

Are copepods viable options as live food ... (Wa Iba)

ARE COPEPODS VIABLE OPTIONS AS LIVE FOOD IN AQUACULTURE HATCHERIES?

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ABSTRACT

This present paper overviews the use of copepods in aquaculture. Some culture methods and nutritional values also described to decide whether copepods are viable and reliable to be used as live food in aquaculture hatcheries. Copepods have been known to have higher nutritional value than *Artemia* and rotifers. In aquaculture, they have been used to fed various species of marine finfish with better results in terms of growth, larval survival and pigmentation compared to some fish larvae fed on other live feeds. However, culturing copepods in intensive systems to harvest high number of copepods is not well established yet due to lack of funding and knowledge. Meanwhile extensive and semi intensive systems are possible to transfer parasites and diseases from wild environment. Furthermore, nutritional value can not be controlled in such systems.

KEYWORDS: copepods, aquaculture, fish larvae

INTRODUCTION

In natural wild ecosystem, almost all fish larvae feed on copepods (Evjemo *et al.*, 2003). In aquaculture hatcheries, live foods for marine fish larvae have been tried particularly rotifers and *Artemia* (McKinnon *et al.*, 2003). However fish larvae fed with these diets exhibited poor survivorship. It is mainly because some marine fishes such as groupers have small mouth at their first larval stages that leads them to unsucceed to ingest rotifers and *Artemia*. This problem have led scientist and aquaculturist to investigate new live foods for fish larvae especially marine fish.

The size of copepods depends on the species as well as on the ontogenetic stage. Vari-

ous copepod sizes are used for specific larviculture applications, assuring an efficient uptake by the target predator at any time during its larval rearing. The harpacticoid, *Tisbe holothuriae* grows from a nauplius size of 55 μm to an adult size of more than 180 μm , *Schizopera elatensis* from 50 to 500 μm , and *Tisbentra elongata* from 150 to more than 750 μm . Sizes for *Eurytemora* sp. (Calanoidea) are on an average of 220 μm , 490 μm , and 790 μm for nauplii, copepodites, and adults, respectively (Lavens & Sorgeloos, 1996).

Some advantages of using copepods as natural prey for marine fish larvae are reviewed by Stottrup (2000). Generally, the benefits of using copepods as live food for early feeding fish larval stage are derived from higher nutri-

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tion value of copepods compared to *Artemia* and rotifers. Furthermore Pinto *et al.* (2001) summarized from Delbare *et al.* (1996) stated that several advantages of the use of copepods as live food for marine fish larvae include the wide range of body size between nauplii and adults; the movement that constitutes a visual stimulus for the larvae; the high amounts of polyunsaturated fatty acids PUFA; the higher levels of digestive enzymes which may play an important role during larval rearing period and some harpacticoid copepods are detritus cleaner of tanks culture. Another advantage is stated by Olsen *et al.* (2004) that calanoid copepods can be used as a source of lipid in salmon aquaculture with regard to their lower content of PCBs and dioxins.

Stottrup (2000) stated that the use of copepods in aquaculture differs from one region to another and species specific despites of their nutritional values. The reason for this is usually associated with high production cost of rearing copepods. Moreover, some genres such as *Acartia* spp. are cannibal which leads to some difficulties to culture them in high density (Mc Kinnon *et al.*, 2003). However, some copepods such as genus *Arcatia*, *Tigriopus japonicus*, *Oithona* spp., *Paracalanus* spp., and *Eurytemnora* spp. have been attempted to be cultured in intensive system to feed larval of early stage (Stottrup & Norsker, 1997). This present paper, thus, overviews the viability of using copepods in aquaculture hatcheries.

HOW TO CULTURE COPEPODS?

In nature, especially in turbid estuarine water, the diet of copepods especially calanoid copepods may not be always optimal to support copepod growth rate, lipid store, reproduction or gut content. Culturing copepods will then bring some benefits for aquaculture because the quantity and quality of algal food can be controlled, along with other conditions such as temperature, salinity, water quality, and photoperiod (Rippingale & Payne, 2001a).

