

Cladocera as a substitute for *Artemia* as live feed in aquaculture practices: a review

Cladocera como sustituto de *Artemia* como alimento vivo en prácticas de acuicultura: una revisión

Souraditya Chakraborty^{1, 2*}, Priyanka Halder Mallick²

¹Department of Zoology, Parimal Mitra Smriti Mahavidyalaya, Malbazar, Jalpaiguri, West Bengal, India, Pin 735221

²Department of Zoology, Vidyasagar University, Midnapore, West Bengal, India, Pin 721102

*Corresponding author: sourachak@gmail.com

ABSTRACT

Rearing larval forms of fishes is a crucial and critical step in aquaculture practices. Proper nurturing of juvenile forms is important to ensure their survival and growth. Supplementary feeds have been used since long to augment growth and productivity of fishes throughout the world. Despite, availability of a variety of advanced artificial feeds; zooplanktons have been preferred over artificial feeds for their qualities like: better digestibility, nutritional levels, preference by fish larvae and economic feasibility, especially for rearing young fin and shell fish species. Conventionally, *Artemia* sp. (brine shrimps) has been preferred among live feed organisms for rearing of fish larvae, but, applicability of cladocerans has been less explored despite their several beneficial features. Studies have reported most cladocerans species (esp., *Moina* sp. and *Daphnia* sp.) are nutritionally rich comprising of a variety of essential fatty acids, amino acids, digestive enzymes and micronutrients etc. Being an important component of trophic structure in water bodies, they form a link between different trophic levels through food chain relationships, ensuring nutrient dynamics. Their small size and jerky movements make them attractive to larval fish forms. Moreover, their cost effective culture process can be an efficient, feasible, economical and sustainable alternative to comparatively costlier and less abundant *Artemia* feed. This article endeavours to review and highlight the efficiency of cladocerans over *Artemia* feed for providing a viable and sustainable diet in commercial aquaculture practice.

Keywords: fish feed, live feed, Cladocera, *Artemia*, nutrition, zooplankton

RESUMEN

La cría de formas larvarias de peces es un paso crucial y crítico en las prácticas de acuicultura. La crianza adecuada de las formas juveniles es importante para asegurar su supervivencia y crecimiento. Los alimentos complementarios se han utilizado desde hace mucho tiempo para aumentar el crecimiento y la productividad de los peces en todo el mundo. A pesar de la disponibilidad de una variedad de alimentos artificiales avanzados, se ha preferido el zooplancton a los alimentos artificiales por sus cualidades como: mejor digestibilidad, niveles nutricionales, preferencia por larvas de peces y viabilidad económica, especialmente para la cría de especies jóvenes de aletas y mariscos. Convencionalmente, *Artemia* sp. (camarones de salmuera) se ha preferido entre los organismos de alimento vivo para la cría de larvas de peces, pero la aplicabilidad de los cladóceros se ha explorado menos a pesar de sus diversas características beneficiosas. Los estudios han informado que la mayoría de las especies de cladóceros (especialmente, *Moina* sp. Y *Daphnia* sp.) Son nutricionalmente ricas y comprenden una variedad de ácidos grasos esenciales, aminoácidos, enzimas digestivas y micronutrientes, etc. Al ser un componente importante de la estructura trófica en los cuerpos de agua, Formar un vínculo entre diferentes niveles tróficos a través de las relaciones de la cadena alimentaria, asegurando la dinámica de los nutrientes. Su pequeño tamaño y movimientos espasmódicos los hacen atractivos para las larvas de peces. Además, su proceso de cultivo rentable puede ser una alternativa eficiente, factible, económica y sostenible a los piensos de *Artemia* comparativamente más costosos y menos abundantes. Este artículo intenta revisar y resaltar la eficiencia de los cladóceros sobre el alimento de *Artemia* para proporcionar una dieta viable y sostenible en la práctica de la acuicultura comercial.

