



*Artemia* culture as live food

# Introduction

---

- Most widely used live food for larviculture of finfish and shellfishes in aqua hatcheries.
- Because of its ready availability in the form of dry cysts containing dormant embryo, which can remain viable condition for years, if stored properly.
- It can be produce on “on demand” through hydration of dormant cysts into nutritious nauplii with in 24 hours.
- More than 85% of marine organisms which are bred in aqua hatcheries world over today are fed with *Artemia* nauplii as a sole diet or in combination with other live or inert food items

# Systematic position

- Phylum: Arthropoda
- Class: Crustacea
- Sub-class: Branchiopoda
- Order: Anostraca
- Family: Artemidae
- Genus: *Artemia*
- Species: *A. salina*
- First report on occurrence of Artemia from saline habitats was reported from Lymington in England in 1755
- However, long before it was known to many ethnic groups as the harbinger of better salt production and hence, was popularly called as **Brine shrimp or Brine worm**
- It is also called **Sea monkey**

# Habit & habitat

---

- *Artemia* is a cosmopolitan organism distributed in the tropical, sub-tropical and temperate zones.
- Although it is highly adaptable organism, capable of living at high water temperature(  $> 35^{\circ}\text{C}$ ), high salinity( upto 340 ppt) and very low dissolved oxygen concentration( $< 1\text{ppm}$ )
- Its global distribution is restricted only to isolated hyper saline bays, salt pans and inland saline impoundments.
- Not found in oceans
- Inhabits in salt pans in different periods of the year. This is mainly dependent on salinity conditions
- Not occur all over the water body

# Locomotion

- On its own, is not able to migrate from one biotope to another.
- Transportation of surface floating eggs (cysts) by adhering to the body of water birds such as flamingoes, shelduck etc or ingested by them while feeding, is perhaps the only way of its natural disposal.
- Artemia occurring in Gulf of Kutch is believe to have been transported from Tuticorin of Tamil Nadu during migration.
- Analysis of birds excreta fed on Artemia cysts showed 65-80% of the ingested cysts remaining viable.
- It was introduced by human into many areas where it has established its respective potentials, presently over 300 habitats are recorded.

# Major habitat in India

---

- 4 major habitats: salt pans in Vadala, Bhaynder, Nalasopara etc in Maharashtra; Sambar lake in Rajasthan; Tuticorin in Tamil Nadu, Sourashtra and Kutch coasts in Gujarat



# Morphology

- *Artemia* cysts measure about 200 micron in diameter and are brown in colour
- It remain covered with hard shell or chorion
- Freshly hatched *Artemia* nauplii are about 0.4 mm in length
- Nauplius has a pair of rudimentary antennae
- It is orange in colour due to presence of yolk, containing all essential fatty acids, amino acids and significant concentration of vitamins, hormones and carotenoids in addition to protein content of nearly 50%
- Adult measures about 1-2 cm in length, it is characterized by the head portion bearing a pair of stalked compound eyes, well develop antennae and the trunk bearing 11 pairs of thoracopods that carry out the function of locomotion, respiration and filter feeding.
- The elongated abdominal portion extends behind the thoracopods and ends into anal furca

# Life history

---

- Males differ from females by two large antenna on the head. Unusually, males have two reproduction organs.
- The females can produce eggs either in a usual way or via parthenogenesis. There are two types of eggs: the thin shell eggs that hatch immediately and the thick shell eggs.
- The thick shell eggs can remain in a dormant state, in cysts (that already consist of multiple cells), for a number of years until they are placed in water.
- Thick shell eggs are produced when the water is drying (the concentration of salt is raising). They need to dry out before they hatch (otherwise they would hatch immediately and no eggs would be left when the water dries up).

# Contts.....

---

- Eggs hatch into nauplius that is about 0.5 mm length. They have one single simple eye that only senses the presence and direction of light.
- Nauplii swim towards the light but adult individuals swim away from it. Later, the two more capable eyes develop but the initial eye also stays, **resulting three eye creatures.** Unlike the most of aquatic species, Artemia swims upside down.

