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ROTIFER CULTURE MANUAL

Prime microalgae

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Obtain the highest growth rates for your rotifers

Proviron Prime microalgae contain freeze dried microalgae ensuring the stable production of rotifers (high fecundity) with high nutritional value (poly-unsaturated fatty acids, proteins, vitamins, antioxidants, etc.). These nutrients are not lost during harvesting and feeding to your larvae. Proviron Prime microalgae rotifer culturing diets are ideal for rotifer batch, semi-continuous and continuous roduction. They are very easy to use and will save on costs and space, eliminating the need for algal production in the hatchery.

Cultivation on NannoPrime (freeze dried *Nannochloropsis* sp.) results in healthy, vigorous rotifers rich in EPA, proteins, minerals and vitamins, ready for the enrichment phase at high densities.

Complementation of a NannoPrime diet with IsoPrime (freeze dried *Isochrysis* "Tahitian strain") at ratios up to 20% gives an extra boost to rotifer growth. The use of IsoPrime is not advised as a sole diet for the cultivation of rotifers.

High efficiency of production

- NannoPrime or the combination of NannoPrime/IsoPrime can be used for low and high density rotifer cultures.
- NannoPrime or the combination of NannoPrime/IsoPrime can be used in batch culture systems, semi-continuous and continuous culture systems.

Rotifers cultured on NannoPrime have:

- A high nutritional value (poly-unsaturated fatty acids (EPA/DHA), minerals, vitamins, antioxidants) for marine fish larvae production
- A low bacterial load
- A high daily egg ratio

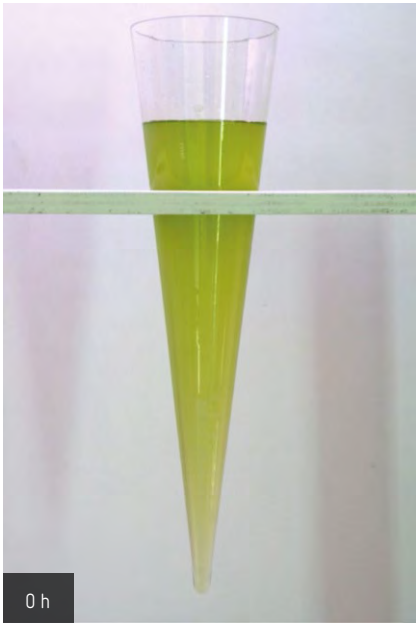
Benefits

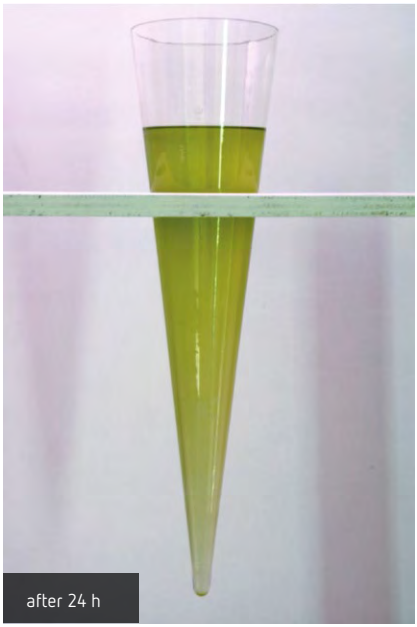
Ready and easy-to-use algae

- Freeze dried microalgae (*Nannochloropsis* sp. or *Isochrysis* "Tahitian strain")
- Packed under protective atmosphere
- Shelf life of 3 years
- Absolute single cell dispersion (photo ①)
- Excellent buoyancy, no sedimentation (photo ②)
- No hassle or costs related to algae cultivation
- Guaranteed pathogen free

①

②





INSTRUCTIONS FOR USE

Preparation of the rotifer culture tank

- Clean and disinfect the culture tank, airstones, air tubing prior to use (use e.g. 200 grams/m³ active chlorine + detergent)
- Disinfect the water of the tank with e.g. 5 grams/m³ of active chlorine and aerate gently for 1 hour
- Deactivate any remaining chlorine by adding 7 grams/m³ sodium thiosulphate
- Suspend airstones 15 cm above the tank floor, along the periphery and also in the center to allow sedimentation and flushing of waste particles
- The use of flock traps to remove eventual waste floccules can help to maintain a clean culture.

