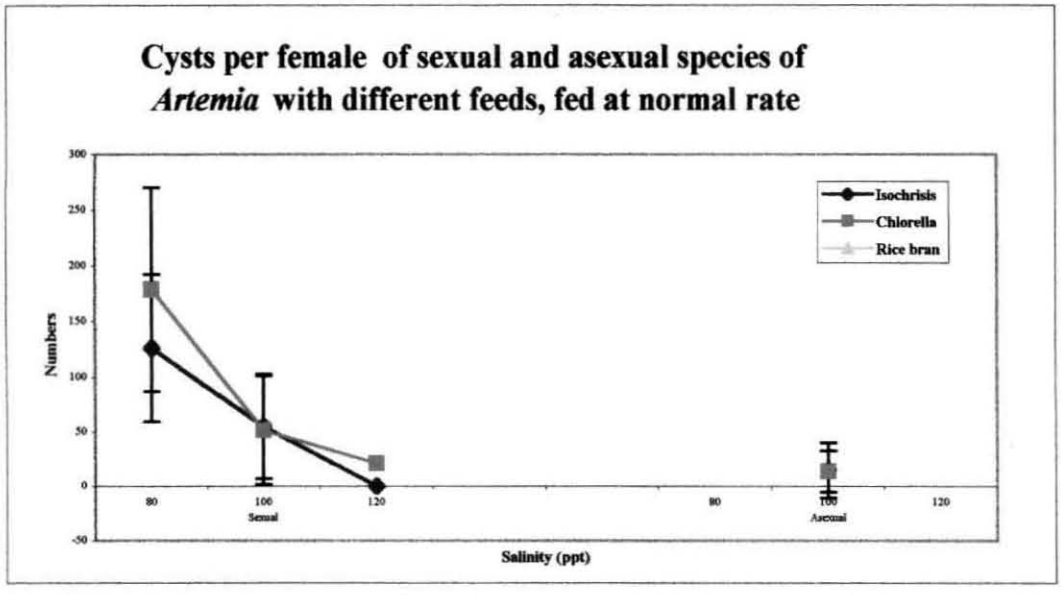


Figure 3cb



at 80ppt to 51.5 cysts per female at 100ppt and at 120ppt it again decreased to 20.67cysts per female. *Isochrisis* sp. fed sexual females also had a similar decrease in the cyst production with an increase in the salinity. Feed type and quality had significantly affected the cyst formation of sexual species ($P<0.01$) (Table 3c). Interactive effects of feed & salinity and feed & species on cyst production were also highly significant. Cyst production was less in case of asexual species compared to the sexual species and the asexual females could produce cyst only at 100ppt with low feeding, at 80 and 120ppt with high feeding, out of the three salinities while the sexual females produced cysts at all the three salinities especially with *Chlorella* sp. as feed. Starved animals never produced any cysts at any of the tested conditions (Figure 3ca). With increased rate of feeding none of the species had any significant increase in cyst production.

3. 3. 2 Nauplii per female

3. 3. 2.1. Effect of salinity

Results on the effect of different salinities on the nauplii production of sexual and asexual species of *Artemia* are listed in Table 3d, Figure 3d. Values of nauplii per female in the Figure 3d are shown in Appendix 3d. At salinities of 20 to 100ppt nauplii production was marginally high for both the species than those between 120 to 195ppt salinities. Productions of nauplii were between 8 to 68 numbers for the sexual strain and 33 to 81 numbers for asexual strain at higher salinities ranged from 120 to 195ppt. Nauplii production at lower salinity range of 20 to 100ppt was 329.67 ± 280 to 1395 ± 161 for the sexual and 126.6 ± 94 to 501.5 ± 98.29 for the asexual animals. Nauplii production was significantly affected by salinity and also differed with species. Interactive effect of salinity and species was also significant ($P<0.01$) (Table 3d). At lower salinities sexual species had more number of nauplii production while at higher salinities asexual species had more nauplii offspring.

Table 3d

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the nauplii per female.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	4837839.7	691120	33.1	0.00**
Species	1	1223046.8	1223046.8	58.6	0.00**
Salinity & Species	7	1899531.3	271361.6	13	0.00**
Error	32	668141.3	20879.4		

Table 3e

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the nauplii per female.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	176120.1	176120.1	10.0	0.00**
Temperature & Salinity	2	19692.4	9846.2	0.6	0.58
Temperature & Species	1	34596.0	34596.0	2.0	0.18
Temperature, Salinity & Species	2	20778.2	10389.1	0.6	0.56
Error	24	424590.0	17691.3		

* Significant
** Highly significant

Figure 3d

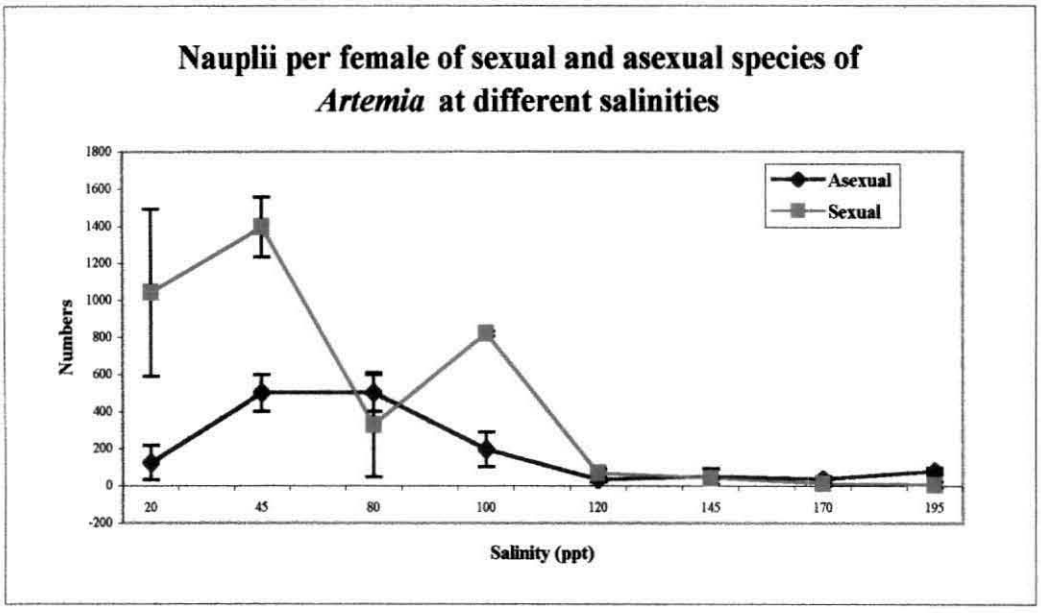
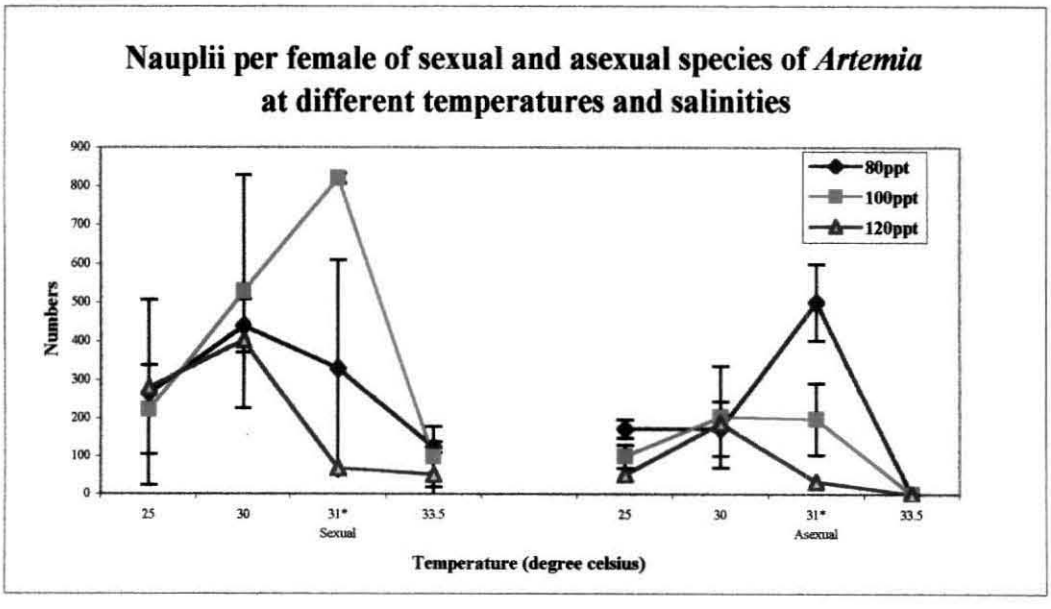


Figure 3e



* Ambient temperature

3. 3. 2. 2. Effect of Temperature

Results on the effect of temperature on nauplii production of sexual and asexual species of *Artemia* are shown in Figure 3e, and Table 3e. Values of nauplii per female in the Figure 3e are shown in Appendix 3e. It was found that temperature had significant effect on the nauplii production per female ($P<0.01$) (Table 3e) and also there was significant difference between the sexual and asexual species ($P<0.01$). Sexual species at 80ppt and 25 °C produced 265.67 numbers of nauplii per female, at 30 °C which increased to 440.0 and then decreased to 124.0 at 33.5 °C and both the species are related parabolically to temperature (excluding the data on ambient temperature). At ambient temperature the nauplii production was 329.67 numbers at 80ppt, 820 at 100ppt and 68.33 at 120ppt. In the case of asexual species at all the conditions the number of nauplii were less compared to that of sexual species. The highest number of nauplii production, 820 was obtained at ambient temperature at 100ppt salinity for sexual species and for the asexual species it was at 80ppt at ambient temperature.

3. 3. 2.3. Effect of quality and quantity of feed

Results on the effect of quality and quantity of feeds on the nauplii production of sexual and asexual species of *Artemia* are shown in Table 3f and Figures 3fa and 3fb. Values of nauplii per female in the Figures 3fa and 3fb are shown in Appendix 3f. At normal feeding sexual females with all the tested conditions produced more nauplii than the asexual females except with *Isochrisis* sp. at 100ppt salinity. Normal feeding with rice bran produced more nauplii than that with high feeding in both the species. Rice bran gave better nauplii production with normal feeding as the nauplii production rate was almost similar to those fed with algal feed. But when the feeding rate was increased rice bran fed females of both the species had lowered nauplii production rate while the algal feeds induced to produce more number of nauplii when the feeding rate was increased. Increased

Table 3f

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the nauplii per female.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	616533.3	616533.3	18.1	0.00**
Feed type	2	888605.1	444302.6	13.1	0.00**
Feed quantity & Feed type	2	695778.2	347889.1	10.2	0.00**
Feed quantity & Salinity	2	154384.2	77192.1	2.3	0.11
Feed quantity & Species	1	612912.0	612912.0	18.0	0.00**
Feed type & Salinity	4	470121.8	117530.4	3.5	0.01**
Feed type & Species	2	136423.5	68211.7	2.0	0.14
Feed quantity, Feed type & Salinity	4	127977.9	31994.5	0.9	0.45
Feed quantity, Feed type & Species	2	250632.1	125316.0	3.7	0.03*
Feed quantity , Salinity & Species	2	2486.2	1243.1	0.0	1.0
Feed type, Salinity & Species	4	675195.1	168798.8	5.0	0.00**
Feed quantity , Feed type, Salinity & Species	4	351612.4	87903.1	2.6	0.04*
Error	72	2450142.7	34029.8		

* Significant
** Highly significant

Figure 3fa

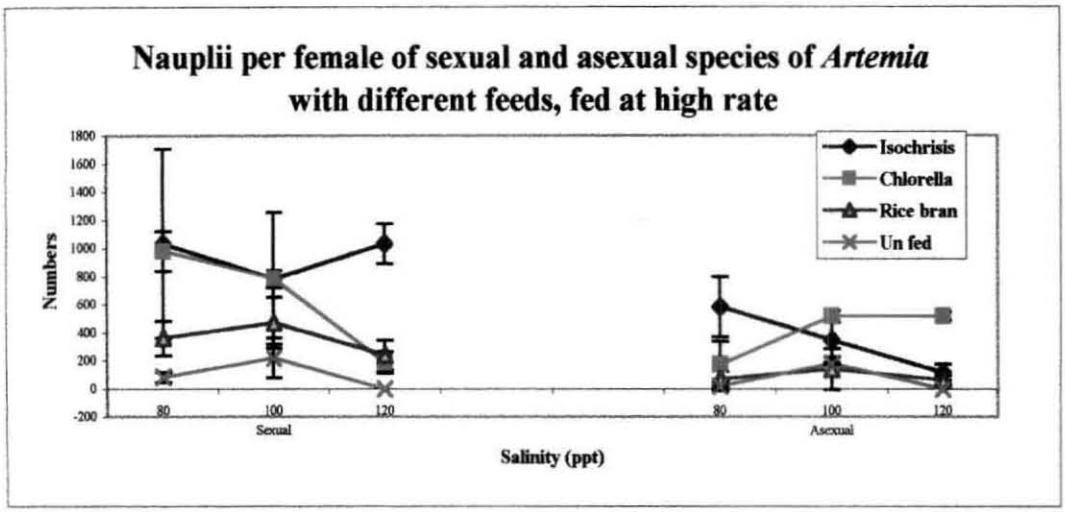
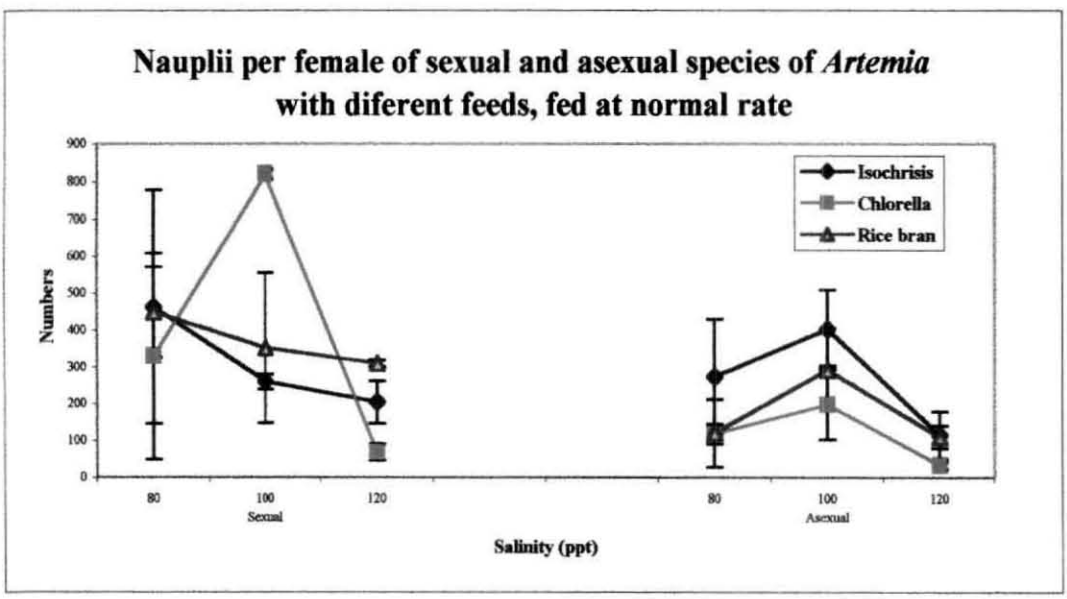


Figure 3fb



feeding with *Isochrisis* sp. and *Chlorella* sp. significantly affected the nauplii production of both the species while increased feeding with rice bran had no effect. Sexual species had their maximum nauplii production with *Chlorella* sp. as feed at 100ppt with normal feeding and with *Isochrisis* sp. as feed at 80ppt when the feeding rate was increased. Increased feeding rate with *Isochrisis* sp. favoured nauplii production of sexual species as well as asexual species and at 100ppt *Chlorella* sp. gave maximum production for both the species.

3. 3. 3. Percentage encystment.

3. 3. 3.1. Effect of salinity.

Results on the effect of different salinities on the percentage encystment of sexual and asexual species of *Artemia* are shown in Figure 3g and Table 3g. Values of 'percentage encystment' in the Figure 3g are shown in Appendix 3g. There was no commendable cyst production in the case of asexual strain but for the sexual strain cyst production showed an increase from 0.53% at 45ppt to 93.7% at 195ppt. At 20ppt there was no cyst production. At 80ppt there was a good encystment rate (41.29). Results support a positive correlation of encystment with increase in salinity only for the sexual strain. Asexual animals were not responding to the given salinity stress and they have produced cysts (5.6%) only at 100ppt. Salinity effects on encystment are highly significant ($P<0.01$). Interactive effect of species and salinity are also highly significant ($P<0.01$) (Table 3g).

3. 3. 3. 2. Effect of temperature.

Effects of three temperatures 25, 30 and 33.5 °C and three salinities 80, 100 and 120 ppt were studied to find out their role in the induction of encystment of the offspring of sexual and asexual species of *Artemia* and the results are shown in Figure 3h and Table 3h. Values of 'percentage encystment' in the Figure 3h are shown in Appendix 3h.

Temperature alone ($P<0.01$) and species and temperature in interaction ($P<0.05$) also had significant effect on encystment of offspring (Table 3h).

Temperature was the most important factor which influenced the encystment rate of both the species (Figure 3h). Salinity also influenced the cyst formation especially in case of asexual species. Asexual females had produced cysts at 25 °C at all the three experimental salinities. Alongwith the increase in salinity percentage of encystment also increased. Asexual animals at 25 °C had the highest percentage of cyst formation (74.5 and 84.5 % at 100 and 120 ppt respectively) than those of sexual females at these conditions (40.27 and 24.45 %). But at 80-ppt salinity sexual females had a little higher encystment rate. Except at 25 °C no other temperature could induce cyst formation in case of asexual females but at ambient temperature at 100 ppt there was 5.67% of cyst formation.

Sexual animals at 30 and 33.5 °C at 80 and 120 ppt salinities respectively showed cyst formation and at 25 °C and ambient temperature sexual females of all the salinities produced cyst (Table 3h) with a maximum at 80 ppt (41.3%). For the asexual females there was a clear increase in encystment rate with increased salinity reaching a maximum at 120ppt which was the upper most salinity tested. For sexual females there was no regular increase or decrease with change in salinities.

In short a decrease in temperature can induce the encystment of offspring for sexual as well as asexual females with a prominent effect on asexual group. The increased salinity is an added advantage as it enhances the encystment rate of asexual females.

Table 3g

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the percentage encystment of offspring.

