# MATERIALS AND METHODS

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# Chapter 3

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## 3. 1 Field collections

The specimens for the present study were collected from seasonal rain fed temporary and permanent water bodies of different localities of Thrissur district, Kerala. The collections were made between 7 and 8 a.m. during the period from June 2003 to May 2006.

Water temperature was measured using a mercury thermometer and the hydrogen ion concentration (pH) with a digital pH meter. The water samples were collected in BOD bottles, fixed on the spot and the dissolved oxygen was estimated following Winkler's method (APHA, 1995).

### 3. 1. 1 Collection Sites

Collections for the present study were made from the following localities in Thrissur district.

**Site 1:** This is a permanent man-made pond located near Poomkunnam fly-over at Patturakkal in Thrissur town, well protected by walls on all sides with an area of 0.12 $km^2$. The pond is being used by local people for washing and bathing. Nearly half of the pond contained aquatic macrophytes like *Utricularia, Hydrilla*, and *Pistia.* The collections were made on 5$^{th}$ March 2004 and cladocerans collected from the pond included *Moina micrura, Chydorus sphaericus* and *Diaphanosoma sarsi.*

**Site 2:** A permanent man-made water body (Vadakkechira pond) located in Thrissur town near North bus stand. The pond has an area of 0.20 $km^2$ and protected on all sides by walls. The pond was partially covered by aquatic macrophytes comprising mainly *Pistia, Salvinia* and *Hydrilla.* The collection was made on 2nd April 2003 and cladocerans collected from the pond included *Latonopsis australis, Oxyurella singalensis, Pseudosida bidentata, Chydorus sphaericus, Ilyocryptus spinifer, Diaphanosoma exciscum* and *Macrothrix spinosa.* The pond was visited by migratory water fowls, especially Common Teal during this period. The other birds such as white-breasted Kingfisher, Little Cormorant and Darter also visited the site. Another collection was also made on 3rd July 2003 and *Daphnia lumholtzi* and *Diaphanosoma sarsi* were abundantly present along with ephippial females and males.

**Site 3:** This is a man-made permanent pond located in Ollur, 5 km away from Thrissur town on the side of NH-47. The pond has an area of 0.14 $km^2$. It is located adjacent to a paddy field and well protected by granite side walls. Local people use this pond for bathing and washing. Aquatic macrophytes were absent and water was green in colour due to the presence of algae like *Chlorella* and *Ankistrodesmus.* The collection was made in 12th August 2003 and cladocerans collected from the pond included *Ceriodaphnia cornuta* and *Moinodaphnia macleayi.*

**Site 4**: It is a temporary water body formed in an abandoned brick-yard with less human interference located at Parappookkara, 17 km away from Thrissur town. It has an area of 0.22 $km^2$. Major aquatic macrophytes included *Salvinia, Hydrilla, Pistia, Nymphea* and *Ipomoea.* The water was turbid at the time of collection. The collection was made on 3rd July 2003 and

cladocerans collected include *Scapholeberis kingi* and *Chydorus sphaericus*. Another collection was also made on 18th November 2003 and cladocerans collected included *Simocephalus serrulatus, Macrothrix spinosa, Macrothrix triserialis, Alona costata* and *Chydorus sphaericus*. Further collection was made on 26th January 2004 when the water body started to dry up and cladocerans collected included *Moina micrura, Macrothrix triserialis* and *Chydorus sphaericus*.

**Site 5:** A shallow man-made pond located at Kalathode, 2.5 km away from Thrissur town. The pond has an area of 0.08 km² and protected on all sides by walls. Aquatic macrophytes were completely absent at the time of collections. The pond is being used by villagers for washing and bathing. Cladocerans collected from this pond in 20th July 2004 included *Ceriodaphnia cornuta* and *Chydorus sphaericus.* The green-coloured water present in the pond was due to dense growth of algae such as *Ankistrodesmus* and *Scenedesmus*.