Tank set up and optimal culture conditions

- Temperature 25-28 °C
- Salinity 20-25 ppt
- Dissolved oxygen 5-7 ppm
- NH_4^+ < 20 ppm; NH_3 < 1 ppm
- pH 7,5-8,5
- Gentle aeration through the use of airstones: sufficient to keep the rotifers in suspension but at the same time allowing debris to sediment

- Batch culture: 3 days, when water quality allows, a 4 day culture can be realized

Mixing instructions

- For NannoPrime:
  - add up to 50 gram to a beaker.
  - pour 1 liter of seawater on top and mix during 5 to 10 minutes with a magnetic stirrer.
- For IsoPrime:
  - mix during 1 minute using a blender. Create a vortex first and slowly add up to 50 gram on top.
  - leave the product to rehydrate (5 minutes or longer) and mix again for 1 minute. Mix for another 30 seconds for every 10 g suspended.
- Optionally, sieve the suspension over a 50 µm sieve before feeding to the tanks.
- The blended feed can be stored up to 48 hours at maximum 4 °C. Shake before use.
- Feed the daily quantities in 4-6 rations or (semi-)continuously using a peristaltic pump.
- Apply the appropriate feeding regime according to your culture strategy to assure the best production output and water quality.

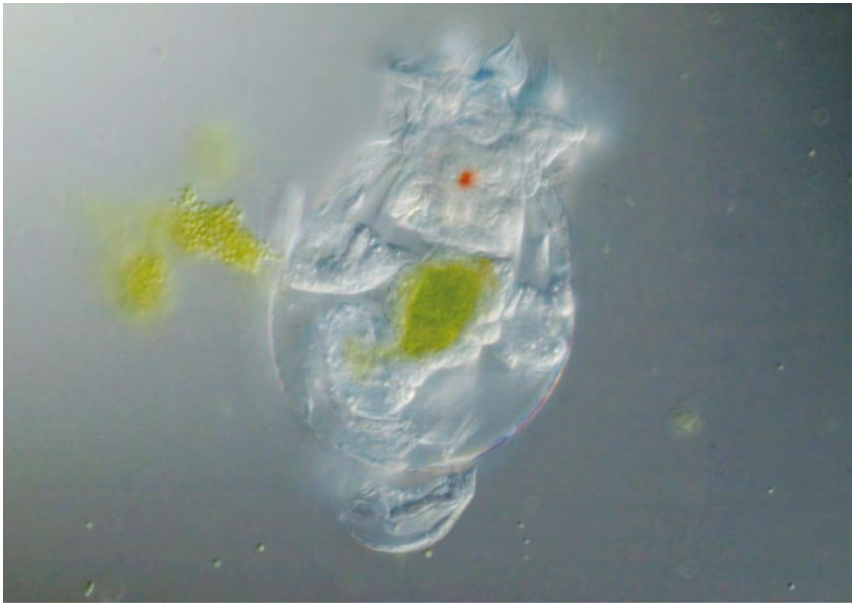
Feeding strategy

An example of a typical feeding regime is given below. The rotifer numbers illustrated in this feeding regime are indicative for *Brachionis plicatilis* 'L-type' rotifers. Results can vary according to your local conditions.
Starter rotifers should be clean, in good nutritional conditions and have an egg ratio of +/- 20%.

Feeding regime*		
Day	rot./mL	g/10^6 rot.
0	350	0,385
1	600	0,385
2	1100	0,385
3	1600	0,385
4	2200	0,385

* background feeding not taken into account

Culture medium should be cloudy, very clear water may be an indication of underfeeding. The application of background feeding is advised to obtain optimal results. Therefore a small extra dosage of 0,02-0,05 g/L of the algae can be added for the first day only.



