

Culture of Rotifer

Introduction

Systematic position:

Phylum : Rotifera

Class : Monogononta

Order : Ploima

Family : Brachionidae

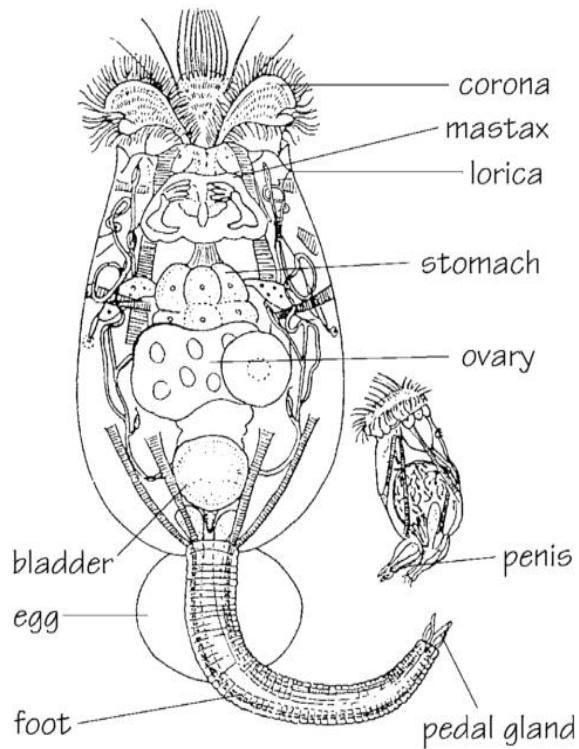
Genus : *Brachionus*

- Rotifers are commonly called as **Wheel animalcules**
- 2200 species of which 90% inhabit in freshwater habitats.
- The rotifers are considered as an important live feed in hatchery operation due to their
 - Planktonic nature,
 - tolerance to a wide range of environmental conditions,
 - high reproduction rate (0.7-1.4 offspring/female/day),
 - small size and slow swimming nature.
 - The possibility of rearing these animals at very high densities (2000animals/ml)
- Their growth is assured by **plasma increase** and **not by cell division**.
- The filter-feeding nature of the rotifers **facilitates the inclusion of specific nutrients** essential for the larval predators through **bioencapsulation into their body tissues**.

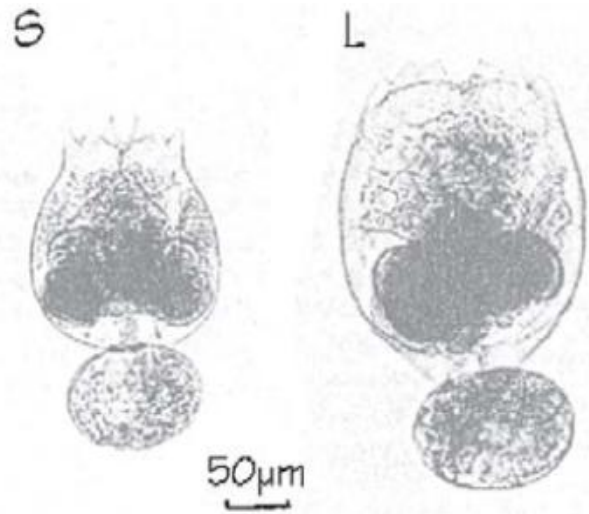
Morphology

- Rotifers are among the smallest filter feeding metazoans. Composed of fixed number of about 1000 cells.
- They filter small particles by means of a **ciliated annular organ**, the **corona**, located in the **anterior part of the body**.
- The corona is also used for its **whirling locomotion**, hence the name of the Class **Rotatoria**.
- ***Brachionus plicatilis*** that is the main species cultured for finfish larval rearing world-wide, is a planktonic.
- The rotifer's body is differentiated into three distinct parts:
 - **Head**
 - The head carries the ring **organ or corona**, which is easily identifiable by its **ring of cilia**.
 - The retractile corona assures locomotion and a whirling water movement which facilitates the ingestion of small food particles (mainly algae and detritus).

