

## Potentiality of natural live food organisms in shrimp culture: A review

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### Abstract

The aquaculture industry is growing quickly due to increased fish consumption and a decline in wild fish catch. About half of the world's seafood demand for human consumption is now met by farmed seafood. The aquafeed market is expanding along with the aquaculture sector. Some of the elements in shrimp feed are derived from terrestrial plants and low-value forage fishes (fish meal). It is impossible to produce more fish meals since doing so would harm the ocean's environment and sustainability. The reduction in shrimp feed cost can also be done by introducing low-cost, environment-friendly ingredients in shrimp feed formulation. Therefore, new and ecologically friendly shrimp (*Litopenaeus vannamei*) feed component sources must be created. Live food organisms are a preferable option for this since they provide a variety of essential amino acids and beneficial triglycerides like fat, vitamins, and colors in their cell metabolites. Microalgae biomasses also represent feasible ingredients for shrimp feed sources. Their distinctive variety of bioactive chemicals can enhance color and pellet quality, act as a bulk element in shrimp feed, and boost the viability of farmed species. Live food organisms have a great economic potential since they have the highest biomass productivity of all photosynthetic organisms. In addition to giving farmers and exporters a better choice for feeding their fish, the availability of on-grown live food would also open up the prospect of improving the performance and quality of the fish and shrimp through bioencapsulation. This review study examines the possibility of generating natural food biomass as a component in shrimp feed.

**Keywords:** Aquaculture, aqua feed, *Litopenaeus vannamei*, Live food organisms, Sustainability

### INTRODUCTION

Aquaculture is the fastest-growing sector of the food industry. According to Marketwatch (2020), the global aquaculture industry is expected to be valued at US\$31.94 billion in 2019. It is predicted to expand at more than 7.1% between 2020 and 2027. Increasing human consumption and commercial acceptability are now driving the expansion of aquaculture (Magoni *et al.*, 2022). In recent years, the industry has introduced several new species. Shrimp is one of the most profita-

ble, lucrative and fastest-growing aquaculture sectors. Several Latin American and Southeast Asian nations, including Ecuador, Thailand, Vietnam, India, Indonesia, and China, accounted for most of the world's production (FAO, 2019; 2020). *Litopenaeus vannamei*, *Penaeus monodon*, *P. merguensis*, *P. chinensis*, *P. japonicus*, *P. indicus*, *P. stylostria*, and *Metapenaeus* spp. are the principal species of shrimp (Alam *et al.*, 2022). Among Penaeid shrimps, *L. vannamei* and *P. monodon* are the most cultured species, comprising around 80 percent of total farmed shrimp production (Food and

Agriculture organization, FAO 2018). In recent decades, a remarkable decline in *P. monodon* production and an increase in *L. vannamei* production have been noticed internationally (Li *et al.*, 2022). *L. vannamei*, a marine species widely cultivated worldwide, is currently the predominant species utilized in shrimp farming (Food and Agriculture organization, FAO 2020); Li *et al.*, 2022).

Farmers prefer shrimp due to its faster growth rate, export potential, resistance to various environmental conditions, tolerance to high stocking density, high meat yield, ability to grow and survive at extreme salinities (Ansari *et al.*, 2021). One of the key barriers to shrimp production is the high cost and poor quality of shrimp feed, which significantly influences shrimp cultivation. The industry's financial viability has increased as a consequence of enhanced shrimp nutrition, which has decreased feed waste. The production, lifespan, and quality of farmed *L. vannamei* are all improving due to diets high in beneficial components, including omega-3 fatty acids, antioxidants, and prebiotic compounds (Rajeev *et al.*, 2021). Fish meal has long been a key component of shrimp feed since it can be manufactured from tiny, leftover fish (Beveridge *et al.*, 2013). Due to its qualities like i) fish meal is a highly sought-after ingredient in shrimp feed, ii) the well-balanced composition and concentrations of protein, minerals, essential fatty acids, and essential amino acids, iii) low feed conversion ratio (i.e., a high percentage of feed is converted into shrimp biomass), resulting in less feed waste; and iv) excellent digestibility and palatability for shrimp, resulting in increased growth; deformities are infrequently reported or not at all (Syanya *et al.*, 2023).

In the recent ten years, the demand for fish meals, a crucial component of feed, has surged by 300% (Indexmundi, 2021). Similar price increases are being seen for other essential commodities like fish oil and soybean meal. Fish farming is anticipated to grow much more in the future (Mandal and Kundu, 2009; Tacon, 2020). Fish meal and fish oil are currently sourced from wild fish, whose harvesting is restricted and uncertain. Pelagic fish are also the source of shrimp feed, and their populations are declining due to exploitation and the El Nino effect (Bakun and Broad, 2003). As a result, several industries and researchers have already started exploring appropriate and environmentally friendly alternatives to fish meals (Table 1), soybeans, and fish oil (Jaseera *et al.*, 2021). This evaluation will quickly cover constraints of substitute sources for fish meals before focusing on natural food organisms and plant-based diet as viable feed components. Aquatic creatures seldom get their food from a single source. This is because a single source does not satisfy complete nutritional needs, including those for carbohydrates, protein, fat, minerals, and vitamins. Ingredients often used in carbs include corn, wheat, rice, maize starch, and potato starch (Guimarães *et al.*, 2019). Typical sources of plant protein are soybean

meal, peas, co-products of cane sugar, macroalgae, canola, corn gluten, potato protein, wheat gluten, guar meal (a byproduct of guar gum), wheat and cassava are two examples (Montoya-Camacho *et al.*, 2019).

The main animal-based proteins in feed include fish meal, feather meal, blood meal, animal waste, marine waste, and fish silage (Mo *et al.*, 2018). The microbiological sources of protein in fish feed are bacteria, yeast, and microalgae (Jones *et al.*, 2020). Fish oil, vegetable oil, soy oil, rapeseed oil, sunflower oil, and algal oil are a few sources of fat or lipid (Allen *et al.*, 2019). While not strictly necessary for shrimp performance, other elements including fiber, vitamins, minerals, and amino acids are important. The alternative feed components described above present a number of advantages, including being nutritive, bioactive substances, and being produced sustainably.