**Site 6:** A shallow man-made pond (Kottamkulam temple pond) located at Urakam 12 km away from Thrissur town. The pond has an area of 0.10 km² and is adjacent to a laterite hillock which drained water to the pond during monsoon. It is protected on all sides by walls and several people use this pond for bathing and washing. *Nymphea* and *Hydrilla* were present on one side of the pond. The collection was made in 5th October 2005 and the cladocerans collected from the pond included *Moina micrura, Alona pulchella* and *Ephemeroporus barroisi.*

**Site 7:** A temporary water body (Chonkulam) with an area of 0.05 km² located adjacent to a paddy field at Mattathurkunnu near Kodakara, 18 kms

away from Thrissur town. Villagers use this water body for bathing and washing. Major aquatic macrophytes included *Salvinia* and *Pistia.* The collection was done using a scoop net. Major cladocerans collected in 22nd January included *Moina brachiata, Diaphanosoma sarsi, Pseudosida bidentata, Macrothrix triserialis, Macrothrix spinosa* and *Ilyocryptus spinifer* while on 2nd March 2005 included *Moina brachiata, Macrothrix triserialis* and *Macrothrix spinosa.* This pond gets completely dried up in summer. Soil samples were also collected on 8th May 2005 for making trials to hatch out resting eggs present in the sample.

**Site 8**: A man-made pond (Peringamkulam pond) with an area of 0.06 $km^2$ located near Kodakara. Villagers use this pond for washing. Although, several collections were made during 5th June to 18th September 2004, cladocerans were found to be absent. However, *Notonecta* and aquatic mites were abundantly represented in the sample. The surface of the pond was completely covered by *Salvinia* and *Pistia.*

### 3. 1. 2 Collection Methods

The collections were made generally with the help of a tow net made of bolting silk of 70μm mesh size with 20 cm mouth diameter. The mouth of the net was tied to a long rope and dragged over a distance of 5 meters at the sub-surface level. In the meanwhile the live animals got trapped in the bottle tied to one end of the net. Care was taken to drag the net at a uniform speed until it was hauled up. This method is mainly employed for the collection of planktonic species.

Collections were also done using 15 cm wide conical scoop net of 70μm mesh size. By means of a handle the net was worked vigorously in beds

of aquatic macrophytes. This was repeated and animals get collected into a bottle tied to one end of the net. The epiphytic species were collected using this method.

The epiphytic cladocerans were also obtained from the above sites by washing the weeds in a bucket of water. The water was again filtered through the bolting silk of 70μm mesh size to obtain the specimens. The specimens associated with the bottom mud were collected using a scoop net after disturbing the bottom. The collected samples were immediately brought to the laboratory in live condition, animals were sorted out for culture and a portion of the sample was preserved for taxonomic study.

### 3. 1. 3 Preservation and Storage

A portion of the collected sample was preserved in 4-6% formalin and kept in the laboratory. Formalin was found to be suitable for storage because chitin does not become too fragile upon storage. For prolonged storage small amount of sucrose (30-40 g/lit) in 5% formalin was added to prevent carapace distortion and egg-loss from the brood pouch following the methodology of Haney and Hall (1973).

### Preparation of Paraffin Mounts

The formalin preserved animal was transferred to a small drop of glycerin placed at the center of a slide. Soft paraffin was melted and applied as a thin border around the specimen using a scalpel. A coverslip was placed supporting on this; and the slide was warmed over a flame. On warming the slide, the paraffin melts and runs in sealing the mount. This slide was labelled and stored for further study.

### 3.1.4 Identification

Species identification was done using standard keys of Michael and Sharma (1988); Battish (1992); and the monographs of Goulden (1968); Smirnov (1974); Idris (1983); Fryer (1987); Dodson and Frey (1991) and Korovchinsky (1992).

## 3. 2 Laboratory culture

The first step in cladoceran culture is to ensure a reliable food source. Unicellular algae especially *Chlorella* has been used as food for freshwater cladocerans in laboratory culture and is known to support growth and reproduction (Nandini and Sarma, 2000). The availability of unicellular algae is required for the successful culture of cladocerans.

### 3. 2. 1 Preparation of Algal culture

An algal stock culture was prepared and maintained in the laboratory to ensure a ready availability of unicellular algae. Water collected from Site 3 containing *Chlorella* sps. was filtered through bolting silk of 70µm mesh size and transferred into an aquarium (40 cm × 20 cm × 20 cm). This water was given a continuous aeration and kept under fluorescent light. Two Gold fishes (*Carassius* sp.) were introduced into this medium so as to maintain the trophic system within the aquarium. These fishes were fed with food pellets available in the market. The algal culture flourished and attained a dark green colour after 15 days. The side walls of the aquarium were always kept clean to ensure sufficient penetration of light. For maintaining this culture for a long period the fish was fed daily, detritus was siphoned out from the bottom and the culture medium was replenished daily by adding aerated-water.

