1. Introduction

Aquaculture provides high quality food for human consumption, and the sector has been constantly growing over the few decades. The total global production of aquaculture reached 87.5 million tonnes (Mt) in 2020, up around 20% from 71.5 Mt a decade ago (FAO, 2022). This increasing trend is predicted to continue, and FAO estimates overall production reaching over 100 Mt in 2030, with both an increase in the production of freshwater and marine fish. However, sustainable development of the sector will be dependent on many factors, such as availability of quality feed ingredients and success in hatchery operations to produce enough quantity and quality of larvae and juvenile fish. Looking forward and considering the little variety in aquacultured species, the aquaculture industry is willing to expend and add new cultured species to their production. However, introducing new species and increasing the production efficiency relies on establishing a good production chain through larval rearing to broodstock management in aquaculture species.

The success of larval and juvenile production is directly affected by several factors, such as the nutritional values of live prey, the quality of formulated feed, labor, broodstock management, and the physical and chemical parameters of the culture water. The most critical issue is the utilization of high-quality live feeds during the larval period. Once marine fish larvae hatch, microalgae are applied in the larval rearing tank using a technique called the greenwater technique.
(Neori, 2011). This method allows live prey, such as rotifers, to be kept alive in the larvae tank by consuming microalgae. The application of microalgae leads to an increase in larval appetite due to the shadow effect in the water column. Another beneficial effect of adding microalgae into the larval tank is a decrease in the number of pathogenic bacteria such as *Vibrio* sp., that led to common disease outbreaks in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) cultures. This chapter summarizes the most commonly utilized live prey, including microalgae, rotifers, artemia, copepods, water fleas, sludge worms, and white worms.

### 1.1. Microalgae

Microalgae are an essential component of the aquatic food web chain in both freshwater and marine environments (Brown, 2002). They are the first feed of several zooplankton species commonly used in aquaculture, such as rotifers (*Brachionus plicatilis*) (Eryalçın, 2019), brine shrimp (*Artemia* spp.) (Turcihan et al., 2021), copepods (*Acartia clausi, Tisbe* sp., *Acartia tonsa, Apocyclops royi*) (Puello-Cruz et al., 2009; Rasdi and Qin, 2018), and water fleas (*Daphnia magna*) (Turcihan et al., 2022). All life stages of bivalve and crustacean species require microalgae due to their filter-feeding ability. Mollusc culture also relies on microalgae production and utilization during the larval culture period. For instance, microalgae are needed for high growth and survival in the production of sea cucumber (Shi et al., 2013) and sea urchin (Carboni et al., 2012).

Microalgae culture practices began in the early 1900s with the development of culture medium formulations based on the requirements of the species of interest. In recent years, microalgae have gained more attention in areas, such as biofuel production, feed ingredients (as essential nutrient sources, including lipids and proteins), wastewater treatments, and CO\textsubscript{2} emissions. The USA and China are heavily carrying out scientific investigations on microalgae (Garrido et al., 2018), and these attempts take place at the applicable industrial level. Moreover, microalgae are widely used as feed ingredients in both the terrestrial and aquatic animal feed industries (Roy and Pal, 2015; Shah et al., 2018). Some microalgae species contain a high level of protein content, such as *Spirulina platensis* (60% of dry weight) and *Chlorella vulgaris* (51-58% of dry weight). On the other hand, some are known for their high lipid contents, such as *Cryptothecodinium cohnii* (40% of dry weight) (Ganuza et al., 2008; Eryalçın et al., 2015). The accumulation of lipid droplets in microalgae mainly depends on species-specific factors and varies under various culture conditions, such as myxotrophic, phototrophic, and heterotrophic culture methods. Microalgae are rich in highly unsaturated essential fatty acids (HUFAs), such as arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), which are essential to many
aquacultured species. For instance, *Nannochloropsis gaditana* has a high EPA (Eryalçın et al., 2015) content, while *Schizochytrium* sp. has a higher amount of DHA (Eryalçın et al., 2013). Both of these microalgae can be used to supply certain fatty acid requirements for fish larvae via the use of live prey enrichments or formulated diets. Microalgae species differ in size, shape, nutrient compositions, chlorophyll contents, polysaccharides, and pigments (Figure 1). In addition, microalgal species show a variety of carotenoids and antioxidant contents. There are several carotenoids purified from microalgae, and some of them are commercially available. The most common carotenoids include astaxanthin, α-carotene, neoxanthin, cryptoxanthin, zeaxanthin, violaxanthin, and lutein. Antioxidant compounds play an important role in the prevention of free radicals and lipid oxidation in cells. Those carotenoids are widely used in feed ingredients for chicken and salmonid diets to sustain desirable egg-yolk and flesh colour, respectively. For instance, Spirulina species are rich in antioxidant compounds such as phycocyanin and alpha-tocopherol (Vitamin E). Freshwater microalgae such as *Chlorella vulgaris* are rich in lutein pigment, which has been found to enhance chicken egg-yolk colour and digestibility (Dineshbabu et al., 2019). Marine microalgae have other carotenoids, such as astaxhantine from *Haematococcus pluvialis* and b-carotene from *Dunaliella salina*, which are the most cultured microalgae for pigment production (Borowitzka, 2013). Additionally, *Porphyridium cruentum* contains a high amount of phycoerythrin and is used in feed ingredients.