Copepods culture can be conducted in either intensive or extensive systems. The culture species are both calanoid or swimming pelagic copepods and harpacticoid or benthic copepods. Generally, copepods reared in extensive or semi intensive systems are more vulnerable to parasitic contamination. It is not an issue in intensive systems. However, high cost of producing copepods in intensive systems still remains a problem.

Producing Copepods in Extensive and Semi Intensive Systems

Some methods of extensive and semi intensive culture of copepods include outdoor pond-based rearing of phytoplankton and zooplankton (hatchery in Denmark) and fish larval rearing lagoon of natural zooplankton (lagoon enclosure system in Norway) (Stottrup, 2000). The culture of copepods in outdoor tanks in Japan is also considered as semi intensive system (Hagiwara *et al.*, 2001). This method allows a large number of copepods to be cultured along with other various species. However, disease transfer and nutritional value can not be controlled in this systems (Knuckey *et al.*, 2005). To prevent parasite transfer into the fish culture systems, Stottrup (2000) suggested to breed adult copepods in holding tanks and then harvest the nauplii to feed fish larvae.

Extensive culture system in Denmark was described by Stottrup (2000). It consisted of 6 outdoor ponds sized 12 m in diameter and 6 m in high. The ponds were emptied during winter and filled with filtered sea water before first fish larvae introduced. The production of natural phytoplankton and zooplankton was monitored daily and if necessary, agricultural fertilizers were added to enhance the growth of the planktons. Meanwhile in Japan, outdoor tanks 24 m³ in volume were used to mass culture of *A. tsuensis*. Some species found in extensive and semi intensive systems are listed in Table 1.

Producing Copepods in Intensive Systems

Some early intensified copepods culture were attempted under laboratory condition for experimental works purposes. Stottrup (2000) described this phase as copepod culture trials which is placed in a very small volume of medium from one to a few litres of water and cultured in limited time (usually weeks to months). Consequently, intensive culture techniques development for commercial market is very slow and fragmented. Hatchery production of copepods is not well established yet despite some real benefits of it. The reason for this might be due to lack of knowledge of mass cultivation of copepods. In contrast with extensive systems, hatchery production of copepods will improve consistency of production, defines nutritional profile more precisely, knows disease status of cultures, and be able to exclude predators and competitors that may

Table 1. Some species cultured in extensive and semi intensive systems (modified from Stottrup, 2000), ER, Exploitation rate, DW, Dry weight

Species	Culture size	Densities	Productivity	Food	Reference
<i>A. tonsa</i>	24 m ³	20% ER=150µg DW/L	20% ER=30µg DW/L	Natural Phytoplankton	Ogle (1979)
<i>A. tsuwensis</i>	1,890 L	232/L	2-75 nauplii/adult	Natural Phytoplankton	Chino, Takahashi & Taki (1990)
<i>E. affinis</i>	30 m ³	300-500/L copepodites and adults >1,000 nauplii/L	7-10 %	Algae and detritus	Nellen, Quantz, Witt, Kuhlmann & Koske (1981)
<i>Tisbe</i> spp.	c.2800 m ³ 1.5 L floating basket in 200 L fish tanks	92±15/ml	not estimated	Natural Phytoplankton Mytilus powder Lettuce pieces	B.Urup (Pers.comm.) Kahan, Uhlig, Schwenzler & Horowitz (1982)
<i>S. ealtensis</i>	210 m ³	29/ml 10-22/ml	4-5 kg at regular intervals	Chlorella minutissima yeast, baker's yeast co culture with rotifers outdoor tanks	Fukusho (1980)

be introduced from pond cultures (Knuckey *et al.*, 2005).

Rippingale & Payne (2001a) stated that intensive cultivation of copepods has succeeded only in small number of species. The easiest copepods to be intensively cultured is harpacticoid copepods such as *Tisbe* spp. and *Tigriopus* spp. *Tisbe* has been cultured in Launceston using batch, semi-continuous and recirculation systems. It is fed with microalgae, fortified yeast, pelleted feeds, vegetables, macroalgae, and associated microflora (Bataglene *et al.*, 2000). However their use in aquaculture is unfavourable for pelagic larvae and only effective for fish who feed on the bottom level of water.

