# CULTURE MEDIA (NATURAL AND SYNTHETIC): ROTIFERA

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## INTRODUCTION

Rotifers are abundant components of almost all fresh-water lakes, ponds, and streams. Two major groups of rotifers have been used in biological studies, The bdelloids, which are usually associated with substrates, have paired ovaries and vitellaria and reproduce exclusively by parthenogenesis. In contrast, monogonont rotifers may either be associated with substrates or be free-swimming members of the plankton. The latter group has a single ovary and vitellarium and may reproduce either by diploid, ameiotic parthenogenesis, or sexually after the production of haploid males. General accounts of both groups may be found in Hyman.[1] An excellent description of monogonont reproductive cycles is presented by Birky and Gilbert.[2]

Individuals of both groups typically have well-developed digestive, nervous, excretory, muscular, and reproductive systems. Many are readily cloned and cultured in the laboratory. Sexual reproduction can be controlled by diet or density in some species, and crosses can be made. Because of these characteristics, as well as their rapid development and short generation times, rotifers have been used in a diverse array of biological investigations.

Littoral rotifers are frequently associated with aquatic plants, and one general collection technique for this group is to fill large jars with loosely packed littoral vegetation and pond water. These jars are brought back to the laboratory and placed under a strong light so that those rotifers having positive phototropism will swim to the surface. Planktonic rotifers are usually collected with #20 plankton nets.

Individuals of the desired species can be removed from aliquots of the field sample in Syracuse watch glasses using micropipettes. Single individuals removed from the field collections are serially transferred through at least ten washes in deep depression slides containing culture medium. Some of the washes may contain antibiotics, as detailed later.

Stock cultures may be maintained in a variety of glassware, ranging from culture tubes to Syracuse dishes. Of particular value in many applications, because of both size and optical properties, is the Bureau of Plant Industry watch glass. Culture dishes are placed in inverted petri plates containing a small amount of water to prevent desiccation. Stock cultures of most species are usually fed two or three times a week and transferred at weekly intervals to fresh media and culture dishes. Most of these manipulations are performed with a stereoscopic microscope (preferably dark field) and magnifications of 6 to 12X. To prevent accidental transfer of individuals from one culture to another, it is convenient to place a small hotplate and beaker of water near the microscope. Several micropipettes can then be placed in the hot water and used in succession. All glassware coming into contact with cultures should be acid cleaned and throughly rinsed in deionized, then glass-distilled, water.

Both bdelloid and monogonont rotifers can be cultured in a variety of media, ranging from lake water to chemically defined media. Many bdelloids are reared using bacteria for food, whereas green algae are employed for most monogononts. A notable exception among monogononts is the important genus *Asplanchna*, a predator that is commonly fed Paramecia in the laboratory. Detailed information on requirements of a number of rotifers is given by Pourriot.[3]

## NATURAL MEDIA

The simplest medium in which to maintain many rotifers for short periods of time is Millipore® filtered water from the lake or pond in which they were collected. However, lake water may change dramatically from one time to the next, and results from investigations using filtered media from different lakes may not be comparable. Consequently, this medium is seldom employed in quantitative or long-term studies.

The euryhaline monogonont *Brachionus plicatilis* is widely cultivated in ponds in Japan as a source of fish food. To culture this rotifer under laboratory conditions, Hirayama and his colleagues[4-7] have employed a dilute (chloride at about 12.8°/$_{oo}$) charcoal-filtered sea water at pH 7.4 to 8.2. Results are reported in the cited papers on the growth of this rotifer on a marine species of *Chlorella* and yeast.

## PARTIALLY PURIFIED MEDIA

A variety of partially purified media have been employed in the laboratory culture of rotifers. Most of these media would be difficult, if not impossible, to standardize, either from time to time or from place to place. Typical of these undefined media is the solution used by Hertel[8] consisting of 70 ml rain water: 30 ml spring water: 20 drops of "stable tea." The "stable tea" was prepared by autoclaving a mixture of 1000 g fresh horse manure in 1200 ml tap water. This medium was used to culture *Epiphanes* (=*Hydatina*) *senta*, a large monogonont.

Recent work has utilized baked lettuce medium[9-12] for *Asplanchna*. This medium is prepared by pulverizing the dried leaves of head lettuce and adding 1.5 g of the resultant powder to one liter of glass-distilled water. The medium is boiled for 10 to 15 min, buffered with 0.2 g $CaCO_3$, filtered, and autoclaved. For use, the baked lettuce medium is inoculated with *Enterobacter* (=*Aerobacter*) *aerogenes* (grown on nutrient agar slants) to form a bacterized broth. After 24 hr, a dense suspension of one of the aurelia group of *Paramecium* species is added to the bacterized broth, which is then placed in the dark at about 28°C. The bacteria should be cleared from the broth in approximately 3 to 4 days, and, subsequently, paramecia can be harvested by either electromigration or centrifugation. Both *Asplanchna brightwelli* and *A. sieboldi* can be reared in either baked lettuce medium or the Pourriot-Gilbert medium described in the next section.

