

# Role of recent feeding protocols, rearing water systems and microbial trends in improving marine larviculture: insights into water quality and larval performance

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**Abstract** Larvae nutrition is considered to be the ‘bottle neck’ for marine finfish culture. Nowadays, marine larviculture can be significantly enhanced by producing new protocols of live feed using modern microbial trends. Primary goals of successful farmed larvae mainly rely on excellent survival and growth, since marine fish larvae record unpredicted high mortality rate and poor performance on designed diets during weaning stage. Adding live or inactive food to the rearing systems is also a widespread technique that has a significant impact on larval survival and performance. The bulk of mass-cultured marine fish larvae still need live feeding species, particularly at the beginning of larval nutrition such as: *Artemia* (brine shrimp), rotifers *Brachionus* spp., and microalgae. Bacteria are also added with live food and microalgae in the larval tanks for the rearing water system. Therefore, this review shed light on the developments of water rearing systems and improvements in marine fish larvae diets by discussing different types of live feeds and formulation of weaning microdiets. Since larval development is the most crucial stage of marine aquaculture production during which the greatest death rates can exceed 70%. Hence, the ontogenic development and digestive physiology of fish model larvae are also highlighted. Beneficial effects of prebiotics and probiotics on improving feed utilization and water quality, promoting larval growth and enhancing disease resistance were also discussed. Moreover, additional case studies about live food enrichment and reared fish larvae were presented to elucidate their effective role in improving water quality and larval growth performance. Despite recent substantial advancements in marine larviculture, many questions about fish larvae nutrition remain unanswered and numerous research avenues remain unexplored. Suggestions, recommendations and future considerations were mentioned to improve diets during larval rearing and pinpoint the research gaps that need to be addressed in manual hatchery operations.

**Keywords** Marine larvae . Ontogenic development . Digestive system . Diets . Water systems . Microbial trends

## Introduction

Aquaculture has witnessed a significant global expansion by providing a vital source of animal protein and key nutrients to the growing world population (El-Zaeem et al. 2024). According to the recent FAO report (2024), aquaculture production (130.9 million tonnes) surpassed capture fisheries for the first time, accounting for 51% of aquatic animal output. This surge highlights aquaculture’s potential to meet rising global demand for aquatic foods. Mostafa et al. (2021) expected this continued growth of aquaculture to surpass 100 million tonnes in 2027 and 202 million tonnes in 2030, which will be primarily responsible for the overall production of aquatic animals, reaching millions of tonnes in 2030. Hence, FAO (2024) claims

that more focused transformative reforms are required as the sector continues to grow to create fisheries and aquaculture sectors that are more sustainable, inclusive, and equitable.

A major obstacle to the rapid growth of aquaculture industry is ensuring that many farmed fish species receive the proper care in their early life stages (Hu et al. 2018; Gallardo et al. 2022). Moreover, the unpredicted high mortality rate of marine fish larvae in the first few weeks after hatching is one of the main barrier for the majority of marine aquaculture species (Bene et al. 2015). This may be due to the difficulty of larvae to first feeding during the weaning stage since they have a small mouth size. In addition, when larvae deplete yolk reserves and start transition from endogenous to exogenous feeding, they do not profit from a well-developed gastrointestinal tract to properly digest the formulated diets (Yúfera and Darias 2007). It is worth to mention also that these young larvae have limited ability of predation due to its immature optical developments, primitive jaws and muscles (Hu et al. 2018). Extensive knowledge about the ontogenic development and digestive system peculiarities of marine larvae make them relevant to overcome high mortality rate to ensure larval performance and to obtain regular good quality of spawning (Roux et al. 2021).

To achieve a successful larviculture production, behaviors and nutritional requirements should be considered when selecting suitable first feeding ingredients (Mejri et al. 2021). Dietary planktons have been recognized as promising exogenous nutrients for marine larvae since they represent more bioavailable nutrients and trigger higher predatory responses (Kandathil Radhakrishnan et al. 2020). These planktons could live with the farmed larvae in the rearing system and be ingested by the larvae whenever need, so they are known as “live feeds”. According to Pan et al. (2022), selection of proper live feed for larval feeding is based on several factors such as morphology, size, nutritional value, growth rate and stock density. Furthermore, bacteria are also added with live food and microalgae in the larval tanks for the rearing water system. Gorrens et al. (2023) pointed out that the availability of organic substrates generally (as prebiotics) or as functional agents (probiotics) with possible effects on the live feeding gut of larvae are both necessary for the persistence of bacterial growth. Recently, dietary prebiotics and probiotics are commonly used because of their significant role in the improvement of overall health profile and growth performance (Selim and Reda 2015), reproductive physiology (Eissa et al. 2024 a,b), feeding parameters (Eissa et al. 2024c), resistance to diseases (Radwan et al. 2023a,b) and the boost of the immune status of numerous aquatic fish species (Yilmaz et al. 2022).

Since marine larvae possess a very sensitive small mouth, therefore, their larviculture are very challenging due to a lack of appropriate first feeding protocols. It is of a crucial concern to enhance aquaculture technologies and innovations of new live feed procedures using modern microbial trends to advance the fast-growing industry of marine larviculture. Therefore, this review highlights the developments of water rearing systems, improvements in marine fish larvae diets and new developments in microbial trends by discussing different types of live feeds (microalge, rotifers, artemia, copepods and drawbacks) and formulation of weaning microdiets. Moreover, the beneficial effects of prebiotics and probiotics and some study cases were reported. Suggestions, recommendations, research gaps and future considerations of its applications are also addressed.

### Water rearing systems

It is worth to point out that bacterial densities, flow rates, and water systems of the process water all impact the constant flow of bacteria that the larvae are exposed to. Bacterial densities in rearing tanks are influenced by different sources of bacteria (bacteria in process water, bacteria introduced with live food and microalgae, and bacterial development in the larval tanks). Furthermore, the treatment of the water supply to the larval tanks will affect the number of bacteria in the water entering those tanks, and the quality of water in the tanks. Finally, the rearing system and the total availability of organic substrates influence the internal processes of bacterial growth (Vadstein et al. 2018).

The bacterial fluxes are measured in larval tanks for three distinct aquaculture systems with varied bacterial concentrations and dynamics. The three distinct aquaculture systems are identified as follows: (1) *Filtered and sanitized intake water*: this system is provided by flow-through systems (FTS). (2) *Flow through matured systems* (MMS): after intake, water is filtered and disinfected and the bacteria are permitted to recolonize the water by allowing it to remain in an aerated biofilter for longer than eight hours. (3)



*Recirculating aquaculture systems* (RAS): in which water is initially cleaned in a manner like that of FTS, but after that, the used water is continuously running through a biological filter and additional water-treatment components (such as protein skimmers and filters).

Microalgae and bacteria from live food have different mechanisms of exposure to larvae than bacteria in process water, encompassing both input and internal bacterial development. If the supply of bacteria came solely from the process water, the bacterial density in the raising tank would be constant over time and would only be impacted by the number of bacteria present in the inflowing water. In this case, the water exchange mechanism would not reduce the number of bacteria in the larval tanks. The density of bacteria in the rearing media will rise with the introduction of germs through internal development or live food, depending on the supply amount and the water exchange rate in the larval tanks (Seychelles et al. 2013).

