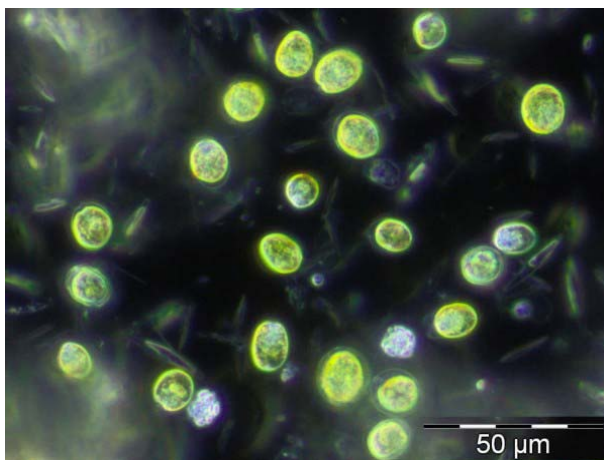


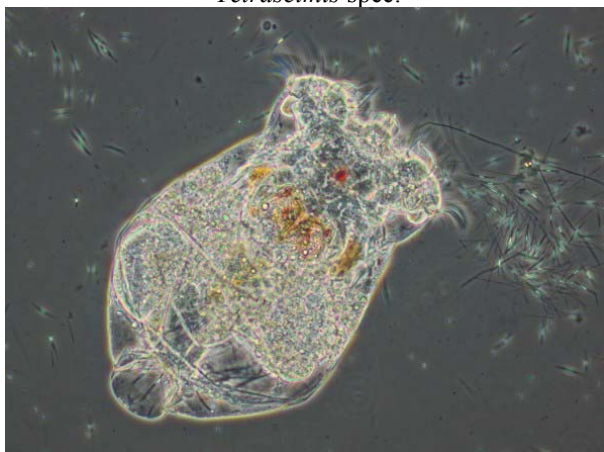
Breeding Plankton



Tetraselmis spec.



Phaeodactylum tricornerutum



Brachionus plicatilis L-type



Aeolidiella stephaniae (*Berghia verrucicornis*)

We take care of it!



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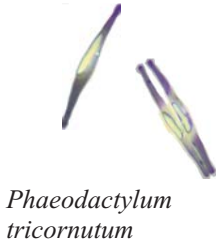
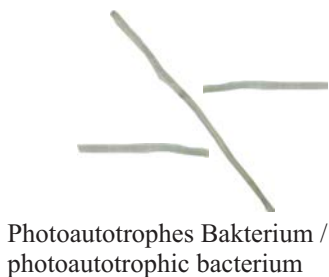
Cultures and media for plankton breeding

Nannochloropsis salina

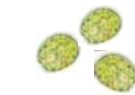
Brachionus plicatilis

Plankton für die Zucht im Größenvergleich / plankton for breeding by size comparison

Phytoplankton



Tetraselmis spec.



