Enhancement of nutritional value on zooplankton by alteration of algal media composition: A review

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Abstract. In aquaculture, fish larvae regularly need a balanced diet according to the timescale because such diets essential for constant growth and reproduction and can avoid malnutrition. Thus, the use of live food organisms is critical as it will first feed for fish larvae. Studies have shown that zooplankton have more excellent digestibility and are suitable as live prey species for different sizes than other live foods (e.g. rotifer and Artemia). However, zooplankton nutrition still needs to improve to meet the nutritional requirement for fish larvae. Feeding zooplankton with well-nourished microalgae is important as it affects the nutritional value of the zooplankton. Algal growth is related to micronutrients (e.g. nitrogen, phosphorus or selenium) supply in the culture medium and the availability of nutrients affects the quality of the algal. Thus, by enriching the algal diet with micronutrients from the culture media, the nutritional value of zooplankton can be improved. This review focuses on the nutritional value of zooplankton through the manipulation of algal media composition as well as wastewater. The relation between the composition of algal media and nitrogen and phosphorus limitation are also discussed. The review links the microalgae nutrient essential with manipulating algal media composition and the change of zooplankton nutrients.

1. Introduction
Aquaculture, sometimes referred to as aquafarm, is among the fastest-growing food sectors in the world. Aquaculture is the practise of cultivating aquatic organisms such as invertebrates, mussels, fishes, and aquatic plants [1]. The critical determinants of effective aquaculture farming are nutrition and feeding to sustain aquaculture improvement, and so aquaculture needs are always dominated by feeds and fertilisers resources [2]. Essential nutrients are critical in the aquaculture sector because they are...
responsible for the entire process. Aquatic creatures require nutrients at all stages of their life cycle, and the source of those nutrients changes as the organism develops, particularly during the larval stage. However, finding acceptable larvae feed has been a significant challenge, as most feeds do not match nutritional requirements or are too enormous for larvae to consume for growth. As a result, live feeds, particularly microalgae and zooplankton, are well-known to be suitable for larvae growth in aquaculture sectors.

Zooplankton is an essential natural food source for a diverse range of aquaculture species' development. Certain fish species, particularly larvae and fry, derive their nutrition predominantly on zooplankton. It provides them with nutritious foods and other substances like as proteolytic enzymes, hormones, and biochemicals that assist in the digestion of juvenile larval gut microbes [3]. Larviculture, particularly the onset of larval feeding, appears to be a vital stage in fish culture. The use of larvae-specific artificial meals demonstrates low digestion. This challenge is overcome through the use of live feed with small dimensions, superior nutritional properties, and movement that gives visual stimulation. However, in order for zooplankton to meet the dietary requirements of fish larvae and fry, specific components such as microalgae must be added. Enrichment of zooplankton is a procedure to enhance the nutritional value of the fish larvae.

The bioencapsulation and enrichment technique is carried out in hatchery circumstances in order to provide more nutritious quality of live food on a big scale in terms of protein and nutritional content for consuming host[4]. The most often used methods of enriching zooplankton include microalgae, fish meal, and canola oil, which might increase unsaturated fatty acid (HUFA) and important amino acid content [5]. However, while it is possible to produce healthy zooplankton nowadays, it is not optimal to obtain the zooplankton with its original nutritional content due to various problems such as poor sanitary and cultural conditions [6]. Additionally, due to the fact that different species of zooplankton have a variable nutrient profile, it is critical to understand the changes in fatty acid composition and the effects of enrichment on essential fatty acids (ARA, EPA, and DHA) for fish larvae [7].

Microalgae are suitable for enrichment because they supply aquatic species including various zooplankton with a nutrient profile that promotes their growth, survival and life table parameter [8]. Microalgae nutritional value varies according to species and is impacted by culture conditions. Due to the simplicity of growing, only a few numbers of species have been utilized [9]. Nevertheless, this small group of microalgae can provide an extraordinary nutritional package for fish larvae. As a main producer in the aquatic food chain, microalgae often contain a high concentration of polyunsaturated fatty acids (PUFAs), including EPA, arachidonic acid (AA), and docosahexaenoic acid (DHA). These are well-known to be necessary for a variety of aquatic animals [10]. The majority of microalgae species contain a high concentration of EPA (7 to 34%). Apart from that, [11] claim that prymnesiophytes (e.g. Isochrysis sp.) contain a high concentration of DHA (0.2 to 11%) and eustigmatophytes (e.g. Nannochloropsis sp.) contain a high concentration of AA (up to 4%). Thus seems precisely Isochrysis sp. and Nannochloropsis sp. have grown in popularity as live feed for aquatic animals, as the essential fatty acids encourage growth, health, and reproduction [12].