Fish are often raised in tanks with continuous water flowing using flow-through systems (FTS). To meet the needs of the cultured species at different developmental stages and to protect the larvae from harmful organisms, the input of water in an FTS is regularly adjusted. Aeration/degassing, disinfection, temperature control, and particle removal are typical treatment techniques for incoming water in an FTS. The treatments' goals for the microorganisms are to lower their general densities and to create a microbial barrier separating the outside world from the rearing facility (Vadstein et al. 2018).

In rearing systems, it is expected to include live or inactive microalgae to enrich the larvae. The community of the rearing tanks consists of three biological compartments with three known interactions (predation) between them. When bacteria and dissolved organic matter (DOM) operate as a growth substrate for the heterotrophic bacteria, the basic food web with three connections becomes a complex food web with countless interactions (Bakke et al. 2015). As a result, the initial feeding and raising tank is probably a complex ecology with hundreds of interactions.

## Larval development

The most crucial stage of marine aquaculture production is the 'larval development', during which the greatest death rates can exceed 70%. Several teleost species have proven their potential as model species for studying the ontogenic development of marine larvae such as medaka (Cresko et al. 2006), cavefish (Jeffery 2008) or stickleback (Merilä 2013). The clownfish *Amphiprion ocellaris* was selected in this review as an experimental model species to describe the marine larval development. This process has been characterized by some morphological criteria (such as notochord flexion, fin ray appearance and white bars formation) and classified into seven distinct stages (Roux et al. 2019). At the beginning, stage 1 corresponds to preflexion larvae without differentiating fins, and characterized by a pigmentation pattern consisting of black melanophores over yellow xanthophores spreaded from the head to the caudal peduncle took place. At stage 2, the notochord is in flexion and enters into post flexion at stage 3. Fins start to develop in these two stages with growing of rays. However, pigmentation patterns remain similar to stage 1. At stage 4, orange xanthophores appear, dorsal and ventral fin rays are fully developed, and pelvic spines continue to grow. Moreover, the overall shape of the larva becomes more ovoid. Transparent white bars on the head and body start to appear at stage 5 and become whiter at stage 6. Stage 7 is characterized by the emergence of the third white bar on the caudal peduncle, and finally, the juvenile stage is reached when fin pigmentation is completed (Roux et al. 2021).

Live feed is required at these early developmental stages due to the larvae's underdeveloped digestive system and small mouth size, which prevents using inert feed. The ability to mimic a species' reproduction and larval development in a controlled setting is necessary for intensive aquaculture output. Fish larvae are extremely fragile creatures whose survival depends on several variables. The most crucial one is nutrition. Newly hatched larvae's yolks include triglyceride-containing fat droplets, free amino acids, and glycogen, among other nutritional reserves (Khairy and El-Sayed 2012). Endogenous feeding is the gradual resorption of these substances. Each species has a different nutritional reserve period (Holt 2011). The larvae depend on capturing and digesting the meal present in the medium when the reserves of yolk have been reabsorbed. The larvae's survival will depend on the transition from endogenous to external feeding, which is a crucial stage.

### Ontogenic development of the digestive system

The development of the alimentary canal of a generalized' fish larva is described in this section. Embryon-



ically, the alimentary canal develops from the involution of columnar endodermal cells that lie above the yolk. At hatching, the alimentary canal appears as a straight tube lying dorsal to the yolk sac, closed at the mouth and anus and is undifferentiated along its length (García-Hernández et al. 2001). The larval alimentary canal remains histologically unchanged until the completion of yolk- and oil globule absorption (several days or weeks), then changes rapidly just before first feeding when the undifferentiated tube becomes segmented by muscular valves into a buccopharynx, fore-, mid-, and hind-gut during transformation of the larval to the juvenile fish (weeks or months). Then, the development of a stomach and pyloric caeca from the posterior foregut accompanies the transformation process constituting the last major morphological change of the alimentary canal. At hatching, the liver and pancreas (along with their ducts) are formed and become functional by the end of yolk absorption (Zambonino-Infante et al. 2008).

Most teleost fish typically have three critical periods (Chen et al. 2006). Hatching: is identified as the first phase which ends until endogenous feeding is complete. Larvae rely on oil globules and the yolk sac's energy stores at this time. They go through a shift from endogenous to exogenous feeding toward the end of the first phase before only consuming external food. Starting external feeding indicates the beginning of the second phase, which is characterized by insufficient digestive capability and ends before the formation of gastric glands in the stomach (Benini et al. 2022). At this stage, pinocytosis, intracellular digestion, and absorption are the main processes used by fish larva (Khojasteh 2012; Jacobsen et al. 2020). Therefore, these fish larvae typically feed on easily absorbed and digestible live foods like rotifers or *Artemia*. The third stage, which lasts until transformation, is marked by the emergence of the pyloric caeca and stomach glands demonstrating the functional maturity of the digestive system (Gomes et al. 2021).

When thinking about larval feeding, changes in mouth size are crucial because they affect the fish's ability to consume food organisms (Qin 2008). When larval fish are given food particles that are too large, they will be unable to eat them, which would result in malnutrition. Therefore, the appropriateness of food particle size to mouth size is one of the most crucial aspects of larval feeding (Holt 2011). Moreover, greater levels of digestive enzymes are created as the mucosa develops, facilitating digestion in the gastric and intestinal tracts (Ianiro et al. 2016).

#### Digestive system capability

The function of the larval digestive system and the metabolic pathways of the assimilated nutrients are significantly influenced by the type of nutrients (protein vs. peptides and amino acids or triglycerides vs. phospholipids), quantities (protein or lipid levels), ratios (DHA (docosahexaenoic acid): EPA: eicosapentaenoic acid ARA: arachidonic acid; or essential fatty acids vs. other fatty acids for metabolic energy), and availability of dietary nutrients. Given the complexity of the metabolic pathways involved, scientists must adopt a more thorough approach to better understand the digestive system and feeding requirements of developing marine fish larvae. More molecular research is needed to find nutrient transporters in the gut lumen throughout ontogeny to establish the absorption capacity of further growing larvae (Padilla and Machado 2020).

#### Nutritional schedule of larvae

One of the critical elements affecting marine fish larvae's survival and growth is their nutritional needs. For the initial days (up to 20–25 days after hatching), larvae are fed live meals such as brine shrimp *Artemia* and rotifers *Brachionus plicatilis*. Then, they get a combination of live and formulated feed until they are entirely weaned (Barakat et al. 2016). Most research focused on providing marine fish larvae with the nutrition they need using inert replacement diets and cultivated live prey that follow a practical feeding schedule. Defining larval digestive capacities and dietary requirements have been advanced significantly by Khairy and El-Sayed (2012), who studied novel methods for feeding enriched live prey with necessary nutrients, and accomplished advancements in preparing and delivering inert meals to larvae. The global shortage of *Artemia* cysts in the mid-1990s led to an increase in the range of commercial microparticulate diets production to partially replace *Artemia* and allow fry to be weaned earlier (Rathore et al. 2016).