The intensive culture of *Amphiascoides atopus* Lotufo and Fleeger, a marine harpacticoid copepods, was described by Sun & Fleeger (1995). This system, basically, was a recirculation system consisted of five components: a centrifugal pump, culture tanks, collectors, filters, and a reservoir tank. This study showed a significant production of this species that might be applied to commercial mariculture.

Some calanoid copepods have been tried to be cultivated but only in small numbers because they can not be kept in high densities such as genus *Acartia*. Species which has showed potential signs to be cultured in intensive systems is *Gladioferens imparipes*, a temperate water calanoid copepods (Rippingale & Payne, 2001b). Some tropical species such as *Bestiolina similis* and *Parvocalanus crassirostris* have been attempted to be cultured in laboratory (Mc Kinnon *et al.*, 2003).

Culture experiments was conducted by Hernandez Molejon and Alvarez-Lajonchere (2003) using *Oithona oculata* Farran, 1913, a coastal water copepod. In this experiment the yield of this species was 115 copepods/mL and a maximum density of 8 copepods/mL, with a daily harvest of 25% of the total volume. A 1-year batch-culture pilot-scale system was also established in indoor concrete 20-m³ tanks using *N. oculata* as food, reaching a mean density of 7 copepods/mL and a maximum density of 10 copepods/mL. Copepod monocultures were maintained in polycarbonate 1000-l outdoor tanks and fed a mixture of five species of microalgae (*Chaetoceros ceratosporum*, *Tetraselmis tetraathele*, *Chlorella* spp., *Dunaliella tertiolecta*, and *N. oculata*). In these nonaerated cultures, final concentrations of

13 copepods/mL were obtained in 15-day cultures.

In intensive calanoid copepod culture systems, regularly removing eggs or young nauplii from broodstock cultures is an effective way. This technique provides quantities of uniform-sized nauplii to feed fish larvae and ensures that most of the food added to cultures does not contain reproductive copepod adults that will be utilised to produce new generation of nauplius instead of somatic growth of fish juvenile stages. Moreover, many omnivorous calanoids e.g. *Acartia* spp. are cannibalistic, so that it is crucial to separate them from the adults as soon as possible. Daily separation of eggs from adult of *Acartia tonsa* is featured in the culture system described by Støttrup *et al.*, 1986. Egg or nauplius separation is important although it is also time consuming (Rippingale & Payne, 2001b).

In intensive culture system, diet and environmental condition are highly controlled. Some studies were conducted to find out diet requirement and water quality parameters that influence the production of copepods (Carli *et al.*, 1995; Lee *et al.*, 2006; Knuckey *et al.*, 2005; Peck & Holste, 2005). Copepods commonly feed on microalgae with species specific diet requirement. Knuckey *et al.* (2005) found that *A. tonsa* would require a binary diet consisting of 1.13 µg AFDW/mL *Cryptomonad* sp. with a lower component (~20%) of *P. salina*. This would ensure that the minimum feed level for maximum development rate is exceeded within a diet rich in PUFA. Cyclopoid copepod, *Paracyclopina nana* Smirnov showed the best growth when fed with TET (*T.suecica*), ISO (*I. galbana*) and the mixed of TET+ISO diet (Lee *et al.*, 2006). *Tigriopus fulvus* Fischer showed satisfactory production with two types of feed i.e yeast and microalgae (*M. lutheri* and *S. cerevisiae*) (Carli *et al.*, 1995).

The other method to obtain copepods nauplii supply rather than relying on culture systems is using copepods diapause eggs. Copepods diapause eggs is an analogous to cyst of *Artemia* and rotifers. Temperature and oxygen determine the survival of diapause eggs in laboratory. Marcus & Murray (2001) stated that copepod diapause eggs can be produced in laboratory, stored, and hatched when nauplii are needed to feed fish larval.

Despite the fact that copepods are beneficial for fish larvae growth, the culture of cope-

Pods is still in developmental stages with lack of progress. Stottrup (2000) attributed this to the lack of economic incentives for the aquaculture industry. To accelerate the development of reliable copepods culture system, some economic studies have to be conducted to prove that additional cost for culturing copepods can be covered by increased revenue from faster growth and high survival rate that will lead to efficient production of fish with short period of grow out.

NUTRITIONAL VALUE OF COPEPODS: what are the differences compared to other live foods?

