





# Artemia Enrichment Strategies: A Comprehensive Review of Nutritional Enhancements With Emphasis on Fatty Acid Profiles in Aquatic Species

Yathish Ramena<sup>1,2,3</sup> | Ram Babu Kurapati<sup>2,3</sup> | Thomas Bosteels<sup>1</sup> | Grace Ramena<sup>2,3</sup>

<sup>1</sup>Great Salt Lake Brine Shrimp Cooperative, Inc., Ogden, Utah, USA | <sup>2</sup>Department of Aquaculture and Fisheries, University of Arkansas at Pine Bluff, Pine Bluff, Arkansas, USA | <sup>3</sup>Aquaculture/Fisheries, Center of Excellence, University of Arkansas Pine Bluff, Pine Bluff, Arkansas, USA

Correspondence: Yathish Ramena (ramenay@uapb.edu)

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#### **ABSTRACT**

Artemia (brine shrimp) is a foundational live feed in global aquaculture, renowned for its adaptability, ease of production, and favorable nutritional profile. However, in its natural state, Artemia contains suboptimal levels of highly unsaturated fatty acids (HUFAs), particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are vital for supporting growth, survival, and immune function in both marine fish and freshwater fish. To enhance its nutritional efficacy, various enrichment strategies have been developed, utilizing oil emulsions, microalgae, yeasts, probiotics, soy lecithin, and trace elements such as selenium and zinc. Each enrichment method offers unique physiological benefits: oil emulsions and microalgae increase PUFA concentrations; soy lecithin enhances fatty acid absorption and digestive efficiency; yeasts improve immune response and pigmentation; probiotics promote gut health and pathogen resistance; and trace minerals contribute to improved metabolic and reproductive performance. Although Artemia is extensively used in crustacean hatcheries, its use as live feed in shrimp larviculture is comparatively limited, despite its well-documented benefits. This review highlights the need for greater inclusion of enriched Artemia in shrimp culture, emphasizing its potential to improve larval performance and overall production outcomes. Optimized enrichment protocols are essential to advancing sustainable and resilient aquaculture systems. Future research should focus on cost-effective, species-specific enrichment strategies to meet the evolving nutritional requirements of modern aquaculture.

# 1 | Introduction: History of *Artemia* and Its Enrichment

Artemia sp. [1] commonly known as "brine shrimp" or "sea monkey," is one of the most widely used live feeds in aquaculture worldwide [2]. As a primitive arthropod closely related to shrimp [3] Artemia belongs to the order Anostraca within the class Branchiopoda [4]. The genus includes multiple species, such as A. franciscana, A. parthenogenetica, A. amati, A. sorgeloosi, A. sinica, A. urmiana, A. tibetiana, A. persimilis, and

A. salina. Artemia reproduces oviparously, producing encysted dormant embryos, and ovoviviparously, directly generating free-living nauplii. Under extreme conditions such as low temperature, dehydration, oxygen stress, and food stress, Artemia produces floating resting eggs (cysts) during dry seasons [5]. These cysts are collected, processed, and stored under dark and cold conditions for later distribution [6]. These dormant cysts are highly resilient against severe conditions and can give rise to new populations once both abiotic and biotic factors in the habitat become favorable again [5].

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Artemia franciscana [7] was first identified in Redwood City, the San Francisco Bay, while the Great Salt Lake in the USA represents one of its most abundant natural habitats [8]. Artemia is widely utilized as an off-the-shelf live feed in the form of dormant cysts that can be hatched on demand [9]. Newly hatched nauplii serve as an ideal larval feed due to their small size, high digestibility, and ease of enrichment [10] Artemia's high moisture content enhances its overall appeal, making it more palatable and easier to digest [11]. Artemia also functions as a potential filter feeder [12] and naturally contains high concentrations of α-linolenic acid (ALA) and linoleic acid (LNA) [13]. Its ability to absorb enrichment materials makes it a universally accepted live feed in hatcheries for various marine and freshwater species [14]. The protein content of Artemia can range from 40% to over 60% of dry weight, while lipid content averages between 15% and 30%, with the remainder consisting of carbohydrates and inorganic materials [15].

Although other live feeds, such as Moina and rotifers, are used, they are often considered less effective than Artemia for reasons related to nutritional quality, growth performance, and rearing efficiency. Alternative live feeds, such as cyclops, Moina, and rotifers, have been explored for larval nutrition in aquaculture. However, their nutritional deficiencies, particularly in essential fatty acids like HUFAs, limit their efficacy for supporting optimal larval growth and development [14, 16, 17]. Although enrichment strategies have been applied to improve their nutritional profiles, these feeds are often less effective when compared to *Artemia*, especially when the *Artemia* is enriched with essential nutrients [18, 19]. Enriched *Artemia* has consistently demonstrated higher nutritional value and superior performance in larval rearing, as evidenced by enhanced growth and feeding responses in fish larvae [2, 20, 21].

The wide-range use of *Artemia* is further supported by its ease of cultivation, stable production protocols, and scalability, which ensure consistent availability and quality in hatchery operations [14, 22, 23]. In contrast, the production of alternative live feeds, such as *Moina*, can be less reliable due to more complex culturing requirements and variability in nutrient content [14, 24]. Moreover, preference trials have shown that marine fish larvae often exhibit higher feeding rates, growth, and survival when fed with *Artemia* over other live feeds [25, 26]. The enhanced palatability, buoyancy, and physical characteristics of *Artemia* nauplii promote efficient ingestion and assimilation, contributing to its dominance as a live feed in larval aquaculture [21, 27].

# 1.1 | Application of Artemia in Early Life Stages of Aquaculture Species

The use of Artemia for shellfish, particularly shrimp such as Pacific white shrimp (*Litopenaeus vannamei*), is less. This is primarily due to shrimp's differing dietary requirements compared to fish. While juvenile fish heavily depend on live feed like Artemia for proper growth, shrimp larvae generally can adapt to other forms of nutrition more readily than fish, reducing the dependence on live feeds such as Artemia [27, 28]. Additionally, shrimp larvae can thrive on formulated diets made from less

expensive ingredients, decreasing the economic viability of using Artemia for their rearing [4, 28]. Thus, economic considerations alongside nutrient availability influence the extent of Artemia's use in aquaculture across different species.

Due to its high nutritional value, *Artemia* is extensively used to feed marine and freshwater fish larvae, serving as a critical live prey species for larviculture [29]. Numerous studies have demonstrated *Artemia*'s role as a supplementary feed for fish species, including *Carassius auratus* [30], *Gadus macrocephalus* [31], *Lates calcarifer* [32], *Dicentrarchus labrax* [33], *Rasbora argyrotaenia* [34], and crustaceans including *Macrobrachium rosenbergii* [35], *Litopenaeus vannamei* [36] and *Scylla paramamosain* [37]. The reliance on *Artemia* is more pronounced in finfish aquaculture due to the specific nutritional needs required for their growth. In contrast, shellfish aquaculture benefits from a broader array of feed options that meet their dietary needs without the high demand for enriched *Artemia*, thereby necessitating less usage of these resources [4, 27, 38, 39].

However, *Artemia* in its natural state contains insufficient concentrations of HUFA, such as EPA and DHA, which are essential for optimal larval growth and development in cultured species [40–42]. This deficiency necessitates enrichment to enhance *Artemia*'s nutritional profile. Research also indicated that relying on non-enriched *Artemia* can result in suboptimal larval growth and increased mortality due to inadequate essential fatty acid (EFA) levels during critical developmental stages [43]. Consequently, Artemia enrichment (Figure 1) has become a standard practice to enhance its biochemical composition, ensuring it meets the nutritional requirements of both finfish and shellfish [14].

Several researchers have investigated different enrichment strategies for *Artemia* (Figure 2), each targeting specific nutritional improvements. A variety of products and substances have been utilized for *Artemia* enrichment (Figure 3) and are commercially available in the market, including oil emulsion [40, 44–49]. Microalgae [15, 30, 50, 51]. Yeasts [30, 52–56]. Probiotics [56–60]. Soy lecithin [61–66]. This review focuses on identifying the most suitable type of enrichment based on the specific aquaculture species that will consume the enriched Artemia.

# 2 | Enrichment of Artemia

# 2.1 | Artemia Enrichment Using Oil Emulsions

Oil emulsion enrichment is a technique used to enhance the nutritional value of live feed organisms such as *Artemia*. Oil-inwater emulsions deliver lipids and micronutrients in a bioavailable form, often containing concentrated omega-3 fatty acids essential for aquatic species' hormonal and metabolic functions [44]. In the context of Artemia enrichment studies, oil emulsions serve as a vehicle for transferring essential HUFA, along with vitamins and other bioactive compounds, to Artemia. Emulsifiers maintain consistent nutrient distribution [45], which improves the absorption of lipophilic nutrients by *Artemia* [46]. These oil emulsions are designed to maximize lipid bio-accessibility while minimizing oxidation, which is critical during the developmental stages of Artemia [67, 68].

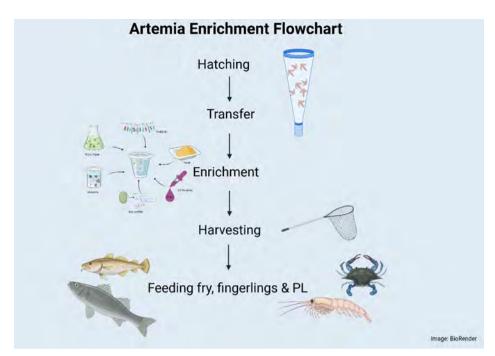


FIGURE 1 | Artemia enrichment process for enhanced nutritional delivery for cultured species.

Different oil types have been employed in *Artemia* enrichment protocols. Oil emulsions are oil-in-water systems formulated to deliver lipids and additional micronutrients in a bioavailable form. In the context of *Artemia* enrichment studies, oil emulsions serve as a vehicle for transferring essential HUFAs, such as DHA and EPA, along with vitamins and other bioactive compounds, to feed live *Artemia* [69]. These oil emulsions are designed to maximize lipid bio-accessibility while minimizing oxidation, which is critical when the *Artemia* are in early developmental stages [67, 68]. Fish oil-based emulsions are the most commonly used, as they provide a rich source of essential fatty acids, particularly DHA and EPA [70, 71]. Additionally, both animal-based and plant-based oils, such as soybean oil, have been reported as alternatives to conventional fish oil emulsions [72, 73].

Artemia enrichment strategies generally involve two types of oil emulsions: simple (single source) and complex (multisource) emulsions. Single-source emulsions consist of one type of oil, either fish oil, algae oil, or any other plant-based or animal-based oil. These single-source emulsions are intended only to develop the fatty acid profile of Artemia. By contrast, complex emulsions may incorporate additional ingredients such as vitamin E, minerals, and immune stimulants alongside the primary lipid source. These complex emulsions are intended not only to enrich the fatty acid profile but also to improve oxidative stability and support overall larval health through enhanced antioxidant protection and immune support [72, 74]. Tehrani et al. reported that enrichment using emulsions prepared from plant-based oils, such as canola oil, when complemented with antioxidants and vitamins, resulted in pronounced improvements in larval performance and environmental-stress resistance in subsequent feeding experiments [75]. Studies on juvenile seahorses and beluga larvae have indicated that a multi-nutrient approach via enriched Artemia enhances not only the fatty acid composition but also

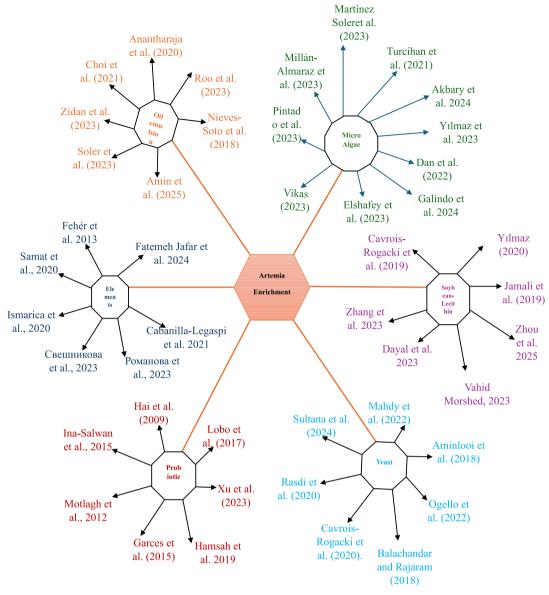
immune resistance and growth performance [76]. Finally, Adloo showed that combining HUFA-rich oils with vitamins (vitamin C and E) resulted in Artemia that could support better larval survival and stress resistance, possibly by limiting oxidative damage during the enrichment process [77].

# 2.1.1 | Mechanism of Oil Emulsion Enrichment

When Artemia ingests oil droplets suspended as an emulsion, the efficiency of nutrient uptake is primarily governed by the properties of the emulsion itself, as well as the environmental conditions during the enrichment process [2, 3]. The uptake mechanism is predominantly a result of the non-selective grazing behavior of Artemia, which enables them to ingest emulsion droplets along with other particulate matter [78]. Once ingested, the emulsified lipids are exposed to digestive enzymes in the gut, thereby facilitating hydrolysis and subsequent absorption of fatty acids [79].

# 2.1.2 | Factors Influencing Oil Emulsion Enrichment

A critical factor influencing enrichment success is the particle size of the emulsion droplets. Smaller droplets offer a greater surface area for enzymatic action, which enhances the dispersion of the oil phase in aqueous media and promotes lipid uptake and bioaccessibility [80–82]. High shear blending methods that generate smaller droplets improve emulsion stability and nutrient bioavailability due to the increased surface area-to-volume ratio [83]. Furthermore, reducing droplet size enhances cellular endocytosis and nutrient assimilation, as droplets below a certain diameter are internalized more efficiently [84]. Thus, careful control of the emulsification process, through methods such as high-pressure homogenization or sonication, is essential to produce droplets of an optimal size for Artemia absorption.



**FIGURE 2** | Schematic overview of recent publications in the field of *Artemia* enrichment. Each node represents a published study, with author names and publication years.

Salinity also critically affects both the emulsion characteristics and the biological response of Artemia. Variations in ionic strength can alter droplet stability by affecting the surface charge and aggregation tendencies of the emulsion particles [83]. Moreover, the salinity of the enrichment medium can directly influence the feeding activity of Artemia, as these organisms have a preferred salinity range where they are optimized [85]. Therefore, adjusting the salinity to align with Artemia's natural habitat conditions helps maintain both the emulsion stability and the overall health of the organisms during enrichment.