## Feeding behavior

A comprehensive understanding of larval digestive systems throughout development concerning food detection *via* the sensory system has to be achieved. It will be substantially assisted in optimizing feeding regimens and substituting inert microdiets for live prey if there is a deeper understanding of the ontogenesis, morphology, and physiology of these sense organs in larvae, juveniles, and adults of reared species. This will allow administration sequences for the species-specific optimization of prey/food item based on organoleptic qualities, size, and detected-ability (i.e. by mechanical or chemical signals, the kind and speed of motions, or buoyancy) (Rønnestad et al. 2013). Regarding confirmation of this situation in other species that have been reared, this information will significantly affect sampling of taxonomical ontogenetic studies, necessitating the collection of reference material from wild populations or the rearing of fish in large systems with natural plankton to understand the typical developmental pattern (Ribeiro et al. 2022).

Although numerous studies have been conducted on the function of proteolytic enzymes, particularly trypsin, in the digestive physiology of larvae, most findings lack any real-world application for the mass rearing of marine fish larvae. Previous studies showed that many marine species' larvae lack tryptic activity after the initial feeding stage (Applebaum and Holt 2003). Even when enough food is available, this is typically accompanied by a large amount of mortality. More research on this apparent ontogenetic deficit is required to determine the root causes of the deficits in larval digestive physiology during this crucial time (Rønnestad et al. 2013). By discovering the elements that make it easier to produce and secrete enough trypsin, for example, and which may eventually be added to the food, this knowledge will help overcome the shortage of proteolytic capacity. It is also unknown whether consequences for protein digestion result from ontogenetic variations in the different trypsin paralogues. Other proteolytic enzymes like chymotrypsin and pepsin may also play role in the growth of marine larvae, although this has not been thoroughly studied (Lazo et al. 2007). Studies on other crucial factors, such as intestinal passage duration and the activity of digestive enzymes throughout a 24-hour cycle, have only been conducted to a limited extent in a few species (Khoa et al. 2020).

Future studies must focus on the mechanics underlying lipid packaging and digestion as well as species-specific knowledge of feeding schedules from the enterocytes to the body during transit. Early in a larval life, the capacity of the triacylglycerol and phospholipid production routes appears to be significantly different, and as the larva develops these two roots dynamically change. These questions need to be clarified to enhance the lipid content of larval diet designs (Hamre et al. 2013).

Cholecystokinin (CCK) is the most researched hormone in larval feeding studies; although not much is understood regarding CCK's dynamics and precise function in fish larvae, much more must be learned about its production, release, and effects. There is some contradictory evidence regarding how fasting, starvation, and feeding regimes affect the regulation of the CCK and trypsin loop. Research should give more attention on the early feeding stages, where CCK seems insufficient to control trypsin secretion (Leet al. 2019).

## Larval dietary requirement

The requirements of marine fish larvae for total dietary lipids and protein have increased (Hamre et al. 2013). It has been shown that the types and amounts of free amino acids (FAA) in rotifers and *Artemia* differ from those in marine fish eggs, larvae, and their natural planktonic diet (Qin 2008). Providing FAA to larvae via prepared microdiets has been difficult because amino acids quickly leach into the environment. Food sources for marine fish larvae such as rotifers and *Artemia*, are deficient in essential fatty acids (EFAs) (Copeman et al. 2002). The interaction of the three essential fatty acids (EFAs), docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6), has been studied in relation to the dietary-essential fatty acid requirements of marine fish larvae. It has been demonstrated that optimal brain development in larvae requires high dietary DHA levels; however, the ideal dietary ratio of EPA to ARA regarding eicosanoid interactions seems to differ depending on the species. The nutritional requirements of fish larvae cannot be assessed using conventional nutritional techniques which make the development of inert meals very challenging (Hamre et al. 2013).



Typically, fish larvae need between 50% and 70% protein for growth, 28% lipids for energy, and 3% highly unsaturated fatty acids (HUFAs) for neurological development, growth, and survival, among other fish health indicators (Cahu et al. 2003). Fish larvae have considerably different dietary requirements compared to juvenile and adult fish (Hamre et al. 2013). Because of this ontogenesis; fish undergo substantial morphological and physiological changes like metamorphosis. Furthermore, fish larvae require high nutrients because they feed constantly and grow swiftly.

The primary reason for the dearth of studies using direct methods is the challenge of planning experiments with complete control over nutrient composition and environmental factors (such as fish density, water quality and renewal, light conditions etc) in every experimental tank. Most new marine aquaculture and aquarium species have sensitive, small-mouthed larval stages that make larviculture particularly challenging because there are not enough appropriate start-feeding methods. Enhancing innovation and diversification within live feed productive programs is vital to develop the quickly expanding marine larviculture business. As a result, live feed production systems are a major emphasis on a global scale in aquaculture (Hansen and Møller 2021).

## Types of live feeds and pertained complications

### Microalgae

Single-celled, phototrophic organisms' microalgae are usually used to nourish zooplankton, which supplies food for the larvae. The most common species in aquaculture include *Nannochloropsis*, *Tetraselmis*, *Isochrysis*, *Skeletonema*, *Thalassiosira*, *Chaetoceros*, *Monochrysis*, and *Haematococcus*. The high amounts of vitamins, vital fatty acids, and necessary amino acids found in microalgae contribute to the nutritional value of zooplankton. They also provide colors that improve the look of meat and skin and aid in developing the reproductive and immune systems (Maguregui 2020).

Microalgae are essential for the function of aquatic food webs because they transform solar energy into trophic resources and bioavailable organic molecules. These tiny autotrophs are utilized as live feeds for a variety of marine animals, including bivalves (Hassan et al. 2022), zooplankton (Dayras et al. 2021), crustacean larvae (Sandeep et al. 2021), and echinodermata (Gomes et al. 2021). Three scenarios about the use of microalgae in marine hatcheries can be explained as follows: (i) using food to stimulate early embryonic stages with nutrients (Dayras et al. 2021); (ii) integrating natural enrichment materials into zooplankton live feed organisms (Fu et al. 2021); and (iii) adding microalgae to produce water conditioners (Basford et al. 2021). Several factors should be considered during applications based on a variety of microalgae traits comprising: cell size, which needs to be in line with the larvae's ability to consume food; (ii) cell structure: the efficiency of ingestion and digestion may be impacted by features of cell walls or skeletons (such as cellulose, SiO<sub>2</sub>, or CaCO<sub>3</sub>); (iii) nutritional profile: taking into account the dietary requirements of their customers, the content (real amount) and composition (%) of different bioactive nutrients also should be taken into consideration (Dayras et al. 2021).

Under different environmental conditions and high cell concentrations, it was found that production of marine microalgae Chlorophytes, such as *Nannochloropsis* sp. and *Tetraselmis* sp., may usually be easily supported. Their strong cellulose cell wall and nutritional deficiencies (i.e., low levels of eicosapentaenoic acid (EPA), 20: 5n-3, or docosahexaenoic acid (DHA), 22: 6n-3) limit their suitability as live feed for animals (Pan et al. 2014). At this peruse, El-Khodary et al. (2021) succeeded to reduce albino phenomena in El-Max well water where *Solea solea* larvae were raised and the microalga *Nannochloropsis* was used as green water in the enrichment of live food by reducing ARA/DHA ratio that induces albinism. The higher digestibility and enhanced nutritional advantages of microalgae are provided by haptophyte and cryptophyte species (such as *Isochrysis* sp., *Tisochrysis* sp., and *Rhodomonas* sp.) with well-balanced polyunsaturated fatty acid (PUFA) profiles and soft cell structures (Mai et al. 2021).