Meadow[13] (cited as a personal communication) cultured the bdelloid rotifer *Philodina* on *Enterobacter* (=*Aerobacter*) *aerogenes* either directly in baked lettuce medium or in a 50% dilution of baked lettuce medium with Pourriot-Gilbert Medium.

## CHEMICALLY DEFINED CULTURE MEDIA

Many of the rotifers commonly used for experimental purposes can be cultured in simple, defined, inorganic media. These media have the advantages of being both reproducible and relatively unfavorable for bacterial growth. Two widely used inorganic media for rotifers are Knop's Solution, which contains per liter 43 mg $KNO_3$, 43 mg $MgSO_4$, and 114 mg $Ca(NO_3)_2$, and Pourriot-Gilbert Medium, which contains per liter 100 mg $KNO_3$, 40 mg $K_2HPO_4$, 62 mg $MgSO_4 \cdot 7H_2O$, and 144 mg $Ca(NO_3)_2 \cdot 4H_2O$. Many modifications of both media are employed in different laboratories; for instance, Halbach[12] substitutes 120 mg/l $CaCl_2 \cdot 2H_2O$ for the $Ca(NO_3)_2$ in Pourriot-Gilbert Medium. All media should be prepared using deionized, glass-distilled water.

A more complete medium (referred to in Reference 14 as Medium 36) was developed for *Daphnia* by Taub and Dollar,[14] but is suitable for many rotifers. This medium, which was designed to be compatible with requirements of both *Daphnia pulex* and *Chlorella pyrenoidosa*, consists of (per liter) 49 mg $MgSO_4 \cdot 7H_2O$, 27 mg $KH_2PO_4$, 4 mg NaOH,

222 mg $CaCl_2$, 85 mg $NaNO_3$, 175 mg NaCl, 3 mg $FeSO_4 \cdot 7H_2O$, and 3 mg EDTA. The basic medium is supplemented with a minor elements solution containing 1.8 mg $H_3BO_3$, 0.3 mg $ZnSO_4 \cdot 7H_2O$, 3 mg $MnCl_2 \cdot 9H_2O$, 0.2 mg $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg $CuSO_4 \cdot 5H_2O$, and 0.03 mg $Co(NO_3)_2 \cdot 6H_2O$.

The four defined media presented above are compared in Table 1.

For most purposes it is important to buffer the culture medium since both fecundity rate and life span are functions of pH. Gilbert[15,16] uses a 0.002 *M* $NaH_2PO_4$-$Na_2HPO_4$ buffer at pH 7.3 for *Brachionus calyciflorus* and at pH 7.5 for *Asplanchna*.

Some investigators have added antibiotics to their defined, inorganic media to suppress bacterial growth. We have cultured *Asplanchna brightwelli* in Pourriot-Gilbert Medium with (singly) 160 units/ml of penicillin G, 100 units/ml dihydrostreptomycin, or 25 μg/ml crystalline tetracycline. In addition, we have used the same doses of penicillin and dihydrostreptomycin together in Pourriot-Gilbert Medium. The antibiotics substantially suppress bacterial growth, but they also reduce the reproductive rate of *Asplanchna* by approximately one third. Consequently, results obtained in the same medium with and without antibiotics may not be comparable.

The result of sexual reproduction in monogonont rotifers is a resting egg. This type of egg is capable of remaining dormant for periods up to several years. Resting eggs can be collected with a micropipette from cultures containing fertilized mictic females and stored for long periods. Birky[11] washed *Asplanchna* resting eggs in Dryl's saline solution[17] with antibiotics (100 to 500 mg/l penicillin or penicillin-streptomycin). Dryl's pH 7.1 solution consists of 20 ml 0.1 *M* sodium citrate, 10 ml 0.1 *M* $NaH_2PO_4$, 10 ml 0.1 *M* $Na_2HPO_4$, 945 ml distilled water, and 15 ml 0.1 *M* $CaCl_2$ ($CaCl_2$ must be added last to avoid precipitation of calcium salts). The eggs were then transferred to fresh saline solution without antibiotics and stored at 4°C. Under these conditions Birky observed hatching of resting eggs after 9 months of storage, but noted a subsequent decrease in percent hatching. Resting eggs of *Asplanchna* can be hatched in about a week by transferring them to Pourriot-Gilbert Medium and warming to room temperature.