The issue now confronting the aquaculture industry is the requirement for a single species of zooplankton that is sustainable and cheap for some hatcheries and farms. The increased demand for Artemia nauplii has increased in the price of getting it to the point where clients cannot afford it. While other zooplankton such as rotifers, copepods, and Moina sp. can be utilized as live feed, Artemia nauplii has a higher nutritional value. Moina sp. and copepods encounter this issue as a result of their biological composition being deficient in key nutrients [13]. The purpose of this review is to gain a better understanding of how to increase the nutritional value of zooplankton by enriching microalgae with altered nutrient composition and how improving micronutrients in algae medium might increase their nutritional value as required by larvae.

2. Enrichment of Zooplankton with Microalgae
Microalgae feeding is an effective method of increasing the nutritional content of zooplankton. This is because microalgae contain nutrients necessary for zooplankton development and survival.
Additionally, microalgae can offer proteins and energy in the form of vitamins, polyunsaturated fatty acids, pigments, and sterols, which contribute to the zooplankton food chain [14]. According to [15], microalgae-fed PUFA-rich diets successfully increased the DHA content of zooplankton. The use of oil emulsions serves as an alternative for the zooplankton enrichment approach. When microalgae are given to zooplankton, however, enough quantities of DHA are obtained. It is comparable to the levels achieved with the commercial oil technique. Therefore, it is considered beneficial for fish larval feeding due to the zooplankton's DHA: EPA ratio [16]. Additionally, [17] showed that microalgae pigments can enhance the nutritional value of zooplankton via an enrichment process. Lutein and astaxanthin are the major pigments in copepod species, although canthaxanthin is found in *Artemia* sp. Both zooplankton species were fed to fish larvae after being treated with microalgae, and the outcome was high for lutein or astaxanthin but not for canthaxanthin. Additionally, recent research by [18] indicated that copepods enriched with *Chlorella* sp. have a substantial effect on the hue color of angelfish fry's body *Pterophyllum scalare*. As a result of this research, it is obvious that the zooplankton ability to convert microalgal pigments must be sufficient to produce a favorable effect for fish larvae.

### Table 1. Current microalgae species being used as enrichment for in aquaculture studies

| Enrichment via microalgae species | Enriched of zooplankton species | Effect study of Larvae species | References |
|----------------------------------|---------------------------------|-------------------------------|------------|
| *Chlorella* sp. | *Moina micrura*, cyclopoid copepod | Growth and coloration of Siamese fighting fish, *Betta splenden* Angelfish, *Pterophyllum scalare* | [19, 18] |
| *Nannocloropsis salina* | Mix of *Brachionus plicatilis* and *artemia nauplii* | Growth, carotenoid and PUFA content of Clownfish, *Amphiprion percula* | [20] |
| *Tetraselmis suecica*, *Cheatoceros calcitran* | *Artemia nauplii* | The development and survival of the river prawn, *Macrobrachium americanum* | [21] |
| *Tetraselmis sp* | Cycloid copepod | Growth, survival and proximate analysis of Tiger prawn *Penaeus monodon* | [22] |
| *Isochrysis galbana* | *Brachionus plicatilis*, *Artemia franciscana* | Survivalship, growth And condition factor of longsnout seahorse, *Hippocampus reidi* | [23] |
| *Isochrysis galbana*, *Thalassiosira weissflogii* | Pseudodiaptomus pelagicus | Stress survival of Florida Pompano (*Trachinotus carolinus*) | [24] |
| *Spirulina plantesis*, *chlorella vulgaris*, *Spirogyra maxima* | Mesocyclops aspericornis | South Asian carp, *Catla catla*, survival, growth, biochemical composition, and energy usage | [25] |

*Moina* sp. and copepod enrichment are not frequently employed in aquaculture. Even though this zooplankton has a high protein content in its nutritional composition, enrichment should be used to ensure the species' mass culture is sustainable [16]. Due to the fact that *Moina* sp. and copepod are filter feeders that graze on phytoplankton, bacteria, and detritus, this zooplankton is primarily enriched by microalgae species [26]. According to [27], microalgae have the ability to enhance the nutritional content of zooplankton by bioaccumulating vital elements such as fatty acids, amino acids, carotenoids, vitamins, and minerals. The use of microalgae to increase the nutritional content of highly unsaturated fatty acids has been successful in large-scale growth of copepod. According to a study on
Macrobrachium rosenbergii, the larvae's growth and survival increased when fed copepod enriched with microalgae contained HUFA [28]. Furthermore, the survival rates of post larvae of tiger prawn, Penaeus monodon shown highest when fed with cycloid copepod enriched with Tetraselmis sp. which shown these microalgae can provide protein and lipid composition as the best among other enrichment regimes [22].