Figure 1. Microalgae species; *Chlorella vulgaris* (A), *Diacronema vlkanium* (B), *Pavlova lutheri* (C) and *Euglena gracilis* (D) (original)
Toxidity, nutrition content, and size should be taken into consideration when microalgae select the species used in aquaculture. In addition, the potential for high biomass production of algae with suitable culture methods and selection is an important consideration. Some of these criteria, along with the usage proposed are summerized in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Microalgae species and products (Yu et al., 2015; Dineshabu et al., 2019; Mansour et al., 2022)</th>
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<tbody>
<tr>
<td><strong>Species</strong></td>
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<td>Feed Ingredients in Aquaculture and Poultry</td>
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<td>Bivalve</td>
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<td>Crustaceans (Artemia, Shrimp)</td>
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<td>Greenwater technique</td>
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<tr>
<td>Molluscs such as Sea cucumber, Abalone, Sea urchins</td>
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<td>Biotechnology &amp; Biofuels</td>
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Moreover, microalgae are promising ingredients for the fish feed industry. Aquaculture is still a growing sector; however, sustainability is a major issue due to the difficulties of supplying raw materials such as fishmeal and fish oil for the production of fish feed. Therefore, there is an increasing number of research projects to replace fishmeal and fish oil with microalgae biomass (Eryalçın et al., 2013; Eryalçın et al., 2015). There are several companies and institutions around the world that produce a variety of microalgae species (Table 2). Currently, in Türkiye, there is only one company, MarinBio, which is located in the west part of Türkiye in Denizli province.

<table>
<thead>
<tr>
<th>Company name</th>
<th>Country</th>
<th>Microalgae species</th>
<th>Purpose</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algaenergy</td>
<td>Spain</td>
<td>Nannochloropsis gaditana, Isochrysis galbana, Tetraselmis suecica, Chlorella vulgaris</td>
<td>Feed Ingredients</td>
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<tr>
<td>Algaspring</td>
<td>Netherlands</td>
<td>Nannochloropsis sp.</td>
<td>Rotifer and Artemia</td>
<td>Nannostar BLUE, Nannostar GREEN, Nannostar RED</td>
</tr>
<tr>
<td>Aquafauna Biomarine Inc.</td>
<td>USA</td>
<td>Schizochytrium sp.</td>
<td>Rotifer</td>
<td>AlgaMac 3050 flake, Red Algamac (for Rotifers), AlgaMac ProteinPlus, AlgaMac Enrich (DHA and Astaxanthin rich)</td>
</tr>
<tr>
<td>BernAqua</td>
<td>Belgium</td>
<td>Spirulina sp., Thalassiosira weissflogii, Thalassiosira pseudonana, Tetraselmis sp.</td>
<td>Fish and Shrimps</td>
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<tr>
<td>BioMar</td>
<td>Norway</td>
<td>-</td>
<td>Fish</td>
<td>AlgaPrime DHA</td>
</tr>
<tr>
<td>Blue Biotech</td>
<td>Germany</td>
<td>Nannochloropsis sp., Isochrysis sp., Haematococcus pluvialis</td>
<td>Astaxanthin Fish coloration</td>
<td></td>
</tr>
<tr>
<td>Corbion</td>
<td>USA</td>
<td>Schizochytrium sp.</td>
<td>Feed Ingredients</td>
<td>AlgaPrime™ DHA</td>
</tr>
<tr>
<td>Cyanotech</td>
<td>USA</td>
<td>Haematococcus pluvialis</td>
<td>Feed Ingredients</td>
<td></td>
</tr>
<tr>
<td>Heliae</td>
<td>USA</td>
<td>-</td>
<td></td>
<td>Nymega</td>
</tr>
<tr>
<td>Innovative aquaculture</td>
<td>Canada</td>
<td>Tahitian Isochrysis, Isochrysis galbana, Pavlova lutheri, Nannochloropsis oculata</td>
<td></td>
<td>IAP Algae Paste</td>
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<tr>
<td>June Spirulina</td>
<td>Myanmar</td>
<td>Spirulina sp.</td>
<td></td>
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<tr>
<td>Mera Pharmaceuticals</td>
<td>USA</td>
<td>-</td>
<td>Astaxanthin for Shrimp and Salmon</td>
<td>AquaXan®</td>
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</tbody>
</table>
Optimal culture methods are crucial for biomass production. Microalgae are mostly cultured in a phototrophic way, where sunlight is used as the primary energy source. Phototrophic culture is the most selected culture method, and it can be set up indoors and outdoors. Higher biomass can be produced in a raceway outdoor system; however, within this method, biomass gain can be limited due to several factors, such as environmental and contamination problems. A photobioreactor system is a good option for mass production because it allows high biomass production in outdoor areas (Figure 2).

