Intensive Indoor and Outdoor Pilot-Scale Culture of Marine Copepods

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Introduction

Copepods are more abundant than any other group of multicellular animals, including the hyper-abundant insects and nematodes. They consume phytoplankton and microorganisms, and they are inturn preyed upon by higher trophic level, animals including fish and whales. In particular, they serve as primary prey for the larval stages of many fish species of economic importance. In aquaculture, copepods have been proven to be the much preferred and most adequate food for many marine fish larvae (Houde 1973; May et al. 1974; Kraul 1983, 1989, 1993) and are also used for the shrimp larvae (Shamsudin and Saad 1993). Good fish productivity of an aquatic ecosystem is related to the presence of copepods and their role as the main food component (May 1970; Bent 1993). The larvae of many marine fish require prey with size of about 50–100 μm wide at their first feeding stage (Detwyler and Houde 1970; Yufera and Pascual 1984). Even the rotifer of type "S" is too large in many cases (Houde 1973; May et al. 1974; Doi and Singhagraiwan 1993). The results concerning first feeding of commercially important fish on dry food organisms are encouraging (Fernandez-Dıaz and Yufera 1997; Cahu and Zambonino Infante 2001). However, live feeds cannot always be substituted because of biochemical and behavioural constraints of the fish larvae (Drillet et al. 2006).

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Global Status

Since the 1960s, culturing copepods have become increasingly more reliable, and approximately 60 copepod species have been successfully raised (Mauchline et al. 1998). The oldest copepod culture is from the Danish Technical University of Denmark (*Acartia tonsa*) (Stottrup et al. 1986). The World Copepod Culture Database was initiated in 2006 at Roskilde University (http://copepod.ruc.dk/main.htm) in an attempt to supply and share information among copepod scientists, aquaculturists and the public at large. The database contains details on various cultures and up-to-date recent knowledge on cultivation procedures. To date, approximately 30 copepod cultures have been referenced in the database (Tables 1, 2).

Copepods have also been satisfactorily used after a period of using rotifers and before introducing *Artemia* nauplii (Kraul 1993; Alvarez-Lajonchere et al. 1996) and are also offered simultaneously (Leu and Chou 1996).

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Table 1 Available reports on copepod culture in foreign countries

