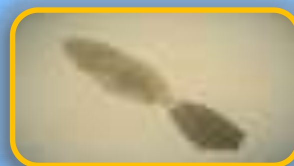
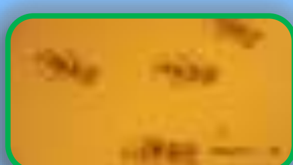
CMFRI Training manual Ser. No.: 9/2016

Training Manual

on

and Shellfish Culture





VISAKHAPATNAM REGIONAL CENTRE
ICAR-CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
OCEAN VIEW LAYOUT, PANDURANGAPURAM
VISAKHAPATNAM - 530003

Live feed for marine finfish and shellfish culture

Shubhadeep Ghosh, Biji Xavier, Loveson L. Edward and Biswajit Dash

Introduction

Live food organisms include all plants (phytoplankton) and animals (zooplankton) which are used in finfish and shellfish larval rearing system. Live foods are able to swim in water column and are available to fish and shellfish larvae thereby stimulate the feeding response (David 2003). In nature, most of the finfish and shellfish larvae feed on small phytoplanktonic and zooplanktonic organisms. Phytoplankton comprises the base of the food chain in the marine environment. Micro algae have an important role in aquaculture as a means of enriching zooplankton for feeding fish and other larvae. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential poly unsaturated fatty acids (PUFA), pigments and sterols, which are transferred through food chain. Zooplankton forms the primary food for fish larvae. The dominant zooplankton groups are rotifers and copepods. These groups are the preferred prey for shrimp and fish larvae and are the most widely used live feeds by aquaculturists. The intensive larval culture of most marine fish depends on large supply of zooplankton.

Phytoplankton

Micro-algae are indispensable in the commercial rearing of various species of marine animals as a food source for all growth stages of bivalve molluscs, larval stages of some crustacean species, and very early growth stages of some fish species. Algae are furthermore used to produce mass quantities of zooplankton (rotifers, copepods, and brine shrimp) which serve in turn as food for larval and early-juvenile stages of crustaceans and fish. Besides, for rearing marine fish larvae algae are used directly in the larval tanks referred as "green water technique", where they are believed to play a role in stabilizing the water quality, nutrition of the larvae, and microbial control. All algal species are not equally successful in supporting the growth and survival of a particular filter-feeding animal. Suitable algal species have been selected on the basis of their mass-culture potential, cell size, digestibility, and overall food value for the feeding animal. Various techniques have been developed to grow these food species on a large scale, ranging from less controlled extensive to mono specific intensive cultures.

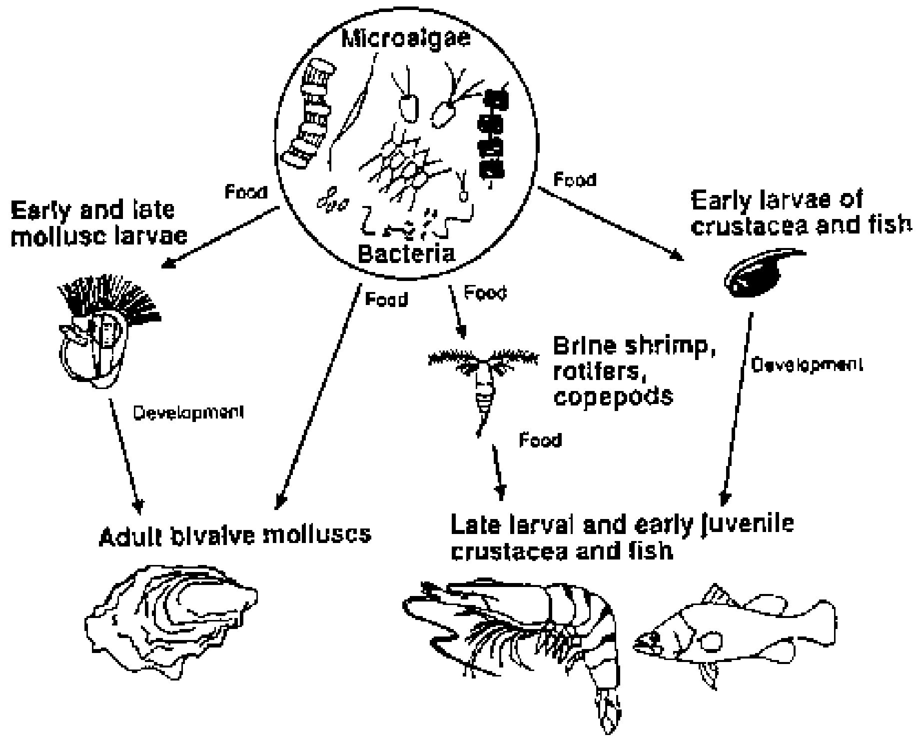


Fig 1. The central role of microalgae in mariculture (Brown *et al*., 1989). Source :FAO 1996

