Potential Species of Copepods for Marine Finfish Hatchery

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Copepods are the most important group of zooplankton which forms the natural food for many fishes and invertebrates. One of the important problems in the marine fish hatchery is the lack of complete balanced larval feed. Copepods, even the newly hatched nauplii are nutritious, rich in PUFA, DHA and EPA, in most desirable ratios (Watanabe *et al.*,1978; 1983; Sargent1986; Watanabe and Kiron, 1994; Sargent *et al.*, 1997; Stottrup, 2000, 2006), easily digestible (Pederson, 1984; Stottrup, 2000) and rich in antioxidants, astaxanthine, vitamin C, D & E (Van der Meeren, 2003; McKinnon *et al.*, 2003). Copepods if fed during the larval phase, reduces malpigmentation and deformity rates, increases the pigmentation and survival (Bell *et al.*, 1997, Bell, 1998, Stottrup, 2000; Hamre *et al.*, 2005). More than 12000 species copepods are there living in a variety of ecological niches. Most of the early fish larvae are evolutionarily adapted for feeding on copepods than on other animals.

Copepods are successfully cultured in fin fish hatcheries of many countries including India, especially for feeding atresial larvae of certain fishes like groupers. Mostly species belonging to the orders Calanoida, Cyclopoida and Harpacticoida are popular for hatchery production as live feed. Species belonging to the genera, *Acartia, Calanus, Temora, Paracalanus, Pseudodiaptomus, Pseudocalanus, Centropages, Eurytemora, Euterpina, Tigriopus, Tisbe, Oithona* and *Apocyclopus* are widely cultured for hatchery use (Stottrup and McEvoy, 2003; Stottrup, 2006). In a study conducted on the wild-caught fish larvae, it was revealed that calanoidcopepod nauplii were an essential item in the early feedof many fish species. Calanoids of the genera *Acartia* and *Gladioferens* have been proved as important live feed for improving survival of some fish species. Harpacticoid copepods also are a good source of larval and juvenile fish feed in aquaculture. According to Turingan *et al.*, (2005) the prey should be of 80% of the mouth gape of the fish larvae. Many copepod nauplii are less than 100μ in size. Alone or as a supplement, in many cases copepods showed to improved primary growth than rotifers and brine shrimps. The smaller size of copepods enables their feeding by mouth gap-limited fish larvae like that of groupers and snappers (Fukuhara, 1989; Doi *et al.*, 1994). Only a few copepods have been continuously and successfully reared in extensive systems (Stottrup *et al.*, 1986; Stottrup, 2000). Most of the copepod rearing trails are done in small scale lasting for a few weeks or months and used only in experimental basis. Modern technologies including mass production of microalgae, with an input of large quantities of sea water copepod culture can be successfully enhanced and meet the required level like that or rotifers for marine finfish hatchery (Van der Meeren and Naas, 1997; Stottrup, 2006).

Species popularly cultured

Of the planktonic copepods in estuarine and coastal habitat, Calanoids are the most abundant taxa of pelagic realm forming an extreme connecting link between phytoplanktons and the fish in this inshore ecosystem. Due to this significance, Calanoids got keen attention from researchers. Among Calanoids, the popular cultivate is the *Acartia* spp. than *Calanus* spp. and *Temora* spp. Most of the species present in Calanoida are of approximately 1.0 mm total length (Mauchline1998). *Acartia clausi* and *Calanus finmarchicus* are the most widely studied Calanoids. (Mauchline 1998), followed by *Temora longicornis*, *Paracalanus parvis*, *Calanushelgo landicus*, *Pseudocalanus elongates*, *Acartia tonsa*, *Centropages hamatus*, *Centropages typicus* and *Temora stylifera*.

Based on the studies conducted by the Japanese scientists on the improvement of copepod mass culture, 13 species were recommended for mass cultivation. These includes *Acartia clausi* (*A. hudsonica* or *A. omorii*, on the basis of current classification), *A.longiremis*, *Eurytemora pacifica*, *Euterpina acutifrons*, *Microsetella norvegica, Oithona brevicornis* (*O. davisae*, based on current classification), *O. nana*, *O. similis*, *Pseudodiaptomus inopinus*, *P. marinus* and *Tigriopus japonicas* (Omori 1973; Nihon Suisanshigen Hogokyoukai 1979). Among these ,*Tigriopus japonicas* is the only one which is produced on a large scale and used in marine fish farming (Kitajima, 1973; Fukusho *et al.,* 1977, 1978; Nihon Suisanshigen Hogokyoukai, 1979). Based on their studies, rearing of the other species was attributed only to laboratory (Iwasaki and Kamiya, 1977; Iwasaki *et al.,* 1977). Maintenance of cultivation has a lot of difficulties including frequent replacement of water, the high demand for cultured algae as well as the low and unstable population growth.