1. Nevertheless, employing such alternatives has some disadvantages (Table 2). For instance, the considerable availability of anti-nutritive elements and indigestible fibers in these plant-based sources is one of their main drawbacks (Kokou and Fountoulaki, 2018). Tannins, saponins, and soluble non-starch polysaccharides are examples of anti-nutritional substances. This hinders fish development and causes food waste (Mo *et al.*, 2018). Proteins derived from insects and bacteria are expensive. Several works have been published due to the rising need for substitute substances in fish feed. The environmental sustainability and commercial viability standards for substitute components must be met. The following criteria can also be utilized to create innovative fish feed (Afewerki *et al.*, 2023): i) The health advantages of shrimp eating that has been substituted out for novel feed as opposed to traditional fish meals should not be denied to humans, ii) Any alternative feed should be high in nutrients, such as omega-3 fatty acids, high in protein, with the right balance of amino acids, and be pleasant and digestible, iii) Anti nutritional elements, non-soluble carbohydrates, fiber, and heavy metals should be present in lower amounts since they impair fish development and cause waste to build up, iv) It is essential to maintain low feed conversion ratios (input/weight gained), v) Sustainability shouldn't be a problem when scaling up a newer stream, vi) The price of feed is one of the most crucial elements, vii) The new feed ought to be affordable on the market, viii) To be exempt from any policy restrictions, such as those set down for Genetically modified organisms (GMOs).

### Live food

The plant (phytoplankton) and animal (zooplankton) life forms commercially significant fish consume are considered live food organisms. Zooplankton often consumes phytoplankton (Mortoja *et al.*, 2023). Live foods can help to encourage larval feeding behavior since they can swim in the water column and are continually accessible to fish and shrimp larvae (Rahimi-Midani,

**Table 1.** Nutritional content of Alternate feed

| Feed ingredient                  | Protein (%) | Lipid (%) | Carbohydrat (%) | References              |
|----------------------------------|-------------|-----------|-----------------|-------------------------|
| <i>Anabaena cylindrica</i>       | 43–56       | 4–7       | 25–30           | Becke, (2007)           |
| Green macroalgae                 | 3.2–35.2    | 0.3–2.8   | 15–65           | Wan et al. (2019)       |
| <i>Saccharomyces cerevisiae</i>  | 50.1        | 1.8       | 4.6             | Nagappan et al. (2021)  |
| <i>Haematococcus</i>             | 30.87       | 23.07     | 37.93           | Madeira et al. (2017)   |
| <i>Spirogyra</i> sp.             | 6–20        | 11–21     | 33–64           | Becker (2007)           |
| <i>Chlorella pyrenoidosa</i>     | 57          | 2         | 26              | Becker (2007)           |
| <i>Botryococcus braunii</i>      | 39.9        | 34.4      | 18.5            | Tavakoli et al. (2021)  |
| <i>Euglena gracilis</i>          | 39–61       | 14–20     | 14–18           | Becker (2007)           |
| <i>Scenedesmus obliquus</i>      | 50–56       | 12–14     | 10–52           | Becker (2007)           |
| <i>Schizochytrium</i>            | 12.5        | 40.2      | 38.9            | Samuelsen et al. (2018) |
| Fish meal                        | 63          | 11        | –               | Hodar et al. (2020)     |
| <i>Nannochloropsis granulata</i> | 33.5        | 23.6      | 36.2            | Tibbetts et al. (2017)  |
| <i>Pavlova</i> sp.               | 24–29       | 9–14      | 6–9             | Madeira et al. (2017)   |
| Corn-gluten meal                 | 62          | 5         | 18.5            | Liu et al. (2020)       |
| Wheat meal                       | 12.2        | 2.9       | 69              | Sørensen et al. (2016)  |
| Azolla meal                      | 30          | 12        | 10              | Khushbu et al. (2022)   |

2023). Most fish and shrimp larvae in nature eat tiny phytoplankton and zooplankton (Kolbasov et al., 2023). Yet, natural fish food species are often more numerous in ponds with greenish water than in clear water (Dennis et al., 2021). The green color shows the existence of phytoplankton and other natural food species. Zooplankton is a significant component of the diet of shrimp larvae in the natural food web (He et al., 2022). Live food organisms are superior to artificial larval feeds in terms of acceptance, nutrition, and other characteristics (Kandathil Radhakrishnan et al., 2020). Many kinds of fish have various feeding habits in water bodies, but all fish need protein-rich live food to grow more quickly, reproduce more successfully, and survive (Mandal et al., 2009). Improvements in live food enrichment technology have contributed to raising the value and potential of live food organisms in the raising of larval aquatic species. The availability of large quantities of live foods organisms such as marine rotifer (*Brachionus spicaticilis* and *B. rotundiformis*) and *Artemia nauplii* to meet nutritional needs of the different stages of shrimp production (Samat et al., 2020).

Nowadays, adding microalgae (also known as "green water") to intensive culture systems together with the zooplankton is a typical method when cultivating fish and shrimp larvae (Prado-Cabrero et al., 2022). Living food creatures are sometimes referred to as "living capsules of nutrition" since they are full of nutrients including vital proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids (Radhakrishnan et al., 2022). The optimum development and survival of the young finfish and shellfish depend greatly on the timely provision of sufficient live food. It is necessary to identify and quantify the nutritional components of natural

foods to maximize output and profitability (Bwala and Omeregie, 2009). Using a variety of enrichment and encapsulation processes, the nutritional quality of living food organisms can be improved (Das et al., 2005). It is readily accepted that the production of live food organisms continues to be a very significant initial step in the intensification of aquaculture, both horizontally as well as vertically (Dhawan and Kaur, 2002).