Treatments	Sum-of-Squares	Degree of freedom	Mean-Square	F-ratio	P
Salinity	7	12702	1814.6	11.18	0.00**
Species	1	12475	12475	76.9	0.00**
Salinity & Species	7	10148.6	1449.8	8.9	0.00**
Error	32	5193.4	162.3		

Table 3h

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the percentage encystment of offspring

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	14715.7	14715.7	61.5	0.00**
Temperature & Salinity	2	796	398	1.7	0.21
Temperature & Species	1	1186.5	1186.5	5.0	0.04*
Temperature, Salinity & Species	2	494.3	247.1	1.0	0.37
Error	24	5740.0	239.2		

* Significant
** Highly significant

Figure 3g

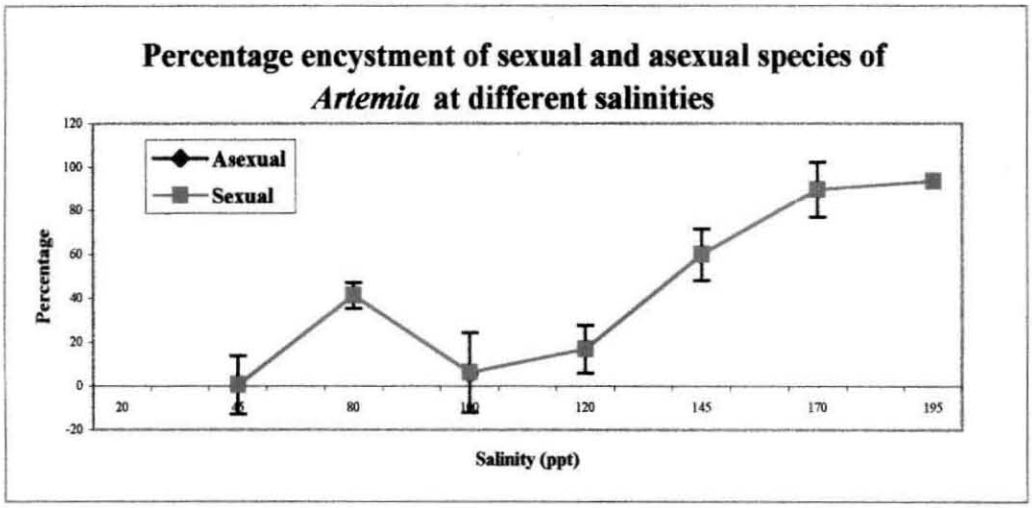
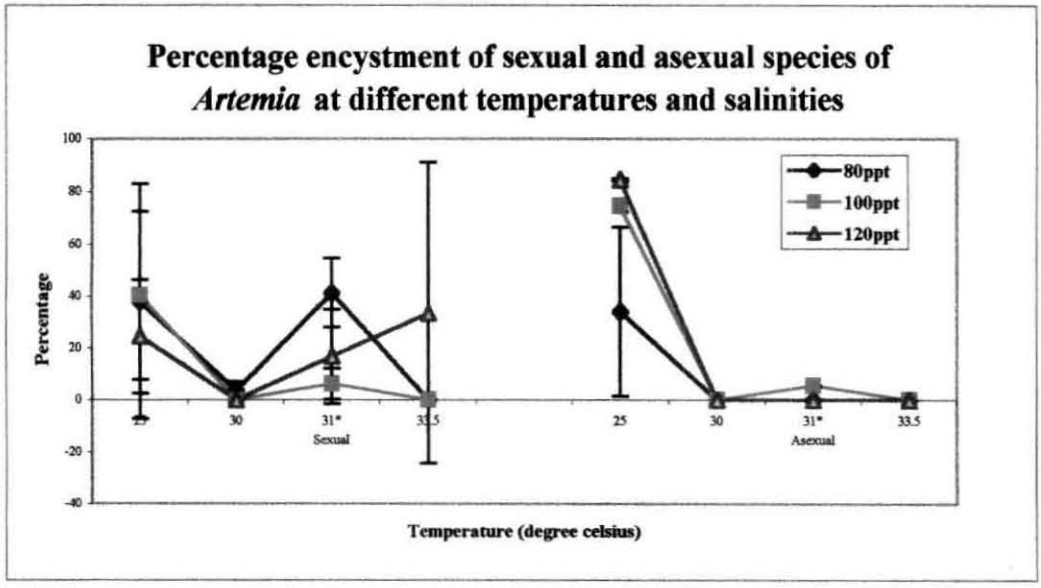


Figure 3h



* Ambient temperature

Table 3i

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the percentage encystment of offspring.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	567.7	567.7	7.9	0.01**
Feed type	2	1605.4	802.7	11.1	0.00**
Feed quantity & Feed type	2	289.2	144.6	2.0	0.14
Feed quantity & Salinity	2	547.4	273.7	3.8	0.03*
Feed quantity & Species	1	609.8	609.8	8.4	0.01**
Feed type & Salinity	4	779.9	195.0	2.7	0.04*
Feed type & Species	2	620.5	310.2	4.3	0.02*
Feed quantity , Feed type & Salinity	4	280.9	70.2	1.0	0.43
Feed quantity, Feed type & Species	2	356.7	178.4	2.5	0.10
Feed quantity, Salinity & Species	2	452.1	226.0	3.1	0.05*
Feed type, Salinity & Species	4	625.7	156.0	2.2	0.08
Feed quantity, Feed type, Salinity & Species	4	419.3	104.8	1.5	0.23
Error	72	5200.3	72.2		

* Significant
** Highly significant

Figure 3ia

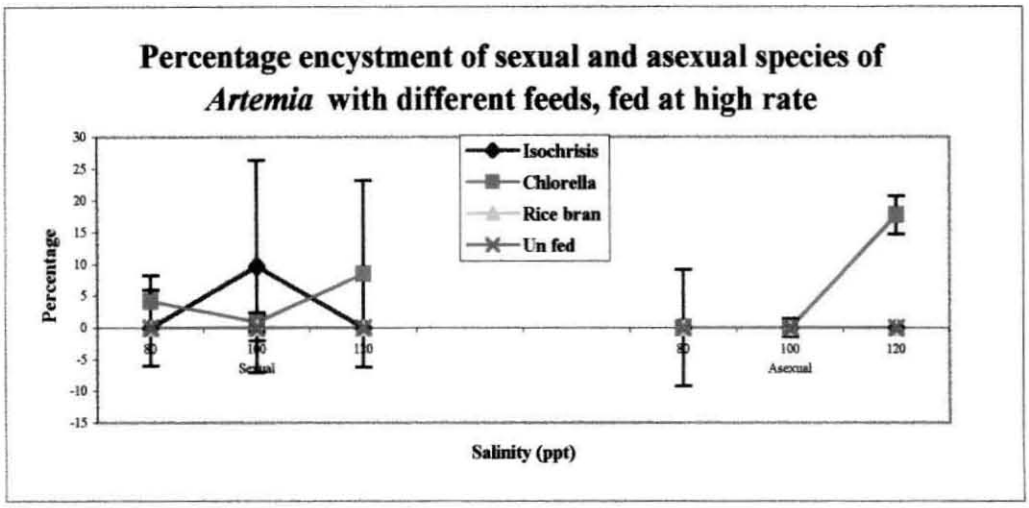


Figure 3ib

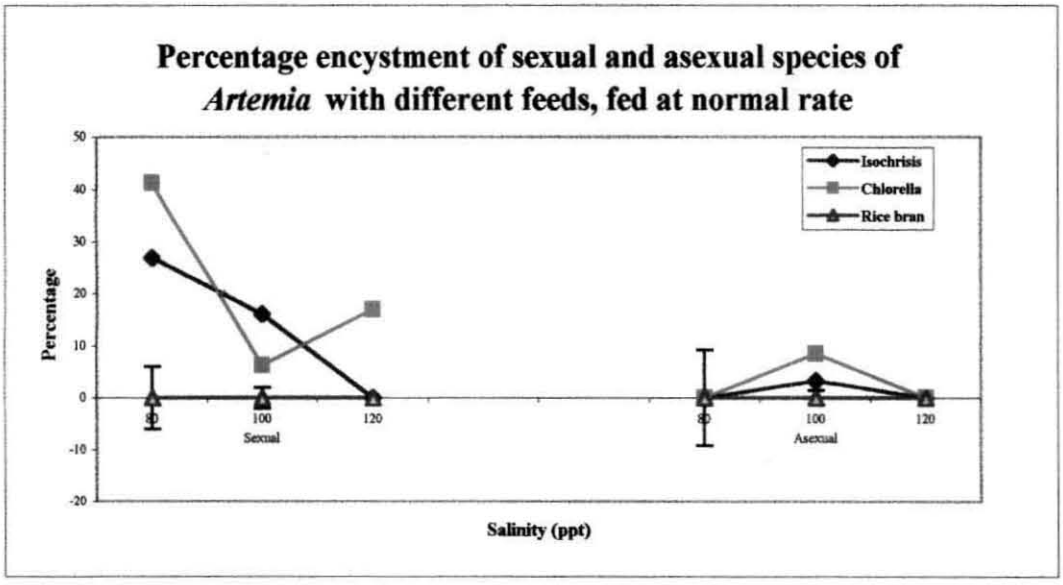


Table 3j

Results from ANOVA showing the effect of different Photoperiodic conditions at different temperatures and their interaction with two different species of *Artemia* on percentage encystment of offspring.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Photoperiodic effect	1	888.5	888.5	2.1	0.16
Temperature	1	2935.1	2935.1	7.0	0.02
Species	1	41.8	41.8	0.1	0.76
Photoperiod & Temperature	1	1861.5	1861.6	4.5	0.05
Photoperiod & Species	1	1035.3	1035.3	2.5	0.13
Temperature & Species	1	61.0	61.0	0.1	0.71
Photoperiod, Temperature & Species	1	354.9	354.9	0.9	0.37
Error	16	6665.1	416.6		

* Significant
** Highly significant

Table 3k

Percentage encystment of sexual and asexual species of *Artemia* species with different photoperiodic conditions

Photo period (hours)	Temperature (^{0}C)	Salinity (ppt)	Sexual	Asexual
12:12 D:L	25	100 ± 3	0	13 ± 18.38
24:0 D:L	25	100 ± 3	56.18 ± 18.63	50 ± 70.71
12:12 D:L	Ambient temperature 31±2	100 ± 3	27.30 ± 3.98	0
24:0 D:L	31±2	100 ± 3	2.65 ± 4.58	0

3. 3. 3. 3. Effect of quality and quantity of feed

Results on the effect of quality and quantity of feed on the percentage encystment of sexual and asexual species of *Artemia* are shown in Figures 3ia and 3ib and Table 3i. Values of 'percentage encystment' in the Figure 3ia and 3ib are shown in Appendix 3i. Among the tested salinities lowest salinity, 80ppt, was found to be optimum for cyst formation especially with *Chlorella* sp. as feed at normal concentration. With *Isochrisis* sp. and *Chlorella* sp. the encystment rates with 80ppt were 26.9 and 41.3% for sexual species and for asexual species there was no cyst production. Ricebran fed females of both the species never produced cyst at any of the tested conditions. In case of asexual species there was cyst formation only at 100ppt with algae fed animals (normal feeding). Encystment rate with unfed animals was also zero for both the species (Figure 3ia). Increasing feeding rate reduced the encystment rate of sexual females at all the cyst-produced conditions. Feed type and quantity had significant effect on the encystment rate of both the species ($P<0.01$) (Table 3i).

3. 3. 3. 4. Photoperiodic effect on encystment.

Results on the effect of photoperiodism on the percentage encystment of sexual and asexual species are detailed in the Table 3j and 3k. Asexual females had encysted offspring only at 25 °C both with 12:12 and 24:0 photoperiodic conditions, while at ambient temperature there was no encystment. In the case of sexual species there was no encystment at 25 °C with 12:12 photoperiod condition while there was encystment at total darkness (56%) at the same temperature. At room temperature also there was cyst production in the case of sexual females both with 12:12 and 24:0 photoperiodic condition.

3. 3. 4. Percentage of encysted broods per female

3. 3. 4.1. Effect of salinity

For sexual species encysted brood appeared only from 80 ppt onwards and for asexual species from 100ppt onwards. Sexual species always had higher number of broods than asexual species at all the salinities at which encystment occurred (Figure 3k).

3. 3.4.2. Effect of temperature

Asexual species had more number of encysted broods than the sexual species. The percentage of encysted broods of sexual species increased from 42 to 64 and then to 87 as the salinity increased from 80 to 100 and then to 120ppt at 25 °C (Figure 3l). At 30 °C only sexual species had encysted broods at 80ppt. While at 25 °C both the species at all salinities could produce encysted broods with asexual species producing more encysted broods of total brood production than sexual species (Figure 3 l).

3. 3. 4.3. Effect of quality and quantity of feed.

Asexual species at 100ppt, with *Chlorella* sp. as feed both at normal as well as high feeding enhanced cyst production. Normal feeding with *Chlorella* sp. produced encysted brood at all 80, 100 and 120ppt salinities. Asexual and sexual species have not produced cyst with rice bran as feed. While they could produce cysts only with *Isochrisis* sp. and *Chlorella* sp. as feed. They produced maximum cysted broods at 120ppt followed by 100 and then 80ppt with *Chlorella* sp. as feed.

3. 3. 4. 4. Photoperiodic effect

Photoperiodic effect on number of broods encysted was studied. Total broods and encysted broods were counted and percentage of encysted broods was recorded with normal light and complete darkness at ambient temperature and 25 °C. Salinity was maintained at 100ppt.

Figure 3k

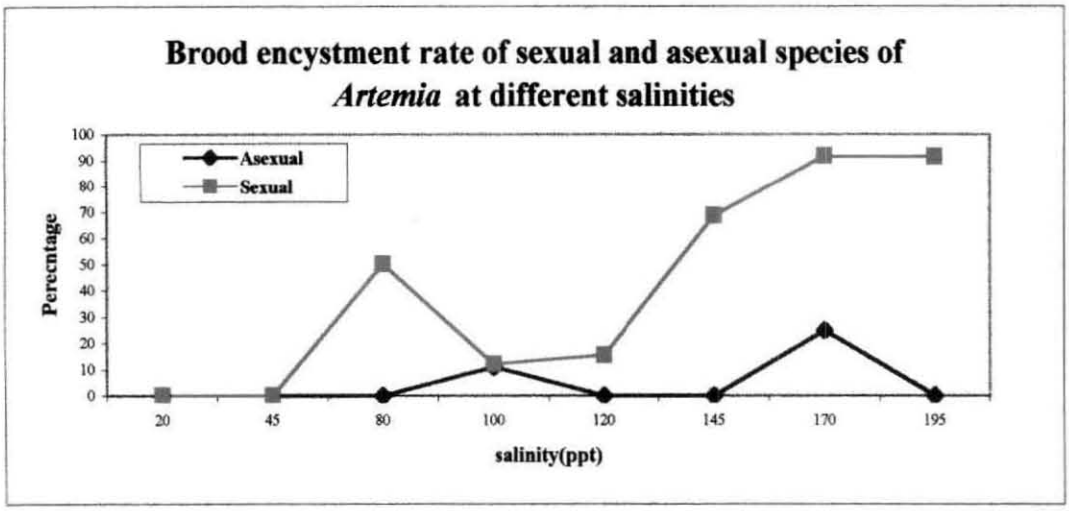
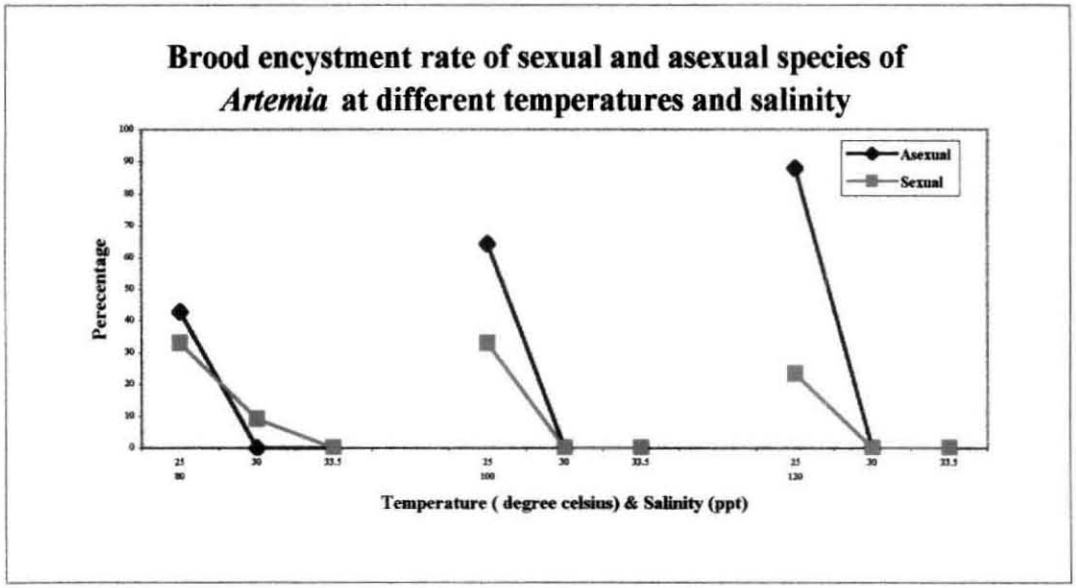


Figure 3l



Sexual species produced encysted broods at ambient temperature while asexual species did not produce encysted brood at this temperature. At 25 °C both the species had encysted broods, maximum with total darkness while asexual species had more encystment at 12:12 photoperiodicity.

3. 3. 5. Hatching rate of cysts obtained from laboratory and wild

Cyst hatching percentage of wild and laboratory reared sexual and asexual species were calculated. Cysts yielded by sexual species from the local salt pans were not available during the study period while those of asexual species were obtained from the Thirespuram salt pans during the month of January 1999. In laboratory only those maintained under lower temperature of 25 °C were found to have produced cysts regularly and enough cysts were obtained for the study which were collected and stored in deep brine and then tested for hatching percentage. The cysts produced at 25 °C and salinity 100ppt were used for the study. Cysts produced in the stock culture at room temperature, which had salinity of 100ppt, were also collected and kept in deep brine so as to terminate its dormancy and later used for hatching experiments.

Hundred numbers of cysts from all the samples were put into sea water, from the deep brine in which it was stored, diluted to 20ppt salinity and was provided with vigorous aeration. No artificial light was given and all the experiments were run in duplicates. Sexual strain which produced cyst in the stock culture gave 0.88% of hatching , and the same at 25 °C gave 0.99% of hatching. Asexual species at all the conditions gave better hatching rate as the cyst produced at room temperature gave 16.67 and those at 25 °C gave 92.16% and those collected from wild gave the maximum of 94.23% of hatching (Figure 3n). When the hatching rate of all the samples were plotted against time maximum hatching was occurred at 21st and 22nd hour (Figure 3m). After 24th hour there was no hatching at all.

Figure 3m

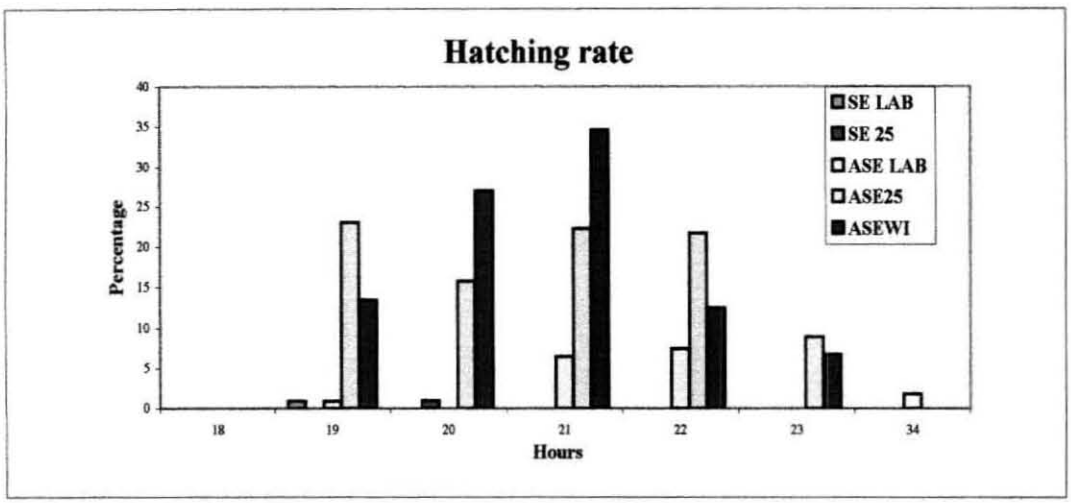
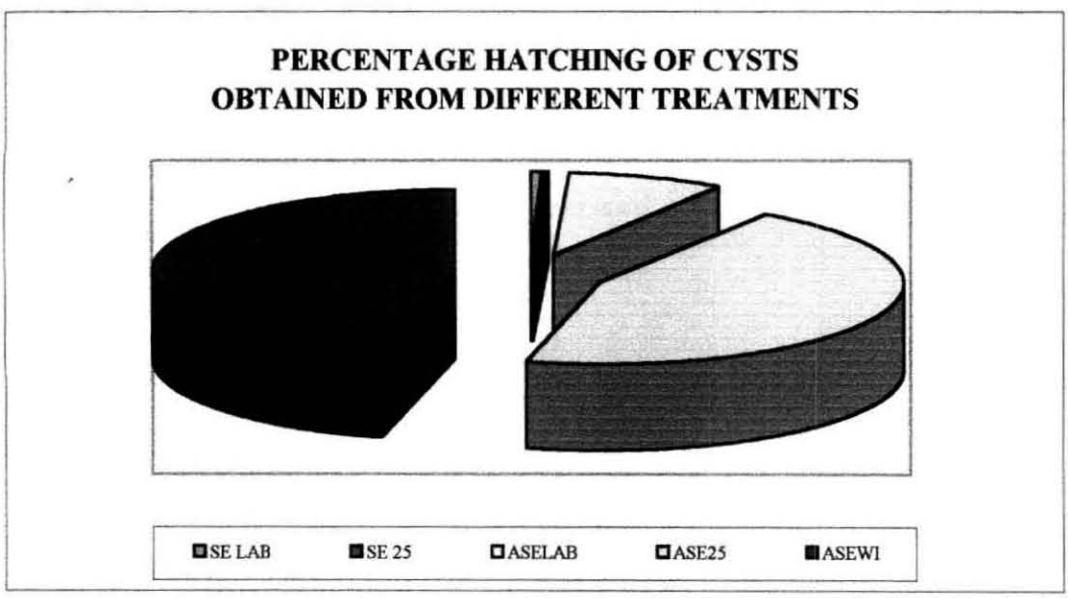


Figure 3n



SE LAB - Sexual cysts from laboratory reared animals at ambient temperature, SE 25 - Sexual cysts from laboratory reared animals at 25 C, ASELAB - Asexual cysts from laboratory reared animals at ambient temperature, ASE25 - Asexual cysts from laboratory reared animals at 25 C, ASEWI - Asexual cysts collected from wild.

3. 4. Discussion

Relative success of an *Artemia* species is mainly determined by 1) Mode of reproduction, sexual or parthenogenetic and 2) Mode of parturition, encysted (Plate 3a) or non encysted (Plate 7c) offspring. Stress is considered as the most important factor, which can trigger oviparity in *Artemia* species and it can be any form of stress like temperature, salinity, photoperiod or food or the combination of all these with other unknown environmental factors (Berthelemy and Hedgecock, 1987). But the physiological process, which leads to the encystment, has to be same irrespective of the stress factor, which induced it. It was found that sudden hypoxic stress and also a sudden salinity variation can cause the accumulation of Lactic acid in *Artemia* (Vos *et al.*, 1979). This excess accumulation of acid can lower the pH of haemolymph and this in turn can induce a decrease in the pH of external medium due to the secretion of lactic acid to the external medium (Conte *et al.*, 1980). All this may act as a causative factor for the encystment induction (Berthelemy and Hedgecock, 1987).

In this study it was observed that ovoviviparous mode of reproduction was the most favoured reproduction of both the species as at all the conditions atleast one ovoviviparous brood was obtained while the oviparous brood was obtained only at certain conditions. With increase of salinity from 120ppt onwards the nauplii production was found to be decreasing in case of sexual species while the encystment had an increased rate from this salinity onwards as shown in Figures 3a and 3g. Asexual species also had less nauplii offspring at higher salinities while at ambient temperature they could produce cysts only at 100 and 170ppt but very few in numbers. In salt pans increased salinity brought by evaporation was known to induce adult *Artemia* for oviparous reproduction (Sorgeloos *et al.*, 1975). In the present study the increased salinity treatment against the sexual species had induced its zygotic encystment as shown in Figure 3g. Salinity increase

from 100ppt onwards was found to have a positive influence on encystment of sexual species. At the same time asexual species was never influenced by the salinity increase or decrease. Amat (1983) found increased oviparity with rising salinities in *Artemia* from Great Salt Lake, Utah (sexual strain). According to Triantaphyllidis *et al.* (1995) San Francisco Bay strain produced higher percentage of encysted offspring than parthenogenetic population from China at all the tested salinities except 100ppt and cyst production was most favoured in salinities 35,140 and 180ppt. But the laboratory studies conducted by Berthelemy and Hedgecock (1987) revealed that increased salinity decreased the chances of oviparity of San Francisco Bay strain (sexual). He has also mentioned that when salinity increased to 15 to 30 then to 40 and 80ppt the oviparity decreased to 44 to 40 then 23 and 0% accordingly.