Harpacticoids of the crustacean class Maxillopoda are easier for culture. These are hardy and can be cultured in high densities than calanoids and cyclopoids. The main disadvantage of harpacticoid copepod is its benthic nature while most of the fish larvae are pelagic in nature. The harpacticoids can be fed using a wide variety of food items including formulated diets. For many marine fish species, they serve as an important food source (Coull, 1990). For some fish species certain harpacticoids serve as prey for their entire life. Species of flatfishes, gobies, salmonids and sciaenids are sometimes considered to be harpacticoid feeders, atleast for a portion of their lives (Coull, 1990; McCall and Fleeger, 1995; Fleeger, 2005). Over 3000 species of Harpacticoids were reported so far (Huys and Boxshall,1991). For the purpose of ecological, physiological and toxicological research, harpacticoid copepods were first isolated from the sediments and cultur Chandler (1986). Later on it was proved that, mass culture of harpacticoids can be easily achieved using wide variety of food items (Battaglia, 1970; Strawbridge *et al.,* 1992; Ingole 1994; Chandler *et al.,* 1997; Lotufo and Fleeger, 1997).

According to Conceicao *et al.,* (2009), the wild zooplankton mostly consisting of copepods are the common food resource for fish larvae in nature.Occurrence of parasitic infections on most of the fish species is the major problem in using wild collected plankton for hatchery. The copepod parasites such as *Lepeophtheirus, Caligus* and *Pseudocaligus* can causes high mortality in fishes if the copepods from the wild collected plankton samples were used for feeding (Chinabut, 1996). Due to risk of parasitic transmission harvesting copepods from natural environmentsis not desirable. In order to avoid this problem Stottrup (2003) was the first to demonstrate the breeding of copepods in a holding tank (as an intermediate host). From tanks, the nauplii can be collected and used as feed for larvae. More than 60 copepod species have been raised in laboratory. For promoting the culture and improving cost-effectiveness of marine copepods in aquaculture industry, the development of appropriate culture techniques is essential. Copepods can be cultured extensively, intensively and semi-intensively. Copepods can be extensively developed in tanks, outdoor ponds, lagoons or enclosed fjords (Coneicao *et al.,* 2009). By using appropriate mesh sizes these cultured copepods can be made available to fish larvae. Planktonic copepods including *Acartia, Centropages* and *Temora* can be cultured in such systems. In extensive systems, culture is done normally on the basis of microalgal booms induced by agricultural fertilizers (Conceicao *et al.,*2009). Inconsistency in the production and domination of undesirable species are the common problem in this method. Rearing copepods in appropriate temperature, sufficient live feed (algae) with frequent exchange of seawater with the use of advanced mesh of varying measurements, continuous and a reliable supply of large scale copepods can be achieved for the use of hatchery without much difficulties.

Nowadays Culture methods for marine copepods are well advanced (Ogle, 1979. Ohno and Okamura 1988, Payne and Rippingale, 2001a,b,c; Santhosh and Anil, 2013). The body of a copepod is elongate, cylindrical, and clearly segmented. Copepods reproduce sexually only. Sexes are separate and can be easily distinguished in most of the cases. Mostly these have six naupliar and copepodite stages each. Two basic types are there, one carry eggsacs and the other scatter their eggs. Mostly these take 10-15days to become adults and live for 25 to 55 days. (Jakob *et al*., 2012; Santhosh and Anil, 2013). In general copepod species that free-spawn their eggs have higher fecundity than species in which females carry their eggs in clusters (Mauchline, 1998). The species that protect their eggs by carrying them in clusters are reported to have lower mortality rates (Kiorboe and Sabatini, 1994). Small pelagic copepod species with short life cycles and fast growth can be cultured with high-yield using semi intensive technologies. In tropical and subtropical countries, with technically qualified labor and facilities, copepods could give good economic results in terms of highly stress-resistant larvae, good survival, growth and biomass productions when compared with enriched *Artemia* or rotifers (Hernandez Molejon and Alvarez-Lajonchere, 2003). Overcrowding (Miralto *et al.,* 1996) and cannibalism (Ohno *et al*., 1990) also have got some influence on fecundity and population growth.