Genus/species	Identification type: morphological or genetical	Geographical origin	Cultivation conditions, Temperature, salinity, light regime, food
Acartia grani	Morphological	Barcelona harbour, Spain (NW Mediterranean)	19 °C/38 ppt/12 L:12D/*Rhodomonas salina*
Acartia sinjiensis	Morphological	Townsville channel, QLD (Australia)	27–30 °C/30–35 ppt/ 18 L:6D/ *Tetraselmis chuii* and T-iso
Acartia southwelli	Morphological	Pingtung - Taiwan	25–30 °C/15–20‰/12 L:12D/*Isochrysis galbana*
Acartia tonsa	Morphological	Origin Unknown	?
Acartia tonsa	Morphological and genetics	Oresund (Denmark)	17 °C/30 ppt/0 L:24D/*Rhodomonas salina*
Acartia tonsa	Morphological	Punta del Este, Uruguay	25–30 °C/17 ppt/indirect natural light/*T-ISO-Tetraselmis*
Ameira parvula	Morphological	Kiel Bight, Germany	18 °C, 17 ppt, 12 L:12D, different algae
Amonardia normani	Morphological	Kiel Bight, Germany	18 °C, 17 ppt, 12 L:12D, different algae
Amphiascoides atopus	Morphological	USA	25 °C, 12/12, cultured phytoplankton
Apocyclops royi	Morphological	Pingtung - Taiwan	25–30 °C/15–20‰/12 L:12D/*Isochrysis galbana*
Centropages typicus	Morphological	Gulf of Napoli Italy (W Mediterranea)	19–21 °C/38 ppt/12 L:12D/*Prorocentrum minimum*/*Isochrysis galbana*/*Tetraselmis suecica*
Eurytemora affinis	Morphological	River Seine Estuary (France)	10–15 °C/15 ppt/12 L:12D/fed *Rhodomonas marina*
Eurytemora affinis	Morphological	Gironde Estuary (France)	10–15 °C/15 ppt 12 L:12D/fed *Rhodomonas marina*
Eurytemora affinis	Morphological	Loire Estuary (France)	10–15 °C/15 ppt 12 L:12D/fed *Rhodomonas marina*
Eurytemora affinis	Morphological	Canada	10–15 °C/15 ppt/12 L:12D/*Rhodomonas marina*
Euterpina acutifrons	Morphological	Mediterranea	19 °C/38 ppt/12 L:12D/*Rhodomonas salina*
Eurytemora affinis	Morphological	Gironde Estuary (France)	10–15 °C/15 ppt 12 L:12D/fed *Rhodomonas marina*
Euterpina acutifrons	Morphological	Mediterranea	19 °C/38 ppt/12 L:12D/*Rhodomonas salina*
Eurytemora affinis	Morphological	Gironde Estuary (France)	10–15 °C/15 ppt 12 L:12D/fed *Rhodomonas marina*
Eurytemora affinis	Morphological	Canada	10–15 °C/15 ppt/12 L:12D/*Rhodomonas marina*
Euterpina acutifrons	Morphological	Mediterranea	19 °C/38 ppt/12 L:12D/*Rhodomonas salina*
Gladioferens imparipes	Morphological	Swan River, Perth Western Australia	23–27 °C, 18 ppt, continuous dark, T-Iso and *Chaetoceros muelleri*
Mesocyclops longisetus	Morphological	Florida/USA	http://edis.ifas.ufl.edu/IN490
Microcyclops albidus	Morphological	Florida/USA	http://edis.ifas.ufl.edu/IN490
Oithona davisae	Morphological	Barcelona harbour, Spain (NW Mediterranean)	20 °C/30 ppt/natural light/*Oxhyris*

(continued)

Table 1 (continued)

Genus/species	Identification type: morphological or genetical	Geographical origin	Cultivation conditions, Temperature, salinity, light regime, food
Pseudodiaptomus annandalei	Morphological	Pingtung - Taiwan	25–30 °C/15–20‰/12 L:12D/*Isochrysis galbana*
Tachidius discipes	Morphological	Kiel Bight, Germany	18 °C, 17 ppt, 12 L:12D, different algae
Temora longicornis	Morphological	North Sea	15 °C/30 ppt/0 L:24D/*Thalassiosiera weissflogii, Rhodomonas salina, Heterocapsa, Prorocentrum minimum*
Temora longicornis	Morphological	Plymouth, Devon, UK	Temp according to current sea temperatures, salinity 30–36 ppt, 12 L:12D, fed mixture of *Isochrysis galbana, Rhodomonas* and *Oxyrrhis*
Temora stylifera	Morphological	Gulf of Napoli Italy (W Mediterranea)	19–21 °C/38 ppt/12 L:12D/*Prorocentrum minimum*/*Isochrysis galbana*/*Rhodomonas baltica*

Table 2 Available reports on copepod culture in India

Culture species	Culture size	Productivity	Food/conditions	Reference
Oithona rigida	25 L	33,867 ind/L	*Chlorella vulgaris, Coscinodiscus centralis, Chaetoceros affinis and Skeletonema costatum*	Santhanam (2002)
Acartia centrura	25 L	41,603 org/l.	*C. marina, Nannochloropsis salina* and *Isochrysis galbana*	Vengadeshperumal et al. (2010)
Acartia southwelli	25 L	55,103 org/l.	*Chlorella marina, Nannochloropsis salina* and *Isochrysis galbana*	Vengadeshperumal et al. (2010)
Tigriopus japonicus	210 m^3	10–22/ml	Chlorella minutissima, x-yeast, baker's yeast co-culture with rotifers;	Fukusho (1980)
Acartia erythreae	25 L	–	Microalgae	Rajkumar (2006)
Macrosetella gracilis	100 L	2.86 ind/ml	Mixed microalgae	Ananth and Santhanam (2011)