- Trunk
 - The trunk contains the **digestive tract**, consisting in a **mastax** that **grinds** the ingested particles, the **oesophagus**, the stomach **with gastric glands** and the **intestine**. The excretory system consists of paired protonephridia with terminal cells (cyrtocytes), the duct and the bladder. The genital organ is unpaired (Monogononta) or paired in the Seisonidea and Bdelloidea. The joint external **opening for bladder** and **oviduct** is called the **cloaca**.
- Foot
 - The foot is ending in **one or four toes** bearing **pedal glands** that secrete an **adhesive substance in crawling and sessile rotifers**.
- **Two different morphotypes** of *B. plicatilis* exist: the **small (S)** type and the **large (L)** type.
- They differ in their **lorica length: 130 to 340 μm** (average 239 μm) for **the L-type** and **100 to 210 μm** (average 160 μm) for **the S-type**.
- **S-type rotifers** are suitable **as first food for fish larvae** with a mouth opening smaller than 100 μm at first feeding, such as gilthead seabream, groupers, and rabbitfish.



Brachionus plicatilis, Male and female



Brachionus rotundiformis and *Brachionus plicatilis*

Rotifer morphotypes, resting eggs and adult female

L-TYPE



S-Type



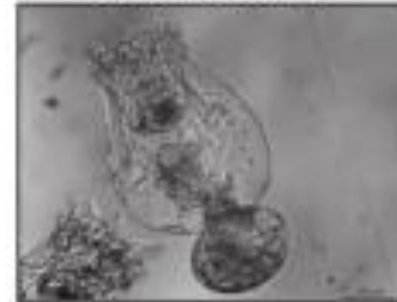
SS Type



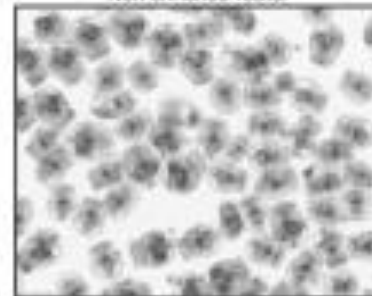
Resting eggs



Egg bearing adult female



Non enriched rotifer



Enriched rotifer



Digestive system

- Coronal cilia **sweeps food into mouth**
- Mouth opens into **chewing structure** called **Mastax**
- Mastax is a powerful muscular wall and contains tiny calcified and jaw like structure called trophi
- Trophi are of different structures depending on the feeding habits
 - For **filter feeding** rotifers – trophi are **covered** in grinding ridges
 - For **carnivorous** rotifers – trophi are **forcep** shaped to bite prey
 - For **ectoparasitic** rotifers – adapted to **grip host**
 - In other forms of rotifers – foot performs to grip prey

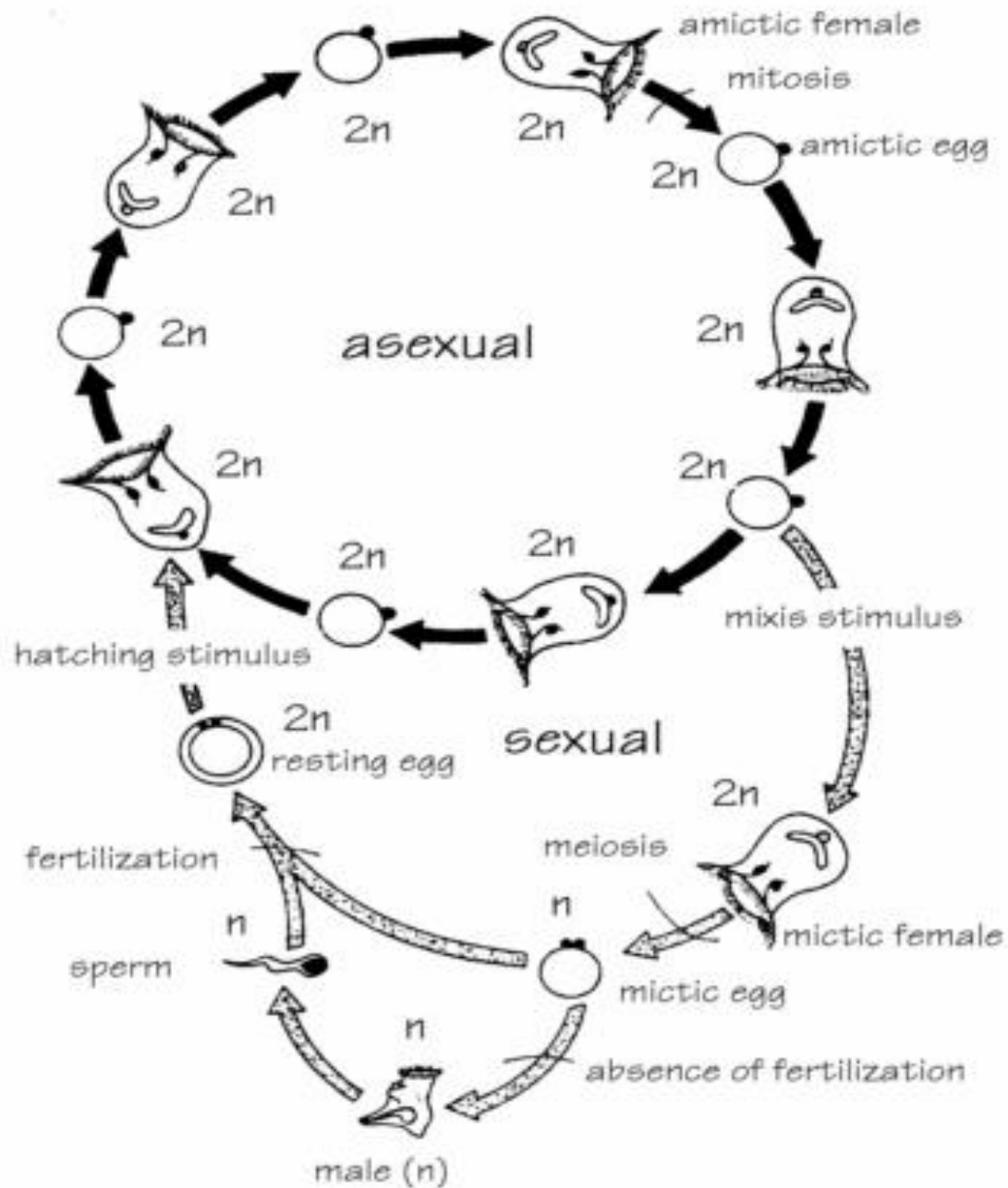
Contd..