TEST RESULTS

Tests were performed at ARC (Artemia Reference Center, Ghent University, Belgium) in which the effect of NannoPrime and the combined use of NannoPrime and IsoPrime on rotifer performance was evaluated.

Aim

Determine the effect of different rotifer feeds/mixtures on rotifer performance (rotifer growth rate and egg percentage) during 2 consecutive batches of 5 days. Analyse the final fatty acid profile in the rotifer tissues.

Experimental set-up

Tanks:	Cylindro-conical 10L, sea water (salinity 25 ppt)
Start density:	± 350 rotifers/mL
Culture period:	2 x 5 days (day 0, 1, 2, 3, 4)
Sampling:	daily, 6 samples of 250 µL/tank
Treatments – feeds:	NannoPrime 100% (A)
	NannoPrime 80% - IsoPrime 20% (B)
	NannoPrime 100% / last 2 days mix B (C)
Dosage:	0,385 g/10^6 rotifers per day for all treatments
Replicates:	3 replicates / treatment
Variables:	Rotifer density, egg percentage: daily and HUFA analyses at the end of batch 2
Set – up:	Temperature 25-28°C
	Dissolved oxygen 5-7 ppm
	NH_4^+ < 20 ppm; NH_3 < 1 ppm

Results

Rotifer density - see figure 1

Batch 1

The rotifer density at the start was 380 ± 28 rot./mL, 374 ± 17 rot./mL and 376 ± 13 rot./mL for feed A, B and C, respectively. The rotifer density increased in all treatments from day 1 onwards. By day 2, the rotifer density further increased; 1372 ± 192 rot./mL, 1396 ± 142 rot./mL and 1559 ± 20 rot./mL, respectively.

On day 3, the density of the rotifers in all treatments continued to increase. Feed A, B and C resulted in similar densities compared to the other feeds: 1953 ± 113 rot./mL, 2072 ± 14 rot./mL and 2166 ± 92 rot./mL for feed A, B and C, respectively.

On day 4, all rotifer densities continued to increase. Feed A, B and C were similar. The final rotifer density was 2556 ± 108 rot./mL, 2882 ± 368 rot./mL and 2622 ± 308 rot./mL for feed A, B and C, respectively (Fig. 1).

Batch 2

The rotifer density at the start was 313 ± 30 rot./mL, 330 ± 12 rot./mL and 348 ± 19 rot./mL for feed A, B and C, respectively. The rotifer density increased on day 1 for all feeds. On day 2, the rotifer density doubled in all treatments; 831 ± 70 rot./mL, 1033 ± 90 rot./mL and 1107 ± 187 rot./mL for feed A, B and C, respectively.

By day 3, the rotifer density kept increasing for feed A, B and C. The density was 1114 ± 90 rot./mL, 1453 ± 73 rot./mL and 1522 ± 237 rot./mL for feed A, B and C, respectively.

On day 4, the rotifer density of all feeds continued to increase. The final rotifer density was 1784 ± 328 rot./mL, 2389 ± 112 rot./mL and 2301 ± 244 rot./mL for feed A, B and C, respectively (Fig. 1).