The main reason of using copepod in finfish aquaculture is its higher nutritional value compared to other live feeds. Southgate (2003) stated that based on some researches, fish larvae fed on copepods showed better growth rate, higher larval survival, desirable pigmentation and better gut development.

Rippingale & Payne (2001a) stated that fish larvae required particular long chain of highly unsaturated fatty acids (HUFAs) in their diet to ensure normal development of their nervous system. These HUFAs are not synthesized by animals but are produced by some species of phytoplankton. Calanoid copepods which feed on phytoplankton are expected to store HUFAs in their body and therefore will be transferred into the fish feeding on them. Harpacticoid copepods which feed by scavenging on detritus or by predating on ciliates and rotifers have larger proportion of fatty acids in their stores which have been synthesized by bacteria rather than phytoplankton. These are less valuable for the diet of fishes.

The fatty acid composition of copepods varies considerably and thus, it will reflect the fatty acid composition in the diet used during the culture. For example, the (n-3)HUFA content of individual adult *Tisbe* fed on *Dunaliella* (low (n-3)HUFA content) or *Rhodomonas* algae (high (n-3)HUFA content) is 39 µg, and 63 µg respectively, and corresponds to 0.8% and 1.3% of the dry weight. Within nauplii, the levels are relatively higher; (i.e. around 3.9% and 3.4%, respectively). Specific levels of EPA and DHA are respectively 6% and 17% in adults fed *Dunaliella*, and 18% and 32% in adults fed *Rhodomonas*. In nauplii, the levels of EPA, DHA and (n-3)HUFA are high, (i.e. around 3.5%, 9.0% and 15%, respectively) (Lavens & Sorgeloos, 1996).

Solbakken *et al.* (2002) found that halibut larvae fed on wild zooplankton i.e. copepods contained higher iodine concentration (700 times higher) than in *Artemia* and threefold higher in Atlantic halibut, *Hippoglossus hippoglossus* larvae fed wild zooplankton than in those fed on *Artemia*. Low dietary iodine may in turn influence the T4 level in larvae at 68 and 77 DPH, and could be related to the different initiation of eye migration and other changes associated with commencement of metamorphosis.

A study conducted by Helland *et al.* (2003) found that protein content in calanoid copepod, *Temora longicornis*, was ranging from 31% to 54% of dry mass (DM) compared to 31% in enriched *Artemia* nauplii. The amount of free amino acids (FAA) in relation to protein was 14% in enriched *Artemia* nauplii and varied between 16% and 27% in copepods. It is suggested that the amount of protein in *Artemia* is too low to support the growth of cod and halibut larvae.

Based on studies conducted by Stottrup *et al.* (1999) and Stottrup & Norsker (1994), Stottrup (2000) stated that HUFA levels especially docosahexanoic acid in copepods *A. tonsa* were higher than in enriched *Artemia*. However studies by Moren *et al.* (2005) suggested that both *Artemia* and copepods did not contain retinoid. Retinoid, derived from vitamin A, is a highly active molecule in the body developmental processes. The deficiency or excess of vitamin A will result in abnormal development during embryogenesis. Implication from this study is that fish larvae have to obtain their retinoid requirements from other sources. Another aspect of nutrition content of copepods is summarized by Mc Kinnon *et al.* (2003) from Sargent *et al.* (1997) as follows:

- 1) A preponderance of phospholipid rather than triacylglycerols in the copepods.
- 2) Levels and ratios of fatty acids that are more closely approximate the natural diet of marine finfish larvae than can be easily generated by less natural feeding regimes.
- 3) The probability of optimal protection of polyunsaturated fatty acids (PUFA) by natural antioxidants against peroxidation and the delivery of optimal levels of natural antioxidants to the larvae.

Fatty acid compositions of *T. Japonicus* and amino acid composition of *Tigropus brevicornis* are shown in Table 2 and 3.