- Mastax opens into Oesophagus, stomach, short intestine and terminates with cloaca and dorsal surface of animal.
- **Seven salivary gland** are present in mouth in front of **oesophagus**.
- Stomach has **two digestive gland** producing digestive enzyme.
- A pair of protonephridia opens into bladder which drains to cloaca.
- Protonephridia **expels water from the body**.

Life history

- Life span of rotifers – 7 days at 25°C.
- Larvae become adult after 0.5 to 1.5 days and females thereafter start to lay eggs approximately every four hours.
- Females can produce ten generations of offspring before they eventually die.
- The life cycle of *Brachionus plicatilis* can be closed by two modes of reproduction:
 - parthenogenesis and
 - sexual reproduction
- During female parthenogenesis, the amictic females produce amictic eggs (diploid, 2n chromosomes) which develop and hatch into amictic females.
- Under specific environmental conditions the females switch to a more complicated sexual reproduction resulting in mictic and amictic females.

- The mictic females produce haploid (n chromosomes) eggs. Larvae hatching out of these unfertilized **mictic eggs develop** into **haploid males**.
- These males are about **one quarter** of the **size of the female**; they **have no digestive tract** and **no bladder** but have an **over-proportionated single testis which is filled with sperm**.
- The resting eggs will develop and hatch into **amictic females** after exposure to **specific environmental conditions** viz. alternations in temperature or salinity or changing food conditions.
- The density of rotifer population also plays an important role in the determination of the mode of reproduction



Parthenogenetical and sexual reproduction in *Brachionus plicatilis*

Food and feeding of rotifers

- Rotifers eats **fish waste, bacteria, algae**
- They feed particles upto **10 μm size**
- Prefers the food items which are simple and well suspended in water
- Obtains food through **ciliated corona**
- Most tested and acceptable food are green algae- ***Chlorella*, *Tetraselmis*, Baker`s yeast**
- Rotifers contribute to nutrient recycling and are grown in tank to keep water clean

Marine Rotifer – ***Brachionus plicatilis***

Freshwater rotifers – ***Brachionus rubens*, *Brachionus calyciflorous***

Environmental conditions

- **Salinity:** Although *B. plicatilis* can withstand a wide salinity range (1 to 97 ppt), the reproduction can take place at salinities **below 35 ppt. better growth within 10-15ppt**
- **Temperature:** It affects reproduction as well as fecundity. Maximum reproduction of *B. plicatilis* occurs at **30° to 34°C**. Low temperature reduces the growth of rotifers whereas high temperature increases the cost of food for rotifers
- **Dissolved Oxygen:** Rotifers can survive in water containing **as low as 2 mg/L. Aeration should not be strong**
- **pH:** Rotifers live at pH-levels **above 6.6 and best results can be obtained at a pH above 7.5.**
- **Ammonia:** High levels of un-ionized ammonia $\text{NH}_3/\text{NH}_4^+$ ratio are toxic for rotifers but rearing conditions with NH_3 concentrations below 1 mg/L appear to be safe.