*Nannochloropsis
salina*

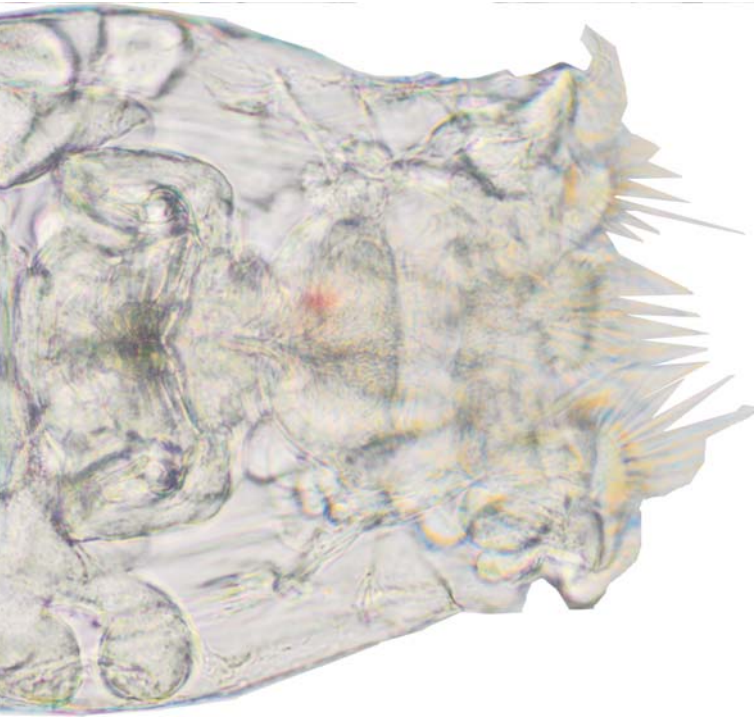


50 µm

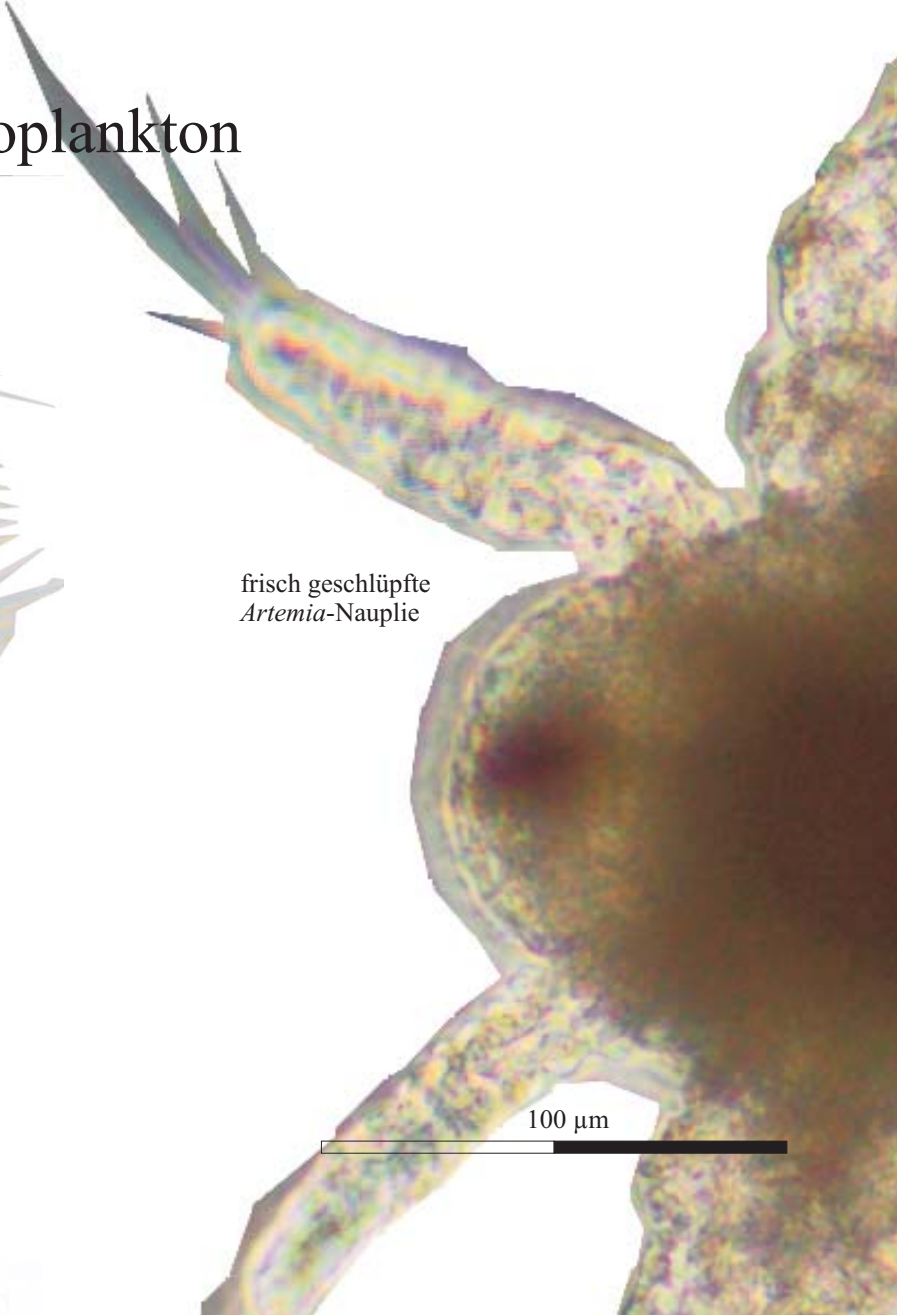


Zooplankton

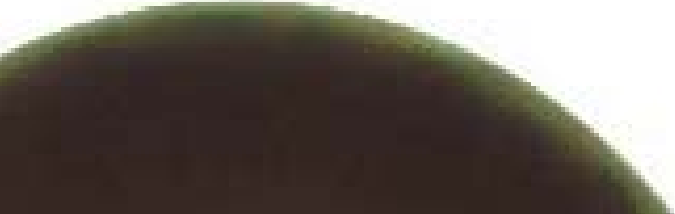
*Brachionus
plicatilis* L-type



frisch geschlüpfte
Artemia-Nauplie



Artemia
Dauercyste



100 µm

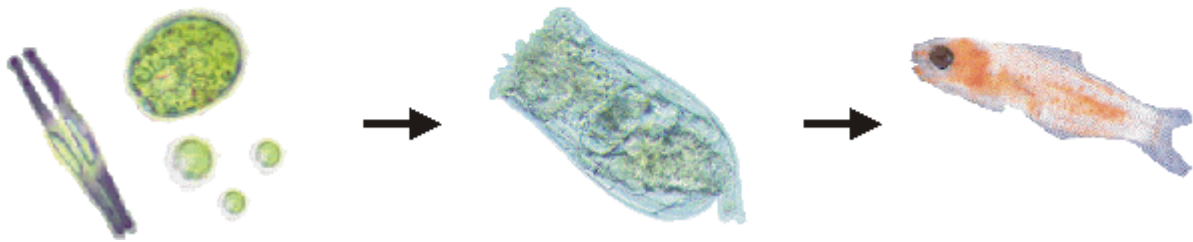


Micro algae - Overview for breeding phytoplankton



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phytoplankton → zooplankton → larvae



Sketch: simplified food chain – from algae to fish larvae

What is plankton?

Plankton are organisms that are drifting in the sea or in fresh water. Although some of them are able to move a little bit, the main motion is done by currents. You can classify plankton in different groups.

By appearance:

- Limnoplankton (in fresh water)
- Potamoplankton (in running waters)
- Mariplankton or neritic plankton (in the sea)
- Haliplankton (at high salt concentrations: salt lakes, sea)
- Saproplankton (in organic polluted water)
- Kryoplankton (in ice and snow)

By nutrition:

- Phytoplankton: organisms that are able to produce or-

ganics by photosynthesis; e.g. algae, phototrophic bacteria

- Zooplankton: organisms that are not able to produce organic by themselves - they need organic substances for getting energy; e.g. small crustaceans, organotroph bacteria, ciliates, etc.
- Bakterioplankton: sometimes bacteria a classified into an own group outside of phyto and zoo plankton.

By size:

- Pikoplankton or oltramicropoplankton: < 2 µm
- Nanoplankton: 2...20 µm
- Mikroplankton: 20 µm...2 mm
- Mesoplankton: 2...20 mm
- Makroplankton or megaplankton: > 20 mm

(other scales are possible)

By stage of development:

- Holoplankton: is living its whole life as plankton
- Mesoplankton: is living as plankton not the whole life time, e.g. fish larvae

The boundaries of the classifications are not exactly defined. For example it is possible that some phytoplanktic organisms are able to live without light by intaking organic material like zooplankton, e.g. Euglena. Some animals like jellyfish start with very small sizes (e.g. nanoplankton) and grow up to some meters (megaplankton). Some diatoms are living as well in fresh water (limnoplankton) as in brackish or sea water (haliplankton, mariplankton).

Why Phytoplankton?

Phytoplankton is the first link in the food chain and is relatively easy to breed. With heterotrophic cultures (e.g. bacteria, yeast) there is the danger of

a culture collapse and the danger of death of the fed animals. In addition autotrophic cultures (algae, phototrophic bacteria) have normally a good composition of essential substances; e.g. highly unsaturated fatty acids (HUFA), vitamins. Yeast and bacteria normally have a lack of these components.

Conditions for a sea water plankton breed

The requirements for an algae breed is not less. Before doing you have to think about costs and man power and space. Half-hearted attempts will fail and this is very disappointing.

Microscope



Professional microscopes with bright field, dark field and phase contrast. For monitoring algae simple microscopes or very strong magnifying glasses (10 to 20-fold magnification) are possible, too. picture: AquaCare

The most expensive unit is the microscope. A 400-fold magnification is necessary to distinguish between the different organisms. If you cannot monitor your cultures it is possible that foreign organisms, that are not suitable for zooplankton or larvae, will overgrow your culture without noticing. As a result you lose the larvae because the foreign organisms will have the wrong size, the wrong nutrients, etc. It is also important to make sure that the algae culture does not contain

its predators, e.g. zooplankton like Brachionus. If the predators will bloom the algae culture is lost. At the beginning of an invasion you can clean the culture by a plankton screen. - Maybe you can lump together with other aquarists or with an involved shop dealer to buy a microscope.

Plankton screens



Plankton screens with 6...200 µm picture: AquaCare

To divide different organisms from each other you can pour the culture through plankton screens. If you want to reject zooplankton from algae you need a screen that is a little bit larger than the size of the algae. For example to clean a Nano-chloropsis-culture (2...3 µm) a screen with 6 µm is perfect, for larger algae e.g. Tetraselmis (10...12 µm) you need a 15 µm screen.

Larger screens are useful for sieving zooplankton. You can use large screen as a pre-filter if a culture is agglutinated.

Media

Micro algae are living in aqueous solutions. Beside the living space (fresh water or sea water) the medium must contain nutrients like nitrate, phosphate, and trace elements. Special species need additional substances like silicic acid (diatoms).

Fertilizers from plant breeder are not useful in every case. Some substances like copper will hinder a good algae

growth. Additionally the proportions between nitrate and phosphate are not optimal.



From stock solutions it is very easy to mix media for algae. picture: AquaCare

Reverse osmosis water



Reverse osmosis unit *Excel* picture: AquaCare

For mixing algae media, for diluting and for cleaning reverse osmosis water (or desalinated water) is necessary.

Meerwasser



Synthetic sea salt picture: AquaCare

For mixing fresh media you need a stock of sea water. The sea water tank should stand as far away as possible from aquaria or plankton tanks. The sea water stock should be closed to prevent contamination by the air (aerosols). Otherwise you must filter the sea water - or if you are using natural sea water. The expense of a micro-filtration unit is very large not suitable for a hobby application.

If you use sea water for algae it is important that the water is minimum three days old. Fresh water contains radicals that destroys sensitive organisms. To mix sea water use a water pump to move the fresh water. Do not use an air pump to aerate the water - you will have too much aerosols that moves in the room.

Disinfection

To use tanks and equipment it is important that they are clean and do not contain organisms and their outlasting stages. So you have to disinfect this items. For hobby aquaristic the only way is the chemical disinfection - others like autoclaves are too expensive.

Note the instruction manual and keep the substances out of the reach of children!



A set for producing a disinfection solution
picture: AquaCare

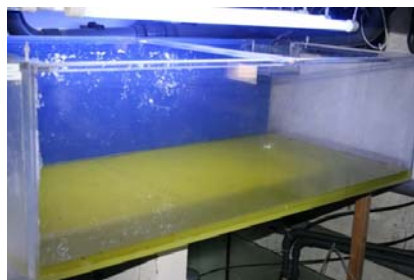
Tanks for breeding algae:



Professional algae breeding unit with some algae tubes (left), algae reactor and the infra-structure
picture: AquaCare

There are many opinions about the right algae tank. All containers have their advantages and their disadvantages. We recommend the algae tubes, because they are not too expensive and because of the closed system they reach good results in handling, needed space, pureness of the cultures and production speed.