Palabras clave: alimento para peces, alimento vivo, Cladocera, *Artemia*, nutrición, zooplancton

INTRODUCTION

In aquaculture practices, rearing of larval forms is the most critical and important step in order to ensure better productivity. Successful rearing of larvae depends mainly on the availability of suitable diets enriched with essential nutrients that readily get consumed and efficiently digested (Giri *et al.*, 2002). Proper nurturing of juveniles requires food supplements with appropriate quantities of lipids, proteins, carbohydrates,

vitamins, and minerals (Watanabe and Kiron, 1994). Zooplankton, since long, have been utilised as live feed for culture of cultivable fin and shellfish species throughout the world, owing to their augmented protein, lipid, carbohydrate, vitamin, mineral, carotenoid, levels (New, 1998; Rajkumar *et al.*, 2008; Das *et al.*, 2012). In aquaculture practices, although live food is difficult to sustain and requires considerable space and expense compared to several advanced formulated diets, however, survivability was found to be higher in larvae reared with live feed organisms (Wang *et al.*, 2005). Moreover, low digestibility, feeding non-preference and nutritional quality have been the major constraints for artificial feed over live feed, especially at larval stages (Carneiro *et al.*, 2003). Live feeds comprising of planktonic organisms are routinely used as starter feed for many culturable finfish and shellfish species, mainly those which cannot be fed upon artificial feeds (Kim *et al.*, 1996). As most natural live feed organisms contain higher nutritional composition of macro and micro nutrients (*viz.*, proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids) they have been referred to as "living capsules of nutrition" (Das *et al.*, 2012; Radhakrishnan Kandathil *et al.*, 2020). Several non-algal live feed organisms including brine shrimps (*Artemia* sp.), rotifers and the freshwater cladocerans, ostracods (*Cypris* sp.), copepods and their larvae etc. are routinely used in aquaculture practices (Das *et al.*, 2012). Most of these natural live feeds can be cultured, have high reproductive potential, and the ability to attain a massive population size in short period (Neelakantan *et al.*, 1988). Rotifers are generally preferred at fry and fingerling stage due to their small mouth size and sluggish movement pattern while with growing size of larvae the food preference changes to brine shrimp, cladocerans and copepods (Velasco and Corredor, 2011; Rasdi and Qin, 2016). Although among zooplanktons, *Artemia* sp. (brine shrimp) is mostly favoured as live feed in culture of fin and shellfishes (Sorgeloos *et al.*, 2001; Rasdi and Qin, 2016); it has been revealed that Cladocerans can be preferred over others as live feed for several reasons, especially as larval feed of fin and shellfishes. Thus, the aim of this paper is to highlight the suitability of cladocerans over *Artemia* sp. (brine shrimp) as a better live feed in pisciculture practices.

NATURAL LIVE FEED IN AQUACULTURE

Natural live feed organisms are an important resource in aquaculture practices. Their capacity to move along all the columns of water, smaller size, reproducibility and better nutritional levels make them a better choice than artificial feed, especially at larval stages. These organisms are enriched with most essential micro and macro nutrients, *viz.*, essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New, 1998), thus are nutritionally balanced. However, achieving optimum growth

and survival of fish larvae is dependent on application of appropriate live feed organism at appropriate life cycle stage, both qualitatively and quantitatively (Akbari *et al.*, 2010). Despite the large scale usage of artificial feed throughout the world, natural live feed has been found to be essential for proper growth of juvenile forms owing to their higher nutritional values and acceptance (Gogoi *et al.*, 2016).