The duration for which Artemia are exposed to enriched emulsion is another important parameter. Sufficient contact time is needed for Artemia to consume an adequate amount of nutrient-loaded droplets, while excessive exposure may lead to oxidative degradation of sensitive PUFAs or other nutrients [79, 86]. Cheban et al. performed a 24-h enrichment, where measurements were taken every 6 h, demonstrating that prolonged

enrichment allows a steady incorporation of nutrients into Artemia [87, 88].

Maganhe and Sanches observed that the lipid content of Artemia peaked at 12 h and then decreased by 24 h [89]. This suggests that while overall lipid content may reach a transient peak earlier, the specific enrichment of long-chain polyunsaturated fatty acids might still benefit from a longer enrichment duration. Viciano et al. reported that under optimal conditions, including a single dose of  $\sim 0.8\,\mathrm{g/L}$  oil emulsion, incubation at 28°C, moderate aeration, and a naupliar density of 300 individuals per mL, the enrichment process could be optimized [71]. Although this study primarily focused on operational conditions, it provides indirect evidence that the duration must be balanced with these parameters to prevent oxidative degradation or suboptimal uptake.

Analyzing these studies suggests that, when using oil emulsions for Artemia enrichment, an enrichment period within the

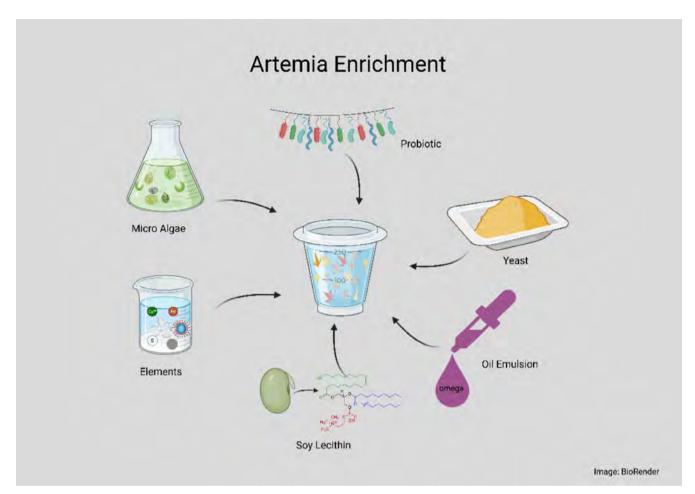


FIGURE 3 | Artemia enrichment strategies using various additives.

range of 12 to 24h appears to be effective. Specifically, a 12-h period might be sufficient for achieving high total lipid content; whereas, a 24-h period tends to favor the maximum incorporation of beneficial HUFAs (EPA and DHA) with diminishing returns and potential oxidative stress occurring beyond that duration. Therefore, the optimal enrichment duration should be chosen based on the targeted nutritional profile of the live prey, with most evidence supporting a 24-h enrichment protocol to maximize HUFA incorporation while being mindful of the physical and chemical stability of the emulsion.

Temperature plays a dual role; elevated temperatures can influence the kinetic motion of oil droplets, increasing collision velocities, and encouraging aggregation, which may lead to reduced bioavailability of the nutrients [84]. In parallel, higher temperatures can enhance the metabolic rate of Artemia, potentially increasing the digestion and assimilation rates of the ingested lipids [48, 73]. Identifying an optimal temperature that maintains emulsion stability and supports efficient metabolic activity is crucial for successful enrichment.

Finally, the concentration of the oil-in-water emulsion is also an essential parameter that must be considered. Optimizing the concentration of oil-in-water emulsions is critical for maximizing lipid uptake while avoiding adverse digestive effects. An increase in emulsion concentration can be beneficial by enhancing the availability of these nutrients to organisms such as Artemia [90]. However, when the concentration is excessively high, there is a propensity for the individual oil droplets to interact and agglomerate. Such droplet agglomeration can reduce the effective surface area available for enzymatic action and uptake, thus impeding efficient lipid absorption [91]. Moreover, aggregated droplets may disrupt normal digestive processes in Artemia, potentially leading to digestive disturbances and suboptimal nutrient assimilation [90]. Therefore, balancing concentration is key to ensuring that Artemia receive an adequate dose of nutrients without compromising the physical properties of the emulsion or their feeding behavior.

#### 2.1.3 | Impact on Aquaculture Species

Recent advances in aquaculture nutrition have underscored the pivotal role of enriching Artemia nauplii with oil emulsions to enhance growth, survival, and stress tolerance of several aquaculture species, as well as to improve their immune responses. Enriching Artemia with oil-based formulations, particularly those rich in PUFAs such as EPA and DHA, has been shown to beneficially alter the lipid profile of the Artemia, thereby enhancing the overall nutritional status of aquatic animals.

Comparable benefits have been observed in sterlet (*Acipenser ruthenus*) larvae, where  $Olio\omega 3$  and Red Pepper commercial oil emulsions enrichment of Artemia nauplii led to increased

levels of PUFAs in the larvae's tissues, correlating with higher growth and survival rates [92]. Furthermore, research on yellow perch (Perca flavescens) larvae confirms that feeding with Artemia enriched with PUFA-oil emulsion helps alleviate osmotic stress and facilitates crucial developmental stages, such as swim bladder inflation, thereby promoting overall larval viability [93]. Similarly, experiments involving Nile tilapia (Oreochromis niloticus) have revealed that supplementation with Artemia enriched using various oil emulsions resulted in measurable improvements in behavioral performance, survival, and specific immune parameters such as lysozyme activity and antioxidant capacity [48]. Moreover, Yin et al. reported that flaxseed oil-enriched Artemia, rich in omega-3 fatty acids, improved survival rates and fatty acid profiles in juvenile lined seahorses. The study highlights flaxseed oil's potential as a valuable enrichment source in aquaculture [94]. An additional study on palm ruff (Seriolella violacea) larvae provided evidence that oil-enriched emulsion containing DHA, EPA, supplemented with vitamin E, astaxanthin, and βglucan in Artemia could yield an 8% increase in survival and a 19% increase in growth, further accompanied by modulation of immune-related factors [95]. Canola oil enrichment can potentially improve larval stress resistance in severum fish, but comparative studies with marine-derived oils (cod liver oil) show conflicting fatty acid profiles [75]. Similar kinds of studies are included in Table 1.

Collectively, these studies highlight the importance of oil-based *Artemia* enrichment in aquaculture, advocating for its wider adoption to improve larval nutrition, stress resistance, and overall performance. Oil emulsion enrichment significantly enhances the growth, nutritional quality, and physiological health of aquaculture species by improving the fatty acid composition of *Artemia*, thereby supporting better growth, immune function, and overall health in aquatic animals reliant on enriched live feeds.

#### 2.2 | Microalgae Enrichment in Artemia

The use of microalgae as an enrichment source for Artemia in larval aquaculture has emerged as a robust strategy to address the nutritional limitations of Artemia nauplii, particularly regarding their inherent deficiency in certain LC-PUFAs, essential amino acids, vitamins, and pigments. Microalgae are recognized for their rich biochemical composition that includes  $\omega$ -3 fatty acids, carotenoids, essential amino acids, high-quality proteins, and vitamins, making them ideal candidates for supplementing the nutritional profile of live feeds [99–101]. Various microalgal species are commonly used due to their varied and complementary nutrient profiles, such as Spirulina, *Chlorella vulgaris* [50], *Dunaliella salina* [50], *Nanochloropsis* [20], *Isochrysis*, and *Aurantiochytrium mangrovei* [62] which are rich in essential nutrients, particularly PUFAs and other beneficial compounds.

Isochrysis galbana is especially valued for its high levels of DHA that significantly enhance the fatty acid profile of Artemia [102]. Nannochloropsis spp., known for its abundance of EPA, further complements these benefits by supplying another critical LC-PUFA, which is vital for the development and survival of fish and crustacean larvae [103, 104]. In addition, species such as

Tetraselmis spp. are incorporated due to their balanced fatty acid composition, good digestibility, and high carbohydrate content; these characteristics aid in the improved assimilation of nutrients during the early stages of larval growth [101, 105]. Furthermore, Schizochytrium spp. is recognized as an exceptional producer of DHA, enhancing the larval diets where DHA enrichment is necessitated [106, 107]. The nutritional enrichment process using microalgae has been shown to yield Artemia nauplii with enhanced quantities of essential biomolecules that are crucial for larval development. Enrichment procedures incorporate microalgae-derived compounds directly into Artemia, thereby augmenting their proximate composition and providing a more balanced blend of proteins, lipids, and micronutrients [108] (Table 8). This enriched Artemia has been associated with improved growth, survival, and stress tolerance in larval aquaculture, as evidenced by studies that document enhanced fatty acid profiles and overall nutritional status following microalgae supplementation [111, 112]. Notably, the enrichment strategy addresses the metabolic constraints of Artemia in desaturating and elongating fatty acids, a process that is inefficient in achieving adequate levels of essential LC-PUFAs for optimal larval nutrition [113].

Recent comparative studies have further underscored the importance of selecting microalgae species with specific nutrient profiles to tailor the enrichment process according to the nutritional requirements of target larvae. For instance, investigations have compared the effects of different microalgal strains on the nutritional enrichment outcomes of Artemia, finding that a combination of microalgae can deliver a more comprehensive nutrient blend than any single species alone [105, 114]. This is particularly critical in larval diets where the developmental stages necessitate a spectrum of fatty acids, pigments, and vitamins for proper growth, immune response, and biochemical functionality [112, 115].

Cumulatively, the use of microalgae in Artemia enrichment not only enhances the nutritional value of the live feed but also contributes to more sustainable aquaculture practices by reducing the reliance on fishmeal and fish oil, which are constrained by availability and economic factors [105]. The integration of microalgae thus plays a dual role in improving larval survival and growth performance while promoting a more environmentally friendly and economically viable aquaculture feed formulation [116].

In conclusion, microalgae serve as an essential enrichment tool for Artemia due to their superior nutrient composition, ease of cultivation, and capacity to provide critical fatty acids, amino acids, vitamins, and pigments. By improving the nutritional profile of Artemia, microalgae contribute significantly to enhancing larval aquaculture diets and overall aquaculture production, which is substantiated by a wide range of recent experimental studies.

## 2.2.1 | Enrichment Protocols

The enrichment of Artemia nauplii with microalgae is a critical process for enhancing their nutritional quality as live feed in aquaculture. The selection of microalgal form, system dynamics,

**TABLE 1** | Summary of studies on oil-emulsion enrichments of *Artemia* in several aquatic animals.

Study	Treatments	Result	References
Pacific cod (Gadus macro cephalus) Artemia enriched with Oil emulsion	alus) Artemia enriched with T2—DHA—33.42%, EPA—1.55% higher survival in Pacific cod		[31]
Macrobrachium rosenbergii Fed with EPA and DHA-enriched Artemia	The PL of <i>M. rosenbergii</i> was fed with T1—freshly hatched, T2—12 h enriched T3—12 h un-enriched T4—24 h enriched T5—24 h un-enriched	Artemia enriched for 24h contained significantly higher levels of EPA (3.76% ±0.11%) and DHA (2.29% ±0.01%), which declined thereafter. A 24-h enrichment period was identified as optimal, enhancing the growth and survival of Macrobrachium rosenbergii larvae	[40]
Amberjack (Seriola dumerili) fed with enriched Artemia using different oil emulsion concentrations	Amberjack (Seriola dumerili) Five experimental emulsions ed with enriched Artemia containing increasing levels of enhances the n-3 LC-HUFA containing different oil emulsion  EPA from 0.8% to 60% of TFA  of Artemia sp., thereby improving		[49]
Nile tilapia with enriched Artemia franciscana using different oil emulsions	T—unenriched Artemia franciscana (G0) (control) T2—soybean oil (G1) T3—sesame oil (G2) T4—rice bran oil (G3)	Soybean oil-enriched $Artemia$ nauplii showed the highest protein (53.6% $\pm$ 0.565%) along with superior weight, length, and survival rates Soybean oil enrichment increased protein to 53.6% and survival to 92%	[96]
Persian sturgeon (Acipenser persicus) larvae. Fed with Artemia enriched with Fish oil (Kilka fish Clupeonella grimmi oil) Soybean oil supplemented with vitamin E	T1 (Control): Non-enriched  Artemia nauplii  T2 (S15): Soybean oil  +15% vitamin E  T3 (S30): Soybean oil  +30% vitamin E  T4 (F15): Fish oil +15% vitamin E  T5 (F30): Fish oil +30% vitamin E	Soybean oil with vitamin E did not change DHA content in <i>Artemia</i> nauplii, but fish oil enrichment significantly increased DHA. Fish larvae fed enriched <i>Artemia</i> (F30 and S30) had higher final weight and weight gain, while vitamin E content in larvae from S15 and S30 was significantly greater than in other treatments	[72]
Octopus bimaculatus paralarvae fed with Artemia enriched with Tuna Orbital Oil (TOO)	T1—non-enriched <i>A</i> .  franciscana (control)  T2—Artemia enriched with TOO	After 5 days of hatching, paralarvae fed the TOO group had a survival rate of $66.5\% \pm 11.3\%$ , compared to $43.7\% \pm 10.4\%$ in the control group. The TOO group also significantly increased total acid activity by 8 and 11 days after hatching	[97]
Artemia enriched with Gamat emulsion, which is an extract of sea cucumber	A—Control (no enrichment) B—1 mL/L gamat C—5 mL/L gamat D—10 mL/L gamat E—15 mL/L gamat F—20 mL/L gamat	Gamat emulsion extract significantly enhanced larval WG, LG, and SR $(p < 0.05)$ , but had no significant effect on SGR $(p > 0.05)$ . The optimal results were at treatment D $(10  \text{mL/L})$ , with WG of $0.00065 \pm 0.000057  \text{g}$ , LG of $3.43 \pm 0.12  \text{cm}$ , and SR of $86.67\% \pm 7.09\%$	[98]

 $Abbreviations: LG, length\ gain; SGR, specific\ growth\ rate; SR, survival\ rate; TOO, tuna\ orbital\ oil; WG, weight\ gain.$ 

feeding duration, density, and the combination of multiple species are parameters that have been extensively researched, as evidenced by recent studies.

Live, paste, and freeze-dried microalgae each present unique advantages and limitations during enrichment. Live microalgae

tend to maintain higher levels of labile compounds, such as vitamins and bioactive pigments, compared to their processed counterparts [117]. Conversely, paste and freeze-dried formats, such as spray-dried products, often exhibit modified physicochemical properties, including reduced particle size, which can enhance nutrient uptake by Artemia [78]. For instance, studies have

demonstrated that processed microalgae, when combined with lipid emulsions, can significantly enhance the incorporation of essential fatty acids like DHA and EPA into Artemia; thus, improving the nutritional profile available to fish larvae [78]. Moreover, the use of freeze-dried or paste formulations may facilitate a more stable product for hatcheries prone to crashes in fresh culture availability [118].