Table 2. Continue

<table>
<thead>
<tr>
<th>Company name</th>
<th>Country</th>
<th>Microalgae species</th>
<th>Purpose</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pacific Bio.</td>
<td>Australia</td>
<td><em>H. pluvialis</em></td>
<td>Astaxanthin for Shrimp and Salmon</td>
<td>ReefAsta™</td>
</tr>
<tr>
<td>Pentair Aquatic Eco-Systems</td>
<td>USA</td>
<td><em>Spirulina sp.</em>, <em>Pavlova sp.</em>, <em>Isochrysis sp.</em>, <em>Thalassiosira sp.</em>, <em>Tetraselmis sp.</em>, <em>Nannochloropsis sp.</em></td>
<td>Feed Ingredients</td>
<td>Hikari® Algae Wafers Spirulina Flake Phyto Feast® Fish Food</td>
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<td>Phytobloom</td>
<td>Portugal</td>
<td><em>Nannochloropsis sp.</em>, <em>Isochrysis sp.</em>, <em>Phaeodactylum sp.</em>, <em>Tetraselmis sp.</em></td>
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<tr>
<td>Reed Mariculture</td>
<td>USA</td>
<td><em>Nannochloropsis sp.</em>, <em>Tetraselmis sp.</em>, <em>Isochrysis sp.</em>, <em>Pavlova sp.</em>, <em>Thalassiosira weissflogii Thalassiosira pseudonana</em></td>
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<tr>
<td>Skretting</td>
<td>Norway</td>
<td></td>
<td>Artemia Fish larvae</td>
<td>ORI N-3 (for <em>Artemia</em>) NEPTUNE (for Green water culture) CLEAN Start (for fish larvae)</td>
</tr>
<tr>
<td>TaiwanChlorella</td>
<td>Taiwan</td>
<td><em>Chlorella sp.</em></td>
<td>Biomass</td>
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<tr>
<td>Tomalgae</td>
<td>Belgium</td>
<td></td>
<td>Feed Ingredients</td>
<td>Thalapure™ Phylavive</td>
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<tr>
<td>MarinBio</td>
<td>Türkiye</td>
<td><em>Schizochytrium sp.</em>, <em>Chlorella sp.</em></td>
<td></td>
<td>AlgomeDHA™ AlgomeGrow™ AlgomeDHA Oil TM</td>
</tr>
</tbody>
</table>
Recently, more studies have focused on heterotrophic culture methods for some microalgae species, such as *Chlorella sorokiniana*, *Chlorella vulgaris*, *Chlorella protothecoides*, *Chlorella zofingiensis*, *Schizochytrium* sp., *Cryptecodinium cohnii*, *Scenedesmus* sp., *Spirulina platensis*, *Isochrysis galbana*, *Euglena gracilis*, *Tetraselmis suecica*, *Nannochloropsis oculata*, *Dunaliella* sp., *Nitzschia laevis*, and *Phaeodactylum tricornatum* (Perez-Garcia et al., 2011; Morales-Sánchez et al., 2017; Hu et al., 2018). These microalgae can be cultured using different organic carbon sources, such as glucose, acetic acid, glycerol, acetate, and ethanol, together with CO$_2$ utilization and without a light source under sterile conditions. In this culture method, cells can accumulate higher lipid molecules than in phototrophic cultivation and are ideal for industrial fermentors and bioreactors. However, despite the high biomass gain, this cultivation method can be suitable for only some microalgal species. Therefore, the selection of microalgal species is very important. Moreover, the expenditures on energy and basic nutrient sources are limiting factors in this technology. For any purpose, microalgal species should be maintained and kept under sterilized conditions, which is called a “pure culture room”. In this way, algae are periodically cultured in petri dishes and test tubes (Figure 3). Sub-cultures are visually and microscopically controlled before up-scaling production is started.
In conclusion, there is still a high demand for microalgae biomass, not only for live prey culture and feed ingredients in aquaculture but also for feed ingredients for poultry and terrestrial animals as a source of protein and lipids. Therefore, in addition to phototrophic production, mixotrophic and heterotrophic cultures of microalgae species are promising production methods in the future for achieving high biomass gain. Moreover, the isolation of new microalgae species and their adaptation into culture systems are also important for the sustainable development of algae culture and biomass production.

1.2. Rotifer culture

Rotifers are the first live prey and are indispensable for fish larval culture. Due to their suitable size, all fish hatcheries should start to feed larvae with this live prey in larval production. Therefore, obtaining this live prey is very critical and vital in marine fish hatcheries. There are several types of rotifers that differ in size, such as *Proales similis* (lorica length 85 µm), SS-type (*Brachionus rotundiformis*; lorica length 100-140 µm), and L-type (*Brachionus plicatilis*; lorica length 250 µm).
The success of rotifer culture mainly depends on the feed, chemical and physical water parameters, and labor capability in hatcheries. Rotifer numbers can be increased in a couple of days under intensive culture conditions by feeding them with baker’s yeast and fresh microalgae (Eryalçın, 2019). However, rotifers lack essential nutrients such as polyunsaturated fatty acids (PUFA), essential amino acids (EAA), vitamins, and minerals (Hamre, 2016). Therefore, the nutritional composition of rotifers should be developed by the enrichment process. This process allows rotifers to be enriched with essential nutrients in a short time before being fed to fish larvae (Eryalçın, 2018). However, there are still some gaps in the cultivation and optimization of the culture process. The main bottleneck to culture success is the nutritional value of feed properties. There are various rotifer diets and enrichment products that vary in nutritional composition. This reality makes the rotifer culture fragile, and hatcheries should develop themselves according to their experiences. In the culture process of rotifers, all rotifers should be female individuals due to the high numbers of rotifers required in larval production. Each female may carry between one and four eggs. Environmental conditions, once broken down or sudden changes in some water parameters, can lead to rotifers sexual reproduction between male and female rotifers, and finally resting eggs occur (Figure 5). Marine fish hatcheries can maintain and survive a small amount of rotifer culture to keep rotifers available for the next production season.
The top priority at commercial marine fish hatcheries is to increasing the number of rotifers in a short period of time is first priority at commercial marine fish hatcheries. However, once reaching a sufficient amount of rotifers, it is important to investigate their nutritional properties before using them to feed fish larvae. Baker’s yeast is the most common diet used for feeding rotifers, but it lacks essential nutrients, which can have a negative impact on the nutritional value of the rotifers (Hamre, 2016; Eryalçın, 2018). Therefore, the selection of suitable microalgae is essential to delivering nutrients to rotifers via microalgae. While fresh microalgae can be difficult to manipulate and culture, commercial products are widely used for feed and enrichment processes, alongside fresh microalgae.