Table 2. Fatty acid composition of total lipids, triglycerides (TG), polar lipids (PL), and free fatty acid fractions (FFA) in *T. japonicus* cultured on baker's yeast and an Omega-yeast (modified from Fukosho *et al.*, 1980) (% DW)

FA	Baker's yeast				Omega-yeast			
	Total	TG	FFA	PL	Total	TG	FFA	PL
14:00	0.6	0.8	0.7	0.6	1.2	1.8	1.7	0.5
15:00	1.8	1.7	0.8	0.5	0.8	0.6	0.6	0.4
16:00	7.1	8.2	8.1	13.2	9.1	10.1	9.9	13.2
16:1n-7	13.9	22.3	12.8	3.2	6.5	7.2	6.6	2.3
18:00	2.5	0.8	2.1	6.6	2.6	1.3	2.5	6.8
18:1n-9	23.7	31.6	20.6	15.7	22.1	32.4	21.8	14.2
18:2n-6	2.9	2.9	2.4	2.2	1.5	1.4	1.7	1.2
18:3n-3	4.4	5.3	3.8	1.2	0.9	0.7	0.7	0.5
18:4n-3	1.1	0.8	0.8	2.3	9.1	11.5	5.6	3.7
20:01	1.4	0.8	0.8	2.3	9.1	11.5	5.6	3.7
20:4n-3	2.1	1.6	2	0.8	0.7	0.4	0.5	0.3
20:5n-3	6	2.9	13.1	8.1	4.7	3.2	7.9	6.4
22:01	0.3	0.7	0.5	0.1	5.4	5.9	3.3	2.2
22:5n-3	1.1	0.8	0.7	1	0.9	0.7	0.6	0.4
22:6n-3	13.8	5.2	16.8	33.2	20.9	15.8	26.2	38.8
(n-3) HUFA	23	10.5	32.6	43.1	27.2	20.1	35.2	45.9

Source : Lavens and Sorgeloos, 1996

The nutritional requirements of marine finfish should be investigate more precisely to determine that copepods are the most suitable food for them. Furthermore, copepods nutritional value has to be carefully monitored during culture period to meet fish larval nutrition requirements.

COPEPOD AS LIVE FOOD IN AQUACULTURE

The use of copepods in aquaculture emerges because other live feeds contain less nutritional value to support optimal production of cultured fish. Another reason is that other live feeds are too large for fish larval with small mouth gap such as groupers. Battaglione *et al.* (2000) reported that striped trumpeted (*Latris lineata*) have been reared successfully using predominantly harpacticoid copepods as a supplementary feed (Figure 1).

Lavens & Sorgeloos (1996) reported from Fukusho *et al.* (1980) that cultured copepods have been successfully used in the larviculture of various flatfish larvae. 30 days-old larvae of the mud dab were fed with *T.*

japonicus cultured on baker's yeast or Omega-yeast, and showed excellent survival and growth rates. For turbot, Nellen *et al.* (1981) demonstrated that the larvae at startfeeding showed a preference for copepod nauplii over *Brachionus plicatilis*; after 14 days culture, their feeding preference shifting towards adult copepods. The survival of the larvae was high (50%), and the fry reached 12 mg DW (17 mm TL) at day 26.

Kuhlmann *et al.* (1981) successfully used 7.5% to 10% harvests of 24 m³ *Eurytemora* cultures to feed turbot larvae. Population densities after 4-6 weeks of culture approximated to reach several hundred adults and copepodites, and several thousand nauplii per litre. Despite these good results, these authors were not able to stabilize production at such levels or to develop a reliable method, and therefore had to add rotifers in addition to the copepod supply. Although the culture was not fully controlled, Kuhlmann *et al.* (1981) estimated the capacity of his 24 m³ copepod culture and came to the conclusion that this capacity should be sufficient to feed a batch of

Table 3. Amino acid composition of *Tigropus brevicornis* cultured on different types of food (g.100g - 1 crude protein) (Vilela, pers.comm.)

<i>T. brevicornis</i> cultured on <i>Platymona sueceica</i> with different additives:				
Amino acid	+ yeast	+ rice bran	+ wheat	+ fish food
Aspartic acid	7.30	6.98	7.08	7.63
Threonine	3.35	3.09	3.53	3.74
Serine	3.37	2.98	3.39	3.59
Glutamic acid	12.05	12.00	11.90	10.62
Proline	5.13	4.49	6.56	4.82
Glycine	4.40	4.24	4.31	4.71
Alanine	5.44	5.45	5.97	5.87
Cystine	0.39	0.84	1.23	1.27
Valine	4.52	4.30	4.21	4.71
Methionine	1.78	1.75	1.64	1.81
Isoleucine	3.35	3.21	3.28	3.48
Leucine	4.79	4.71	6.24	6.73
Tyrosine	3.89	3.99	3.21	3.87
Phenylalanine	2.64	2.67	3.37	3.44
Histidine	1.94	1.75	1.78	1.33
Lysine	4.81	4.65	4.81	4.92
Arginine	6.52	6.34	5.76	6.11
Total	75.67	73.44	78.27	78.65
Protein (%)	51.1	48.6	43.9	46.5