The basic morphology is similar to most of the crustacean type with large cephalosome and a small urosome, large first antennae and small second antennae with typical crustacean mouth parts. There are 4 pairs of two-branched swimming legs and the fifth pair mostly unbranched; each pair fused at the base by a plate which powers the legs to move organized and this is a highly successful evolutionary design. (Dussart and Defaye 2001). The calanoids are mainly herbivorous and the harpacticoids are mainly omnivorous. Most of the cyclopoids are predators. The smaller species tend to be plankton feeders, whereas the larger species tend to be aggressive predators, consuming protozoans, rotifers, and small aquatic animals (Fryer 1957; Hutchinson 1967).

Calanoids

The calanoid copepods are predominantly pelagic, occurring at all depths, with some near-bottom and benthic species. They are mainly feeding on small phytoplankton cells by filtration, rarely predators feeding on a variety of animal prey including copepod eggs. These can be easily distinguished by their long antennules, mostly as long as the body itself or sometimes, even longer, with up to 27 segments and biramous antennae mostly used as accessory locomotor appendages. Usually the antennule of male will be modified (Huys & Boxshall 1991; Dussart & Defaye 2001) and the position of the prosome–urosome articulation is between the fifth and sixth postcephalosome somite (Mauchline 1998; Dussart & Defaye 2001).

Among the calanoid copepods, the genera *Acartia*, *Pseudodiaptomus*, *Sinocalanus*, *Eurytemora*, *Centropages*, *Gladioferens*, *Parvocalanus*, *Bestiolina*, *Temora* and *Labidocera* were popularly proposed for hatchery production. Nauplii of some *Acartia* spp. are as small as 100 μm in length and 50-60 μm in width, making them suitable for first feeding of fish larvae. *Labidocera* sp. found to grow successively larger and produced more eggs in an established laboratory culture. Several Paracalanoid species are larger, thus are more suitable, for feeding to larger fish larvae. Paracalanid copepods are very common in coastal areas, while *Acartia* spp. found almost everywhere (Cheng-sheng Lee, *et al.,* 2005).

Among the families of larval fish that feeds on copepod prey, a vast majority shown clear preference for calanoid copepods and more specifically for small calanoid species (Sampey *et al.,* 2007) are hence considered to be the most promising order of copepod for production and used as live prey items for marine hatcheries (Doi*et al.,* 1997;Stotttrup, 2000). Specifically, pelagic calanoid species from coastal waters with high tolerance to wide ranges of environmental conditions are preferred live prey candidates (Stottrup, 2003; Conceicao*et al.,* 2010). Popular calanoid species reported as ideal live feed are *Acartia grani* (Spain), *A. sinjiensis* (Australia), *A. southwelli* (Taiwan, India), *A. spiniauda* (India), *A. tonsa* (Denmark, Uruguay), *A. parvula* (Germany), *A. centrura* (India), *A. erythreae* (India), *Centropages typicus* (Italy), *Eurytemora affinis* (France, Canada, Mediterranean sea), *Gladioferens imparipes* (Australia), *Pseudiaptomus annandalei* (Taiwan), *P. serricaudatus* (India), *Temoralongicornis* (North Sea, UK), *T. stylifera* (Italy) and *T. turbinata* (India).

Harpacticoids

The harpacticoids, comprises over 50% of copepod species, are primarily marine, free living, benthic organisms rarely represented in pelagic water samples. These are common in sediments occupying spaces between sand particles (interstitial), burrowing into sediment (burrowers), or living on sediment or plant surfaces (epibenthic). They are distinguished by their short and streamlined body, antennules with fewer than 10 segments and biramous antennae. The position of the prosome–urosome articulation is between the fourth and fifth postcephalosome segment (Dussart & Defaye 2001). Usually there is only one egg pouch and males are smaller with a specialized antennule.

Harpacticoid copepods are popular in culture for their higher population density but their benthic nature makes them less available to the fish larvae. However, the nauplii of some harpacticoids exhibit positive photo taxis so that they can be harvested easily and fed separately to fish larvae (Stottrup and Norsker, 1997). Harpacticoids copepod species can be reared to high production rates, are generally not cannibalistic and can be raised on formulated feeds. Two species or more species also can be cultured at high densities. Often harpacticoid come as a contaminant species along with ciliates in the culture of calanoid copepods. The nauplii of *Nitokralacustris* are benthic but the copepodid stages (90 μm in length and 30-40 μm in width) are mostly in column water and are suitable for feeding fish larvae. Nauplii of hapacticoid copepod speciesare difficult to separate from the culture and harvesting from the sediments is also not easy (Cheng-sheng Lee *et al.,* 2005).