Enrichment products and application methods, including intervals and usage time, play a vital role in the accumulation of essential nutrients in rotifers. The protocols for enrichment differ among hatcheries. Additionally, cultivation type is another important factor that affects rotifer culture performance and nutritional value. Batch, semi-continuous, and high-density culture methods are applied depending on the hatchery’s facilities procedure, tank shape and volumes, and the types of feed used in rotifer production. In the batch culture, harvested rotifers are separated into two parts: one part is used for new inoculation to continue the rotifer culture, and the other part is used to feed fish species. Commercial feeds are added to the

**Figure 5.** Life cycle of Rotifer (*Brachionus plicatilis*) (Lavens and Sorgeloos, 1996)
culture tank for a short time, typically 3-4 days. Semi-continuous culture differs from batch culture in that it involves periodic harvesting and washing of the rotifer cultures, which can take longer to complete. Another culture method is high-density culture, which involves the application of concentrated microalgal biomass. However, maintaining water quality and stability is a major challenge with this method. To address these issues, protein skimmers, filtering, and partial water renewals can be applied in the high-density culture method (Yoshimatsu and Hossain, 2014).

Continuous rotifer is essential in marine fish hatcheries, whether during periods of intense culture or the drying season. Rotifer feeds are also crucial, and companies can either produce them themselves via microalgal production or obtain them from commercial companies. When introducing new rotifer diets, it is important to try them out in small amounts before implementing them on a large scale. This helps to ensure that the new diets are suitable and effective before investing significant resources in their production and use.

1.3. Artemia culture

Artemia is a filter-feeding crustacean brine shrimp that is found in salt lakes throughout the world (Madkour et al., 2022). It was discovered in the early 1970s for its high nutritional value and ease of hatching cysts, making it a valuable resource for fish culture purposes (Figure 6) (Sorgeloos et al., 1977). In these years, Artemia nauplii has been widely used in the cultivation of marine and freshwater larvae and juvenile fish (Sorgeloos et al., 2001). Although artemia is rich in protein, it contains fewer lipids. Therefore, Artemia nauplii should be enriched before being fed to fish larvae, just like rotifers.

Dried cysts are the inactive embryos of Artemia in the late gastrula stage. These cysts are obtained from nature using plankton nets, then dried and packed (Figure 7). However, collecting these cysts is not sustainable for further live prey utilization in aquaculture. The hatching rate of Artemia cysts varies depending on their geographic origin. Currently, artemia
cysts are mostly obtained from Salt Lake, Utah, USA, followed by China, Russia, Kazakhstan, Uzbekistan, Vietnam, Thailand, Argentina, and Brazil, respectively (Litvinenko et al., 2015).

Fish production hatcheries require live prey that is similar in size to their natural prey, which is typically larger than 1 mm in total length, such as copepods, for feeding to larval fish. Recent studies have focused on improving the production performance of Artemia through the use of freshly cultured microalgae or commercial diets. Artemia metanauplii can reach to 2–3 mm length and has a high nutritional value, making it essential for the nutrition of newly cultured aquatic organisms. For example, *Artemia franciscana* metanauplii is an appropriate size for seahorse (Vite-Garcia et al., 2014), clownfish (Chen et al., 2020), anemones, crustaceans (Nelson et al., 2002), fish (Lim et al., 2001), soft corals (Tsounis et al., 2010), and for use in cephalopod culture (Guinot et al., 2013). Therefore, larger sizes of *Artemia* spp. are also needed for feeding new marine and freshwater larvae and juveniles.

*Artemia* spp. is a fast-growing brine shrimp that primarily feeds on small particles such as bacteria (Toi et al., 2013), microalgae (Zhukova et al., 1998), and organic matter in the water column (Maldonado Montiel et al., 2003). Among these, microalgae are the primary food source for *Artemia* spp. and are essential for its efficient growth (Turcihan et al., 2021).

The limited availability of *Artemia* cysts is a significant challenge for sustainability in larval feeding, which is why alternative live prey options must be explored for both freshwater and marine environments. Türkiye has several *Artemia* sources located in different regions, with İzmir Çamaltı (Saygi, 2004) and Gökçeada (Eskandari, 2014) being the most well-known salt lakes that provide *Artemia* cysts. However, to improve the efficiency and sustainability of *Artemia* production, further research is needed to optimize these sources.
1.4. Copepod culture

The primary live prey for marine fish larvae during their initial stages of development are rotifers (*Brachionus plicatilis*) and *Artemia* spp., which are suitable for their small mouth sizes. For the first 20–30 days after hatching, marine fish larvae require live prey to develop properly because their digestive enzymes are insufficient and their digestive systems are not fully developed (Kolkovski et al., 1997; Kolkovski, 2001). However, in terms of nutritional value, rotifers and *Artemia* spp. are nutritionally inadequate compared to copepods, which are the primary natural live prey for marine fish larvae in their natural ecosystems. Copepods contain higher levels of essential amino acids, fatty acids, vitamins, and minerals (Rasdi and Qin, 2016). For fast-growing species like grouper, successful larval culture requires the use of copepods as the sole live prey or co-feeding with *Artemia* spp. (Burgess et al., 2020; Ranjan et al., 2022).