**Culture of *Chlorella***

Unicellular algae especially *Chlorella* forms one of the most important food items that can be easily ingested by most of the cladocerans. For developing a culture, the sample taken from the stock culture was centrifuged and algal cells were isolated carefully under a stereoscopic microscope using a micropipette. The isolated *Chlorella* cells were inoculated into the culture medium.

The culture medium was prepared using tap-water aerated for a period of 5 to 6 days; nourished with finely powdered groundnut cake at a rate of 500 mg/litre (Nayar and Babu, 2002). A mixture of super-phosphate (200 mg /litre) and urea (100 mg/litre) was added to this medium as an additional nutrient. The isolated live *Chlorella* cells were inoculated separately into three separate glass jars (30 cm × 15 cm × 15 cm) containing the medium. The inoculum was continuously aerated and kept in the laboratory under fluorescent light of uniform illumination (500 lux). This was again enriched by the addition of 20 ml of the same nutrient medium on every alternate day. James *et al.* (1988) have reported a considerably higher algal production when the medium is slightly acidic (pH 6.5) and the same condition was maintained by adding suitable buffer capsules. The culture was examined on alternate days until the appearance of a dark green colour and the *Chlorella* density was estimated using a haemocytometer. For maintaining this culture, a portion of the culture medium was replaced every day and replenished with the same quantity of water. For prolonged storage without contamination the culture was centrifuged and subsequently transferred to test tubes plugged with cotton. These test tubes were placed in a refrigerator at 10-15°C. It was again re-suspended in water when required for routine feeding.

### 3. 2. 2 General Rearing Methods

Cladocerans for the biological studies were collected from the field as mentioned in section 3. 1. 2 and reared in laboratory.

**(1)** ***Pseudosida bidentata*** Herrick, 1884 **var.** ***szalayi*** (Daday, 1898)

This species was obtained from the collection made at Site 7, on 22nd January 2005 when the water temperature was 26°C, pH 7.5 and dissolved oxygen 6.8 mg/litre. The collected population of *P. bidentata* comprised of parthenogenetic females. A stock culture of this species was developed by isolating the parthenogenetic egg-bearing females from this sample. The culture was maintained in the laboratory upto October 2005 for detailed studies on their life cycle.

**(2)** ***Latonopsis australis*** Sars, 1888

This species was obtained from among the littoral weeds at Site- 2 from the collection made on 2nd April 2003 when the water temperature was 29°C, pH 7.2 and dissolved oxygen 6.6 mg/ litre. The collected population of *L. australis* comprised of only parthenogenetic females. The live specimens were brought to the laboratory and egg-bearing females were isolated for developing a stock culture, which was maintained in the laboratory upto August 2003 for detailed studies on the life cycle.

**(3)** ***Diaphanosoma sarsi*** Richard, 1895

This species was obtained from the collection made at Site 1, on 5th March 2004 when the water temperature was 28°C, pH 6.2 and dissolved oxygen 4.4 mg/ litre. A good number of adult parthenogenetic females and

ephippial females were present in the sample. A stock culture was developed by isolating the egg-bearing females from this sample. The stock culture was maintained in the laboratory upto November 2004 for life cycle studies.

**(4) *Ceriodaphnia cornuta*** Sars, 1885

This species was obtained from the collection made at Site 5, on 20th July 2004 when the water temperature was 24°C, pH 6.8 and dissolved oxygen 6.1 mg/ litre. The population of *C. cornuta* comprised only parthenogenetic females. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory upto February 2005 for the detailed study of life cycle.

**(5) *Scapholeberis kingi*** Sars, 1903

*S. kingi* formed a compact aggregation on the surface film at Site 4 and were collected using a scoop net on 3rd July 2003 when the water temperature was 24.5°C, pH 6.0 and dissolved oxygen 7.2 mg/ litre. The aggregation comprised only parthenogenetic females. A stock culture was developed by isolating the egg-bearing females from this sample and was maintained in the laboratory upto February 2004 for life cycle studies.