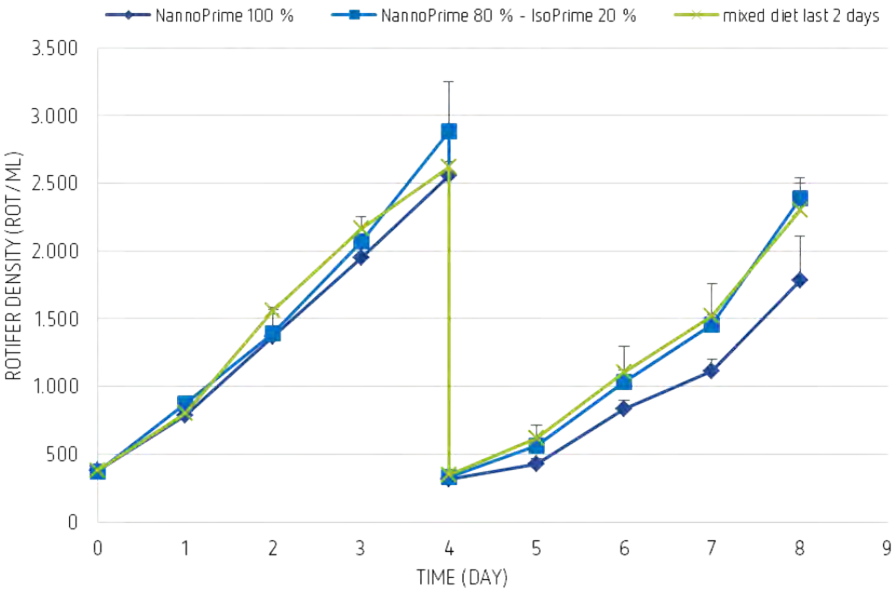


Fig. 1: Rotifer density during the two consecutive batch cultures. n=3 with each 6 countings / replicate (mean + SD)

Egg percentage - see figure 2

Batch 1

The egg percentage was around 23% at the start of the experiment.
The egg percentage decreased in treatment B to 7 ± 2 %, while in the other treatments, it remained similar 18 ± 7 % and 23 ± 5 % for feed A and C, respectively on day 1.
On day 2, the egg percentage decreased significantly in treatment C to 7 ± 1.
On day 3, the egg percentage kept decreasing in feed A and B, but increased again in feed C.
On the last day, the egg percentage increased in all treatments.
The final egg percentage was 24 ± 3 %, 26 ± 12 % and 29 ± 19 % for feed A, B and C, respectively (Fig.2).

Batch 2

The egg percentage was 17 ± 3 %, 24 ± 1 % and 20 ± 2 % for feed A, B and C, respectively at the start.
The egg percentage increased sharply in feed A, but remained similar in feeds B and C on day 1: 52 ± 8 %, 24 ± 1 % and 27 ± 6 % for feed A, B and C, respectively.
On day 2, the egg percentage decreased in all treatments.
On day 3, the egg percentage remained similar in all treatments: 13 ± 5 %, 5 ± 1 % and 4 ± 1 % for feed A, B and C, respectively.
The final egg percentage was 14 ± 6 %, 7 ± 2 % and 16 ± 16 % for feed A, B and C, respectively (Fig. 2).

Conclusions

In both batches, feeds A, B and C resulted in very high rotifer densities. The freeze dried products all lead to similar rotifer densities and egg ratios. No foaming was observed

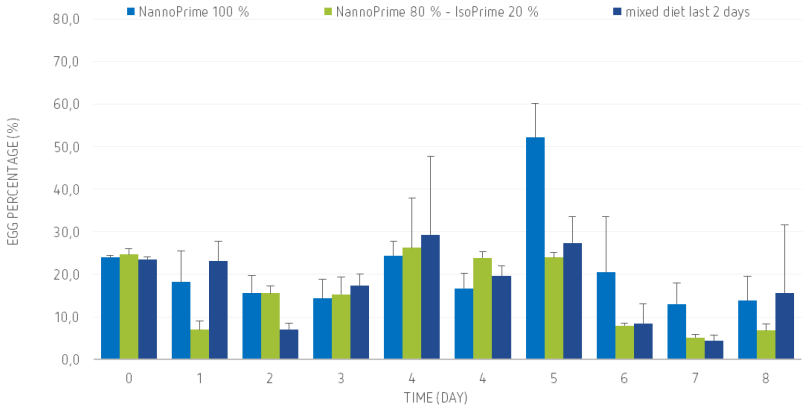


Fig. 2: Egg percentage during the two consecutive batch cultures. n=3 with each 6 countings / replicate (mean + SD)