1. Open tanks / aquaria:



A plexi-glass tank as algae breeding tank
picture: AquaCare

Open tanks or old aquaria are used very often, because they have large volume and they are not expensive. But the open construction have a big disadvantage: because they are an open system contaminants (other algae, zooplankton) easily will introduce themselves and destroy the culture. Open tanks are only useful for very rigid species like *Nannochloropsis salina* are kept. The high reproduction of this algae will overgrow most of other contaminating algae. These systems are not useful for very sensitive algae. The

grow speed is normally limited by the light, so you can normally have the same algae growth in intensive algae tubes compared to open aquaria.

2. Algae tubes



Algae tubes with minimum 4 liters (1 US gal) volume are the minimum for breeding algae for zooplankton
picture: AquaCare

Algae tubes are closed systems that needs less space, e.g. you can mount them at a wall. If the algae tube is as long as the used light bulb (e.g. T5) you can reach very high illuminance levels and consequentl high algae growth rates. If the inlet air is filtered over a sterile filter (e.g. 0,45 µm) and the outlet air is connected to a collecting bottle with its own sterile air filter a contamination is unlikely. You can increase very good slowly growing species in algae tubes. Volumes below 4...5 liters (1...1.3 US gal) are not useful - otherwise the yield is too low for feeding zooplankton. - Depending on the lights it is possible to harvest 10time more phytoplankton in algae tube compared to open aquaria systems (see ahead). With optimum conditions it is possible

to harvest 50% of the volume per day.

3. Algae reactors:



Professional algae reactors are expensive, but they have maximum capacity. picture: AquaCare

Algae reactors are similar built as algae tubes. But the control of the environmental parameter are better controlled. With a temperature and pH controller very high algae densities and high growth rates are possible. Because of the very strong lights normally these reactors have to be chilled - at least in summer. Otherwise the upper temperature limit of the algae is overshooted. It is possible to run algae reactors continuously to get the same algae quality. Because of the the high invest algae reactors are normally only for professional breeders and zoos.

Useful accessories

Following items are very useful for breeding algae:



Bottles with R.O. water for cleaning and dilluting, and disinfecting fluid picture: AquaCare



Pasteur pipette and syringes or suck up of samples picture: AquaCare



Lots of different bottles with caps made of plastic or glass for storing media and other fluids; scaled bottles are very practical picture: AquaCare



Bottles brushes in different sizes picture: AquaCare

Algae stock cultures

Last but not least you need algae stock cultures. It is better to buy them in a fresh condition to get active algae. If mirco algae are sleeping you need more time to wake them up.



Phytoplankton stock cultures, here *Nannochloropsis salina* stock „Nan4“.

Cultures are available in different volumes and - most important - with different cell density: the darker the colour the more cells are in the water. Before using the algae you may check if the right culture is sent and if there are contaminants, e.g. Brachionus, in the culture. Open strictly minimum two backup cultures. Is one culture is contaminated with other algae or predators you will loose it very fast.

Working „sterile“ (semi-sterile)

To work with phytoplankton and zooplankton it requires neat and clean working. Otherwise the cultures are contaminated very fast and you will lose them. It is not possible to work sterile for hobby aquarists. But follow the next points to avoid contamination as far as possible.

- Before doing: thinking.
- Label all bottles and cultures you work with: otherwise you will mix up cultures and media.
- Write a protocol: only with it you work methodically.
- Separate phytoplankton cultures from zooplankton cultures: otherwise aerosols may transport unpopular guests from one system to another.
- If you aerate a culture the air inlet should be protected with a sterile filter (max. 0.3 µm): otherwise contaminants have no problems.
- Before using bottles, tanks and tools sterilize them: some algae, cysts and eggs from zooplankton are tough and they are able to withstand rough conditions. If the conditions get better - new medium - they will grow rapidly again.
- If you work with a new culture separate it from the others - all devices should be sterilized carefully.
- If possible: store backup cultures in another room without aquaria or other open water surfaces.
- Avoid draughts - do not breeze directly into the cultures.

- Close algae tubes and algae reactors carefully, do not operate with open tops; cover aquaria.

Methods for algae breeding

Strain cultures / preservation cultures / back up cultures:



Strain cultures with different algae; here cultivated in Erlenmeyer flasks with sterile plugs on a shaker.
picture: AquaCare

Open a strain culture of every organism you get. Do not work with cultures without create a backup culture / preservation culture. These cultures may be cultured a long time at sub-optimal conditions: cool, less light. Work with minimum two back up cultures per strain. If you divide the cultures you can work with one part to open a mass culture - the other part is for back up again. Divide the cultures every some weeks to months (depending on strain) and fill up with fresh medium. The storing of cultures depends on the strains. Some organisms should be stored in a refrigerator, others with less light and higher temperature (e.g. window seats of a north window). Some autotrophic microorganisms change their colour if they are stored for longer time without nutrients (nitrogen, phosphorus): the green pigments (chlorophyll) are reduced and yellow or reddish pigments dominate.



The green culture (*Nannochloropsis salina*, strain Nan-4) has all nutrients, the yellow and orange live without nitrogen and phosphorus since 12 resp. 26 days.
pictures: AquaCare

Mass culture

To establish a mass culture you need on one hand a starting culture (strains are available in aquaristic shops or at AquaCare) and on the other hand a breeding system (algae tube, aquarium, algae reactor, see above).








Before starting a new mass culture, make sure that the strain contains what it should contain: microscopical control. Take a part of a back up culture, fill it into the breeding system and fill up with fresh medium. The dilution with fresh medium depends on several factors:

- the more active the start culture the higher the possible dilution

- the more different the medium of back up culture and fresh medium the less fresh medium may be used: especially pH value, salinity and temperature are important

- the maximum dilution depends on the strain
- If you do not know something about a new strain take on on part back up culture maximal

on part fresh medium. If the culture gets darker and more intensive you may dilute more.

<p>Start of a culture (unknown "micro algae" strain Xxx-8) Culture system: 2 litres glass cylinder with sterile aeration Medium: AquaCare algae medium A6, 10fold Lighting: 39 W JBL Solar Ultra Natur T5, 9000 K, 2200 lm Temperature: approximal 25°C, pH: ca. 8,5-9,0 you can see clearly: the start culture has a yellow colour (less nutrients); within two days the colour changes to green</p>						
						
1. Tag: 100 ml Startkultur + 100 ml Medium	2. Tag + 200 ml Medium	3. Tag + 400 ml Medium	4. Tag + 800 ml Medium	5. Tag auf 2 Liter aufgefüllt	6. Tag	7. Tag

If the intensity of a culture does not change anymore the maximum is reached. Now you can use the culture, e.g. to feed zooplankter like *Brachionus spec.*. If you need micro algae regularly use only half of the culture and add the rest with fresh medium to 100%. Monitor the culture regularly by microscopical control. Contaminated cultures should be drained. You may estimate the condition of the culture by eyes or with a strong magnifying glass, too. Many micro algae are growing free suspended, that mean the grow a single algae and not on surfaces or together in networks.

If you notify growth at the system walls or agglutination in the culture it is often a sign for contamination.

If you want to start with algae breeding, try a rigid strain at

first, e.g. *Nanochloropsis salina*, to get fast a success and routine in handling with algae.

If you have fish larvae and you need absolutely algae for feeding you should have two mass

cultures to make sure that every day algae are available - redundant principle.