Many biomass production programs are currently applied to extract bioactive compounds. Various approaches, including ultra-thin mesh layers, tubular glass, plastic bags, and flat-plate photobioreactors (PBRs), are being used more frequently (Sandmann et al. 2021; Wurm and Sandmann 2021). These meticulously designed systems may exceptionally produce high cell densities for aquaculture applications, even though the PBR can raise production costs (Leal et al. 2021).



Using nongenetic and genetic alterations; strain selection for microalgal applications facilitate the production of beneficial bioactive chemicals (such as antipathogenic and antioxidant) for aquatic larvae growth on farms (Kiataramgul et al. 2020). However, transgenic microalgae should thoroughly assess their biosecurity before their large-scale utilization (Figure 1).

### Rotifers

Rotifera is a phylum of multicellular microbes known as rotifers. Since the 1970s, species and strains of the genus *Brachionus* have been used for the early feeding of marine larvae during the first three to ten days after hatching (dph) (Lubzens et al. 2001). The taxonomy of the *Brachionus plicatilis* and *Brachionus rotundiformis* complex is currently being debated; however, according to size, they are commonly referred to as SS-, S-, and L-type rotifers. In addition, rotifers are in high demand in the contemporary larviculture sector due to the following factors: (1) First feeding of commercially relevant fish species (e.g., sea bass and sea bream) requires a moderate cruising swimming style and an appropriate size range (100–250  $\mu\text{m}$ ) (Conceição et al. 2010). (2) Rotifers exhibit cyclic reproduction in their life cycle, with a parthenogenetic period during which only amniotic females are present. These females have remarkable fertility and can breed without the assistance of males.

### Artemia

The *Artemia* genus is an aquatic crustacean that predominates in hypersaline settings belonging to Branchiopoda class (e.g., inland salt lakes). Due to excessive hypersaline stress, *Artemia* forms floating resting eggs (also known as cysts) during dry seasons. The cysts are gathered, purified, and dehydrated before being processed into canned goods under cold, dark storage conditions. Even though most marine or brackish larvae cannot naturally reach *Artemia*, they are widely employed in the larviculture business for the following reasons: The initial naupliar stage of several *Artemia* species ranges from 400  $\mu\text{m}$  to 570  $\mu\text{m}$  and provides numerous benefits such as: (i) size suitability; (ii) vector of nutrients or medicine delivery systems; and (iii) preferable size for second-stage larval feeding (7–14 dph). Nauplii are also obtained at desired times for larval feeding (enrichment required before usage) to achieve persistent cysts and manipulated hatching (Eryalcin 2018).

### Copepods

This category serves as the primary prey for larval feeding in the wild. Copepods have a nutritional makeup,



**Fig. 1** Modeling of indoor and outdoor microalgae culture in the marine hatchery of NIOF Egypt (2023) offers recommendations for cost-cutting and optimization techniques for microalgae production.

in contrast to rotifers and *Artemia* (Table 1), that fully adapts to the needs of fish larvae. They also have a size range (between 45 and 600 microns) that fits the various fish mouth sizes, ensuring their ingestion. Aquaculture is primarily interested in three species of copepods: cyclopoids, harpacticoids, and calanoids. Some *Acartia* spp. nauplii are appropriate for initial feeding because they are only 50 to 60  $\mu\text{m}$  wide and 100  $\mu\text{m}$  long. Harpacticoid copepod species are distinguished by their widespread distribution, high production rates, and lack of cannibalism. Furthermore, several genera in this category probably can be raised on formulated foods. *Tisbe holothuriae* and *Nitokra lacustris* are two species that may be cultivated at great densities. The genera *Oithona* and *Dioithona* were listed as potential candidates for first-feeding larvae among the cyclopoid copepods (Maguregui 2020).

Due to their availability and preference as live feeds for fish or invertebrate larvae in the marine environment, planktonic copepods are used as live feeds in aquaculture hatcheries (Fernández-Ojeda et al. 2021). Species belonging to the orders Calanoida, Cyclopoida, and Harpacticoida are commonly selected and cultivated for larval feedings. Copepods come in twelve developmental phases, from which six species (nauplii, copepodites, and adults), with a wide range of prey sizes (60–1000  $\mu\text{m}$ ). More fish larvae are encouraged to become predators by their jerky swimming style (Burbano et al. 2020). Remarkably, even without the need for an additional enrichment process, the high amounts of these zooplankters of 3 HUFA provide nutritional benefits that make them acceptable for larviculture (Matsui et al. 2021).

The larviculture of marine ornamental fish has shown great promise, especially with regard to the generation of “micro-sized” copepods, such as those produced by the species *Parvocalanus* sp., *Bestiolina* sp., and *Paracyclops* sp. (Dayras et al. 2021; Wang et al. 2021). They are regarded as difficult but essential for commerce and conservation requirements. Cryopreservation and resting eggs are substitute methods for obtaining live copepods instead of maintaining culture (Pan et al. 2018; Wilson et al. 2021). Nevertheless, creating a particular *Acartia tonsa* copepod in cold storage appears feasible and marketed, and its industry should continue to improve the induction and storage protocols of other latent copepods.

#### Live feed drawbacks

Like other animal industries, the ability to get larvae in sufficient quantity and quality to maintain productivity is a prerequisite for the intensive production of aquaculture species (Kaleem and Sabi 2021). Intensive culture of many species, including sea bass, sea bream, and turbot, becomes possible by the evolution of larval nutrition using *Artemia* and rotifers. However, there are several drawbacks to this kind of diet. First, keeping these auxiliary foods in addition to fish farming is very expensive (Maguregui 2020). In addition, because they are living organisms, particularly rotifers, the preservation of the same amount of nutrients is challenging because it depends on several factors, such as the kind and concentration of microalgae they eat and the water quality (Penglase et al. 2011).

#### Formulation of weaning microdiets

Since feeding with most inert diets, especially those that are semi-purified, are poorly tolerated by most animals, the use of experimental microdiets is considered as a challenge. Their effects might also be masked by shortages in some particular nutrients. Therefore, numerous attempts have been exerted to advance this topic. Hamre et al. (2011; 2013) pointed out that *Senegalese sole* larvae microdiets designed with two protein levels (55% and 62%) gave the higher protein content of larvae showing a faster rate of eye migration but modestly improved growth and survival. As far as we know, no valid dose-response study has been conducted with fish larvae that employed a macronutrient composition variation of more than two levels.

**Table 1** Comparative analysis of the several live feed varieties (copepods, rotifer, and *Artemia*)

Parameter	Rotifer	Artemia	Copepods (natural zooplankton)
Size	90–350 $\mu\text{m}$	400.0 to 570.0 $\mu\text{m}$	<60–1000 $\mu\text{m}$
Crop density	Tolerant to high densities	Tolerant to high densities	Sensitive to high densities
Supply	Easy to control	Direct dependence	Erratic and unpredictable
Quality of nutrients	Low	Low	High (PUFA)
Bioencapsulation	Yes	Yes	Unlikely





Conversely, the experimental microdiets provide the opportunity to evaluate various dietary macronutrient compositions allowing to investigate the potential of macronutrient preferences. Juvenile and adult fish can choose the right diet based on their macronutrient needs. Trials using labeled food microparticles on gilthead sea bream (*Sparus aurata*) larvae yielded conflicting results, suggesting that the larval stage does not yet possess the ability to select macronutrients. These results may affect gut development and feeding behavior (Rathore et al. 2016).