Cultured algal species - major classes and genera

Today, more than 40 different species of micro-algae, isolated in different parts of the world, are cultured as pure strains in intensive systems.

The list includes species of diatoms, flagellated and chlorococcalean green algae, and filamentous blue-green algae, ranging in size from a few micrometers to more than 100 µm.

Table 1. Major classes and genera of micro-algae cultured in aquaculture (modified from De Pauw and Persoone, 1988). Source: FAO 1996

Class	Genus	Examples of application
Bacillariophyceae	*Skeletonema*	PL,BL,BP
	Thalassiosira	PL,BL,BP
	Phaeodactylum	PL,BL,BP,ML,BS
	Chaetoceros	PL,BL,BP,BS
	Cylindrotheca	PL
	Bellerochea	BP
	Actinocyclus	BP
	Nitzchia	BS
	Cyclotella	BS
Haptophyceae	*Isochrysis*	PL,BL,BP,ML,BS
	Pseudoisochrysis	BL,BP,ML
	dicrateria	BP
Chrysophyceae	*Monochrysis (Pavlova)*	BL,BP,BS,MR
Prasinophyceae	*Tetraselmis(Platymonas)*	PL,BL,BP,AL,BS,MR
	Pyramimonas	BL,BP
	Micromonas	BP
Cryptophyceae	*Chroomonas*	BP
	Cryptomonas	BP
	Rhodomonas	BL,BP
Cryptophyceae	*Chlamydomonas*	BL,BP,FZ,MR,BS
	Chroomonas	BP
Xanthophyceae	*Olisthodiscus*	BP
Chlorophyceae	*Carteria*	BP
	Dunaliella	BP,BS,MR
Cyanophyceae	*Spirulina*	PL,BP,BS,MR
Eustigmatophyceae	*Nannochloropsis*	FL

PL, penaeid shrimp larvae; BL, bivalve mollusc larvae; ML, freshwater prawn larvae; BP, bivalve mollusc postlarvae; AL, abalone larvae; MR, marine rotifers (*Brachionus*); BS, brine shrimp (*Artemia*); SC, saltwater copepods; FZ, freshwater zooplankton ,FL, fish larvae

The most frequently used species in commercial mariculture operations are the diatoms *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *C. calcitrans*, the flagellates *Isochrysis galbana*, *Tetraselmis suecica*, *Monochrysis lutheri* the chlorococcalean *Chlorella* spp. and eustigmatophycean, *Nannochloropsis* spp.

Nutritional value of micro-algae

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Although there are marked differences in the compositions of the micro-algal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. Expressed as percentage of dry weight, the range for the level of protein, lipid, and carbohydrate are 12-35%, 7.2-23%, and 4.6-23%, respectively.

Table 2. Concentrations of chlorophyll a, protein, carbohydrate and lipid in 16 species of micro-algae commonly used in aquaculture (modified from Brown, 1991).Source: FAO 1996

Algal class Species	Dry weight (pg.cell-1)	Chl a	Protein	Carbohydrate	Lipid
	Weight of constituent (pg.cell-1)				
Bacillariophyceae					
Chaetoceros calcitrans	11.3	0.34	3.8	0.68	1.8
Chaetoceros gracilis	74.8	0.78	9.0	2.0	5.2
Nitzchia closterium	-	-	-	-	-
Phaeodactylum tricornutum	76.7	0.41	23.0	6.4	10.7
Skeletonema costatum	52.2	0.63	13.1	2.4	5.0
Thalassiosira pseudonana	28.4	0.27	9.7	2.5	5.5
Chlorophyceae					
Dunaliella tertiolecta	99.9	1.73	20.0	12.2	15.0
Nannochloris atomus	21.4	0.080	6.4	5.0	4.5
Cryptophyceae					