Recent efforts have been made to isolate local copepods from the Marmara Sea, including *Acartia clausi*, *Penilia avirostris*, and *Paracalanus parvus*, which were successfully cultured under laboratory conditions by feeding them with different microalgae, such as *Chlorella vulgaris*, *Rhodomonas* spp., *Rhinomonas reticulata*, *Isochrysis galbana*, and *Thlassiosira pseudonana*. Researchers investigated the effects of these microalgae on the survival rates and fatty acid composition of the cultured copepods. The results showed that a combination of *Chlorella vulgaris* and *Rhodomonas* species resulted in the highest survival rate for *Acartia clausi* (Eryalçın et al., 2022, unpublished data) (Figure 8). The utilization of *Isochrysis galbana* as a feed source resulted in a higher accumulation of n-3 HUFA in *Acartia clausi* compared to other microalgal species. It is evident that the survival rates and growth parameters of copepod species vary depending on the species of copepod and the specific microalgal used as feed.

![Figure 8. Acartia clausi (A), Paracalanus parvus (B), Penilia avirostris (10X; Leica DM1000) (original)](image)

In recent years, there has been an increase in studies on culture techniques, optimization of culture conditions, and feeding of copepods (Chintada et al., 2022). For instance, when *Acartia ohtsukai* was cultured at different salinities and temperatures below 10 °C, low survival
rates were observed, but survival rates were not affected by a wide range of salinities (Choi et al., 2021). In another study, the effects of different temperatures on the egg productivity of the calanoid copepod *Acartia amboinensis* were examined, and an optimum temperature of 27 ºC was determined for this species; when the culture temperature was increased to 30 ºC and 33 ºC, egg production decreased (El-Sherbiny and Al-Aidaroos, 2021). Copepod eggs are currently sold by commercial companies for marine fish larval production, and the collection and storage of these eggs require experienced labor and equipment. Several studies have been carried out to optimize egg collection, such as examining the effects of different salinities and storage temperatures on the hatching and survival of eggs in *Acartia sinjiensis*, which showed that eggs could be stored for up to 180 days at 4°C and 1 ºC (Choi et al., 2022). From these findings, it is clear that optimizing the culture temperature is crucial for successful copepod culture performance.

The aquaculture sector requires large numbers of live prey with essential nutrients, and the focus of large-scale copepod cultivation is on calanoid species due to their high egg production and culture success. However, the main challenges in this effort are the high demand for fresh microalgae and the optimization of environmental conditions such as pH, dissolved oxygen, salinity, temperature, and water exchange rate selected for the cultured copepod species (Sarkisian et al., 2019). Egg productivity is a key factor in managing high copepod biomass under controlled conditions. However, some copepod species, such as *Acartia tonsa*, exhibit low hatching rates, which are attributed to the effects of temperature and salinity. It has been observed that hatching rates of this species decreased after 8 weeks of egg storage, with varying hatching rates at a salinity of 30 ppt at a temperature of 18 ºC (Torres et al., 2021). Despite studies on culture parameters, research on the nutritional requirements of copepods is scarce in both the scientific and aquaculture areas.

In a feeding experiment on *Gladioferens imparipes*, it was observed that copepods fed with the microalgae *Isochrysis galbana* exhibited increased egg productivity. It is well-known that survival rates and fatty acid contents directly correlated with nutrient concentrations (El-Tohamy et al., 2021). The cultivation period of *Tisbe sp.* and *Apocyclops sp.* showed that fatty acid profiles are affected by time, and it was concluded that the long-term culture of copepods increased their total fatty acids by storing more lipid (Alejos-Cabrera et al., 2022). The amount of lipid storage in copepods is known to be directly related to the feed they consume. The fatty acid composition of *Apocyclops royi* and *Pseudomonas annandalei* fed with *Dunaliella tertiolecta*, *Rhodomonas salina*, and baker’s yeast was investigated. According to this study, the level of EPA was enhanced by the microalgae *Rhodomonas salina* compared
to baker’s yeast. The same study showed that *Pseudomonas annandalei* fed with *Dunaliella tertiolecta* exhibited a high level of another important fatty acid, ARA (Nielsen et al., 2020).

It is possible that there is a cross-effect between microalgal diets and copepods, as some algae contain specific long-chain fatty acids such as ARA, EPA, and DHA, which are essential nutrients for copepods. Additionally, selecting the appropriate copepod species for cultivation can also be challenging, as each species may have different preferences for certain algae and nutrients. To address this issue, several studies are being conducted to investigate the effects of different microalgal species alone or in combination. For instance, the effect of three microalgal species, *Rhodomonas salina*, *Tisochrysis lutea*, and *Pavlova lutheri*, on *Paracyclopinana nana* culture was examined, and it was found that *Rhodomonas salina* was the best diet for this copepod species (Dayras et al., 2021). In another study, the copepod *A. bilobata* was fed solely or in combination with *Isochrysis galbana*, *Chaetoceros muelleri*, and *Nannochloropsis oculata*, and copepods fed with *I. galbana* showed increased egg production, hatching rate, and adult individuals (Chintada et al., 2022). These studies have provided valuable data on copepod culture. Currently, copepod eggs are available commercially from some institutes and companies worldwide (https://algova.com/en/Copepod-Eggs-Cysts-Acartia-tonsa-Starter-Feed-for-Fish-Larvae/COP0025M). In Türkiye, copepod culture production at an industrial level is still not common. However, some new marine species, such as the white grouper (*Epinephelus aeneus*), require a copepod mixture for their larval stage nutrition. Therefore, in order to contribute to the aquaculture sector with new species, copepod culture should be successfully managed and applied.