#### Importance of live feed

The supplemented artificial feed cannot provide all the nutrients needed for shrimp growth. Thus, live food must be supplied to fish and shrimp. Fish and shrimp larvae should be provided with a nutrient-rich diet to get the most out of their growth. Although larval rearing is one of the riskiest aspects of aquaculture, it also has the potential to be one of the most lucrative endeavours (Das et al., 2012). Special planning and techniques are needed to reduce the possibility of a high mortality rate at this stage of culture. For nutrition, they require small-sized living meals. Fish and shellfish can readily digest live foods, which provide a high-protein diet (Schwepe et al., 2022). This will also boost the production cost. Yet it is simple and affordable to culture these living foods (Martínez-Córdova et al., 2015).

In contrast to zooplankton, which consists of plankters of animal origin, phytoplankton is made up of creatures that do not photosynthesize, such as bacteria and fungus as well as chlorophyll-bearing species like *Microcystis*, *Volvox*, *Eudorina*, and *Oscillatoria*. It consists primarily of planktonic crustaceans (*Artemia* sp.), Cladocerans (*Moina* sp., *Daphnia* sp., and *Ceriodaphnia* sp., etc.), Ostracoda (*Cypris* sp., *Stenocypris* sp., and *Eucypris* sp., etc.), and copepods (*Mesocyclops*

**Table 2.** Advantages and disadvantages of alternate fish feed

| Particulars              | Advantages                                                                                                  | Disadvantages                                                                                                                                                         | References                                                                                  |
|--------------------------|-------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Soybean meal (SBM)       | High protein (44% -48%)                                                                                     | Also contains anti-nutritional substances like lectin that reduced feed intake<br>Amino acids like cystine methionine, lysine, and threonine and tyrosine are limited | Goda <i>et al.</i> (2007);<br>Zhou <i>et al.</i> (2018)                                     |
| Macroalgae               | Highly nutritious and contains variety of pigments, and secondary metabolites that may benefit farmed fish. | poor digestibility and also contains excess heavy metals, anti-nutritional factors like phlorotannins, lectins, and phytic acids                                      | Hayes and Garcia-Vaquero (2016);<br>Nagappan <i>et al.</i> (2021)                           |
| Yeast                    | Rapid growth rate, can be grown on lignocellulosic wastes and contains favorable amino acids                | Production cost is high and very low in methionine and cysteine amino acids                                                                                           | Blomqvist <i>et al.</i> (2018);<br>Marques <i>et al.</i> (2004);<br>Li <i>et al.</i> (2022) |
| Microalgae and Algal oil | Rich in omega fatty acids, high in antioxidants and have probiotic effect                                   | Poor digestibility<br>High production cost                                                                                                                            | Arun <i>et al.</i> (2020);<br>Katiyar and Arora (2020);<br>Madeira <i>et al.</i> (2017)     |
| Guar meal                | Could replace soy meal                                                                                      | Contains antinutritional and anti-digestive components like saponins, phytate                                                                                         | Nidhina and Muthukumar (2015);<br>Ullah <i>et al.</i> (2016)                                |
| Wheat                    | Good digestibility<br>Low in protein and high starch content                                                | Lysine is limiting amino acids                                                                                                                                        | Sørensen <i>et al.</i> (2011)                                                               |
| Fish by-products         | Good palatability and digestibility                                                                         | Potential viruses and contaminants that are toxic to both fish may be present.                                                                                        | Alam <i>et al.</i> (2022);<br>Brunel <i>et al.</i> (2022)                                   |
| Canola meal              | High protein content                                                                                        | Low in phosphorous                                                                                                                                                    | Wickramasuriya <i>et al.</i> , 2015                                                         |

*leuckarti*, *M. hyalinus*, *Microcyclops varicans* and *Heliodiaptomus viduus*, etc.) and their larvae (Allen *et al.*, 2019). In aquaculture, microalgae are used as live feed for bivalve mollusks in all development stages, abalone, crustaceans, certain fish species, and zooplankton during their larval and early juvenile stages (Haoujar *et al.*, 2022). Over the past forty years, over a hundred different types of microalgae have been investigated as food, but probably fewer than twenty have found widespread usage in aquaculture. For microalgae to be useful as aquaculture species, they must have several essential qualities (Gomez *et al.*, 2023). Aquaculture relies on microalgae to enhance zooplankton, which is then fed to fish and shrimp larvae. They supply vital elements such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments, and sterols, passed down the food chain, energy and protein (necessary amino acids). The zooplankton groups Rotifera (rotifers) and Copepoda are two of the most prevalent (copepods) (Bwala and Omoregie, 2009). These two species are the most often utilized live feeds by aquaculturists since they are the preferred prey for shrimp and fish. The majority of marine fish require an abundant supply of zooplankton for their intense larval

development. The primary component of the diet of cultured finfish and shellfish larvae is live feeds, such as microalgae, rotifers, *Artemia*, and copepods. Compared to hard, dry prepared food, live prey with a thin exoskeleton and a high water content have a lower nutrient concentration and may be more appetizing to the larvae once taken into the mouth (Bauer, 2023). In zooplankton culture, such as rotifers and *Artemia*, microalgae are also used as a secondary food source. Commercially accessible items include live microalgae concentrates, dried microalgae, frozen and freeze-dried microalgae, microcapsules, yeasts, or diets based on yeast, bacteria, and algal pastes. Due to its size, this provides a suitable first meal after many species have exhausted their vitelline reserves. According to Lubzens and Zmora (2003), the dry weight of rotifer contains between 28% and 63% protein, 9% to 28% fat, and 10.5% to 27% carbohydrate (Dry weight). Compared to rotifers and *Artemia*, copepods and other natural zooplankton species typically produce significantly better results regarding larval survival rates, growth, and quality. In actuality, they make up the majority of shrimp larvae's diets in nature. Comparing copepods to *Artemia* and rotifers, they also have more protein and