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) and Watanabe et al. (1983) reported that culture media did not influence the copepod chemical composition, although Delbare et al. (1996) reported that copepod n-3 HUFA reflected the culture diet. A positive correlation of the n-3 HUFA levels and highest nauplii production was reported by Norsker and Stottrup (1994). Other good characteristics of copepods are their swimming movements as a larval visual stimulus; their tank-cleaning performance primarily by benthic harpacticoids, which are grazers (Stottrup et al. 1995); their high digestive enzyme content (Delbare et al. 1996); and a possible enhancement of their feeding rates with improved growth and survival (Støttrup and Norsker 1995; 1997).

Indian Scenario

In India, work on copepod culture is very scanty. Merrylal James and Martin Thompson (1986) cultured the copepods belonging to the genera *Cyclops*, *Oithona* and *Pseudodiaptomus* and introduced them in mariculture. Santhanam

(2002) has studied the culture and utilization of *Oithona rigida* in seabass *Lates calcarifer* and tiger shrimp *Penaeus monodon* larval rearing. Rajkumar et al. (2004) cultured the copepod *Acartia clausi* in laboratory condition. Recently, marine copepods, viz., *Nitocra affinis* and *Oithona rigida*, have been successfully cultured with the density of 1,30,000 ind./l and 1,15,000 ind./l, respectively, fed with mixed marine microalgae such as *Chlorella marina*, *Isochrysis* sp., *Dunaliella* sp. and *Nannochloropsis* sp. The initial stocking density of both species ranged between 100 and 200 individuals, and the culture duration lasted for 45 days. Used plastic cans bought from shops served as culture vessels. This is the first successful achievement on *Nitocra affinis* and *Oithona rigida* for high-density culture in low volume of seawater at lower cost. The culture technology has been developed for marine copepods *Nitocra affinis* and *Oithona rigida* in the Marine Planktonology and Aquaculture Laboratory of the Department of Marine Science, Bharathidasan University, Tiruchirappalli, by Dr. P. Santhanan, Assistant Professor, and his team for the first time in India.

Need for Marine Copepods Culture

Aquaculture is considered to be one of the lucrative industries due to the high market price (of shrimp) and the unlimited demand for it in the international market. By virtue of its geographical location in the Indian Ocean, India possesses a rich fishing ground in the sea and offers immense potential for aquafarming (Varghese 1995). For a sustainable growth of the aquaculture industry, regular supply of adequate quantities of quality fish seeds is one of the prerequisites. Quality seeds are those that ensure high growth rate and low mortality and that can withstand stress during culture (Santhanakrishnan and Visvakumar 1995). To produce good quality of adult fish, an effective larval rearing is necessary to produce quality fish fry. A reduction in the use of chemicals and drugs or addition of hormones would increase the natural immunity of fish larvae (Sorgeloos and Leger 1992), though providing sufficient nutrients to the larvae and preventing bacterial infections are still the most important requirement.

Therefore, the importance of live feeds in fish and shrimp culture has been well documented. The use of live feeds for larval fish is well established with brine shrimp, *Artemia* and rotifer, *Brachionus plicatilis* being the most common among them. While brine shrimps are very amenable to commercial culture (Loya-Javellana 1989), difficulties have been reported on the use of rotifers because of their small size, their nutritional variability and their culture susceptibility to crashing (Kovalenko et al. 2002). Although the *Artemia* nauplii have been widely used as live food, by no means it is the optimal live food organism in terms of nutritional requirement of fish and shrimp larvae. The main disadvantages of *Artemia* are marked variation in cost, physical properties and nutritional quality among different sources. Hence, the production of very small, rapidly developing and highly vulnerable larvae remains a bottleneck in the commercially successful culture of many marine fish species (Shields et al. 1999). The bio-enrichment of *Artemia* has been widely adopted to overcome the problem of inferior nutrition supply. But there are still other nutrient deficiencies in the enriched *Artemia* nauplii, such as free amino acids availability (Helland et al. 2003), and the biological composition of *Artemia* is not stable after enrichment (Olsen et al. 2000; Olsen et al. 1999). In addition, common lipid enrichment actually reduces the relative protein content and alters the amino acid profile of *Artemia* nauplii (Helland et al. 2003). Losses of nutrients may take place if the live food is not fed to the fish larvae immediately after enrichment (Olsen et al. 2000). Moreover, enrichment of *Artemia* with commercial emulsifier is increasing the cost of production of fish larvae.