# Food & feeding

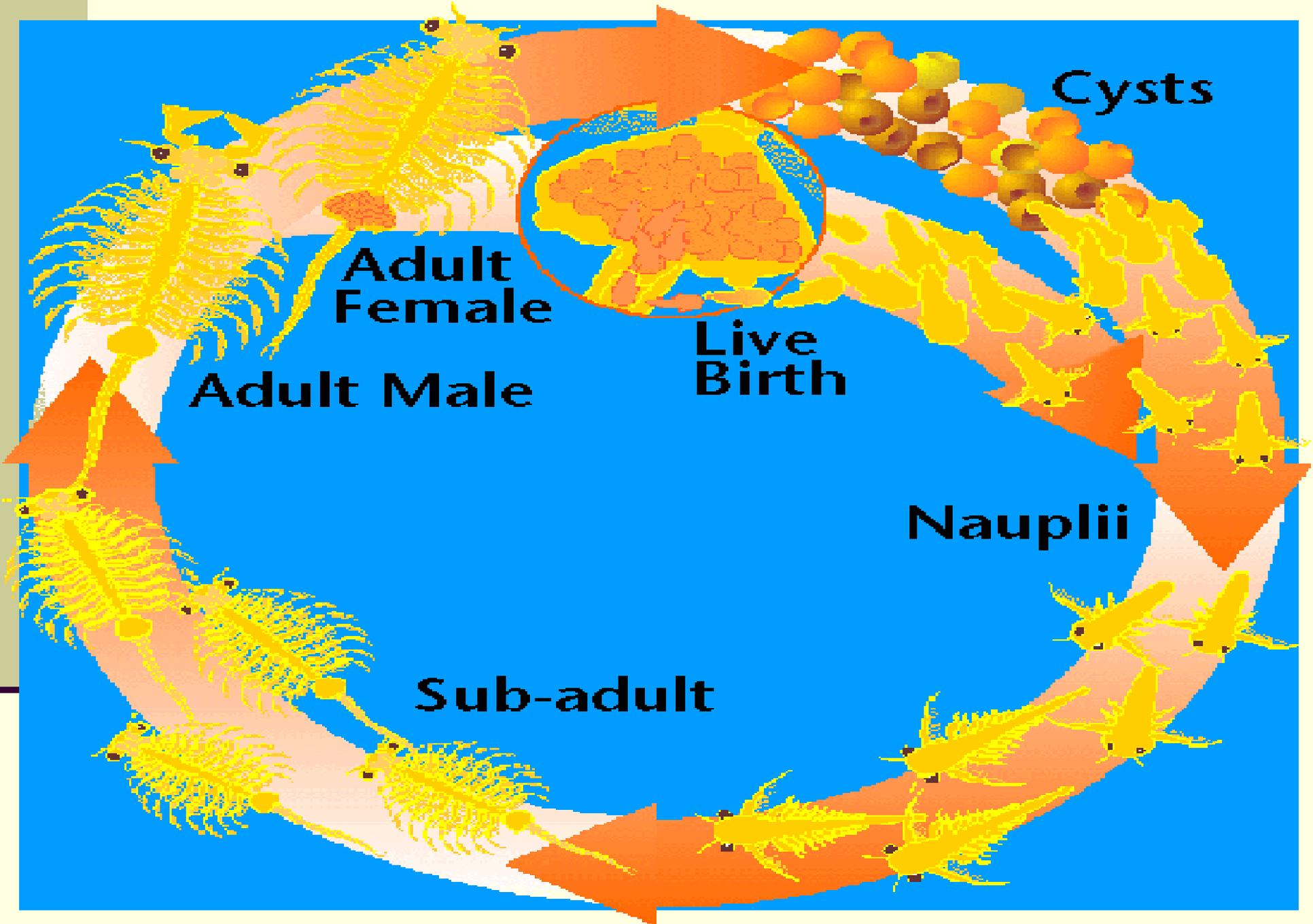
---

- In nature basically feeds on algae.
- Prefers to take and digest *Dunaliella* and *Scenedesmus* but cant digest species like chlorella due to thick cell wall in laboratory, it takes any particulate food such as bacteria, pig manure, phytoplankton, rice bran, shrimp head and copra meal
- Also fed with a mixture of bread yeast and *Spirulina* in 1:1 ratio
- Food particles are brought to the mouth by constant movement of its appendages

