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Review

Functional Additives as a Boost to Reproductive Performance in Marine Fish: A Review

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Abstract: This contribution brings together current knowledge on the use of functional food additives affecting marine fish reproductive performance. This article reviews published studies by several authors who have worked with specialized diets and focused on the dietary needs of brood fish, with the objective of identifying the relevant functional additives with potential to improve reproductive performance. The use of commercial and experimental diets that may have an effect on egg viability, quantity, and quality are discussed, with reference to hatching rates, larval survival, and compositions of fatty acids and amino acids after feed supplementation with various nutritional compounds. The intention of this review is to highlight the benefits of the use of vitamins, carotenes, fatty acids, and proteins of animal origin in broodstock nutrition, all of which have been shown to improve the quality of progeny under captive conditions. Finally, consideration is given to future perspectives on the use of additives in marine fish nutrition.

Keywords: broodstock; nutrition; vitamin; carotene; protein; fatty acids

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1. Introduction

Aquaculture is a heterogeneous practice worldwide in terms of the number of species with culture potential. Thus, diversification by cultivating and commercializing new species has a positive impact on annual increases in production. The success of diversification is directly related to captive broodstock, and involves partial or total closure of the life cycle, control of environmental variables such as photoperiod and temperature, management of sex ratio (females and males), selection of the best broodstock in terms of embryo, sperm, and water quality, and determining the best diet to ensure a healthy broodstock [1]. Certain species of marine fish that are currently cultured have huge potential for diversification. For example, fish of the families Sparidae, Carangidae, and Lutjanidae, among others, have been domesticated; examples include *Sparus aurata*, *Seriola rivoliana*, *Seriola dorsalis*, *Seriola dumerili*, *Seriola quinqueradiata*, *Pagrus major*, *Pagrus pagrus Acanthopagrus latus*, *Chano chanos*, *Gadus morhua*, *Hippoglossus hippoglossus*, *Lutjanus argentimaculatus*, *Lutjanus campechanus*, *Paralichthys olivaceus*, *Pseudocaranx* and *Dicentrarchus labrax*.

Currently, aquaculture research seeks to improve broodstock rearing and spawning techniques, as companies in the aquaculture sector aim to produce in a consistent and controlled manner the greatest number of high-quality eggs to meet their production needs and sales targets. One of the main problems encountered in marine fish reproduction under culture conditions is obtaining viable gametes that meet the characteristics required to produce strong and well-nourished larvae capable of surviving the ontogenetic process. The ongoing search for improved understanding of the nutritional requirements of broodstock continues, and research has expanded to consider specific dietary needs associated with improvement of reproductive performance. Experimental testing has included implementation of functional additives in diets to enhance reproductive performance [2]. The objective of this current review is to analyze contributions related to the nutritional aspects of marine fish broodstock, highlighting the effect of implementing functional additives in broodstock diet and their influence on progeny quality and maturation in captivity, as well as suggesting areas where more research is needed.

2. Importance of Nutrition in Brood Fish

Nutrition is a vital factor affecting fish in the reproductive stage. This is especially the case among fish that have been recently introduced into aquaculture, because their reproductive performance can be highly variable. In these cases, nutrition represents a significant component that can limit maturation, fecundity, and larval survival [3]. In captive fish, the quality of the diet administered can affect gonadal development and fertility rate if there is a deficiency of essential nutrients [4]. Likewise, the nutrients supplied in the diet have an effect on maturation, egg viability, and larval survival [5–7]. For example, vitamin E deficiency in the diet was found to decrease the percentage of normal eggs and the fecundity of *Sparus aurata* [3].

Increased attention has been focused on understanding the effects of nutrition and feeding during fish reproduction, including its effects on egg quality, because of the increasing demand for adequate and consistent egg production to meet the needs of aquaculture farms. Thus, it is important to understand whether a possible nutritional deficiency could be the main cause of reproductive failure in wild fish [8].

3. Nutritional Requirements for Brood Fish

The quality and quantity of nutrients in fish feed play important roles in growth, reproduction, and other physiological functions. These organisms in their different stages require specific proteins, fatty acids, vitamins, and minerals. Macronutrients may come from fresh or frozen food (fish, squid, etc.), processed food (pellets), or a mixture of both. However, it is sometimes necessary to supplement certain functional feed additives, among which probiotics, prebiotics, fatty acids, amino acids, and vitamins are the most commonly applied. Because the aquaculture industry keeps fish in captivity in ponds or cages, the organisms may be limited in their ability to obtain optimal nutritional requirements such as they may receive in the natural environment. Therefore, diets should be supplemented with proteins, lipids, vitamins, and inorganic components, in order that the development and survival of the fish are not damaged [6,9,10].

4. Functional Additives and Reproductive Performance

Aquaculture researchers have realized that not only is it essential to know the percentage of lipids and proteins in diets, but also the levels of functional additives that play a crucial role in maintaining high-quality spawning. In this context, functional additives are defined as substances that exert a benefit for a certain biological function.

The provision of functional additives in conventional broodstock diets is intended to improve progeny quality, among other benefits. The quality of larvae and eggs is a very important parameter in the aquaculture industry, because a large quantity of eggs of optimum quality is necessary to allow further development of the cultured organism [11]. The main parameters considered in the evaluation of progeny quality are egg viability, hatching rate, and survival. However, other markers exist to establish quality during embryo development or in the vitelline larval stage, for example, egg morphometry, oil drop, biochemical composition of yolk reserves, and malformations in larvae.

4.1. Proteins, Main Food Additives

Proteins are the most abundant nutrients found in eggs [12] and play a fundamental role during all stages of fertilization, embryonic development, growth, and reproduction [13–15]. The protein requirements of fish depend on the availability of protein sources, energy in the diet, and water temperature [16].

Feeding the optimal amounts of protein allows the formation of lipoproteins, hormones, and enzymes, which are essential in the formation of oocytes and spermatozoa. Proteins such as lipoproteins, hormones, and enzymes are found in fish eggs, determining egg quality and, therefore, the availability of juveniles for large-scale production. Multiple studies have demonstrated that when broodstock are fed a protein-rich diet, the benefits include enhanced gonadal maturation, improved spawning, high hatching rates, and improved larval egg survival [3,17].

Table 1 shows the protein values used in different fish species diets in the reproductive stage.

Table 1. Suggested crude protein percentages in different diets of marine broodstock fish. CP: percentage of crude protein; CL: percentage of crude lipids.