Micro algae

Culture tanks and technique



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Small aquaria

Necessary equipment:

- aquarium made of glass with cover, about 10...100 liters (the faster the cultivated algae sediments the smaller the aquarium).
- air supply (membrane pump, small compressor).
- if you supply more than one aquarium each tanks should be saved with a check valve.
- sterile filter with maximum 0.3 pore size (use only the hydrophobic

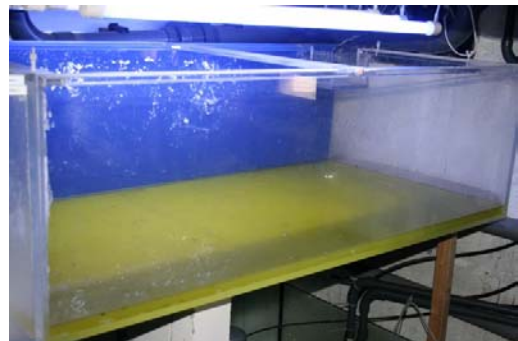
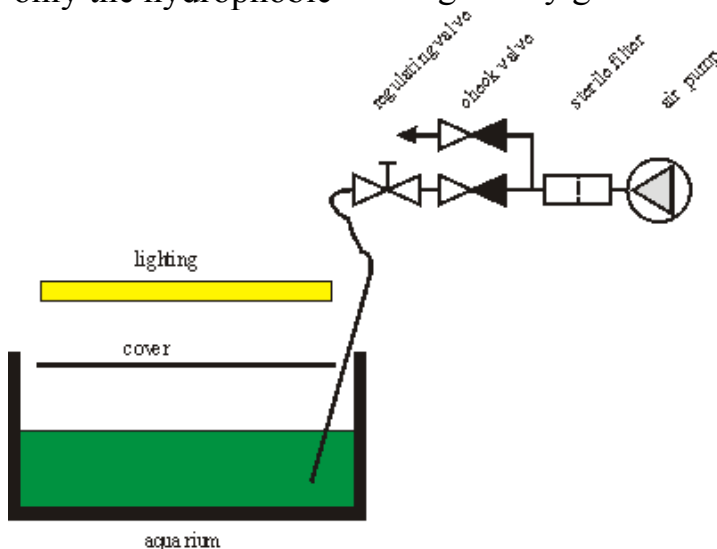
types); as an alternative use a lidded glass or bottle, put in filter floss and mount an air inlet and outlet.

- lighting: normally fluorescent lamps are the best choice, e.g. T5 with daylight spectrum.
- general equipment: see overview about breeding phytoplankton.

Functionality

To enable photosynthesis the micro algae are illuminated. Only with light the algae may grow and proliferate.

The incoming air has two jobs: first the ascending bubbles generate a current that prevents settling down of the algae. Second CO₂ gets into the culture to supply the algae with carbon. The aquarium should be far away from every zooplankton culture and normal aquaria. It happens very easily that contaminants (undesired foreign organisms) pollute the culture and you must pour it away.



Algae tubes

Necessary equipment:

- minimum two tubes with each minimum 4...5 liters volume;
- air supply (membrane pump, small compressor);
- if you supply more than one aquarium each tanks should be saved with a check valve;
- sterile filter with maximum 0.3 pore size (use only the hydrophobic types); as an alternative use an lidded glass or bottle, put in filter floss and mount an air inlet and outlet.
- lighting: normally fluorescent lamps are the best choice, e.g. T5 with day-light spectrum.
- general equipment see Overview about breeding phytoplankton.

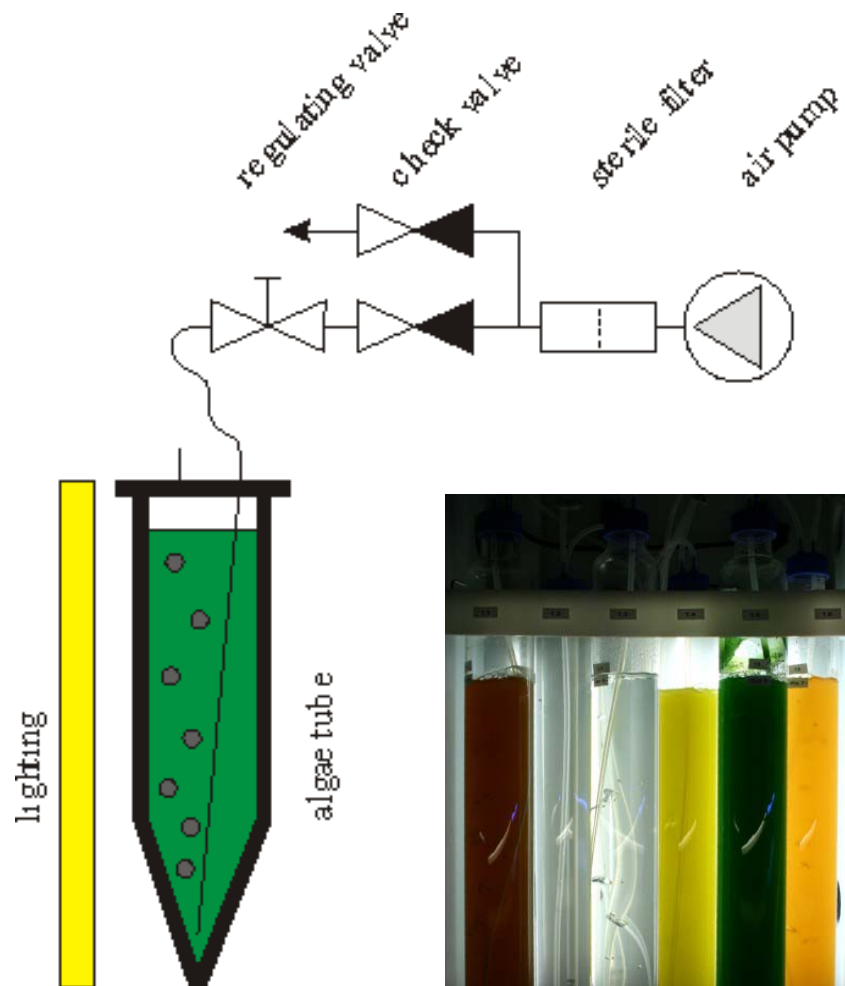
Functionality:

The principle is the same. Because of the very low water depth (diameter of the tube) the algae are illuminated better and therefore you can harvest the algae earlier. A very good mixing is possible with very low air input (1..2 bubbles per second is

enough). So it is possible to cultivate very sensitive algae, too. The closed system is a very good protection against contaminants. If the air outlet is connected to a bottle with sterile filter, it is possible to work absolutely sterile. But you must take care to the temperature. The intensive lighting warms up the water. The algae tube work best in cool rooms, or you aerate



with chilled air. Alternatively you can put the algae tube into a temperature regulated glass tank. The aquarium water should be mixed with a disinfecting fluid to prevent algae growth in the aquarium.



Design of the operation parameters

Lighting

- Fluorescent bulbs with daylight spectrum are the best choice as well as energy saving bulbs. Metal halids or mercury-vapour lamps are not practical because of their

very high heat input. The future will show if LED lamps will work, too.

- The numbers of lighting bulbs is important for the temperature: the low the room temperature, the more bulbs may be in-

stalled. The temperature of the algae culture depends on the species.

- If a lighting interval is not known, start with 12 hours lights on and 12 hours light off. Many robust species will grow

very good with 10 hours light on and 2 hours light off. The harvest is higher.

Air supply

- Work only with big air bubble. Small bubbles generated by air stons are producing a lot of aerosols and the algae will grow unchecked at places you do not want.
- The more sensitive the algae the lower the air input. Rigid algae will grow best at high air inputs. The more air is blown in the more CO₂ will reach the algae. If the algae settle down you must raise the air inlet.

Temperature

- Very seldom you need a heater. Normally you have too high temperatures in the culture. To prevent high temperatures you should choose the coldest room, you may take less lights or work with chilled air. Do not chill with fans. In open cultures (aquarium) the danger of contamination is too high and in closed systems (algae tube, algae reactor) the fan will not chill.

pH value

- Normally the pH is not regulated. Caused by photosynthesis CO₂ is needed and raise the pH - possibly up to 9.5.
- The carbonate hardness of the medium (sea water plus nutrients) should be at 7°dH (alkalinity 1.2). Higher values will lower the pH oscillation between night and day. To raise the KH you may use KH-plus or triple buffer.

Salinity

- The salinity has to be adjusted to the need of the cultivated algae. If a species may stand very high salinity, too, it is better to cultivate at higher salinities to prevent the culture of contamination.

Concentrations of nutrients

- The more sensitive the algae the lower the nutrient concentration. Please dose the medium correspondingly to the cultivation instruction.
- The higher the level of the algae aquarium (larger the diameter of the algae tube or reactor) the lower the concentration of nutrients. If the culture gets too green the distant algae

are not illuminated properly. This effect is only reversible with more lights.