Harpacticoid copepods can be cultured easily in high densities than calanoids. Many species are epibenthic in nature with reduced size and can be utilized for feeding fish larvae. Their nutritional value is similar to that of calanoids. Some models are there with semi-automated system for feeding and harvesting of nauplii will minimize the labour using harpacticoid culture (Stottrup, 2006). Usual epibenthic nature of harpacticoids makes them less suitable for the fish larval feed except for some pelagic harpacticoids like *Euterpina acutifrons* which is used in the rearing of pelagic larvae of *Coryphaena hippurus* (Kraul *et al.,* 1992). Popular harpacticoid species in the aquaculture system are *Ameira parvula* (Germany), *Amonordianormani*, *Amphiascoidesatopus* (USA), *Euterpina acutifrons* (Mediterranean sea, India), *Trachidiusdiscipes (*Germany), *Tigriopus japonicus* (India) and *Macrosetell agracilis* (India).

Cyclopoids

The cyclopoids include pelagic, epibenthic, benthic and parasitic species and are more abundant in freshwater environments(Huys & Boxshall 1991). The antennules in cyclopoids are shorter than in calanoids, rarely reaching beyond the cephalothorax and usually have six to 17 segments. Unlike calanoids and harpacticoids, cyclopoids have uniramous antennae which is modified for catching food (Huys & Boxshall 1991; Dussart & Defaye 2001). Like that of harpacticoids, the position of the prosome–urosome articulation is between the fourth and fifth postcephalosome segment (Dussart & Defaye 2001).

Among the cyclopoid copepods, the genera *Oithona* and *Dioithona* are popular in culture. Nauplii of these are mostly less than 100 μm in length and are negatively phototactic, and can be collected in plankton nets. Nauplii of the cyclopoid copepod *Apocyclopsroyi* develop from eggs in 4-5 days and are used for first feeding practices in Taiwan (Cheng-sheng Lee *et al.*, 2005). The swarm forming cyclopoid copepod *Dioithona oculata* culture experiments suggest that this species has good potential for the high density culture (Hernandez Molejon and Alvarez-Lajonchere, 2003). Cyclopoids can be easily cultured and can obtain higher densities than calanoids. They can be fed with a variety of foods but popularly cultured using phytoplankton in intensive systems (Stottrup, 2006). Herna´ndezMolejo´n and Alvarez-Lajonche‘re (2003) reported culture experiments with *Oithona oculata* and with a final population of 13 copepods /ml in 15-day cultures without aeration. Larvae of symbiotic poecilostomatoid copepods, which are swimming but non feeding, reported to be co cultured with mussels (the host of their adult stages) for feeding fish larvae in Taiwan (Cheng-sheng Lee *et al.,* 2005). Ho (2005) reported that a symbiotic (with bivalve mollusk) copepod namely *Pseudomyicola spinosus* belonging to the family Myicolidae, can be used as live feed in marine finfish rearing. The first seven stages in the life cycle of the species are planktonic and can be used as the live feed.

Fisheries agency of Japan has recommended 13 species of copepod for the mass culture as a part of a project entitled "searching for suitable species and mass culture of zooplankton as food for the early stage of fish seed in marine fish farming" for Japan. The species includes *Acartia hudsonica*, *A. longiremis*, *Eurytemora pacifica*, *Euterpina acutifrons*, *Microsetell anorvegica*, *Oithona davisae*, *O. nana*, *O. similis, Pseudodiaptomus inopinus, P. marinus* and *Tigriopus japonicus* (Omori 1973; Nihon Suisanhigen Hogokyoukai 1979) and concluded that among these, *T. japonicus* was the most ideal species to be cultured on a large scale and which can be economically used as larval feed in marine fish farming (Kitajima 1973; Fukusho*et al.,* 1977;Nihon SuisanhigenHogokyoukai, 1979). Popular cyclpoid species in the live feed purpose are *Apocyclops royi* (Taiwan), *A. panamensis* (UK), *Mesocyclops longisetus* (Florida), *Microcyclops albidus* (Florida), *Oithona davisae* (Spain) and *O. rigida* (India).

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