Species	CP %	CL %	Weight (Kg) *	Size (cm) **	Reference
Seriola dumerili	54–58.9	18.3–24.8	11.1–14	-	[18–20]
S. rivoliana	69.3	21	10.321	-	[21]
S. quinqueradiata	43.7–65	6.7–24.9	6.1–7.6	69.1–71.4	[22–26]
Sparus aurata	48.9–56.3	14.7–19.1	1.2–2	37.1–41.2	[27–29]
Pseudocaranx dentex	50.8	14	3.5	-	[30]
Dicentrarchus labrax	-	22	-	-	[31]
Paralichthys olivaceus	53.1–52.3	16–17.5	1.3–2.2	-	[32,33]
Lutjanus argentimaculatus	39.4	8.6	3.24	58.9	[34]
Lutjanus campechanus	83.5	2.4	3	-	[35]
Pagrus pagrus	45	10	3.07	-	[36]

* Average weights; ** average sizes; - data not reported.

Reproductive performance can be conditioned by the protein source and concentration provided to broodstock, which are kept in captivity and are usually fed raw fish or artificial diets. In many cases, the protein level in the diet may not be sufficient, which could reduce reproductive performance. Possible solutions are to increase protein levels or modify the protein source.

Various researchers reported good results regarding egg quality when broodstock were fed different protein sources, in terms of fertility, egg hatching, and larval survival (Table 2). Introducing cuttlefish meal into the diet of *Pagrus major* was demonstrated to generate better reproductive performance compared to other protein sources, such as whitefish meal or krill [37–39]. Likewise, use of the same protein source for *Dicentrarchus labrax* broodstock at different concentrations affected the quality of spawning, which improved when high protein concentrations were used [40].

Regarding *Seriola quinqueradiata*, no significant differences in spawning were observed when frozen fish and pellets were used as the only food sources [41]. Nevertheless, those results were contradicted by reports of a significant improvement in *S. quinqueradiata* reproductive performance with the use of dry pellets in contrast to raw fish, indicating that implementing different types and quantities of proteins in the diets can play an important role in reproductive performance [42]. Furthermore, *Pseudocaranx dentex* spawn demonstrated better quality in terms of viable hatching rate when the fish were fed a mixture of raw fish that included mackerel, squid, and shrimp in a 2:2:1 ratio, compared with those fed pellets [43]. In the 2000s, Emata and Borlongan researched diets beneficial to obtaining better spawning quality in *Lutjanus argentimaculatus*, using raw fish and a mixture of flours (fish, soybean, and wheat) as the main protein sources. The authors demonstrated that the use of raw fish improved the fertilization rate and quantity of larvae obtained [34]. In addition, the reproductive performance of *Acanthopagrus latus* was explored by applying three different protein concentrations in the broodstock diet. The diet included fish and soybean meal as the main proteins, alongside casein. The authors reported that viability and hatching were slightly better at 60% dietary protein concentrations than at 40 or 50% [44]. In another study, Abrehouch et al. evaluated *Pagrus pagrus* spawning after fish were fed diets with different protein sources. The authors found significant differences in the hatching rate when using bogue (*Boops boops*) and squid, in contrast with sardine and pellets [36].

Similarly, when *S. rivoliana* broodstock were fed with mackerel, the number of eggs significantly increased and the hatching rate improved, in contrast with treatments of mussel with squid, and mackerel and pellets with squid meal. However, no significant differences were found when fertilization rates were compared [21]. Finally, Sarih et al. supplemented the diet of *S. quinqueradiata* with a raw protein increase of 56%. The improvements in hatching rate and percentages of viable eggs were significant [19].

Table 2. Reproductive performance of different species fed different protein sources and concentrations. C: Concentration; V: Viable eggs; F: Fertilization; H: Hatching; LO: Larvae obtained.

Species	Source	C (%)	V (%)	F (%)	H (%)	LO (%)	Reference
Pagrus major	White fish	72	-	-	26.3	6.2	[37]
	Casein	51	-	-	0.9	-	
	Cuttlefish meal	57	-	-	93	97	
P. major	Fish meal	67	37.5	-	56.9	14.3	[38]
	Cuttlefish meal	61	98.4	-	95	92	
	Raw krill	100	86.2	-	93.4	75.8	
P. major	Fish meal	67	65.6	-	57.1	89.5	[39]
	Defatted krill	64	69.2	-	59.7	92.3	
	Cuttlefish meal	61	99.3	-	84.5	97.8	
Dicentrarchus labrax	Fish meal	51.3	50.3	94.3	4.04	1.9	[40]
	Fish meal	32.6	34.7	85.8	0.04	0.16	
Seriola quinqueradiata	Raw fish	-	57.7	77.5	43.1	90.4	[41]
	Wet pellet	-	58.7	71.1	36	91.2	
	Dry pellet	-	56.6	70	31.3	90.8	
S. quinqueradiata	Raw fish	-	35.7	3	-	-	[42]
	Wet pellet	75	39	33.8	17.7	-	
	Dry pellet	65	59	56.9	46.9	-	
Pseudocaranx dentex	Dry pellet	-	71	55.6	45.9	-	[43]
	Raw fish mix	-	83	66.2	60.3	-	
Lutjanus argentimaculatus	Flour mix	78.9	-	76.9	74.	71.2	[34]
	Raw fish	-	-	72.6	70.4	69.3	
Acanthopagrus latus	Pellet	40	58.7	82.3	47.5	-	[44]
	Pellet	50	57.7	76.9	40.3	-	
	Pellet	-	60.9	77.2	48.5	-	
Pagrus pagrus	Sardine–pellet	95	85	-	48.	-	[36]
	Bogue–squid	83	86	-	67.5	-	
S. rivoliana	Squid–mussel	59.8	-	99.5	72	74.3	[21]
	Mackerel	62.8	-	99.7	92.4	83.7	
	Squid meal	64.9	-	99.8	89.1	56.5	
S. dumerili	Squid and fish meal	58.3	90	56.21	87.6	-	[19]

- Data not reported.

The data obtained reveal that soybean and wheat meal are very common ingredients in pelleted feeds, which are intended to complement or replace fishmeal-based feeds. Different studies have shown that vegetable meals are not a good substitute for fishmeal, as they decrease the reproductive performance of fish. In contrast, the best protein source for broodstock is frozen raw fish.