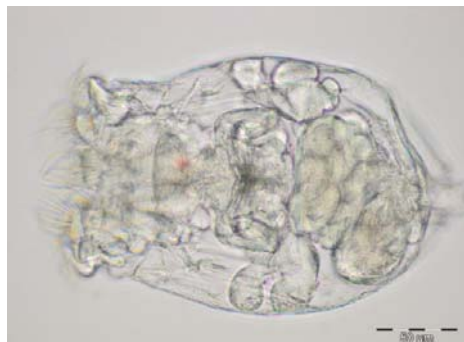
- If green cultures get yellow or orange with the time the medium has any nutrients. It is better to harvest algae before starving.
- Before using the algae for feeding you must check the nutrient concentration (nitrate, phosphate, if necessary silicic acid). Too high concentrations will fertilize the aquarium or zooplankton tank. At high concentration the culture has to be filtered or kept some days longer in the algae tank.
- The AquaCare algae media are composed in that way, that phosphate is totally used if nitrate is left a little bit. So you can check the nitrate concentration very fast with nitrate test sticks. If the concentration is below 10 mg/l you can use the culture without scruple.

Zooplankton -

An overview about breeding zooplankton



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Brachionus plicatilis L-type
(rotifer)



Artemia-Nauplie
(hatched)



Artemia „salina“ sub-adultus

Requirements for breeding zooplankton

To get high-quality zooplankton normally a phytoplankton breed is necessary. It is possible to feed zooplankton with substitutes (e.g. yeast), but important ingredients like high unsaturated fatty acids (HUFA) are missing or their composition is in a poor combination. Only if zooplankton has perfect combination and concentration of all essential ingredients fish larvae or others will grow without malformations. To cultivate phytoplankton the overview about breeding phytoplankton may help. Only some additional things are needed:

- Plankton screens with 50 to 150 µm, to catch the wanted zooplankton organisms;
- additional breeding flasks or tanks;
- a sheltered place (far away from algae cultures) to prevent contamination of the al-

gae culture with their "enemies";

- equipment for enrichment the zooplankton (concentrates of essential substances like vitamins, fatty acids, minerals, antioxidants).

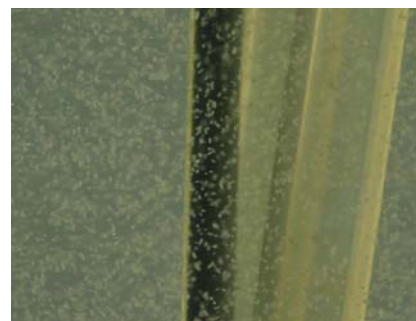


Artemia „salina“ fed with *Phaedactylum tricornerutum* in a zooplankton tube

Enrichment of feeder animals

Even if zooplankton is fed with high-quality micro algae it makes sense to enrich the zooplankton with essential ingredients before feeding. Many of the dissolved substances penetrate during enrichment through the body of the zoo-

plankton (see refrigerator method) or are actively incorporated by the organisms (flask method). Both methods are quoted from MAI 2004.



Brachionus plicatilis (fed with *Nanochloropsis salina*) in a zooplankton tube (the vertical stick has a diameter of 3 mm)

Refrigerator method (for very fresh hatched nauplia, that cannot eat)

1. Fresh hatched nauplia (e.g. *Artemia*) should be best concentrated: pour the culture through a 100-150 µm screen and wash the remaining nauplia with very less water from the screen).
2. Mix them with enrichment food (see MAI 2004) - the

proportions have to be determined by yourself.

3. Spread the nauplia-pulp in a flat flask with cover, e.g. petri dish.
4. Store it for 24...48 hours in a refrigerator and feed the living nauplia to your larvae. Never feed dead zooplankton!

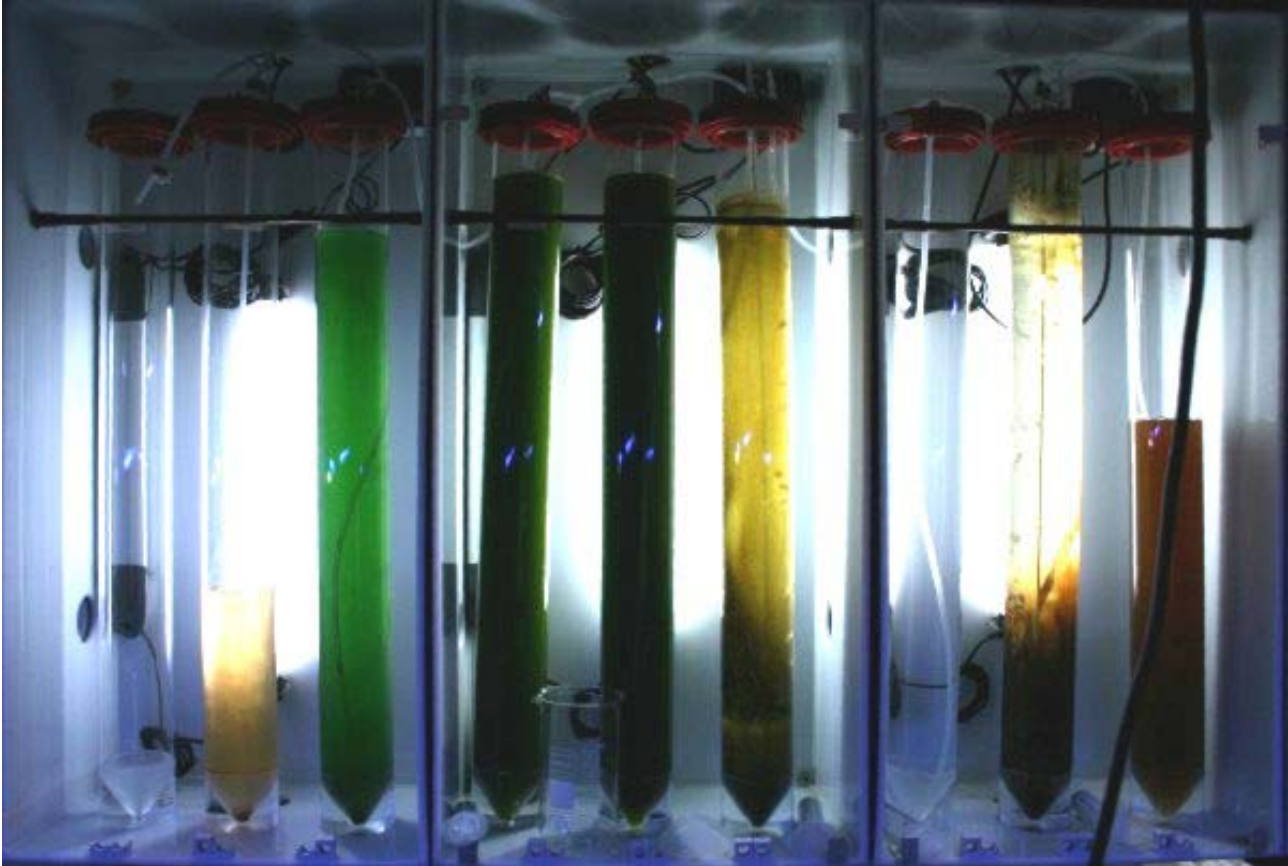
Flask method (for older nauplia, that ingest food actively)

1. Concentrate 24...36 hour old *Artemia* nauplia and wash them from the screen with less sea water; fill them into a clean *Artemia* flask.
2. Add an enrichment concentrate (see MAI 2004) - the proportions have to be determined by yourself.
3. After 6...8 hours (not longer for *Artemia*; for others the time may vary) the nauplia are well-fed and should be fed to the larvae.
4. You can use surplus nauplia later on by a new enrichment process.

Zooplankton - culture tanks and necessary equipment



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Eine Zooplanktonanlage mit mehreren Planktonröhren

Zooplankton aquarium

Necessary equipment

- Glass aquarium with glass cover, about 20...100 litres.
- Air supply (membrane pump or small compressor); if you supply more than one tank each line should be protected with a check valve.
- Lighting: not necessary but useful, because a part of the produced pollutants are eliminated by feeder algae, if they have light.

- General equipment see overview about breeding phytoplankton.