3. Nutritional Content of Microalgae

The nutritional content of microalgae species varies substantially. Microalgal diets have the potential to improve the nutritional value of zooplankton and larval animals [29]. Numerous factors, including a nutrition structure, form, digestion, and biochemistry content, all contribute considerably to its nutritional value [10]. Because microalgae are the principal producers in the aquatic food web, they can offer phytonutrients such as eicosapentaenoic acid (EPA), arachidonic acid (AA), and docosahexaenoic acid (DHA). These phytonutrients are required to grow and develop aquatic organisms [30].

Antioxidants are critical in protecting zooplankton and fish larvae from disease, damaged cells, and the acquisition of the organism's characteristic. Carotenoids especially are quantified differently for each algae species. This nutritional value provided by microalgae is advantageous compared to the fish pellets used in hatcheries [31]. Artificial diets frequently lack this nutrient; which certain aquatic creatures require to increase their value. [19] reported that the improved red hue on the body of B. splendens fed with M. micrura supplemented with Chlorella sp. was observed in comparison to other feeding regimes, indicating that the carotenoid composition supplied by microalgae was superior to that supplied by other feeding regimes. This also shows microalgae can boost carotenoid composition in zooplankton and fish larvae consume, especially in ornamental fish industries. Hatcheries that employ artificial diets can compensate for this deficiency of carotenoid in the diet by incorporating microalgae into the fish feed.

The next key ingredient contained in microalgae that zooplankton and fish larvae require is amino acids. The amino acid composition of microalgae is generally identical and is unaffected by growth circumstances [32]. Because microalgae contain sufficient essential amino acids for every species, amino acids have the slightest likelihood of affecting the nutritional value of microalgae [33]. Microalgae comprise 30-40% protein, 10-20% lipids, and finally 5-15% carbs during their development phase [9]. Microalgae's nutritional content will alter in the presence of stress, such as nitrogen or phosphorus limitation. As glucose levels rise dramatically, this situation doubles the proximate composition. However, earlier research indicates that high carbohydrate levels can sustain maximum growth in aquatic organisms [34]. Finally, all nutritional components such as fatty acids, amino acids, antioxidants such as vitamins, and carotenoids are critical in improving the nutritional performance of zooplankton and fish larvae in aquaculture [35].

4. Relationship Between Microalgae Media Composition, Zooplankton Nutritional Value on Microalgae Growth

A culture medium's chemical composition supplies vital nutrients for zooplankton growth and development. The more complex the fatty acid profile of the microalgae, the more nutritious the zooplankton or fish larvae can be due to direct or indirect intake of enriched microalgae [36]. When ingested, altering the mineral composition of algal media directly affects the zooplankton or fish larvae. According to [37], altering the nitrogen and phosphorus ratios have an effect on EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) at 20:1 and 30:1 ratio, respectively. Thus, these nutrients are altered in such a way that they promote increased fatty acid synthesis. Additionally, mineral alteration has been demonstrated to have a beneficial effect on the biological processes of zooplankton, resulting in a higher rate of protein synthesis [38].

The culture medium can be improved in terms of the nutritional value as a feed for zooplankton or microalgae by the addition of certain micronutrients. On the other hand, altering the mineral concentration of algal media has a favourable effect on algal development [39]. The ratios of mineral content rise in proportion to the density of microalgae growth. These characteristics can be detected in microalgae cultures when the mineral content of nitrogen and phosphorus is altered. Additionally, it has
an effect on zooplankton, since it consumes enriched microalgae with a variety of beneficial properties for industry [40]. Additionally, changing the minerals in these microalgae enables the production of a large volume of oil at various concentrations [41]. According to [42], different culture mediums generate varying quantities of biomass depending on the nutritional content. Conway medium (600x10⁴ cells/ml) grew microalgae exponentially, followed by Miquel medium (500x10⁴ cells/ml), TMRL medium (470x10⁴ cells/ml), and TMRL urea medium (450x10⁴ cells/ml) on only one species of microalgae Skeletonema costatum. It is understood that a single species of microalgae can provide a high stocking density without altering the mineral content.