System design during the enrichment process is equally important. Static systems, where Artemia are enriched in relatively still water, can sometimes lead to sedimentation of microalgal cells and uneven nutrient availability. Dynamic systems that incorporate water movement or aeration can improve the contact rate between Artemia and microalgal cells, thereby enhancing the bioencapsulation of nutrients [119]. Such systems ensure a homogenous suspension of microalgae, prevent cellular aggregation, and consequently improve the retention of essential nutrients [119]. The choice between static and dynamic systems must consider the hatchery infrastructure, economic constraints, and desired enrichment outcomes.

The duration of the enrichment process is another critical parameter. Most protocols recommend a feeding duration in the range of 2–6 h, which is sufficient for Artemia to accumulate optimum levels of PUFAs and other essential nutrients [120]. Notably, Millán-Almaraz et al. observed that a short enrichment period (~6–8 h) improved the PUFA profile of Artemia, although the exact duration may need to be adjusted based on environmental conditions and the specific microalgal species used [15]. Similarly, experiments that track enrichment time intervals (from 0 to 9 h) have helped establish benchmarks for achieving an optimal balance between nutrient uptake and potential declines in microalgal viability over time [102].

Microalgal density and concentration during enrichment are fundamental to ensuring adequate nutrient uptake. Using concentrations such as  $10^7$  cells mL<sup>-1</sup>, as seen in several studies, achieves a sufficient dose of microalgal biomass to bioencapsulate vital nutrients [121]. In some protocols, concentrations are tailored based on the microalgae species' growth rate and nutrient content, with studies demonstrating that even slight variations in cell density can markedly affect Artemia's biochemical composition [102]. These optimized concentrations facilitate efficient nutrient transfer from the algae to Artemia, thereby enhancing the overall quality of the live feed.

Co-enrichment or co-feeding with multiple microalgal species has emerged as a promising strategy to achieve a broader nutrient spectrum. The rationale for combining species is to exploit the complementary nutritional profiles offered by different microalgae. For example, Planas et al. described an enrichment protocol that incorporated live microalgae (*P. tricornutum* and *I. galbana*) along with dried Spirulina, thus providing a diverse profile of carotenoids, essential fatty acids, and vitamins [121]. The synergistic effect of such combinations has been shown to improve not only the concentration of LC-PUFAs within Artemia but also their antioxidant properties [122]. These improvements in the nutritional quality of Artemia can lead to better growth, survival, and immune responses in the subsequent larval stages of cultured species [4].

In conclusion, these studies underscore the importance of considering microalgae form (live vs. paste vs. freeze-dried), system dynamics (static vs. dynamic with aeration), feeding duration, and optimal algal densities in designing effective enrichment protocols for Artemia. Additionally, combining multiple algal species provides a multifaceted nutrient profile that further enhances the nutritional value of Artemia. Collectively, these findings help refine enrichment strategies to support the healthy development of marine larvae in aquaculture.

#### 2.2.2 | Mechanism

The mechanisms by which Artemia ingest and assimilate microalgae during enrichment involve a complex interplay of physical filtration, biochemical digestion, and subsequent nutrient transfer influenced by multiple factors. In Artemia, filterfeeding is mediated by specialized appendages that generate water currents to capture and carry along microalgal cells; these cells are captured by the setae and transported toward the mouth, where they enter the digestive tract [102, 123]. This feeding mechanism is effective for capturing microalgal cells. The physical properties of microalgae, such as cell size and morphology, directly affect the capture efficiency and subsequent assimilation of essential fatty acids and other nutrients [117].

#### 2.2.3 | Factors Influencing Microalgal Enrichment

Algal density (cells/mL) is a critical factor determining the ingestion rate and overall nutritional benefit during enrichment. When the density of microalgal cells increases, the encounter rate between Artemia and the cells rises, thereby enhancing filtration rates and nutrient uptake [102]. However, excessive cell density may lead to an increased organic load in the rearing medium, raising the bacterial load, as observed that organic inputs and excretions during enrichment can stimulate opportunistic bacteria proliferation [124]. Thus, an optimal density must balance maximal ingestion with minimal adverse microbial effects.

Feeding duration is another key parameter affecting the assimilation process. Experimental studies indicate that short-term enrichments (3 to 7 h) can optimize the lipid profile of Artemia by maximizing the deposition of PUFAs such as EPA and DHA, which are vital for the development of fish and crustacean larvae [102]. Prolonged enrichment may initially promote body size and lipid content increases, but can eventually lead to metabolic catabolism of the ingested nutrients, reducing the overall quality of the enrichment if not consumed rapidly by the larvae [102].

Algal cell size and structure also play important roles. Smaller microalgal cells are generally ingested more readily due to easier passage through the filtering apparatus; for instance, species like Spirulina, which lack rigid cellulosic walls, are rapidly digested and assimilated [125]. Conversely, larger cells or those with thicker cell walls may persist longer in the gut, affecting both retention time and assimilation efficacy [117]. This size dependency highlights the need to select microalgal species based not only on their intrinsic nutritional content but also on their physical properties.

In conclusion, optimized Artemia enrichment depends on balancing algal density, feeding duration, and microalgal type. Too much algae concentration or an overly long enrichment duration can harm water quality or nutrient value. By carefully managing algal density, enrichment duration, and the type of microalgae used, we can significantly enhance the nutritional quality of Artemia, ultimately supporting healthier growth in fish and crustacean larvae.

#### 2.2.4 | Impact on Aquaculture Species

Research has shown that microalgae enrichment enhances the biochemical composition of Artemia nauplii, which, in turn, improves the growth and survival of fish and shrimp larvae. For example, Sanudin et al. demonstrated that enriching Artemia with specific microalgal species positively influenced larval morphogenesis and survival in Scylla tranquebarica, highlighting that the nutrient profile of enriched Artemia contributes directly to improved larval growth [117]. Similarly, Vikas indicated that Artemia enriched with a balanced blend of microalgae species provides a more complete fatty acid profile, including EPA and DHA, which is critical for early developmental stages [102]. Dan et al. revealed that Artemia effectively digested the microalgae, Chlorella vulgaris and Nannochloropsis oculata, and the combined supply of these species with additional digestible materials supported continuous Artemia production while enhancing its nutritional composition. The findings suggest that strategic enrichment can improve both digestibility and nutrient uptake in Artemia [51]. Similarly, Chakraborty et al. found that Artemia enriched with microalgae displayed improved fatty acid profiles suitable for larviculture. This indicates that a range of aquatic species can benefit from enriched Artemia as feed [3].

Furthermore, microalgae enrichment has been linked with improved immune parameters in larvae, notably by increasing antioxidant activity and lysozyme activity. Eryalcin reported that specific microalgal mixtures not only enriched the fatty acid composition of Artemia but also enhanced metabolites associated with immune metabolism and stress resistance [119]. The enhanced transfer of HUFAs from microalgae to Artemia, supported by findings from [15]. In addition to growth and immune improvements, enriched Artemia have also been shown to have beneficial effects on pigmentation and stress tolerance in post-larvae. El-Khodary et al. observed that different microalgal species used in enrichment protocols significantly affected pigmentation in *Solea aegyptiaca* larvae, with better pigment retention and expression leading to more robust post larvae [104].

In summary, the integration of different microalgae species into Artemia enrichment protocols establishes an effective strategy to optimize the nutritional quality of live feed. This strategy benefits aquaculture species across multiple dimensions: improved growth and survival rates through enhanced nutrient profiles; boosted immune parameters such as antioxidant and lysozyme activities through better transfer of essential fatty acids; and enhanced pigmentation and stress tolerance in post larvae, a critical factor for long-term aquaculture success. The convergence of evidence from these recent studies confirms that microalgae enrichment of Artemia represents a sustainable and efficacious approach to improving larval quality in aquaculture systems.

These studies (Table 2) collectively highlight the benefits of microalgal enrichment in *Artemia*, demonstrating growth, survival, and biochemical composition improvements. A systematic understanding of *Artemia* digestion, growth metrics, and biochemical changes in response to microalgae enrichment enhances our knowledge of aquaculture nutrition and live feed optimization.

# 2.3 | Soy Lecithin Enrichment

Soy lecithin, a plant-derived material extracted from soybeans, serves as a cost-effective source of phospholipids and fatty acids for enriching Artemia. Its composition, including high levels of phosphatidylcholine, phosphatidylethanolamine, and linoleic acid, enhances the nutritional quality of Artemia nauplii used in aquaculture [43, 61, 62, 131, 132].

The enrichment process typically utilizes soy lecithin's emulsifying properties to integrate lipid molecules into aqueous enrichment media. Research has reported using doses of soy lecithin ranging from 2 to 12g/kg in Artemia culture systems [69]. These methodologies not only boost the phospholipid content in Artemia but also allow for modulation of fatty acid profiles, resulting in increased levels of n-6 PUFAs and adjustments in other essential fatty acids [62, 65]. Such modifications are important because they positively affect the growth, survival, and reproductive performance of cultured species that consume these enriched diets [63]. The benefits of soy lecithin-based enrichment are extensive. Economically, its plant origin ensures affordability and wide availability, making it an attractive alternative to more expensive marine-derived phospholipids [132]. Biologically, enriched Artemia have demonstrated improved nutritional outcomes that support early developmental stages and enhance reproductive capacity in aquaculture species [43, 63].

Additionally, soy lecithin poses a lower microbial and pathogen risk compared to some other dietary supplements, further highlighting its utility [132]. However, because soy lecithin inherently has lower levels of LC-PUFAs, careful optimization, and potential supplementation with marine-derived LC-PUFAs are necessary for maintaining optimal fatty acid profiles [62, 65]. Despite these advantages, challenges exist in using soy lecithin for Artemia enrichment. One concern is its sensitivity to processing conditions, such as heating and prolonged aeration, which can alter its emulsifying properties and modify its phospholipid composition [133]. Moreover, variations in the biochemical and fatty acid profiles from its plant origin necessitate precise dosage and formulation optimization to prevent adverse effects, such as potential reductions in lipid digestibility in certain species [134]. Balancing the increase in n-6 PUFA levels and maintaining an optimal overall fatty acid profile continues to require further research [65, 69].

## 2.3.1 | Extraction of Soy Lecithin

A fundamental step in this process is the extraction and supply of soy lecithin. Industrially, soy lecithin is typically obtained as a by-product of soybean oil processing. The extraction involves

 TABLE 2
 Summary of studies on microalgae enrichments of Artemia in several aquatic animals.

Study	Treatment (T)	Result	References
Greater Amberjack (Seriola dumerili) fed with artemia enriched with Nannochloropsis, Spirulina	Artemia were separately enriched with Nannochloropsis, Algamac 3080, and Spirulina, and unenriched Artemia nauplii served as control feed	Significant differences in growth and survival in larvae fed enriched Artemia compared to the control	[20]
Penaeus vannamei postlarvae with enriched Artemia using microalgae	A—Artemia + microalgal emulsion A + dry diet B—Artemia + microalgal emulsion B + dry diet C—Non-enriched Artemia (Control)	DHA content was higher in treatments A and B (9.80% $\pm$ 0.71% and 9.75% $\pm$ 0.44%) than in C (5.78% $\pm$ 0.68%) ( $p$ < 0.05). EPA content was greater in A and B (0.815% $\pm$ 0.06% and 0.86% $\pm$ 0.08%) than in C (0.43% $\pm$ 0.02%) ( $p$ < 0.05)	[47]
Goldfish (Carassius auratus) with enriched Artemia using microalgae spirulina and canthaxanthin	T1—Commercial diet (Control)  T2—Commercial diet +  un-enriched Artemia  T3—Commercial diet +  Spirulina-enriched Artemia  T4—Commercial diet +  Canthaxanthin-enriched Artemia  T5—Commercial diet + Spirulina and  Canthaxanthin-enriched Artemia	The highest carotene content was in <i>Artemia</i> enriched with Spirulina and Canthaxanthin (T5), and the lowest in T2. Goldfish fed <i>Artemia</i> diets (T3–T5) showed significant increases ( $p < 0.05$ ) in RBCs, Hb, WBCs, and lymphocytes, peaking in T5. Total protein, albumin, and globulin were highest in T5 and T4, while digestive enzyme activity showed no significant differences ( $p > 0.05$ ) among treatments	[30]
Effect of feeding with different microalgae on survival, growth, and fatty acid composition of <i>Artemia franciscana</i> metanauplii	T1—Amphora viridis (AV) T2—Chlamydomonas reinhardtii (CR) T3—Chlorella vulgaris (CV) T4—Dunaliella salina (DS) T5—Combination of all four microalgae (MX diet)	The MX group had greater total length and n-3 fatty acid content ( $p < 0.05$ ). Total n-3 HUFA levels were significantly higher in <i>Artemia franciscana</i> fed DS and AV diets ( $p < 0.05$ )	[50]
Co-culturing microalgae Navicula salinicola & Isochrysis galbana with oil emulsion	T1—Fresh Navicula salinicola (NFRE) T2—Frozen Navicula salinicola (NFRO) T3—Spray-dried Navicula salinicola (NSD) T4—Spray-dried Isochrysis galbana (ISD) mixed with a commercial oil concentrate (Incromega) or marine lecithin (LC 60)	Survival of <i>Artemia</i> was over 92% and significantly enhanced by microalgae and lipid emulsions. A mixture of <i>Isochrysis galbana</i> and lipid emulsion yielded higher DHA/EPA and EPA/ARA ratios, while combining microalgae with LC 60 lipid emulsion improved polar lipid and DHA incorporation	[78]
Artemia franciscana nauplii fed with Schizochytrium sp.	24-h-old <i>Artemia</i> nauplii were fed 400 mg/L of the algae for 24 h	DHA—undetectable levels to 0.8% of dry weight EPA—0.1%–0.5% of dry weight ARA—trace to 0.3% of dry weight	[126]
Co-culturing microalgae with Roseobacter clade bacteria for Vibrionaceae control in microalgae-enriched Artemia	Two microalgae combined with four bacteria, co-cultured in 24-well plates Medium: 2 mL SSW + 0.5 mL of 2-week-old microalgae (10 <sup>6</sup> -10 <sup>7</sup> cells/mL)  Control: Microalgae grown without bacteria (parallel setup)	Probiotics did not affect microalgae growth or significantly alter the associated bacterial community composition, according to DGGE analysis. Ruegeria-inoculated Phaeodactylum tricornutum cultures reduced the total Vibrionaceae count in Artemia by 2 log units	[127]