**Table 1**
**COMPARISON OF DEFINED MEDIA DESCRIBED IN TEXT**

(Concentrations Indicated are m*M*)

| | Medium | | | |
|---|---|---|---|---|
| | Knop's | Pourriot-Gilbert | Halbach | Taub-Dollar-36 |
| $KNO_3$ | 1.13 | 0.99 | 0.99 | – |
| $MgSo_4 \cdot 7H_2O$ | 0.36 | 0.25 | 0.25 | 0.20 |
| $Ca(NO_3)_2 \cdot 4H_2O$ | 0.26 | 0.61 | – | – |
| $CaCl_2$ | – | – | 0.82 | 2.00 |
| $K_2HPO_4$ | – | 0.23 | 0.23 | – |
| $KH_2PO_4$ | – | – | – | 0.20 |
| NaOH | – | – | – | 0.10 |
| $NaNO_3$ | – | – | – | 1.00 |
| NaCl | – | – | – | 3.00 |
| $FeSO_4$ | – | – | – | 0.01 |
| EDTA | – | – | – | 0.01 |
| Minor elements | No | No | No | Yes |

## DIETS

Most rotifers are capable of filtering bacteria from their media, and for some species bacteria constitute a sufficient food resource. Dougherty et al.[18] describe techniques for dixenic culture of the monogonont rotifer *Lecane inermis* on *Escherichia coli* and an unidentified, Gram-negative, rod-shaped bacterium. The bdelloid *Philodina acuticornis* var. *odiosa* has been maintained in monoxenic culture on *E. coli.* Meadow and Barrows[19] found that the same species of *Philodina* could also be cultured on *Aerobacter* (=*Enterobacter*) *aerogenes, Pseudomonas* sp., *Chlorella vulgaris,* and *Chlamydomonas reinhardti.* Dougherty and his colleagues succeeded in culturing *L. inermis* axenically on a heated lamb liver extract,[20] but this culture could not be maintained indefinitely.[21]

Other species, including the widely investigated monogonont rotifers *Euchlanis dilatata*[22] and *Brachionus calyciflorus,*[15] are best reared with algae. A variety of chlorophytes such as *Chlamydomonas reinhardti, Chlorella vulgaris,* and *Stichococcus bacillaris* and euglenophytes such as *Euglena gracilis* and *E. geniculata* have been used for these rotifers. The cited algae are available at modest cost from the Culture Collection of Algae at Indiana University.

The predatory rotifer *Asplanchna* is usually cultured on *Paramecium,* which in turn can be cultured in bacterized perth grass, available from M. McIntyre Co., Edinburgh, Scotland,[16] Cerophyl, available from Cerophyl Laboratories, Inc., Kansas City, Missouri,[13] or baked lettuce medium.[9] *Asplanchna* can also be cultured using other rotifers, conspecific neonates, or the colonial green alga *Eudorina* for food. These food items, however, are more difficult to culture or produce slower growth rates in *Asplanchna* than Paramecium.

Diet has been demonstrated to play an important role in determining reproductive mode in some rotifers. Parthenogenetic females of *Epiphanes senta* can be induced to produce oocytes meiotically by switching the major food source from the colorless alga *Polytoma* to the green alga *Chlamydomonas.*[2] However, a similar change in the diet of *Brachionus calyciflorus* does not alter the mitotic mode of oocyte maturation and parthenogenetic reproduction.[15] In the genus *Asplanchna,* Gilbert[16] and Gilbert and Thompson[23] have clearly demonstrated the induction of sexual reproduction by dietary vitamin E ($\alpha$-tocopherol). Vitamin E is also thought to be required for male fertility.

Diet can also have a marked affect on the morphology of some rotifers. A striking dietary polymorphism has been described by Gilbert and Thompson[23] for *Asplanchna sieboldi.* These rotifers exist in three morphs: (1) saccates, which are about 600 $\mu$m long as adults and are the dominant form in cultures fed paramecia exclusively; (2) cruciforms, which are about 850 $\mu$m long and possess four body wall outgrowths; and (3) campanulates, which are about 1200 $\mu$m long and lack body wall humps. The increase in size and change in shape of *Asplanchna* is induced by $\alpha$-tocopherol and appears to be the result of both increased nuclear number and amount of cytoplasm (involved tissues may be syncitial). A small increase in size has been observed in *Brachionus calyciflorus*[24] when $\alpha$-tocopherol is added to the diet.

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