Nutritional compounds such as n-3 fatty acids, essential amino acids (EAA) and protein content of live feeds are critical factors for the survival and optimal growth of larval finfish and crustaceans. Hence, the need for the production of quality copepod gains importance. Copepods

are very nutritious larval feeds, containing more EPA and DHA. Copepods also have the highest DHA to EPA ratio (Nanton and Castell 1999; Toledo et al. 1999). On the contrary, rotifers and *Artemia* are poor in polyunsaturated fatty acids (PUFAs) and need to be enriched before feeding, which in turn has its own shortcomings, as mentioned before. Among the very many benefits that application of copepod food could have in aquaculture industries are improved larval survival (Shields et al. 1999), higher growth rates (Stottrup and Norsker 1997), better pigmentation (Spenelli 1979; Støttrup et al. 1998), improved gut development (Luizi et al. 1999) and a source of exogenous enzymes (Munilla-Moran et al. 1990).

The marine copepods are considered to be "nutritionally superior live feeds" for commercially important cultivable species, as they are a valuable source of protein, lipid (especially HUFA, 20:5 n-3 and 22:6 n-3) and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval survival, growth, digestion and metamorphosis (Stottrup 2000; Kleppel 1993). Copepods are known to have greater digestibility (Schipp et al. 1999) and a relatively high weight-specific caloric content (Sun and Fleeger 1995). In addition, the growth stages of copepods from first nauplius to adult form a broad spectrum of prey sizes. This makes them suitable prey for a similarly broad range of developing fish sizes (Schipp et al. 1999). It is well known that the red pigment astaxanthin is one of the strongest antioxidants in nature (Edge et al. 1997) and is abundant in crustaceans (Matsuno 1989; Matsuno et al. 1990). It is well established that the pigmentation of copepods can improve the survival and growth of larvae. Furthermore, carotenoids are important antioxidants and often play other biological functions, such as regulatory effects on intra- and intercellular signalling and gene expression (Sies and Stahl 2005).

HUFAs are essential for marine fish larvae (Watanabe et al. 1983; Witt et al. 1984; Sorgeloos et al. 1988; Watanabe and Kiron 1994; Kanazawa 1995). 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 even enriched *Artemia* nauplii which give better results in terms of survival, growth and stress resistance in fish larvae (Fujita 1979). Superior larval stress resistance can be achieved with copepods, even when DHA content is less than in enriched *Artemia* nauplii (Kraul et al. 1992). Although *Artemia* enrichment has greatly helped in improving commercial aquaculture, the most advanced rotifer and *Artemia* bioencapsulation techniques have not matched the good results of copepods, and their composition was subsequently taken as the standard for improving enrichment techniques for rotifers and *Artemia* (Kraul et al. 1988, 1992; Sorgeloos and Leger 1992). 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 (Stottrup et al. 1986; Stottrup and Norsker 1997).

