# Copepod Culture

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## Introduction

Copepods represent about 80% of zooplankton in the ocean and are the natural food source for many marine fish larvae. Copepods can adapt to fluctuating environmental conditions and the resting eggs some copepods produced can survive for years. This makes it a suitable group as live feed in aquaculture.

Live prey is necessary for fish larvae for many reasons. The larvae of many marine fish require prey about 50–100 μm wide at first feeding (Detwyler and Houde, 1970; Yufera and Pascual, 1984). Fish larvae with very small eggs and little vittelin cannot survive on the yolk available for many days and such larvae are called the altresial larvae. Such larvae need small feed depending on the smaller mouth size. Also for this type of larvae the stomach is not fully developed and they obtain digestive enzymes from the live feed they prey upon. Another advantage of live feed is that fish larvae prefer moving feed rather than inert feed during early stages of development.

## Profile

Most adult copepods have a length between 1 and 5 mm. The body of most copepods is cylindroconical in shape, with a wider anterior part. The trunk consists of two distinct parts, the cephalothorax (the head being fused with the first of the six thoracic segments) and the abdomen, which is narrower than the cephalothorax. The head has a central naupliar eye and uniramous first antennae that are generally very long. Planktonic copepods are mainly suspension feeders on phytoplankton, yeast and/or bacteria; copepods are therefore filter feeders.

Copepods cultured or wild collected have a very good nutritional profile suitable for fish larval rearing. Copepods offer a great variety of sizes, species and qualities (Kinne, 1977; Yufera and Pascual, 1984; Delbare *et al*., 1996), and have high levels of protein, highly unsaturated fatty acids (HUFA), carotenoids and other essential compounds (Kraul *et al*., 1992). In general copepods have high protein content (44-52%) and a good amino acid profile, with the exception of methionine and histidine. The fatty acid composition of copepods varies considerably, since it reflects the fatty acid composition of the diet used during the culture. HUFA are essential for marine fish larvae and Docosahexaenoic acid (DHA; 22:6 (n:3)) has a significant influence on larval stress resistance (Kraul *et al*., 1991, 1993). DHA content is higher in copepods than in recently hatched Artemia nauplii and gives better results in terms of survival, growth and stress resistance (Fujita, 1979). Superior larval stress resistance can be achieved with copepods, even when DHA content is less than enriched Artemia nauplii

(Kraulet *al*., 1992). Other good characteristics of copepods are their swimming movements as a larval visual stimulus, the tank-cleaning performance primarily by benthic harpacticoids, which are grazers (Støttrup *et al*., 1995), their high digestive-enzyme content (Delbare *et al*., 1996) and a possible enhancement of feeding rates with improved growth and survival (Støttrup and Norsker, 1997).The passage of copepods in the alimentary canal of fish larvae is slower than artemia which makes its digestion and absorption more efficient (Pederson, 1984). This is due to the fact that copepods contain more digestive enzymes which are a suitable source of exoenzymes for fish larvae.

Several candidate species of copepods belonging to both the calanoid and the harpacticoid groups have been studied for mass production. Calanoids can be easily recognized by their very long first antennae (16-26 segments), while the harpacticoids have only a short first antennae (<10 segments). Cyclopoid species which has culture potential is *Oithona* sp. Some calanoids that are mass cultured are: *Acartia tonsa, Eurytemora affinis*, *Calanus finmarchicus, C. helgolandicus and Pseudocalanus elongates. Cultured* harpacticoids are: *Tisbe holothuriae, Tigriopus japonicas*, *Tisbenta elongate and Schizopera elatensis*. In general, it may be stated that harpacticoid copepods are less sensitive and more tolerant to extreme changes in environmental conditions (i.e. salinity: 15-70 $g.l^{-1}$; temperature: 17-30 °C) than calanoids and thus are easier to rear under intensive conditions. Moreover, harpacticoids have a higher productivity than calanoids and can be fed on a wide variety of food items, such as microalgae, bacteria, detritus and even artificial diets. However, as mentioned previously, care should be taken in this respect as the lipid and (n-3) HUFA composition of the copepods is largely dependent on that of the diet fed.