Chroomonas salina	122.5	0.98	35.5	11.0	14.5
Eustigmatophyceae					
Nannochloropsis oculata	6.1	0.054	2.1	0.48	1.1
Prasinophyceae					
Tetraselmis chui	269.0	3.83	83.4	32.5	45.7
Tetraselmis suecica	168.2	1.63	52.1	20.2	16.8
Prymnesiophyceae					
Isochrysis galbana	30.5	0.30	8.8	3.9	7.0
Isochrysis aff.	29.7	0.29	6.8	1.8	5.9
Galbana(T-so)	102.3	0.86	29.7	9.1	12.3
Pavlova lutheri	93.1	0.34	24.2	6.9	11.2
Pavlova salina					

The content of highly unsaturated fatty acids (HUFA), in particular eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA), and docosahexaenoic acid (22:6n-3, DHA), is of major importance in the evaluation of the nutritional composition of an algal species to be used as food for marine organisms. Significant concentrations of EPA are present in the diatom species (*Chaetoceros calcitrans*, *C. gracilis*, *S. costatum*, *T. pseudonana*) and the prymnesiophyte *Platymonas lutheri*, whereas high concentrations of DHA are found in the prymnesiophytes (*P. lutheri*, *Isochrysis* sp.) and *Chroomonas salina*. Micro-algae can also be considered as a rich source of ascorbic acid (0.11-1.62% of dry weight). The nutritional value of micro-algae can vary considerably according to the culture conditions like media in which the micro algae are cultured. The protein content per cell, which is considered as one of the most important factors determining the nutritional value of micro-algae as feed in aquaculture, was found to be more susceptible to medium-induced variation than the other cellular constituents. Moreover, the growth of animals fed a mixture of several algal species is often superior to that obtained when feeding only one algal species. A particular algae may lack a nutrient, while another algae may contain that nutrient and lack a different one. In this way, a mixture of both algal species supplies the animals with an adequate amount of both nutrients.

Use of micro-algae in aquaculture

Micro-algae are an essential food source in the rearing of all stages of marine bivalve molluscs (clams, oysters, and scallops), the larval stages of some marine gastropods (abalone, conch), larvae of several marine fish species and penaeid shrimp, and zooplankton.

Bivalve mollusks

Intensive rearing of bivalves depend on the production of live algae, which comprises on average 30% of the operating costs in a bivalve hatchery. The relative algal requirements of the various stages of the bivalve culture process depend on whether the operation aims at the mass-production of larvae for remote setting or growing millions of seed till planting size.

Penaeid shrimp

Algae are added during the non-feeding nauplius stage so that algae are available immediately upon molting into the protozoea stage. Algal species most often used are *Tetraselmis chui*, *Chaetoceros gracilis*, and *Skeletonema costatum*. As feeding preference changes from primarily herbivorous to carnivorous during the mysis stages, the quantity of algae is reduced. Nevertheless, a background level of algae is maintained as this may stabilize water quality.

Marine fish

Apart from the requirement for micro-algae for culturing and/or enriching live prey organisms such as *Artemia* and rotifers, algae are often used directly in the tanks for rearing marine fish larvae. This "green water technique" is part of the commonly applied techniques for rearing larvae of gilthead seabream *Sparus aurata* (50,000 cells $ml^{-1}$of *Isochrysis* sp.+ 400,000 cells.ml^{-1} of *Chlorella* sp. per day), milkfish *Chanos chanos* (between 500 and 3,500 *Chlorella* cells.ml^{-1} are added from hatching till day 21), Mahimahi *Coryphaena hippurus* (200,000 cells.ml^{-1} of either *Chaetoceros gracilis*, *Tetraselmis chui*, or *Chlorella* sp.), halibut *Hippoglossus hippoglossus* (*Tetraselmis* sp.), and turbot *Scophthalmus maximus* (60,000 cells.ml^{-1} of *Tetraselmis* sp. or 130,000 cells.ml^{-1} of *I. galbana*).

The effects of the presence of micro-algae in the larval rearing tank are still not fully understood and include:

- Stabilizing the water quality in static rearing systems (remove metabolic by-products, produce oxygen)
- A direct food source through active uptake by the larvae with the polysaccharides present in the algal cell walls possibly stimulating the non-specific immune system in the larvae;
- An indirect source of nutrients for fish larvae through the live feed (i.e. by maintaining the nutritional value of the live prey organisms in the tank);
- Increasing feeding incidence by enhancing visual contrast and light dispersion, and Microbial control by algal exudates in tank water and/or larval gut.

