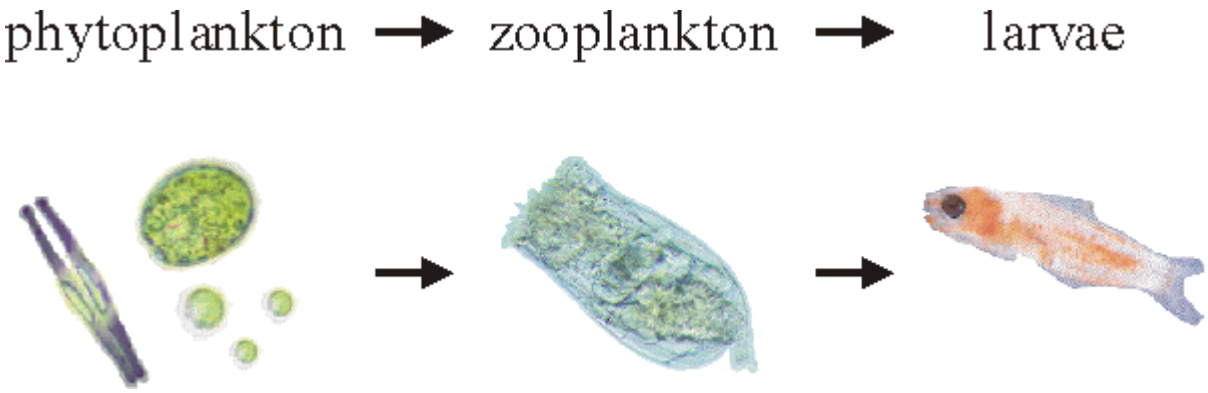
Micro algae -
Overview for breeding phytoplankton

AquaCare GmbH & Co. KG
Am Wiesenbusch 11 - D-45966 Gladbeck - Germany
☏ +49 - 20 43 - 37 57 58-0 • 🖷: +49 - 20 43 – 37 57 58-90
www.aquacare.de • e-mail: info@aquacare.de



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. Allthough 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 organics 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 oltramicroplankton: < 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 (megaplankon). 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 ralatively 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 additon 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 you 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 loose 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 wil bloom the algae culture is lost. At the beginning of an invasion you can clean the culture by an plankton screen. - Maybe you can lump together with other aquarists or with an involved shop dealer to by a microscope.

Plankton screens

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

To divide different organismen 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 large than the size of the algae. For example to clean a Nannochloropsis-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. Additional 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 dilluting 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-filtraton unit is very large not suitable for a hobby application.

If you use sea water for algaes 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 childen!

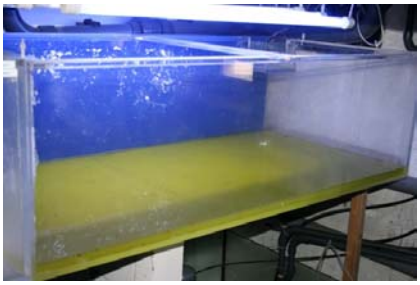
A set for producing a disinfection solution
picture: AquaCare

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

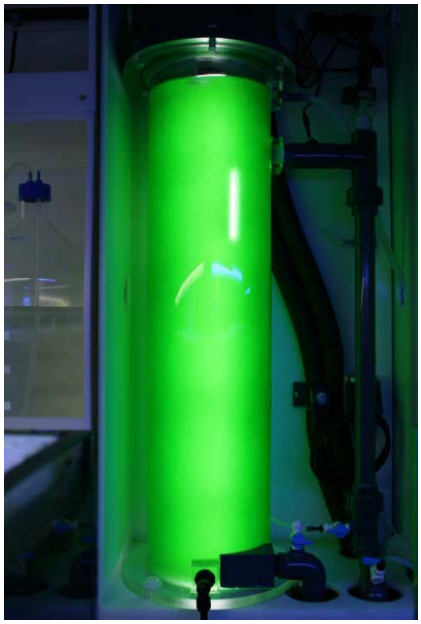
2. Algae tubes

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 consequentyl 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 steril 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 paratmeter 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

Battery driven milk frothers are very useful to homogenize food.
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“ and *Brachionus* spec. “Bra9”.

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 loose 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.
- Lable all bottles and cultures you work with: otherwise you will mix up cultures and media.
- Write a protocoll: only with it you work methodically.
- Seperate phytoplankton cultures from zooplankton cultures: otherwise aerosols may transport unpopular guest 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 zooplankter are tough and they are able to withstand rough conditons. If the conditions gets better - new medium - they will grow rapidly again.
- If you work with a new culture seperate it from the others - all devices should be sterilzed carefully.
- If possible: store backup cultures in another room wihout 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 / perservation culture. These cultures may be cultured a long time at suboptimal 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 reducted and yellow or redish pigements 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 is 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.

Start of a culture (unknown "micro algae" strain Xxx-8)

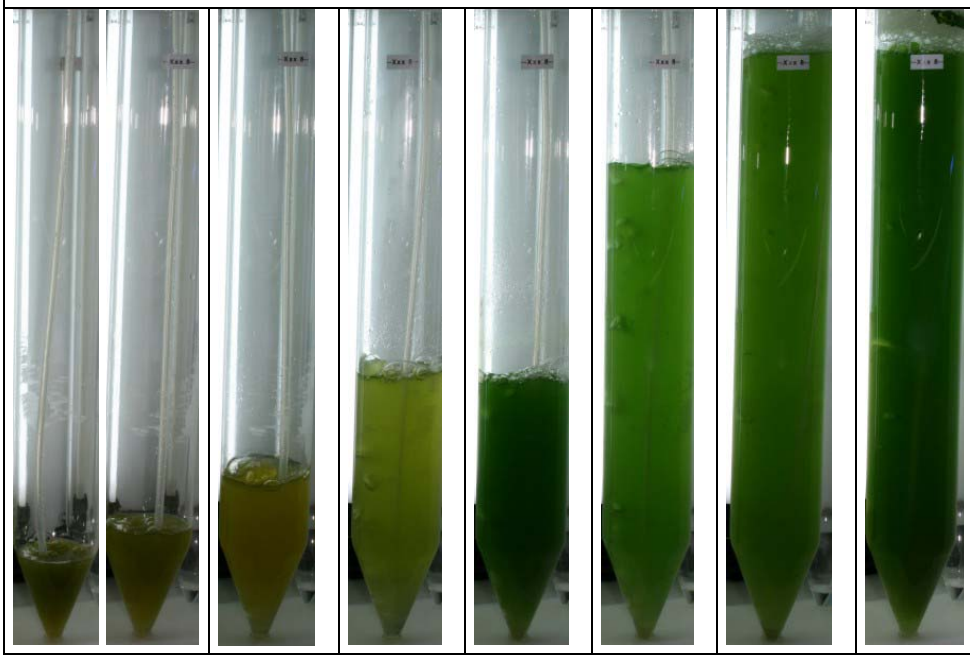
Culture system: 2 litres glass cylinder with sterile aeration

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



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	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 microscopial 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.