In the tropical countries, including India, natural live feeds mainly comprise of two components: algal and non-algal. Non-algal components comprise of brine shrimps (*Artemia* sp.), rotifers (eg., *Brachionus plicatilis*, *Brachionus rotundiformis*, *Keratella* sp., *Asplanchna brightwelli*, *Polyarthra vulgaris*, *Filinia opoliensis* etc.) and the freshwater cladocerans (eg., *Moina mongolica*, *Moina micrura*, *Daphnia carinata*, *Ceriodaphnia* sp.) ostracods (*Cypris* sp.), and copepods (*Mesocyclops leuckarti*, *M. hyalinus*, *Microcyclops varicans*, *Heliodiaptomus viduus* etc.) and their larvae (Palanichamy, 1996; Gogoi *et al.*, 2016; Radhakrishnan Kandathil *et al.*, 2020). Choosing appropriate live feed organism at optimum life cycle stage of fish larvae requires the consideration of the following criteria: size of the feed, gape-size of the fry or fingerling's mouth; the nutritional quality of the feed and nutritional requirement of the larvae; the feed should essentially be rich in highly unsaturated fatty acids (HUFA); feed should be perceivable and preferred by the juveniles; should be easily digestible; feed organism should reproduce fast and increase in number; and also should be sturdy and eurytolerant (Anuraj *et al.*, 2015). Although in aquaculture practices brine shrimps and copepods are successfully utilised at different life cycle stages, however, easily culturable cladocerans, owing to their jerky movement pattern, are preferred by juvenile fish groups (Akbari *et al.*, 2010; Gogoi *et al.*, 2016). Rotifers being small sized are also utilized at larval stages, however, their labour intensive culture process have been a constraint for their wide usage. Copepods are routinely used successfully in cold water fish culture (Dhont *et al.*, 2013). Thus, a comparison between two major live feed organisms *Artemia* sp. and Cladocera is pertinent to highlight their suitability in commercial aquaculture.

COMPARISON OF ARTEMIA AND CLADOCERANS AS LIVE FEED IN AQUACULTURE

Artemia sp. (Leach, 1819), commonly known as "brine shrimps" or "sea monkeys", are the most routinely used live feed in aquaculture throughout the world (Radhakrishnan Kandathil *et al.*, 2020) (Figure 1). It is a primitive arthropod, closely related to shrimp family belonging to the order – Anostraca of the class - Crustacea. Their reproduction process depends on prevailing environmental condition, either by producing nauplii (ovoviviparous mode) or by producing cysts (oviparous) (Criel and Macrae, 2002). Harvested cysts can be preserved for years and can be reutilised according to need. If hydrated in saline water, the quiescent larvae resumes arrested

metabolism and hatches out as nauplii, which is used as feed (Dhont *et al.*, 2013). Although more than 50 strains of *Artemia* sp. have been identified few species are routinely used viz., *Artemia salina* (Leach, 1819), harvested from Mediterranean area and *Artemia franciscana*, collected from North, Central and South America (Sorgeloos *et al.*, 2001; Das *et al.*, 2013; Dhont *et al.*, 2013; Radhakrishnan Kandathil *et al.*, 2020) . They are mainly found in hyper saline habitats (Dhont *et al.*, 2013). Around 90% of brine shrimps are supplied around the world from Great Salt lake, Utah, USA (Das *et al.*, 2012). However, the main disadvantage is its cost, unavailability (Akbar *et al.*, 2010) and specific nutritional deficiencies (Radhakrishnan Kandathil *et al.*, 2020).

Cladocera, generally called 'water fleas', is a superorder belonging to the class Branchiopoda, super class Crustacea and phylum Arthropoda (Smirnov, 1971). Freshwater cladocerans like *Moina mongolica*, *Moina micrura*, *Daphnia carinata*, *Daphnia lumholtz*, *Ceriodaphnia* sp. etc. are commonly used as live feed (Palanichamy, 1996) (Figure 1). Having high reproduction rates, jerky movements, wide temperature tolerance and the ability to thrive in eutrophic water and organic wastes, they qualify as an important live feed for aquaculture process (Mayer and Wahl, 1997). Inability to tolerate salinity is one of the drawbacks of cladocerans feeds (Das *et al.*, 2012). Comparison between brine shrimps and cladocerans are highlighted to discuss suitability of them as live feed in aquaculture.