(Continues)

TABLE 2 | (Continued)

Study	Treatment (T)	Result	References
Artemia salina co-culturing microalgae with oil emulsion	T1-Nanochloropsis T2-Isochrysis T3-Pavlova T4-Cod liver oil	Survival of <i>Artemia</i> was highest with cod liver oil (95%), followed by <i>Nanochloropsis</i> , <i>Isochrysis</i> , and <i>Pavlova</i> . Cod liver oil also had the highest energy content (19 kJ) compared to other feeds (7.7 kJ to 11.6 kJ)	[128]
Alga Aurantiochytrium mangroves FIKU008-enriched Artemia fed for green tiger shrimp, Penaeus semisulcatus	Four different enrichment levels of AUR  T1—0.0  T2—0.6  T3—0.8  T4—1.0 g/L  T5—commercial solution  (S-presso) (Control)	DHA and EPA levels were significantly enhanced with AUR at 0.6 and 0.8 g/L. This enrichment effectively improved the growth and survival of the early larval stages of <i>Penaeus semisulcatus</i>	[129]
Microalgae premix (Padina australis, Sargassum ilicifolium, and Stoechospermum marginatum enriched Artemia urmiana in Litopenaeus vannamei)	T1—non-enriched metanauplii (MPE0) T2—Meta nauplii enriched with 200 mg/L (MPE200) T3—Metanauplii enriched with 400 mg/L (MPE400) T4—Metanauplii enriched with 600 mg/L (MPE600)	The highest SGR, percentage weight gain, PER, and dry matter were observed in the group fed <i>MPE600</i> -enriched metanauplii	[130]

Abbreviations: DGGE, denaturing gradient gel electrophoresis; Hb, hemoglobin; PER, protein efficiency ratio; RBCs, red blood cells; SGR, specific growth rate; WBCs, white blood cells.

solvent-based degumming processes, which separate phospholipids from neutral lipids, followed by purification steps that can include bleaching and deodorization [135]. Studies in food science have characterized the physicochemical properties of soy lecithin, especially through its role in emulsification and stabilization techniques [136, 137]. These investigations confirm the efficacy of extraction methods and provide insights into the behavior of soy lecithin when combined with other bioactive compounds. Such a background is crucial as the extracted soy lecithin is then supplied in an aqueous suspension or emulsion form that is directly used to enrich Artemia cultures.

### 2.3.2 | Mechanism

Once supplied, the mechanism by which Artemia nauplii absorb and incorporate soy lecithin has been elucidated by several research works that focus specifically on soy lecithin enrichment. Studies by Zhou et al. demonstrate that incubation of Artemia nauplii in soy lecithin-supplemented seawater results in a significant increase in phospholipid content. In these experiments, appropriate concentrations ( $10\,\mathrm{g/m^3}$ ) as reported by Panagiotakopoulos et al. [138] or varying percentages as investigated by Yilmaz [62] and incubation times (up to 12 h) have been optimized to maximize uptake. The primary route of incorporation is via the ingestion of fine suspensions of soy lecithin particles. Once ingested, the lecithin is assimilated into the digestive tract and subsequently deposited into various tissues, thereby elevating the overall phospholipid profile.

Cavrois-Rogacki et al. compared soy lecithin with marine lecithin in Artemia enrichment protocols. Their results indicate that while marine lecithin can enhance both the phospholipid and HUFA fractions (notably DHA), soy lecithin predominantly boosts the phospholipid content. This difference is attributed to the inherent absence of LC-PUFA in soy lecithin, underlining that the nutritional profile of the enrichment diet is intimately linked to the source of lecithin used [65]. In a related study, Guinot et al. have shown that when soy lecithin is combined with HUFA-rich emulsions, the metabolic machinery of ongrown *Artemia* meta nauplii can efficiently incorporate these components, suggesting that soy lecithin is not only a phospholipid source but can also facilitate the assimilation of complementary essential fatty acids [70].

The mechanistic insights into soy lecithin incorporation also extend beyond ingestion. Several studies examining lipid enrichment in Artemia suggest that surface adsorption may play a role. Once soy lecithin particles adhere to the outer body surfaces, they can be gradually ingested during routine feeding movements, a process that reinforces the importance of particle size and dispersion stability within the enrichment medium [65, 138]. Investigations into the emulsifying properties of soy lecithin, under its amphiphilic structure, demonstrate that it forms stable micelles in aqueous environments [136, 137] thereby enhancing its bioavailability for uptake by the nauplii.

A broader context is provided by reviews on the role of phospholipids in aquafeed development [139] and meta-analyses

comparing various live-prey enrichment protocols [140]. Although some studies have focused on marine lecithin [70], such works help in drawing comparisons that underscore the unique contributions of soy lecithin. While marine lecithin may deliver additional LC-PUFA, soy lecithin, due to its ready availability, cost-effectiveness, and consistent quality, is particularly efficient at elevating the phospholipid content of Artemia nauplii [65, 138]. Moreover, studies focused on the emulsion stability of soy lecithin [136, 137] corroborate that formulation parameters (surfactant concentration, particle size distribution, and processing conditions) are critical determinants of its performance in the enrichment process.

#### 2.3.3 | Parameters

A critical parameter is the concentration of soy lecithin in the enrichment medium. Studies have shown that varying lecithin levels can significantly influence the uptake of essential phospholipids and fatty acids in Artemia. For example, Yılmaz evaluated enrichment levels of 0%–4% soy lecithin and reported that even small increments improved the nutritional profile of Artemia and enhanced survival and growth of shrimp postingestion [62]. Similarly, Jamali et al. noted improvements in the biochemical composition of broodstocks and their progeny when using soy lecithin-enriched Artemia [43].

The duration of the enrichment period is another determining factor. Optimization studies suggest that short-term exposure can be sufficient to enrich Artemia nauplii with key phospholipids and highly unsaturated fatty acids without compromising their viability [65]. Lipid uptake during enrichment is time-dependent, where an optimal window exists for maximizing nutrient incorporation. Hence, precise calibration of the enrichment duration is vital to balance lipid loading and maintain the vitality of Artemia, which is essential for successful nutrient transfer in a trophic cascade.

Ambient environmental factors, such as temperature and salinity, play pivotal roles in the enrichment process. Temperature affects both the emulsification process and the physiological response of Artemia. Cavrois-Rogacki et al. showed that short-term chilling did not compromise the enriched nutritional profile of Artemia nauplii, maintaining the bioavailability of enriched lipids under modified thermal conditions [65]. While salinity was not explicitly studied in this context, it is well recognized as a critical parameter in Artemia cultivation and may interact with emulsion stability and nutrient uptake. Jamali et al. maintained controlled salinity conditions, which likely contributed to the reproducibility of their enrichment outcomes [141]. Furthermore, broader emulsion research illustrates that modifications in ionic strength or pH can affect potential and the stability of lecithin-based emulsions, thereby influencing enrichment efficiency.

In conclusion, successful enrichment of Artemia with soy lecithin relies on carefully orchestrating formulation (concentration and particle size), exposure time, and environmental control. Research suggests that optimal enrichment enhances the nutritional profiles necessary for the growth, survival, and reproductive success of cultured aquatic species. Further research should focus on refining these parameters, particularly

the interplay between salinity and emulsion stability, to maximize the benefits of soy lecithin-enriched Artemia in aquaculture.

## 2.3.4 | Impact on Aquaculture Species

The application of soy lecithin as an enrichment agent in Artemia has been studied extensively, and its efficacy has been demonstrated across multiple aquaculture species. Research comparing independent studies on soy lecithin-enriched Artemia indicates that such enrichment protocols can significantly enhance the nutritional value of live prey, thereby improving larval growth, survival, stress resistance, and digestive efficiency. For instance, Yılmaz demonstrated that shrimp larvae (*Penaeus semisulcatus*) exhibited better growth and survival when fed soy lecithin-enriched Artemia, aligning with other studies exploring phospholipid supplementation in live feeds [62].

Soy lecithin enrichment is associated with improvements in fatty acid composition, crucial for optimal development and metabolic regulation in larval fish and shrimp. Guinot et al. detailed how incorporating fatty acids from soy lecithin into Artemia nauplii facilitates the conversion of phospholipids to triacylglycerols, a process that may be instrumental in delivering highly unsaturated fatty acids required by many marine larvae [70]. Similarly, Jamali et al. reported that soybean lecithin enrichment enhanced the biochemical composition of Artemia and improved the fatty acid profile of eggs in cichlid broodstocks, suggesting enhanced reproductive performance alongside larval development [43].

The optimization of enrichment conditions is essential for realizing the benefits of soy lecithin. Estévez and Papiol investigated the emulsion properties and enrichment protocols, noting that while the total lipid content of Artemia did not always increase with higher concentrations of soy lecithin, the quality of the phospholipid profile and the balance of fatty acids improved [69]. These alterations in nutritional quality are central to achieving enhanced intestinal development and nutrient absorption, as observed in studies such as that by Zhou et al., which showed improvements in intestinal morphology and desiccation stress tolerance in yellow drum larvae following soy lecithin enrichment [138].

The benefits extend to reproductive performance and overall physiological resilience. In addition to improvements in growth and nutrient absorption, enriched Artemia has been linked to enhanced stress resistance parameters, such as increased tolerance to thermal and salinity stress. Jamali et al. corroborated that lecithin-enriched Artemia can induce improvements in digestive enzyme activities and reproductive biomarkers in both fish and shrimp larvae [141]. Liu et al. further demonstrated that younger larvae, which have a limited capacity to synthesize essential phospholipids de novo, particularly benefit from enriched live feeds, emphasizing the age-dependent nutritional requirements fundamental to early development [142].

Moreover, studies examining aquaculture species such as rock bream and yellowfin seabream have confirmed these positive

trends. Zhang et al. compared growth performance, body composition, and liver metabolic profiles in rock bream larvae reared on diets incorporating soybean lecithin-enriched Artemia and found that these larvae exhibited better overall performance than those fed traditional microdiets [66]. Similarly, Morshedi et al. reported that yellowfin seabream larvae fed soy lecithin-enriched live prey not only grew faster but also developed a more robust stress resistance and digestive enzyme profile [143]. These findings have implications for improved management practices in shrimp, as improvements in white shrimp (*Litopenaeus vannamei*) and other economically important species such as seabass and grouper have also been indirectly supported via analogous mechanisms in larval nutrition.

This enrichment strategy leads to improved growth and survival, enhanced stress resistance, and better intestinal development and nutrient absorption in fish and shrimp larvae. The convergence of results across various experimental models suggests that soy lecithin-enriched Artemia is a promising tool for the development of cost-effective, nutritionally robust aquaculture practices. Future research may focus on optimizing soy lecithin inclusion levels and enrichment times to further refine feeding regimes for high-value species such as seabass, grouper, and white shrimp. These studies (Table 3) highlight soy lecithin enrichment's significant nutritional and physiological advantages, making it a promising additive for optimizing live feed nutrition in aquaculture systems.

# 2.4 | Yeast Enrichment in Artemia

The use of yeasts as an alternative or supplement for Artemia enrichment has attracted considerable attention because they provide a multifaceted nutritional boost that can help overcome the limitations of conventional live feeds. Yeasts deliver high-quality protein and essential amino acids while also offering bioactive compounds such as  $\beta$ -glucans, mannan-oligosaccharides, vitamins (B-complex), and antioxidant molecules that together contribute to improved health and performance in cultured aquaculture species [146]. These bioactive components serve as immune boosters, facilitating the development of a more resilient immune system in aquaculture species [147]. This enrichment strategy potentially enhances the immunological competence of the prey, which in turn may support faster growth rates and higher survival in the target aquaculture species [52].

Among the yeast species used, *Saccharomyces cerevisiae* (baker's yeast) has been widely studied for its high protein content and its capacity to yield immunostimulatory  $\beta$ -glucans. These compounds have been shown to modulate immune responses in diverse aquatic organisms, as evidenced by improvements in immune parameters in both vertebrates and invertebrates [146, 148]. *Candida utilis*, also known as Torula yeast, is another yeast species that has been employed in artemia enrichment studies. It is particularly valued for its high nucleic acid content, which can play a role in promoting growth and immune health by supporting nucleic acid metabolism in developing larvae. In feeding trials with species such as rainbow trout, partial replacement of fishmeal with torula yeast led to improved growth performance and enhanced antioxidant capacity [149].

Phaffia rhodozyma, frequently used as a source of astaxanthin, represents a specialized case whereby the enrichment not only supplies macronutrients but also imparts potent antioxidant benefits [150]. Astaxanthin, a carotenoid with strong free-radical scavenging abilities, has been demonstrated to improve the antioxidative capacity and overall health status of aquaculture species [151, 152]. This dual role as both nutrient and bioactive compound makes Phaffia rhodozyma particularly advantageous for Artemia enrichment protocols aimed at bolstering the oxidative stress resistance of subsequent fish or shrimp larvae. Similarly, the marine yeast Debaryomyces hansenii attracts researchers due to its inherent salt tolerance and rich profile of Bvitamins and amino acids; these characteristics align well with the saline rearing conditions of Artemia, making it a promising candidate for live feed enrichment, although direct reports on its use in Artemia are not as prevalent as those for baker's yeast.

In addition to whole yeast cells, the use of commercial yeast extracts, whether hydrolyzed or autolyzed, has proven beneficial by providing easily digestible peptides, free amino acids, and smaller bioactive molecules [2]. These extracts have been incorporated into fish diets, leading to favorable outcomes such as enhanced growth and improved immune status, further highlighting their potential as a supplement in Artemia enrichment strategies [153].

Overall, the diversity of yeast species available, each characterized by unique nutritional and bioactive profiles, offers aquaculture practitioners a versatile tool kit. Enriching Artemia with these yeasts not only improves the basic nutritional quality but also introduces immunostimulatory compounds ( $\beta$ -glucans and mannan-oligosaccharides), vitamins, and antioxidants that are critical in sustaining the health and performance of aquaculture species [154]. Such an integrated nutritional approach is likely to mitigate common deficiencies and stressors encountered in larval rearing environments.

#### 2.4.1 | Mechanism of Enrichment of Artemia With Yeast

Artemia are filter feeders that generate water currents using their feeding appendages, allowing them to capture suspended particles efficiently. Yeast cell walls are inherently robust and primarily composed of components such as  $\beta$ -glucans, mannans, and chitin. When yeast cells are provided to Artemia in an intact form, the tough cell wall can hinder the availability of intracellular nutrients because the digestive enzymes of Artemia may not break down the cell wall efficiently [155]. Conversely, pre-treating yeast to disrupt or break the cell wall can lead to improved digestibility, thereby enhancing the nutritional quality of the bioencapsulated feed. This distinction between intact and broken yeast cells directly affects how Artemia metabolizes the ingested particles, influencing growth performance and overall health [155–157].