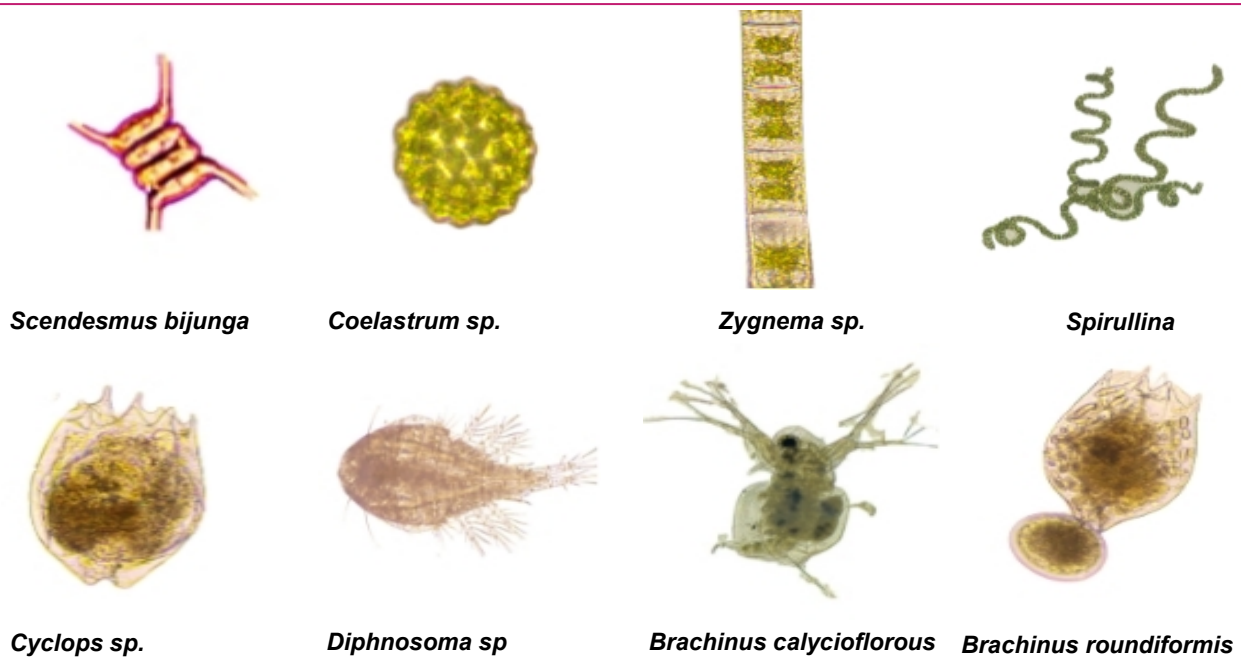


Fig. 1. Plate showing phytoplankton and zooplankton species

free amino acids. Protein and free amino acids comprised 32.7 to 53.6% and 4.3 to 8.9%, respectively, of the copepod (Mandal *et al.*, 2009).

#### Microbes

Microorganisms are also used as live food. Bacterial cells often have significant nutritional value because they include proteins, polysaccharides, and vital amino acids. In addition to aiding in the digestion and absorption of food by reducing larger food molecules to smaller ones in the stomach of larvae, the bacterium is a rich source of exogenous enzymes (Jaseera *et al.*, 2021). Additionally, bacteria can be utilized as probiotics and have been added to fish culture feed. Such probiotics allow shrimp to grow well without additional medication. *Bacillus* is the genus that most probiotics used in aquaculture (Liu *et al.*, 2020). Yeast may be directly utilized as a major food source for various larvae but is generally used as a feed for zooplankton cultivated for use in larviculture. It plays a significant role in artificial larval diets (Martínez-Córdova *et al.*, 2015). Moreover, yeast has been investigated as an additive to or a replacement for algae in the post-larval penaeid shrimp diet. In contrast to the utilization in terrestrial animals, probiotic microorganisms are also being used in fish meals (Nagappan *et al.*, 2021). In fish culture, probiotic materials have been employed as dietary supplements. Readily available packets of commercial preparations of beneficial bacteria like *Bacillus subtilis*, *B. polyriyxa*, *B. negaterium*, etc. are offered (Vázquez *et al.*, 2005; Wang, 2017). Protozoans, rotifers, and copepods are just a few of the zooplankton species that eat these bacteria. According to Yamasaki and Hirata (1990), *B. plicatilis* may thrive by consuming microorganisms like yeast and bacteria.

#### Microalgae

Algae are multicellular or unicellular plants that carry chlorophyll. They can be colonial or filamentous when multicellular. They are mostly aquatic and exhibit distinct carotenoid pigments in addition to chlorophyll, which gives them varied colors. Algae are further divided into three groups based on the makeup of their photosynthetic pigments, including Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) (Rashad and El-Chaghaby, 2020). Whereas green algae or Chlorophyta, is mostly freshwater and free-floating in nature, brown and red algae are primarily marine in nature. Brown algae contain iodine and algin, agar jelly, which is used to make ice cream and culture medium, is made from certain red algae. In freshwater and marine settings, Chlorophyta is the primary food producer and the first link in the aquatic food chain (Das *et al.*, 2012). Researchers became aware of the utilization of microalgae as a source of protein food in the middle of the 20th century. Unicellular algae are now the most expensive and least understood live food. The importance of microalgae as live feed is due in part to their nutritional qualities but also to their small size, which ranges from 5 to 25 microns and perfectly satisfies the optimal food size requirements for young aquatic creatures (Baweja and Sahoo, 2015). *Cheatoceros*, *Skeletonema*, *Scendesmus*, *Coelastrum sp*, *Zygnema sp*, *Spirulina*, *Isochrysis*, *Monochrysis*, *Tetraselmis*, *Dunaliella*, *Nannochloris*, *Chlorella*, *Brachionomonas*, and other species of microalgae are frequently cultured for larval rearing (Fig. 1). The normal composition of microalgae in the late logarithmic phase is 30–40% protein, 10–20% lipids, and 5–15% carbs (Hussain *et al.*, 2020). When used as shrimp larval food, microalgae accelerate enzymatic synthesis, the