Functionality

Fill the aquarium preferably with phytoplankton and aerate them with large bubbles (all algae should be in motion). The added zooplankton culture (e.g. *Brachionus* or fresh hatched *Artemia*) subsist on the phytoplankton. If the coloured turbidity diminishes, add additional algae or harvest the zooplankton. Different sizes (stages of the zooplankton) will be separated with different screens. Add the harvested zooplankton

to your fish larvae. Possibly enrich the zooplankton with essential nutrients before.

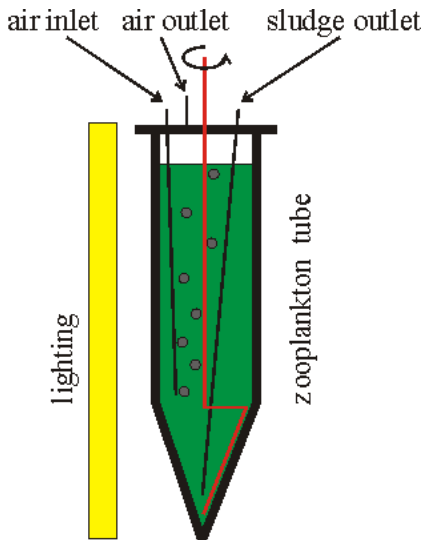
Zooplankton tube

Necessary equipment

- Zooplankton tubes with each 4...5 litres volume; but with more hose connectors than algae tubes. At cultures that generates lots of detritus a paddle scraper is useful.
- Air supply (membrane pump or small compressor; if you supply more than one tank each line should be protected with a check valve.

- Lighting: not necessary but useful, because a part of the produced pollutants are eliminated by feeder algae, if they have light.
- General equipment see overview about breeding phytoplankton.

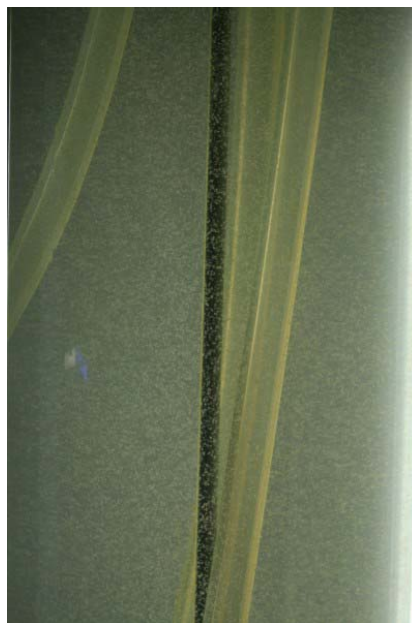
Functionality



The principle is the same like the zooplankton aquaria. But the system is nearly air-tight to prevent contamination and you can drain the forming detritus. For this purpose the delivered paddle scraper will loosen deposits (detritus) of the cone. Then you can suck the detritus with a hose. The air inlet is mounted about 5 cm over the bottom (only with little sedimenting algae like *Nannochloropsis* spec.). So the algae are in abeyance and the mud sinks into the cone. With strongly sedimenting algae the air should be bubbled at the bottom; about 10...20 minutes before sucking the detritus you must stop the air input to give the detritus time for sedimenting.



A *Brachionus plicatilis* culture, fed with *Nannochloropsis salina* at complete mixing mode (air inlet at the bottom): the medium is turbid by the produced detritus (rest of algae, faeces of the rotifers, dead animals)



The same conditions but not at completed mixing mode (air inlet is about 5 cm over the bottom): the white dots in the water column are *Brachionus* – there are very less other particles in the medium. You can regularly suck the sedimented detritus with a hose. The partition of culture and detritus is only working with algae that sediment less, e.g. *Nannochloropsis salina*.

Dimensioning the operation parameters

Lighting

- The best are fluorescence lamps with day light spectrum, but energy saving bulbs are useable, too. Compared to algae tubes the light intensity may be very low. So normally you do not get temperature problems.

Air supply

- Use only large bubbles, because fine bubbles produced with air stones are generating much aerosols. Then the danger of contaminating other systems (e.g. algae culture) with predators (e.g. rotifers) is large.
- The more sensitive the organism the lower the air input to reduce powerful currents with strong shearing forces.

Temperature

- Normally too high temperatures are avoided by using less light. But if you raise up organisms with a low temperature maximum you must use a chilling method.
 - Establish the system in a cool room.
 - Work with chilled air.
 - Work with a cooling coil that is installed in an aquarium that contains the zooplankton tube.
 - Do not chill with fans, because closed system will not cool down with fans and at open cultures the danger of drifting predators into algae cultures is too high.

pH value

- Normally the pH is not controlled.
- If very high pH oscillations occur measure the alkalinity (KH value) and add a pH

buffer like AquaCare Triple Buffer when indicated.

Salinity

- The salt content (salinity) should be adapted to the organism you would like to cultivate (zooplankton and phytoplankton). If a species or strain is able to survive high salinities choose a high salinity. The higher the salinity the less contaminants will occur. The culture will be clean for a longer time.
- The more the air input the more water will evaporate. Check the salinity regularly and add distilled or R.O. water as needed.

Concentration of feeder organisms and waste products

- The more sensitive the cultivated species, the less concentration of the feeder organism, e.g. algae. Too high algae densities cause high growing rates of the zooplankton. In the process high ammonia concentrations are produced and the plankton animals will die. The tolerance against ammonia is very different: *Artemia* “salina” stands concentrations to 391 µg/l (this is 4,08 mg/l Ammonium at 20.5°C, 35/1000 and pH 8,63), others will die faster.

Cultures and media for plankton breeding


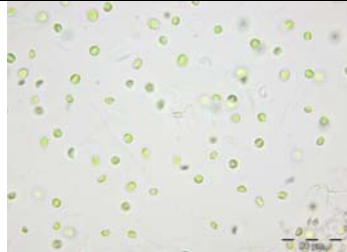




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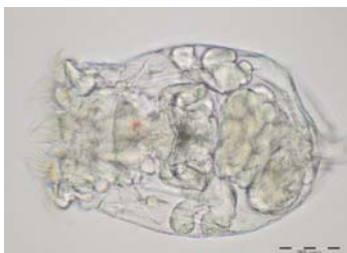


Algae cultures

The cultures of AquaCare are delivered in 1 liter flasks. There is a high purity level (microscopic control), but we cannot guarantee axenic culture (free of any other organisms). The transport (within Germany) take place only Wednesdays and not at very hot weather. Detailed information are only available in the internet.

<i>Nannochloropsis salina</i> , Strain Nan-4			
		Cell length: 2...5 µm Cell wide: 70...100% of cell length Order number 1 litre: klt-nan4-010	<i>Nannochloropsis salina</i> is an extreme small micro algae for feeding very small bis larger zooplankton organisms. It is very rigid and prevails easily in mixed cultures at non optimal conditions. Ideal for beginners. Medium: algae medium 14:1
<i>Phaeodactylum tricornutum</i> , Strain Pha-7			
		Cell length: 25 µm Cell wide: 2.5 µm Order number 1 litre: klt-pha7-010	Pennate diatom; easy to grow. It should be fed only indirect (e.g. with <i>Brachionus</i>) to larvae. Medium: algae medium 7:1


Zooplankton cultures

<i>Brachionus plicatilis</i> L-type, Strain Bra-9			
		Cell length: 200...400 µm Cell wide: ca. 50...75% of length Order number 1 litre: klt-bra9-010	Ideal "transport container" for nutrients for feeding small and medium larvae. The nutrient concentration of <i>Brachionus</i> is very low, but it is possible to enrich these rotators very easily. Therefore a high-grade nutrient concentrate and/or fresh micro algae have to be fed. After it <i>Brachionus</i> should be used.

Breeding media (concentrates)

Breeding media of AquaCare are designed exactly for the needs of the different algae. To prevent bacterial growth only mineral substances are used as far as possible. The pH of the ready solution is not influenced. To validate if the medium contains still enough nutrients the nitrate concentration should be controlled regularly by test sticks. If nitrate is diminished the phosphate concentration is low, too. The finished algae culture is ready for use in the next plankton level without cleaning of centrifugating. The direct feeding to zooplankton (if they need phytoplankton) is possible, too. Phosphate and nitrate concentrations are below harmful concentration all the time. Eutrophication or undesirabel development are expelled.