GENERAL QUALITIES OF ARTEMIA AND CLADOCERANS AS LIVE FEED

Artemia sp. or brine shrimps are the most widely used non-algal live feed organism. The main advantage for their wide usage is its storage capacity, as live nauplii can be produced instantaneously "on demand" (Dhert and Sorgeloos, 1995; Sorgeloos *et al.*, 2001) from dry powder like form. In general, around 2, 00,000 to 3, 00,000 nauplii can be hatched per gram of high quality cysts (Treece *et al.*, 2000). Dormant forms of brine shrimp seeds release free swimming nauplius larvae when kept in water and sodium/calcium hypochlorite solution for standard time periods, (approximately 12-24 hours) (Anuraj *et al.*, 2015). The free swimming nauplii regain their metabolic activities and are of 0.14 mm in length on an average (Das *et al.*, 2012). All stages of life cycle of *Artemia* larvae can be used as feed for different fish species. Freshly hatched *Artemia* nauplii appear to be a better food for the larvae of *Penaeus monodon*, *P. indicus*, *P. kerathurus*, *Metapenaeus monoceros*, *M. ensis*, *M. endevouri* and *Macrobrachium rosenbergii* (Neelakantan *et al.*, 1988). Cryopreserved *Artemia* seeds can be stored for long periods and transported long distances. It has been recorded that feeding fish larvae with nauplii increases fish growth, development and survival chances (Gopalakrishnan *et al.*, 1976; Kadhar *et al.*, 2014). It has been seen that size of *Artemia* larvae varies at

different geographical locations. Indian variety of the species could not be successfully employed owing to its smaller size. Thus, in India, *Artemia* seeds have to be imported, increasing the cost of aquaculture (Das *et al.*, 2012).

Cladocerans or water fleas are abundantly found in freshwater ponds with moderate to high productivity levels. Two important genera, *Daphnia* and *Moina*, have been successfully used as live feed in culturing fish larvae since long (Alikunhi, 1952; Das *et al.*, 2012).

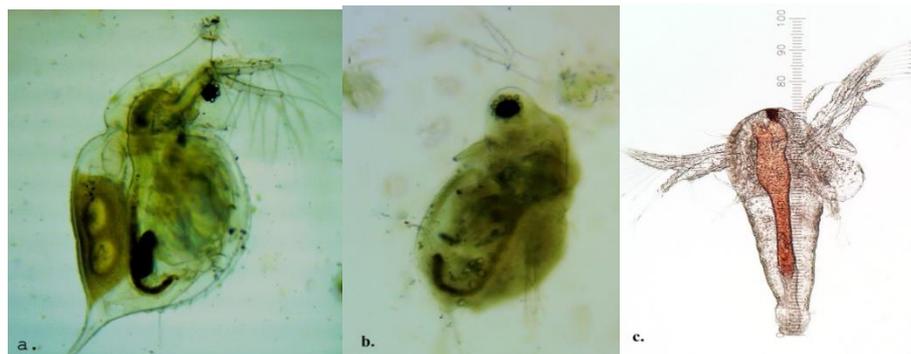


Figure 1. Live feed organisms, a. *Daphnia lumholtzi*, b. *Moina micrura*, c. *Artemia* nauplii (University of Alberta, 2018)

Use of *Moina* sp. (especially, *Moina macrocopa* and *Moina salina*) in culture of larval and juvenile stage for finfish and shellfish has been increasing worldwide owing to their rapid growth rate and nutritional quality (especially protein content) (He *et al.*, 2001; Ingram *et al.*, 2009; Peña-Aguado *et al.*, 2009; Poynton *et al.*, 2013). *Moina* sp. has been a successful replacement of *Artemia* sp. for larval feed of finfish and shellfishes (Dodson *et al.*, 2010). *Moina* sp. can be found worldwide and cultured inexpensively. *Moina micrura* is also used as supplement to artificial feed as well. They have been routinely used in hatcheries and also for ornamental fish culture (Martin *et al.*, 2003). *Daphnia* sp., on the other hand, due to their hops and jumps in water are easily predated and preferred by fish larvae. Most encountered species, *Daphnia magna* with a body length of 5mm serves as a preferred food of planktivorous fishes (Lauridsen and Lodge, 1996; Ebert *et al.*, 2005). Cladocerans, being filter feeders prey upon, nanoplankton, phytoplankton, bacteria, algae etc. while themselves being devoured by fish larvae help in recycling of nutrients and transference of energy to higher trophic level through trophic chain relationship (Gogoi *et al.*, 2014). Thus, they are an integral component for trophic dynamics. Their high fecundity, ability to reach high densities in a short span, broad level of tolerance, low productivity cost and ability to thrive in waste water makes them beneficial as a live feed organism.