# 2.4.2 | Factors Influencing Yeast Enrichment in Artemia

The enrichment of Artemia nauplii with yeast is influenced by several interrelated factors that determine both the nutritional

**TABLE 3** | Summary of studies on soybean-lecithin enrichments of *Artemia* in several aquatic animals.

Study	Treatments	Result	References
Cichlid green terror (Aequidens rivulatus) fed with enriched Artemia franciscana with soy lecithin	Ten dietary treatments at five different replacement levels (0%, 25%, 50%, 75%, and 100%) of the CD with either UA or EA	Soy lecithin enrichment of $Artemia$ $franciscana$ significantly increased body lipid content in green terror broodstock at 25%, 50%, and 75% EA ( $p$ < 0.05), with no changes in dry matter, protein, or ash content The highest total polar lipid (18.26%) and elevated PUFA and DHA levels were observed in the 50CD:50EA group ( $p$ < 0.05)	[43]
Yellow Drum ( <i>Nibea</i> albiflora) larvae fed with <i>Artemia</i> nauplii enriched with soy lecithin	T1—SL group T2—NH group (Control)	The SL group had significantly greater body weight and standard length than the NH group ( $p$ <0.05) Soy lecithin-enriched <i>Artemia</i> improved growth, desiccation stress tolerance, lipid catabolism, intestinal structure, and immune responses in yellow drum larvae	[138]
Soy lecithin-enriched Artemia nauplii and microdiet in rock bream (Oplegnathus fasciatus) larvae	T1—SL-enriched Artemia nauplii T2—SL-enriched MD	Feeding with MD increased larval standard length and growth rate compared to live prey MD-fed larvae had reduced lipase activity and lower alanine and aspartate aminotransferase levels, indicating decreased amino acid catabolism	[66]
Soy lecithin-enriched <i>Artemia</i> nauplii in yellowfin seabream ( <i>Acanthopagrus latus</i> ) larvae	T1—very low (2%, N) T2—low (4%, L) T3—medium (8%, M) T4—high (12%, H)	Larvae fed live prey enriched with 8% and 12% SL had higher survival rates ARA and DHA accumulation increased in larvae fed high SL-supplemented foods Moderate SL levels (4%–8%) are recommended for enriching live food in <i>Acanthopagrus latus</i>	[144]
Soy lecithin-enriched <i>Artemia</i> Indian black tiger shrimp <i>Penaeus monodon</i> , reared under hyperosmotic stress conditions	DL-1 (Control), DL-1.5, DL-2 and DL-2.5 were formulated by including soy-lecithin at the rate of 1%, 1.5%, 2%, and 2.5%, respectively	RGR was significantly higher in DL-2 and DL-2.5 groups ( $p < 0.05$ ) Body lipid content was also higher (3.66%) in DL-2 and DL-2.5 compared to DL-1 and DL-1.5 (3.25%-3.42%) ( $p < 0.05$ ) Soy lecithin is effective under hyperosmotic stress and is recommended at $> 2.5\%$ in $P.$ monodon diets	[145]

Abbreviations: ARA, arachidonic acid; CD, commercial diet; EA, lecithin-enriched *Artemia*; MD, microdiet; NH, newly hatched; SL, soy lecithin-enriched; UA, unenriched *Artemia*.

quality of the live feed and its subsequent performance during larviculture. One of the key parameters is the yeast concentration, generally applied in the range of  $0.5-5\,\mathrm{g/L}$ . This concentration window ensures that enough yeast cells are available to be assimilated by the Artemia while avoiding any negative impacts of oversupply that might interfere with Artemia survival or lead to excessive nutrient deposition [158]. Precise calibration of the yeast dosage is essential to achieve optimal uptake and retention of beneficial compounds, as observed by studies evaluating probiotic yeast enrichment [159].

Feeding or enriching time is another critical parameter. Although enrichment durations in the literature span from 2 to 6 h, the optimal period is determined by balancing sufficient ingestion of yeast cells against prolonged exposure that might compromise Artemia viability or lead to nutritional imbalances. Experimental work suggests that relatively short enrichment

times (between 2 and 4h) can maximize yeast uptake while maintaining Artemia vitality, a concept reflected in evaluations of the best time and concentration for yeast probiotic enrichment [159]. This timeframe allows for the direct ingestion of yeast cells without relying predominantly on yeast adherence to the Artemia surface, a factor that plays only a minor role in overall enrichment [158].

The physiological status of the yeast (live versus inactivated) further contributes to the efficacy of enrichment. Studies comparing live yeast cultures with inactivated (dead) cells indicate that live yeasts may confer additional benefits, such as improved immune responses and enhanced reproductive indices in fish when delivered via enriched Artemia [160]. For instance, the use of  $\beta\text{-ME-treated}$  yeast resulted in significant improvements in immune parameters in ornamental fish fed with enriched Artemia, pointing toward potential benefits related to yeast

metabolic activity and cell wall integrity that are lost upon inactivation [160]. Conversely, direct feeding of yeast suspensions, even when using inactivated cells, is effective for delivering nutritional benefits, suggesting that the viability of yeast may be less critical if processed appropriately [161].

In addition to manipulating concentration and exposure time, the strategy of co-enrichment, by combining yeast with other enrichments such as microalgae or oil emulsion, offers a method for further enhancing the nutritional profile of Artemia. Co-enrichment can improve the fatty acid profile (increasing n-3 HUFAs) and supply other essential micronutrients, thereby benefiting larval growth and survival [20]. Such strategies leverage the complementary benefits of different enrichment agents, allowing for a more balanced nutritional input than yeast enrichment alone.

Taken together, the optimal design for Artemia enrichment with yeast involves careful control of the yeast concentration within the 0.5–5 g/L range, a precisely timed enrichment process (typically 2–6h), and consideration of the yeast's viability. While live yeast cultures may offer superior benefits over inactivated cells because of their metabolic activity and subsequent health-promoting properties, direct feeding of yeast suspensions and coenrichment approaches present viable alternatives for achieving desired outcomes in larval rearing systems [20, 158, 160, 161].

#### 2.4.3 | Impact on Aquaculture Species

Several studies have demonstrated that larvae fed with yeastenriched Artemia experience improved survival and faster growth. For instance, research on larval Korean rockfish indicates that enrichment strategies, including those utilizing yeast components such as yeast, enhance nutritional quality and thus result in higher survival and faster growth rates [162]. This improvement in early life stage performance is crucial because better nutritional provisioning during the larval phase translates into a more robust establishment of cultured populations. A key advantage of yeast enrichment is its role in pigment synthesis, particularly melanin, which influences aquatic species' coloration and overall health. Research has demonstrated that Artemia enriched with specific yeast strains contributes to increased melanin synthesis, subsequently enhancing the pigmentation of fish species such as Siamese fighting fish (Betta splendens) [53].

Moreover, yeast enrichment is crucial for improving lipid profiles. Sultana et al. reported substantial enhancements in the fatty acid profiles of yeast-enriched *Artemia*, noting the critical role of these improved compositions in fostering growth and development in aquatic species [52]. Balachandar and Rajaram demonstrated that yeast supplementation enhances protein and lipid content in live feeds, improving nutritional quality. This cost-effective approach supports the health and growth of aquatic species, making it ideal for resource-limited aquaculture systems [54]. Similarly, Aminlooi et al. found that *Saccharomyces cerevisiae*-enriched *Artemia* improved reproductive performance, immune response, and disease resistance in *Poecilia latipinna*. Enhanced survival and growth without adverse effects highlight yeast's value as a safe, effective dietary

supplement in aquaculture [160]. Moreover, Elshafey et al. reported that Artemia enriched with yeast improved growth,  $\beta$ -carotene content, pigmentation, and immune responses in  $Carassius\ auratus$ . These benefits were attributed to the high polyunsaturated fatty acid (PUFA) content, enhancing larval development [30].

Similarly, yeast enrichment has been linked with enhanced immune responses in aquatic larvae. A study on live-bearing ornamental fish (Poecilia latipinna) demonstrated that the supplementation of Artemia with Saccharomyces cerevisiae led to significant increases in lysozyme activity as well as disease resistance, likely due to the bioactive compounds (e.g.,  $\beta$ -glucans and yeast-released polypeptides) that function as immunostimulants [160]. These compounds promote innate immune defenses and prime the larvae for better pathogen resistance. Furthermore, Wang et al. demonstrated that yeast-based diets improve immune responses due to their rich content of proteins, vitamins, and bioactive compounds [163]. Similarly, Abdel et al. highlighted yeast's role as a feed additive in aquaculture, improving immune responses, growth, and overall health. The enriched Artemia's fatty acid profile further enhances diet quality, promoting better growth and health in aquatic animals [164]. Moreover, Khanjani et al. reported increased lysozyme activity and improved immune defense in fish larvae fed yeast-enriched Artemia. The β-glucans in yeast were key to the enhanced immune responses observed [56].

The collective findings from the reviewed studies support the assertion that yeast enrichment effectively enhances the nutritional and immunological profile of live aquaculture feeds, particularly Artemia. Specifically, yeast supplementation has been shown to improve key performance indicators in aquaculture species, including increased survival, accelerated growth, enhanced pigmentation through melanin synthesis, and bolstered immune responses, thereby establishing more robust cultures during critical early life stages. The bioactive compounds present in yeast, such as β-glucans and polypeptides, stimulate innate immunity and contribute to improved lipid and protein profiles, which are essential for the healthy development of aquatic larvae. These documented benefits make yeast enrichment a promising, cost-effective strategy, especially in resourcelimited aquaculture systems, warranting further research to optimize its application and maximize benefits across diverse species.

These studies (Table 4) highlight yeast enrichment's significant nutritional, immunological, and physiological advantages, making it a promising additive for optimizing live feed nutrition in aquaculture systems.

# 2.5 | Probiotic Enrichment in Artemia

Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts [166]. The use of probiotic-enriched Artemia as a vehicle to deliver beneficial microbes to fish and shrimp larvae has garnered increasing attention in aquaculture due to its potential to enhance larval health and performance of Artemia, which benefits larviculture practices in aquaculture. Traditionally, Artemia

**TABLE 4** | Summary of studies on yeast enrichments of *Artemia* in several aquatic animals.

Study	Treatments	Result	References
Influences of Baker's yeast (Saccharomyces cerevisiae) 10 <sup>6</sup> to 10 <sup>8</sup> cells/mL on Artemia Salina Nauplii to improve the PUFA Composition	T1—Saccharomyces cerevisiae T2—Chlorella salina T3—Chaetoceros calcitrans T4—Nannochloropsis salina	Enrichment of <i>Artemia</i> with <i>Chlorella</i> salina and Baker's yeast resulted in a reduction in PUFA content, even after 6 h of enrichment	[3]
Use of natural enrichment diets such as yeast, microalgae, and herbal extract	Yeast, microalgae, herbal extract growth and improves the nutritional profile of Artemia, positively influencing the fatty acid composition essential for larval development		[14]
Artemia supplementation with treated yeast cells, Saccharomyces cerevisiae, on Poecilia latipinna	T1—Commercial food T2—Unsupplemented Artemia T3—Artemia supplemented with $\beta$ -ME-treated yeast cell (4×10 $^7$ CFU/L)	Treated yeast cell supplementation in <i>Artemia</i> significantly improved reproductive indices and immune responses in ornamental fish, suggesting yeast as an immunostimulant in aquatic nutrition	[160]
Use of yeasts in aquaculture nutrition	Artemia enriched with yeast	Yeast supplements and yeast-containing feed improve disease protection and productivity in fish, boosting the aquaculture industry. Some yeasts in probiotic products enhance fish immunity and water quality, resulting in positive production outcomes	[164]
Influence of two different yeasts on <i>Artemia</i> enrichment	T1—a commercial product from specific strains of <i>S. cerevisiae</i> and <i>C. utilis</i> T2—only <i>S. cerevisiae</i>	Larvae fed with hydrolyzed yeast had higher weights and specific growth rates than those fed <i>Artemia</i> without yeast. Yeast-enriched <i>Artemia</i> enhanced growth and immunity, with benefits lasting 10 days post-supplementation, and improved stress tolerance in <i>pejerrey</i> larvae	[165]

Abbreviation: CFU, colony-forming units.

have been used primarily for their nutritional value in larval rearing; however, their native microbiota often lacks the specific beneficial properties necessary to optimally support early gut development and immune competency [167]. Probiotic supplementation, defined by FAO as live microorganisms that confer health benefits when administered in adequate amounts, is now being integrated with Artemia to overcome these limitations [161]. This integration facilitates the introduction of targeted microbial species into the gut ecosystem of larval fish and shrimp during critical developmental phases [168]. Different classes of probiotics, including lactic acid bacteria (LAB), Bacillus species, Vibrio-related strains, yeast-based probiotics, and other bacterial strains, have been investigated for use in Artemia enrichment, each with specific advantages and considerations (Table 5).

A variety of methods have been developed to enrich Artemia with probiotics. Bioencapsulation is the most commonly used strategy, wherein probiotic strains such as *Lactococcus lactis* and various Bacillus spp. are adhered to or ingested by Artemia nauplii, thereby enabling delivery directly into the larval gut [176].

Other approaches include co-culturing Artemia with probiotic-producing bacteria or yeast, which allows for a more natural colonization process with the added benefit of modulating the host's microflora [177]. Recent innovations also involve incorporating advanced encapsulation techniques such as microencapsulation, which provide controlled release of the probiotic and protect the microorganisms from the harsh environmental conditions of the aquatic rearing system [178]. These methodologies ensure that beneficial microbes are present at effective dosages during larval feeding, thereby enhancing the efficiency of probiotic administration [176–178].

The effects of probiotic enrichment of Artemia on larval development are multifaceted. Experimental studies have indicated that the use of probiotic-encapsulated Artemia leads to increased growth rates, typically in the range of 20%–23% higher than controls in shrimp postlarvae [168]. Improved digestive enzyme activity and enhanced nutrient absorption in larval stages have also been documented following probiotic-enriched feedings [179]. Furthermore, immunomodulatory effects have

TABLE 5 | Summary of probiotic strains applied in Artemia enrichment and their functional roles in enhancing larval health.