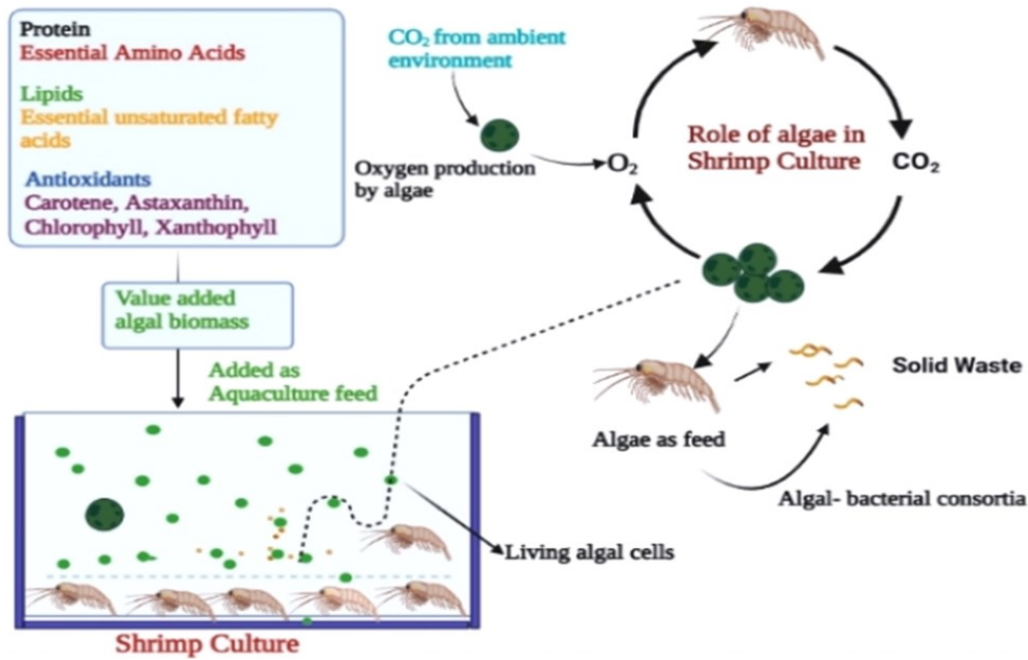


Fig. 2. Role of microalgae in shrimp culture

start of eating in young larvae, and also functions as a water conditioner (Fig. 2) by removing nitrogenous materials (Martínez-Córdova *et al.*, 2015). Other live food creatures like Rotifers, Copepods, Cladocerans, Artemia, etc. depend on microalgae as a major source of food. Today, microalgae are employed as a crucial food source for raising marine bivalve mollusks, gastropods, fish larvae, and shrimp larvae in all stages.

### Rotifers

Rotifers are sometimes known as animalcules with wheels. They are an essential category of live food organisms for use in hatcheries. The most popular type of rotifer, *Brachionus*, is an excellent beginning food for many fish and shrimp species in freshwater and marine environments (Dhert *et al.*, 2001). There is a good representation of *Brachionus* species (Brachionidae: Rotifera) in various bodies of water (Zhang *et al.*, 2023). Depending on the mouth size of the grown organisms, little (50 to 110 micron length) or giant (100 to 200 micron length) rotifers are utilized. Over 2,500 different species of rotifers have been identified in freshwater, brackish water, and ocean waters worldwide (Fontaneto *et al.*, 2008). The species most frequently utilized to feed shrimp larvae in hatcheries worldwide is *B. plicatilis*. It is a tiny, slowly swimming euryhaline species with high nutritional value. It is prolific and tolerant of various climatic conditions, adapting well to mass culture. For the mass larval rearing of several aquatic animals, the rotifer species *B. plicatilis* and *B. rotundiformis* have proved essential as a live food source (Han *et al.*, 2023). The need for rotifers is increasing due to advancements in shrimp larval-rearing techniques. Rotifers' nutritional value for fish larvae relies on their source of food. Shrimp larvae depend on highly unsatu-

rated fatty acids (HUFA) to survive and develop. For marine shrimp larvae, rotifer diets containing DHA (docosahexaenoic acid, 22:6n-3) and EPA (eicosapentaenoic acid, 20:5n-3) may be beneficial. Rotifers range from 52 to 59% protein, up to 13% fat, and 3.1% n-3 HUFA, depending on their dietary source. Several growing techniques are utilised to increase their nutritional quality, such as feeding them with various algae, baker's yeast, and artificial meals. Managing large rotifer cultures and their predictable output is a significant challenge. The main component in their mass manufacturing seems to be their food. Nowadays, the major dietary component for rotifers is often fresh baker's yeast. Various steps are taken to address the issue, including adding microalgae to baker's yeast, boosting the nutritional value of rotifers with vitamin C, treating with antibiotics to avoid bacterial infection, and using probiotics, or adding helpful bacteria to rotifer cultures (Lubzens *et al.*, 2003).

### Culture of rotifers

Stock cultures of *B. plicatilis* must be produced in order to get pure cultures. Using a scoop net with a mesh size of 50 to 100 microns, *B. plicatilis* are gathered from saltwater or brackish water bodies to begin stock culture. After gathering, they are put in a plastic bucket and transported to the lab. Add fresh, clear water comparable in salinity to the field sample water containing *B. plicatilis* to the sample to dilute it. Using a microscope, the sample is examined; whenever *B. plicatilis* is seen, it is removed with a tiny dropper and inoculated it into a 10 ml glass tube with 5 ml of water (Arun *et al.*, 2020). *B. plicatilis* chlorella was fed at a cell density of  $10 \times 10^6$  cells per milliliter or yeast at 200 ppm. Daily serially dilute the cultures in test tubes using multiple