**(6) *Simocephalus serrulatus*** (Koch, 1841)

This species was obtained from the collection made at Site 4, on 18th November 2003 when the water temperature was 25°C, pH 5.8 and dissolved oxygen 5.6 mg/litre. The aquatic plants were collected from the site and rinsed in water to collect the specimens into a bottle. The collected population of *S. serrulatus* comprised only parthenogenetic females. A stock culture was

developed by isolating the species from this sample and was maintained in the laboratory from upto May 2005 for detailed studies on life cycle.

(7) ***Moina brachiata*** (Jurine, 1820)

The top soil samples were collected from the Site 7, when the pond got dried up in May 2005. The soil sample was passed through proper sieves to remove the algal matter and other unwanted particles. This sample was dried and stored in polythene packets.

Two litres of the culture medium containing algae was transferred into a glass jar and kept it under mild aeration. A portion of the stored soil sample was introduced into this culture medium and kept in laboratory at 28-30°C, pH 6.8 and dissolved oxygen 7.6 mg/litre. Further, 100 ml of algal culture was added on every alternate day. On 6$^{th}$ day the neonates of *M. brachiata* appeared in the culture. These neonates were sorted out and reared to maturity in separate glass vessels. This was kept as stock culture and maintained in the laboratory upto December 2005 for life cycle studies.

(8) ***Moinodaphnia macleayi*** (King, 1853)

*M. macleayi* was obtained from the collection made at Site 3, on 12$^{th}$ August 2003 when the water temperature was 25 °C, pH 6.5 and dissolved oxygen 5.4 mg/ litre. The collected *M. macleayi* population comprised only parthenogenetic females. A stock culture was developed by isolating the species from this sample, in which a good number of males and ephippial females were developed. The stock culture was maintained in the laboratory upto December 2003 for detailed studies on the life cycle of female.

**(9) *Ilyocryptus spinifer*** Herrick, 1882

This species was obtained from the collection made at Site 7, on 22nd January 2005 using a scoop net when the water temperature was 26°C, pH 7.5 and dissolved oxygen 6.8 mg/ litre. The *I. spinifer* population collected from the site comprised only of parthenogenetic females. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory upto November 2005 for detailed studies on the life cycle.

**(10) *Macrothrix triserialis*** (Brady, 1886)

*M. triserialis* was obtained from the collection made at Site 4, on 18th November 2003 when the water temperature was 25°C, pH 6.0 and dissolved oxygen 5.9 mg/ litre. The aquatic plants were collected from the site and rinsed in water to collect the specimens into a bottle. The collected *M. triserialis* population comprised only parthenogenetic females. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory upto January 2005 for detailed studies on the life cycle.

**(11) *Alona pulchella* King, 1853**

*A. pulchella* was obtained from the collection made at Site 6, on 5th October 2005 using a scoop net, when water temperature was 26°C, pH 5.6 and dissolved oxygen 4.2 mg/ litre. A good number of adult parthenogenetic females were present. A stock culture was developed by isolating the egg-bearing females from this sample and was maintained in the laboratory upto May 2006.

**(12) *Oxyurella singalensis*** (Daday, 1898)

This species was obtained from the collection made at Site 2, on 2nd April 2003 using a scoop net when the water temperature was 29°C, pH 7.2 and dissolved oxygen 6.6 mg/ litre. A good number of adult parthenogenetic females were present in the collection. A stock culture was developed by isolating the species from this sample and was maintained in the laboratory up to October 2003.

**Cladoceran Stock culture**

The stock culture of the above species was developed under laboratory conditions using the following methodology.

Ten egg-bearing parthenogenetic females of a particular species were sorted out from the field sample using a sterilized pipette under a stereoscopic microscope. These adult females were immediately transferred into a beaker containing filtered pondwater and kept undisturbed for 2-3 days to enable them acclimatize the laboratory conditions.

The egg bearing females were again transferred into an aquarium (40 cm × 20 cm × 20 cm) containing culture medium, prepared by adding finely powdered groundnut cake (500 mg/litre) into 4 litres of aerated tap-water. A mild aeration was given throughout the culture period and kept it in the laboratory. *Chlorella* culture (100 ml) was added daily to ensure a sufficient supply of food and to compensate the water lost by evaporation. The culture was examined periodically. When the culture becomes overcrowded, about one tenth of this stock culture was daily removed to reduce the population density and replenished with an equal amount of water. By this periodic

removal and renewal of water the stock culture could be maintained for a longer duration.