Probiotic	Effects	References
Bacillus subtilis, Bacillus licheniformis	Enhance nutrient assimilation and pathogen protection; spore-forming, and heat-resistant for enrichment	[19, 108, 169]
Lactobacillus rhamnosus IMC 501	Reduces Vibrionaceae pathogens; improves intestinal microbial balance and immunocompetence in Artemia	[170, 171]
Enterococcus spp.	Exhibits immunomodulatory properties and competitive exclusion; potential for improving Artemia health	[170-172]
Non-pathogenic Vibrio alginolyticus	Reduces ambient pathogenic Vibrio levels; requires careful strain selection to avoid toxicity	[173–175]

been observed when beneficial bacteria modulate the gut microbiota. For instance, supplementation with strains such as *Shewanella putrefaciens* can foster an intestinal environment that not only promotes growth but also enhances resistance to common pathogens by stimulating the innate immune system [180]. These improvements are critical during the early phases of development when larvae are particularly susceptible to stress and pathogenic invasions [179].

The importance of a balanced and beneficial gut microbiota in early larval development cannot be overstated. During these critical developmental windows, the establishment of symbiotic microbial communities plays a central role in metabolic programming, nutrient assimilation, and immunological maturation [181]. Probiotic-enriched Artemia have shown potential in creating a more stable and health-promoting gut microbiota, thereby reducing the incidence of dysbiosis that is often associated with traditional Artemia feeding practices [161, 181]. In doing so, these practices contribute significantly to improved larval survival, reduced pathogen load, and ultimately enhanced production performance.

Despite the promising outcomes, there remain challenges and limitations associated with traditional Artemia in delivering health-boosting microbial benefits. The native microbial populations in Artemia can be inconsistent and may not confer the robust probiotic effects needed to combat pathogenic outbreaks [167]. Moreover, without targeted enrichment strategies, the beneficial microbes may not be present in sufficient numbers, or they may be outcompeted by undesirable bacteria in the rearing environment [168, 178]. Future prospects in this field involve refining bioencapsulation techniques and exploring innovative delivery systems such as alginate-based microbeads, which have shown promise in controlled probiotic release in other aquaculture species [178]. Additionally, advancements in molecular diagnostic techniques are likely to allow for precise monitoring of gut microbiota dynamics in response to probiotic treatments, thereby optimizing formulations and dosing strategies [177, 178].

In conclusion, probiotic-enriched Artemia represent a cuttingedge approach to improving the health and survival of fish and shrimp larvae. The strategy capitalizes on the dual role of Artemia as both nutritional feed and a vector for beneficial microbes, thus addressing the inherent limitations of traditional live feeds. Future research should continue to optimize enrichment methods and explore novel probiotic candidates to further enhance larval development and robustness in aquaculture systems.

#### 2.5.1 | Mechanism of Enrichment

A primary mechanism of enrichment involves immersing Artemia nauplii in water laden with probiotic bacteria at defined concentrations, approximately 10<sup>7</sup> CFU ml<sup>-1</sup> as reported by Panah et al. [182]. In the early hours post-hatching, the nauplii's integument is soft and permeable. During this period, although the digestive system is not yet fully developed, the thin cuticle allows for some degree of passive adsorption of probiotic cells onto the surface of the nauplii [183]. However, as the nauplii develop and reach instar stages when the mouth opens (Instar III), active ingestion becomes the principal route of probiotic uptake. In addition to ingestion, the adhesion of probiotic bacteria to the external surfaces of Artemia plays an important role. The exoskeleton, along with residual organic matter and mucus produced by the nauplii, provides ample binding sites for probiotics. Such bioencapsulation of probiotics not only promotes growth and survival in aquatic larvae but also helps mitigate pathogenic challenges in aquaculture systems [171, 183].

# 2.5.2 | Key Benefits Provided by Probiotics

Probiotics provide several key benefits in aquaculture. They colonize the gut and outcompete pathogens for nutrients and adhesion sites, preventing infections such as those caused by Vibrio spp. Additionally, probiotic enrichment enhances digestive enzyme activities like protease, amylase, and lipase, improving feed conversion efficiency, nutrient absorption, and growth performance [169, 184]. Probiotics also boost immune responses by increasing lysozyme activity, stimulating immune-related enzymes, and strengthening systemic defenses [185]. Furthermore, they promote a balanced and resilient gut microbiota, supporting digestion and reducing the risk of opportunistic infections. Lastly, probiotics enhance antioxidant enzyme activities, including superoxide dismutase, catalase, and glutathione peroxidase, helping larvae withstand oxidative stress and improving survival under challenging conditions [186].

#### 2.5.3 | Impact on Aquaculture Species

The use of probiotic-enriched Artemia as a live feed in aquaculture has been shown to improve larval performance across diverse species, including various aquatic animals. The ability of probiotics to enhance the nutritional quality of Artemia by enriching its profile with essential nutrients and beneficial microorganisms thereby stimulates appetite and improves feed conversion efficiency. Lobo et al. demonstrated that *Artemia* enriched with *Shewanella putrefaciens* exhibited significantly higher levels of HUFAs, which contributed to improved larval development in Senegalese sole [180]. Moreover, the enhanced nutrient uptake is partly due to the bioencapsulation mechanism by which Artemia transfers both the probiotics and their metabolic by-products to the larvae, stimulating digestive enzyme activity and overall metabolism [48, 184].

In addition to growth improvements, the gut morphology of larvae benefits markedly from the administration of probioticenriched Artemia. Studies have demonstrated that probiotics promote an increase in the length of intestinal villi and the density of goblet cells, which are critical determinants of nutrient absorption as well as mucosal barrier function [187]. An increase in villus length translates to a higher absorptive surface area, while enhanced goblet cell density supports improved secretion of mucus, thereby offering protection against pathogens. This combination fosters a microenvironment conducive to both efficient digestion and robust health [187]. The scope of probiotic action extends beyond individual strains. Xu et al. showed that Lactiplantibacillus plantarum and Pediococcus acidilactici exhibited antagonistic effects against pathogenic bacteria, reducing disease incidence and fostering microbial community stability within aquaculture environments [57]. Similarly, Touraki et al. highlighted that probiotic-enriched Artemia not only boosts immune responses but also contributes to the development of a beneficial intestinal microflora in fish, which is crucial for nutrient uptake and host defense [58].

Furthermore, the immunomodulatory effects associated with probiotic supplementation via Artemia are of significant importance. The innate immune system of larval species is often characterized by parameters such as respiratory burst activity and nitric oxide production. Probiotic integration into the Artemia diet has been correlated with enhancements in these immune parameters, leading to better defense mechanisms against potential pathogens [188]. The augmented innate immune response not only aids in immediate pathogen clearance but also contributes to long-term disease resistance, a vital attribute for high-density aquaculture systems where disease outbreaks can otherwise be catastrophic [48].

Collectively, the evidence underscores that probiotic-enriched Artemia positively influences growth performance, gut structural development, and innate immune competence in aquaculture larvae. These improvements contribute to enhanced larval survival rates and overall aquaculture productivity, making probiotic enrichment a promising strategy for sustainable aquaculture practices.

These studies (Table 6) underscore the multifaceted benefits of probiotic-enriched Artemia, including enhanced growth

performance, improved immune function, and increased pathogen resistance; making it an indispensable strategy for sustainable aquaculture.

# 2.6 | Elements, Minerals, and Vitamins Enrichment in *Artemia*

The enrichment of *Artemia* with essential micronutrients, including vitamins, minerals, and trace elements, represents a promising strategy to enhance larval performance and address the nutritional deficiencies commonly found in conventional live feeds used in aquaculture. Early life stages of fish and shrimp are particularly sensitive to micronutrient imbalances, which can lead to poor growth, skeletal deformities, reduced stress tolerance, and high mortality. Micronutrients such as vitamin C, B-complex vitamins, fat-soluble vitamins (A, D, E, K), and minerals like calcium, phosphorus, magnesium, selenium, and zinc play crucial roles in skeletal development, immune function, metabolic regulation, and oxidative stress protection.

Numerous studies have demonstrated that micronutrient-enriched *Artemia* improve larval growth, skeletal integrity, stress resistance, antioxidant defenses, and immune responses across various aquaculture species [4, 14, 193]. Selenium enrichment, for example, enhances antioxidant enzyme activities and survival rates, while vitamin C fortification supports collagen synthesis and tissue repair [194]. Advanced enrichment techniques such as bioencapsulation and nano-encapsulation have further improved the bioavailability and stability of micronutrients in *Artemia*, allowing for consistent delivery and maximizing physiological benefits.

In conclusion, micronutrient enrichment of *Artemia* provides a scientifically validated approach to enhancing larval quality by ensuring balanced nutrient intake, supporting robust development, and strengthening resistance to environmental and pathogenic challenges. Future research should focus on refining enrichment methodologies, exploring synergistic effects of micronutrients, and developing integrated systems that mimic the nutritional profiles of natural prey, ultimately enhancing the efficiency and sustainability of aquaculture systems.

# 2.6.1 | Impact on Aquaculture Species

Research on trace element enrichment has shown significant benefits for aquaculture. Zinc-enriched *Artemia* enhances bone formation and growth in *Anabas testudineus* larvae [195], while iron bioencapsulation improves mineral assimilation and growth in fish [122]. This illustrates how trace element enrichment can be crucial for enhancing mineral absorption, which in turn promotes healthier and more robust larval development. Furthermore, integrating selenium, iodine, and manganese into *Artemia* diets supports metabolic regulation and reproductive performance, ensuring optimal larval development.

The mineral and vitamin enrichment of Artemia has been shown to exert positive impacts on aquaculture species. In

**TABLE 6** | Summary of studies on probiotic enrichments of *Artemia* in several aquatic animals.

Study	Treatments	Result	References
Pacific white shrimp (Litopenaeus vannamei) larvae administered probiotic Pseudoalteromonas piscicida	T1—probiotic <i>Pseudoalteromonas</i> piscicida 1Ub—106 CFU/mL T2—prebiotic MOS—12 mg/L T3—synbiotic (probiotic <i>P.</i> piscicida 1Ub 106 CFU/mL and 12 mg/L prebiotic MOS)	THC, PO activity, and RB activity in shrimp larvae given probiotics, prebiotics, and synbiotics were higher ( $p < 0.05$ ) than in the control group	[189]
Shewanella putrefaciens Pdp11 probiotic supplementation as an enhancer of Artemia in Senegalese sole larvae culture	T1—Probiotic cells were supplied in a dose (2.5×107 CFU/mL)  T2—No probiotic cells were supplied to the preenriched <i>Artemia</i> metanauplii used as (Control)	Probiotic administration significantly increased total lipids, particularly n-3 HUFA levels, in Pdp11-enriched <i>Artemia</i>	[180]
Paenibacillus pabuli against Vibrio alginolyticus in Artemia culture	The strains used were <i>Paenibacillus</i> D12 and <i>Paenibacillus</i> D14, which were retrieved from the intestines of healthy red tilapias	The inclusion of <i>Paenibacillus</i> pabuli has shown potential in enhancing the health and disease resistance of <i>Artemia</i> cultures against <i>Vibrio</i> alginolyticus	[190]
Marine Lactobacillus pentosus H16 protects Artemia franciscana from Vibrio alginolyticus pathogenic effects	The study aimed to evaluate the probiotic properties and protective action of <i>Lactobacillus pentosus</i> H16 against <i>V. alginolyticus</i> 03/8525, used in vitro and in vivo studies with <i>Artemia franciscana</i>	Increased survival and growth rates were observed in fish or crustacean larvae fed enriched <i>Artemia</i>	[191]
Modulating gut microbiota of Artemia urmiana by administration of different levels of Bacillus subtilis and Bacillus licheniformis	Three levels of probiotics:  T1—10 <sup>2</sup> CFU/g feed  T2—10 <sup>4</sup> CFU/g feed  T3—10 <sup>6</sup> CFU/g feed  T4—No probiotics in the diet (Control)	Probiotics like <i>Bacillus subtilis</i> and <i>Bacillus licheniformis</i> improved gut microbiota and digestive enzyme activities in <i>Artemia</i> , enhancing the growth of shrimp and fish	[192]

Abbreviations: CFU, colony-forming units; MOS, mannan-oligosaccharide; PO, phenol oxidase; RB, respiratory burst; THC, total hemocyte count.

particular, larvae fed enriched Artemia exhibit improved growth and skeletal development, higher survival rates, enhanced stress resistance, and an upregulated immune response [77, 196]. The biofortification of live feed with vitamins such as ascorbic acid (vitamin C) and tocopherol (vitamin E) promotes optimal metabolic functions in larvae, which are crucial during the rapid growth phases experienced early in development [77, 197].

Building upon this, Rizk et al. explored the potential of enriching local *Artemia* strains, observing that incorporating vitamins and minerals not only improved the proximate composition but also significantly increased the EPA content, a crucial fatty acid for aquatic animals [198]. This finding emphasizes that *Artemia* enrichment with essential nutrients can boost the overall nutritional value of live feeds. Similarly, Sveshnikova et al. highlighted that mineral and vitamin-enriched *Artemia* positively impact the nutritional profiles of nauplii, improving growth and survival rates in fish and other aquatic species [108].

In terms of growth promotion and skeletal development, the incorporation of vitamin C into Artemia enrichments has been associated with increased ossification and a reduction in skeletal anomalies. Studies have demonstrated that dietary supplementation with L-ascorbic acid improves not only general growth

performance but also specific aspects of morphological development, which are critical for species transitioning to formulated diets later in life [197]. Similarly, the presence of vitamin E, a lipid-soluble antioxidant, helps protect cell membranes during periods of intense metabolic activity, thereby contributing to improved skeletal integrity and overall development [196].

Further supporting the importance of nutritional enrichment, Zhang et al. demonstrated that vitamin E-enriched *Artemia* significantly improved growth performance and reproductive capabilities in Nile tilapia. The study also noted that vitamin E acted as an antioxidant, enhancing stress resilience and overall health. This reinforces the idea that specific vitamins can have a profound impact on aquatic species' health, particularly in enhancing their ability to withstand environmental stresses [199].

In addition to vitamins, Nikapitiya et al. found that enriching *Artemia* with phages and other specific nutrients significantly improved the health and disease resistance of cultured aquatic animals. This highlights the dual benefits of enriched *Artemia* in providing both nutrition and health support, especially during the early life stages of fish and crustaceans [178]. Furthermore, Mulyani et al. evaluated the impact of vitamin C-enriched *Artemia* on milkfish larvae, reporting significant improvements

in growth, survival, and immune response, reinforcing the vital role of vitamins in supporting larval development and overall health [200]. In conclusion, incorporating trace elements and vitamins into *Artemia* not only enhances its nutritional value but also supports better health outcomes in aquaculture species, promoting sustainable practices in aquaculture systems.