### 1.5. Water flea culture

Water fleas are freshwater cladoceran that are widely distributed in all freshwater ecosystems (Yıldız et al., 2022). They are the main target diets of fish, birds, and turtles in lakes and rainwater reservoirs (Cox et al., 2018). *Daphnia pulex* and *Daphnia magna* are the most common species that are widely used for aquaculture purposes (Ashforth and Yan, 2008; Turcihan et al., 2022) (Figure 9). Daphnia species are not only used for aquaculture but also for wastewater treatments (Ra et al., 2008), ecotoxicology, ecology (Ebert, 2022), and evolutionary biology studies (Stollewerk, 2010).
The culture performance of Cladocerans is strongly related to diet quality, physical and chemical environmental conditions. In their natural habitat, they consume microalgae without selection. Daphnia individuals can convert organic material consumed by microalgal production from ponds and wastewaters (da Silva Campos et al., 2020). Recently, it has been shown that these cladocerans are unable to convert long-chain essential fatty acids. However, daphnia can accumulate essential nutrients through their feeds, such as fatty acids, amino acids, and minerals. For instance, the levels of oleic acid, Σ n-9, and Σ MUFAs in Daphnia biomass were correlated with their diets (Turcihan et al., 2022). Moreover, some microalgal-based powder diets can also improve Daphnia’s nutritional components, including not only fatty acids but also essential amino acids (Zeybek and Eryalçın, unpublished data 2023).

Daphnia individuals are of suitable size for the first feeding of fish larval nutrition. Recent studies have revealed that daphnia can be a substitute for Artemia, which is a very limited source in nature worldwide (Chakraborty and Mallick, 2023). Due to their high protein content, Daphnia biomass can also be utilized in the form of powder as a dietary supplement in carp (Abdel-Tawwab et al., 2020; Bogut et al., 2010; Suantika et al., 2016), barramundi (Chiu et al., 2015), grey mullet (Abo-Taleb et al., 2021), and kuruma shrimp (Mona et al., 2017) diets. Therefore, the successful culture of Daphnia is more important than ever before. Growth performance in Daphnia culture is evaluated by several parameters. First of all, the Daphnia stock culture should survive and produce some females that carry eggs for future production. Sudden and extreme changes from optimum levels of culture water conditions, such as pH (optimum pH:}
6–7), oxygen (O\textsubscript{2} > 5.5 ppm), minerals (Ca\textsuperscript{2+} is essential for the exoskeleton), and temperatures (20–22 °C), can lead to Daphnia creating resting eggs called epiphia. This event will affect the total Daphnid production in a closed environment. Water fleas can be cultured with various microalgae with gentle aeration and daily water renovation. They reproduce parthenogenetically when physical and chemical conditions are optimal, and diets are available (Figure 10).

![Daphnia life cycle](image)

**Figure 10.** Daphnia life cycle (Ebert, 2005)

There is still a high demand for Daphnia culture, with large production for both aquarium and freshwater fish feeding. Moreover, recent studies have revealed that water flea species can be used as feed ingredients in formulated microdiets. Therefore, large-scale production techniques should be applied, and the private sector should produce water fleas to meet the demand for aquaculture.

### 1.6. Worms

The Food and Agriculture Organization (FAO) has predicted that by 2025, 1.8 billion people in countries worldwide will experience water scarcity (Van Huis et al., 2013). Therefore, the potential use of insects as a sustainable protein source for the rapidly growing population has been the subject of increasing debate. Edible insects, in particular, have gained attention due to their low requirements for feed, land, and water compared to traditional sources of protein. Furthermore, insects emit less CO\textsubscript{2} and greenhouse gases than cattle and small livestock, have a high feed conversion efficiency, and are easy to store on a large scale (Premalatha et al., 2011; Van Huis et al., 2013; Dobermann et al., 2017).
In recent years, there has been a growing interest in exploring alternative protein sources to meet the increasing demand for food in a sustainable and environmentally friendly manner (Rumpold and Schlüter, 2013). Yellow mealworms, in particular, have been shown to be effective in the biological transformation of organic waste, and they can convert about 1.3 billion tons of bio-waste annually (Veldkamp et al., 2012). *Tenebrio molitor* larvae exhibit a significant abundance of protein, fat, and indispensable amino acids, endowing them with great potential as a nutritional protein source for human consumption as well as animal feed. In addition, the cultivation of mealworms requires less water and land compared to traditional livestock production, and their waste products can be used as fertilizer (Lundy and Parrella, 2015). Another nutrient source, *Zophobas morio*, has been shown to consume and biodegrade plastics, such as polystyrene or polyethylene, and may be effective in waste management (Rumbos and Athanassiou, 2021). Overall, *Zophobas morio* and *Tenebrio molitor* have significant potential as sustainable sources of protein and as a means of reducing food waste and plastic pollution. However, further research is needed to fully explore their potential and to develop efficient and cost-effective methods for their cultivation and use.