**Ephippium collection**

The stock culture was kept under constant observation to find out the appearance of ephippial females. When ephippial females appeared, they were carefully collected with the help of a brush and were transferred to separate petri-dishes containing fresh culture medium for further study. Periodic examination is required for the collection of ephippia cast off by these females. The ephippia were collected, dried and stored without damage.

### 3. 2. 3 Rearing methods for Life cycle studies

**Parthenogenetic females**

The egg-bearing parthenogenetic females were isolated from the stock culture and introduced individually into beakers containing 100 ml of culture medium. The adult females were removed from the culture soon after the release of the first generation of neonates. These neonates were again reared to maturity in fresh medium until they released the second generation.

Immediately after the release, twenty neonates were individually sorted out and reared in separate glass vessels. Cladocerans such as *P. bidentata, L. australis, D. sarsi, C. cornuta, S. serrulatus, M. brachiata, M. macleayi, M. triserialis* and *O. singalensis* were cultured in test tubes of 10 ml capacity while *S. kingi, I. spinifer* and *A. pulchella* were cultured in petri-dishes of the same capacity.

Each individual was supplied with *Chlorella* ($2 \times 10^6$ cells $ml^{-1}$) at an

interval of 24 hrs while changing the culture medium. An equal quantity of the algal medium was added to each test tube. The medium change was necessary to ensure sufficient food as well as for the removal of neonates and the exuvia cast off during moulting. All the studies were made in the laboratory where the water temperature varied from 26-30°C and pH 6.2 to 6.8. All the culture tubes were kept partially immersed in another tray of water to minimize temperature fluctuations.

The moulting, instar duration, egg production, embryonic development, life cycle and life span were individually recorded until death of the last individual. The size measurements were done in each instar to record the growth, the details of which are given in section 3.3. 3.

All the twenty neonates for life cycle studies were reared individually as mentioned above, while another set was also reared simultaneously providing the same conditions for dissections and identification of developmental stages.

**Culture of males**

The stock culture was examined periodically to find out the appearance of males. When males made their appearance 20 neonates of less than 12 hrs of age were sorted out. Males could be identified mostly based on morphological features and their relative size.

The life cycle was studied by rearing them under the same laboratory conditions provided for the culture of females as mentioned above. The size measurements were done daily and the growth was recorded until death of each individual. The life cycle of four cladoceran males such as *Pseudosida*

*bidentata, Moinodaphnia macleayi, Macrothrix triserialis* and *Oxyurella singalensis* were studied.

### 3. 3 Studies on individual species

The following aspects were studied by culturing each species under similar laboratory conditions.

### 3. 3. 1 Morphology

The general morphology of the three different morphotypes viz. the parthenogenetic female, ephippial female and male were studied under a microscope based on temporary mounts made in glycerol. The characters studied included the body form and size, head morphology, nature of postabdomen, setation in antennae, antennule and the shell.

The dissections were done when needed using tungsten micro-needle fitted on handles. Drawings were made using camera lucida and measurements were done using calibrated micrometers. Microphotographs were taken using stereoscopic zoom-microscope fitted with Sony digital camera of 7.2 mega pixels.

### 3. 3. 2 Reproduction and Life cycle

Twenty neonates were sorted out and reared in separate glass vessels as mentioned in section 3. 2. 3. The events during the life cycle were followed by periodic observation until death of the last individual.

The mean value of initial size is taken as Size at Birth (SaB). The stage between hatching of a neonate and the moult preceding the first appearance of

eggs in the brood pouch is considered the pre-adult stage. The mean time required for pre-adult stage is taken as Pre-adult Instar Duration (PID).

The time of moulting was determined by examining for the presence of exuvium in the medium. The developmental stage between successive moults is defined as an instar and the time between successive moults as the instar duration. This includes the time required for egg transfer, embryonic development and release of young ones followed by moulting.

Age at First Reproduction (AFR) is the time taken for the completion of pre-adult moults and the attainment of sexual maturity which is calculated in number of days. The first appearance of mature eggs in the brood pouch is considered the initiation of adulthood and the mean size during this stage is expressed as Size at First Reproduction (SFR). This stage is designated as the primiparous stage and the succeeding instars are considered the adult instars.