If feed is supplemented with animal meals, such as krill or cuttlefish meal, reproductive performance benefits from better quality eggs and larvae. It is important to consider that each farmed species has different nutritional requirements. Thus, further studies should be performed to confirm the effects of different types of proteins added to broodstock diets, to establish the sources and amounts of protein optimal for improving the reproductive performance of different species. Nevertheless, the research to date has not consistently identified the optimal percentages of total protein for different fish species. Hereinafter, this review discusses how to modify the percentages of specific amino acids that affect reproductive performance, indicating the specific nutritional demands that each species has.

4.2. Amino Acids as Functional Additives

Amino acids are the basic protein components necessary for fish at all their development stages, acting as energy substrates and participating in different metabolic pathways. At least 50% of the amino acids consumed by fish are deposited in muscle [45]. However, fish are unable to synthesize all the amino acids they require, so these must be provided in the supplied diet [14]. Taking into account requirements that vary according to species and growth stages, the National Research Council (NRC) has made recommendations on essential amino acids considered important for fish [16] (Table 3).

Table 3. National Research Council requirements of essential amino acids in fish diet in relation to stage of life. Adapted from Andersen et al. [46].

Amino Acids	Stage of Life			
	Larvae-Alevine	Juvenile	Adult	Average
	% of Diet			
Arginine	1.7	1.7	1.5	1.6 ± 0.11
Histidine	0.8	0.76	0.6	0.72 ± 0.10
Leucine	2.3	2.1	2	2.1 ± 0.15
Isoleucine	1.3	1.2	1.5	1.3 ± 0.15
Lysine	2.5	2.3	2	2.2 ± 0.25
Methionine + Tyrosine	1.2	1.2	1.5	1.3 ± 0.17
Methionine + Cysteine	2.7	2.4	2	2.3 ± 0.35
Threonine	1.5	1.5	1.3	1.4 ± 0.11
Tryptophan	0.3	0.3	0.4	0.3 ± 0.05
Valine	1.7	1.67	1.5	1.6 ± 0.10

Broodstock diets should be supplied with essential amino acids either in the form of protein or other supplements. Amino acid composition has been analyzed in broodstock diets (Table 4), such as *S. dumerili* [19], *S. dorsalis* [47] and *Sparus aurata* [48]. Regardless of the essential amino acids required by the fish, the implementation of certain non-essential amino acids (e.g., taurine) plays an important role in reproductive performance, as reported for *S. quinqueradiata* by Matsunari et al. [22]. However, information about the addition of this non-essential amino acid into broodstock diet is poorly documented. To validate the dosage and confirm its efficiency in diets, more research is necessary to fully understand the effect of taurine in other fish species.

Table 4. Amino acid content and composition (% dry matter) in different experimental diets for broodstock.

Amino Acids	*Seriola dumerili* [19]			*Seriola dorsalis* [47]		*Sparus aurata* [48]	
Essential Amino Acids (EAA)	**A1**	**A2**	**A3**	**B1**	**B2**	**C1**	**C2**
Arginine	2.6	2.7	2.9	2.3	2.2	3.4	3
Histidine	1.5	1	1.0	0.9	0.9	1.3	1.2
Isoleucine	1.8	1.9	2.1	1.7	1.6	2.1	2.1
Leucine	3.4	3.5	3.8	3.1	3.03	3.2	3.9
Lysine	2.9	3	3.2	2.5	2.5	2.9	2.3
Methionine	1.2	1.2	1.3	-	-	1.1	0.9
Phenylalanine	1.9	2	2.1	1.8	1.8	2.4	2.5
Threonine	1.8	1.9	2	1.5	1.5	1.9	1.7
Tryptophan	-	-	-	0.4	0.4	0.6	0.6
Valine	2	2.1	2.2	2	1.9	2.5	2.4
Non-Essential Amino Acids (NEAA)							
Cysteine	0.5	0.5	0.6	0.5	0.4	0.6	0.6
Tyrosine	1.3	1.4	1.4	1.3	1.3	1.8	1.8
Alanine	2.4	2.5	2.7	2	2.2	-	-
Aspartic acid	3.9	4.0	4.2	3.1	3	-	-
Glutamic acid	8.9	9.2	10.	7.5	7.3	-	-
Glycine	2.8	2.9	3.1	2.6	2.5	-	-
Proline	2.9	2.9	3.1	2.8	2.7	-	-
Serine	2.1	2.2	2.3	1.6	1.6	-	-
Taurine	0.3	1.1	0.3	0.2	2.6	-	-
Total EAA	19.4	19.6	20.9	16.	16.1	21.4	20.6
Total NEAA	25.4	27	28.1	22.2	24.0	2.4	2.4

A1: Diet with histidine; A2: Diet with taurine; A3: Diet with increased protein. B1: Control diet; B2: Diet with taurine. C1: Fish meal and fish oil diet; C2: Vegetable meal and fish oil diet.

Taurine, an essential amino acid, possesses unique properties because it is not included in protein composition, as a peptide bond is not added to the protein. Thus, it is the freest acid in animal tissue. The physiological role of taurine in fish was investigated by Divakaran [49], who reported its involvement in numerous physiological processes, highlighting its neuromodulatory actions, calcium absorption, fat modulation by emulsion binding with bile acids, osmoregulation, metabolite reproduction, and detoxification. Consequently, taurine is essential for various functions of the organism [49]. *Seriola quinqueradiata* fed a taurine-free diet were reported to have lower growth efficiency and feed conversion rate, higher mortality, increased anemia, and higher incidence of green liver disease [50]. Similarly, taurine administered to catfish (*Ameiurus catus*) as a dietary supplement had an anti-stress effect, demonstrating increased catalase and superoxide dismutase activity, and decreased levels of lipid peroxidation [51].

The effect of taurine administration on marine fish spawning was studied in species of the genus *Seriola* (Table 5). The null addition of taurine to the broodstock diet for *S. quinqueradiata* was found to decrease spawning success. Conversely, fish spawned eggs with higher hatching success and showed better quality spawning when taurine was supplemented at 1.0% [22]. In the case of *S. dorsalis* broodstock supplemented with taurine at 2.67%, significant improvements in their reproductive performance were observed compared with those receiving no exogenous supply [47]. However, supplementation in *S. dumerili* of histidine at 0.5 to 1.5% showed better results than taurine on reproductive performance. Nevertheless, the addition of taurine in the diet at levels from 0.3 to 1.1% revealed an improvement in fecundity in comparison to broodstock fed a higher protein diet [19].