Oligochaetes worms are considered one of the most cost-effective live feeds for fish and prawns (Marian and Pandian, 1984). *Enchytraeus albidus* is a small, white, soil-dwelling worm that is commonly used as a model organism in ecotoxicology studies due to its sensitivity to environmental pollutants (Schmelz, 2003; Spurgeon, 2010). In addition, it has been shown to play an important role in soil processes such as nutrient cycling, decomposition, and soil structure formation (Nielsen et al., 2020). *Tubifex tubifex*, on the other hand, is a freshwater oligochaete worm that is widely used as a live food source for fish and other aquatic organisms in aquaculture (Marian and Pandian, 1984; Hossain et al., 2012). It is a hardy and easy-to-rear species that can be cultured on a variety of organic substrates, making it an economical and sustainable alternative to conventional artificial feeds (Phillips and Buhler, 1979; Mollah et al., 2009). However, it has been found that *T. tubifex* from polluted waters can harbor human pathogens responsible for diseases such as hepatitis (Jewel et al., 2016), highlighting the importance of developing pollution-free culture technologies. Therefore, *Enchytraeus albidus* and *Tubifex tubifex* are two species of oligochaete worms that have significant ecological and practical importance. Studying their biology and ecology can provide valuable insights into the functioning of the soil and aquatic ecosystems, while their practical applications in ecotoxicology and aquaculture can have economic and environmental benefits.
1.6.1. Sludge worm (*Tubifex tubifex* Muller, 1774)

*Tubifex tubifex* is an aquatic oligochaete species classified in the Animalia kingdom, Clitellata class, and Tubificidae family according to the taxonomic hierarchy (*Tubifex tubifex* Müller, 1774) (Lucan-Bouché et al., 1999; Kolesnyk et al., 2019) (Figure 11). It is a benthic organism found in freshwater ecosystems worldwide, particularly in sediment-rich habitats such as rivers, streams, and lakes. It is also commonly found in sewage treatment plants and other areas with high levels of organic matter (Şahin et al., 2011).

![Sludge worm (*Tubifex tubifex*) individuals (Mandal et al., 2018)](image)

**Figure 11.** Sludge worm (*Tubifex tubifex*) individuals (Mandal et al., 2018)

Sludge worms, also known as Tubifex tubifex, live in silt and slime-lined tubular burrows, which gave them their name, forming large clusters in sludge-rich environments (Ikhsan et al., 2021). These pinkish-red, thread-like creatures are approximately 8 cm long and 0.6-0.7 mm thick and have four chaetae on each body segment, except for the first two segments in front of the mouth (Snimshhikova and Linevich, 1987). *T. tubifex* is a hermaphrodite species, with both male and female reproductive organs located in segments 10 and 11. However, individuals must exchange sperm with another individual to reproduce (Van Haaren and Soors, 2013). The clitellum, located in the anterior third of the body, secretes the cocoon during mating, and interpersonal sperm transfer takes place within this structure. The cocoon also provides nutrients for the embryological development of the fertilized egg until it hatches into a worm (Van Haaren and Soors, 2013). Sludge worms can produce up to four cocoons per year (Kaster, 1980). These creatures constantly swallow sludge with their front end as they reside at a depth of 5-10 cm in the soil, aiding in the mineralization of the soil by excreting simple minerals (Verdonschot, 1989; Reynoldson et al., 1991; Fedonenko et al., 2017).
Sludge worms, also known as Tubifex tubifex, are commonly used as an inexpensive live food for fish and other aquatic animals due to their high protein and essential fatty acid content. However, their exact nutrient content can vary depending on factors such as species, diet, and rearing conditions (Herawati et al., 2020). Sludge worm is characterized by its high proximate composition, specifically crude protein (52.11-65.30%), crude lipid (7.62-12.29%), crude fiber (4.07-9.55%), and crude ash (4.31-11.82%) (Herawati et al., 2020). Additionally, they have a significant content of n-3 fatty acids (18%) and n-6 fatty acids (22%). The amino acid profile of the protein in sludge worms is ideal for fish, with lysine and leucine being the most abundant amino acids. Furthermore, these worms contain carotenoids at a concentration of 15.02 mg/kg (Yanar et al., 2003).

Harvesting sludge worms in natural conditions can be risky due to their preference for contaminated waters (Reynoldson et al., 1991). To mitigate this, sludge worms should be grown in a controlled environment with steady water flow and high organic detritus. Environmental variables such as water temperature, oxygen, and substrate properties play a role in reproduction and growth, with temperature and oxygenated water being important factors (Jewel et al., 2016). The ideal temperature for sludge worm production is 22°C, and they can tolerate temperatures between 20 and 27°C but should be kept away from temperatures over 30°C. To culture sludge worms, a container with 50 to 75 mm of thick pond mud blended with decaying vegetable matter and bread can be used. The system should be inoculated with sludge worms (62.5 g/m²) obtained from muddy canals or sewage canals, and clusters of sludge worms will develop within 15 days. The most efficient production time is 20 days with a continuous mild water flow and a suitable drainage system (Fedonenko et al., 2017). Feed for sludge worms is organic matter, such as bread or manure, given once every 3-4 weeks. When mud worms are deprived of oxygen, they come to the surface (Das et al., 2012). Therefore, before feeding to fish, sludge worms must be washed and cleaned to remove accumulated pollutants and reduce the risk of disease and parasite transmission.