Source: Lavens and Sorgeloos, 1996

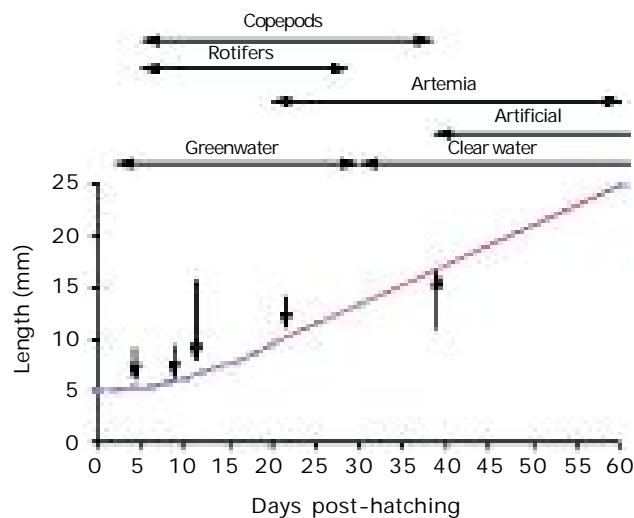


Figure 1. Feeding regime and development of striped trumpeter larvae (*Latris lineata*) reared in green-water tanks using *Tetraselmis suecia*

4000 freshly-hatched turbot larvae until metamorphosis (Lavens & Sorgeloos, 1996).

Mc Kinnon *et al.* (2003) found that copepods (especially *Acartia spp.*) have a proven track record in maximizing the larval survival of high-value tropical finfish species. The results of their studies also indicate that other species of tropical copepods, such as *B. similis*, may have more desirable characteristics as larval diets than *Acartia spp.* for fish species with small larvae. Furthermore Evjemo *et al.* (2003) stated that the content of n-3 HUFA, and in particular the DHA content in the live prey, may be a critical factor for halibut larvae. The lipid and fatty acid compositions in copepods can be considered as an important reference for optimising the lipid composition of cultivated live food organisms like *Artemia sp.* with a low DHA content that has negative effects on the larvae. Whether the qualities of the copepods can be assigned to the n-3 HUFA and DHA content alone, or to a combined effect of other factors, remains the subject for continuing research. Furthermore, McEvoy *et al.* (1998) assumed that since evolution has equipped halibut larvae with the means to efficiently capture and assimilate natural marine zooplankton, providing captive halibut larvae with a diet of copepods will alleviate stress to a considerable degree, thus reducing the incidence of malpigmentation.

Some examples given above are evident that copepods can be used effectively and efficiently for larval rearing purposes. However future researches are still needed to find out other nutritional values of copepods that are essential to maintain the normal development of marine fish.

CONCLUSION

Until recently, copepods have been used to feed various species of marine finfish larvae with promising results. Some fish farms obtain copepods from the wild or culture them in extensive or semi intensive systems. This kind of practice will lead to disease transfer and uncontrolled nutritional value of copepods. On the other hand, intensive systems have not well established yet due to lack of funding and knowledge. However some pelagic copepod species with short life cycles and fast growth rates could be cultured with high-yield semi-intensive technologies. This systems can be applied in tropical regions in which coun-

tries benefited from low costs of technically qualified labors and land availability, can develop copepods cultures that could give good economic results in terms of highly stress-resistant larvae, good survival, growth and biomass productions compared to enriched *Artemia*.

In terms of nutritional value, copepods might serve as another important alternative live feed for fish hatchery culturing small larvae and small mouth opening of fish species. However, the reliable method for mass culture of copepods has to be established first in order to ensure continuous supply of this live feed for fish larvae.

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