These studies (Table 7) underscore the multifaceted benefits of elements, minerals, and vitamins enriched *Artemia*, including enhanced growth performance, improved immune function, and increased pathogen resistance, making it an indispensable strategy for sustainable aquaculture (Table 8).

# 3 | Influence of Enrichment on the Fatty Acid Composition of *Artemia* Nauplii

The enrichment of *Artemia* with targeted nutrients significantly alters their fatty acid profile, a critical factor in enhancing their value as live feed for marine fish and crustacean larvae. Fatty acids, particularly LC-PUFAs like EPA and DHA, are essential for the optimal development, growth, and immune response of aquatic animals (Table 9).

Enrichment with high-DHA formulations [69] significantly enhanced DHA content (16.2%), making it the most effective treatment for increasing this essential omega-3 fatty acid. This enrichment also provided substantial levels of alpha-linolenic acid (ALA; 21.7%) and EPA (6.0%), aligning with the nutritional requirements of many marine species. HUFA + Vitamin C enrichment [207] demonstrated a broad enhancement of omega-3 and omega-6 fatty acids. Notably, it resulted in the highest ALA content (36.43%) and considerable EPA (7.72%), along with elevated levels of linoleic acid (11.33%) and oleic acid (16.67%). This combination suggests its effectiveness in supporting membrane fluidity and oxidative balance in developing larvae.

Red pepper enrichment [92] showed elevated levels of ALA (30.44%), linoleic acid (5.94%), and oleic acid (19.53%), along with moderate levels of DHA (3.84%) and EPA (2.80%). Although lower in LC-PUFAs compared to high-DHA or HUFA products, red pepper-enriched Artemia may contribute beneficial antioxidant properties in addition to fatty acids. Commercial enrichment using DC Super Selco [71], a commonly used product in hatcheries, delivered the highest EPA concentration (12.0%) and a balanced amount of DHA (2.4%), positioning it as a reliable choice for enhancing omega-3 LC-PUFAs in live feed. In contrast, lecithin-enriched A. franciscana [208] all treatments contained the highest EPA level (15.37%) but relatively low DHA (1.53%). Lecithin also contributed to elevated levels of cis-vaccenic acid (14.10%) and linoleic acid (12.25%), reflecting its phospholipid-rich composition, which supports membrane structure and function. Finally, Spirulina enrichment [30] resulted in high ALA (24.07%) and oleic acid (22.39%) but minimal EPA (1.57%) and no detectable DHA. While Spirulina may enhance antioxidant and immune-stimulating properties, its utility for LC-PUFA enrichment appears limited.

These findings demonstrate that the "choice of enrichment substance has a direct and significant influence on the fatty acid profile of *Artemia*". High-DHA and HUFA-based enrichments are particularly valuable for species requiring high levels of omega-3 LC-PUFAs. In contrast, lecithin and plant-based enrichments may be used strategically depending on the cultured species' specific nutritional goals and developmental stage. The ability to tailor fatty acid profiles through targeted enrichment supports the broader goal of optimizing live feed quality in hatchery systems.

# 3.1 | Comparative Analysis of EPA and DHA Enrichment Using Various Lipid Sources

The nutritional value of *Artemia* is highly dependent on enrichment with LC-PUFAs, particularly EPA and DHA. Various studies have reported enrichment outcomes using different lipid sources, with EPA and DHA levels typically measured after 24h of exposure. According to Figure 4, marine-derived oils such as squid oil and cod liver oil consistently result in the highest EPA deposition, reaching ~9.29 and 8.59 mol%, respectively. Plantbased sources like linseed oil and microalgal products such as *Schizochytrium* (at 400 mg/L) offer moderate EPA enrichment, while unenriched *Artemia* and treatments with yeast show minimal EPA content.

In terms of DHA enrichment, the reviewed studies highlight that *Schizochytrium* at higher concentrations (400 mg/L) is particularly effective, achieving DHA levels of up to 7.3 mol%, which surpasses all other enrichment sources. Squid oil contributes moderate DHA enrichment (around 4.27 mol%), while cod liver oil delivers lower levels (1.35 mol%). Other sources, including linseed oil, yeast, and lower doses of *Schizochytrium*, have been found to contribute little to no measurable DHA (Figure 4).

These findings from multiple sources suggest that while marine oils are efficient for EPA enrichment, microalgal *Schizochytrium*, especially at higher concentrations, offers superior potential for DHA accumulation. Notably, only *Schizochytrium* at elevated doses supports meaningful enrichment of both EPA and DHA. This highlights the importance of choosing appropriate enrichment media in aquaculture hatchery protocols to ensure the production of nutritionally superior live feeds, ultimately supporting better growth and health of aquatic species.

## 3.2 | Knowledge Gaps

Artemia enrichment is essential for enhancing its nutritional profile, yet several knowledge gaps persist. These gaps highlight areas where further research is needed to optimize enrichment protocols and improve larval outcomes in cultured species.

There are several larval stages in Artemia nauplii, each with a distinct nutritional profile. However, a research gap remains in determining which naupliar stage is most suitable for enrichment. Limited data exist on how enriched Artemia withstands environmental fluctuations. Microalgae-enriched nauplii show 40% longer survival in low-salinity conditions compared to unenriched counterparts, but broader environmental tolerance profiles are lacking. Preliminary work with *Pediococcus* 

**TABLE 7** | Summary of studies on the enrichment of *Artemia* with elements, compounds, and minerals in several aquatic animals.

Study	Treatments	Result	Citations
Orange-spotted rabbitfish (Siganus guttatus) larvae fed sodium iodide-supplemented brine shrimp (Artemia sp.)	T1—unsupplemented $Artemia$ (control)  T2—sodium iodide (0.8 g/L)  T2—sodium iodide (0.8 g/L)  Significantly higher mean body (0.20 $\pm$ 0.01 g) and lower muscle count (0.003 $\pm$ 0.001 MF/ $\mu$ m²) the control (0.14 $\pm$ 0.01 g and 0.009 $\pm$ MF/ $\mu$ m², respectively) ( $p$ < 0.  Iodine supplementation significantly higher mean body (0.20 $\pm$ 0.01 g) and lower muscle count (0.003 $\pm$ 0.001 MF/ $\mu$ m²) the control (0.14 $\pm$ 0.01 g and 0.009 $\pm$ MF/ $\mu$ m², respectively) ( $p$ < 0.  Iodine supplementation significantly higher mean body (0.20 $\pm$ 0.01 g) and lower muscle count (0.003 $\pm$ 0.001 MF/ $\mu$ m²) the control (0.14 $\pm$ 0.01 g and 0.009 $\pm$ 0.01 in control (0.14 $\pm$ 0.01 g and 0.009 $\pm$		[201]
Enrichment of Artemia nauplii with selenium for marine fish larvae	T1—Control (unenriched Artemia)  T2—Artemia enriched  with sodium selenite  T3—Artemia enriched with selenoyeast  (Sel-Plex) at varying concentrations	Sodium selenite did not improve selenium levels in <i>Artemia</i> nauplii. In contrast, Sel-Plex enrichment showed a dosedependent increase in selenium content (1.7 to 12.4 mg/kg). Sel-Plex had no negative effect on lipid and fatty acid enrichment. This confirms its suitability for refining live prey enrichment protocols	[193]
Effects of cobalt, manganese, and zinc enrichment in <i>Artemia</i> on growth and survival of barramundi ( <i>Lates calcarifer</i> ) larvae	$\label{eq:total_control} \begin{split} &\text{T1Control (unenriched $Artemia$)} \\ &\text{T2Artemia} \text{ enriched with} \\ &50\text{mg/L CoCl}_2\text{(Co-1)} \\ &\text{T3100mg/L CoCl}_2\text{(Co-2)} \\ &\text{T450mg/L MnCl}_2\text{(Mn-1)} \\ &\text{T5100mg/L MnCl}_2\text{(Mn-2)} \\ &\text{T650mg/L CoCl}_2 + \text{MnCl}_2\text{(CoMn-1)} \\ &\text{T7100mg/L CoCl}_2 + \text{MnCl}_2\text{(CoMn-2)} \\ &\text{T850mg/L CoCl}_2 + \text{ZnSO}_4\text{(CoZn-1)} \\ &\text{T9100mg/L CoCl}_2 + \text{ZnSO}_4\text{(CoZn-2)} \end{split}$	Cobalt and manganese enrichment significantly improved larval growth.  Combined cobalt and manganese treatments increased mortality and cannibalism. Zinc and cobalt showed no competitive interaction, while cobalt and manganese displayed competitive retention in larvae. Strong correlations were found between trace element concentrations in <i>Artemia</i> and larvae	[202]
Effects of potassium iodide- enriched <i>Artemia</i> on iodine uptake, growth, and thyroid status in larval zebrafish ( <i>Danio rerio</i> )	T1—Unenriched Artemia (control) T2—Artemia enriched with potassium iodide wax spray beads (KI WSB)	Zebrafish larvae fed KI WSB-enriched Artemia showed a 10-fold increase in total iodine, improved survival, and reduced epithelium-to-colloid (v:v) ratios at 38 dpf. Iodine from enriched Artemia was bioavailable, and Artemia also contained significant exogenous thyroid hormones and deiodinase. KI WSB had no impact on marine bacterial levels	[203]
Effects of dietary iron and vitamin C supplementation via Artemia enrichment on development, gene expression, and antioxidant status in Senegalese sole (Solea senegalensis) larvae	T1—Control: Artemia enriched with Tisochrysis lutea  T2—F group: Artemia enriched with T. lutea + iron (Fe)  T3—FP group: Artemia enriched with T. lutea + iron (Fe) + ascorbyl palmitate (vitamin C)	Larvae in the FP group had the highest Fe and AA levels, faster growth, earlier metamorphosis, and significantly improved antioxidant status. Gene expression analysis showed differential regulation of stress, metabolic, antioxidant, osmoregulation, and iron homeostasis genes. Similar skeletal development was observed across treatments, with increased collagen fibers in the FP group	[204]
Effects of copper-enriched <i>Artemia</i> on growth, antioxidant activity, and salinity stress tolerance in Chinese mitten crab ( <i>Eriocheir sinensis</i> ) larvae	T1—Control: Artemia with 0 mg Cu/mL T2—Artemia enriched with 0.1 mg Cu/mL T3—Artemia enriched with 0.2 mg Cu/mL T4—Artemia enriched with 0.4 mg Cu/mL	Cu enrichment did not significantly improve survival but enhanced growth and larval stage index in treatments 1 and 2. Antioxidant enzyme activity (SOD, CAT) was highest in treatment 3. Salinity stress tolerance improved in treatments 1 and 2. Optimal Cu range in <i>Artemia</i> for benefits was 33–52 µg/g dry matter	[205]

(Continues)

TABLE 7 | (Continued)

Study	Treatments	Result	Citations
Effects of zinc (Zn) and manganese (Mn) supplementation in <i>Artemia</i> on growth, survival, body composition, and skeletal deformities of red sea bream ( <i>Pagrus major</i> ) larvae	T1—Control (Artemia without Zn or Mn) T2—Zn-enriched Artemia (Z) T3—Mn-enriched Artemia (M) T4—Zn + Mn enriched Artemia (ZM)	Mn supplementation (M group) significantly improved growth (TL = $15.60 \pm 0.45$ mm) versus control (TL = $14.90 \pm 0.41$ mm); survival was not affected by Zn or Mn. Mn and ZM groups showed increased crude lipid content and Mn levels; the Z group had the highest Zn content. Skeletal deformities were significantly reduced in Zn, Mn, and ZM groups, with the highest deformities in the control group, especially in the vertebral column. Mn level in <i>Artemia</i> between $12$ and $42.8 \mu\text{g/g}$ DM is beneficial for growth and skeletal development	[206]

Abbreviations: CAT, catalase; Cu, copper; DFP, days post-fertilization; DM, dry matter; KI, potassium iodide; Mn, manganese; SOD, superoxide dismutase; TL, total Length; v:v, volume-to-volume ratio; WSB, washed soluble brain extract; Zn, zinc.

acidilactici reveals viable bacterial enrichment within 4 to 6 h, but long-term impacts on larval microbiomes remain unstudied [57].

While zinc and manganese enrichment reduced skeletal deformities in red sea bream larvae [193], iodine enrichment showed minimal impact on Atlantic halibut and cod [210]. The mechanisms behind inconsistent mineral retention in Artemia remain poorly understood. Optimal mineral concentrations for different fish larvae are not well established, particularly for selenium and iodine. Many of these studies rely on controlled lab conditions, neglecting practical challenges in large-scale aquaculture settings.

While numerous studies have investigated the effects of enriched Artemia on the early stages of fish, limited research has been conducted on the adult stages. Ismarica et al. indicated that the size and mouth opening of fish larvae influence their capacity to ingest suitable food, underscoring the importance of Artemia, which is appropriately sized and nutritionally adequate for early larval development [211]. As fish reach juvenile and adult stages, their dietary requirements shift. Adult fish often require a more complex and diverse diet than what is provided by Artemia alone. Recent studies found that a mixed diet comprising various feed types resulted in better overall growth rates for common carp than a diet solely based on Artemia, indicating that adult fish should be offered more formulated or varied feeds that better align with their dietary needs [212]. Furthermore, Sautter et al. suggested that while live feeds such as Artemia are beneficial for juvenile growth, they might not provide a complete dietary solution as fish mature, necessitating the integration of high-quality artificial feeds to meet their evolving nutritional demands [213].

The limited effectiveness of Artemia in meeting adult fish dietary needs is partly due to the larger quantities of macro and micronutrients required at this stage. Elshafey et al. found that while Artemia enriched with supplements can enhance growth and physiological conditions in fish, adult fish tend not to thrive solely on such diets due to their larger requirements [30].

Additionally, the metabolic rates in adult fish are more complex, necessitating effective nutrient absorption, which complicates reliance on a single food source like Artemia [214]. In summary, while enriched Artemia serves as an excellent live feed for fish larvae due to its appropriate nutritional content and size, its role diminishes as fish transition to adulthood, where diverse and nutritionally complete diets become essential for optimal growth and health.

# 3.3 | Vinh Chau's Artemia, Great Salt Lake Artemia, and Bohai Bay Artemia

In natural environments, Artemia species exhibit considerable size diversity, often linked to their specific geographic distribution and environmental conditions. Various ecological factors, environmental conditions, and nutritional strategies significantly influence these size variations. Populations from Great Salt Lake (*A. franciscana*) typically reach a larger size at maturity compared to those found in Vinh Chau and Bohai Bay, which are characterized by distinct climatic and salinity conditions affecting maturation rates [215, 216]. Specifically, *A. franciscana* has been documented to grow up to 18 mm in length, whereas Vinh Chau's and Bohai Bay's Artemia populations generally exhibit smaller sizes, often around 10–15 mm<sup>244</sup>.