# Reproduction

---

- Attain sexual maturity in 2 weeks time after hatching and reproduces continuously throughout its life span of 30-40 days.
- It occurs in two stains, one bisexual and the other parthenogenetic( only female)
- Adult male can be identified by the presence of pair of large sized claspers in its anterior portion.
- Adult females are devoid of suck claspers and possess an ovisac and uterus.



# Life cycle

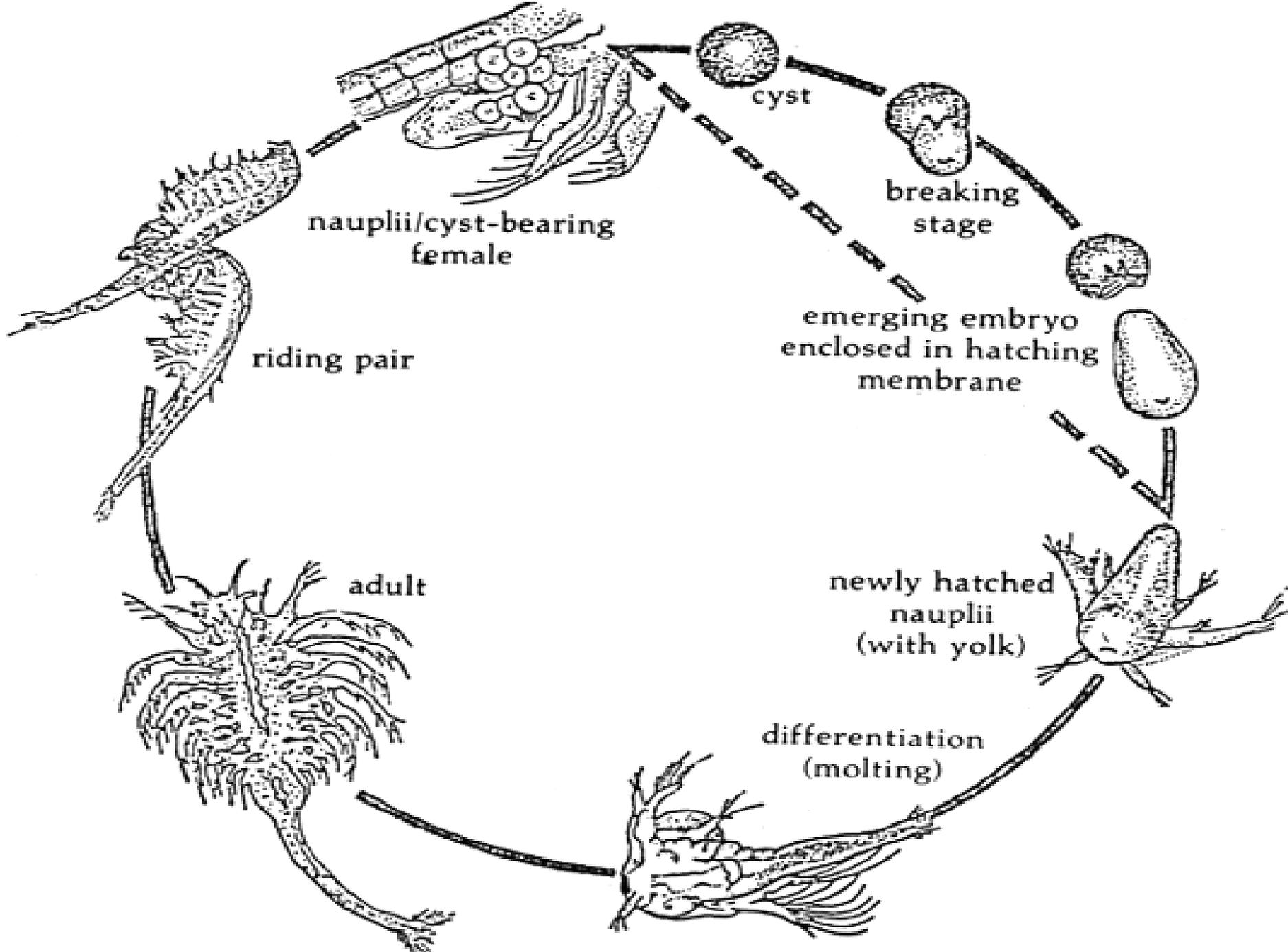
---

- Sexually copulated or parthenogenetic female when becomes gravid, carries about 100-110 eggs in the brood sac
- Eggs are generally released in 4 batches at the interval of 4-5 days