Overall, sludge worms have the potential to be a valuable food source for aquatic animals, but their culture requires careful attention to environmental variables and proper cleaning before use.

1.6.2. White worms (Enchytraeus albidus Henle, 1837)

The white worm, also known as Enchytraeus albidus, is a terrestrial species and is one of the first enchytraeids ever described that belongs to the Annelida phylum, Clitellata class, and Enchtraeidae family (Bunke, 1998; Erséus et al., 2019) (Figure 12). This species is found
along the coasts of northern Europe and the Arctic and is viable in both fresh and salty water as well as in soil (Stephenson, 1930; de Boher et al., 2018; Erseus et al., 2019).

![White worms (Enchytraeus albidus)](https://cdn.shopify.com/s/files/1/0522/7623/2391/products/grindalworms_540x.jpg?v=1609851985)

**Figure 12.** White worms (*Enchytraeus albidus*)

*Enchytraeus albidus* is a relatively larger species within the genus, exhibiting a length ranging from 10 to 35 mm and a diameter of 0.5 to 1.0 mm. It features a pure white coloration (occasionally with a yellowish hue) and is generally transparent when viewed through a microscope (Bell, 1958) (Figure 12). The segment count of *Enchytraeus albidus* ranges from 52 to 74. The presence of a clitellum is a characteristic of adult individuals. The clitellum spans the entirety of segments 12th and 13th (Bell, 1958). The clitellum of *Enchytraeus albidus* is a specialized structure in the epidermis responsible for secreting a cocoon that serves as the site for depositing eggs and extruding spermatozoa received from a mating partner into the cocoon to facilitate fertilization (Jamieson and Ferraguti, 2006). *Enchytraeus albidus* has a hermaphroditic reproductive system, but most species show sexual reproduction (Hönemann and Nentwig 2009). During mating, the sperm is transferred from one worm to another through copulation (Jamieson and Ferraguti, 2006). The fertilized eggs are laid in cocoons that are produced by the clitellum. The cocoons are usually deposited in soil or aquatic environ-
ments, where the young hatch and develop (Maraldo and Holmstrup, 2009). The lifespan of a white worm is between 2 to 9 months, during which it can produce up to 1000 viable eggs (Hönemann and Nentwig, 2009; Fairchild et al., 2017). Egg diameter in *Enchytraeus albidus* varies between about 300 and 500 µm (Jamieson and Ferraguti, 2006). Sexual maturity is reached 5–7 weeks after hatching from the egg (Hönemann and Nentwig 2009).

*E. albidus* is known to contain a crude protein content that ranges from 45-70% of dry weight and a crude lipid content of approximately 15-20% of dry weight (Holmstrup et al., 2020; Dai et al., 2021). The ash content of this species is typically around 6% (Fairchild et al., 2017). White worms are known to have lower levels of n-3 fatty acids (11-23 mg/g dry weight) and higher levels of n-6 fatty acids (31-126 mg/g dry weight) (Fairchild et al., 2017). Furthermore, certain literature recognized that the total fatty acid levels are approximately 15-20% of the dry weight (Dai et al., 2021). Lysine and arginine are the most abundant amino acids, with percentages of 7.2% and 6.0%, respectively (Holmstrup et al., 2022). These worms have a high glycogen content of 20-25% of dry weight (Dai et al., 2021).

For cultivating white worms, it is necessary to maintain a temperature of 17-18°C, a humidity of 23-25%, and use slightly acidic to neutral soil. The worms are cultivated in soil with a soft texture, high porosity, and water-holding capacity in moistened plastic boxes, and poured to a height of 10-15cm (Walsh et al., 2015). White worm culture is added to the soil at a depth of 3–4 cm at a rate of 200–250 g/m². Following this, 2-3 ditches with a depth of 5cm are dug in the ground, where some of the food is placed and then covered with soil. In a thriving culture, the worms concentrate on the soil thickness near the bait (Springett, 1964). Different cereals, flour, bran, vegetables, roots, green herbaceous plants, berries, fruits, and yeast are used for feeding, which is carried out once a week (Walsh, 2012; Fairchild et al., 2017). To control pests such as mites and fly larvae, the culture boxes should be kept covered, and any mold-like particles in the food should be removed immediately (Fedonenko et al., 2017). The use of worm culture begins during the period of maximum increase in their biomass, that is, within 40-50 days from the moment of cultivation beginning (Springett, 1964; Fedonenko et al., 2017). It should be refreshed every six months by changing the substrate in the culture medium and creating new cultures (Fairchild et al., 2017; Fedonenko et al., 2017). For the harvest of the white worms, a container of water can be used along with a light source. The soil containing the worms is placed in the container, and the worms will gradually move to the surface and form dense tangles. The tangled worms can then be transferred to another container of water to remove any remaining soil. Sometimes special heaters are used that generate heat (Fedonenko et al., 2017).
Consumption of white worms may increase the risk of obesity due to their high glycogen content, despite their beneficial metabolic fuel properties. Hence, it is important to regulate the number of worms given to fish during feeding. Furthermore, detailed cultivation methods are necessary to improve the HUFAs content of white worms during the production process. This can help address the issue of imbalanced fatty acid composition, which is a concern associated with using white worms as fish feed.

References


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