**Reproduction**

Parthenogensis is not there in copepods. Since both males and females are present, sexual reproduction is present. The fertilized eggs are attached to female's abdomen and the eggs hatch out into naupliar larvae with 3 pairs of legs. They grow and moult several times to become copepodite. Copepodites further grow and moult before becoming adult copepods. Copepods need longer period of time to grow from egg to adult than rotifers and cladocerans. Some species have resting eggs or diapause in egg or copepodite stage, which can be effectively utilized in culture of copepods by storing eggs.

**Criteria for selection of copepod for culture**

Natural occurrence, type of spawning (free spawner/ egg carrying), daily egg production and fecundity (eggs per female)

**Culture Techniques**

The culture techniques followed for copepods are as follows:

| Culture techniques | Culture volume | Productivity (eggs/ day) (l) |
|---|---|---|
| Semi extensive | Large ponds/ lagoons1.200-10.000 $m^3$ | <50 |
| Semi intensive | Tanks:200-300 $m^3$ | <100 |
| Intensive | Flasks or tanks 5-110L | 500-6000 |

Most of the large-scale copepod culture systems are based on outdoor semi-controlled polyculture techniques, although several attempts have been made to culture some species in intensive systems (Støttrup *et al*., 1986; Støttrup and Norsker, 1997).

**Collection**

Zooplankton samples are to be collected from the estuarine waters using plankton net of 200 micron mesh size on full moon day early morning hours. The samples have to be transported to the laboratory and thoroughly rinsed with filtered water of same salinity. From the samples copepods are identified under microscope using the keys (Kasturirangan, 1963).

**Isolation**

After collection the zooplankters are screened to isolate size fraction containing adult and later stage copepodites of the desired species. The zooplankton samples are first sieved through a 500 micron mesh to remove the larvae of fish or shrimp. The samples are again sieved through 200 micron mesh to remove smaller zooplankters like rotifers. Copepod nauplii and molluscan larvae. This is repeated 3-4 times by rigorous washing with fresh seawater. After repeated washing, the remaining adult copepods and later stage copepodites are used as stock cultures.

**Stock cultures**

Stock cultures are maintained in 5-20 litre glass containers with continuous aeration. Periodic water exchange with filtered seawater reduces contamination of the culture. Each time the contents were filtered through 200 micron mesh before water change and the adults that are retained were further reared by feeding on microalgae. The water quality parameters are to be maintained by regular monitoring of pH, salinity, temperature, dissolved oxygen etc. The algal feeds include *Chlorella marina, Isochrysis galbana*, *Chaetoceroscal citrans*. Further scaling up of culture is done by inoculating into bigger containers. Algal stock cultures also have to be maintained for its continuous supply for mass culture. Standard methods are available for algal stock culture.

**Production of copepods**

Batch culture of copepods is relatively straight forward once proper environmental and nutritional conditions are met. The culture flasks are stocked with adult copepods (10-25 individuals/ml). The stock cultures maintained in 1L conical flasks containing filtered sea water (20-35 ppt) are fed with microalgae in the ratio 1:25 (v/v). The copepods are fed with algae on alternate days. The stock culture is maintained by rinsing with filtered sea water and the eggs, nauplii, and adults separated and put into fresh culture flasks every week. The adult would begin producing eggs/ sperms in 9-12 days; thereafter egg production would initially rise, then reaches the peak and finally falls. Once the hatching success falls below 75%, it is time to terminate the culture batch. For continuous production of nauplii, sequential batch cultures have to be initiated at every 5-7 day intervals. When timed correctly, one tank of a series will be at the maximum productivity at any given time.

**Mass culture**

For mass culture chlorinated and dechlorinated (using sodium hypochlorate and sodium thiosulphate) sea water is used. The cultures can be maintained in 50L-3000L out–door culture tanks with continuous aeration.Copepods are daily fed with a mixture of micro algal diet (*T. gracilis, C. calcitrans, I. galbana.*) and baker's yeast.