- The life cycle of *Artemia* pass through 13 instars( larval stages)
- The first instar( nauplii) is the best larval feed.



A close-up photograph of a female Artemia uterus, showing the formation of cysts. The uterus is a long, thin, yellowish structure. At the top left, there are two small, dark, circular structures. The main body of the uterus is a long, thin, yellowish tube. At the bottom right, there is a cluster of small, reddish-brown, oval-shaped structures, which are the cysts. The background is a light, yellowish color.

Close-up of female *Artemia* uterus showing oviparous formation of cysts.



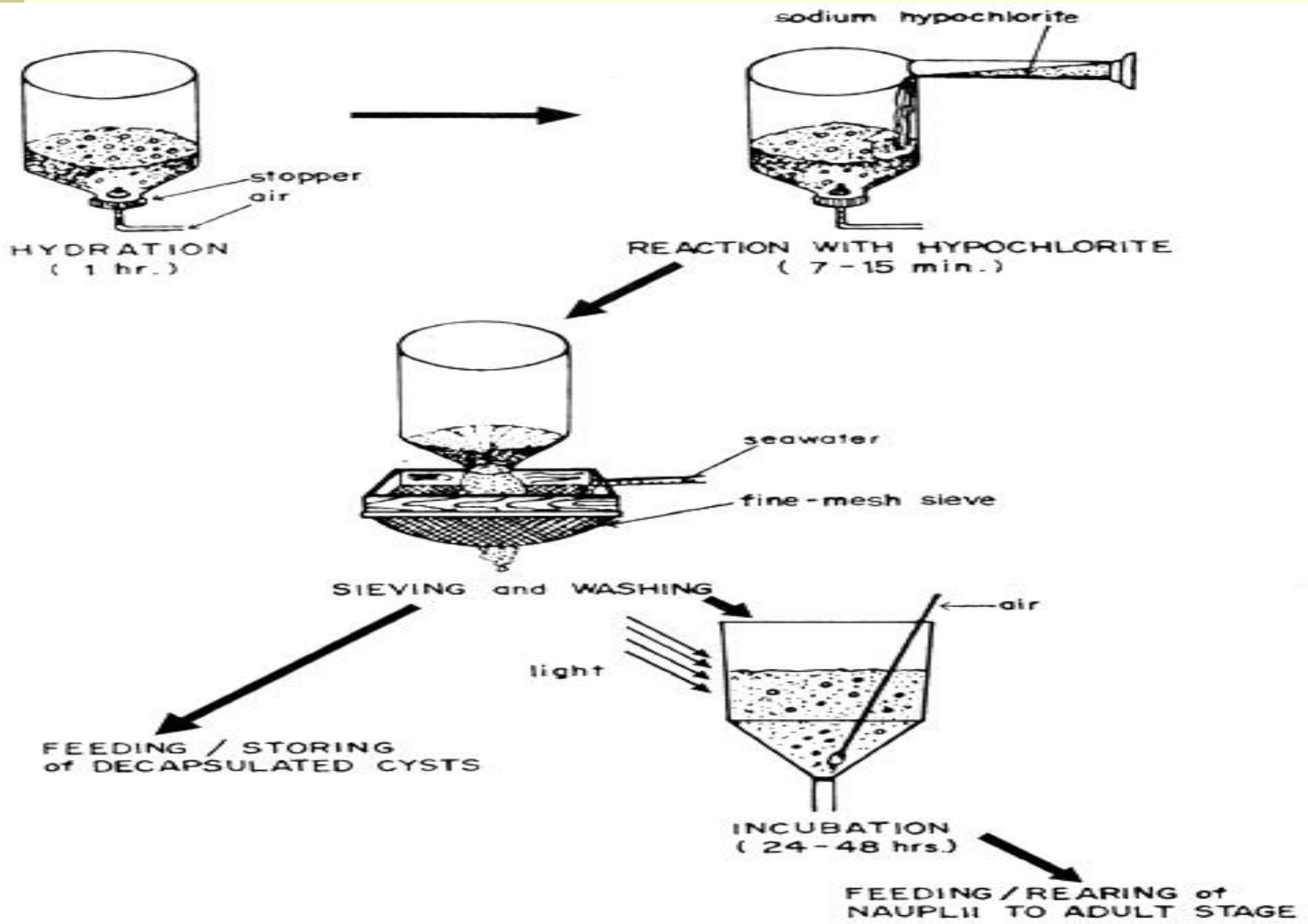
# Production of *Artemia* Nauplii for Hatcheries