Dutrieu (1960) was the first to mention the effect of quality of feed on cyst formation. In her cultures, *Artemia* raised on yeast diet was mostly ovoviviparous while others fed algae had cyst broods. Present study also supports the need of algae for encystment for both the species. In the feeding experiments there was commendable cyst production only with algal feeds while with rice bran there was no cyst production at all for both the species. But the encysted offspring were a small percentage of the total offspring as the majority of the offspring were nauplii in all the feeding experiments.

The increased or decreased feeding rate ~~or the unfed conditions~~ were found to have significant influence on the encystment (Table 3c). Berthelemy and Hedgecock (1987) had indicated that yeast diet can support oviparity under some circumstances as the *Artemia* fed with yeast when kept at 12:12 L:D photoperiod was found to develop cyst broods. Provasoli and Pintner (1980) described that a short day light can induce oviparity.

Since unfed conditions at any of the three tested salinities 80, 100 and 120ppt had not induced encystment of any of the species it is wrong to conclude that salinity above 100ppt do increase the encystment of sexual species as mentioned in the beginning. It is assumed that salinity can only enhance the encystment rate in a conducive atmosphere for cyst production like surplus algal feeds. Berthelemy and Hedgecock (1987), had also reported that scarcity of food can not induce oviparity in *Artemia* significantly.

Percentage of females reproducing oviparously varies significantly among temperature treatments. Oviparity of sexual and parthenogenetic tetraploid females are found inversely related to temperature. According to Barata *et al.* (1996b), parthenogenetic tetraploids had no significant change in mode of parturition with temperature while sexual and parthenogenetic diploids had significant difference and it can be concluded that Tuticorin strain is diploid genetically as they produced encysted offspring with lowered temperature. Zang and King (1993) reported that cyst production is related to geographic origin and degree of ploidy of parthenogenetic population. Tetraploid parthenogenetic female produced high percentage of encysted offspring than parthenogenetic diploid and sexual population (Barata *et al.*, 1996b). But Browne *et al.* (1984,1988) and Browne(1992) reported high nauplii production for all Polyploid strains tested. Browne (1980a) has also reported that viviparity is the favoured mode of reproduction in all the strains and Kutch strain showed relatively low constant encystment rate while San Francisco Bay strain produced distinct cyst brood and produced majority of its cysts in middle life (broods 6 to 10) and are contradictory to the present study as in the present case almost all the broods had cysted offspring irrespective of the brood age especially at lower temperature of 25 °C. Furthermore environmental factors are known to play a large role in determining whether offspring produced are nauplii or cysts (Versichele and Sorgeloos, 1980; Lenz and Browne, 1991).

At 25 °C, which was the lowest temperature tested the nauplii production of sexual species was equally good as that of cyst production at all the salinities while the asexual species had a great decrease in the nauplii offspring as the encystment rate had an increase with salinity at this temperature, (Figure 3b and 3h). The temperature of 25 °C was found to have induced cyst production for both the species while the asexual species responded more positively than the sexual species towards lowering of temperature. Interaction of salinity with temperature was also highly significant for asexual species but not in the case of sexual species (Figure 3h). In the experiments of Wear *et al.* (1986) there was no constant and predictable pattern of cyst production but there was 83% cyst production at 26 and 32 °C over all the tested salinity ranges and 60% cyst production at 200 to 260ppt salinity over complete range of temperature. High temperature and high salinity in combination accounted for 58% of cyst production. Effect of photoperiod was found strong only at low temperature and temperature in turn was found to be influenced by salinity and photoperiod (Berthelemy and Hedgecock, 1987). This was found true also in the present study as reduced light at low temperature induced the encysted broods of both the species as shown in Table 3k. But at ambient temperature the reduction of light had not induced encystment for sexual species while for asexual species there was no encystment at all with 12:12 or 24:0, D:L condition. So it is assumed that photoperiodism by itself has no influence in the encystment while it can enhance the cyst production once the temperature is low. According to Bethlemy and Hedgecock (1987) sexual strain of San Francisco Bay was influenced by the interaction of temperature and salinity while in the present study the sexual species had no influence but the asexual species was highly influenced by the interaction. According to Berthelemy and Hedgecock (1987) females maturing late have a tendency to become oviparous than those mature faster. This may be true as at 25 °C the pre reproductive period was longer and the females of both the species took longer days for maturation

(Figure 1b, Chapter 1) and there was commendable encystment only at this temperature.

Certain chemicals like FeEDTA have been reported to induce cyst formation (Berthelemy and Hedgecock,1987; Bowen *et al.*, 1969) but it was not tested in the present study as only effect of ecological factors on the zygotic encystment were concentrated. Browne (1980a) reported effect of brood number on cyst formation with oviparity rising to 10-60% between third and fifteenth brood depending on geographic origin of strain. According to him majority of cysts were produced in middle of lifespan of *Artemia franciscana* species (brood 6-10) while Indian strain towards late in its life cycle (brood 11 to 15). Sexual species of the present study produced cyst with response to salinity and also with low temperature. With higher salinity 145, 170 and 195ppt they started giving cysted brood from their first brood itself and rarely produced viviparous brood in between. While at 25 °C they have produced irregular encysted brood (Figure 3h) with ovoviviparous brood in-between but they have also produced encysted brood from the beginning itself for a few occasion. But in case of asexual species they have produced regular encysted brood only at 25 °C, and it started appearing only from the second or third brood onwards and once the encystment started the females never again produced the viviparous brood. But sexual species had turned back to viviparity for a number of times even after started producing cysted broods. It may be because of that the parthenogenetic species are totally unfamiliar with the cold conditions as they belong to tropical region, the lowered temperature could have acted as an induction for oviparous mode of reproduction. At the same time sexual species which belong to the temperate areas are familiar to the cold conditions and prefer oviparous mode.

In a relatively stable environment where there is a possibility of competition from other related species it is always advantageous for *Artemia* to produce live offspring to maximise their success in the habitat. There is a

chance of delay in the population growth if the encystment rate of the population is high (Browne, 1980b). There are reports to show that cyst production is a costly mode of reproduction in metabolic terms (Browne, 1980a) with 22% of dry mass of encysted embryo utilised for encapsulation (VonHentig, 1971). The mid temperature 30 °C selected for the study was found to be the most suitable for nauplii production for both the species and sexual species had higher number of nauplii at all the experimental conditions.

In the present study it seems the temperature had acted as a stress factor for asexual species as well as the sexual species but in nature possibility of having such a lower temperature is very rare as throughout the year Tuticorin area is always reported to have higher ranges of temperature. Sexual species which is assumed to have come from the San Francisco Bay population seems to have acclimatised to higher temperature conditions of Tuticorin as it responded to the lower temperature during the present study along with asexual species.

During December- January, temperature at Tuticorin was found to be low and during these months cysts were available in salt ponds where there was asexual *Artemia* species, which support the finding that temperature greatly influence the encystment rate. Plenty of wild cysts were available during this period of the year. Usually during these months salt production in the salt pans of Tuticorin is suspended because of the onset of rain and extra income can be obtained from them by converting them to *Artemia* culture ponds during this sterile period of salt production.

Control of reproductive mode in the intensive system is possible as reported by Lavens and Sorgeloos (1984). But cyst production in large pond with the environmental manipulation is not possible, as salinity is the only factor, which can be manipulated in a pond habitat. Bringing salinity to

the normal sea water level was found to have increased the cyst induction in the case of sexual strain (Berthelemy and Hedgecock, 1987).

Cysts obtained from the sexual species had a low hatching rate compared to those obtained from the asexual species under similar conditions (Figure 3n). Once the cysts are put for hatching maximum hatching were obtained at the 22nd hour for both the species (Figure 3m).

CHAPTER 4

4. 2.2. First nauplii size

Size of the first nauplii, in microns was measured using an ocular micrometer, which was pre calibrated with stage micrometer. Six numbers of nauplii were measured collecting randomly from each of the experimental jars.

4. 2.3. Size at first maturity

Adult size in millimetre are measured using Camera Lucida, once the brooder release the first batch of nauplii. It was measured using a dissection microscope and was spread on a glass using a brush. The microscope was attached with a Camera Lucida so as to measure the animal size .The length from the eye to the tail end of the animal was measured. Minimum of five numbers of adults were taken for measurement from each of the experimental tanks.

4. 3. Results

4. 3.1. Cyst size

4. 3.1.1. Effect of salinity

Results on the effect of different salinities on the cyst size of sexual and asexual species of *Artemia* are shown in Table 4a and Figure 4a. Values of 'cyst size' in the Figure 4a are shown in Appendix 4a. Cyst sizes of both sexual and asexual species at different salinities are listed in the Figure 4a. Cyst size of sexual strain varied between 222.24 and 249.93 microns and that for the asexual strain varied between 222.16 to 244.46 microns, for the entire salinities tried (Figure 4a). Salinity difference affected the cyst size of sexual species significantly ($P<0.01$).

4. 3.1.2. Effect of temperature

Results on cyst sizes of sexual and asexual species of *Artemia* at varied temperature and salinity combinations are shown in Figure 4b. Values of 'cyst size' in the Figure 4b are shown in Appendix 4b. Size of sexual species varied between 216.6 to 249.93 microns, while that of asexual species varied between 222.16 to 252.44microns. There was a slight decrease in cyst size of sexual species at the highest salinity of 120ppt as compared to the size recorded at 80 and 100ppt at 25 °C while the cyst size of asexual species at 25 °C increased with increase in salinity with a maximum at 120ppt salinity.

4. 3.1.3. Effect of quality and quantity of feed.

The cyst production was noticed only with algal feed while those fed with rice bran never produced any cysts. Cyst size with different feeds are shown in Appendix 4c. Sexual species produced cysts with both the algal feed at all the tested salinities while asexual species produced cysts only at 100ppt with normal feeding and at 120ppt with higher feeding. In case of sexual species there were differences in the cysts size between *Chlorella* sp. fed and *Isochrisis* sp. fed animals as the *Chlorella* sp. fed animals had comparatively larger cyst diameter than that of the *Isochrisis* sp. fed animals. Sexual cyst size varied between 216.6 to 249.9 microns and that of asexual cyst between 222.2 to 277.7 microns.

4. 3. 2. Nauplii size

4.3.2.1. Effect of Salinity

Results on the effect of salinity on nauplii size of sexual and asexual species of *Artemia* are shown in Table 4d and Figure 4d. Values of 'nauplii size' in the Figure 4d are shown in Appendix 4d. Size variation of the sexual strain was between 491.13$\pm$22.29 to 538.97$\pm$0.33 and that of asexual strain between 505.9$\pm$9.64 to 544.29$\pm$3.90. There was significant difference between the salinities ($P<0.01$) and also between the species ($P<0.05$).

Table 4a

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on cyst size

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	6	2116.5	352.8	7.8	0.00**
Error	14	630.6	45		

Table 4d

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on nauplii size.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	7	6778.1	968.3	4.1	0.00**
Species	1	1234.3	1234.3	5.2	0.03**
Salinity & Species	7	5290.1	755.7	3.2	0.01**
Error	32	7578	236.8		

Table 4e

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the nauplii size.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	4039.3	4039.3	10.2	0.00
Temperature & Salinity	2	563.5	281.8	0.7	0.5
Temperature & Species	1	694.3	694.3	1.8	0.2
Temperature, Salinity & Species	2	810.1	405.0	1.0	0.38
Error	24	9517.8	396.6		

* Significant
** Highly significant

Figure 4a

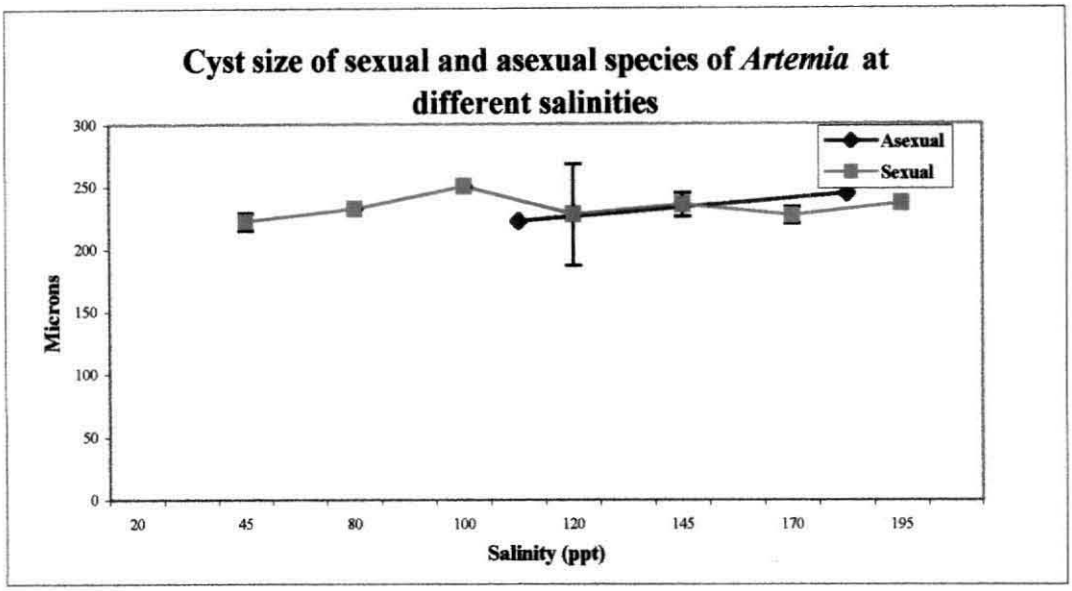
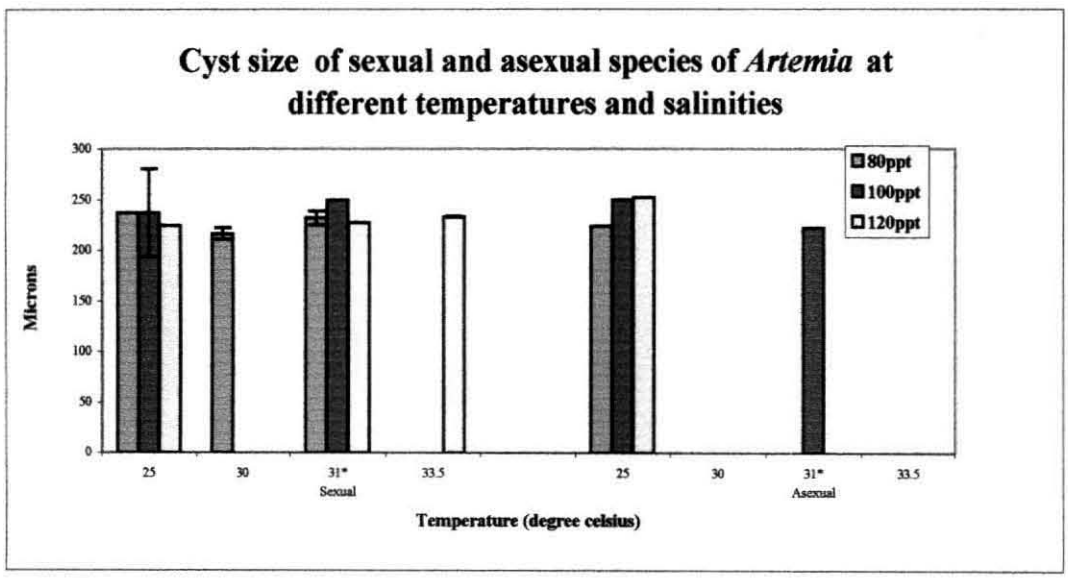


Figure 4b



* Ambient temperature

Figure 4d

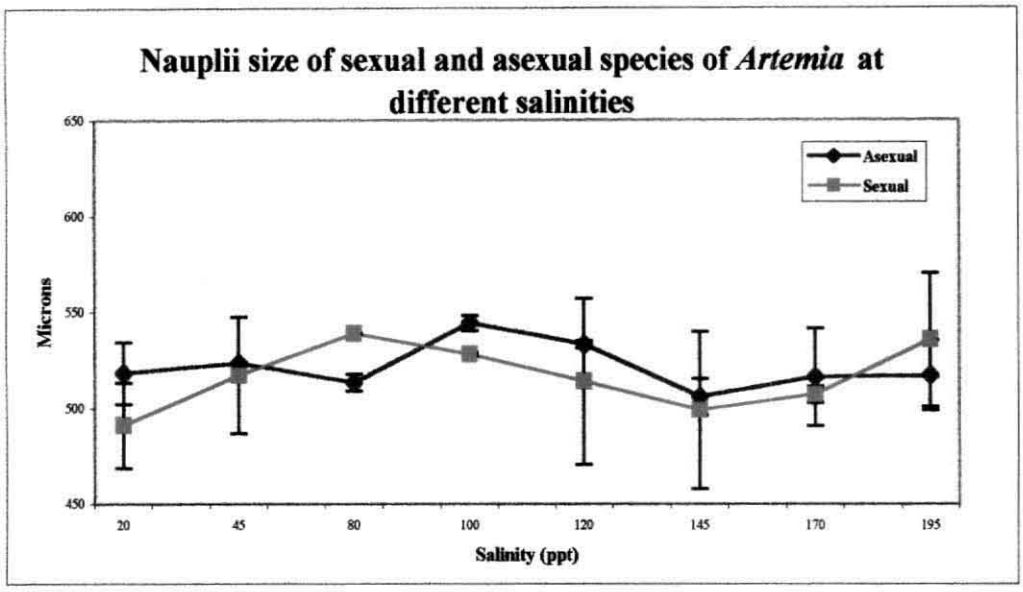
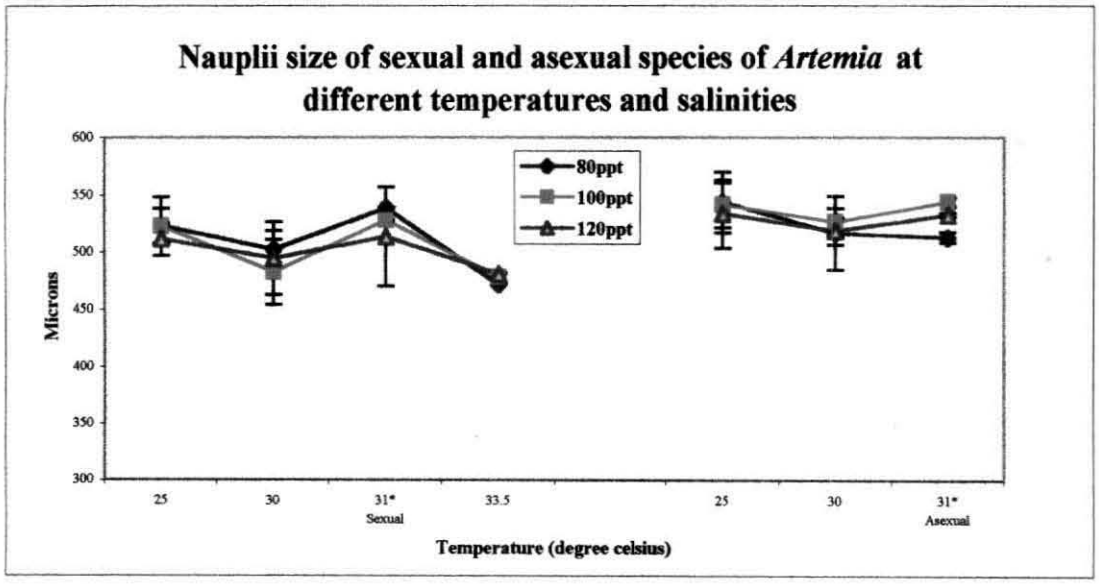


Figure 4e



* Ambient temperature

Salinity effect was common for both the species as the nauplii size was lower at lower salinities and increased with increase in salinity up to 80ppt in the case of sexual and 100ppt in the case of asexual species and then decreased till 145ppt for both the species with a further increase in size with increase in salinity (Figure 4d).

4. 3. 2. 2. Effect of temperature

Results on effects of temperature on the variation in the size of nauplii of sexual and asexual species of *Artemia* at different salinities are listed in Table 4e and Figure 4e. Values of 'nauplii size' in the Figure 4e are shown in Appendix 4e. Temperature had significant effect on both the species at 25 and 30 °C ($P<0.01$) as size decreased with increase in temperature and was true in the case of both the species (Figure 4e). Sexual species had slightly smaller nauplii size compared to that of asexual species. In case of sexual species nauplii size ranged between 472.26 microns (at 33.5 °C and 80ppt salinity) to 538.97 microns (at ambient temperature and 80ppt salinity) and for asexual species nauplii size ranged between 513.39microns (ambient temperature and 80ppt) to 544.29 microns (at ambient temperature and 100ppt salinity). There was no significance in interactive effect of temperature and salinity.

4. 3. 2. 3. Effect of quality and quantity of feed.