Table 5. Reproductive performance of different species fed different protein sources and concentrations. C: Concentration; V: Viable egg; H: Hatching; S: Survival.

Species	Source	C (%)	V (%)	H (%)	S (%)	Reference
Seriola dumerili	Histidine	1.5	97	96.1	50	[19]
	Taurine	1.1	81.9	77.9	31.	
	Protein	56	90	87.6	46.85	
S. dorsalis	Taurine	2.6	57.4	62	-	[47]
	Taurine	0	30	61.7	-	
S. quinqueradiata	Taurine	0	-	-	-	[22]
	Taurine	0.5	14	6.1	-	
	Taurine	1	81.7	33	-	

The addition of essential amino acids such as taurine in broodstock diet is important because fish are not able to synthesize this amino acid, so an external supply is necessary. Addition of this amino acid into diets has contributed to increased fertilization rates, production of more eggs, and improved larval survival. However, it is important to continue generating knowledge about amino acid supplementation, because comparatively little information is available about their potential effects on the reproductive performance of marine fish.

4.3. Lipids and Fatty Acids as Functional Additives

Lipids are a diverse group of organic compounds that are insoluble in water but soluble in organic solvents, and are involved in a wide variety of biological functions vital to all organisms. For example, they are considered among the structural and scaffolding elements of cell membranes, while metabolically they function as energy reserves and hormone precursors, participating in various signaling pathways, contributing to digestive capacity and overall health [52–54]. Lipids are present in a wide variety of molecules, including phospholipids, sterols, terpenes, and fatty acids, among many others. However, the most widely implemented within fish are polyunsaturated fatty acids [52] which include omega-3 and omega-6. From the omega-3 fatty acids, some of the most commonly used in this context are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). According to the data reported in this review, linoleic acid and arachidonic acid (AA) are among the omega-6 lipids most frequently used as dietary supplements in farmed fish.

Essential fatty acids (EFAs) are the major nutrients affecting fish development and reproductive performance [3]. Most fish in the reproductive stage use these acids for the production of cell membranes, necessary for somatic growth and successful larval development. Therefore, providing sufficient EFAs in adequate quantities is important for producing strong and healthy cells [3,4,17]. Consequently, the diet provided to broodstock should contain abundant fatty acids to meet their energy needs, increase gamete production, and improve larval quality [3,4,17].

Carnivorous species such as *Seriola* sp. require EFA supplementation, specifically LC-PUFA [55,56], because they cannot optimally convert linoleic acid (18:2 n-6) to ARA, or α-linolenic acid (18:3 n-3) to EPA and DHA [53]. The EFA profile of dietary ingredients can differ considerably, depending on the type of food fed to the organisms. For example, fish meal and fish oil contain high levels of highly unsaturated long chain omega-3s (LC n-3 HUFA) such as eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA), and the LC n-6 HUFA arachidonic acid (20:4 n-6, ARA) [57].

Table 6 shows amounts of fatty acids reported in different diets used for broodstock.

Table 6. Total amounts of fatty acids in broodstock diets.

Species	∑Saturated	∑Monounsaturated	∑n3-HUFA	Reference
Seriola quinqueradiata	30.3–30.6	30.5–34.3	4.5–25	[23–25,42]
Pseudocaranx dentex	26.9	34.5	3.8–22.7	[30,58]
S. dumerili	29.2–31.4	24–32.9	29	[18,20]
S. dorsalis	30.6	29.6	25.6	[59]
Sparus aurata	21.7–33.4	31.9–33.7	20.4	[10,60]
Paralichthys olivaceus	39.7	34.7	2.36	[61]
Acanthopagrus latus	23.9	16.9	37.7	[44]
Pagrus pagrus	27.8	29.1	33.64	[36]
Hippoglossus hippoglossus	25.3	34.2	-	[62]

In terms of lipids, fatty acids are one of the nutrients most frequently used to evaluate improvement of egg quality. Several studies have observed egg condition to examine the effects of increasing and decreasing quantities of fatty acids in the diet (Table 7). As mentioned above, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), n-3 highly unsaturated fatty acids (n-3 HUFA), and n-3 long chain polyunsaturated fatty acids (LC n-3 PUFA) are among the most studied fatty acids supplied to broodstock.

S. aurata is among the best studied species with respect to dietary fatty acid supplementation. Increasing n-3 HUFA in the diet evidently improves egg lipid content; a positive correlation was found between dietary and egg levels of n-3 HUFA [63]. The incorporation of squid oil had a positive effect on the total number of eggs produced. However, n-3 HUFA content at 1.08% was confirmed to be insufficient to meet the requirements of *S. aurata* [10], which include 1.6% n-3 HUFA [63]. A deficiency of n-3 HUFA present in the diet leads to lower fatty acid composition in eggs. However, when fish were fed with 1.8% n-3 HUFA, they showed better egg production [64]. The inclusion of fatty acids in the diet affects egg composition and quality, i.e., increasing the levels of 18:1 n-9, 18:3 n-3, and 20:3 n-3 in eggs improves their chemical composition [65].

As research on *S. aurata* continues, reproductive performance in terms of egg viability, hatching rate, and fertility has been improved by adding n-3 HUFA to broodstock diet. Moreover, supplementing diets with high levels of HUFA was found to cause no negative effects on the eggs [28]. *Spaurus aurata* broodstock performance was affected when fish oil was replaced entirely by rapeseed oil [66]. The implementation of diets rich in α-linolenic acid caused a negative effect on progeny, compared to broodstock fed diets high in fish meal and fish oil, which had higher levels of saturated, monounsaturated, and n-3 LC PUFA (20:5 n-3, 22:6 n-3) [29]. Likewise, the production of viable eggs and larvae per spawning significantly improved when broodstock were fed a diet based on fish oil [29,60].

Supplementation with vegetable oils in broodstock diets resulted in a decrease in reproductive performance, reducing female fecundity, and lowering the EPA and DHA content of eggs [48]. Progeny from broodstock supplemented with rapeseed oil showed higher PUFA content in the liver and muscle, as well as increased growth as when high acyl desaturase 2 (*Fads*2) activity was found [67].