Free-living copepods have been intensively studied because of their impact as key players in the marine pelagic environment. In terms of biomass, copepods can represent up to 80 % of the mesozooplankton (Mauchline et al. 1998). They are an important food source for planktivorous fish and fish larvae in general (e.g. Fox et al. 1999; Mollmann et al. 2004). The life cycles and physiology of copepods have been intensively studied in relation to various environmental conditions on the pelagic ecosystem. Experimental studies such as feeding behaviour, fecundity, developmental biology, nutritional composition of copepods with reference to environmental parameters and feeds have benefited from the small-scale cultivation of copepods. Also, when compared to field-sampled specimens, the advantage is that the history and consistent condition of cultivated copepods are well known and thus can even be manipulated. The data generated during various culture experiments could be applied in a variety of research areas such as copepod genetics, feeding behaviour, population dynamics, parameterization of standing stocks, production rates for ecosystem models, etc.

Microalgal Culture

The amount and quality of algal feeds are essential parameters that enhance the production of copepod. Hence, a series of preliminary trials were carried out to determine a suitable diet for copepods. Our results confirmed that copepods benefit from mixed algal cultures with *Isochrysis* being the important constituent. Unialgal cultures of *Isochrysis* sp., *Chlorella* sp. and *Tetraselmis* sp. were maintained as live feeds for copepods. The algae were maintained at an optimum temperature of 24–28 °C, salinity of 28–30 ppt and with 12:12 h light and dark conditions.

Indoor algal stock culture was maintained in special air-conditioning room using Conway's medium. About 10 ml of the inoculum in the growing phase was transferred to the culture flask provided with 12:12 h. light and dark cycle. Outdoor mass culture was maintained in large volume of FRP tanks using commercial fertilizers, viz., ammonium sulphate/urea/super phosphate, in the ratio of 10:1:1 g.

Water Quality

Copepods were reared in clean, filtered seawater diluted to approximately 24 ppt filtered through filter bags (5 μm). This salinity was maintained at 26–28 ppt during most part of the study. Aeration was achieved via air pumps with air stones inserted from the top of the vessels. Ammonia, nitrite, pH and dissolved oxygen were measured at periodic intervals in all cultures.

Copepods Source

Copepods for culture were collected from Muthupet and Muttukadu estuaries (Southeast coast of India). Copepods were collected using plankton net (158 μm) at dawn. Contamination from other zooplankters was reduced by thorough rinsing and filtering with meshes of various sizes upon return to the laboratory. The majority of copepods collected were found to be calanoids. *Pseudodiaptomus* sp. being positively phototactic was easily separated and isolated in the presence of lamp using a hand net. *Oithona rigida* was the second best in terms of density from our samples followed by *Nitocra affinis*.

Isolation of Copepods

After collection, the zooplankton was screened to isolate the size fraction containing predominantly adult and late-stage copepodids. This was achieved by first removing fish and prawn larvae. Grading was accomplished by using a set of superimposed sieves with varying mesh sizes and with decreasing mesh size from upstream to downstream. Copepod samples were screened coarsely through a 500 μm mesh to remove fish and prawn larvae. Then the samples were screened through 190 μm mesh to remove rotifers, nauplii of copepods and barnacles. After grading, copepods were identified using standard manuals, monographs and text books (Kasturirangan 1963; Perumal et al. 2000; Santhanam and Perumal 2008).

Indoor Stock Culture of Marine Copepods

After the species confirmation, known numbers of gravid females were isolated using capillary tubes, fine brush and needle and were stocked initially in low volume of glass beakers and conical flasks provided with microalgae without aeration. Later, the copepods were subcultured into average volume of plastic containers filled with filtered seawater and were provided with vigorous aeration. The optimum water quality conditions like temperature, salinity, pH and dissolved oxygen were maintained. Copepods were fed with a daily ration of microalgae diet in the constant concentration.

Copepods were generally cultured in seawater of 26–28 ‰ salinity. The stock was maintained mostly at a temperature of 29 °C. Copepod stock cultures were maintained in cylindrical,

flat-bottomed, polyethylene 5 or 7 l plastic cans at air-conditioning room. Initially the stock was maintained with 50–80 adult copepods. The microalgae cultures grown non-axenically in 5-l flasks were provided as food.