Replacement diets for live algae

The high costs associated with algal production, the risks for contamination, and temporal variations in the algal food value still pose problems for any aquaculture operation depending on the mass-cultures of unicellular algae. In order to overcome or reduce the problems and limitations associated with algal cultures, various investigators have attempted to replace algae by using artificial diets either as a supplement or as the main food source. Different approaches are being applied to reduce the need for on-site algal production, including the use of preserved algae, micro-encapsulated diets, and yeast-based feeds.

Preserved algae:

A possible alternative to on-site algal culture could be the distribution of preserved algae that are produced at relatively low cost in a large facility under optimal climatological conditions and using the most cost-effective production systems. Centrifugation of algae into a paste form and subsequent refrigeration until required is widely applied in North America by oyster hatcheries using remote setting techniques. However, the limited shelf-life and/or the high prices of the presently available algal pastes (US$ 200 or more per kg dry weight) have discouraged many growers from using them.

Micro-encapsulated diets:

Through micro-encapsulation techniques dietary ingredients can be encapsulated within digestible capsules and delivered to suspension-feeders without losses of nutrients to the aqueous medium. Possible problems arising from the use of micro particulate feeds include settling, clumping and bacterial degradation of the particles, leaching of nutrients, and low digestibility of the cell wall material. In this regard, low susceptibility to bacterial attack and high digestibility for the filter-feeder may be conflicting requirements for a capsule wall.

Yeast-based diets:

Because of their suitable particle size and high stability in the water column yeasts can easily be removed from suspension and ingested by filter-feeding organisms. Furthermore, as opposed to most of the other alternatives to live algae, yeasts can be mass-produced at a relatively low cost. The potential of yeasts as a food in aquaculture has been proven by their successful application in the rearing of rotifers and some species of penaeid shrimp. However, a limited nutritional value of yeasts was reported for various species of filter feeders and attributed to their nutritionally deficient composition and/or undigestible cell wall. Despite this, the nutritional value and digestibility of yeast-based diets can be improved through the addition of limiting essential nutrients and the chemical treatment of the yeast cell wall, respectively. In this way, about 50% of the algae can be substituted by yeast-based diets with minimal effects on the growth of juvenile hard clam, *Mercenaria mercenaria* (Coutteau *et al.*, 1994).

Zooplankton

Rotifers

The Rotifera (="wheel bearers") are a group of tiny animals first observed by early microscopists in the late 1600s. They are relatively small group of minute, unsegmented, pseudocoelomate, aquatic invertebrates with bilateral symmetry. Around 2,000 rotifer species have been described. Most species are in the range of 0.1 to 1 mm in length, although a few species may reach 2 to 3 mm. Different rotifer species display a striking variety of body forms and the morphology of individuals may be further altered (e.g, by growth of spines) in response to ecological cues indicating the presence in the environment of particular types of prey or predators (Wallace and Snell, 1991). Most rotifers are solitary, but there are a small number of colonial (mostly sessile, i.e. attached to the substrate) species. Although there are both solitary and colonial sessile rotifers, most rotifers are motile and very active (Brusca and Brusca, 2003).

Importance in mariculture

Rotifers are best suitable for early life stages of fish and methodologies have been devised for reliable supply of rotifers in large quantities. High numbers of rotifers, easily reaching several billions, may be required each day for raising fish larvae in commercial hatcheries. The amount needed ranges from 20000 to 100000 rotifers per fish larvae during the 20 – 30 days of culture.

Table 3. Characteristics of rotifers (Stottrup and McEvoy, 2003)

Size	90 – 350 µm
Body shape	Round and flat without spines
Distribution in water column and swimming	Usually planktonic and relatively slow growing
Density	Tolerance of high densities
Salinity	Tolerance to a wide range of salinities
Supply	Manipulated and regulated; reliable depending on culture facilities
Nutritional quality	Can be manipulated and regulated
Digestibility	Lorica and eggs not digested
Transmission of parasites and predators of fish larvae	Minimal
Transmission of therapeutic agents and probiotics	Feasible

Major fish species produced today using rotifers during the early developmental stages include yellowtail, red sea bream, Asian seabass, turbot, mullet, gilthead sea bream and European sea bass (FAO, 1998). Rotifers are also used as food for culturing penaeid shrimp (Samocha et. al., 1989) and crabs (Keenan and Blackshaw, 1999). Rotifers serve as “living capsule” providing the nutrients required by the cultured marine fish larvae for proper development. Research on *B. plicatilis* and *B. rotundiformis* has increased enormously during the past three decades and these two species are the best-studied rotifers so far. Rotifers will probably maintain their role as food organism for fish larvae, in spite of attempts to replace them with more accessible formulated food.