When lipids were added to their diet through trash fish, *Dicentrarchus labrax* showed improved reproductive performance and the fatty acid composition of their eggs showed a significant increase in arachidonic acid (22:5 n-6) and DHA, compared with fish that had been supplemented with a combination of fish oil and corn oil [68]. However, the addition of lipids is not always the best approach. Pelleted feed has been supplemented with different quantities of lipids. Simply using trash fish (*Boops boops*) provided results showing better fecundity, egg viability, and hatching rate [69]. The implementation of tuna orbital oil in the diet was proven to be better than fish oil for reproductive performance, improving viability, hatching, and survival; likewise, the implementation of this oil generated higher concentrations of PUFAs and lower concentrations of monounsaturated acids in the eggs [70]. Asturiano et al. reported no significant differences in fecundity when using fish oil or tuna orbital oil, but a slight difference in larval survival was observed with the use of orbital oil due to its high percentage of n-6 PUFAs [31].

The study of the genus *Seriola* has offered increasing promise for improving reproductive performance. The composition of fatty acids in the ovaries of *S. dumerili* was analyzed, contrasting the use of fish oil against an experimental diet composed of different lipid sources, such as fish oil, rapeseed oil, and Algaritum DHA70. The ovaries of the fish receiving the experimental diet showed higher proportions of oleic acid (18:1 n-9) and vaccenic acid (18:1 n-9), reducing occurrence of fertilization. In contrast, the fish fed with commercial diet showed the highest values of total HUFA and PUFA in ovaries, indicating better quality [20]. Stuart et al. supplemented the diet of *S. dorsalis* with arachidonic acid (ARA). Fish without exogenous addition of ARA (1.4%) in their diet spawned 23 times more than those supplemented with 4.6% ARA; however, egg quality was much better in the 4.6% treatment group, and the final 20:4 n-6 content of the eggs was 4.8 higher than those receiving the 1.4% treatment (1.9, 20:4 n-6) [59]. Finally, the synergistic effects of different fish and vegetable oil concentrations have been reported, indicating better fecundity in *S. dumerili* broodstock fed with linseed (3%), palm (4%), and fish (3.9%) oils, as well as higher fertilization rates and egg viability, indicating that the ideal levels of n-3 LC-PUFA range from 1 to 1.7% [18].

Although most relevant studies have focused on *S. aurata*, *D. labrax*, and species of the genus *Seriola*, the addition of fatty acids to diets has also been analyzed in other marine-spawning fish species. In *Acanthopagrus latus*, for example, three different lipid concentrations were added to the diet using a mixture of fish oil and sunflower oil, with results indicating that lipid concentrations at 15% expressed better results in terms of fecundity, viability, hatching, and larval survival [44]. Zakeri et al. evaluated different sources of n-3 HUFA, using fish oil, sunflower oil, and a mixture of both. An improvement in the quality of progeny was corroborated in terms of viability, hatching, and survival, and the highest amounts of DHA (29.3), EPA (10.07), and ARA (2.84) were observed in the eggs of fish with 10% pure fish oil in their diet [71].

The diet of *Paralichthys olivaceus* has been supplemented with different levels of HUFA n-3 (0.4, 0.8, and 2.1%), and the best spawning parameters were obtained with the addition of 10% haddock visceral oil, achieving higher concentrations of HUFA n-3 in eggs and a higher percentage of lipids (23.46%) [61]. Arachidonic acid (AA) was added at different concentrations (0.1, 0.6, and 1.2%) using AA ethyl esters (SUNTGA 40S and ethyl oleate), where all the parameters of egg quality measured were higher in fish supplemented with 0.6% AA. The highest lipid concentration occurred at 2.1% AA. Nonetheless, excess of AA can negatively affect reproduction, and its increase does not improve egg quality [32].

The addition of menhaden oil (*Brevoortia* sp.) compared with the addition of a mixture of two commercial products (DHA Gold® and ARASCO®, Riyadh, Saudi Arabia) was evaluated in *Lutjanus campechanus*. The results revealed that eggs showed significant changes in their composition of fatty acids when commercial supplements were used, and that none of the oils improved reproductive performance [35].

In conclusion, supplementation or addition of lipid sources, especially fatty acids, plays a key role in reproductive performance. Several investigations have demonstrated that the egg quality of different species can be improved in terms of fertilization, viability, hatching, and larval survival by using different sources, such as HUFA n-3 or LC-PUFA n-3 (EPA, DHA). Enrichment of diets with lipids consequentially alters in the fatty acid profile of eggs, as lipid quantity and quality can increase or decrease the quality of the eggs. The origin of these lipids is very important, and using only vegetable oils is not the best option. Results have shown that fish oil is superior to vegetable oils, as it improves egg quality. Finally, the data provided in this review represent an excellent starting point for the further development and improvement of diets in future.

Table 7. Egg fatty acid composition and reproductive performance with use of different lipid sources. C: Concentration; V: Viability; H: Hatching; S: Larval survival; Sat: Saturated.