Additionally, it is essential to identify culture media based on microalgae species, as not all culture media support the growth of all algal species [43]. Investigated the growth potential of a variety of microalgae on F/2 media. The results indicate that the F/2 medium is capable of supporting only Chlorella sp., Chaetoceros sp., and Dunaliella sp., but not Nannochloropsis sp. or Tetraselmis sp. Thus, culture medium selection is critical for microalgae growth, as variations in the recipes affect algal growth and chemical composition. Subsequently, when fed this enhanced-microalgae, it carries the impacts of zooplankton or fish larvae.

5. Macronutrient Improvement and Manipulation of N: P Ratios in Algal Medium
Microalgae require three essential nutrients to grow and function: carbon, nitrogen, and phosphorus [43]. The overall mix of nutrients accessible in the culture medium significantly affects the digestion of these nutrients. Microalgae's nutrient utilisation rates are strongly correlated with their growth, and a scarcity of a major nutrient can dramatically affect their growth rate [44, 45]. Nitrogen is critical because it is necessary for protein and amino acid synthesis, whereas phosphorus is essential for nucleic acid synthesis and energy metabolism in algae [37]. Both of these nutrients are provided by the culture medium to the zooplankton. The nutritional composition of live food, microalgae, can significantly affect the growth and reproduction of animals [46]. The polyunsaturated fatty acids (PUFAs) responsible for this include 20:5(n-3) eicosapentaenoic acid (EPA) and 22:6(n-3) docosahexaenoic acid (DHA) [37].

The adjustment of nitrogen (N) and phosphorus (P) concentrations in the algal medium demonstrates changes in the biochemical composition and aids in the analysis of microalgal nutrient uptake. Limiting nutrients can affect the growth of microalgae by creating and accumulating carbon (C), which eventually results in the microalgae utilising less nutrients [47]. By providing a nitrogen-deficient environment for microalgae, it is possible to influence the protein content, resulting in an excess of carbohydrates and a high lipid storage capacity [48], whereas when phosphorus is lacking in the microalgae, the opposite occurs. According to past study, a particular nutrient can alter an algal cell's biochemical and physiological composition [49]. Nitrogen and phosphorus manipulations in algal medium provide information on the fatty acid components such as eicosapentaenoic acid (EPA), linoleic acid (ALA), arachidonic acid (ARA), and docosahexaenoic acid (DHA) [37] that are necessary for mass culture of microalgae to be sustained. Research from [49] also mentioned that, nitrogen and phosphorus concentrations at various molar ratios (16:1) and (40:1) resulted in relatively high algal growth. However, an overabundance of these nutrients (200:1) does not sustain growth, as seen by a reduction in the algae M.aeruginosa. As a result, the nitrogen and phosphorus ratios are affected by the concentrations of these nutrients in the culture medium. The ratio, concentration, and quantity of yield all correspond even with the growth of microalgae.

6. Enrichment of Microalgae with Micronutrients
Micronutrients are often essential for a good microalgae population and often cofactors in a variety of microalgae development-critical biochemical activities. Furthermore, these micronutrients have a key role inside the genetic and functional properties of growth components [50]. Manganese is a critical component in plant tissue culture media and is present in trace amounts Mn ions also can emitted by the culture media as manganese and molybdenum have a reciprocal relationship in which high Mn levels in plant roots and culture can compensate for low molybdenum levels [51]. Mn includes enzymes necessary for the deactivation of oxidase inhibitors [52]. According to [53], manganese ions enhance...
IAA levels in tissue by inactivating indole-3-acetic acid oxidase, which results in increased algal growth and output. After that, copper (Cu) consumption is crucial in aquatic species' and works as a nutrient in a variety of ways, including skeletal development, pH regulation, and enzyme activity [54]. Copper can be used as an enrichment in microalgae and can benefit the majority of zooplankton by providing a more nutritious food source for fish larvae. [55] shown that when Chinese mitten crab larvae are fed copper-enriched rotifers, copper-enriched microalgae increase growth and development. Thus, copper-enriched microalgae can be fed to a variety of zooplankton species to raise their nutritional value and health, but only in sufficient numbers to minimize culture toxicity. Other than that, boron in sufficient levels can alter the metabolism and consumption of growth and development-related microalgae molecules [59]. It is a great microalgal enrichment candidate due to its multiple favorable effects on fish larval nutrition. According to [56], supplementing boron with microalgae will result in a favorable link because boron is a critical component of primary producers' growth and development.