The larvae of gilthead sea bream do not appear to get full even if the food is available until the stomach is completely developed and serves as a food reservoir. Moreover, the gut passage starts to be better regulated when more effective acidic proteolysis is produced allowing nutrient digestion and absorption to become more efficient (Rønnestad et al. 2012). When food is available, fish larvae tend to feed continuously, which will define how long food will remain in their guts. This is probably going to have an impact on nutrient availability and on the optimal macronutrient balance. The applied protocols used in nutrients administration will also affect the ideal food composition.

### Formulated diets

One of the main goals of research on marine fish larvae nutrition is the development of formulated feeds (microdiets) that may be used in their early feeding period. This will reduce the requirement of live feed organisms, which are labor-intensive and challenging to be cultivated. For improving larval nutrition, it is reciprocally vital to have dependable feeds with well-known formulated compositions. This was viewed as utopian in the 1970s and 1980s, but today, its realization and widespread commercial use are closer. In fact, a complete and successful replacement has been achieved at the experimental scale (Cahu et al. 2003), and the general results of progressive replacement in numerous species are highly promising (Hamre et al. 2013).

Worth to point out that feed particles recognized as food attractants may be added to the diet. Certain amino acids are potent stimulants for fish as illustrated in Table 2. When these attractants are incorporated into food particles, eating is stimulated during the food search. Furthermore, several digestive hormones that help larvae to digest and assimilate food are activated by amino acids (Sandel et al. 2010). The amino acids that cause fish olfaction are species-specific and differ significantly in quantity, kind, and concentration—even in mixes. Gilthead sea bream larvae (20 dph) increased their rates of microdiet ingestion up to 120% when exposed to the visual and chemical stimuli (working synergistically) of different concentrations of *Artemia nauplii* (Rathore et al. 2016).

Various chemical cues triggered the larval reaction, including the substance betaine and several free amino acids (FAAs) such as arginine, glycine, and alanine. These were chosen from a list of 14 metabolites present in the *Artemia*-rearing media, and their selection was confirmed by observing how each one affected the place at which microdiet (MD) digested food. Additionally, it was discovered that age affected how

**Table 2** Amino acids as feed attractant in marine organisms (Rathore et al. 2016)

Marine fish	Type of amino acid
Rainbow trout ( <i>Salmo gairdneri</i> )	Mixture of L-amino acids
Atlantic salmon ( <i>Salmo salar</i> )	Glycine
Sea bass ( <i>Dicentrarchus labrax</i> )	Mixture of L-amino acids
Pig fish ( <i>Orthopristis chrysopterus</i> )	Glycine, betaine
Red sea bream ( <i>Chrysophrys major</i> )	Glycine, betaine, Glycine, alanine, lysine Valine, glutamic acid and arginine
Gilthead sea bream ( <i>Sparus aurata</i> )	Glycine, betaine, alanine, Arginine
Turbot ( <i>Scophthalmus maximus</i> )	Inosine and IMP
Dover sole ( <i>Solea solea</i> )	Glycine, betaine
Puffer ( <i>Fugupardalis</i> )	Glycine, betaine
Japanese eel ( <i>Anguilla japonica</i> )	Glycine, arginine, alanine, proline
Cod ( <i>Gadus morhua</i> )	Arginine
Herring ( <i>Clupea harengus</i> )	Glycine
Glass eel ( <i>Anguilla anguilla</i> )	Glycine, arginine, alanine, proline, Alanine, glycine, histidine, proline
Lobster ( <i>Homarus americanus</i> )	Glutamate, betaine, taurine, ammonium chloride
Western Atlantic ghost crab ( <i>Ocypode quadrata</i> )	Butanoic acid, carboxylic acid, trehalose, carbohydrates, homarine, asparagine
Freshwater prawn ( <i>Macrobrachium rosenbergii</i> )	Taurine, glycine, trimethylamine, betaine
Abalone ( <i>Haliotis discus</i> )	Mixture of L-amino acids and lecithin and lecithin



much of an impact these FAAs and betaine had on the pace at which larvae ingested food (Kolkovski et al. 2009). The effectiveness of alanine, glycine, and betaine as feeding stimulants in gilthead sea bream larvae that were seven days old was investigated by Rathore et al. (2016). After adding the amino acids to MD, the ingestion rate was measured both with and without rotifers. When rotifers were present, the pace at which the entire MD was consumed decreased.

#### Types of formulated microdiets

The terminology for these various technologies might be complicated. Many distinct forms of microparticles have been generated utilizing multiple approaches. However, the preparation techniques are outside the purview of this analysis, where micro-bound and micro-encapsulated diets are the two basic forms of microdiets with full formulation (Langdon et al. 2008). Particles generated using one of the aforementioned techniques can go through a coating procedure. However, lipid beads and liposomes, as well as specialized lipid formulations, have been utilized to distribute hydro-soluble micronutrients such as vitamins, amino acids, and minerals (Monroig et al. 2006). The majority of the feeds that are now on the market are micro-bound, meaning that a binder connects the feed's various elements into a network. The finished dry mixture is crushed and sieved to get the necessary particle size. Like some commercial beginning feeds, it is possible to extrude or mechanically agglomerate the micro-bound diet.

The particles are generated separately with a spherical or nearly spherical shape using various micro-encapsulation technologies. The production of complex particles, which can be formed from polymers such as alginate, chitosan, gelatine, zein, carboxymethyl-cellulose, or cross-linked protein, is the most recent experimental development in simulated feeds (El-Sayed and Barakat 2016). This kind includes very tiny particles inside a particle with a normal diameter. These complex particles are designed to halt water-soluble nutrient loss more efficiently. For this, lipid spray beads (LSB) and liposomes can both be used (Langdon et al. 2008; Hamre et al. 2013).

#### Advances of probiotics in aquaculture

Non-digestible food ingredients, known as 'prebiotics' aid in the growth of beneficial bacteria in the intestines. They could be a tool for the beneficial bacterial selection that benefits the host. Prebiotics are originally created for humans, unlike probiotics. They have widely been investigated in aquaculture (Hasan and Banerjee 2020). Previous studies have reported the advantages of using prebiotics in diets of aquatic species demonstrating an increase in growth rates, an enhancement of gut health and nutrient availability, modifications of acquired and innate immune responses, control of microbial establishment, strength in defenses against the expansion of possible pathogens, absorption of mycotoxin containing nutrients as well as reduction in adverse drug and vaccine reactions (Selim and Reda 2015; Yilmaz et al. 2022). However, these prebiotics mostly favor lactic acid bacteria, which are not the most prevalent species in fish's gut microbiota and might not be the best choice.