**Table 3.** Effect of microalgae based feed on growth performance

| Shrimp Species                            | Algae species        | Replacing Pellet         | Weight gain (%) of algae based feed | Weight gain (%) of Reference | % difference between weight gain of algal and that of Reference diet | Specific growth of algae based feed | Specific growth rate of reference diet | Reference diet % difference between Specific growth rate of algal and that of reference diet | References              |
|-------------------------------------------|----------------------|--------------------------|-------------------------------------|------------------------------|----------------------------------------------------------------------|-------------------------------------|----------------------------------------|----------------------------------------------------------------------------------------------|-------------------------|
| <i>Litopenaeus vannamei</i> (Post larvae) | Schizochytrium sp.   | Fish Oil Extrusion       | 637                                 | 562                          | 13                                                                   | 2.36                                | 2.24                                   | 3                                                                                            | Allen et al. (2019)     |
| <i>Litopenaeus vannamei</i> (Juvenile)    | Aurantiochytrium sp. | Fish Oil Cold Pelletized | 293                                 | 297                          | -1                                                                   | 5.66                                | 5.38                                   | 5                                                                                            | Guimarães et al. (2019) |
| <i>Penaeus monodon</i> (Juvenile)         | Aurantiochytrium sp. | Wheat Extrusion          | 1217                                | 971                          | 25                                                                   | 9.21                                | 8.81                                   | 5                                                                                            | Jaseeraet al. (2021)    |
| <i>Litopenaeus vannamei</i> (Juvenile)    | Aurantiochytrium sp. | Fish Oil Cold pelletized | 293                                 | 297                          | -1                                                                   | 5.66                                | 5.38                                   | 5                                                                                            | Guimarães et al. (2019) |
| <i>Penaeus monodon</i> (Post larvae)      | Aurantiochytrium sp. | Wheat Extrusion          | 1217                                | 971                          | 25                                                                   | 9.21                                | 8.81                                   | 5                                                                                            | Jaseeraet al. (2021)    |
| <i>Penaeus monodon</i> (Post larvae)      | Aurantiochytrium sp. | Wheat Extrusion          | 1094                                | 971                          | 13                                                                   | 8.85                                | 8.47                                   | 4                                                                                            | Jaseeraet al. (2021)    |
| <i>Litopenaeus vannamei</i> (Post larvae) | Tetraselmis suecica  | Cold pelletized          | 16,255                              | 12,529                       | 30                                                                   | 5.56                                | 5.38                                   | 3                                                                                            | Sharawy et al. (2020)   |
| <i>Litopenaeus vannamei</i> (Juvenile)    | Schizochytrium sp.   | Fish Oil                 | 2988                                | 2869                         | 4                                                                    | 5.63                                | 5.38                                   | 5                                                                                            | Wang et al. (2017)      |

big 20 ml test tubes with 10 ml of water. Gradually raise the volume to jars or beakers with a 50 to 10 ml capacity, then 1 to 2 litres (Dhert et al., 2001). This manner produces stock cultures with 100 to 150 individuals per milliliter. These processes serve as the seed for popular culture. *B. plicatilis* is often bulk cultivated in 10 to 15 ppt salty water to achieve maximal reproduction. The continuous culture method is one of the many widely employed techniques (Lubzens,1987).

This approach involves inoculating the culture tank with chlorella after fertilizing it with ammonium sulfate (100 gm/1000 l), single super phosphate (10 g/1000 l), and urea (10 g/1000 l). *Chlorella* is re-fertilized with the same media as before once it reaches its maximal density (10 ×10<sup>6</sup> to 20 ×10<sup>6</sup> cells/ml). Baker's yeast is added at a rate of 1.0 gm/million *B. plicatilis*/day when ingests chlorella cells (Koste and Shiel, 1987). Around 25% of the culture is collected and moved to a different tank when the *B. plicatilis* population reaches 100 to 150 organisms per 1 ml. With the aid of this process, *B. plicatilis* may be supplied to hatcheries continuously. Around 25% of the water in the tank's bottom is replenished every five days with fresh water to sustain a long-term culture in the same tank (Nidhina and Muthukumar, 2015).

**Cladocerans**

Cladocerans are frequently referred to as "water fleas." *Daphnia* and *Moina*, two cladocerans, are significant as live foods. All around the world, freshwater ponds and lakes are home to *Daphnia* (Rottmann, 1992). Protease, peptidase, amylase, lipase, and even cellulose, which act as exogenous enzymes in the gut of fish and shrimp and improve their digestibility, are among the broad spectrum digestive enzymes found in *Daphnia* (Sørensen et al., 2016). Because it is bigger than moina, it is used as live food for fish at higher developmental stages. In temporary ponds and ditches, moina can be found. They are smaller than *Daphnia* and a good alternative for *Artemia* in hatcheries since they have 50–60% protein and 20–30% lipids (Ansari et al., 2021). The most popular live feed organism for feeding young postlarvae larvae used to be moina. Cladocerans benefit from rapid reproduction, a broad range of temperature tolerance, and the capacity to subsist on phytoplankton and organic waste. *Diaphanosoma celebensis* cannot survive in brackish water with a salinity of more than 3 ppt. This species is being used more and more throughout Asia. This water flea, which measures 400 to 800 micrometers, is saline-tolerant (1 to 42 ppt) and has been successfully raised in backyard hatcheries (Arora, 2020). The cladoceran species *Evandne tergestina*, *Penilia avirostris*, and *Podon polyphemoides* are also promising. In Southeast Asia, sea bass fry have been fed the cladoceran *Moina macrocopa* after being weaned from *Artemia* and before being given minced fish meat. *Moina salina*, a similar cladocera,

has been employed in fish farming. Depending on their age and the food they eat, *Moina* has a wide range of nutritional needs. *Moina* often contains 50% or more protein by dry weight. Typically, adults have more fat in their bodies than children do. The total fat content per dry weight for mature females is 20-27%, but it is 4-6% for youngsters. *Moina* used to be the most popular live food source for ornamental fish (Liu *et al.*, 2020).

### Artemia

A zooplankton, *Artemia* is often called a sea monkey or brine shrimp. The most common organism utilized as live feeding in aquaculture hatcheries is *Artemia*. It is a creature that has a close relationship with shrimp and is a member of the order Anostraca, class Crustacea, and phylum Arthropoda. The main benefit of employing *Artemia* is the ability to instantly manufacture living food from dry, storable powder, specifically from dormant *Artemia* cysts that, when submerged in saltwater, reawaken their metabolism and, within 24 hours, release free-swimming nauplii of roughly 0.4 mm length (Wickramasuriya *et al.*, 2015). Due to their accessibility and simplicity, *Artemia* naupli are the most commonly utilized food among the live diets used in fish farming. Its propensity to create cysts—dormant eggs—has led to widespread use. The Great Salt Lake in Utah produces around 90% of the brine shrimp used for commercial purposes worldwide (Becker, 2007). For every gram of high-quality cysts, between 2,000 and 3,000 naupli hatch. Cysts, naupli, juveniles, and adults from all phases of *Artemia* are employed as live feed (Wang *et al.*, 2017). Histidine, methionine, phenylalanine, and threonine are lacking in *Artemia* naupli, but all the amino acids are present in mature brine shrimp. *Artemia* is now the only diet utilized in many commercial aqua hatcheries because of its excellent nutritional value and conversion efficiency. All *Artemia* life stages—cysts (after decapsulation), nauplii, juveniles, and sub-adults are fed to livestock.