Results on the effect of quality and quantity of feed on the nauplii size of sexual and asexual species of *Artemia* are shown in Figures 4fa and 4fb. Values of 'nauplii size' in the Figure 4fa and 4fb are shown in Appendix 4f. Sexual as well as asexual females fed with ricebran had produced nauplii of smaller size as indicated in Figures 4fa and 4fb. The nauplii size of sexual species size ranged from 442.47 microns to 538.97 microns, while that of asexual species ranged from 472.98 to 545.7 microns. *Chlorella* sp. fed animals had comparatively larger nauplii size than both ricebran fed and *Isochrisis* sp. fed females except at 120ppt where *Isochrisis*

Table 4f

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the nauplii size.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	566.0	566.0	1.9	0.18
Feed type	2	25981.1	12990.1	42.6	0.00**
Feed quantity & Feed type	2	334.3	167.1	0.5	0.58
Feed quantity & Salinity	2	336.8	168.4	0.6	0.58
Feed quantity & Species	1	95.1	95.1	0.3	0.58
Feed type & Salinity	4	5146.1	1286.5	4.2	0.00**
Feed type & Species	2	1204.0	602.0	2.0	0.15
Feed quantity, Feed type & Salinity	4	1990.4	497.6	1.6	0.18
Feed quantity, Feed type & Species	2	69.5	34.8	0.1	0.89
Feed quantity, Salinity & Species	2	2612.2	1306.1	4.3	0.02*
Feed type, Salinity & Species	4	7239.0	1809.7	6.0	0.00**
Feed quantity, Feed type , Salinity & Species	4	1857.5	464.4	1.5	0.20
Error	72	21941.7	304.7		

* Significant
** Highly significant

Figure 4fa

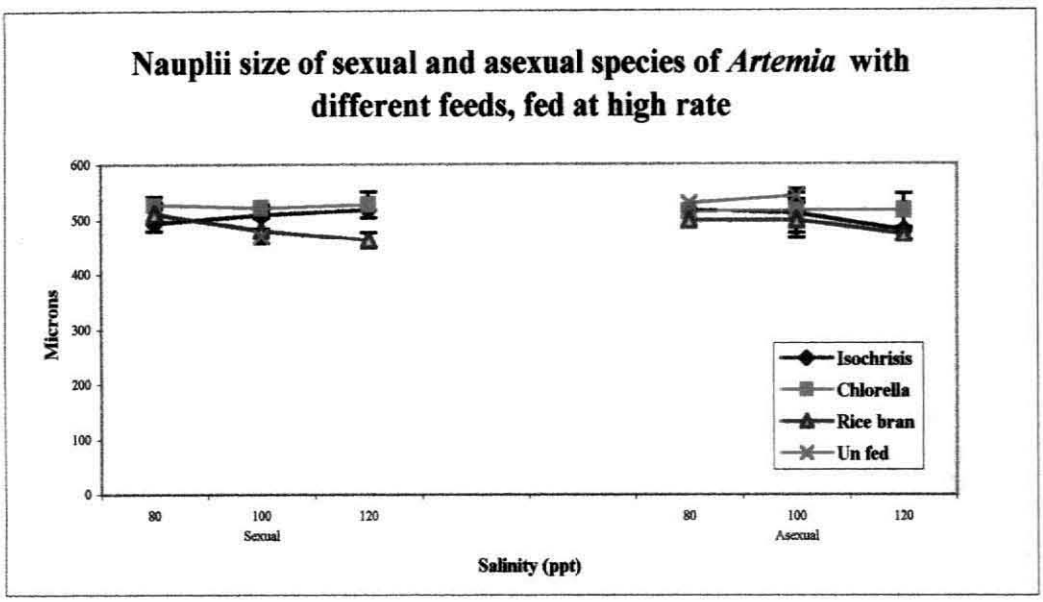
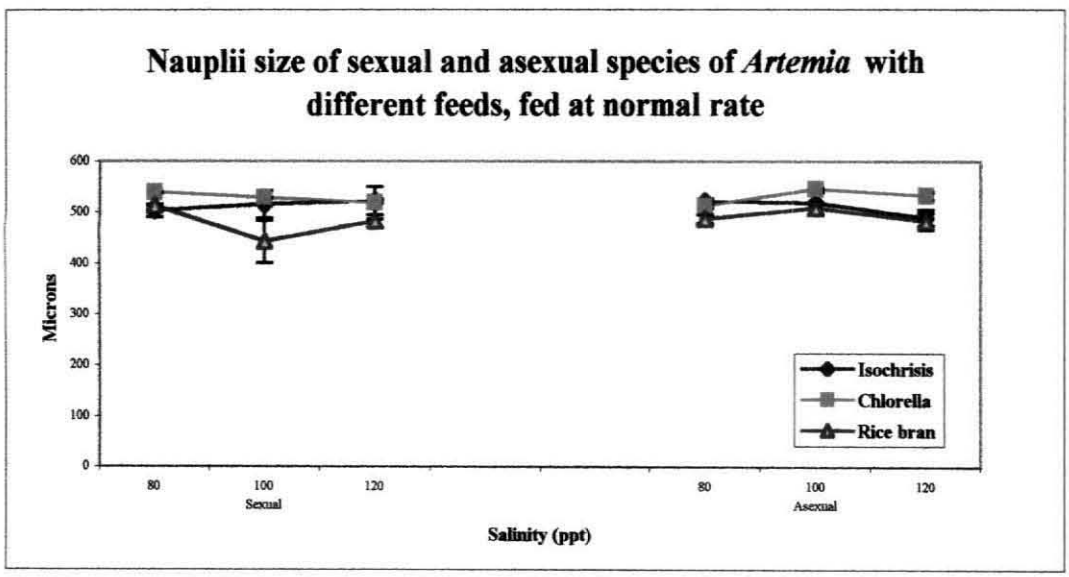


Figure 4fb



sp. fed sexual females produced larger nauplii than those fed with *Chlorella* sp. (Figures 4fb). Effect of different feeds on the size variation in the nauplii was highly significant (P<0.01) (Table 4f). Increased feeding rate with any of feeds such as *Isochrisis* sp., *Chlorella* sp. or ricebran had no significant effect on nauplii size of any of the species (Table 4f).

4. 3. 3. Adult size on first maturity

4. 3. 3.1. Effect of salinity

Results on the effect of salinity on size of maturity of females of both the species of *Artemia* are given in the Table 4g and Figure 4g. Values of 'adult size' in the Figure 4g are shown in Appendix 4g. In the sexual strain males were having a size lesser than the females, Figure 4g. Among the females of both the species sexual species was smaller in size compared to the asexual females (Figure 4g) except at 145ppt. In sexual female the size ranged between 6.32mm and 8.37mm and in the asexual female the size ranged between 7.8mm and 9.91mm. Salinity had significant effect on the adult size and size between the species also differed significantly (P<0.01).

4. 3. 3. 2. Effect of temperature

Results on the effect of temperature on the adult maturity size is shown in Table 4h and Figure 4h. Values of 'adult size' in the Figure 4h are shown in Appendix 4h. Adult maturity size had a parabolic relation (excluding the ambient temperature) with increased temperature in both the species as shown in Figure 4h. There was an increase in size with increase in temperature from 25 to 30 °C but decreased when temperature was further increased to 33.5 °C. Sexual species had lesser maturity size at higher temperature as increased temperature facilitated the maturation process. Effect of temperature on adult size was highly significant (P<0.01). Interactive effect of temperature and salinity on adult size was also significant (P<0.05).

Table 4g

Results from ANOVA showing the effect of different salinities and also the salinities in interaction with two different species of *Artemia* on the adult size at maturity.

Treatments	Degree of freedom	Sum-of-Squares	Mean-Square	F-ratio	P
Salinity	5	4.7	0.9	5.4	0.00**
Species	1	6.9	6.9	39.6	0.00**
Salinity & Species	5	4.9	1.0	5.6	0.00**
Error	24	4.2	0.2		

Table 4h

Results from ANOVA showing the effect of different temperatures and salinities and also their interaction with two different species of *Artemia* on the adult size at maturity.

Treatments	Degree of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Temperature	1	22.0	22.0	75.0	0.00**
Temperature & Salinity	2	2.3	1.1	3.9	0.04*
Temperature & Species	1	5.1	5.1	17.3	0.00**
Temperature, Salinity & Species	2	1.8	0.9	3.0	0.07
Error	24	7.0	0.3		

* Significant
** Highly significant

Figure 4g

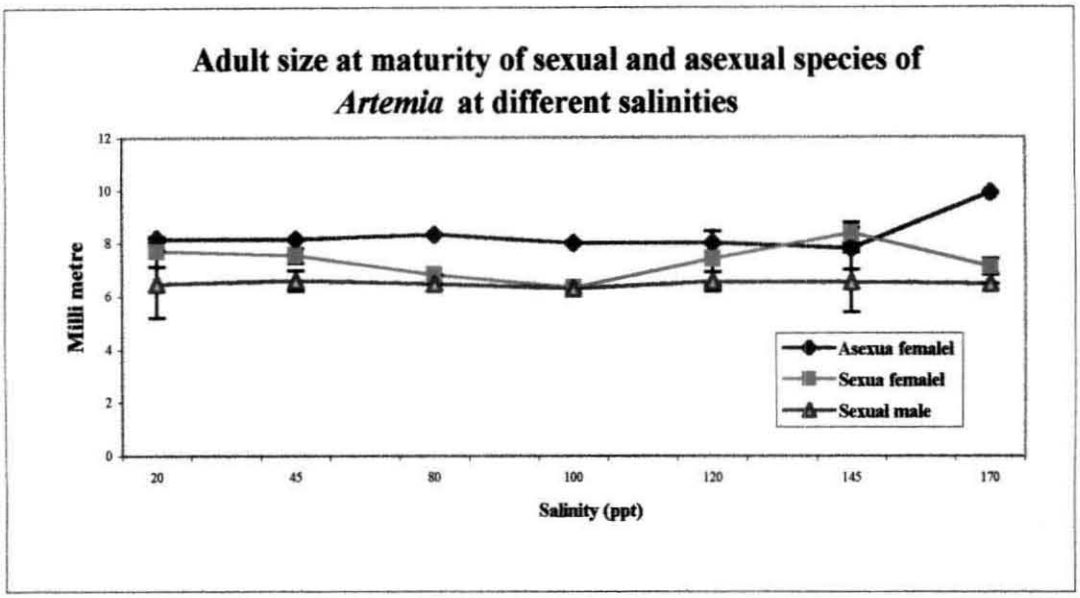
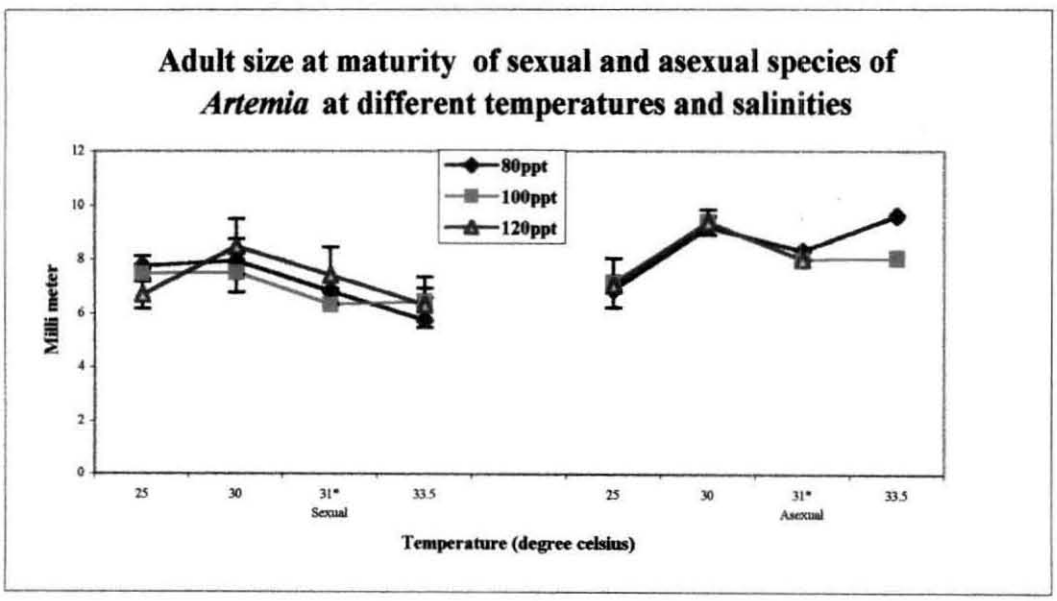


Figure 4h



* Ambient temperature

Table 4i

Results from ANOVA showing the effect of different feeds at two different concentrations at different salinities and also their interaction with two different species of *Artemia* on the adult size at maturity.

Treatments	Degrees of freedom	Sum-of-Squares	Mean-square	F-ratio	P
Feeding quantity	1	14.2	14.2	59.7	0.00**
Feed type	2	39.7	19.8	83.5	0.00**
Feed quantity & Feed type	2	4.4	2.2	9.3	0.00**
Feed quantity & Salinity	2	0.8	0.4	1.6	0.20
Feed quantity & Species	1	0.26	0.3	1.1	0.30
Feed type & Salinity	4	9.9	2.5	10.4	0.00**
Feed type & Species	2	50.1	25.1	105.4	0.00**
Feed quantity, Feed type & Salinity	4	6.3	1.6	6.6	0.00**
Feed quantity, Feed type & Species	2	7.7	3.9	16.3	0.00**
Feed quantity, Salinity & Species	2	2.3	1.1	4.8	0.01**
Feed type, Salinity & Species	4	7.2	1.8	7.6	0.00**
Feed quantity, Feed type , Salinity & Species	4	0.3	0.1	0.3	0.84
Error	72	17.1	0.2		

* Significant
** Highly significant

Figure 4ia

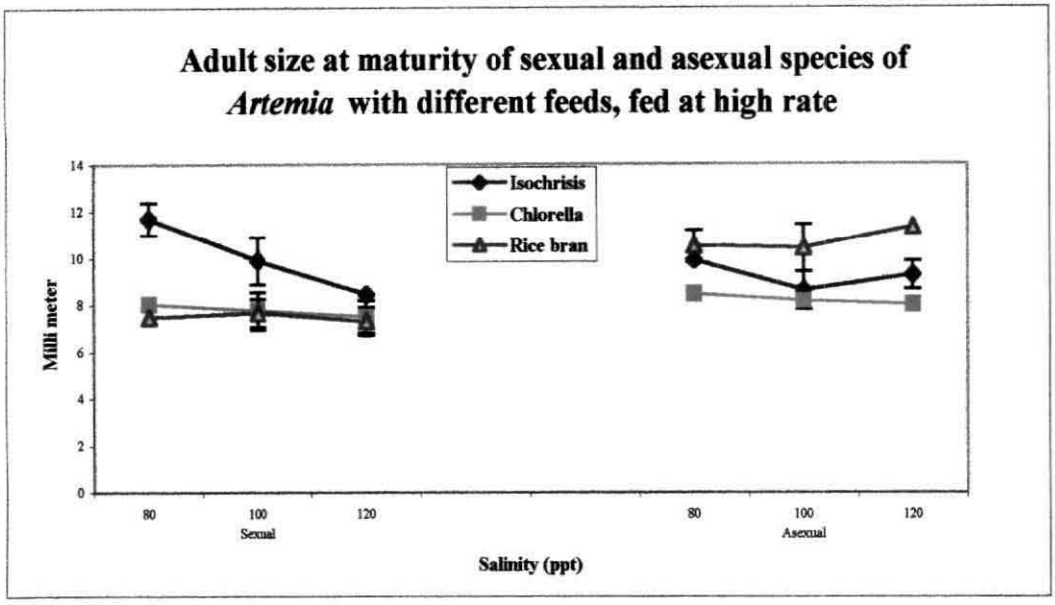
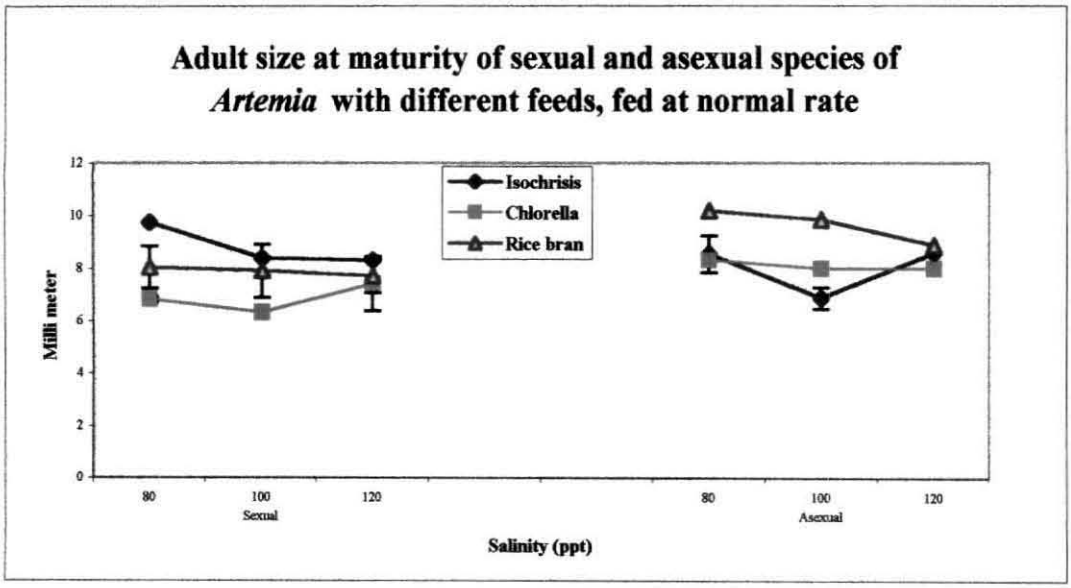


Figure 4ib



4. 3. 3. 3. Effect of quality and quantity of feed

Results on the effect of quality and quantity of feed on maturity size of sexual and asexual species of *Artemia* are listed in the Table 4i and Figures 4ia and 4ib. Values of 'adult size' in the Figure 4ia and 4ib are shown in Appendix 4i. Sexual females fed with *Isochrisis* sp. were found to have larger size at the time of maturation at all the three salinities while in case of asexual females it was rice bran fed animals which had larger size, (Figure 4ia and 4ib) at normal as well as high feeding. Animals fed on *Chlorella* sp. had lesser size compared to *Isochrisis* sp. and ricebran fed animals at most of the conditions. The size variations among males at different feeding regimes are not very clear.

The size of sexual females fed with *Isochrisis* sp. as well as ricebran were found to decrease with increased salinity, rice bran fed asexual species had a decrease in its first adult size with increase in salinity and increased with high feed concentration. There was significant difference on adult size with feed type and quantity ($P<0.01$). Interactive effect of feed type and quantity were also highly significant at both the feeding concentrations ($P<0.01$). With increased feeding rate of *Isochrisis* sp. , *Chlorella* sp. and rice bran, maturity size of both the species had significant increase ($P<0.01$).

4. 4. Discussion

In the present experiment the asexual parthenogenetic females were found to have reached larger size at the time of maturity than the sexual females at almost all the tested conditions except with *Isochrisis* sp. fed animals at 80 and 100ppt salinities (Figures 4ia and 4ib). According to Amat, 1980b, Tobias *et al.*, 1980 and Vanhaecke and Sorgeloos, 1980b *Artemia parthenogenetica* females often attain larger size than sexual females. With *Isochrisis* sp. as feed asexual females had comparatively smaller size than the sexual females especially with high feeding as shown in the Figures 4ia,

4ib. The prereproductive period of asexual females when fed with *Isochrisis* sp. at the tested salinities was longer (Chapter 1, Figures 1ca and 1cb) than that of the sexual females indicating that the growth rate of parthenogenetic females are low with *Isochrisis* sp. as feed.

Asexual females reached maturity at almost same size irrespective of salinity as shown in Figure 4g, but the extreme high salinity of 195 ppt was found to have effected the maturation process of asexual and sexual females as they failed to mature at this condition. In sexual females the size at maturity decreased as salinity increased from 20 to 100ppt and then increased till 145ppt. However, at 170ppt a decrease was noticed in the size. The male size were not found to be influenced with the difference in salinity but the males always had smaller size than that of females in the sexual species at all the experimental conditions. The data on pre reproductive period of both the species at various salinities (Chapter 1, Figure, 1a) indicate that both the lower as well as higher salinity delay the development of both the species but the growth of the animal seem to have been affected only at higher salinities as it had smaller size at higher salinity ranges as reported here. These findings are in agreement with the findings of Wear *et al.* (1986) and Triantaphyllidis *et al.* (1995) who have observed that adult size are inversely proportional to salinity especially above 140ppt.

Temperature had a parabolic relation with the adult size at maturity of both the species as at lower temperature of 25 °C and the higher most temperature of 33.5 °C the females of both the species had comparatively smaller size while at the mid temperature of 30 °C the females had their maximum size as indicated in the Figure 4h. The prereproductive periods (Chapter 1, Figure 1b) of both the species at 30 °C were shorter than those at 25 °C and the larger size of animals at 30 °C indicate that the growth rate of animals are maximum at this temperature.

The quality of feed also found to have affected the size of maturity as the sexual females had smallest size when fed with ricebran and *Chlorella* sp. as feed while the *Isochrisis* sp. fed females were larger at the time of maturity at 80 and 100ppt. At the same time rice bran fed asexual females had largest size at maturity than the animals fed with algae. Between the salinities there was no much variation in the size of both the species. Since the pre reproductive periods of both the species fed with rice bran were comparatively longer as discussed in the Chapter 1, the smaller size of sexual species fed with rice bran indicates that rice bran is inferior to algal feeds. Eventhough the prereproductive period of asexual species was comparatively longer than the sexual females the asexual females had significantly larger size than sexual females. Therefore, it is assumed that ricebran is a better feed for asexual females. Prereproductive periods of sexual (except at 120ppt) (Figures 1ca and 1cb) species with the algal feeds were slightly shorter than asexual species, but size difference of both the species were not that prominent, indicating better growth rate of sexual species. The *Isochrisis* sp. fed animals had larger size at the time of maturity than the *Chlorella* sp. fed animals of both the species indicating better growth rate with *Isochrisis* sp.