---

- *Artemia* lays tiny brown coloured round eggs in high saline waters which float on water surface.
- These eggs( cysts) are collected, washed, dried and after sieving, packed in air-tight tins and sold in markets under different brand names.
- For use in aquahatcheries, *Artemia* cysts are hatched into nauplii following the standard technique



# Steps in decapsulation and hatching of Artemiacysts



# Hydration of cysts( Steps I)

---

- For hydration, required amount of dry cysts are put in a container containing saline water or fresh water( 20 ml for every one gm of cyst)
- Vigorous aeration provided
- In about one hour, the cysts get hydrated and turn spherical
- The hydrated cysts are filtered on 100 micron mesh bolting silk cloth

# Decapsulation of cysts ( Steps II)

---

- Decapsulation of hydrated cysts of *Artemia* is carried out by treating these with chemicals which dissolved the shell.
- Two types of chemicals are available for the purpose a) Commercial grade sodium hypochloride (NaOCl) and b) commercial bleaching powder.

# Sodium hypochloride (NaOCl) method

---

- In this method, the hydrated cysts are kept in 5% sodium hypochloride solution @ 15 ml for every one gm of cysts
- The oxidation process start immediately as a result of which heat is produced and the temperature raises beyond 40 °C
- In order to prevent damage of embryo from the rising temperature, the container containing the hydrated cysts are placed inside a trough containing cool water or ice cubes

# Contts.....

---

- During the period, the cysts are constantly stirred using a glass rod to facilitate uniform cooling.
- As the chorion dissolves, a gradual change in the colour of cysts can be noticed from dark brown to white
- In about 5-10 minutes, the chorion gets dissolved and the decapsulated cysts are filtered on the 100 micron mesh bolting silk cloth
- The decapsulated cysts are then thoroughly washed in freshwater or saline water to remove the traces of toxic chlorine.
- In order to ensure complete removal of chlorine, the decapsulated cysts are given a dip in 0.1% sodium thiosulphate.

# Decapsulation of cysts using Bleaching powder

- In this method of decapsulation of *Artemia* cysts, commercial bleaching powder, containing 30-33% active chlorine concentration is used.
- The hydrated cysts are filtered into a scoop net made of 100 micron bolting silk cloth.
- The scoop net containing the cysts are then kept in a bucket filled with water containing 200-250 ppm of chlorine prepared by using commercial bleaching powder.
- After having kept in chlorinated for 15-20 minutes, the chorion of cysts get dissolved and the colour of the cysts turn white
- The scoop net along with the decapsulated cysts is henceforth removed from the water.
- The traces of chlorine are then also removed same method as stated in previous method.