Species	Source	C (%)	∑Sat	∑MUFA	n-3 HUFA	∑PUFA	V (%)	H (%)	S (%)	Reference
Sparus aurata	Sardine oil	0	2.6	2.8	1.4	-	70.8	96.9	47.5	[63]
	Sardine oil	2.31	2.8	2.5	1.7	-	74.8	96.4	46.9	
	Sardine oil	4.57	3.1	3.1	1.7	-	77.1	94.8	36.4	
	Sardine oil	8.35	3.02	2.7	1.9	-	78.5	96.8	37.3	
S. aurata	Sardine oil	2.31	33.4	33.7	20.4	-	75.1	96.6	24	[10]
	Squid meal	61.7	34.3	33.8	21	-	76.8	96	24.3	
	Squid oil	8.5	23.7	31.8	22.5	-	80.8	91	19.1	
	Sardine oil	7.2	20.7	55.9	13.1	-	78.6	95.2	24.9	
	Oil free	0	32.7	32.8	18.4	-	76.3	91.6	20.1	
S. aurata	Cod liver oil	6.5	25	-	17.7	-	90	42	-	[64]
	Olive and flaxseed oil	7, 4.2	18.9	-	11.5	-	40	30	-	
S. aurata	Cod liver oil	6.5	33.3	-	-	-	-	-	-	[65]
	Olive and flaxseed oil	7, 4.2	21.9	-	-	-	-	-	-	
S. aurata	n-3 HUFA	2.4	28.9	32	29.5	63.9	86.4	92.9	83.8	[28]
	n-3 HUFA	3.8	30.7	22.8	36.8	64.05	89.6	96.2	85.1	
	n-3 HUFA	2.7	29.1	30.9	31.5	65.4	83.5	95	86.2	
	n-3 HUFA	4	31.5	22.94	35.4	62.8	95.3	97.2	84.6	
S. aurata	Fish oil	-	18.1	25.6	43.9	-	78.6	96	81.7	[66]
	Rapeseed oil	-	17.3	34.55	31.1	-	66.5	90.2	76.2	
	Fish oil	-	29.3	28.6	27.6	-	65.3	89.5	75.6	
	Rapeseed oil	-	23.3	32.7	22.8	-	62.2	90.6	71.9	
S. aurata	Fish oil	8	-	-	-	-	92.6	-	-	[29]
	Vegetable oil	5.6	-	-	-	-	89.9	-	-	
S. aurata	Fish oil	9.9	25.3	31.8	-	29.6	81.3	92.2	93.6	[60]
	Vegetable oil	9.9	18.7	35.4	-	25.8	54.3	91.1	87.2	
S. aurata	Fish oil	9.8	-		-	-	59.63	49.6	38.2	[48]
	Fish oil	10.9	-		-	-	53.9	50.2	38.9	
	Vegetable oil	8.2	-		-	-	53.6	49.3	39	
Dicentrarchus labrax	Fish and corn oil	1.3	28.7	19.1	-	48.7	45.2	28.7	-	[68]
	Trash fish	4.8	31.1	15.9	-	48.7	25.8	8.1	-	
D. labrax	Fish oil	-	19.5	37.5	-	38.3	40	9	18	[70]
	Tuna orbital oil	-	18.5	31.9	-	45	62	29	60	
D. labrax	Trash fish	20	-	-	-	-	45.22	28.7	-	[69]
	Fish oil	10	-	-	-	-	2.59	0.45	-	
	Fish oil	22	-	-	-	-	10.68	3.55	-	
D. labrax	Fish oil	22	-	-	-	-	-	-	13.9	[31]
	Tuna orbital oil	21	-	-	-	-	-	-	20.9	
Acanthopagrus latus	Fish and sunflower oil	15	-	-	-	-	58.9	50.6	90.9	[44]
	Fish and sunflower oil	20	-	-	-	-	59.5	43.8	62.1	
	Fish and sunflower oil	25	-	-	-	-	58	41.9	58	
A. latus	Fish oil	10	29.3	28.6	39.3	-	60.2	59.8	89.	[71]
	Sunflower oil	10	34.6	36.3	22.7	-	58.4	39.1	64.5	
	Fish and sunflower oil	5,5	34.3	33.2	27.8	-	57.7	43.5	59.12	
Seriola dumerili	Fish oil	13	25.6	24.5	-	47.36	-	-	-	[20]
	Fish oil, rapeseed oil, and Algaritum DHA70	2.1, 8.2, 1.7	25	25.9	-	44.23	-	-	-	
S. dumerili	Flaxseed oil, palm oil, and fish oil	4.5,5.9, 0.6	26.8	24.8	-	24.6	86.	80.6	33.3	[18]
	Flaxseed oil, palm oil, and fish oil	3, 3.9, 4	27.7	23.3	-	24.93	84.9	80.9	29.3	
	Flaxseed oil, palm oil, and fish oil	1.5,2, 7.4	27.5	23.3	-	24	84.9	83.2	35	
	Fish oil	10.9	27.5	22.4	-	23.5	84.5	78	32.8	
S. dorsalis	*Mortierella alpina* oil	1.4	22.2	30.5	-	45.1	33.7	52.4	-	[59]
	Mortierella alpina oil	4.7	21.6	28.9	-	43.1	72.1	25.6	-	

Table 7. *Cont.*

Species	Source	C (%)	∑Sat	∑MUFA	n-3 HUFA	∑PUFA	V (%)	H (%)	S (%)	Reference
Paralichthys olivaceus	Palm olein	10	29.1	22.6	10.1	-	62.2	76.8	67.8	[61]
	Haddock visceral oil and palm olein	2, 8	32.4	19.4	11.5	-	46	53.1	76.9	
	Pollock visceral oil,	10	30.7	24.1	16.55	-	67.3	89.2	94.1	
P. olivaceus	SUNTGA 40S, and ethyl oleate	0, 6	24.3	44.5	14.9	-	42.1	50.6	47.7	[32]
	SUNTGA 40S and ethyl oleate	1.2, 4.8	26.2	40.7	15.9	-	44.5	79.4	79.3	
	SUNTGA 40S and ethyl oleate	2.4, 3.6	27	39.3	15.2	-	33.5	27.2	0	
Lutjanus campechanus	*Brevoortia*	3.6	36.7	27.9	-	36.4	-	88.2	70	[35]
	DHA Gold and ARASCO	2.4, 1.2	39.8	37.8	-	22.4	-	-	-	

4.4. Vitamins and Carotenoids as Functional Additives

Vitamins are organic compounds essential for proper growth, reproduction, and health, and should be supplied in the diet in small amounts [14]. Lack of vitamins is the most common cause of deficiency in commercial aquaculture [72]; the signs include abnormal swimming, skin disease, bone deformities, edema, eye and gill pathology, hemorrhage, liver disease, and growth retardation [14]. The most relevant vitamins to consider in the diets of fish are A, D, E, and K (fat-soluble), along with vitamin C and those of the B complex (water-soluble) [14].

During fertilization, other specific nutrients are important for reproduction in fish, such as vitamin E (alpha-tocopherol), vitamin C (ascorbic acid), and carotenoids [73,74]. In salmon, vitamin C has shown to play an important role in reproduction [75,76], as well as in steroidogenesis and yolk sac production [77]. Vitamins C and E have antioxidant properties that help protect sperm during spermatogenesis and fertilization, reducing the risk of lipid peroxidation that is detrimental to sperm motility. In addition, vitamin C deficiency reduces sperm motility during egg laying [3].

Sparus aurata diet supplementation with between 22 and 125 mg·kg^{-1} of vitamin E (α-tocopherol) was shown to reduce significantly the incidence of abnormal eggs [10]. In addition, fortification with this vitamin can improve fertility as well as vitality. In salmon, for example, the optimal vitamin E requirement for successful spawning is 250 mg·kg^{-1} [78]. Conversely, fish supplemented with low levels of vitamin E (<22 mg·kg^{-1}) were reported to have lower reproductive and breeding rates [3]. Vitamin C content in the broodstock diet is essential for collagen synthesis during embryonic development, and improves embryonic survival. Vitamin A is also important for egg laying as well as larval development and gonadal maturation, and plays an important role during embryo development [79].