The growth of non-pathogenic species may be aided by oligo- and polysaccharides because many marine diseases predominantly require proteins and amino acids as carbon sources. Numerous researches on beneficial effects of oligosaccharides on fish species' growth has been conducted on adults (Eissa et al. 2024a,c), but few have been applied on larvae (Ringø et al. 2010). It was reported that oligosaccharides promote growth, enhance food conversion, influence pathogen colonization leading to an increase in immune response in several fish species, as well as act as pathogen adhesion inhibitors to intestinal cells (Hahor et al. 2019). After exposure to stressful situations, it was indicated that cobia larvae's survival was increased by mannan oligosaccharides provided *via* enriching live food (Salze et al. 2008). Moreover, Eissa et al. (2024a) demonstrated that reproductive performance of red tilapia (GSI, total number of fries per female and mean fry weight) was enhanced with increasing dietary supplementation of mannan-oligosaccharides (MOS) to 1.5 g kg<sup>-1</sup>. They also reported that reproductive hormones and gonadal histology were significantly improved in fish fed diets treated with 1.5 g MOS kg<sup>-1</sup>. In addition, the prebiotic  $\beta$ -glucan possess several biological properties, comprising antibacterial, antioxidant, immunostimulant and antitoxic effects (Zeng et al. 2016). Fath El-Bab et al. (2022) informed that  $\beta$ -glucan supplement improves the performance of some fish species. Furthermore, the dietary inclusion of oligosaccharides and  $\beta$ -glucan at a dose of 1.5



and 2 g/kg significantly improved the survival rates of Nile tilapia during challenge with *Streptococcus* and enhanced fish growth, feed parameters, body composition, blood profile, and growth-associated genes (Eissa et al. 2024c). Do-Huu et al. (2019) also found that pompano fish fed with 0.1–0.2%  $\beta$ -glucan had higher survival rates than those in fish fed with untreated ones.

### Benefits of probiotics in aquaculture

Beneficial effects of probiotics on aquaculture are demonstrated in Figure 2 and elaborately discussed as follows:

#### Improving feed utilization

Zheng and Wang (2016) mentioned that diet supplemented with *Lactobacillus pentosus* increased the expected amount of feed consumed by the white prawn *Litopenaeus vannamei*. Moreover, the use of heat-killed *Lactobacillus plantarum* for 12 weeks at concentrations of 50, 100, or 1000 mg/kg significantly enhanced the amylase, lipase, and protease activity of Nile tilapia (Dawood et al. 2020). The application of *Lactobacillus plantarum* at doses of  $10^7$ ,  $10^8$ , and  $10^9$  CFU/gm also resulted in an increase in the activities of lipase, protease, amylase, and alkaline phosphatase in narrow-clawed crayfish *Astacus leptodactylus* (Valipour et al. 2019). In addition, Ramos et al. (2015) evaluated the impact of introducing two types of endospore bacillus to four isonitrogenous diets for rainbow trout (*Oncorhynchus mykiss*) fry and the results indicate that both probiotics, in a dose-dependent way, improves immunological humoral responses and zootechnical performance without obviously changing intestinal shape.

According to several studies on immunostimulation, fish fed on probiotics showed improvements in their immunological responses (Waiyamitra et al. 2020). Ibrahem (2015) conducted a study to assess the impact of specific probiotics on fish growth and gut bacterial ecology, focusing on dangerous bacteria. His findings showed that the fish groups fed probiotics showed a significant increase in body weight gain, feed conversion ratio, protein efficiency ratio, and other essential growth indicators such as Gram-negative *Aeromonas*, *Alteromonas*, and *Photorhodo* and Gram-positive *Bacillus*, *Lactococcus*, *Micrococcus*, *Carnobacterium*, *Enterococcus*, *Streptococcus*, and *Weisslla*. In addition to increasing feed conversion ratio and feed utilization, these probiotics generated extracellular antibiotic-like substances or iron binding agents (siderophores) that prevent the formation of some pathogenic bacteria. These probiotics also showed the ability to adhere to the intestinal mucosa and prevent harmful germs from adhering (Rusu et al. 2020). Probiotics also improved water quality (bioremediation) and addressed the red tide plankton issue (Helmy and Kardenia 2019).

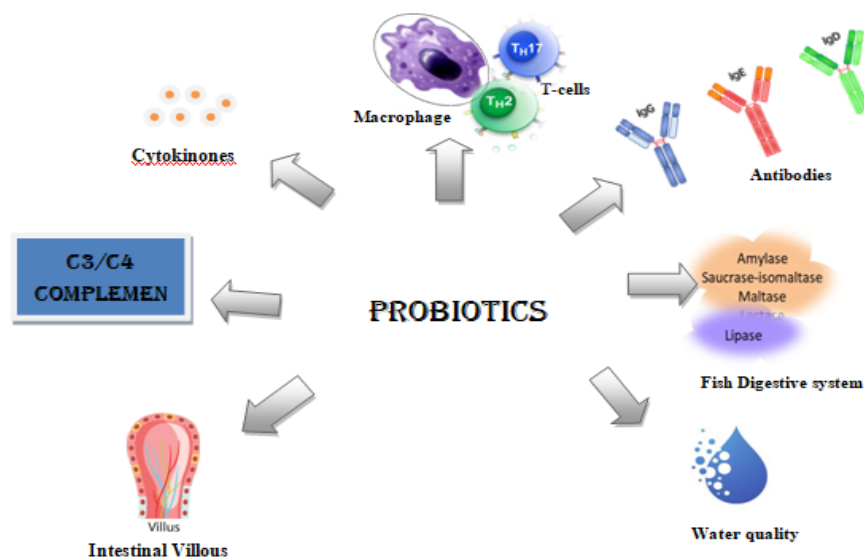


Fig. 2 Beneficial effects of probiotics on aquaculture



### Promoting growth

Fish development performance is directly affected by using bacterial probiotics, which is one of the most anticipated outcomes, either by increasing their ability to directly absorb nutrients or supplying them with nutrients (Joel et al. 2020). Probiotic supplementation significantly improves growth performance, which may be attributed to the increased release of digestive enzymes that boost hunger, produce vitamins, break down indigestible components, and improve gut shape (Doan et al. 2018). The use of *Bacillus subtilis* feed supplementation at  $10^7$  and  $10^9$  CFU/kg food for five weeks significantly increased the growth of the Pacific white shrimp *Litopenaeus vannamei* (Kewcharoen and Srisapoom 2019). Adding *Enterococcus faecalis* and *Pediococcus acidilacti* to mud crab, *Scylla paramamosain* diets dramatically increased weight gain and specific growth rate (Yang et al. 2019). Moreover, probiotics are similarly employed in ornamental fish. Zebrafish (*Danio rerio*) grew more quickly after eating *Pediococcus acidilactici* without losing their appetite (Ahmadifar et al. 2020).

### Enhancing disease resistance

The host's resistance to disease is increased by the capacity of probiotic microorganisms to release substances that have a bactericidal or bacteriostatic effect on pathogenic bacteria in the intestine of the host, such as bacitracin and polymyxin produced by *Bacillus* sp (Cruz et al. 2012). In addition, the probiotic *Enterobacter* sp. supplementation enhanced the rainbow trout's (*Oncorhynchus mykiss*) resistance against *Flavobacterium psychrophilum* illness (LaPatra et al. 2014). *Aeromonas veronii* and *Flavobacterium sa-sangense*, two isolated gut autochthonous probiotic bacteria, also increased common carp illness resistance to *Aeromonas hydrophila* (Chi et al. 2013). Furthermore, Nile tilapia was given  $10^7$  cells/gm of *Lactococcus garvieae* (isolated from raw cow milk) for 10 days to improve their resistance to *Staphylococcus aureus* (Abdelfatah and Mahboub 2018). *Cromileptes altivelis* also increased resistance to *Vibrio harveyi* after receiving  $10^6$ ,  $10^8$ , or  $10^{10}$  CFU/g of *Lactococcus lactis* isolated from the animal's stomach for four weeks (Sun et al. 2018). According to Baños et al. (2018), administration of an *Enterococcus faecalis* (isolated from a commercial probiotic) for 30 days at a dose of  $10^8$  CFU/g enhanced rainbow trout illness resistance to *Lactococcus garvieae*. *Pediococcus pentosaceus* also possessed exceptional antibacterial activity against numerous serious fish illnesses, including several *Aeromonas* species (Gong et al. 2019).