Different species of algae have very different needs of nitrogen and phosphorous - the N:P ratio may differ extremely from the so-called REDFIELD ratio. AquaCare has optimized the media to the cultivated algae species to make sure that after the breeding time both nutrients N and P are nearly consumed. If you want to cultivate an algae with unkown requirements choose the medium with a N:P ratio of about 16:1. To create any N-P ratio you can use the N- and P-additives.

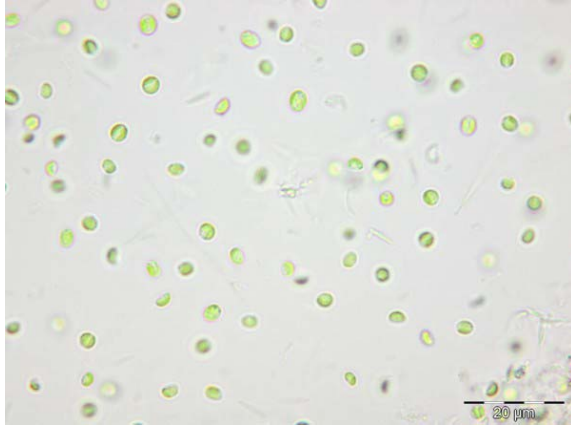
		1 litre lasts for: 10,000 litres 1fold Medium 1,000 litres 10fold Medium 100 litres 100fold Medium contains 16 trace elements, nitrogen and phosphorous
N:P = 14:1	Order number 1 litre: klt-14-010	e.g. for <i>Nannochloropsis salina</i> ,
N:P = 7:1	Order number 1 litre: klt-07-010	e.g. for <i>Phaeodactylum tricornutum</i> ,
N-additive	Order number 1 litre: klt-N-010	for mixing any N-P ratio: contains 23.5 g N/l (1.685 M) resp. 3.3 g P/l (0.1053 M)
P-additive	Order number 1 litre: klt-P-010	at dosing 1 ml per litre yields 104 mg/l nitrate resp. 10 mg/l phosphate in the ready algae medium

Cultures for breeding micro algae

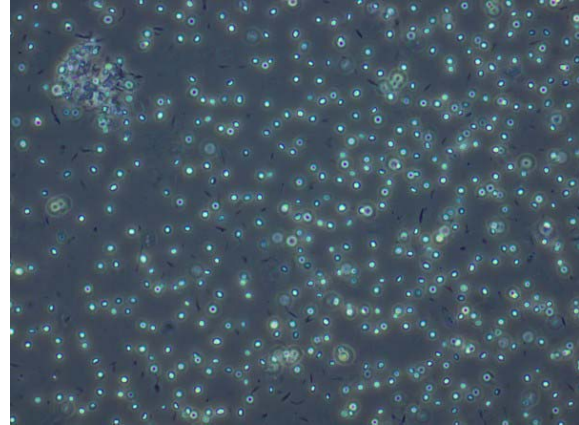
Nannochloropsis salina



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Nannochloropsis salina (bright field)



Nannochloropsis salina (phase contrast)

Version	10.2010
Species	<i>Nannochloropsis salina</i>
Class	Eustigmatophyceae
General description	green, spheroidally to light oval, very small micro algae
Size	length: 2-5 µm; wide: 70-100% of length
Ingredients	<p>Fatty acids: EPA and AA; content of EPA up to 40% of fatty acids (ZVI COHEN 1999) depending on culture conditions; any DHA; max. HUFA concentration at ca. 25°C; at other authors 8...16°C (ZVI COHEN 1999); optimal nitrogen supply increases HUFA part (ZVI COHEN 1999); part AA = 9,5%, EPA = 25,8% and DHA = 4,18% (CHAKRABORTY et al. 2007);</p> <p>Pigments: Chlorophyll a but not chlorophyll b or c, (ZVI COHEN 1999); β-Carotin, Violaxanthin, Zeaxanthin + Anthraxanthin (at strong lights) (ZVI COHEN 1999);</p> <p>Sugar: Glucose, Fucose, Galactose, Manose, Rhamnose, Ribose, Xylose, but any Arabinose; (ZVI COHEN 1999)</p> <p>Amino acid: less Methionin, Trpytophan, Cystin, Histidin, Hyroxylprolin, but high concentration of Aspartat, Glutamat, Prolin (ZVI COHEN 1999);</p>
Colour of culture	green-yellow; at N-P-lack yellow to orange
Effort of cultivation	low
Characteristic of cultivation	is growing fast and is able to overgrow other algae cultures; is sedimenting very bad; accept relative high concentration of pesticide DCMU (ZVI COHEN 1999);
Cultivation in	algae aquarium algae tube

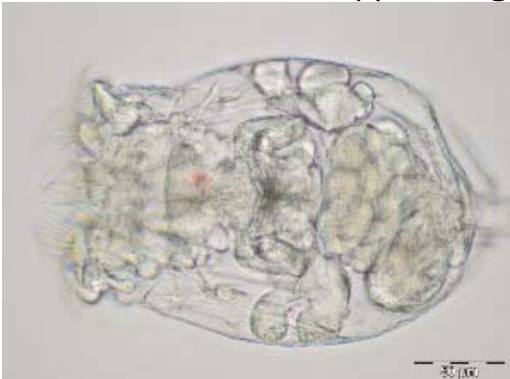
	algae reactor
Lighting	fluorescen lights, energy saving lamps, daylight, different spectra possible day / night from 10:2 h12:12 H; at strong lighting protection pigments of VAZ cycle (Violaxanthin/Antheraxanthin/Zeaxanthin) are formed, but not of DT cycle (Diadinoxanthin/Diatoxanthin) (LOHR 2000);
Aeration / circulation	less ... strong extrem rigid against shear forces
CO ₂ fertilization	possibel (best grow) but not necessary (less effort)
Range of pH value	7.5 ... 8.5
Range of temperature	20 ... 25°C; below 10°C and over 38°C any growth (ZVI COHEN 1999)
Range of salinity	AquaCare is cultivating at 35/1000
Kind and concentration of medium	alage medium (16:1): 1 ... 100fach; for normal growth 10fold medium is best
Backup culture	Window seat culture: cool; dived and fill up with 10fold medium every some month, shaking daily; shaker culture: dived and fill up with 10fold medium every some month; refrigerator culture: wake up the culture very carefully: equal temperature for minimum 24 hours at diminished lights, after it dilute the culture carfully with 1fold medium
Suitable for	<i>Artemia</i> spec., all stages from nauplius to adultus <i>Brachionus</i> spec. SS- to L-type

Cultures for breeding zooplankton

Brachionus plicatilis strain Bra-9 (L-type, large)



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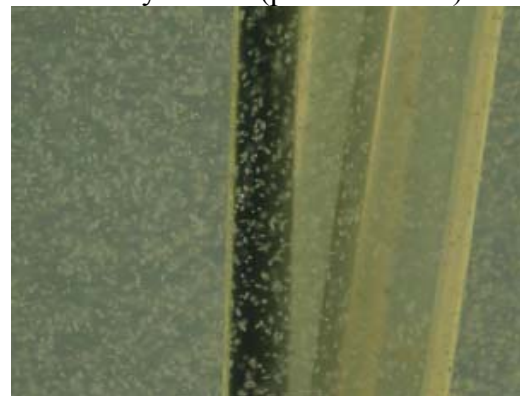
Brachionus plicatilis, Bra-9, L-type (bright field)



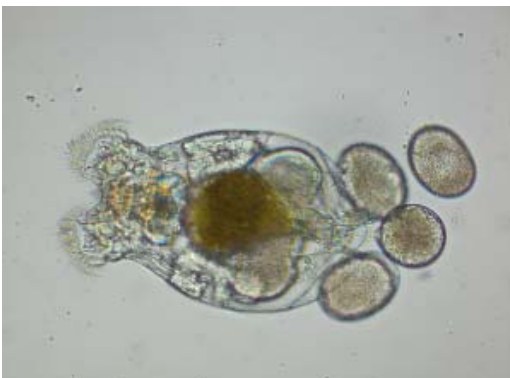
Brachionus plicatilis, Bra-9, L-type, with subitaneous egg: the red eye spot in the egg is clearly visible (phase contrast)



Brachionus plicatilis, Bra-9, L-type, fed with *Phaeodactylum tricornerutum* (phase contrast)


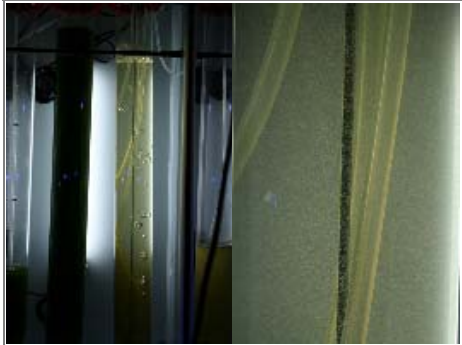


Brachionus plicatilis, Bra-9, L-type in mass culture (ca. 250 animals per ml), raised in the AquaCare zooplankton tube and fed with *Nannochloropsis salina*.