Culture conditions for freshwater rotifers

- Can tolerate temperature between 15-31⁰C
- Optimum pH: 6-8 DO Level : 1-2 ppm
- Free ammonia should be less. More than 3 ppm inhibits reproduction
- Feeding rate of *B. rubens* is higher than *B. plicatilis*
- Can be grown in synthetic media as follows:
 - NaHCO₃ : 96 mg
 - CaSO₄, 2H₂O : 60 mg
 - KCl : 4 mg
 - De-ionised water: 1 litre

Culture procedures

- Intensive production of rotifers is usually performed in batch culture within indoor facilities;
- **For stock cultures:**
- The rotifers can be obtained from the wild, or from research institutes or commercial hatcheries.
- Before being used in the production cycle the inoculum should first be disinfected.
- The most drastic disinfection consists of killing the free-swimming rotifers but not the eggs with a cocktail of antibiotics (e.g. erythromycin 10 mg.l⁻¹, chloramphenicol 10 mg.l⁻¹, sodium oxolate 10 mg.l⁻¹, penicillin 100 mg.l⁻¹, streptomycin 20 mg.l⁻¹) or a disinfectant.
- The eggs are then separated from the dead bodies on a 50 µm sieve and incubated for hatching and the offspring used for starting the stock cultures.

Maintenance of Stock culture

- The culture water (seawater diluted with tap water to a salinity of 25 ppt) is aerated, pre-filtrated over a 1 μm filter bag and disinfected overnight with 5 mg/L NaOCl.
- The next day the excess of NaOCl is neutralized with Na₂S₂O₃ and the water is filtered over a 0.45 μm filter.
- Stock culture maintained in small vials can be inoculated with initial density of 2 rotifers/ml and can be fed marine Chlorella every day.
- After one week the rotifer density should have increased from 2 to 200 individuals/ml. The rotifers are rinsed and a small part of this is used for subsequent maintenance of the stock, and the remaining rotifers can be used for upscaling.

Upscaling of stock cultures to starter cultures

- The upscaling of rotifers is carried out in 500 ml erlenmeyers flask or conical flask and placed 2 cm from fluorescent light tubes (5000 lux).
- The temperature in the erlenmeyers should not be more than 30°C.
- The rotifers are stocked at a density of 50 individuals/ml and fed 400 ml freshly-harvested algae approximately 50 ml of algae being added every day to supply enough food.
- Within 3 days the rotifer concentration can increase to 200 -300 rotifers/ml. During this short rearing period no aeration is applied.

Contd..

- Once the rotifers have reached a density of 200-300 individuals/ml they are rinsed on a submerged filter consisting of 2 filter screens.
- The upper mesh size (200 μm) retains large waste particles, while the lower sieve (50 μm) collects the rotifers. The concentrated rotifers are then distributed in several 15 L bottles or columns filled with 2 L water at a density of 50 individuals/ml and a mild aeration is provided and algae are supplied daily.
- Every other day the cultures are cleaned (double-screen filtration) and restocked at densities of 200 rotifers/ml.
- After adding algae for approximately one week, the 15 L bottles are completely full and the cultures can be used for inoculation of mass cultures.

Mass culture of rotifers

Mass culture parameters and condition

Parameter	Acceptable range	Preferable range
Temperature (°C)	20-30	25-27
Salinity (ppt)	1-60	18-25
Dissolved oxygen (ppm)	> 4	5-7
NH ³ /NH ⁴⁺ (mg/l)	6-10	-
NH ³ (mg/l)	-	< 1
pH	5-10	7.5 - 8.5
Light (lux)	-	2000

1. Daily tank transfer method
2. Continuous culture
3. Method developed at CIFE, Mumbai

Enrichment “rotifers are what they eat”

- Improves nutritional status by using Omega-3
 - Micro algae which is rich in Omega-3 fatty acids are fed to the Brachionus
- Ascorbylpalmitate can be used as the Vit.-C source