Carotenoids administered in the diet can also play an important role in animal health, operating as antioxidants and scavenging free radicals that are generated by normal cellular activity and various stressors [80]. The antioxidant activity of carotenoid molecules depends on their structure and the nature of the oxidant itself. Thus, carotenoids have been reported to be the strongest inhibitors of simple oxygen, while astaxanthin, a member of the carotenoid family, reduces the rate of oxidation [81].

Carotenoids may also contribute to hatching success, larval viability, and egg pigmentation. They are known to alter the immune system of fish and improve larval survival, because they are precursors of vitamin A [82,83]. Carotenoids reach the ovary and larvae [84,85] in less than 48 h and influence egg pigmentation [86]. Astaxanthin is one of many carotenoids that influence egg quality [87].

Diets supplied to broodstock are frequently supplemented with commercial vitamin complexes. Certain vitamins or antioxidants have been increased in the diet, resulting in better reproductive performance. Some concentrations of vitamins and carotenes used in broodstock diets are shown in Table 8.

Table 8. Amounts of vitamin A, vitamin E, and carotenes in different diets.

Species	Vitamin A ($IU \cdot g^{-1}$)	Vitamin E ($\mu g \cdot g^{-1}$)	Carotenes	Reference
Seriola quinqueradiata	21.4–26	0.21–471.8	0.3–3 ($g \cdot kg^{-1}$)	[23–26,42]
Sparus aurata	-	-	60 ($mg \cdot kg^{-1}$)	[28]
Pseudocaranx dentex	15.5–16.5	177–327	0.04–3.6 ($g \cdot kg^{-1}$)	[30,43,58]
Pagrus major	-	-	4 ($g \cdot kg^{-1}$)	[88]

- Data not reported.

Spawn quality has been analyzed while using different vitamins (Table 9). Watanabe et al. added vitamin E to the diet of *Pagrus major*; supplementation of 200 mg of α-tocopherol improved the production of normal larvae by 39% compared with treatment without vitamin E [39]. Mangor-Jensen et al. evaluated the effects of vitamin C at different dietary concentrations (0, 50, and 500 $mg \cdot kg^{-1}$) on the maturation and egg quality of *Gadus morhua*. The authors found no significant differences ($p > 0.05$) between the groups with respect to lengths, weights, gonad sizes, and fertilization rates after the addition of vitamin C [89].

The joint and individual effects of dietary supplementation of vitamins C and E (0.1% and 0.05%, respectively) on *Chano chanos* reproduction were examined. Supplementing broodstock with vitamin C resulted in higher egg viability, hatching, and survival rates compared with organisms that were not supplemented. The broodstock supplemented only with vitamin E completed fewer spawnings, and those supplemented with vitamin C produced better quality eggs [90]. However, these results differed from those reported by other researchers, where the use of higher concentrations of vitamin E produced better results in terms of egg quality [39]. In the same context, the addition of vitamin C at low concentrations did not improve reproductive performance [89].

Regarding the supplementation of vitamin A (retinol palmitate) in the diet of *Platichthys stellatus* broodstock, the inclusion of this vitamin was observed to slightly improve the percentage of viable eggs, the hatching rate, and level of larval survival. However, higher retinol concentrations were observed in the ovaries of females supplemented with vitamin A (49.7 $\mu g \cdot g^{-1}$) compared to those not supplemented (5.1 $\mu g \cdot g^{-1}$), as well as darker pigmentation in the skin of broodstock in the supplemented group [91]. Subsequently, the inclusion of vitamin A for *Paralichthys olivaceus* resulted in a slight improvement in reproductive performance, but no significant differences were found [33], so it can be concluded that supplementation with vitamin A has no effect on egg quality. Finally, the effects of dietary vitamins A (retinol acetate), E (α-tocopherol acetate), and C (L-ascorbyl-2-phosphate) were evaluated in terms of the gonadal development and reproductive performance of *Platichthys stellatus*. The gonadal index (GSI), as well as absolute and relative fertility were higher when diets were supplemented with vitamin A, followed by the inclusion of vitamins E and C, respectively [92].

Table 9. Reproductive performance in different fish using different sources of vitamins in diets. V: Viability; H: Hatching; NL: Normal larvae.

Species	Vitamin	Quantity	V (%)	H (%)	NL (%)	Reference
Pagrus major	α-tocopherol α-tocopherol	50 mg·100 g^{-1} 200 mg·100 g^{-1}	65.6 95.1	57.1 77.8	89.5 97.1	[39]
Gadus morhua	Ca-ascorbate-2-monophosphate	0 mg·kg^{-1}	-	-	-	[89]
	Ca-ascorbate-2-monophosphate	50 mg·kg^{-1}	-	-	-	
	Ca-ascorbate-2-monophosphate	2 mg·kg^{-1}	-	-	-	
Chano chanos	Vitamin E Vitamin C Vitamins C and E Vitamins C and E	0.05% 0.10% 0.1%, 0.05% 0%	55.5 50 50 32.2	51.6 42.8 56.5 34.7	16.1 0 13 8.7	[90]
Platichthys stellatus	Retinyl palmitate Retinyl palmitate	0% 0.30%	86 88.9	80.4 89.8	68.7 75.6	[91]
Paralichthys olivaceus	Vitamin A Vitamin A	0.77 mg·kg^{-1} 16.9 mg·kg^{-1}	76 87.8	79.5 77.6	52.8 69.4	[33]
P. stellatus	Retinyl acetate α-tocopherol acetate L-ascorbyl-2-phosphate	8000 IU·kg^{-1} 250 mg·kg^{-1} 500 mg·kg^{-1}	- - -	- - -	- - -	[92]

Few studies have been conducted regarding vitamin supplementation in the diet of marine broodstock, with vitamins A, E, and C being the most investigated in the existing literature. However, the effects of these three vitamins on reproductive performance are highly variable. The implementation of these vitamins has been reported to affect spawning quality, both positively and negatively. The results suggest that when added at specific concentrations these different vitamins can play an important role in the reproductive process. It is important to take into consideration that each species has its specific requirements, so that certain concentrations of these vitamins may be beneficial for one species but not for another. Further research should continue to assess more accurately the vitamins required by broodstock, to develop an understanding of which vitamins play the most important roles in reproductive performance.