### Boosting the immune response

Probiotics can improve numerous immune indices of aquaculture animals. They can prevent pathogen infection by boosting the host immune system and cellular and non-specific immunity in the body (Hamka et al. 2020). Lactic acid bacteria, including *Lactococcus lactis*, *Lactobacillus sakei*, and *Leuconostoc mesenteroides*, increased the fraction of phagocytic active head kidney cells and activated the complement receptor expression in rainbow trout, improving both cellular and hormonal immune capabilities (Balcazar et al. 2007). The host-associated probiotics *Lactobacillus plantarum* and *Bacillus velezensis* enhanced the innate immune markers, including skin mucus lysozyme and peroxidase activity, serum lysozyme, serum peroxidase, alternative complement, phagocytosis, and respiratory burst activities (Doan et al. 2018). Supplementing *Bacillus pumilus*, a probiotic produced from the host gut, with young golden pompano (*Trachinotus ovatus*), boosted the fish's total protein and lysozyme activity (Liu et al. 2019). Supplementing the diet with *Lactobacillus plantarum* at  $10^8$  and  $10^9$  CFU/g after 15 days resulted in a considerable increase in complement activity. Black-eared catfish's respiratory burst activity and lysozyme activity were considerably elevated by *Lactobacillus plantarum* at  $10^8$  and  $10^9$  CFU/g after 30 and 45 days of feeding *Pangasius larnaudii* (Silarudee et al. 2019). When fed shrimp continuously for five weeks a diet supplemented with the chosen probiotic *Bacillus subtilis* at  $1 \times 10^7$  and  $1 \times 10^9$  CFU/kg food, it effectively enhanced growth and markedly boosted immunological responses. Additionally, *B. subtilis* clearly enhanced midgut features by thickening the intestinal wall and microvilli. Lastly, it is clear that this probiotic increased resistance to *V. parahaemolyticus* (Kewcharoen and Srisapoom 2019).



## Improving water quality

Probiotic bacteria can quickly absorb or decompose organic or hazardous materials from the water, improving its quality. Probiotics break down organic items such as leftover food, plankton, fish or prawn excreta, and other organic materials into CO<sub>2</sub>, nitrate, and phosphate, which help to enhance the nutrient cycle that sustains a healthy water quality habitat for cultured animals (Manam 2022). According to the findings of Nimrat et al. (2012), introduction of mixed *Bacillus* probiotics significantly enhanced the water quality of culture water regarding pH, ammonia, and nitrite levels while cultivating white prawns. In shrimp farming, a probiotic water injection of *Bacillus subtilis* at 10<sup>3</sup>–10<sup>5</sup> CFU/ml was found to dramatically reduce total ammonia and enhance water quality (Kewacharoen and Srisapoom 2019).

## Stress tolerance

The stress tolerance of aquatic animals is considerably improved by probiotic supplementation. Since cortisol is a stress hormone, research on fish's stress tolerance to ammonia found that fish fed *Lactobacillus plantarum* probiotics had lower cortisol levels compared to fish fed on a control diet (Nguyen et al. 2019). Moreover, the defense against hypoxic stress in Nile tilapia is considerably improved using *Aspergillus oryzae* as a probiotic (Dawood et al. 2020). Compared to greater and lower doses, pabda catfish have significantly higher saline water stress tolerance when dietary commercial probiotics are supplemented at 0.2% (Chowdhury et al. 2020).

## Case studies and recommendations

It is obvious that commercially producing microdiet has not been completely replaced by rotifers and *Artemia*, the finfish larvae's primary food sources, since it does not compromise the required growth and survival rates. Therefore, a variety of aspects and disciplines needed to be taken into account when employing an integrative approach to address the causes behind this lack of success. The particles must, first and foremost, be appealing to the larvae. As a result, the particles must integrate or be covered with a feed attractant. This should entail dietary production methods that prevent leaching, especially of amino acids. Several nutritional case studies carried out on different species of fish larvae to improve water quality, larval growth performance and survival rates are illustrated in Table 3 and discussed as follows:

**Table 3** Cases studies on the effects of different feeding strategies and/or water system on marine larviculture

Feeding/ water technique	Fish larvae	Effects	References
<i>Bacillus</i> sp. R2 & <i>Planococcus</i> sp. R11	<i>Sparus aurata</i>	- protease activity increased - histology of the digestive system was improved - rise in the numbers and length of villi and goblet cells - improve growth - lower mortality	Ghoname et al. (2020)
<i>Cyclops abyssorum</i> divergens	<i>Dicentrarchus labrax</i>	-improve growth (17%; 20.8%), -Increase survival rate (27%–57%) & biochemical composition.	El-Sayed et al. (2021)
Water quality enriched with <i>Nanochloropsis oculata</i>	<i>Solea aegyptiaca</i>	- prevent albinism -increase morphometric parameters -High growth rate	El-Khodary et al. (2021)
Silver nanoparticles synthesized by <i>Aspergillus flavus</i> amylase	<i>Dicentrarchus labrax</i>	-reduce mortality - improve growth performance	Barakat et al. (2022)
<i>Spirulina</i> and <i>Tetraselmis</i> additives to formulated diet or decapsulated <i>Artemia</i> cysts	<i>Solea solea</i>	-67% survival rate -highest body protein contents & lipid level	Shawky et al. (2021)
Magnetic water devices with different intensities	<i>Dicentrarchus labrax</i>	-better larval survival (10.78%–30.30%) -higher growth performance -increased dissolved oxygen and stress resistance	El-Sayed et al. (2022a)
Effects of ocean acidification on <i>Ulva fasciata</i> used as a nutrient supplement	<i>Dicentrarchus labrax</i>	- the highest survival rate - the largest reduction in the pathogenic bacteria community	Barakat et al. (2021)
AF- <i>Artemia</i> (during 1 <sup>st</sup> critical days) followed by <i>Rhodomonas salina</i> alga (until 1 month of age)	<i>Amphiprion bicinctus</i>	-Early pigmentation -Metamorphosis improvement	El-Sayed et al. (2022b)



### Using probiotics through live food enrichment and in rearing water systems

The use of probiotic bacteria to increase sea bream (*Sparus aurata*) larval production was investigated by Ghoname et al. (2020). These authors studied the effects of a combination of two probiotic bacterial strains (*Bacillus* sp. R2 and *Planococcus* sp. R11) that were isolated from the guts of mother fish (natural microbiota in the gastrointestinal cavity) and tested on gilthead sea bream larvae up to 40 days after hatching (DAH), as well as application of live food cultures (rotifers and *Artemia*). Probiotics were introduced in triplicates into the larval-rearing system using various techniques. The first approach was exogenous feeding, in which live food was provided by bio-encapsulation, tank water, or both. Compared to the control, the R11 probiotic supplement showed higher performance than its corresponding in the bio-encapsulation process. Sea bream fed *Planococcus* sp. (R11) showed a considerable increase in protease activity, with the highest value of enzyme activity (8.95 0.21 U/mg) compared to the control (1.3 0.03 U/mg). On the other hand, the histo-morphometric analysis of the digestive systems of larvae treated with bacteria revealed a rise in the numbers and length of villi (35, 9 m) as well as goblet cells (37). This research study confirmed the application of probiotics in aquaculture to improve growth and to lower mortality.