# Advantage of Decapsulation process

---

- Though *Artemia* cysts can be hatched into nauplii, but the decapsulation process is considered desirable because it helps in improving the hatching percentage and also in eliminating the chances of disease contamination into aqua hatcheries

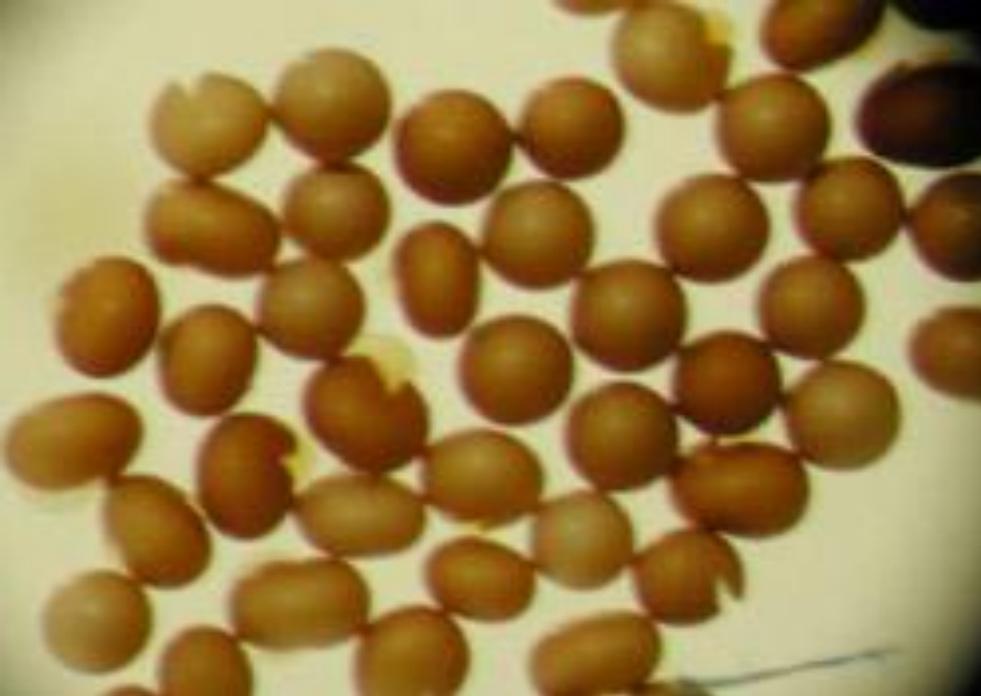
# Hatching of Decapsulated cysts ( Steps III)

---

- Decapsulated *Artemia* cysts are hatched in cylindroconical FRP jar of varying capacity ( 5-500 lits) depending on the requirement.
- The optimum water quality required for hatching of decapsulated *Artemia* cysts are:
- Temperature: 17-30 ° C
- pH: 7.5-8.5
- Salinity: 25-30ppt
- Light: 1000 lux
- Dissolved oxygen: saturation point

# Contts....

- Initially, the jars are properly disinfected with 200 ppm chlorine or 100 ppm formalin.
- Finally, the jars are rinsed with the same saline water which is to be used for hatching of decapsulated cysts.
- Accordingly, jars are now filled with 25-30 ppt of filtered sea water (saline water)
- On the top of the hatching jar, light is provided with 1000 lux intensity.
- The light might have helps the dormant embryo in waking its metabolic activity
- The decomposed cysts in the jar are stocked @ 0.5-1.0g/lit
- The cysts hatch into nauplii in about 12-24 hours, depending on the strain of Artemia, quality of cysts and water temperature



# Harvesting of Nauplii

---

- The freshly hatched *Artemia* nauplii are harvested in a 100 micron mesh net by taking advantage of their photostatic nature.
- For that act, the aeration of hatchling jar is stopped and the jar is closed with lid.
- After 2-3 minutes, the outlet valve is opened for a while carefully to remove the unhatched cysts settles in the cone.
- Now, the transparent part at the bottom portion of the jar is illuminated so as to attract nauplii to concentrate near outlet