After enrichment with nutrient sources, juvenile Artemia's growth can be significantly increased, impacting their final size. Research indicates that the enrichment type and size of food particles are crucially influential on ingestion rates and, consequently, growth [51, 157]. Studies demonstrate that Artemia enriched with smaller microalgae, such as *Nannochloropsis*, have shown improved growth rates due to better digestion and nutrient absorption compared with those that feed on less nutrient-dense alternatives [51]. Thus, when similar enrichment protocols are used, it is reasonable to anticipate that the initial size advantage seen in Great Salt Lake Artemia persists; but the research gap narrows as Vinh Chau and Bohai Bay Artemia, which are initially smaller, show more pronounced growth

TABLE 8 | Average proximate composition (in % ± SD) of Artemia nauplii enriched with Chaetoceros, lecithin, rice bran, red algamac.

Parameter	Naupli (unenriched)	Chaetoceros (microalgae)	Rice bran	Lecithin- enriched <i>Artemia</i>	Red algamac (biomarine- aquafauna)
Citations	[109]	[54]	[54]	[63]	[110]
Crude protein (%)	$52.2 \pm 8.8$	$55.55 \pm 0.55$	$59.43 \pm 0.02$	$50.2 \pm 2.6$	$40.74 \pm 1.02$
Crude lipid	$18.9 \pm 4.5$	$19.38 \pm 0.06$	$19.90 \pm 0.02$	$19.7 \pm 1.1$	$16.98 \pm 1.15$
Ash (%)	$9.7 \pm 4.6$	$8.87 \pm 0.02$	$5.53 \pm 0.01$	$17.0 \pm 3.2$	$6.07 \pm 0.35$
Carbohydrate (%)	$14.8 \pm 4.8$	$5.16 \pm 0.08$	$15.11 \pm 0.01$		

Source: Data compiled from [54, 63, 109, 110].

 TABLE 9
 Fatty acid composition (% total fatty acids) of the various enrichments for Artemia culture.

n 11	Spirulina- enriched	High		n 1	DC Super Selco (commercial- enrichment	Lecithin- enriched A.
Fatty acids	Artemia	DHA	HUFA+vitamin C	Red pepper	fluid)	franciscana
References	[30]	[69]	[207]	[92]	[71]	[208]
C14:0 (Myristic acid)	1.65	0.2	$1.30 \pm 0.20$	$1.19 \pm 0.091$	$1.0\pm0.1$	$0.93 \pm 0.33$
C16:0 (palmitic acid)	11.86	7.7	$15.50 \pm 1.95$	$13.93 \pm 0.339$	$10.0 \pm 0.5$	$12.21 \pm 1.50$
C16:1 (Palmitoleic acid)	3.94	0.6	_	$2.30 \pm 0.074$	_	_
C18:1 (Vaccenic acid)	_	_	$4.53 \pm 0.07$	7.22	_	_
C18:1n-9 (Oleic acid)	22.39	19.0	$16.67 \pm 0.43$	$19.53 \pm 0.096$	$16.4 \pm 0.8$	$12.8 \pm 0.04$
C18:1n-7 (Cis-Vaccenic acid)	_	8.6	_	$8.12 \pm 0.068$	$6.7 \pm 0.5$	$14.10 \pm 0.19$
C18:2n-6 (Linoleic acid)	6.1	6.0	$11.33 \pm 0.54$	$5.94 \pm 0.027$	$5.9 \pm 0.4$	$12.25 \pm 0.87$
C18:3n-3 (Alpha-linolenic acid)	24.07	21.7	$36.43 \pm 0.37$	$30.44 \pm 0.153$	$22.0 \pm 0.8$	$5.93 \pm 0.21$
C20:4n-6 (Arachidonic acid)	1.1	1.2	_	$1.06 \pm 0.023$	$1.0\pm0.2$	$2.29 \pm 0.69$
C20:5n-3 (Eicosapentaenoic acid)	1.57	6.0	$7.72 \pm 0.32$	$2.80 \pm 0.017$	$12.0 \pm 2.6$	$15.37 \pm 0.83$
C22:6n-3 (Docosahexaenoic acid)	_	16.2	_	$3.84 \pm 0.146$	$2.4 \pm 0.3$	1.53±0.44

responses due to potentially higher nutrient retention rates from enriched diets [216–218].

Additionally, sexual dimorphism influences size variations in Artemia species. Generally, males are smaller than females, and this pattern can vary across different Artemia populations. For instance, studies have noted consistent trends in size dimorphism among Artemia species, suggesting that environmental stressors, such as salinity and temperature, may shape these size differences [215–217]. Bohai Bay Artemia, which often experiences different environmental stressors, can exhibit unique responses to enrichment, sometimes resulting in a dramatic

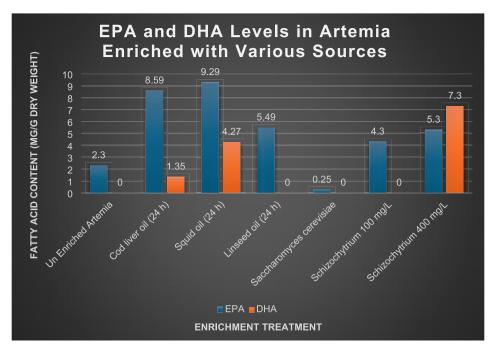


FIGURE 4 | EPA and DHA Levels in *Artemia* enriched with Unenriched *Artemia* [126], Cod liver oil (24h) [209], Squid oil (24h) [209], Linseed oil (24h) [209], *Saccharomyces cerevisiae* [3], Schizochytrium 100 mg/L. *Source*: Data compiled from [126], *Schizochytrium* 400 mg/L [126].

increase in female sizes relative to males after targeted nutritional interventions [215–217].

# 4 | Summary of Key Findings

The enrichment of Artemia with yeast, probiotics, and essential elements significantly improves its nutritional profile, enhancing aquaculture productivity (Table 10). Key findings include that oil-emulsion enrichment improves the bioavailability of essential fatty acids such as DHA and EPA, promoting optimal growth in species like Penaeus vannamei. Microalgae enrichment enhances LC-PUFA content, supporting growth and survival rates. Soy lecithin enrichment increases essential fatty acid and phospholipid content while enhancing digestive enzyme activity. Yeast enrichment enhances the bioavailability of proteins, essential fatty acids, and micronutrients, thereby improving immune function and pigmentation. Probiotic enrichment enhances nutritional quality, pathogen resistance, and survival rates in fish larvae. Enrichment with selenium, zinc, iodine, manganese, vitamins, and minerals supports immune responses, metabolic function, and reproductive performance.

# 5 | Conclusion

The strategic enrichment of *Artemia* as a live-feed organism has evolved into a cornerstone practice in modern aquaculture, playing a critical role in enhancing the nutritional and immunological status of larval fish and crustaceans. As aquaculture systems grow in intensity and complexity, optimizing early-stage nutrition becomes paramount, especially considering the high sensitivity of larvae to dietary deficiencies and environmental stressors. The integration of various enrichment modalities, namely oil emulsions, microalgae, yeast, probiotics, soy lecithin,

**TABLE 10** | Comparative summary of *Artemia* enrichment methods.

<b>Enrichment method</b>	<b>Key findings</b>
Oil emulsion	Improves fatty acid profile and <i>Artemia</i> quality [46], enhances shrimp growth [47], boosts fish health markers [48].
Microalgae	Supports better digestion and nutrition in <i>Artemia</i> [51], increases protein and carotenoids [15], and raises PUFA and fish growth [102].
Soy lecithin	Enhances digestive enzymes in <i>Artemia</i> [63] improves fish growth and lipid profiles [66, 70].
Yeast	Enriches <i>Artemia</i> with proteins and micronutrients [52], improves fatty acids and coloration in fish [219, 220].
Probiotics	Raises HUFA in <i>Artemia</i> and supports larval development [180], reduces pathogens and boosts survival [191, 221].
Mineral elements	Zinc and iron improve fish larval growth and mineral uptake [108].

and essential micronutrients, has been extensively studied and has shown promising results in improving larval survival, growth, stress tolerance, and disease resistance across a wide range of aquaculture species. This conclusion synthesizes current findings across these enrichment domains and highlights

their individual and synergistic contributions to sustainable and efficient larviculture.

Oil emulsions enriched with HUFAs, including EPA and DHA, have demonstrated significant efficacy in enhancing the lipid profile of Artemia. These essential fatty acids are critical for membrane fluidity, visual acuity, neural development, and immunomodulation in larval fish and shrimp. Studies have shown that the use of commercial or custom-prepared oil emulsions (fish oil, algal oil, krill oil) can result in the successful encapsulation of HUFAs into Artemia nauplii, significantly improving larval growth performance, survival, and resistance to stressors. Emulsion particle size, oil concentration, emulsifier type, enrichment duration, and oxygen availability during enrichment are key parameters influencing the efficacy of HUFA transfer. Significantly, using stabilized emulsions and time-controlled enrichment protocols has minimized oxidative degradation of fatty acids, ensuring optimal delivery of intact lipids to target species. The impact of such enrichment is particularly pronounced in marine finfish and crustaceans with limited capacity for de novo synthesis of long-chain polyunsaturated fatty acids.

The use of microalgae in Artemia enrichment strategies serves a dual purpose: providing essential fatty acids and pigments while simultaneously introducing bioactive compounds that improve digestive physiology and immunity. Species such as Isochrysis galbana, Nannochloropsis oculata, Chaetoceros muelleri, and Tetraselmis suecica are frequently used due to their rich profiles of EPA, DHA, sterols, and antioxidants. Microalgal-enriched Artemia enhances pigmentation, digestive enzyme activity, and larval viability, particularly in marine species requiring high levels of essential fatty acids and carotenoids for proper development. The co-feeding of microalgae with Artemia, or pre-enrichment of Artemia in dense algal cultures, has proven effective in improving nutrient bioavailability. Additionally, particular microalgal species contribute immunostimulatory compounds such as β-glucans, which fortify larval defense mechanisms. These benefits underscore the role of microalgae not only as a nutritional source but also as a functional feed additive within integrated hatchery systems.

Yeast-based enrichment has emerged as an economical and potent strategy to enhance Artemia's nutritional and immunological value. Strains like Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansenii, and Phaffia rhodozyma are used for their content of proteins, B-complex vitamins, carotenoids (astaxanthin), and immunostimulants such as β-glucans and mannan oligosaccharides. Yeast enrichment has been linked to improved growth rates, pigmentation, stress resistance, and non-specific immune responses in fish and shrimp larvae. Optimal enrichment depends on yeast viability, enrichment concentration, duration, and delivery method (bioencapsulation vs. microencapsulation). Co-enrichment with yeast and other agents, such as microalgae or micronutrients, has also demonstrated synergistic effects on larval development and resistance to pathogens. The role of yeast in enhancing mucosal immunity and gut microbiome modulation offers an exciting avenue for future probiotic-yeast combined enrichment strategies.

Probiotic enrichment of *Artemia* introduces beneficial microorganisms into the larval gut at early developmental stages,

establishing a favorable microbiota and enhancing immune surveillance. Probiotic strains such as Lactobacillus rhamnosus, Bacillus subtilis, Enterococcus spp., Vibrio alginolyticus, and Saccharomyces cerevisiae have been successfully delivered via Artemia using bioencapsulation, co-culturing, or microencapsulation techniques. Probiotic-enriched Artemia has been associated with improved intestinal morphology, higher digestive enzyme activity, enhanced survival, and better resistance to bacterial and viral infections. The immunomodulatory role of probiotics is particularly crucial in reducing reliance on antibiotics and enhancing larval resilience during stress-inducing hatchery phases. Successful probiotic enrichment depends on strain compatibility, colonization potential, and retention time in Artemia. Notably, the delivery of probiotics via live feed ensures more efficient gastrointestinal tract colonization compared to waterborne or formulated feed administration.

Soy lecithin, a rich source of phospholipids and choline, has been widely used to enhance lipid digestion, membrane integrity, and energy metabolism in larval diets. Lecithin-enriched *Artemia* provides essential phospholipids that promote emulsification and absorption of dietary lipids, a crucial benefit for species with underdeveloped digestive systems. The enrichment improves growth performance, stress tolerance, reproductive health, and intestinal development in shrimp and fish larvae. Furthermore, including soy lecithin reduces the incidence of fatty liver syndrome and promotes hepatopancreatic health. Factors influencing enrichment efficacy include lecithin concentration, emulsification stability, and duration of enrichment. The benefits of lecithin are most pronounced when combined with other lipophilic nutrients or antioxidants, making it a key component of multi-nutrient enrichment protocols.

Micronutrient enrichment of Artemia with essential vitamins and minerals such as vitamins A, C, D, E, B-complex, selenium, zinc, iron, calcium, phosphorus, and magnesium is critical for correcting common deficiencies in conventional live feeds. These micronutrients support key physiological functions, including skeletal formation, immune competence, antioxidative defense, and metabolic regulation. Enrichment through bioencapsulation or nanoencapsulation techniques has dramatically improved the bioavailability and stability of these nutrients within Artemia, enhancing their efficacy upon ingestion. Research has shown that selenium enrichment enhances antioxidant enzyme activity; vitamin C supports collagen synthesis and wound repair; and vitamin E protects lipid membranes during oxidative stress. Similarly, mineral supplementation, particularly with zinc and calcium, has shown marked improvements in bone development, immune responses, and survival in species such as Anabas testudineus, milkfish, and tilapia. Future directions should aim at fine-tuning enrichment durations and exploring synergistic effects among micronutrients, especially in species with complex nutrient requirements.

## 5.1 | Final Remarks

The enrichment of *Artemia* remains a dynamic and multifaceted approach to overcoming nutritional bottlenecks in early aquaculture. When strategically formulated and scientifically validated, enriched *Artemia* improves larval performance and

contributes to more resilient, sustainable, and antibiotic-free production systems. Its role as a functional feed extends beyond nutrition into health management, making it a vital component in the future of global aquaculture.

#### **Author Contributions**

Yathish Ramena: conceptualization, investigation, methodology, visualization, writing – original draft. Ram Babu Kurapati: writing draft, resources, software, visualization, writing – review and editing. Thomas Bosteels: funding acquisition, project administration, resources, review and editing. Grace Ramena: supervision, funding acquisition, project administration, resources, review and editing.

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#### **Conflicts of Interest**

The authors declare no conflicts of interest.

#### **Data Availability Statement**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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