Between the sexual and asexual species from Tuticorin salt pans there was significant variation in the nauplii size at different temperature and salinity conditions studied as the asexual nauplii had comparatively larger size at most of the experimental conditions. The size of the sexual nauplii varied between 442.47 microns to 538.97 microns while that of asexual nauplii between 472.98 to 545.70 microns. Vanhaecke and Sorgeloos (1980b) compared nauplii of different strains. Nauplii size of parthenogenetic strain from India is recorded as 390 $\pm$ 0.12 (Royan *et al.* 1987) while that from China and Italy varied between 493 to 517 microns and was the largest of all the strain studied while those of San Francisco Bay

strain varied between 428 to 431 microns and was the smallest nauplii of the tested strains.

Nauplii size and temperature had a linear relation as shown in Figure 4e as increased temperature reduces the nauplii size of both the species . At salinities 20ppt and also at 145 and 170ppt both the species had produced nauplii of smaller size (Figure 4d). The quality of feed also was found to have some effect on nauplii size as the *Chlorella* sp. fed animals of both the species had produced larger nauplii than that of *Isochrisis* sp. fed and rice bran fed animals as indicated in Figures 4fa and 4fb.

In the present study the cyst size from all the experiments of asexual species ranged from 222.16 to 277.7 microns while that of sexual species ranged from 216.2 to 249.93 microns. There was significant difference between the cyst size of the two species at 25 °C the only temperature at which there was significant cyst production by both the species. According to Vanhaecke and Sorgeloos (1980b) cyst size of sexual population of San Francisco Bay, USA range from 224.3 to 228.7 microns with the thickness of the chorion varying between 7.05 to 8.3 microns whereas the cysts size of Tuticorin parthenogenetic population varied between 282.9 to 283.8 microns with a chorion thickness of 10.1 to 10.9 microns. Also the mean size was found to vary among different batches of the same strain and was thought to be due to changing environment conditions in the salt ponds. They have also observed that a drop in the salinity could increase cyst size by a few microns and the role of food conditions on the cyst size diameter also could not be ruled out.

At room temperature only the sexual species had produced cysts at almost all the tested salinities but the asexual species could produce cyst only at 100 and 170ppt salinities. There was no much difference between the size of cysts produced at different salinities. Considering the feeding

effect on cyst size only the algal feeds were found to have significant effect on cyst production that too only on sexual species and the cysts produced with *Chlorella* sp. as feed had larger size than that produced with *Isochrisis* sp. feed. Asexual species had cysts only at 100ppt with algae, when fed at normal level and cysts produced with *Isochrisis* sp. had larger size than those with *Chlorella* sp. fed animals.

CHAPTER 5

CHAPTER 5.

Electron microscopic study on the cysts, of sexual and asexual species of brine shrimps

5. 1. Introduction

Taxonomy of *Artemia* population is always problematic, as there is no distinct morphological differences among the population. In the beginning *Artemia* was named as *Artemia salina* in general as there was no efficient taxonomic system developed for *Artemia*. Later depending on the geographical origin they were named as *Artemia franciscana*, *A. persimilis, A. monica* and *A. parthenogenetica*. Some species, which are isolated geographically are also found to have some minor morphological difference. In order to distinguish the cysts of sexual species from that of asexual attempts were made to identify the ultrastructural differences of the cyst of the two species of *Artemia* available at Tuticorin.

Cysts released by *Artemia* and other anostracan can remain dormant without any development for a long period even when the environmental conditions are favourable for hatching. This is because the cysts possess endogenous control over their own development which can hardly be altered with environmental manipulations and the cyst is said to be in 'Dormancy' at this stage.

However, this dormancy can be terminated and the cysts can be induced to develop by different methods like 1. dehydration 2. low temperature and 3. anoxic conditions. By following either of these methods the cysts can be brought to a stage called 'Quiescent' and the same can be

later hatched by providing favourable conditions. The quiescent state reached by adopting dehydration method is known as ' Anhydrobiosis' and that of by low temperature as ' Cryptobiosis' and the quiescent stage due to anoxic conditions are known as 'Anoxibiosis' (Crowe *et al.*, 1987).

Dormant stage →	1 Dehydration 2 Low temperature 3 Anoxic condition	→	Quiescent stage →	Favourable conditions → Hatching

Intensive studies on Branchiopod resting eggs were started since the mid 1980's. There were studies aimed to detail the ultrastructure of *Artemia franciscana* (Lee *et al.*, 1994). Vanhaecke and Sorgeloos (1980b) had found that cyst diameters could be used as a useful tool for separating *Artemia* species of different geographic origin. As there are variations in cyst among the sexual and asexual species of *Artemia* , which belong to the anostracans as detailed in the previous chapter a detailed investigation on the structure of the cysts by electron microscope was also conducted as this study will certainly cast more light on species differentiation.

Many have tried to correlate the cyst diameter with other morphological as well as geographic characters of the population. Mura (1986) and Belk *et al.* (1990) found there was a correlation between the cyst diameter and female length of *Chirocephalus* sp. and *Streptocephalus seali*. Mura (1991b) in his experiments tried to correlate altitude and cyst diameter. Recently Hill and Shepard (1997) conducted detailed study of Californian anostracan cyst and found all genera and some species also can be identified using external and/or internal cyst wall characters and they found difference in the external morphology of the cysts of *A. franciscana* and *A. monica*. Mazzini (1978) and Morris and Afzelius (1967) have studied the ultra structure of desiccated cysts of *Artemia* using SEM. According to Mazzini (1978) the different cyst layers of anostracans can be named as the cortical layer, upper surface of alveolar layer with polygonal septa, alveolar layer and the

fibrous layer. But according to Spotte and Anderson (1988) the polygonal ornamentation in tertiary base of *Artemia franciscana* cyst are embedded in the fibrous layer, which is located beneath alveolar layer.

Biochemical adaptations of encysted embryo of *Artemia salina* to survive the extreme environmental conditions are described by Clegg (1974). Ultrastructure of cyst wall of *Artemia franciscana* has been studied by Anderson *et al.* (1970). In the present work an attempt has been made to study the morphological as well as ultrastructural differences of the cysts of the sexual and asexual *Artemia* species of Tuticorin salt pans. As it has been observed in the present investigation that at lower temperature especially at 25 °C, chances of cyst production is higher, the cysts produced at this temperature were also taken for the study.

5. 2. Materials and Methods

5. 2.1. Scanning Electron Microscopy (SEM)

The eggs for the SEM study were stored in deep brine. Some of the respective samples were collected and were taken in a 3ml centrifuge tube and the contents of each vial were fixed in cold gluteraldehyde solution (3%) and was given three buffer wash with 0.1 molar sodium phosphate buffer solution and the vials were refrigerated at 6 °C for 24h and later again treated with osmium tetroxide and were dehydrated with 30, 50, 75, 95 and 100% acetone at an interval of one hour. The cysts were then allowed to dry and were allowed to stick on double-sided tape and was observed after sticking on a stub which in turn is coated with gold.

5. 2. 2. Transmission Electron Microscopy (TEM)

A few numbers of cysts collected from deep brine were taken in 3ml plastic centrifuge tubes and were fixed in 3% buffered gluteraldehyde solution for 3 hours at 4 ^{O} C following immersion fixation technique. Decanned tissues were treated for buffer wash (0.1m Sodium cacodylate) for fifteen

minutes with three changes and was post fixed in 1-% osmium tetroxide for two hours at 4 °C. The cysts were then dehydrated in acetone in different concentrations, 30, 50, 70 and 90% each of 10 minutes duration at 4 °C and finally in 100% with two changes 20 minutes each at room temperature. The infiltration is done in Spurr's resin (Spurr, 1969) combined with acetone in the ratio of 1:3,1:1, 3:1 one hour in each. After infiltration the cysts were made in to blocks in plastic vials and kept in the 'Cintex' incubator setting the temperatures at 70 °C for 9 hours. From the polymerised blocks ultra thin sections were cut, in the LKB ultratome NOVA and were stained in Uranyl acetate and Lead citrate, double stage staining to enhance the contrast. The ultrathin sections were mounted on the grids and the image observed was recorded in the Hitachi H 600 transmission electron microscope.

5. 3. Results and discussion

The size variation of the cysts of the two species were clear as indicated in the previous chapter. There are reports which indicate that the size variation between the cysts of asexual and sexual species exists (Vanhaecke and Sorgeloos, 1980b). During the present study, difference in the hatching rates of both the species was observed, see Chapter 3. Cysts of the asexual species collected from wild as well as laboratory samples showed good hatching response while that of the sexual species were poor. Scanning electron microscopic photo of both the asexual and sexual *Artemia* species showed little variation (Plates 8a and 8b). The beginning of hatching process, half-hydrated (Plate 8c) and fully hydrated cyst with the oozing hatching membrane (Plate 8d) are shown in Plate 8. Of all the samples studied irrespective of the species and source the cysts were having an outer tertiary envelop (Plates 9e, 10a, 10d and 10e) of uniform structure. Anostracan cysts in common have a tertiary envelop, consisting of cortical layer and an

PLATE 8

a) Electron micrograph (SEM) of asexual dehydrated cyst.

b) Electron micrograph (SEM) of sexual dehydrated cyst.

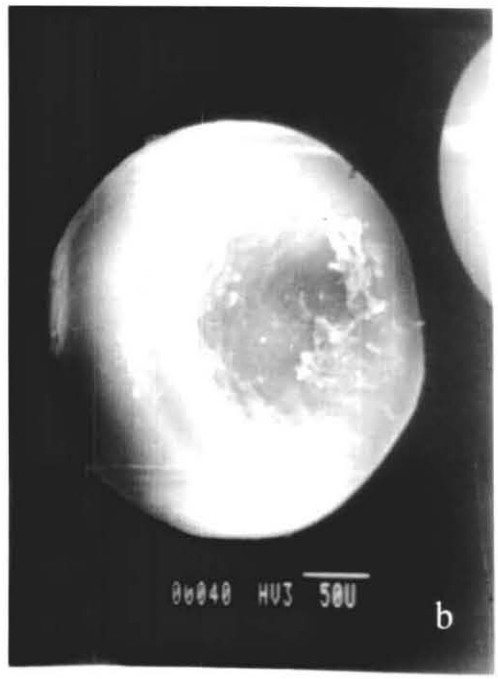
c) Electron micrograph (SEM) of hydrating asexual cyst.

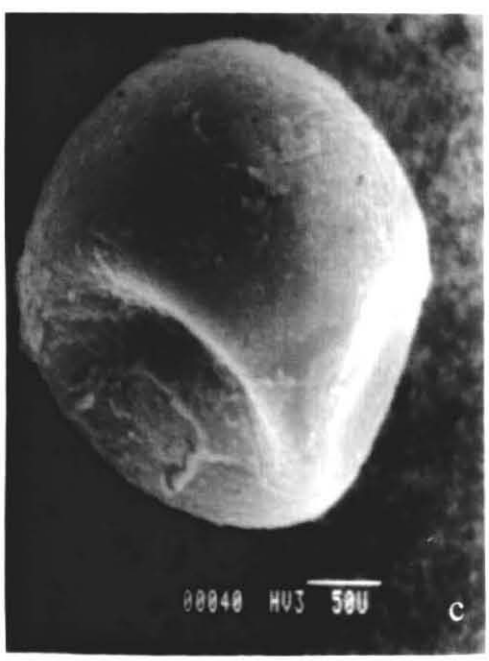
d) Electron micrograph (SEM) of asexual cyst, beginning of hatching process

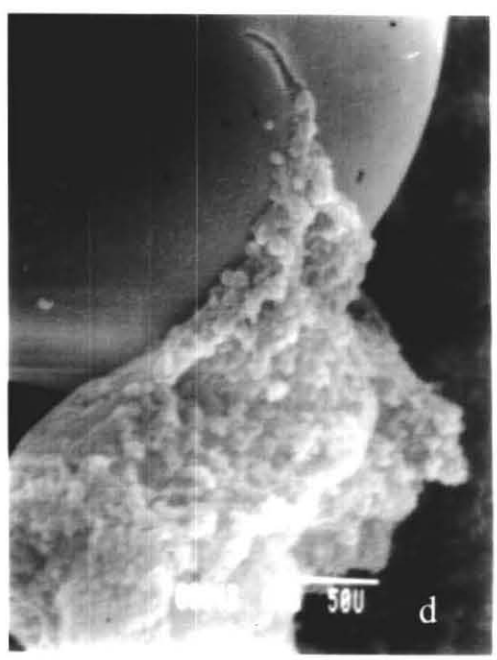
e) Electron micrograph (TEM) of asexual cyst from stock culture
   1 – Undulated outer cuticular layer
   2 – Alveolar layer
   3 – Subcortical space

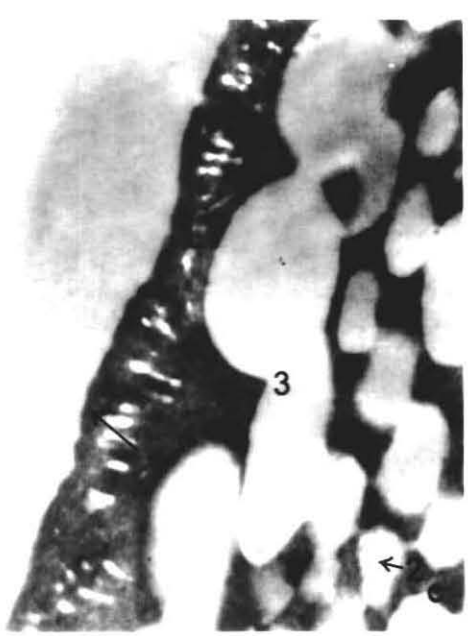
f) Electron micrograph (TEM) of sexual cyst from stock culture
   1 – Smooth cuticular layer
   2 – Alveolar layer
   3 – Subcortical space

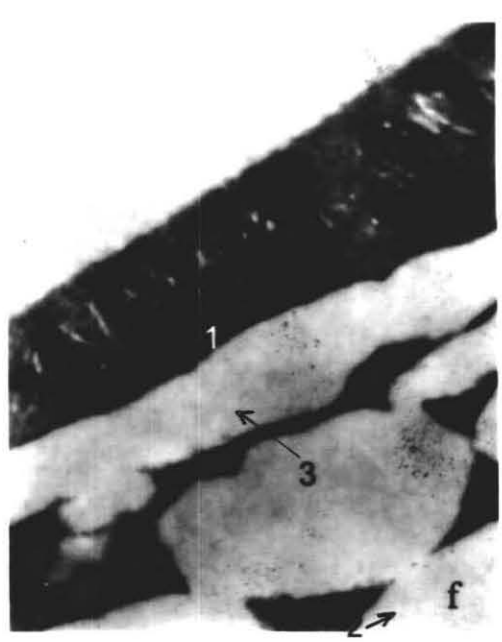
Plate 8











alveolar layer (Gilchrist,1978) and are generally made of the secretions from the shell gland (Mawson and Yonge, 1938; Linder, 1960; Anderson *et al.*, 1970).

Cyst wall of *Artemia* as well as other anostracans are to protect the enclosed embryo from physical as well as other possible damages during the unfavourable conditions and yet allow rehydration once the conditions are favourable. Lee *et al.* (1994) provided a detailed external and internal morphology of *Artemia franciscana.* The cyst wall is a tertiary envelop, mistakenly called as a chorion (Spotte and Anderson, 1988) is nonchitinous and is secreted by shell glands of maternal reproductive tract. In case of insects which has a chorionic layer around their eggs, chorion is being secreted by follicle (Gilchrist, 1978) and thus differ from the crustacean tertiary structure in origin. The tertiary outer covering of anostracan cysts comprise of lipoprotein complexes (Brown, 1950), but the embryonic layer is chitinous in nature (Linder, 1960) and is produced by the embryo.

The cyst wall of all the samples had an outer hard layer followed by a vacuolar layer with spongy appearance and membranous layer next to that. The details of tertiary layers of sexual cyst from the stock (Plates 9e and 9f) asexual cyst from the stock (Plates 10a, 10b and 10c), sexual cysts collected from experimental unit (Plate 10d) and asexual cyst from the experimental unit (Plates 10e and 10f) are shown in Plates 9 and 10. The middle layer was thicker than the other two. The vacuoles of the middle layer were inter-connected through small channels (Plates 8e, 8f, 9a, 9b and 9e). Function of this layer may be to help the embryo for respiration and also as a flotation device (Gilchrist, 1978). It may also protect the embryo from physical shocks.

Scanning microscopic study of *Artemia* cyst by Wheeler *et al.*

PLATE 9.

a) Electronmicrograph (TEM) of asexual cyst from wild

1 - Undulated cuticular layer.

2 - Alveolar layer.

3 - Sub cortical space.

b) Electronmicrograph (TEM) of asexual cyst from wild

c) Electronmicrograph (TEM) of asexual cyst from an experimental unit of 80ppt salinity and 25 °C temperature.

1- Undulated outer cuticular layer.

2- Alveolar layer.

3- Sub cortical space.

d) Electronmicrograph (TEM) of sexual cyst from an experimental unit of 80ppt salinity and 25 °C temperature.

1- Smooth outer cuticular layer.

2- Alveolar layer.

3- Sub cortical space.

e) Electronmicrograph of (TEM) outer tertiary layer of asexual cyst (stock culture)

1 – Smooth outer cuticular layer

2 – Interconnected alveolar layer

3 – Inner layer

4 – Embryonic layer

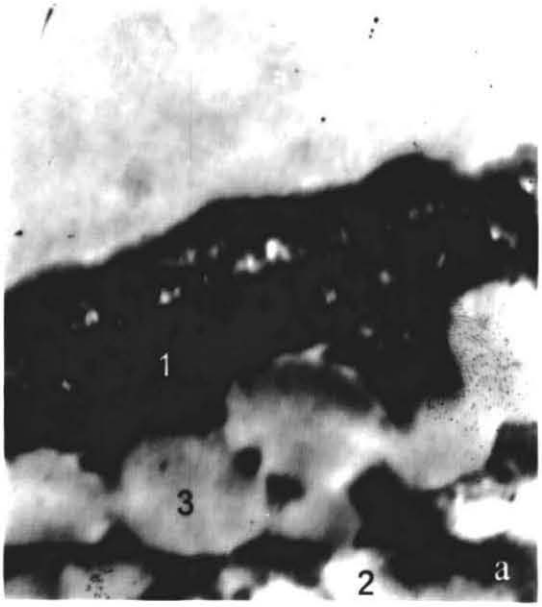
f) Electronmicrograph of cyst (sexual species) showing embryo

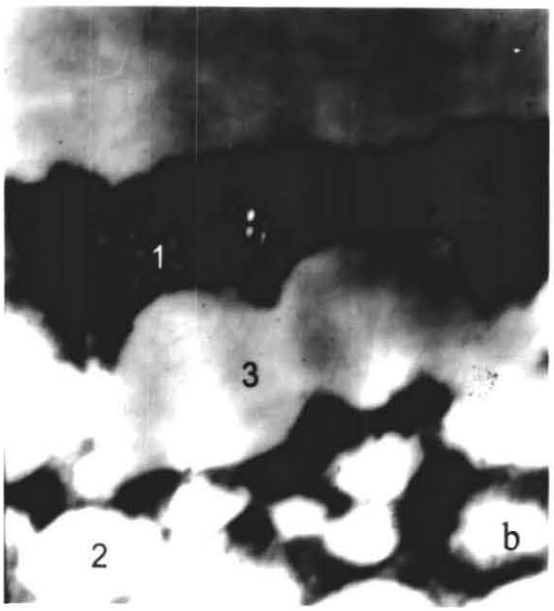
1 – Alveolar layer

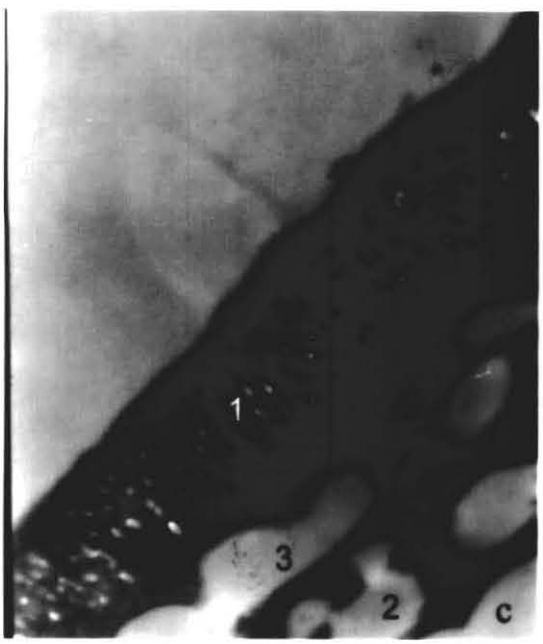
2 – Embryonic layer

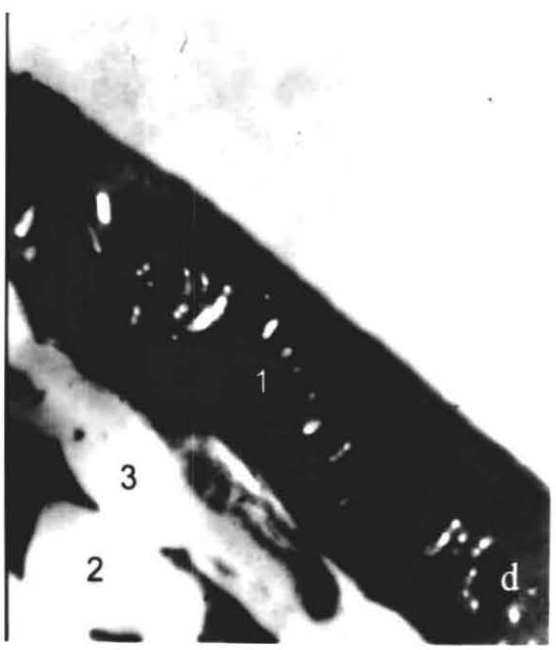
3 - Embryo

Plate 9









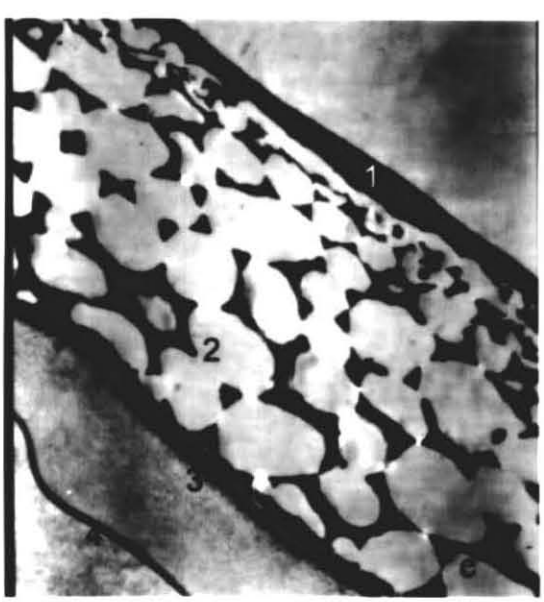


PLATE 10.