Outdoor Pilot-Scale Culture of Marine Copepods

Pilot-scale copepods culture was started with clean FRP tanks, algae and a filtered, UV-treated seawater. Tanks were stocked with known numbers of gravid females. Gravid females had released the nauplii within 36–42 h. The detritus in the tanks were removed daily using graded sieves connected with siphon hose, and then the adults and nauplii were introduced back to the tank. The sequential batch cultures were started at 5–7-day intervals for continuous copepods production.

Air Quality Control

The cool, filtered, interior air was used for aeration. The disposable in-line 0.2 μm pore antibacterial air filters were used for providing aeration. Aeration was used to maintain the algae culture in a condition of CO_2 saturation, pH stability and uniform mixing.

Water Quality Control

The seawater conditions like salinity, pH, DO, colour and scent (particularly the "rotten egg" smell of H_2S) were checked prior to collection and treatment. The seawater was serially filtered through 50, 10 and 1 μm mesh bags and then passed through an ultraviolet sterilizer. Filter bags were cleaned and then sanitized overnight in hypochlorite solution once a week under normal usage. Filtered, UV-treated seawater was used directly to culture copepods. The filtered seawater was treated with 10 % commercial hypochlorite solution at 0.2 ml/l and stand for overnight without aeration. After that, seawater was dechlorinated with thiosulfate solution volume for volume (V/V) at 0.2 ml/l. The dechlorinated water was used for filling all wash bottles, stacked-sieve holders, harvest samples, population counts, etc.

System and Equipments Preparation

The fibreglass tank was washed with a low-residue laboratory detergent (e.g. Alconox or Sparkleen) and water followed by thorough rinsing. Then the tank was treated with 100 % muriatic acid (HCl) solution in the outdoors followed by thorough rinsing with filtered seawater. Tanks were leached three times (24 h each time) to remove all water-soluble remnants of the manufacturing process. Tanks were filled to the rim with filtered, UV-treated seawater and adjusted the salinity as needed, and water was chlorinated with 60 ml (0.2 ml/l) commercial 10 % hypochlorite solution per litre. The treated system was allowed to stand for 24 h. Thereafter, the system was dechlorinated with 60 ml of stock thiosulfate solution. The vigorous aeration was started. After an hour, "free chlorine" test strip was dipped and zero "free chlorine" remaining was confirmed. The treated seawater was aerated to at least 6 mg/l DO.

Harvest Stipulations

The stacked-sieve holder and wash bottles were washed with treated seawater at culture tank temperature. The siphon hose were connected to the siphon head and the stacked sieves. The copepods were harvested and filtered onto a wet freestanding sieve. As the tank water level drops, frequent rinsing was done to rinse down copepods stuck to the sidewalls. 30 % of the tank volume was exchanged weekly with new treated seawater.

Conclusion

The fact that copepods are highly valuable source of live food for fish larval rearing is well established. However, despite significant progress in copepod cultivation, their use is still sporadic. This can be attributed to rare use of copepods in commercial settings. Thus, conscious efforts need to be made to upscale the copepod culture to commercial levels in order to ensure production of a reliable, continuous supply of copepods on a large scale. Different species of copepods provide us with different characteristic, i.e. differences in shape, size and movement.

Annual sales of *Artemia* cysts marketed for crustacean and larval fish food have increased to more than 2,000 t (Sorgeloos et al. 1994). This figure may be underestimated considering the report of Stael et al. (1995) that 600 t was used in Thailand just for shrimp culture in 1993. These high consumption levels can lead to a situation where the demand exceeds the supplies (Stael et al. 1995), with consequent costs escalation. It is expected that this situation will not improve much during the next few seasons due to rather unfavourable conditions for *Artemia* production in the lake as well as due to increasing hatchery demand (Lavens and Sorgeloos 1998). This is known as the *Artemia* crisis (Sorgeloos 2000). Hence, the use of copepods at a commercial level needs to be addressed urgently.

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