The rotifer *Brachionus rotundiformis* can be mass cultivated in large quantities and is an important live feed in aquaculture. This rotifer is commonly offered to larvae during the first 7–30 days of exogenous feeding. Variation in prey density affects larval fish feeding rates, rations, activity, evacuation time, growth rates and growth efficiencies. *B. rotundiformis* can be supplied at the food concentrations required for meeting larval metabolic demands and yielding high survival rates. Live food may enhance the digestive processes of larval predators. Larvae are first fed on a small strain of rotifers, and as larvae increase in size, a larger strain of rotifers is introduced. Rotifers are regarded as living food capsules for transferring nutrients to fish larvae. These nutrients include highly unsaturated fatty acids (mainly 20: 5 n−3 and 22: 6 n−3) essential for survival of marine fish larvae. In addition, rotifers treated with antibiotics may promote higher survival rates. The possibility of preserving live rotifers at low temperatures or through their resting eggs has been investigated.

An investigation of body size variability among 13 strains of the rotifer *Brachionus plicatilis* was conducted under controlled laboratory conditions. Lorica lengths ranged from 123 to 292 µm and lorica widths from 114 to 199 µm. An 85% increase in lorica length was recorded as females grew from birth to adulthood. Manipulation of rotifer size by culture conditions was investigated for various salinities, diets, and temperatures. In extreme cases, diet and salinity produced a 15% and 11% change in lorica length, respectively.

An investigation of body size variability among 13 strains of the rotifer *Brachionus plicatilis* was conducted under controlled laboratory conditions. Lorica lengths ranged from 123 to 292 µm and lorica widths from 114 to 199 µm. An 85% increase in lorica length was recorded as females grew from birth to adulthood. Manipulation of rotifer size by culture conditions was investigated for various salinities, diets, and temperatures. In extreme cases, diet and salinity produced a 15% and 11% change in lorica length, respec

Copepods

Copepods are the most important group of zooplankton which forms the natural food for many fishes and invertebrates. Copepods, even the newly hatched nauplii are nutritious, rich in PUFA, DHA and EPA, in most desirable ratios (Watanabe et al., 1978, 1983; Sargent,1986; 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 (Va der Meeren, 1991; 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). 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). 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).

References

Bell, J. G., Stottrup, J. G. and Shields, R.J. 1997. Utilization of copepod diets for larviculture of halibut, cod and grouper. *Aquaculture Asia.*1998, 42-49.

Bell, J. G. 1998. Current aspects of lipid nutrition in fish farming. In: Biology of farmed fishes (K. Black and A.D. Pickering, Eds.), Sheffield, UK, Shelfield Academic Press, pp. 114-145.

Brown, M.R. 1991. The amino acid and sugar composition of 16 species of microalgae used in mariculture. *Aquaculture*, **145**: 79-99.

Coutteau, P., Hadley, N., Manzi, J. and Sorgeloos, P. 1994. Effect of algal ration and substitution of algae by manipulated yeast diets on growth of juvenile *Mercenaria mercenaria*. *Aquaculture,* **120**: 135-150.

David, A. B., 2003. Status of marine aquaculture in relation to live prey: past, present and future. In*:* Live feeds in marine aquaculture Josianne, G. S., and Lesley, A.M. (Eds.). Blackwell publishing, UK, pp. 1-16.

De Pauw, N. and Persoone, G. 1988. Micro-algae for aquaculture. In: Micro-algal Biotechnology. Borowitzka, M.A. and L.J. Borowitzka (Eds.). Cambridge University Press, Cambridge, U.K., pp. 197-221.

Doi, M., Ohno, A. and Taki, Y. 1994. Development of mixed feed state larvae of red snapper, *Lutjanus argentimaculatus*, *Suisanzoshoku* **42**: 471-476.