a) Electronmicrograph of cyst of asexual species from stock culture showing outer tertiary cell wall.
   1 – Undulated outer cuticular layer
   2 – Alveolar layer
   3 – Inner layer
   4 – Embryonic layer

b) Electronmicrograph of inner and alveolar layer of asexual cyst from stock culture
   1 – Alveolar layer
   2 – Inner layer

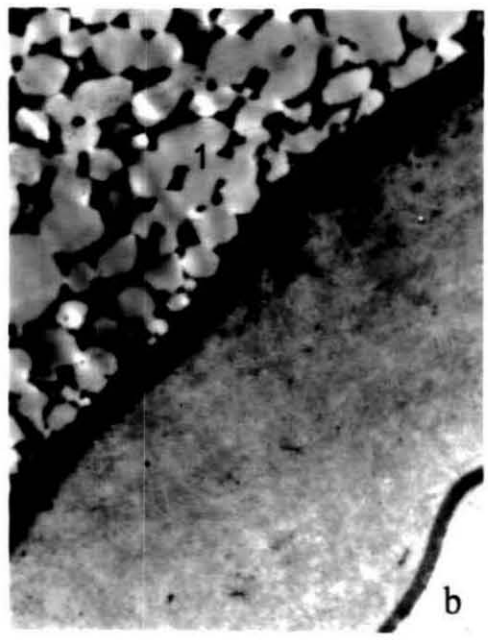
c) Electronmicrograph of asexual cyst, showing cell wall and embryo
   1 – Alveolar layer
   2 – Inner layer
   3 – Embryonic layer
   4 – Embryo

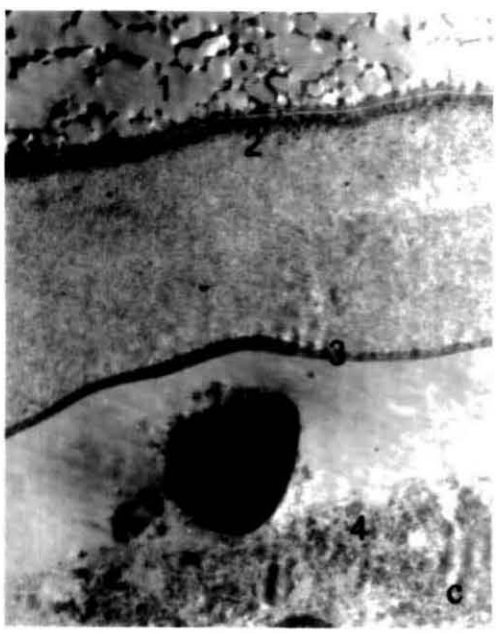
d) Electronmicrograph of sexual cyst, from an experimental unit of 80ppt salinity and 25 °C temperature showing tertiary cell wall
   1 – Cuticular layer
   2 – Alveolar layer
   3 – Inner layer
   4 – Embryonic layer
   5 – Embryo

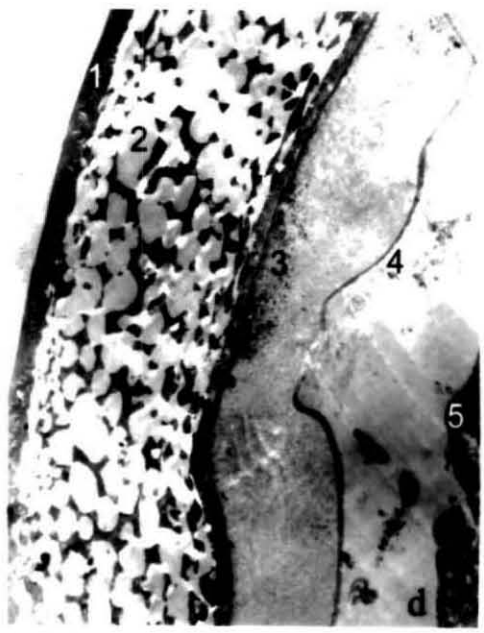
e) Electronmicrograph of asexual cyst from an experimental unit of 80ppt salinity and 25 °C temperature, showing tertiary cell wall.
   1 – Undulated outer cuticular layer
   2 – Subcuticular space
   3 – Alveolar layer
   4 – Inner layer

f) Electronmicrograph of asexual cyst from an experimental unit of 80ppt salinity and 25 °C temperature showing the embryo
   1 – Embryonic layer
   2 - Embryo

Plate 10







(1979) reveals three distinct region, as noted by TEM study of Morris and Afzelius (1967) and Anderson *et al.* (1970). According to them outer most region of shell is smooth in texture, and have one outer membrane and cortical layer. The middle layer appears spongy in morphology composed of outer cuticular, fibrous and an inner cuticular membrane. Vanhaecke and Sorgeloos (1980b) have also mentioned a possibility that anostracan cyst size varies significantly among the batches from the same strain and may be due to changing environmental conditions in the salt ponds. According to them tertiary envelop of cysts of *Artemia* vary in thickness which is strain dependent. D'Agostino (1980) speculated that structural difference also may exist between different strains. Previously it was thought that *Artemia* has no inter-specific differences with their cyst morphology (Browne and MacDonald 1982). But according to Spotte and Anderson (1988) differences in the thickness of the two layers and in the amount of space separating them (sub cortical space) varies by species. In the present case the close observation of the tertiary cell walls of both the species showed some differences between the two. Among the two species of Tuticorin, the cuticular layer of the asexual species had irregular (undulating) appearance with its cross sections (Plates 8e, 9a, 9b and 9c) while the sexual species had a smooth cuticular layer (Plates 8f and 9d) with almost uniform thickness. Sub cortical layer of the asexual species was more prominent than that of the sexual species. But intra sexual differences were not noticed in both sexual and asexual species with change in temperature (Plate 10e for asexual and Plate 10d for sexual species).

In the present experiment the cross section of outermost cortical layer shows some fissures (Plates 9a, 9b, 9c and 9d) indicating the possibility of pores connecting middle layer with the surroundings. But *Artemia* is known to have smooth surface devoid of any pores (Spotte and Anderson, 1988). Egg shell structure of *S. dichotomous* comprise mainly two layers, an outer thick cortex and an inner alveolar layer, the SEM study of

them shows presence of pores on its surface. The sub cortical space was found to be present in both sexual as well as the asexual species, but its thickness varied with the species which was also noticed by Gilchrist (1978). Subcortical space between cortex and alveolar layer was found discontinuous. This has been absent in case of *Artemia salina* (Morris and Afzelius, 1967). The studies of Morris and Afzelius (1967) indicated the formation of sub cortical layer when the cysts were hydrated and reported that the layer is not present in the dried cysts.

SUMMARY

SUMMARY

A comparative study of reproductive biology of two *Artemia* species available at Tuticorin salt pans, one the exotic sexual, *Artemia franciscana* and other the indigenous asexual, *Artemia parthenogenetica* were conducted at different ecophysiological conditions such as salinity, temperature, feed quality, feed concentration and also under starvation. Objective of the study was to have a detailed investigation on the reproductive biological aspects of the two species so as to find out the possibility of competitive exclusion of either them from the salt pans and also the viability of selecting either of these species to culture in the salt pans of Tuticorin.

Higher as well as lower salinities caused prolonged prereproductive period in both sexual and asexual species, while the mid salinities of 80 and 100ppt were the most ideal.

At ambient temperature, salinity increase caused the animal to have shorter reproductive period but at 25 and 30 °C with increased salinity reproductive period also increased.

Percentage reproductive period of the total lifespan was greater at 30°c for sexual population but at 25 °C for asexual population.

Prereproductive periods of both the species were shortest at 30 °C than at 25 and 33.5 °C temperature.

Brood number, fecundity and brood size of the sexual females were more than that of the asexual females at most of the experimental conditions. Salinity, temperature and also the quality of feed influenced fecundity.

Higher salinity and higher temperature reduced the fecundity of both the population and the total fecundity of asexual population decreased linearly with increase in temperature.

Isochrisis sp. was found to be the ideal feed for sexual as well as asexual population at lower salinities while at higher salinity the asexual population performed well with ricebran as feed.

The brood number and inter brood period were also markedly influenced more by temperature than salinity and feed. With an increase in salinity there was an increase in the inter brood period of both the populations.

Feed also had some effect on the inter brood period as the *Isochrisis* sp. fed sexual females had shorter inter brood period than those fed with other feeds.

Temperature was found to be the primary factor, responsible for oviparous mode of reproduction.

At 25 °C which was the lowest temperature selected for the present study there was maximum encystment for both the sexual as well as asexual population.

Photoperiod also had an interactive effect with temperature but found to have significant effect only with the asexual population.

For sexual population salinity also acted as an inducing factor for encystment of broods, at salinities above 145ppt. In case of feeds, *Chlorella* sp. fed animals only could produce encysted offsprings as with rice bran encystment rate was nil.

The size of females of asexual population at maturity was bigger than that of sexual females at almost all the tested condition.

Higher salinities and both higher and lower temperatures negatively affected the growth rate of both the populations resulting in smaller size at maturity.

Rice bran was the better feed for asexual population especially at higher salinities and *Isochrisis* sp. the best for sexual population for maximum growth.

The nauplii and cyst size of asexual population was higher than those of sexual population.

Temperature also affected the nauplii size as both the population produced smaller nauplii at higher temperature and *Chlorella* sp. fed animals had comparatively larger nauplii size than those fed with *Isochrisis* sp. and rice bran as feed.

Hatching rate of cysts of both the population obtained from different experimental units were found to show variations and an electron microscopic study of the cyst wall of these samples were conducted.

Cyst wall of both the populations had same tertiary structure as reported by previous workers. But the outer cuticular layer of both the populations had differences in its structure and can be considered as a useful tool in species differentiation and subcortical space of both the species also had inter-species variations.

Sub-cortical space was found to be present for dehydrated as well as hydrated cysts contradictory to some reports that it appeared only after dehydration of the cysts.

Inter-connecting pores were present in the alveolar layer in the cysts of both the population.

Since there are no much morphological variations between the two population the difference in the hatching rate between the population is presumed to be due to some strain specific biochemical differences of the cysts.

Rather than a complete dominance of either of these species the sexual species may dominate in the winter/rainy season and the asexual one in the summer season. Because the pre reproductive period of asexual species at higher salinities was less than the sexual species while at lower salinities the sexual had shorter pre reproductive period. The brood sizes of asexual species were also large than the sexual species at higher salinities while at lower salinities sexual species had larger broods. The oviparous mode of reproduction by the asexual species at lower temperature also support this view as the sexual species could produce nauplii offspring even at this temperature.

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APPENDICES

Appendix 1a

Pre reproductive period of sexual and asexual species of *Artemia* at different salinities			
Salinity (ppt)	Ambient temperature (^{0}C)	Sexual	Asexual
20 (20 ± 3)	27 ± 2	17.50 ±3.54	26 ± 2.83
45 (46 ± 3)	27 ± 2	16.50 ±2.12	22 ± 0.00
80 (80 ± 2)	31 ± 3	12.33 ±1.15	17 ± 0
100(102 ± 3)	31 ± 3	13.67 ±0.58	17 ± 0
120(121 ± 3)	31 ± 2	23.00 ±0.00	18 ± 0
145(145 ± 4)	30 ± 3	32.00 ±0.00	27 ± 0
170(169 ± 4)	30 ± 3	*	*
195(195 ± 3)	30 ± 3	*	*

Appendix 1b

Pre reproductive period of sexual and asexual species of *Artemia* with different temperature and salinities			
Temperature (^{0}C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	27.50 ± 0.71	29.33 ± 1.15
30 ± 0.5	80(80 ± 2)	12.00 ± 1.41	28.50 ± 0.71
31 ± 3	80(80 ± 2)	12.33 ± 1.16	17.00 ± 0.00
33.5 ± 0.5	80(80 ± 2)	16.00 ± 0.00	*
25 ± 0.5	100(102 ± 3)	27.50 ± 0.71	29.00 ± 0.00
30 ± 0.5	100(102 ± 3)	16.00 ± 0.00	29.00 ± 0.00
31 ± 3	100(102 ± 3)	13.67 ± 0.58	17.00 ± 0.00
33.5 ± 0.5	100(102 ± 3)	16.00 ± 0.00	*
25 ± 0.5	120(121 ± 3)	28.00 ± 0.00	37.00 ± 0.00
30 ± 0.5	120(121 ± 3)	18.00 ± 0.00	31.50 ± 4.95
31 ± 3	120(121 ± 3)	23.00 ± 0.00	18.00 ± 0.00
33.5 ± 0.5	120(121 ± 3)	19.00 ± 0.00	*

*Animals failed to mature

Appendix 1c

Pre reproductive period of sexual and asexual species of *Artemia* with different quality and quantity of feed						
Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ±3	11.33 ± 0.58	11.00 ± 0.00	15.33 ± 0.58	14.00 ± 0.00
CHL	80(80 ± 2)	31 ±3	11.67 ± 0.58	12.00 ± 0.00	18.00 ± 0.00	17.00 ± 0.00
RBRAN	80(80 ± 2)	29 ±2	18.50 ± 0.71	31.00 ± 0.00	19.50 ± 0.71	20.00 ± 0.00
ISO	100(102 ± 3)	31 ±3	10.00 ± 0.00	10.00 ± 0.00	15.00 ± 0.00	16.50 ± 0.71
CHL	100(102 ± 3)	31 ±3	10.50 ± 0.70	13.50 ± 0.00	18.50 ± 0.70	17.00 ± 0.00
RBRAN	100(102 ± 3)	31 ±3	13.50 ± 2.12	12.00 ± 0.00	19.00 ± 0.00	20.00 ± 0.00
ISO	120(121 ± 3)	30 ±2	11.50 ± 0.71	16.00 ± 0.00	16.00 ± 1.00	17.50 ± 0.71
CHL	120(121 ± 3)	30 ±2	19.00 ± 1.73	23.00 ± 0.00	18.50 ± 0.71	18.00 ± 0.00
RBRAN	120(121 ± 3)	31 ±3	15.00 ± 0.00	13.00 ± 0.00	19.00 ± 0.00	19.00 ± 0.00

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 1d

Reproductive period of sexual and asexual species of *Artemia* with different salinities			
Ambient temperature (°C)	Salinity (ppt)	Sexual	Asexual
20 (20 ± 3)	27 ± 2	24.33 ± 7.37	8.68 ± 5.50
45 (46 ± 3)	27 ± 2	46.67 ± 8.50	27.00 ± 1.41
80 (80 ± 2)	31 ± 3	27.33 ± 10.02	18.00 ± 2.82
100(102 ± 3)	31 ± 3	36.50 ± 4.95	15.00 ± 2.80
120(121 ± 3)	31 ± 2	18.33 ± 2.52	7.33 ± 2.89
145(145 ± 4)	30 ± 3	20.33 ± 0.58	7.00 ± 3.46
170(169 ± 4)	30 ± 3	20.00 ± 0.00	10.33 ± 0.58
195(195 ± 3)	30 ± 3	18.75 ± 7.41	18.75 ± 3.93

Appendix 1e

Reproductive period of sexual and asexual species of *Artemia* with different temperature and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	31.33 ± 14.01	20.67 ± 4.04
30 ± 0.5	80(80 ± 2)	33.00 ± 4.00	9.33 ± 5.51
31 ± 3	80(80 ± 2)	27.33 ± 10.02	18 ± 2.82
33.5 ± 0.5	80(80 ± 2)	9.50 ± 2.12	0
25 ± 0.5	100(102 ± 3)	45.67 ± 5.69	34 ± 2.83
30 ± 0.5	100(102 ± 3)	29.00 ± 16.09	4 ± 1
31 ± 3	100(102 ± 3)	36.50 ± 4.95	15 ± 2.8
33.5 ± 0.5	100(102 ± 3)	7.33 ± 3.06	0
25 ± 0.5	120(121 ± 3)	52.33 ± 1.53	43.67 ± 8.62
30 ± 0.5	120(121 ± 3)	54.00 ± 4.24	12.33 ± 5.03
31 ± 3	120(121 ± 3)	18.33 ± 2.52	7.33 ± 2.89
33.5 ± 0.5	120(121 ± 3)	6.67 ± 4.04	0

Appendix 1f

Reproductive period of sexual and asexual species of *Artemia* with different qualities and quantities of feed

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	20.00 ± 8.49	30.00 ± 7.07	29.33 ± 6.35	16.00 ± 1.000
CHL	80(80 ± 2)	31 ± 3	34.33 ± 11.59	27.33 ± 10.02	14.50 ± 9.19	18.00 ± 2.830
RBRAN	80(80 ± 2)	29 ± 2	21.00 ± 1.41	21.00 ± 5.57	6.00 ± 1.73	16.00 ± 9.900
UNFED	80(80 ± 2)	31 ± 3	1.33 ± 0.58		0.33 ± 0.58	
ISO	100(102 ± 3)	31 ± 3	19.67 ± 5.03	15.67 ± 6.11	18.47 ± 5.13	21.30 ± 6.500
CHL	100(102 ± 3)	31 ± 3	27.67 ± 12.66	36.50 ± 4.95	12.67 ± 12.67	15.00 ± 2.830
RBRAN	100(102 ± 3)	31 ± 3	23.00 ± 6.00	14.33 ± 6.43	11.50 ± 4.95	22.00 ± 0.000
UNFED	100(102 ± 3)	31 ± 3	16.00 ± 22.52		14.50 ± 3.53	
ISO	120(121 ± 3)	30 ± 2	43.00 ± 2.00	28.33 ± 5.51	11.25 ± 2.36	10.00 ± 5.560
CHL	120(121 ± 3)	30 ± 2	20.67 ± 7.23	18.33 ± 2.52	5.00 ± 1.00	7.33 ± 2.890
RBRAN	120(121 ± 3)	31 ± 3	23.67 ± 5.86	15.50 ± 2.12	9.50 ± 5.32	12.25 ± 3.300
UNFED	120(121 ± 3)	30 ± 2	0		0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 1g

Post reproductive period of sexual and asexual species of *Artemia* with different salinities			
Salinity (ppt)	Ambient temperature (°C)	Sexual	Asexual
20 (20 ± 3)	27 ± 2	0.67 ± 0.58	0.67 ± 1.16
45 (46 ± 3)	27 ± 2	1.00 ± 0.00	0.50 ± 0.71
80 (80 ± 2)	31 ± 3	0.33 ± 0.58	9.00 ± 4.24
100(102 ± 3)	31 ± 3	1.50 ± 2.12	3.00 ± 1.00
120(121 ± 3)	31 ± 2	2.67 ± 1.53	1.33 ± 2.31
145(145 ± 4)	30 ± 3	2.33 ± 1.53	2.67 ± 1.53
170(169 ± 4)	30 ± 3	5.33 ± 0.58	2.67 ± 2.08
195(195 ± 3)	30 ± 3	0	0

Appendix 1h

Post reproductive period of sexual and asexual species of *Artemia* with different temperatures and salinities			
Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	2.00 ± 1.73	7.17 ± 6.71
30 ± 0.5	80(80 ± 2)	3.67 ± 2.08	1.33 ± 2.31
31 ± 3	80(80 ± 2)	0.33 ± 0.58	9 ± 4.24
33.5 ± 0.5	80(80 ± 2)	1.00	0
25 ± 0.5	100(102 ± 3)	3.00 ± 3.46	4 ± 2.83
30 ± 0.5	100(102 ± 3)	2.33 ± 0.58	4 ± 1
31 ± 3	100(102 ± 3)	1.50 ± 2.12	3 ± 1
33.5 ± 0.5	100(102 ± 3)	3.33 ± 3.21	0
25 ± 0.5	120(121 ± 3)	0.33 ± 0.58	2.33 ± 3.21
30 ± 0.5	120(121 ± 3)	0.50 ± 0.71	2 ± 1.73
31 ± 3	120(121 ± 3)	2.67 ± 1.53	1.33 ± 2.31
33.5 ± 0.5	120(121 ± 3)	6.67 ± 4.04	0