*Artemia* nauplii is almost the only food source in most commercial aqua hatcheries. Aquarists, fish breeders, and aquaculturists frequently employ frozen adult *Artemia*. Moreover, *Artemia* biomass is used to extract medicinal compounds, make food items that are high in protein, or as a food supplement for domestic cattle. In certain nations, it is even consumed by people. Because of its enormous utility, *Artemia* trade is a flourishing industry across the world (Hodar *et al.*, 2020).

### Culture of Artemia

The normal procedure (Van Stappen, 1996) calls for the hydration of cysts, decapsulation of cysts, and hatching of decapsulated cysts before *Artemia* cysts develop into nauplii. *Artemia*-hydrated cysts are decapsulated together with a chemical treatment that dissolves the shells. The hydrated cysts are stored in 5% sodium hypochlorite solution @15ml for every one gram cyst. The oxidation process begins immediately,

producing heat and raising the temperature above 40°C (Hodar *et al.*, 2020). The containers containing the hydrated cyst are set within a trough filled with chilly water to prevent embryo harm from the growing warmth. The cysts are continuously agitated with a glass rod to promote consistent cooling (Zhou *et al.*, 2018). Cysts gradually transition from a dark brown color to white when the chorion dissolves. The chorion dissolves in 5 to 10 minutes, and the decapsulated cysts are then filtered through a 100 micron screen. Once the decapsulated have been properly rinsed in freshwater, any remaining hazardous chlorine is removed. The decapsulated cysts are submerged in a sodium thiosulphate solution at a concentration of 0.1% to confirm that all chlorine has been removed. Despite the fact that *Artemia* cysts can hatch directly into nauplii, decapsulation is preferred since it increases the hatching percentage and reduces the risk of disease contamination in hatcheries. The decapsulated cysts are stocked at 0.5 to 1.0 g/L of sea water. With active aeration, the cysts are retained in suspension (Fontaneto *et al.*, 2008). Depending on the strain of *Artemia*, the quality of the cysts, and the water temperature, the cysts hatch into nauplii in between 12 and 24 hours. Temperature between 27 and 30 °C, salinity between 25 and 30 ppt, pH between 7.5 and 8.5, light intensity between 1000 and 1500 lux, and dissolved oxygen up to saturation point is the ideal environmental conditions needed for effective hatching (Han *et al.*, 2023; Das *et al.*, 2012).

### Copepods

Copepods like Cyclops and Eucyclops are typical zooplankton found in both fresh and brackish water. Almost all fish and shrimp larvae naturally prey on copepods in the wild, especially the *Copepod naupli* (van der Meeren *et al.*, 2008). Copepods are generally able to provide for the nutritional needs of larvae (Samat *et al.*, 2020). Copepods make a great first food supply for larvae because they are high in HUFAs and rich in EPA and DHA fatty acids (Dey *et al.*, 2022).

### Tubifex

*Tubifex* is a particular kind of worm belonging to the phylum annelida, class oligochaeta (Hirabayashi *et al.*, 1998). These worms typically congregate in sewer drains. For many ornamental fish brooders, *tubifex* makes an excellent food. They are abundant in amino acids and extremely necessary fatty acids. Fish fed on them can develop quickly, but they might not be as vibrant or healthy as fish with more well-balanced diets (Bhat *et al.*, 2023). According to Mahmut *et al.* (2022), *T. tubifex* had crude protein, lipid, ash, and moisture contents that were, respectively, 11.02, 0.58, 2.14, 1.83, and 18.78. Total fatty acid content was 7.28 mg/100 mg dry weight, and  $\omega$ -3 (C18:3n-3 and C20:5n-3) and  $\omega$ -6 (C18:2n-6c and C20:4n-6) fatty acids made up 18% and 22% of the total, respectively (Das *et al.*, 2012). Lysine and leucine were the most prevalent amino ac-



ids (amino acid g/100 g protein), followed by arginine (5.390.04), valine (4.920.09), threonine (4.810.09), phenylalanine (4.360.09), isoleucine (4.310.08), tyrosine (2.740.07), histidine (2.670.03), and methionine (1.82±0.04) The amount of total carotenoids in tubifex is 15.020.80 mg/kg (Beauchamp *et al.*, 2001).

### Chironomid larvae

Chironomids fall within the phylum Arthropoda, order Diptera and class Insecta. Due to the haemoglobin in their bodily fluids, they are often called "Blood worms." Chironomids are highly digestible and contain high levels of lipids, proteins, vitamins, and minerals (Das *et al.*, 2012). Numerous fish and invertebrates, like shrimp are known to eat chironomids, which are considered significant food. Many small fishes, insects, and soil worms are added to fish's diet since they are thought to satisfy their nutritional needs (Bhat *et al.*, 2023; Board *et al.*, 2017).