Brachionus plicatilis, Bra-9, L-type, with four eggs (one just detached)

Version	11.2010
Species	<i>Brachionus plicatilis</i> strain Bra-9, L-type Because of the many different species and the fact that most experiments are done under the name <i>B. plicatilis</i> , there are no clear indications about breeding - especially temperature and salinity range differs by the species and you must do your own experiences for best breeding. Instructions by AquaCare are valid only for the marked strain (e.g. Bra-9).
Family	Rotatoria (rotifers); a splitting into three different species is possible: <i>B. plicatilis</i> (Müller) = L-type, <i>B. rotundiformis</i> (TSCHUGUNOFF) = S-type, <i>B. ibericus</i> (CIROS-PÉREZ et al.2001) <i>B. plicatilis</i> (numbers of chromosome 2n=22), <i>B. rotundiformis</i> (2n=25), SS-type is probable subspecies of <i>B. rotundiformis</i> (KOREA-US AQUACULTURE) GÓMEZ et al. 2002 investigate with nucleus and ribosome DNA analysis 9 species in the complex of <i>B. plicatilis</i> ; maybe more than 14 species
General description	oval body, in the back a food for paddling or holding on a substrate; carries one to two seldomly four subitane eggs outside of its body in the near of the food; in the front is a ciliary collar = wheel organ (in situ it looks like rotating wheels), that ends in the buccal tubus; red eye spot; The food is uptaken by the external cilia and is transferred by the cilia of the buccal tubus to the mastax. <i>Brachionus</i> identifies particles of the wrong size or already digested but not crushed particles (e.g. yeast cells) and spits them out. Afterwards accepted particles will be crushed by the mastax (gizzard) and directed through the oesophagus into the stomach. 2 minutes later the sphincter muscle opens and the stomach content reaches the midgut = intestinum. After another 10-20 minutes the gut content is discharged through the anus; new stomach content reaches the midgut. Following passages through the digestive tract are faster. Starving animals (> 48 h without food) need more time for digesting (30-90 min). (LINDEMANN 2001) Exact description see STORCH & WELSCH 2009. <i>Brachionus</i> is used as a indicator for environmental toxins (acute toxicity after ASTM)
Size	the size depends on factors like salinity and from type of strain; strain Bra-9, L-type: 200...400 µm (AquaCare) S-type: 99-281 µm (THEILACKER & MCMASTER 1971)
Ingredients	<i>Brachionus</i> has less nutrients but it is a ideal transport container for essential substances. If <i>Brachionus</i> is fed with valuable food, e.g. micro algae, or if it is enriched with essential substances you get high quality food for your larvae. 16% of the brought in nitrogen is needed for growth and reproduction, the rest is excreted (TANAKA 2007)
Colour of culture	depending on food from white to coloured turbid
Effort of cultivation	Bra-9: very less, very rigid against environmental changes

<p>Characteristic of cultivation</p>	<p><i>Brachionus</i> is contaminating very easily other cultures like algae cultures. Bacteria and fungi that may harm will establish very easily if <i>Brachionus</i> is fed with non-algae food.</p> <p>Do not starve <i>Brachionus</i> too long; best feed them daily - next day the food turbidity should be disappeared (you see it easily if you feed algae: if the colour of the algae is disappeared you can feed <i>Brachionus</i> again, see pictures below).</p> <p><i>Brachionus</i> cultures produce lot of detritus (excrements, dead animals, agglutinated algae): it is best to suck all sediments daily (very easily to do with a zooplankton tube with paddle scraper.</p> <p>The higher the particle density and the lower the <i>Brachionus</i> density the better the intake of particles (LINDEMANN 2001).</p> <p>If you feed bakery yeast take 0.2 g with 30 ml sea water, mix it well; feed only as much that the turbidity is gone next day; aerate the culuture well.</p> <p>Possible rotifer densities: In AquaCare 4 litre zooplankton tube (see picture below) at room temperature and daily harvesting of 400 ml (10% per day) densities of 100...150 / ml are easily possible - so you can get 40,000...60,000 animals per day. 200-700/ml (LINDEMANN 2001); 500-1,500/ml (PFEIFFER & LUDWIG 2007); 20,000/ml (KOREA-US AQUACULTURE);</p> <div style="display: flex; justify-content: space-around;"> <div data-bbox="384 1014 847 1357">  </div> <div data-bbox="884 1014 1347 1357">  </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 5px;"> <div data-bbox="384 1357 847 1469"> <p><i>Brachionus</i> fed with <i>Nannochloropsis salina</i>: green turbidity</p> </div> <div data-bbox="884 1357 1347 1469"> <p>24 hours later: the algae are eaten; the white turbidity (small white dots) is induced by <i>Brachionus</i></p> </div> </div>
<p>Cultivation in</p>	<p>Zooplankton aquarium Zooplankton tube (recommended)</p>
<p>Lighting</p>	<p>Not absolutely necessary but lights make sense if you feed micro algae: the algae take in the excreted nitrogen and phosphate, additional the algae produces oxigen for <i>Brachionus</i>. A lighted culure fed with algae is more stabel. Avoid UV radiation (direct sun light).</p>
<p>Aeration / circulation</p>	<p>less ... medium</p>
<p>Range of pH value</p>	<p>at pH 6.0 hardly activity, above 7.0 <i>Brachionus</i> are eating (LINDEMANN 2001); avoid pH oscillations; at pH 6.5...8.5 any differences in activity and respiration; too high pH values have worse effects than too low. (KOREA-US AQUACULTURE);</p>
<p>Range of temperature</p>	<p>At 20...25°C the stomach is filled after 5 minutes, at 10...15°C it needs 120 minutes. at 5°C considerably longer. at 0°C food is not uptaken and damages</p>

	occur; above 30°C <i>Brachionus</i> stops eating, too. (LINDEMANN 2001). max. growths at 30...34°C (THEILACKER & MCMASTER 1971); avoid temperature oscillations; temperature maximum for <i>B. rotundiformis</i> higher than for <i>B. plicatilis</i> (KOREA-US AQUACULTURE); temperature minimum for <i>B. Rotundiformis</i> 20°C, <i>B. plicatilis</i> 10°C (KOREA- US AQUACULTURE);
Range of salinity	59...957 m-osmol/l, equates to: 2...32/1000 (converted by WEAST 1985); the inner salt concentration of the animal equals the salt concentration of the medium (EPP & WINSTON 1977); Bra-9: 35/1000 (AquaCare)
Range of oxygen	> 1 mg/l (KOREA-US AQUACULTURE);
Kind and concentration of medium	Sea water with the same salt concentration as the feeder algae, but not without the range of its tolerance (see range of salinity). Heterotrophic growths with glucose possible; glucose is intaken actively by the animal (LI et al. 1993);
Backup culture	Backup culture fed with micro algae (no additional organic substances), to prevent contamination with bacteria and fungi; The generation of mictic eggs depends on e.g. population density: > 0.1 female per ml (STELZER & SNELL 2003)
Suitable for feeding	medium and large fish larvae; the quality of <i>Brachionus</i> depends extremely on kind of food and method of enrichment