Rotifer fatty acid profile - see figure 3

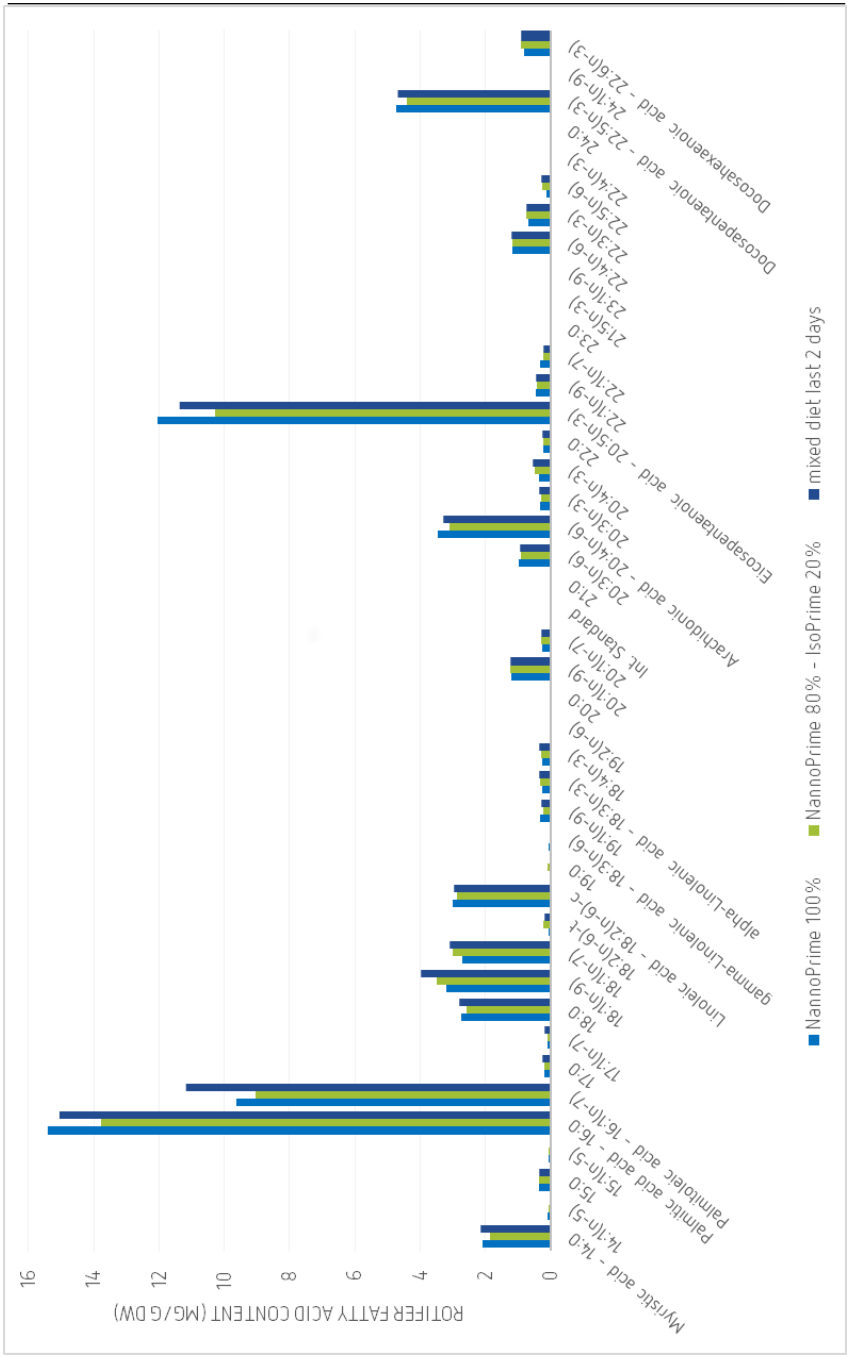


Fig. 3: Fatty acid profile of rotifers

The rotifer fatty acid profile was determined by means of FAME analysis according to the method described by Lepage and Roy (1984). Rotifers cultivated on NannoPrime or on a NannoPrime/IsoPrime (80/20) mixture contained high levels of the omega-3 LC-HUFA's eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA). The presence of IsoPrime in the diet is reflected to some extent in the presence of docosahexaenoic acid (DHA) in the rotifer tissues.

References: Lepage, Guy and Roy, C.C. (1984). Improved recovery of fatty acid through direct transesterification without prior extraction or purification, J. Lip. Res., 25, 1391-1396

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