Annex A
Live food production for fish larviculture

Extracted from Agh & Sorgeloos (2005) and Lavens & Sorgeloos (1996)

ROTIFER PRODUCTION

1. Water disinfection

The culture water (seawater diluted with tap water to a salinity of 25 ppt) is aerated, prefiltrated over a 1 µm filter bag and disinfected overnight with 5 mg/l NaOCl. The next day the excess of NaOCl is neutralized with $Na_2S_2O_3$ and the water is filtered over a 0.45 µm filter.

2. Upscaling of stock cultures to starter cultures

a. The upscaling of rotifers is carried out in static systems consisting of erlenmeyers of 500 ml 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 rotifers/ml. During this short rearing period no aeration is applied.

b. Once the rotifers have reached a density of 200-300 individuals.ml^{-1} 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. If only single strainers are available this handling can be carried out with two separate filters. Moreover, if rinsing is performed under water the rotifers will not clog and losses will be limited to less than 1%.

c. The concentrated rotifers are then distributed in several 15 l bottles filled with 2 l water at a density of 50 individuals/ml and mild tube aeration provided. In order to avoid contamination with ciliates the air should be filtered by a cartridge or activated carbon filters. Fresh algae (*Chlorella* 1.6 × 10^6 cells/ml) 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.

3. Mass production on algae and yeast

The mass production on algae and yeast is performed in a batch or semi-continuous culture system:

a. Batch culture system

The tanks (1 200 l capacity) are half filled with algae at a density of 13-14 × 10^6 cells/ml and inoculated with rotifers at a density of 100 individuals/ml. The salinity of the water is 23 ppt and the temperature maintained at 30°C. The first day active baker's yeast is administered two times a day at a quantity of 0.25 g/10^6 rotifers. The next day the tanks are completely filled with algae at the same algal density and 0.375 g baker's yeast per million rotifers is added twice a day. The next day the rotifers are harvested and new tanks are inoculated (i.e. two-day batch culture system).

b. Semi-continuous culture

In this culture technique the rotifers are kept in the same tank for five days. During the first two days the culture volume is doubled each day to dilute the rotifer density in half. During the next following days, half the tank volume is harvested and refilled again to decrease the density by half. On the fifth day the tank is harvested and the procedure started all over again (i.e. five-day semi-continuous culture system).

4. Enrichment

Rotifers can either be enriched for highly unsaturated fatty acids (n-3), vitamin C, and protein:
HUFA enrichment can be done with algae, formulated feeds or home-made and commercial oil emulsions.

a. Enrichment with algae

The most commonly used algae for growing rotifers are Nannochloropsis and Isochrysis. Rotifers incubated in these algae cultures (at approximately 5-25.106 algae/ml) are incorporating the essential fatty acids in a few hours time. Storable algal products such as algal pastes and frozen algae can also be used for rotifer cultures.

b. Enrichment with oil emulsions

For the enrichment or boosting of rotifers several approaches can be followed: 1) the adjustment of the lipid and vitamin content of the rotifers just before feeding them to other organisms is referred to as short term enrichment (generally less than 8 h exposure) and 2) the feeding of rotifers on a complete diet or long term enrichment (rearing of the rotifers on the enrichment diet for more than 24 h).
The short term enrichment technique has the advantage of being fast and flexible, but very often produces lower quality rotifers with a too high lipid content and poor hygienic quality. The biggest problem in this enrichment technique resides in the fact that a lot of rotifers are lost when they are concentrated (sticking of the rotifers) at high density. Moreover, the retention time of the nutrients, which are mainly accumulated in the digestive tract of the rotifers, is very short and can create problems when the rotifers are not eaten immediately.

ARTEMIA (*nauplii*) PRODUCTION

1. Desinfection

a. Prepare 200 ppm hypochlorite solution: ±20 ml liquid bleach (NaOCl)/10 l.
b. Soak cysts for 30 minute at a density of ± 50 g cysts/l.
c. Wash cysts thoroughly with tap water on a 125 µm screen.

2. Hydration

Hydrate cysts by placing them for 1 h in water (≤ 100 g/l) with aeration at 25°C.

3. Decapsulation

a. Collect cysts on a 125 µm mesh sieve, rinse, and transfer to the hypochlorite solution.
b. The hypochlorite solution can be made up (in advance) using liquid bleach NaOCl (fresh product; activity normally =11-13% w/w) or bleaching powder $Ca(OCl)_2$ (activity normally ± 70%) in the following proportions:
    i. 0.5 g active hypochlorite product (activity normally labelled on the package, otherwise to be determined by titration) per g of cysts.
    ii. an alkaline product to keep the pH>10; per g of cysts use:
        - 0.15 g technical grade NaOH when using liquid bleach.
        - Either 0.67 $NaCO_3$ or 0.4 g CaO for bleaching powder; dissolve the bleaching powder before adding the alkaline product; use only the supernatants of this solution.
        - Seawater to make up the final solution to 14 ml per g of cysts.
c. Cool the solution to 15-20°C (i.e. by placing the decapsulation container in a bath filled with ice water). Add the hydrated cysts and keep them in suspension (i.e. with an aeration tube) for 5-15 min. Check the temperature regularly, since the reaction is exothermic; never exceed 40°C (if needed add ice to decapsulation solution). Check evolution of decapsulation process regularly under binocular.

4. Washing

When cysts turn grey (with powder bleach) or orange (with liquid bleach), or when microscopic examination shows almost complete dissolution of the cyst shell (= after 3-15 min.), cysts should be removed from the decapsulation suspension and rinsed with water on a 125 µm screen until no chlorine smell is detected anymore. It is crucial not to leave the embryos in the decapsulation solution longer than strictly necessary, since this will affect their viability.

5. Deactivation

Deactivate all traces of hypochlorite by dipping the cysts (< 1 min.) either in 0.1 N HCl or in 0.1% $Na_2S_2O_3$ solution, then rinse again with water. Hypochlorite residues can be detected by putting some decapsulated cysts in a small amount of starch-iodine indicator (= starch, KI, H_2SO_4 and water). When the reagent turns blue, washing and deactivation has to be continued.

6. Incubation

Incubate cysts 15-20 hours (at 25°C) with seawater 5-32 ppm at density of 2 g/l for smaller volumes (<20 l) a maximal cyst density of 5 $g.l^{-1}$ can be applied. Required amount of cysts depends on hatching efficiency of cyst batch (number of nauplii per gram) and required amount of nauplii.

7. Enrichment (triplicate)

a. Take 200,000 nauplii.
b. Rinse nauplii on sieve with filtered seawater.
c. Stock in 1 l cone with point and airstone aeration at 200 nauplii/ml.
d. Count initial density (3 × 250 N/ml) add 2 × 0.2 g of HUFA emulsion (2 × 2 ml of 5 g/ 50 ml diluted emulsion) over 24 h (t = 0 h and t = 10-12 h).
e. 24 h at 28°C, monitor O_2 regularly!

8. Harvesting

a. Count survival, *i.e.* count dead nauplii (no lugol) and total nauplii (+lugol) from 3 × 250 µl sample per cone.
b. Remove all aeration.
c. Concentrate nauplii using light.
d. Siphon nauplii on sieve.

References/Literature cited

Agh, N. and Sorgeloos, P. (eds.), 2005. Handbook of Protocols and Guidelines for Culture and Enrichment of Live Food for Use in Larviculture, (www.urmia.ac.ir/PubFiles/203140_handbook%20final.pdf), 60 pp.

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