Appendix 1i

Post reproductive period of sexual and asexual species of *Artemia* with different qualities and quantities of feed

Feed	Salinity (ppt)	Ambient temperature (^{0}C)	Sexual H	Sexual L	Asexual H	Asexual
ISO	80(80 ± 2)	31 ± 3	1.67 ± 1.15	1.67 ± 1.15	0 ± 0.00	1.00 ± 1.00
CHL	80(80 ± 2)	31 ± 3	2.00 ± 2.00	0.33 ± 0.58	0.33 ± 0.58	9.00 ± 4.24
RBRAN	80(80 ± 2)	29 ± 2	4.00 ± 1.41	1.00 ± 1.00	2 ± 2.00	2.50 ± 0.71
UNFED	80(80 ± 2)	31 ± 3	1.67 ± 2.89			
ISO	100(102 ± 3)	31 ± 3	3.33 ± 2.31	1.33 ± 0.58	2.33 ± 2.30	3.00 ± 2.00
CHL	100(102 ± 3)	31 ± 3	1.00 ± 0.00	1.50 ± 2.12	2 ± 1.73	3.00 ± 1.41
RBRAN	100(102 ± 3)	31 ± 3	2.00 ± 1.73	3.67 ± 4.62	3 ± 0.00	1.00 ± 1.41
UNFED	100(102 ± 3)	31 ± 3	4.67 ± 0.58		0.33 ± 0.58	
ISO	120(121 ± 3)	30 ± 2	4.00 ± 1.73	0.33 ± 0.58	4 ± 2.00	1.67 ± 1.15
CHL	120(121 ± 3)	30 ± 2	1.33 ± 0.58	2.67 ± 1.53	2.67 ± 1.53	1.33 ± 2.31
RBRAN	120(121 ± 3)	31 ± 3	0.00 ± 0.00	0.00 ± 0.00	1 ± 0.00	2.50 ± 1.30
UNFED	120(121 ± 3)	30 ± 2	0		0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 1j

Total life span of sexual and asexual species of *Artemia* with different salinities

Salinity (ppt)	Ambient temperature (^{0}C)	Sexual	Asexual
20 (20 ± 3)	27 ± 2	42.67	35.33 ± 5.69
45 (46 ± 3)	27 ± 2	63.33 ± 8.33	49.50 ± 2.12
80 (80 ± 2)	31 ± 3	39.00 ± 9.54	44.00 ± 7.07
100(102 ± 3)	31 ± 3	48.00 ± 2.83	35.00 ± 4.25
120(121 ± 3)	31 ± 2	44.00 ± 1.00	26.66
145(145 ± 4)	30 ± 3	54.00 ±	36.67

Appendix 1k

Total life span of sexual and asexual species of *Artemia* at different temperatures and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	61.00 ± 14.80	50.67 ± 5.51
30 ± 0.5	80(80 ± 2)	52.67 ± 2.08	39.17 ± 3.21
31 ± 3	80(80 ± 2)	39.00 ± 9.54	44 ± 7.07
33.5 ± 0.5	80(80 ± 2)	26.00 ± 1.41	3 ± 1.73
25 ± 0.5	100(102 ± 3)	75.67 ± 5.86	67 ± 5.66
30 ± 0.5	100(102 ± 3)	47.33 ± 16.56	53.33 ± 20.03
31 ± 3	100(102 ± 3)	48.00 ± 2.83	35 ± 4.25
33.5 ± 0.5	100(102 ± 3)	26.67 ± 6.03	2 ± 1.73
25 ± 0.5	120(121 ± 3)	80.67 ± 2.08	83 ± 8.18
30 ± 0.5	120(121 ± 3)	73.50 ± 3.54	45.83 ± 3.79
31 ± 3	120(121 ± 3)	44.00 ± 1.00	26.66
33.5 ± 0.5	120(121 ± 3)	28.00 ± 6.24	3

Appendix 1l

Total life span of sexual and asexual species of *Artemia* with different quality and quantity of feeds

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	32.97 ± 2.33	43.00 ± 7.07	44.66 ± 8.10	31.50 ± 0.71
CHL	80(80 ± 2)	31 ± 3	48.00 ± 4.30	39.00 ± 9.54	32.83 ± 3.50	44.00 ± 7.07
RBRAN	80(80 ± 2)	29 ± 2	43.50 ± 1.11	53.00 ± 6.00	27.50 ± 2.22	38.50 ± 9.19
ISO	100(102 ± 3)	31 ± 3	33.00 ± 5.23	20.00 ± 8.00	35.80 ± 2.20	40.00 ± 7.54
CHL	100(102 ± 3)	31 ± 3	39.17 ± 4.10	48.00 ± 2.83	33.17 ± 5.20	35.00 ± 4.24
RBRAN	100(102 ± 3)	31 ± 3	38.50 ± 4.30	28.00 ± 2.00	33.50 ± 5.20	43.00 ± 1.41
ISO	120(121 ± 3)	30 ± 2	58.50 ± 3.22	44.67 ± 5.77	31.25 ± 6.60	28.66 ± 5.51
CHL	120(121 ± 3)	30 ± 2	41.00 ± 2.32	44.00 ± 1.00	26.17 ± 6.30	26.66 ± 0
RBRAN	120(121 ± 3)	31 ± 3	38.67 ± 3.52	28.00 ± 0.00	29.50 ± 3.25	*

*Females failed to mature

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 1m

Reproductive period in percentage to total life span of sexual and asexual species of *Artemia* with different salinities		
Salinity (ppt)	Asexual	Sexual
20 (20 ± 3)	23.22	62.79
45 (46 ± 3)	54.66	72.88
80 (80 ± 2)	41.03	68.57
100(102 ± 3)	43.37	77.25
120(121 ± 3)	30.77	35.03
145(145 ± 4)	16.20	35.84

Appendix 1n

Reproductive period in percentage to total life span of sexual and asexual species of *Artemia* with different temperatures and salinities			
Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	51.36612	40.79337
30 ± 0.5	80(80 ± 2)	62.65823	23.81925
33.5 ± 0.5	80(80 ± 2)	36.53846	*
25 ± 0.5	100(102 ± 3)	60.35242	50.74627
30 ± 0.5	100(102 ± 3)	61.26761	7.500469
33.5 ± 0.5	100(102 ± 3)	27.5	*
25 ± 0.5	120(121 ± 3)	64.87603	52.61446
30 ± 0.5	120(121 ± 3)	73.46939	26.90377
33.5 ± 0.5	120(121 ± 3)	23.80952	*

* No reproduction

Appendix 1o

Reproductive period in percentage to total life span of sexual and asexual sp species of *Artemia* with different quality and quantity of feed

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	60.96	69.76	65.66	50.79
CHL	80(80 ± 2)	31 ± 3	71.53	70.08	43.94	40.9
RBRAN	80(80 ± 2)	29 ± 2	48.28	39.62	21.82	41.56
ISO	100(102 ± 3)	31 ± 3	59.60	78.35	52.77	53.25
CHL	100(102 ± 3)	31 ± 3	70.64	76.04	38.78	42.86
RBRAN	100(102 ± 3)	31 ± 3	59.74	51.18	34.33	51.16
ISO	120(121 ± 3)	30 ± 2	59.74	63.42	36.29	34.89
CHL	120(121 ± 3)	30 ± 2	50.41	41.66	19.11	27.49
RBRAN	120(121 ± 3)	31 ± 3	61.21	55.36	32.20	36.3

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 2a

Broods per female of sexual and asexual species of *Artemia* at different salinities

Salinity (ppt)	Ambient temperature (°C)	Sexual	Asexual
20 (20 ± 3)	27 ± 2	8.33 ± 2.08	3 ± 1
45 (46 ± 3)	27 ± 2	11.33 ± 0.58	7 ± 0
80 (80 ± 2)	31 ± 3	10.00 ± 4.00	3.5 ± 0.7
100(102 ± 3)	31 ± 3	12.50 ± 0.71	4.5 ± 0.7
120(121 ± 3)	31 ± 2	4.67 ± 0.58	2.33 ± 0.6
145(145 ± 4)	30 ± 3	5.33 ± 0.58	2 ± 1
170(169 ± 4)	30 ± 3	6 ± 0.00	2.67 ± 0.6
195(195 ± 3)	30 ± 3	5.25 ± 2.06	4.5 ± 0.6

Appendix 2b

Broods per female of sexual and asexual species of *Artemia* at different temperature and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	8.00 ± 1.73	4.67 ± 0.58
30 ± 0.5	80(80 ± 2)	11.00 ± 1.00	3 ± 1
31 ± 3	80(80 ± 2)	10.00 ± 4.00	3.5 ± 0.7
33.5 ± 0.5	80(80 ± 2)	3.00	0
25 ± 0.5	100(102 ± 3)	9.00 ± 1.00	7 ± 1.41
30 ± 0.5	100(102 ± 3)	9.00 ± 3.61	5.67 ± 4.23
31 ± 3	100(102 ± 3)	12.50 ± 0.70	4.5 ± 0.7
33.5 ± 0.5	100(102 ± 3)	2.67 ± 1.53	0
25 ± 0.5	120(121 ± 3)	10.00 ± 1.73	8.33 ± 1.53
30 ± 0.5	120(121 ± 3)	14.50 ± 0.71	3.33 ± 2.08
31 ± 3	120(121 ± 3)	4.67 ± 0.57	2.33 ± 0.58
33.5 ± 0.5	120(121 ± 3)	1.67 ± 1.15	0

Appendix 2c

Broods per female of sexual and asexual species of *Artemia* with different quality and quantity of feed

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	6.00 ± 3.61	6.33 ± 4.04	7.67 ± 1.56	4.50 ± 0.70
CHL	80(80 ± 2)	31 ± 3	11.33 ± 4.04	10.00 ± 4.00	5 ± 2.83	3.50 ± 0.71
RBRAN	80(80 ± 2)	29 ± 2	6.50 ± 0.71	7.67 ± 1.53	2 ± 1	4.50 ± 2.12
UNFED	80(80 ± 2)	31 ± 3	1.33 ± 0.58		0.33 ± 0.58	
ISO	100(102 ± 3)	31 ± 3	6.67 ± 1.53	5.67 ± 2.31	5 ± 1	6.33 ± 1.50
CHL	100(102 ± 3)	31 ± 3	9.00 ± 3.46	12.50 ± 0.71	3.33 ± 0.58	4.50 ± 0.71
RBRAN	100(102 ± 3)	31 ± 3	7.33 ± 1.53	5.00 ± 1.73	3.5 ± 2.12	6.00 ± 0.00
UNFED	100(102 ± 3)	31 ± 3	4.00 ± 3.46		4	
ISO	120(121 ± 3)	30 ± 2	14.33 ± 0.58	9.33 ± 1.15	2.5 ± 0.07	3.67 ± 1.53
CHL	120(121 ± 3)	30 ± 2	7.00 ± 0.00	4.67 ± 0.58	1.67 ± 0.58	2.33 ± 0.58
RBRAN	120(121 ± 3)	31 ± 3	7.00 ± 1.00	5.00 ± 0.00	2.75 ± 1.5	3.25 ± 0.96
UNFED	120(121 ± 3)	30 ± 2	0		0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 2d

Inter brood period of sexual and asexual species of *Artemia* at different salinities			
Salinity (ppt)	Ambient temperature (°C)	Sexual	Asexual
20 (20 ± 3)	27 ± 2	3.30 ± 0.093	3.94
45 (46 ± 3)	27 ± 2	3.93 ± 0.612	4.33
80 (80 ± 2)	31 ± 3	2.88 ± 0.120	4.5 ± 0.7
100(102 ± 3)	31 ± 3	2.95 ± 0.071	3.88 ± 0.18
120(121 ± 3)	31 ± 2	5.28 ± 1.040	4.71 ± 0.29
145(145 ± 4)	30 ± 3	4.42 ± 0.320	4.5 ± 0.71
170(169 ± 4)	30 ± 3	3.87 ± 0.120	4.5 ± 0.5
195(195 ± 3)	30 ± 3	4.92 ± 1.220	4.46 ± 0.36

Appendix 2e

Inter brood period of sexual and asexual species of *Artemia* at different temperatures and salinities			
Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	4.61 ± 0.67	5.6 ± 0.24
30 ± 0.5	80(80 ± 2)	3.37 ± 0.65	4.28 ± 0.25
31 ± 3	80(80 ± 2)	2.88 ± 0.12	4.5 ± 0.7
33.5 ± 0.5	80(80 ± 2)	3.50 ± 0.71	0
25 ± 0.5	100(102 ± 3)	4.22 ± 0.31	6.2 ± 0.23
30 ± 0.5	100(102 ± 3)	3.22 ± 0.30	4.6 ± 0.4
31 ± 3	100(102 ± 3)	2.95 ± 0.07	3.88 ± 0.18
33.5 ± 0.5	100(102 ± 3)	2.92 ± 0.59	0
25 ± 0.5	120(121 ± 3)	5.66 ± 0.52	5.96 ± 0.06
30 ± 0.5	120(121 ± 3)	3.58 ± 0.58	4.29 ± 0.06
31 ± 3	120(121 ± 3)	5.28 ± 1.04	4.71 ± 0.29
33.5 ± 0.5	120(121 ± 3)	3.00	0

Appendix 2f

Inter brood period of sexual and asexual species of *Artemia* with different quality and quantity of feed

Feed	Salinity (ppt)	Ambient temperature (^{o}C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ±2)	31 ±3	2.65 ± 0.59	3.55 ± 0.42	4.14 ±0.14	4 ±0.00
CHL	80(80 ±2)	31 ±3	2.89 ± 0.05	2.88 ± 0.12	4.25 ±0.35	4.5 ±0.71
RBRAN	80(80 ±2)	29 ±2	3.17 ± 0.23	3.17 ± 0.29	4.67 ±0.58	4.55 ±0.07
UNFED	80(80 ±2)	31 ±3	2.00		0	
ISO	100(102 ±3)	31 ±3	2.88 ± 0.29	2.63 ± 0.04	4.23 ±0.25	3.86 ±0.23
CHL	100(102 ±3)	31 ±3	2.83 ± 0.29	2.95 ± 0.07	4.03 ±0.45	3.88 ±0.18
RBRAN	100(102 ±3)	31 ±3	3.43 ± 0.21	3.13 ± 0.23	4.00 ±0.00	4
UNFED	100(102 ±3)	31 ±3	3.00 ± 1.73		3.17 ±0.23	
ISO	120(121 ±3)	30 ±2	3.12 ± 0.04	3.38 ± 0.22	3.88 ±0.25	3.47 ±0.71
CHL	120(121 ±3)	30 ±2	3.19 ± 0.20	5.28 ± 1.04	4.33 ±0.58	4.17 ±0.29
RBRAN	120(121 ±3)	31 ±3	3.50 ± 0.10	3.63 ± 0.00	4.44 ±0.59	4.21 ±0.71
UNFED	120(121 ±3)	30 ±2	0		0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 2g

Offsprings per brood of sexual and asexual species of *Artemia* at different salinities

Salinity (ppt)	Ambient temperature (^{o}C)	Sexual	Asexual
20 (20 ± 3)	27 ±2	121.05 ± 32.50	51.72 ± 40.07
45 (46 ± 3)	27 ±2	126.66 ± 3.95	59.59 ± 23.11
80 (80 ± 2)	31 ±3	45.67 ± 19.01	33.53 ± 2.58
100(102 ± 3)	31 ±3	65.50 ± 4.95	48.9 ± 24.5
120(121 ± 3)	31 ±2	19.58 ± 8.18	16.61 ± 5.88
145(145 ± 4)	30 ±3	22.4 ± 2.51	25.78 ± 8.88
170(169 ± 4)	30 ±3	15.59 ± 1.07	18.56 ± 1.71
195(195 ± 3)	30 ±3	20.54 ± 4.57	81 ± 17.45

Appendix 2h

Offsprings per brood of sexual and asexual species of *Artemia* with different temperatures and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	52.33 ± 1.13	74.87 ± 22.06
30 ± 0.5	80(80 ± 2)	41.46 ± 6.69	56.71 ± 12.77
33.5 ± 0.5	80(80 ± 2)	41.34 ± 4.72	0
25 ± 0.5	100(102 ± 3)	45.06 ± 1.19	55.28 ± 10.57
30 ± 0.5	100(102 ± 3)	55.67 ± 17.11	45.75 ± 31.7
33.5 ± 0.5	100(102 ± 3)	31.17 ± 14.63	0
25 ± 0.5	120(121 ± 3)	36.33 ± 6.23	40 ± 3.69
30 ± 0.5	120(121 ± 3)	27.84 ± 2.14	66.6 ± 25.27
33.5 ± 0.5	120(121 ± 3)	43.00 ± 16.64	0

Appendix 2i

Offsprings per brood of sexual and asexual species of *Artemia* with different quality and quantity of feed

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ±3	168.96 ±42.83	93.87 ±18.19	75.00 ± 29.96	70 ±15.7
CHL	80(80 ± 2)	31 ±3	95.21 ±19.82	45.73 ±19.11	37.00 ± 12.02	33.4 ±2.58
RBRAN	80(80 ± 2)	29 ±2	66.06 ±3.10	61.82 ±5.79	33.00 ± 8.89	32.6 ±6.5
UNFED	80(80 ± 2)	31 ±3	61.00 ±7.00		34.06 ± 0	
ISO	100(102 ± 3)	31 ±3	130.17 ±16.50	61.83 ±23.25	67.97 ± 11.19	66.9 ±16.5
CHL	100(102 ± 3)	31 ±3	81.27 ±28.31	65.50 ±4.95	47.47 ± 18.17	48.9 ±24.5
RBRAN	100(102 ± 3)	31 ±3	62.78 ±12.07	65.26 ±22.74	32.95 ± 21.85	48.8 ±2
UNFED	100(102 ± 3)	31 ±3	73.63 ±13.96		1.77 ± 0	
ISO	120(121 ± 3)	30 ±2	72.54 ±11.34	21.67 ±3.98	44.38 ± 12.41	29.6 ±5.96
CHL	120(121 ± 3)	30 ±2	29.33 ±9.24	19.58 ±8.18	31.00 ± 6.50	16.6 ±5.58
RBRAN	120(121 ± 3)	31 ±3	38.79 ±11.03	62.10 ±1.83	6.28 ± 0	34.2 ±4.17
UNFED	120(121 ± 3)	30 ±2	0.00		0.00	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 2j

Total offsprings per female of sexual and asexual species of *Artemia* at different salinities

Salinity (ppt)	Ambient temperature (°C)	Sexual	Asexual
20 (20 ± 3)	27 ±2	1041.67 ± 450.73	126.66 ± 94.07
45 (46 ± 3)	27 ±2	1395.00 ± 161.08	501.5 ± 98.29
80 (80 ± 2)	31 ±3	508.33 ± 371.12	118 ± 32.5
100(102 ± 3)	31 ±3	820.00 ± 12.73	211.5 ± 76.66
120(121 ± 3)	31 ±2	89.00 ± 47.84	33 ± 10.58
145(145 ± 4)	30 ±3	111.67 ± 19.66	53.67 ± 40.2
170(169 ± 4)	30 ±3	93 ± 7.21	49.33 ± 11.02
195(195 ± 3)	30 ±3	98.25 ± 35.26	81 ± 17.45

Appendix 2k

Total offsprings per female of sexual and asexual species of *Artemia* at different temperature and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	401.67 ± 111.11	358.33 ± 139.1
30 ± 0.5	80(80 ± 2)	457.67 ± 79.78	173.33 ± 72.28
31 ± 3	80(80 ± 2)	508.33 ± 371.12	118 ± 32.5
33.5 ± 0.5	80(80 ± 2)	124.00 ± 14.14	0
25 ± 0.5	100(102 ± 3)	374.67 ± 62.61	394 ± 152.03
30 ± 0.5	100(102 ± 3)	528.00 ± 301.08	203 ± 133.83
31 ± 3	100(102 ± 3)	820.00 ± 12.73	211.5 ± 76.66
33.5 ± 0.5	100(102 ± 3)	98.00 ± 79.73	0
25 ± 0.5	120(121 ± 3)	361.00 ± 64.47	329.67 ± 29.57
30 ± 0.5	120(121 ± 3)	403.00 ± 11.31	187 ± 79.67
31 ± 3	120(121 ± 3)	89.00 ± 47.84	33 ± 10.58
33.5 ± 0.5	120(121 ± 3)	63.67 ± 28.54	0

Appendix 2l

Total offsprings per female of sexual and asexual species of *Artemia* with different quality and quantity of feed

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ±3	1034.00 ±671.08	588.33 ±355.44	583.00 ± 213.16	273.67 ± 156.00
CHL	80(80 ± 2)	31 ±3	1027.00 ±183.39	508.33 ±371.12	188.00 ± 185.26	118.00 ± 32.53
RBRAN	80(80 ± 2)	29 ±2	360.00 ±123.04	449.67 ±122.68	72.00 ± 53.03	121.00 ± 91.90
UNFED	80(80 ± 2)	31 ±3	81.33 ±35.91		19.67 ± 34.06	
ISO	100(102 ± 3)	31 ±3	851.00 ±105.50	315.33 ±51.54	347.30 ± 125.00	417.67 ± 107.90
CHL	100(102 ± 3)	31 ±3	795.33 ±479.93	820.00 ±12.73	156.67 ± 54.24	211.50 ± 75.66
RBRAN	100(102 ± 3)	31 ±3	472.00 ±181.41	352.33 ±203.97	138.50 ± 146.30	292.50 ± 12.02
UNFED	100(102 ± 3)	31 ±3	219.67 ±142.22		181.00 ± 7.00	
ISO	120(121 ± 3)	30 ±2	1037.00 ±138.92	204.67 ±57.76	116.75 ± 57.56	114.67 ± 65.16
CHL	120(121 ± 3)	30 ±2	205.33 ±64.69	89.00 ±47.84	51.67 ± 22.12	33.00 ± 10.58
RBRAN	120(121 ± 3)	31 ±3	240.00 ±103.33	310.50 ±9.19	62.50 ± 33.57	109.75 ± 31.03
UNFED	120(121 ± 3)	30 ±2	0		0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 3a