- 
- After 5 minutes, the outlet is opened and nauplii are collected into a net made of 100 micron bolting silk cloth kept in the bucket having an outlet at the upper edge to facilitate drainage of excess water
  - After collection, the nauplii are thoroughly washed and stocked in a container containing seawater( saline water) of similar salinity at that of hatching jar and used as live food for the young stages of finfish and shell fish species in hatcheries as required

# Culture techniques of *Artemia*

- Presently in India, demand met through import of exotic strains of *Artemia* cysts.
- As the local strains are not able to meet nutritional requirements of the larval and post larval stages of the fin and shell fishes as exotic strains do.
- There are also problem on account of improper processing and packaging of local strains of cysts
- Hence, it is desirable to have a refined culture meteorology for Indian *Artemia* strains

# Salient feature of culture of *Artemia*

---

- **Selection of pond:**
- Need availability of water with high salinity
- Such saline water are only available in salt pans occurring plenty along the vast expanse of coastal belt of the country
- In a typical salt pan, the salt is produced during dry season
- Three types of ponds are usually involved in production of salt namely, reservoir, evaporation pond and crystallization ponds
- Sea water taken into reservoir, wherever form, water is pumped into evaporation ponds as and when required
- From evaporation ponds, water is pumped into crystallization ponds where the salinity of the water reaches to 240 ppt
- In this set up, it is evaporation tanks having a salinity range of 30-200 ppt which offer an excellent sites for *Artemia* culture

# Selection of pond

---

- The size of pond varies from 0.25 to 0.50 ha.
- The pond should hold about 0.5 m depth of water
- For easy collection of cysts, the longer side of the pond should be parallel to the prevailing wind direction
- This facilitates the cysts to accumulate at the side of the bank opposite to wind direction

# Pond preparation

- Pond should be drained and dried thoroughly during the beginning of the dry season, this helps in oxidation of metabolic wastes and eliminate the predators, if any, in the pond bottom soil.
- The pond dykes should be checked for leakage and seepage
- Inlets and outlets should be provided with 500 micron nylon screen
- If soil pH is less than 6.5, lime should be provided to neutralize acidity
- Normally, to raise soil pH by 0.1 unit, about 500 kg of agricultural lime( $\text{CaCO}_3$ ) per hac is used
- Now, the pond is ready to fill with sea water
- As the salinity of the pond gradually rises to 100ppt or above and the depth reaches 40 to 50 cm, the pond is ready for inoculation with *Artemia nauplii*

# Fertilization of pond

---

- To maintain optimum density of phytoplankton, pond should be fertilize 5-7 days prior to inoculation of Artemia nauplii
- The transparency of the pond water should be checked before fertilization
- If the transparency is 30 cm and above, it would require the application of fertilizations
- Rate of manure:Poultry manure@ 0.5-1.0 ton/ha; Pig manure@ 1.5-2.0 ton/ha
- Inorganic fertilizers are applied in a combination of 100 kg/ha mono-ammonium phosphate(16-20-0) and 50 kg of Ammonium –nitrate(30%)

# Inoculation

---

- *Artemia* nauplii are inoculated @ 80- 100 nauplii/lit of the pond water.
- In order to avoid transport of *Artemia* nauplii, the cysts are hatched near the pond in a shaded place
- The quantity of the cysts required would depend on percentage of hatching rate.
- Inoculation is done in the early morning hours when the pond water temperature is low.
- *Artemia* nauplii are inoculated after 24 hours of hatching.

# Culture period

---

- Culture duration: **5 months** of which first two are defined as grow-out period.
- The third month as the inducement period and the last two months as harvest period.
- During the grow-out period, the optimal environmental conditions have to be maintained.
- In order to maintain optimum depth, water is taken into the pond regularly.
- Salinity should be maintained at around 100-110 ppt for the production of biomass and about 120-150 ppt for the production of cysts
- Source of water to be free from pollutants.