Selenium can be employed to enhance the growth of microalgae, necessary for the nutrition of animals, the generation of thyroid hormones, and the development of fish or zooplankton [57, 58]. When administered to zooplankton or fish larvae, sodium selenite can be added to a variety of microalgae species to boost their nutritional value. According to [58], *Chlorella vulgaris* may absorb sodium selenite fully, resulting in an increased sodium selenite content in the culture media, the efficiency of sodium selenite absorption by microalgae can increased, hence improving the zooplankton's quality. [59] also established that Se was a required trace element for daphniid development. The use of sodium selenite in conjunction with other trace elements the enrichment process, can increases the growth and survival of fish, particularly marine fish larvae [37]. Iron (Fe) is the most critical micronutrient in terms of quantity and the concentration of dissolved Fe and its bioavailability in the system may be critical in appropriately controlling the growth and adaptability of microalgae [60]. Apart from that, improved biomass production in marine strain *C. vulgaris* was observed when a Fe instantly enriched the medium during the late exponential growth phase of cells, however in the late exponential growth phase, when cells have been collected, high lipid synthesis was noticed and relayed to a new nutrient medium with particularly higher Fe levels [9]. Thus, supplementing microalgae with Fe can improve lipid production since it can be transmitted to zooplankton via the enrichment process.

7. Wastewater as Algal Source of Nutrients
Wastewater from industry contains effluents that can be recycled or repurposed for microalgae growth since it has the ability to absorb and repurpose effluents as nutrients. Agriculture is one of the biggest effluent-producing industries, and its wastewater can be recycled to grow microalgae [61]. The use of wastewater as a microalgal nutrient provides a number of advantages for effluent cleanup and algal biomass production. Wastewater is primarily composed of nutrients such as nitrogen (N), phosphorus (P), and carbon (C), each of which has a distinct tolerance for concentrations [62]. According to [63], microalgal species such as *Chlorella* sp. and *Scenedesmus* sp. may develop at high ammonium concentrations, which can be detrimental to other microalgal species. The amount of nitrogen (N) and phosphorus (P) in various forms of wastewater varies. Previous study from [64], stated that animal dung has emerged as the most productive wastewater source for microalgae production due to its high nutritional content in terms of nitrogen and phosphorus.

The N:P ratio in wastewater has an effect on the algae's ability to absorb these nutrients for growth. According to the Redfield ratio, microalgae have a stable molar ratio of 16:1 for N: P. When the molar ratio of a nutrient exceeds its initial value, it becomes a limiting factor in the microalgae's growth [65]. Additionally, restricting these nutrients has an effect on the microalgae's biochemical composition, as restricting nutrients might result in an accumulation of carbs and lipids, but without restricting nutrients, the microalgae's protein content will be high [66]. The balance of nitrogen (N) and phosphorus (P) in the nutrient ratio can result in a high protein content, which is advantageous as animal feed in the aquaculture or agriculture industries. The N: P ratio of wastewater does not entirely depend on the capacity of the culture medium to absorb nutrients; as different species of microalgae absorb nutrients at varying rates. Additionally, vital nutrients are present in wastewaters; some wastewaters, such as palm
oil mill effluent (POME), contain trace metal elements such as iron, magnesium, and potassium, promoting the growth of microalgae efficiently [67].

8. Conclusion
This review has an outrageous influence on zooplankton quality of alteration of algal media composition. By altering the ratio of nitrogen (N) and phosphorus (P) in algal media's biochemical makeup, it is possible to evaluate the positive and negative effects on the quality of microalgae. In the process of manufacturing enhanced microalgae as food for zooplankton or fishery larvae, the involvement of micronutrients is equally crucial. These micro-nutrients are often the minor factor that increases the quality of microalgae as it will be used in a modest amount. Furthermore, because of the accessibility of critical nutrients wastewater can be a major fertiliser supply for mass cultivation of microalgae. Effluents produced by the major firms might contribute to avoiding the polluting of natural water bodies using microalgae for the elimination from the waste-water of nitrogen(N), phosphorus (P) and carbon(C). But microalgae enrichment serves to increase the nutritional content of live feeds such as the fatty acid profile and amino acids. The fish larvae are actually essential for the growth and reproduction of these components. Thus, altering and enriching microalgae medium for zooplankton can have a positive impact, especially in larvae breeding, on aquaculture development. Additional study should be done to detect and strengthen this component of the knowledge.

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