Cysts per female of sexual and asexual species of Artemia at different salinities

Salinity (ppt)	Ambient temperature(°C)	Sexual	Asexual
20(20 ± 3)	27 ± 2	*	*
45 (46 ± 3)	27 ± 2	0.69	*
80 (80 ± 2)	31 ± 3	178.67 ± 91.500	*
100(102 ± 3)	31 ± 3	51.50 ± 50.205	9 ± 15.6
120(121 ± 3)	31 ± 2	20.67 ± 27.592	*
145(145 ± 4)	30 ± 3	68.66 ± 22.230	*
170(169 ± 4)	30 ± 3	82.67 ± 17.160	13 ± 22.52
195(195 ± 3)	30 ± 3	90.25 ± 29.770	*

* No cyst production

Appendix 3b

Cysts per female of sexual and asexual species of *Artemia* at different temperatures and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	136.00 ± 171.02	186 ± 161.08
30 ± 0.5	80(80 ± 2)	17.67 ± 19.14	0
31 ± 2	80(80 ± 2)	178.67 ± 91.50	0
33.5 ± 0.5	80(80 ± 2)	0.00	0
25 ± 0.5	100(102 ± 3)	152.33 ± 115.02	295.5 ± 120.91
30 ± 0.5	100(102 ± 3)	0.00	0
31 ± 2	100(102 ± 3)	51.50 ± 50.21	9 ± 15.6
33.5 ± 0.5	100(102 ± 3)	0.00	0
25 ± 0.5	120(121 ± 3)	81.00 ± 70.15	278.67 ± 25.5
30 ± 0.5	120(121 ± 3)	0.00	0
31 ± 2	120(121 ± 3)	20.67 ± 27.59	0
33.5 ± 0.5	120(121 ± 3)	12.00 ± 20.78	0

Appendix 3c

Cysts per female of sexual and asexual species of *Artemia* with different quality and quantity of feeds

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ±2)	31 ±3	0	126.00 ± 66.7	0	0
CHL	80(80 ±2)	31 ±3	47.67 ±48.00	178.67 ± 91.5	10.3 ± 17.9	0
RBRAN	80(80 ±2)	29 ±2	0	0	0	0
UNFED	80(80 ±2)	31 ±3	0		0	
ISO	100(102 ±3)	31 ±3	70.33 ±121.82	55.00 ± 48.2	0	14.7 ± 25.400
CHL	100(102 ±3)	31 ±3	10.00 ±17.32	51.50 ± 50.2	0	13.5 ± 19.090
RBRAN	100(102 ±3)	31 ±3	0	0	0	0
UNFED	100(102 ±3)	31 ±3	0		0	
ISO	120(121 ±3)	30 ±2	4.67 ±4.04	0.00	0	0
CHL	120(121 ±3)	30 ±2	15.67 ±27.14	20.67	8.66 ± 15.1	0
RBRAN	120(121 ±3)	31 ±3	0.00	0	0	0
UNFED	120(121 ±3)	30 ±2	0		0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 3d

Nauplii per female of sexual and asexual species of *Artemia* at different Salinities

Salinity (ppt)	Ambient temperature(^{o}C)	Sexual	Asexual
20(20 ± 3)	27 ± 2	1041.67 ± 450.731	126.66 ± 94.07
45 (46 ± 3)	27 ± 2	1395.00 ± 161.081	501.5 ± 98.29
80 (80 ± 2)	31 ± 3	329.67 ± 280.022	501.5 ± 98.29
100(102 ± 3)	31 ± 3	820.00 ± 12.728	198 ± 94.7
120(121 ± 3)	31 ± 2	68.33 ± 22.591	33 ± 10.58
145(145 ± 4)	30 ± 3	43.33 ± 3.210	53.67 ± 40.2
170(169 ± 4)	30 ± 3	12.33 ± 10.790	36.33 ± 14.57
195(195 ± 3)	30 ± 3	8 ± 16.000	81 ± 17.45

Appendix 3e

Nauplii per female of sexual and asexual species of *Artemia* at different temperature and salinities

Temperature (^{o}C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	265.67 ± 241.33	172.33 ± 23.8
30 ± 0.5	80(80 ± 2)	440.00 ± 68.55	173.33 ± 72.3
31 ± 2	80(80 ± 2)	329.67 ± 280.02	501.5 ± 98.29
33.5 ± 0.5	80(80 ± 2)	124.00 ± 14.14	0
25 ± 0.5	100(102 ± 3)	222.33 ± 116.34	99 ± 31.11
30 ± 0.5	100(102 ± 3)	528.00 ± 301.08	203 ± 133.8
31 ± 2	100(102 ± 3)	820.00 ± 12.73	198 ± 94.7
33.5 ± 0.5	100(102 ± 3)	98.00 ± 79.73	0
25 ± 0.5	120(121 ± 3)	280.00 ± 119.88	51 ± 4.36
30 ± 0.5	120(121 ± 3)	403.00 ± 11.31	187 ± 79.67
31 ± 2	120(121 ± 3)	68.33 ± 22.59	33 ± 10.58
33.5 ± 0.5	120(121 ± 3)	51.67 ± 47.35	0

Appendix 3f

Nauplii per female of sexual and asexual species of *Artemia* with different quality and quantity of feeds

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	1034.00 ± 671.08	462.33 ± 316.00	583.00 ± 213.16	273.67 ± 156.10
CHL	80(80 ± 2)	31 ± 3	979.33 ± 141.74	329.67 ± 280.02	172.50 ± 163.34	118 ± 26.56
RBRAN	80(80 ± 2)	29 ± 2	360.00 ± 123.04	449.67 ± 122.68	72.00 ± 53.03	121 ± 91.92
UNFED	80(80 ± 2)	31 ± 3	81.33 ± 35.91		19.67 ± 34.06	
ISO	100(102 ± 3)	31 ± 3	780.67 ± 60.04	260.33 ± 20.40	347.30 ± 125.00	403 ± 106.80
CHL	100(102 ± 3)	31 ± 3	785.33 ± 469.39	820.00 ± 12.73	515.60 ± 39.70	198 ± 94.75
RBRAN	100(102 ± 3)	31 ± 3	472.00 ± 181.41	352.33 ± 203.97	138.50 ± 146.37	292.5 ± 12.02
UNFED	100(102 ± 3)	31 ± 3	219.67 ± 142.22		181.00 ± 7.00	
ISO	120(121 ± 3)	30 ± 2	1032.33 ± 142.96	204.67 ± 57.76	116.75 ± 57.56	114.67 ± 65.16
CHL	120(121 ± 3)	30 ± 2	189.67 ± 76.97	68.33 ± 22.59	516.06 ± 30.80	33 ± 10.58
RBRAN	120(121 ± 3)	31 ± 3	240.00 ± 103.50	310.50 ± 9.19	62.50 ± 33.57	109.73 ± 31.03
UNFED	120(121 ± 3)	30 ± 2			0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 3g

Percentage encystment of sexual and asexual species of *Artemia* at different salinities

Salinity (ppt)	Ambient temperature (°C)	Sexual	Asexual
20(20 ± 3)	27 ± 2	*	*
45 (46 ± 3)	27 ± 2	0.53	*
80 (80 ± 2)	31 ± 3	41.29 ± 13.27	*
100(102 ± 3)	31 ± 3	6.24 ± 6.01	5.67
120(121 ± 3)	31 ± 2	16.80 ± 18.18	*
145(145 ± 4)	30 ± 3	59.9 ± 11.01	*
170(169 ± 4)	30 ± 3	89.65 ± 11.67	*
195(195 ± 3)	30 ± 3	93.7 ± 12.60	*

* There was no cyst formation at these conditions

Appendix 3h

Percentage encystment of sexual and asexual species of *Artemia* at different temperatures and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	37.92 ± 45.04	34.1 ± 32.53
30 ± 0.5	80(80 ± 2)	3.63 ± 3.51	0
31 ± 2	80(80 ± 2)	41.29 ± 13.27	0
33.5 ± 0.5	80(80 ± 2)	0	0
25 ± 0.5	100(102 ± 3)	40.27 ± 32.35	74.5 ± 1.98
30 ± 0.5	100(102 ± 3)	0.00 ± 0.00	0
31 ± 2	100(102 ± 3)	6.24 ± 6.01	5.67
33.5 ± 0.5	100(102 ± 3)	0	0
25 ± 0.5	120(121 ± 3)	24.45 ± 21.87	84.5 ± 0.46
30 ± 0.5	120(121 ± 3)	0	0
31 ± 2	120(121 ± 3)	16.80 ± 18.18	0
33.5 ± 0.5	120(121 ± 3)	33.33 ± 57.74	0

Appendix 3i

Percentage encystment of sexual and asexual species of *Artemia* with different quality and quantity of feeds

Feed	Salinity (ppt)	Ambient Temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	0.00 ± 0.00	26.93 ± 15.27	0	0
CHL	80(80 ± 2)	31 ± 3	4.17 ± 4.15	41.29 ± 13.27	0.03	0
RBRAN	80(80 ± 2)	29 ± 2	0	0	0	0
UNFED	80(80 ± 2)	31 ± 3	0			
ISO	100(102 ± 3)	31 ± 3	9.67 ± 16.74	16.14 ± 13.98	0	3.3
CHL	100(102 ± 3)	31 ± 3	0.87 ± 1.50	6.24 ± 6.01	0	8.5 ± 12.020
RBRAN	100(102 ± 3)	31 ± 3	0	0	0	0
UNFED	100(102 ± 3)	31 ± 3	0			
ISO	120(121 ± 3)	30 ± 2	0.00 ± 0.00	0.00 ± 0.00	0	0
CHL	120(121 ± 3)	30 ± 2	8.50 ± 14.72	17.01 ± 18.14	17.7 ±3	0
RBRAN	120(121 ± 3)	31 ± 3	0	0	0	0
UNFED	120(121 ± 3)	30 ± 2	0		0	

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 3j

Percentage encystment of sexual and asexual species of *Artemia* at different photoperiodic conditions				
Photo period (hours)	Temperature (°C)	Salinity (ppt)	Sexual	Asexual
12:12 D:L	25	102 ± 3	0	13 ± 18.38
24:0 D:L	25	102 ± 3	56.18 ± 18.63	50 ± 70.71
12:12 D:L	Ambient temperature 31±2	102 ± 3	27.30 ± 3.98	0
24:0 D:L	31±2	102 ± 3	2.65 ± 4.58	0

Appendix 4a

Cyst size of sexual and asexual species of *Artemia* at different salinities			
Salinity (ppt)	Ambient temperature (°C)	Sexual	Asexual
20(20 ± 3)	27 ± 2	*	*
45 (46 ± 3)	27 ± 2	222.24	*
80 (80 ± 2)	31 ± 3	232.00 ± 6.93	*
100(102 ± 3)	31 ± 3	249.93 ± 0.00	222.16
120(121 ± 3)	31 ± 2	227.48 ± 0.33	*
145(145 ± 4)	30 ± 3	235.36 ± 40.83	*
170(169 ± 4)	30 ± 3	226.81 ± 9.29	244.46
195(195 ± 3)	30 ± 3	236.35 ± 6.54	*

* No cyst production

Appendix 4b

Cyst size of sexual and asexual species of *Artemia* species at different temperature and salinities

Temperature (°C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	237.00 ± 0	224.02 ± 5.9
30 ± 0.5	80(80 ± 2)	216.60 ± 5.56	*
33.5 ± 0.5	80(80 ± 2)	*	*
31 ± 3	80(80 ± 2)	232.00 ± 6.93	*
25 ± 0.5	100(102 ± 3)	237.43 ± 43.29	250.14 ± 0.69
30 ± 0.5	100(102 ± 3)	*	*
33.5 ± 0.5	100(102 ± 3)	*	*
31 ± 3	100(102 ± 3)	249.93	222.16
25 ± 0.5	120(121 ± 3)	224.38 ± 3.04	252.44 ± 2.97
30 ± 0.5	120(121 ± 3)	*	*
33.5 ± 0.5	120(121 ± 3)	233.35	*
31 ± 3	120(121 ± 3)	227.48 ± 0.33	*

* Cysts were not produced at these conditions

Appendix 4c

Cyst size of sexual and asexual species of *Artemia* with different qualities and quantities of feeds

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	*	225.86 ± 3.2	*	*
CHL	80(80 ± 2)	31 ± 3	222.16	232.00 ± 6.93	*	*
RBRAN	80(80 ± 2)	29 ± 2	*	*	*	*
UNFED	80(80 ± 2)	31 ± 3	*		*	
ISO	100(102 ± 3)	31 ± 3	216.61	231.25 ± 12.9	*	277.70
CHL	100(102 ± 3)	31 ± 3	242.99	249.93	*	222.16
RBRAN	100(102 ± 3)	31 ± 3	*	*	*	*
UNFED	100(102 ± 3)	31 ± 3	*		*	
ISO	120(121 ± 3)	30 ± 2	222.24	*	*	*
CHL	120(121 ± 3)	30 ± 2	227.76	227.73	233.27	*
RBRAN	120(121 ± 3)	31 ± 3	*	*	*	*
UNFED	120(121 ± 3)	30 ± 2	*		*	

*Cysts were not produced at this conditions

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 4d

Nauplii size of sexual and asexual species of Artemia at different salinities

Salinity (ppt)	Ambient temperature(^{0}C)	Sexual	Asexual
20(20 ± 3)	27 ± 2	491.13 ± 22.29	518.37 ± 16.03
45 (46 ± 3)	27 ± 2	517.29 ± 30.45	523.47 ± 0
80 (80 ± 2)	31 ± 3	538.97 ± 0.32	513.39 ± 4.4
100(102 ± 3)	31 ± 3	528.10 ± 0.65	544.29 ± 3.9
120(121 ± 3)	31 ± 2	513.79 ± 43.26	533.19 ± 1.86
145(145 ± 4)	30 ± 3	498.87 ± 40.97	505.9 ± 9.64
170(169 ± 4)	30 ± 3	507.05 ± 4.31	516.16 ± 25.35
195(195 ± 3)	30 ± 3	535.5 ± 34.65	517.17 ± 18.24

Appendix 4e

Nauplii size of sexual and asexual species of Artemia at different temperature and salinities

Temperature (^{0}C)	Salinity (ppt)	Sexual	Asexual
25 ± 0.5	80(80 ± 2)	523.16 ± 15.28	544.09 ± 26.42
30 ± 0.5	80(80 ± 2)	502.61 ± 16.38	517.26 ± 32.28
31 ± 2	80(80 ± 2)	538.97 ± 0.33	513.39 ± 4.4
33.5 ± 0.5	80(80 ± 2)	472.26 ± 0.00	
25 ± 0.5	100(102 ± 3)	522.86 ± 25.66	541.52 ± 19.64
30 ± 0.5	100(102 ± 3)	482.95 ± 28.40	526.55 ± 12.2
31 ± 2	100(102 ± 3)	528.10 ± 0.66	544.29 ± 3.9
33.5 ± 0.5	100(102 ± 3)	478.28 ± 4.58	
25 ± 0.5	120(121 ± 3)	511.52 ± 14.21	533.8 ± 29.76
30 ± 0.5	120(121 ± 3)	494.90 ± 31.88	518.76 ± 12
31 ± 2	120(121 ± 3)	513.79 ± 43.27	533.19 ± 1.86
33.5 ± 0.5	120(121 ± 3)	481.52 ± 0.00	

Appendix 4f

Nauplii size of sexual and asexual species of *Artemia* with different quality and quantity of feeds

Feed	Salinity (ppt)	Ambient temperature (°C)	Sexual H	Sexual L	Asexual H	Asexual L
ISO	80(80 ± 2)	31 ± 3	494.77 ± 10.42	503.10 ± 12.18	516.82 ± 7.32	521.6 ±5.600
CHL	80(80 ± 2)	31 ± 3	526.96 ± 5.72	538.97 ± 0.33	513.75	513.4 ±4.410
BRAN	80(80 ± 2)	29 ± 2	511.16 ± 31.43	513.42 ± 0.56	498.70	487.4 ±8.510
NFED	80(80 ± 2)	31 ± 3	*		529.63	
ISO	100(102 ± 3)	31 ± 3	508.66 ± 0.65	515.29 ± 27.29	510.65 ± 3.1	518.4 ±22.200
CHL	100(102 ± 3)	31 ± 3	521.41 ± 5.49	528.10 ± 0.66	515.60 ± 39.7	545.7 ±1.970
BRAN	100(102 ± 3)	31 ± 3	479.87 ± 23.23	442.47 ± 41.68	498.47 ± 31.42	508.9 ±0.000
NFED	100(102 ± 3)	31 ± 3	470.24 ± 13.98		542.09 ± 5.7	
ISO	120(121 ± 3)	30 ± 2	518.13 ± 13.33	521.78 ± 4.58	480.42 ± 24.53	489.7 ±15.790
CHL	120(121 ± 3)	30 ± 2	527.16 ± 23.90	518.30 ± 31.50	516.06 ± 30.8	533.1 ±7.860
BRAN	120(121 ± 3)	31 ± 3	462.80 ± 14.30	482.51 ± 12.76	472.98 ± 11.57	483 ±16.350
NFED	120(121 ± 3)	30 ± 2	*		*	

* Nauplii were not produced at these conditions

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran

Appendix 4g

Size at maturity of sexual and asexual species of *Artemia* at different salinities

Salinity	Ambient temperature	Sexual		Asexual
		Female	Male	
20(20 ± 3)	27 ± 2	7.7 ± 0.57	6.46 ± 1.26	8.17 ± 0.08
45 (46 ± 3)	27 ± 2	7.54 ± 0.28	6.60 ± 0.38	8.16 ± 0
80 (80 ± 2)	31 ± 3	6.82 ± 0.00	6.46 ± 0.00	8.33 ± 0
100(102 ± 3)	31 ± 3	6.32 ± 0.00	6.30 ± 0.00	8 ± 0
120(121 ± 3)	31 ± 2	7.42 ± 1.04	6.55 ± 0.36	8 ± 0
145(145 ± 4)	30 ± 3	8.37 ± 0.41	6.53 ± 1.12	7.8 ± 0.79
170(169 ± 4)	30 ± 3	7.11 ± 0.30	6.46 ± 0.00	9.91 ± 0
195(195 ± 3)	30 ± 3	*	*	*

* females died before reaching maturity

Appendix 4h

Size at maturity of sexual and asexual species of *Artemia* at different temperature and salinities

Temp. (°C)	Salinity (ppt)	Sexual female	Sexual male	Asexual
25± 0.5	80(80 ± 2)	7.75 ± 0.38	5.60 ± 0.81	6.9
30± 0.5	80(80 ± 2)	7.94 ± 0.81	6.97 ± 0.30	9.19
33.5± 0.5	80(80 ± 2)	5.75 ± 0.81	5.85 ± 0.04	9.62
25± 0.5	100(102 ± 3)	7.46 ± 0.20	6.61 ± 0.81	7.13 ± 0.92
30± 0.5	100(102 ± 3)	7.51 ± 0.74	6.46 ± 0.20	9.38 ± 0.46
33.5± 0.5	100(102 ± 3)	6.41 ± 0.93	5.87 ± 5.87	8.04
25± 0.5	120(121 ± 3)	6.68 ± 0.51	5.60 ± 0.20	7.04 ± 0.21
30± 0.5	120(121 ± 3)	8.47 ± 1.02	7.61 ± 7.61	9.38 ± 0.22
33.5± 0.5	120(121 ± 3)	6.32 ± 0.61	5.96 ± 0.91	*

*Female died before reaching maturity

Appendix 4i

e at maturity of sexual and asexual species of *Artemia* species with different quality and quantity of feeds

eed	Salinity (ppt)	Ambient temperature (°C)	Sexual H Female	Sexual H Male	Sexual L Female	Sexual L Male	Asexual H	Asexual L
SO	80(80 ±2)	31 ± 3	11.7 ± 0.7	6.96 ± 0.92	9.76	8.33	9.91	8.55 ±0.71
HL	80(80 ±2)	31 ± 3	8.04	7.75	6.82 ± 0.10	6.46	8.47	8.33
RAN	80(80 ±2)	29 ± 2	7.47	6.80	8.04 ± 0.81	6.89	10.52 ± 0.68	10.20
SO	100(102 ±3)	31 ± 3	9.90 ± 1.0	6.97 ± 0.71	8.40 ± 0.03	6.89	8.62 ± 0.81	6.88 ±0.41
HL	100(102 ±3)	31 ± 3	7.76 ± 0.8	7.75 ± 0.81	6.32	6.03	8.18	8.00
RAN	100(102 ±3)	31 ± 3	7.66 ± 0.6	6.80 ± 0.10	7.90 ± 1.01	6.89	10.43 ± 1	9.86 ±0.46
SO	120(121 ±3)	30 ± 2	8.47	7.90	8.30	6.55	9.28 ± 0.6	8.60
HL	120(121 ±3)	30 ± 2	7.51 ± 0.7	6.60 ± 0.81	7.41 ± 1.04	6.55	8	8.00
RAN	120(121 ±3)	31 ± 3	7.32 ± 0.6	6.46	7.71 ± 0.65	7.18	11.34	8.90 ±0.41

ISO – *Isochrisis* sp., CHL – *Chlorella* sp., RBRAN – Rice bran