# Biomass harvest

---

- As the *Artemia* population in production pond increases beyond 250 numbers pr lit, the biomass should be harvested
- The harvesting is done with help of a hand net made up of 500-1000 micron mesh nylon netting
- The harvesting should be stopped when *Artemia* density in the ponds reduces to 100 individual per litre
- The harvested biomass should be cleaned thoroughly with fresh water to eliminate attached salt, debris etc.
- The biomass should be put in thick plastic bag, in a thin layer
- Then the bags are spread in plastic trays and followed by quick freezing at  $-25^{\circ}\text{C}$
- This frozen biomass is used as feed to the post-larval stages and brood stock of finfish and shell fish in hatcheries

# Cyst production

---

- For the production of cysts, the population should be allowed to reach a density of more than 200 adults/litre
- The reproductive behavior of the population has to be subjected to **environmental stress** so as to enable them to switch over to cyst-bearing from that of nauplius-producing form
- A drastic reduction in **dissolved oxygen content**( below 1ppm) creates the stress condition in the population
- As the saline nature of the water has direct bearing on dissolved oxygen content of the water, the increase in saline results in lowering of oxygen level
- During 3<sup>rd</sup> month of culture, the salinity of pond water is increased from 100-200 ppt by pumping high saline water
- This would result in the release of cysts from the female population

# Cysts

---

- The liberated cysts float on water surface and are driven towards inner rim of the pond by wind action
- Collection of cysts should be undertaken daily, otherwise may get mixed up with sand, dried and blown away by the wind
- The cysts are best harvested with double screen dipnet made up of outer and inner screens of 150 micron and 350 micron nylon netting respectively
- The tail end of the outer net is kept open to collect the cysts after every harvest
- This helps in assorted collection of cysts and adult *Artemia* separately

# Cysts

---

- The harvested cysts are emptied in large buckets filled with saturated brine water by opening the knot made at the tail end of the outer net
- The biomass in the inner net is collected and processed for quick freezing
- The cysts are to be engineered through various processes to enhance the shelf life

# Processing of cysts

---

- The cysts stored in brine solution float on the water surface while the heavy debris sink to the bottom.
- When considerable quantity has been collected, the floating cysts are scooped out and transferred to another container with brine
- A stick could be used to mix the water half way through the container to remove other debris that may be sticking to the cysts
- Aerating the container at half its height proves beneficial in cleaning the debris attached to the cysts
- The brine transfer also help in hastening dehydration process of the cysts

# Processing of cysts

---

- After 24 hours the cysts are scooped out and washed with fresh water for about 5 minutes to remove the salt
- The cysts are then placed in a bucket containing fresh water
- This would separate the cysts from light debris and some empty cysts shells
- The full cysts would sink in freshwater
- The process should not take more than 15 minutes otherwise the cysts would hydrate.
- The cysts are then collected in a 100 micron bag net and finally washed with freshwater.

# Drying

- The cysts are spread in thoroughly cleaned and dried trays
- The cysts are either sun dried on out door racks under the shade or in drying boxes
- Drying is complete when the cyst lumps collapses into powder when touched
- **Storage of Cysts**
- The cysts are stored in air-tight tin containers for keeping viable condition for a year
- Commercial quantities should be packed in vacuum-sealed or nitrogen flushed cans for longer storage and easy transport
- Cysts can also stored in brine for shorter duration, not require the drying process

# Production

- About 30-50 kg of cysts and about 200-500 kg of live biomass could be produced from **1.0 ha pond**
- The cyst and live biomass production would yield a profit if two crops per year is achieved from 1 ha pond with a pay back period being 3 years and 44.59%(CIBA,1997) internal rate of return.
- **Major Production Centres of World**
- **USA: Great salt Lake( 90%); San Francisco bay at California(3%)**
- **Macau, Brazil, Mekong Delta, Vietnam,Philippines, Canada(Caplin lake), Australia (Strak Bay)**