### Enrichment of live food organisms

The essential fatty acid content, notably that of the highly unsaturated fatty acids eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), determines the nutritional quality of live feeds for aquaculture activities (HUFA) (Yadav *et al.*, 2020). With different strategies of enrichment and bioencapsulation, great attention has been placed on improving the nutritional value of living food organisms in recent years (Balla *et al.*, 2022). By enabling them to develop for a set amount of time in a medium containing the necessary nutrients in the right amounts, the nutrients that are missing or present in insufficient amounts in food organisms can be made available (Szopa *et al.*, 2022). Several emulsified formulations and micro particle materials are employed to add vitamins, color, and vital fatty acids to these meals. Artemia's filter feeding system makes it very simple to change the biochemical makeup. Researchers (Tacon, 2020) have created a variety of enrichment products, such as unicellular algae, compound meals, micro particle diets, etc., to increase the lipid composition of both Artemia nauplii and juveniles. Many benefits and opportunities are offered by using liposomes as enrichment products. This extraordinary feat of larviculture has improved survival rates, growth rates, and disease and stress resistance. The enrichment approach is being used more frequently to intensify culture procedures and, in the future, it will aid in the beginning of commercial marine fish culture of new species (Sadeghi *et al.*, 2023). Live feed enrichment techniques may also be effective for preventative and therapeutic measures of fish and shrimp larviculture to provide medications and vaccinations to culture organisms (Zhou *et al.*, 2018). This will reduce the pricey pharmaceuticals that leak into the environment, which may otherwise harm human and animal health. In order to increase stress and disease resistance when raising larvae in hatcheries, ascorbyl palmitate, an optimal source of vitamin C sup-

plementation, can be used.

### Major problems with live food

Live food is still the most feasible method for raising larvae for aquaculture species when numerous criteria are considered (Romanova *et al.*, 2020). In contrast, it might be challenging to have an adequate amount of live feed available at the right moments in intensive culture systems (Wan *et al.*, 2019). The main barrier to producing live food organisms is its high cost, particularly in smaller hatcheries. Some major areas of concern are the difficulty obtaining pure strains and the absence of infrastructural facilities such as controlled environmental laboratories for culture upkeep (Zhou *et al.*, 2018). In order to prevent disease transmission to fish and shellfish larvae, live feed must be kept clean at all times while it is being produced. For low and medium-level farmers, the new technology of the enrichment process is a pricey business. Similarly, the high infrastructure and personnel need and the variable cost for live feed production demonstrate the necessity of designing suitable modified culture technology (Draganovic *et al.*, 2013). Although several Artemia strains exist in India, aqua hatcheries increasingly depend on imported cysts due to selection and compatibility issues with indigenous strains (Valenti *et al.*, 2021). For feeding various larval stages of fish and shellfish, the nutritional quality of the live feed organisms must be summarized. Thus, additional study efforts should be made on the compatibility of many of the existing living food species (Fontaneto *et al.*, 2008).

### Future prospects

The aquaculture industry has access to a wide variety of live food organisms. Nevertheless, new species with improved nutritional quality or growth characteristics might boost hatchery efficiency for some specialized uses for industry sectors (Detkeow and Subsoontorn, 2022) The use of microalgae either as a full or partial enrichment should be considered for improving the nutritional quality of zooplankton. Enhancing their live feed production facilities' caliber, volume, and economic viability concerns fish farmers. To enhance feed quality, many of them now supplement cultures with omega yeast, vitamins (E, D, C, and B<sub>12</sub>), marine oils or other HUFA sources, and bacteria that produce vitamin B<sub>12</sub> (Bature *et al.*, 2022). Live feeds for fish larvae are being enhanced by modifying their biochemistry via managing their diet and supplementing the cultures with microencapsulated feeds or emulsified oils. Although algae and rotifers are the most popular living foods, their usage has drawbacks and restrictions. Rotifer and copepod cultures are subject to collapse or "crash". Producers are finding new species of live food organisms better suited for specific culture situations. It is advised to co-feed live and artificial feeds to many commercial species while they are still in the larval stage. Microencapsulated diets do have one very positive attribute - they

are an alternative way to administer vaccines and therapeutic agents to larvae.

The production of live food organisms continues to be a crucial first step in aquaculture, even though the large-scale, intensive production of microalgae and rotifers is costly and frequently unreliable (Lall and Dumas, 2022). Ciliates are consumed by larval fish and crustaceans in the wild and are considered promising candidates for mass production. Ciliates are microscopic, soft-bodied, and nutrient-rich (Syanya *et al.*, 2023). Earthworms are excellent food for cultured fish species; they can be used alone and in combination with other foods. Earthworm has been discovered to be an excellent source of protein. Earthworm accelerates growth, improves sexual performance, stimulates the appetite, and makes feeds more attractive, so the animals come to feed better and waste is avoided (Pinto *et al.*, 2023). Fairy shrimp, the freshwater relatives of the more popular *Artemia*, offer interesting possibilities as live food in larval culture.. They are probably more appropriate for freshwater fish and crustacean cultures that depend on live foods. Their high carotenoid content makes them a candidate for color enhancement in ornamental fish culture (Xu *et al.*, 2023) Moreover, they are receptive to bio-encapsulation and enrichment, making them an ideal candidate for the delivery of valuable nutrients and other molecules to the young larvae. The cysts of fairy shrimps contain 45-50% protein; and 5-6% of lipids. Live fairy shrimp, both the larvae and the adults, can be fed to various organisms used for aquaculture. There is a wide scope of further research for the perfection and standardization of different cultural techniques of live food organisms.

## Conclusion

The demand for aquaculture products and aquafeed is rising globally. However, traditional fish feed that uses fish meal and soybean meal as its main ingredients is unsustainable and unable to fulfil consumer demand. Producing these feeds is resource intensive, as it means that wild-caught fish are caught for feed production. Natural live food organisms have a lot of potential to replace fish meal and soybean meal. It contains characteristics like a quicker pace of growth and the capacity to grow and create high-value goods without using fresh water and arable land. These are a source of protein, lipids, and carbs, but they also include a variety of useful substances. Cheaper alternative diets with similar nutritional quality are needed to maintain the shrimp's cost competitiveness in the global market. The industrial development of aquaculture has been hampered by the lack of suitable live feeds for feeding the shrimps at their various production stages. Here, an effort has been made to raise awareness about recent advancements in using various live food organisms in intensive fish and shellfish culture. In addition to giving farmers and exporters a better choice for feeding their

fish, the availability of on-grown live food would also open up the prospect of improving the performance and quality of the fish and shrimp through bioencapsulation.

## Declaration of Competing Interest

The authors declare they have no known competing financial interests.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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