

Ingrid Lein and Synnøve Helland

1. Introduction

One of the most important bottlenecks in the larval and juvenile production of marine fish species is assurance of the nutritional quality of the live feed. It is evident that there are species and life stage specific nutritional requirements of marine fish larvae and juveniles. The nutritional requirements of marine fish larvae has induced an extensive international research effort to obtain nutritionally adequate standards for the live prey organisms most commonly used in commercial hatcheries; these are rotifers (*Brachionus* sp.) and *Artemia* sp. (the most commonly used is *Artemia franciscana*) (Figure 1). Even small differences in the nutritional quality of the live feed organisms can have a large impact on the quality and robustness of the fish at later stages.

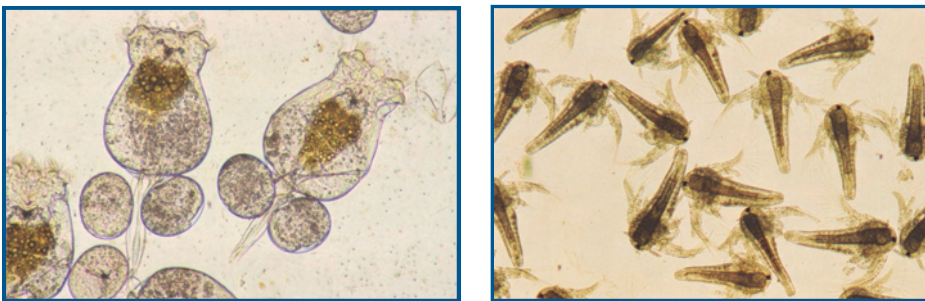


Figure 1. The most commonly used life feed organisms; the rotifer (*Brachionus* sp., left image) and the *Artemia* sp (right image). Photos: Nofima Marin AS

There are several methods employed for producing rotifers such as batch, semi-continuous, and continuous cultivation. Also, the scale of production varies from small indoor tanks to large outdoor tanks.

Artemia are bought as dried cysts that must be hydrated and hatched, and the cyst shells are often chemically removed before hatching, a process that also reduces the bacterial load. Most often *Artemia* are fed as newly hatched nauplii or they are enriched for about 20 to 24 hours.

High quality live feed production requires strict control of the nutritional quality of the enrichment mediums, the microbial environment and of the physical parameters like oxygen, temperature, salinity, pH, and of the turbulence created during mixing.

This protocol is a general method for sampling and transport of samples of live feed organisms for chemical analysis. For specific analyses there may be other protocols and, therefore, the protocol must be discussed with the laboratory that will analyse the samples—before sampling and shipping of samples— whether it is a commercial laboratory or a collaborating research and development partner.

2. Sample size and number of samples:

Before starting the sampling of the different live feeds, the required amount of rotifers or *Artemia* per analysis must be defined.

In order to reflect variations in different production batches, it is a good idea to take samples over several days. These may be analysed as one pooled sample or as separate samples, which mainly will be a question of cost. The same tube might also contain live feed samples from several days sampling.

Most relevant parameters to analyse:

- Dry matter
- Energy
- Total lipids and fatty acids
- Lipid classes
- Total protein
- Vitamins
- Minerals and trace elements

Sampling:

- Harvest the decided amount (wet weight in grams) of enriched rotifers or *Artemia* on a sieve. Rinse well with fresh water (temperature ~20-30°C) in order to remove all enrichment products on the surface of the live feed. *This is very important, because the results will be completely masked if some enrichment medium remains on the surface of the live feed .*
- Dry the underside of the sieve with a dry paper cloth to remove as much water as possible.
- Carefully transfer the sampled live feed to a vial with a small clean spoon or spatula.
- Label the samples with a PENCIL. — Ballpoint or markers may be unreadable if a sample leaks.
- Freeze the samples. Store them at least as cold as -20°C, preferably as cold as -80 for lipid analysis.
- Do not fill the vials completely, they will burst when freezing!

3. Transport/Shipping:

- Put samples in ice packs (ice elements) in at least -20°C .
- Just before sending: wrap the frozen vials with insulating plastic foil around the ice elements. You can use tape to stick the plastic foil to itself (do not freeze the plastic foil)
- Put everything in a Styrofoam box
- Leave the Styrofoam box in the refrigerator until the transport company picks it up.
- Make sure that the transport box has been properly labelled with the name and address of the receiver
- Include an overview of the samples together with the shipment and also send this by e-mail when you notify the receiver about the shipment
- It is very important to notify the receiver before sending the samples!

4. Labeling:

Include on all labeling, the following information

- Company name
- Name of contact person
- Address
- Appropriate telephone number
- "Biological material – for science only"