### Using cyclopoid copepods in combination with a mixed algal diet instead of *Artemia*

Using cyclopoid copepods as live food is considered to be an alternative to *Artemia* in larval feeding. El-Sayed et al. (2021) studied this application by evaluating the potential use of *Cyclops abyssorum* divergens as a natural live food choice for *Dicentrarchus labrax* larvae instead of the costly conventional *Artemia franciscana* as a biotechnological tool in larval rearing to develop the aquaculture industry. An equal portions (1:1:1:1) of significant mixed algae strains (*Isochrysis galbana*, *Tetraselmis chunii*, *Chlorella marina*, and *Nannochloropsis oculata*) were given to the cyclops *C. abyssorum* divergens and/or *A. franciscana*. This study aimed to determine which methods of larval rearing—using appropriate nursery feed—should be prioritized to improve the growth and survival of fish larvae. The results showed that the amino acid and fatty acid profiles (EA) of enriched *C. abyssorum* divergens (EC) differed significantly from those of enriched *A. franciscana*. Due to the bioconversion of alpha-linolenic acid, (ALA, C18:3 n-3), and further metabolism of palmitic acid (C16:0), there is a significant accumulation of DHA fatty acid in EC and lesser levels in EA. Compared to larvae fed EA, larvae fed EC had greater specific growth rates (SGR) and higher survival percentages (S%). Higher quantities of DHA fatty acids were found in the final carcass composition of fish fed EC representing a DHA/EPA ratio of 2.95% (>1), whereas larvae fed EA had a ratio of 0.90% (1). The higher concentrations of amino acids in the EC given to the larvae functioned as a tool for the faster growth and survival rate of the tested species. These findings suggested that *Cyclops abyssorum* divergens, could be used as a natural live food candidate rather than the more expensive standard *Artemia* in marine larvae feeding strategies to improve growth (17%–20.8%), survival (27%–57%), and biochemical composition. Furthermore, much research is required to determine whether alternative Cyclopoids or Harpacticoids are suitable, as well as how to produce eggs in a resting state. Besides the drawbacks of commonly used live feeds (*Brachionus rotifers* and *Artemia*), development of new live feed cultures, such as micro-sized copepods and their diversification are recommended to encourage commercial uses. An ongoing program to produce dormant live feed, such as copepod eggs at rest, is anticipated to open doors for the marine larviculture.

### Species-specific nutrient affinity can be exploited as a solution for larvae albinism

According to El-Khodary et al. (2021), larval rearing problems such as albinism in aquaculture can be prevented by maintaining water quality with specific live food enrichment using *Nannochloropsis oculata*. By carefully adjusting the diet of *Solea aegyptiaca* with *N. oculata*, these authors also reported an increase in growth rate and morphometric parameters of *Solea* during their larval stages.

### Fungal nanoparticle delivery of particular larval digestive enzymes through live feeding

By examining the ability of marine *Aspergillus flavus* amylase (Amy) to synthesize silver nanoparticles



(AgNPs), a reduction in mortality rate and an enhancement in growth performance were reported on sea bass (*Dicentrarchus labrax*) larvae (Barakat et al. 2022). Hence, it is possible to use nano-form to administer digestive enzymes through larval feeding.

### Weaning

Shawky et al. (2021) found that the use of *Spirulina* and *Tetraselmis* additives to formulated diet or decapsulated *Artemia* cysts gave a 67% survival rate of post-weaned common sole larvae, exhibited the highest body protein contents and lipid level compared to their corresponding in other treatments. Hence, they recommended that the lack of acceptable diets that can be used for weaning an emerging aquaculture species such as *Solea* species, needs more research on larval survival and development.

### Using magnetized water at different strength to improve marine larviculture

Magnetized water is a new application for water treatment alternative to other cost systems used to improve water quality (Fadel et al. 2024). European sea bass larvae were treated by different tesla as a source of magnetic power used in the deteriorated conditions at the end of the spawning season. Authors recommended that the magnetic water devices with different intensities achieved better larval survival (10.78%–30.30%), higher growth performance, a rise in dissolved oxygen (DO) content *via* water molecule expansion, and an increase in stress resistance (El-Sayed et al. 2022a).

### Beneficial physiological effects of ocean acidification on *Ulva fasciata* during larval rearing

The drawbacks of the effects of ocean acidification with larval rearing can be solved by enabling the use of CO<sub>2</sub>-treated macroalgae as nutritional supplements to improve sea bass aquaculture performance. Consequently, it was reported that sea bass larvae have the highest survival rate and the largest reduction in the pathogenic bacteria community using algal methanolic extract treated with different CO<sub>2</sub> concentrations (Barakat et al. 2021).

### Marine ornamental fish larval rearing

El-Sayed et al. (2022b) showed that AF-*Artemia* was selected as the first feed to facilitate rearing intricacy of the clownfish *Amphiprion bicinctus* larvae in their 1<sup>st</sup> critical days (8 days) and recommended the use of *Rhodomonas salina* alga in larval rearing water and live food enrichment till one month of age as a prime candidate for developing early pigmentation and improving metamorphosis in the ornamental fish industry.

## Conclusion

The authors of this review demonstrated new developments and applications in larval rearing systems, especially live prey period and water management systems with microbial control trends. Additionally, authors addressed suggestions and recommendations to try for improvements in the length of larval rearing and pinpoint the gaps and bottlenecks that need to be accomplished in both manual hatchery operations as well as future studies that were devised in their recent approaches for improving larval survival, growth, and quality of different marine fish species raised in hatcheries. These future considerations comprised that scientists must adopt new approaches to better understand the digestive system and feeding requirements of developing marine fish larvae. Even when enough food is available, this is typically accompanied by a large amount of larval mortality. Hence, more research is required to determine the root causes of this apparent ontogenetic deficit in larval digestive physiology during this crucial stage. Moreover, authors elaborately discussed the beneficial effects of prebiotics probiotics applications on larval performance and feed digestibility enhancing water quality. More molecular research is also needed to find nutrient transporters in the gut lumen throughout ontogeny, the mechanics underlying lipid packaging and species-specific knowledge of feeding schedules during food passage from the enterocytes to the body in order to increase further



absorption capacity of growing larvae. These questions need to be clarified to enhance the lipid content of larval diet designs. Another aspect that should be taken into account is the real-world application for the mass rearing of marine fish larvae. To maximize the performance of both prey and larval culture, future programs should also focus on both indoor and outdoor aquaculture systems using appropriate robotics and autonomous systems approaches with artificial intelligence (AI) technology.

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