NUTRITIONAL QUALITIES OF *ARTEMIA* AND CLADOCERANS AS LIVE FEED

The growth of any cultured organism depends on the nutritive value of the feed, i.e., the quantity of protein, lipid, carbohydrate, vitamins and minerals (Neelakantan *et al.*, 1988). The significant nutritional property of *Artemia* nauplii is its essential fatty acid (EFA) content, especially eicosapentaenoic acid (EPA) content. The value of EPA levels in *Artemia* varies on the biogeographic region, climatic features, basis of diet provided, from one strain to another, even between batches of a single strain, thus, estimation of (n-3) HUFA EPA need to be given priority before selecting *Artemia* nauplii as live feed for specific fishes. Otherly, nutrient enrichment of *Artemia* with EFA can be an alternative step (Sorgeloos *et al.*, 2001; Copeman *et al.*, 2002; Zakeri *et al.*, 2011; Navarro *et al.*, 2014). This may add or increase the levels of HUFAs, especially EPA (20:5n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Smith *et al.*, 2002). *Artemia* nauplii also lack long-chain PUFA and naturally low in essential HUFAs (Akbari *et al.*, 2010). Ahmadi *et al.*, (1990) reported that *Artemia* nauplii contains a good percentage of C18:3n3 and very low amount of C20:5n3 (EPA); therefore, it is considered useful for fresh water applications (Ahmadi *et al.*, 1990). The variation in total lipid and protein composition from different strains of *Artemia* sp. is reported to be due to their genetic structure or variation of their diet nutrients (Schauer *et al.*, 1980; Agh and Hosseini Ghatre, 2002, Agh and Sorgeloos, 2005). *Artemia* sp. is subjected to different natural algae populations, marine oil emulsion combinations and other supplementary diets enrich their nutrient levels (Woods, 2003; Palma *et al.*, 2011; Figueiredo, 2012). Thus, several enrichment technics (eg. microalgae, yeasts, (heterotrophically grown) bacteria, microencapsulated products, and emulsified products) are required for enhancing the nutritional quality of *Artemia* sp., making it labour intensive and costly.

Table 1: Major nutritional components of *Artemia* sp, *Moina* sp and *Daphnia* sp (Gladyshev *et al.*, 2016; Cheban *et al.*, 2017; Rocha *et al.*, 2017).

Composition	<i>Artemia</i>		<i>Moina</i>	<i>Daphnia</i>
	Nauplii	Adult		
Protein	52.2	56.4	66.33	39.68
Lipid	18.9	11.8	10.82	24.99
Carbohydrate	14.8	12.1	19.83	-
Ash	9.7	17.4	3.02	28.15

The nutritional quality of commonly used Cladoceran genus *Daphnia* and *Moina* varies depending on their life cycle stage, ingested diet, habitat type etc. In general, among Cladocerans 50 % of the body dry weight is found to be protein, while fat content