FAO. 1998. The State of World Fisheries and Aquaculture. Food and agriculture Organizations of the United Nations, Rome.

Fukuhara, O. 1989. A review of the culture of grouper in Japan. *Bulletin of Nansei Regional Fisheries Research Laboratory*, **20**:47-57.

Hamre, K., Moren, M., Solbakkan, J., Opstad and I., Pittman, K. 2005. The impact of nutrition on metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture* **250**: 555-565.

Keenan, C. P. and Blackshaw, A. 1999. Mud crab Aquaculture and Biology. Proceedings of International Science Forum in Darwin, Australia, 21-24 April, 1997. *ACIAR* Proceedings No. 78.

Mckinnon, A. D., Duggan, S., Nichols, P. D., Rimmer, M. A., Semmens, G. and Robino, B. 2003. The potential of tropical Paracalanid copepods as live feeds in aquaculture. *Aquaculture* **223**: 89-106.

Pederson, B. H., 1984. The intestinal evacuation rate of larval herring (*Clupea herengus* L.) predating on wild zooplankton. *Dana*, **3**: 21- 30.

Samocha, T. M., Uziel, N. and Browdy, C. L. 1989. The effect of feeding two prey organisms, nauplii of *Artemia* and rotifers, *Brachionus plicatilis* (Muller), upon survival and growth of larval marine shrimp, *Penaeus semisulcatus* (de Hann). *Aquaculture* **77**: 11-19.

Sargent, J. R. and Henderson, R. J. 1986. Lipids. In *The Biological Chemistry of Marine Copepods*, Corner, E.D.S. and O,Hara, S.C.M. (Eds.), Oxford, England: Clarendon Press. pp. 59-108.

Sargent, J. R., McEvoy, L. A. and Bell, J. G., 1997.Requirements, presentation and sources of poly unsaturated fatty acids in marine fish larval feeds. *Aquaculture* **155**: 117-128.

Stottrup J. G. and N. H. Norsker, 1997. Production and use of copepods in marine fish larviculture. *Aquaculture* **155**: 231-247.

Stottrup, J. G. 2006. A Review on the status and progress in rearing copepods for marine Larviculture. Advantages and disadvantages among Calanoid, Harpacticoid and Cyclopoid copepods. In: L. Elizabeth Cruz Suarez, Denis Rique Marie, Mireya Tapia Salazar, Martha G. Nieto Lopez, David A Villarreal Cavazos, Ana C. Puello Cruz and Armando Garcia Ortega, (Eds.). *Advances en Nutrition Acuicola VIII. VIII Symposium International de Nutrition Acuicola.* 15-17 November.

Universidad Autonomy de Nuevo Leon, Monterrey, Nuevo Leon, Mexico. pp. 62-83.

Stottrup, J. G. and McEvoy, L. A. 2003. Live Feeds in Marine Aquaculture. Ames, Iowa, USA: Iowa State Press. pp. 336.

Stottrup, J.G. 2000. The elusive copepods: their production and suitability in marine aquaculture. *Aquaculture Research* **31**: 703-711.

Vander Meeren, T. 1991. Selective feeding and prediction of food consumption in turbot larvae (*Scophthalmus maximus* L.) reared on the rotifer *Brachionus plicatilis* and natural zooplankton. *Aquaculture* **93**: 35-55.

Wallace, R. L. and Snell, T. W. 1991. *Rotifera*. In: Ecology and Classification of North American Freshwater Invertebrates (Ed. by J. H. Thorpe and A. P. Covich), Academic Press, New York. pp. 187 – 247.

Watanabe, T. and Kiron, V. 1994. Prospects in larval fish dietetics, *Aquaculture* **124**: 223-251.

Watanabe, T., Arakawa, T., Kitajima, C., Fukusho, K. and Fujita,S. 1978. Nutritional quality of living feed from the viewpoint of essential fatty acids for fish. *Bulletin Japanese Society for Scientific Fisheries* **44**:1223-1227.

Watanabe, T., Kitajima, C. and Fujita, S. 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture* **34**: 115 – 143.

Training Programme on

"Live Feed for Marine Finfish and Shell fish Culture"

17th - 22nd March 2016

Visakhapatnam Regional centre of CMFRI

Ocean View Layout

Pandurangapuram, Visakhapatnam