The period from birth till the release of first generation of neonates is taken as First Generation Time (FGT), which is calculated by adding the mean duration of pre-adult and the primiparous instar (first egg bearing instar). The Relative Length (RL) and Relative Height (RH) were calculated by dividing Size at Birth (SaB) by Size at First Reproduction (SFR). The mean duration of the adult instar is taken as the mean Adult Instar Duration (AID).

**Egg production**

Egg production in Cladocera is associated with the termination of each adult instar. The number of eggs present in a single brood during an instar is designated as a clutch. The number of eggs/ brood was counted from

the primiparous instar onwards till the end of the life span of an individual. In terms of number it is designated as the clutch size. The maximum clutch size (Emax) is the mean highest number of eggs produced during the life cycle.

**Fecundity**

Fecundity is defined as the potential reproductive capacity of an organism during its life time measured by the number of eggs produced. In Cladocera, it specifically refers to the number of eggs produced per female, starting from the primiparous instar until the death of the animal. In the present study it is estimated by enumerating the number of eggs in the brood pouch of the parthenogenetic female after dissecting out the eggs using micro-needles and counting them under the microscope. This could also be done by counting the number of eggs in live condition since the eggs are visible through the carapace.

The total number of eggs produced during the life is a measure of reproductive potential and expressed as cumulative fecundity ($\Sigma mx$). The number of eggs produced per individual per day of adult life was also calculated by dividing the cumulative fecundity by total duration of adult instar in days.

The Rate of Egg Production (REP) is estimated by plotting the cumulative egg production against the instar number following the methodology of Navaneethakrishnan and Michael (1971), in which the angle of slope of regression line is taken as the rate of egg production.

### 3. 3. 3 Growth

The progressive increase in mean size of the individual during each instar is a measure of growth rate and hence important in studies on biology (Edmondson, 1955). Growth rate is an important life history trait because it is intrinsically linked to and directly affects other life history traits such as Age at First Reproduction (AFR), Size at First Reproduction (SFR), First Generation Time (FGT) and Rate of Egg Production (REP).

The pattern of growth was studied by measuring the size of the individual at each instar. Size of the animal refers to the Total Length (TL) extending from the anterior margin of the head to the tip of the tail spine. The maximum distance between the dorsal and ventral margin of the carapace is taken as the Carapace Height (CH).

Size measurements of each individual were done using calibrated micrometers at regular 24 hrs interval until their death. The mean value of the measurements in each instar and its standard error (SE) was calculated. The growth rate was represented by plotting instar number on x-axis against mean size.

The size increment during each instar is also taken as an index of the growth rate. Size increments of the females were calculated for each individual by subtracting its size at instar-X from its size at instar X +1. The percentage of size increment was calculated with respect to the size at each succeeding instar. Correlation between size and a few selected life history characters were also analyzed.

### 3. 3. 4 Embryonic Development

The sequence of morphological changes during the embryonic development was documented related to time duration in hours. The deposition of eggs into brood pouch could be regarded as the first event which initiates the embryonic development. The morphological changes in the embryo could be visible through the carapace even when the embryo developed *in vivo.* The end of embryogenesis was determined by the completion of development and subsequent release of a batch of neonates from the brood pouch.

Green (1956) has earlier divided the embryonic development mainly into 3 stages viz: the early, middle and late stages. During the present study attempts were done to find out other clearly identifiable sub-stages. The adult females reared for the study of embryonic development were periodically dissected out under stereoscopic microscope to expose the embryos for photography and measurements. At least 10 embryos were measured to determine the size range.

### 3. 3. 5 Life span and Survivorship

The life span or longevity is the period of time between the birth and death of an organism. The life span was calculated by recording the duration of life starting from 1$^{st}$ day till the death of the last individual.

The maximum Life span (Lmax) observed in each species is expressed as duration of time in days. The mean life span ($\Sigma$lx) of each species was also calculated and expressed in terms of days. Handling during the early stages was done with great care since this may increase chances of mortality.

The term survivorship refers to the number of individuals survived after each day. This was estimated by recording the number of individuals survived at an interval of 24 hrs starting from 1$^{st}$ day until death of all the individuals cultured for life cycle studies. The percentage survival was calculated as the percentage of their initial number. Age in days was plotted in x-axis against percentage survival to represent survivorship curves.