is 20-27% for adult forms (Rottmann *et al.*, 2003; Gogoi *et al.*, 2016). A comparison of essential nutrients between *Artemia* sp. and cladocerans (*Moina* sp. and *Daphnia* sp.) is depicted in Table 1. Comparison reveals *Moina* sp. contains better protein and carbohydrate level whereas, *Daphnia* sp. contains better percentage of lipid and ash content. In a study conducted on *Moina macrocopa*, fed with yeast diet, the protein content was found to be 70.87% to 76.26% (Manklinniam *et al.*, 2018). However, for commercial production *Moina* sp. cultured with *Chlorella* gives the best nutritional enrichment followed by yeast. Bogut *et al.*, (2010) in a study on *Daphnia magna* as feed of common carp reported the proportions of saturated and unsaturated fatty acids in lipids as 18.70% and 66.20%, respectively. Among the unsaturated fatty acid types, the omega-3 was recorded to be 27.30%. The ratio between omega-3 and omega-6 fatty acids ratio was 5.68:1, while protein contents amounted to 39.24% of dry weight. This satisfies the nutritional requirements of carp fishes (Bogut *et al.*, 2010). In a study conducted from samples collected from a natural reservoir the protein, carbohydrate and fat content of *Moina micrura* was found to be 20.65%, 19.6%, 8.7% respectively (Dube *et al.*, 2017). In another study by Ovie and Ovie (2006), the amino acid profile, moisture content and crude protein level was enumerated in *Moina micrura*, *Diaphanosoma excisum* and *Brachionus calyciflorus*. According to the study, a total of 17 amino acids (including 9 essential) were recorded. The crude protein levels were recorded as 52.4%, and 57.3%, and 50.3%, respectively in the three cladocerans. In another study by Tong *et al.*, (1988), the composition of essential amino acids were found to be lower in *Moina mongolica* than commonly used live feeds such as *Artemia* nauplii or fairy shrimps, however the methionine composition was higher in the former (1.5% of total amino acid content). Thus, *M. mongolica* can be a good source of methionine amino acid for fish larvae. Similarly, the content of 20:5 ω 3 (EPA) in *M. mongolica* was higher (12.7%) compared to other routine used live feeds (Tong *et al.*, 1988). A comparison between *Artemia* sp. and cladocerans (*Moina* sp. and *Daphnia* sp.) in fatty acid composition between and percentage reveals *Moina* sp. and *Daphnia* sp. contains most essential fatty acids components, which are important for fish growth in better amount compared to *Artemia* nauplii (Table 2).

Table 2: Composition and percentage of fatty acids in *Artemia* sp., *Moina* sp. and *Daphnia* sp. (Gladyshev *et al.*, 2016; Rocha *et al.*, 2017; Singh *et al.*, 2019).

Fatty acids	<i>Artemia</i> (%)	<i>Moina</i> (%)	<i>Daphnia</i> (%)
C14:0	0.47	4.25	0.19
C16:0	10.5	10.53	17.83
C16:1	1.46	21.67	3.05
C18:1	6.57	9.1	4.94
C18:1n-9	18.9	11.83	6.40
C18:1	5.34	-	3.64
C18:2n-6	5.29	2.35	12.05
C18:3n-3	31.4	20.19	26.22
C20:4n-6	0.48	2.66	1.20
C20:5n-3	2.19	3.04	0.65
C22:6n-3	0.39	1.31	0.05
C22:5	0.01	-	-

CONCLUSION

Thus, live feed organisms are an essential component for larval stage of fin and shellfish culture. The proper growth, survival and nutrition depend on the choice of live feed used at specific stages of life cycle of fishes. Live feed organisms have many beneficial features which make them a better choice over artificial feeds in aquaculture practices. Routinely used live feeds include *Artemia* nauplii, fairy shrimps, rotifers, cladocerans, copepods etc. Although, *Artemia* sp. has been the most preferred live feed organism throughout the world, but due to high cost and low availability, other suitable replacements, including cladocerans, have been studied for a considerable period. Studies suggest, cladocerans have higher nutritional levels, better economic values, better availability, high reproductive rate compared to *Artemia* nauplii. In addition to that, due to jerky movement pattern and small size of cladocerans they are preferred more by larval stages of fishes. They can be grown easily in eutrophicated water bodies and also through mass culture techniques. Levels and variety of essential fatty acids, amino acids, digestive enzymes and micronutrients are reported to be better in different cladocerans species compared to *Artemia* feed. Moreover, survivability and growth rate of frequently cultured fishes have been found to be better with cladocerans. Hence, with inclusion of some diet enrichments, like use of different oil emulsions, algae, vitamin sources etc., cladocerans can become a feasible replacement of costly *Artemia* sp. in

freshwater aquaculture practices and their usage must be encouraged at all levels. Culture and cultivation of cladocerans can thus be an economically beneficial, sustainable and worthy practice in terms of live feed supply in commercial aquaculture. Thus, more emphasis should be given to develop better mass culture and cultivation techniques of cladocerans for betterment of aquaculture throughout the world.

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