The use of carotenes in diets has been evaluated in different species (Table 10). For *S. quinqueradiata*, 10% krill meal was added to their diet, resulting in eggs with a strong yellowish color and high content of zeaxanthin and lutein [26]. Nonetheless, total egg production, hatching rate, fertilized eggs, and larval survival were higher in broodstock fed without krill meal, compared with those receiving diets containing 20 or 30% krill meal. One potentially influential factor could have been that the diet not supplemented with krill contained 20% more fish meal [24]. Similarly, broodstock fed dry pellets supplemented with 30 ppm astaxanthin showed improved reproductive performance [25]. Likewise, egg production tended to increase and better survival rates were observed when different carotenoid sources were used, such as dry pellets with squid meal and paprika, compared with dry pellets with astaxanthin or dry pellets with paprika [23].

The performance of *Pseudocaranx dentex* was studied after feeding 2% *Spirulina* pellets vs. raw fish, and *Spirulina* was observed to have no significant effect on reproductive performance, as larval buoyancy, fertilization, and hatching rates were approximately 20% higher without it [58]. Vassallo-Agius et al. evaluated the effect on *P. dentex* of squid meal and astaxanthin in dry pellet form, compared with a group fed mackerel, squid, and shrimp in a 2:2:1 ratio. The groups showed no significant differences with respect to spawning parameters such as viability and hatching, indicating that the frozen food contained a suitable quantity of carotenoids, resulting in very similar spawning parameters as when astaxanthin was added exogenously [30].

The addition of 73.7 mg·kg^{-1} astaxanthin to the diets of *Gadus morhua* broodstock led to an improvement in egg production and quality, and an increase in numbers of viable and fertilized eggs, showing enhancement after 15 days of egg production, thus improving reproductive performance [93]. Broodstock fed with diets containing higher water content and higher astaxanthin (100 ppm·kg^{-1} with 30% water) showed higher egg production, lower mortality, and higher fertilization compared with fish that were fed lower amounts of astaxanthin and water (50 ppm·kg^{-1} with 30% water and 50 ppm with 10% water) [94].

The percentage of fertilized eggs was found to increase after the addition of β-carotene, canthaxanthin, or astaxanthin to the basal diet of *Pagrus major*. However, the hatching rate did not improve with the addition of these pigments, although a reduction of the lipid droplet anomaly was observed [95]. The addition of carotenoids from animal sources, such as 10% krill meal, was shown to provide better egg and larval quality, and egg numbers improved when 10% squid was added to the diet [42].

Table 10. Reproductive performance in different species with the addition of carotenoids in the diet. C: Concentration; V: Viability; H: Hatching; L: Larvae.

Species	Source	C (%)	Carotenes	V (%)	H (%)	L (%)	Reference
Pagrus major	Oils extract	10	3.2 mg·100 g^{-1}	18.2	27.3	24	[95]
	Krill	-	108 mg·100 g^{-1}	82.7	90	91.2	
Seriola quinqueradiata	Fish	-	-	35.7	-	-	[42]
	Pellet	-	30 g·kg^{-1}	39	17.7	-	
	Krill	10	3%	59.2	46.9	-	
S. quinqueradiata	Fish	-	0.04 g·100 g^{-1}	-	-	-	[26]
	Squid	10	86.7 mg·100 g^{-1}	-	-	-	
	Krill	10	2.59 mg·100 g^{-1}	-	-	-	
S. quinqueradiata	Krill	0	0.63 mg·100 g^{-1}	57.6	51.3	50	[24]
	Krill	20	2.16 mg·100 g^{-1}	47.9	29.8	32.7	
	Krill	30	3.53 mg·100 g^{-1}	52.4	10.8	11.9	
S. quinqueradiata	Astaxanthin	0	2.4 ppm	31.4	21.2	19.4	[25]
	Astaxanthin	20	17.3 ppm	51.6	40	36.9	
	Astaxanthin	30	32.9 ppm	52.6	45.2	42.6	
	Astaxanthin	40	39.6 ppm	21.2	13.7	12.5	
Pseudocaranx dentex	*Spirulina*	2	46.1 mg·kg^{-1}	28.9	23.2	60.7	[58]
	Squid and shrimp	-	6.4 mg·kg^{-1}	52.6	46.6	81.9	
S. quinqueradiata	Squid and shrimp	-	30 ppm	92.6	73.1	-	[30]
	Squid meal	31	16.4 ppm	94.7	77.9	-	
P. dentex	Astaxanthin	30 ppm	36.6 ppm	85.4	64.4	-	[23]
	Paprika	2	38.1 ppm	93.9	94.9	-	
	Squid meal and paprika	28, 2	35.9 ppm	92.7	92.3	-	
Gadus morhua	Astaxanthin	-	100 ppm	92	-	75	[94]
	Astaxanthin	-	50 ppm	88	-	84	
	Astaxanthin	-	50 ppm	59	-	75	
G. morhua	Carophyll Pink	-	-	31.5	11	-	[93]
	Astaxanthin	-	73.7 mg·kg^{-1}	33	13.5	-	-

- Data not reported.

Studies on the addition of carotenoids to broodstock diets have shown promising results, suggesting that the inclusion of these pigments can favor reproductive performance, increasing the number of fertilized eggs and the rate of hatching, as well as leading to a significant reduction in larval mortality. However, further research is required to gain a more complete understanding of all the effects that carotenoids can have on broodstock diets. It can be inferred from the data reported so far that the origin of carotenoids must be considered and they must be provided in adequate amounts to secure positive results with respect to the quality of the eggs and larvae obtained.

4.5. Minerals as Functional Additives

Aquatic organisms exhibit a special physiological mechanism for absorbing minerals from their diet and the aquatic environment. Research on mineral implementation in fish has been relatively slow [96]. Ten minerals (i.e., calcium, phosphorus, magnesium, potassium, copper, iron, zinc, manganese, selenium, and iodine) have been identified and shown to be essential within the fish diet [96,97].

The supply of macro-minerals such as calcium, magnesium, phosphorus, potassium, and sodium is vital for the development of bones and other hard tissues such as fins, scales, and exoskeleton [14]. Currently, information is scarce regarding dietary mineral requirements in fish diets. These inorganic elements are very abundant in the aquatic environment, so fish can obtain their requirements from their natural surroundings [14]. If their diet is deficient in certain minerals, various physiological alterations may result. For example, reduction of phosphorus, magnesium, and zinc results in bone deformation [96]; a deficiency of copper decreases growth, as well as promoting the formation of cataracts [96,98].

Because of the current lack of information on mineral requirements for marine broodstock, information is provided about certain minerals that are considered relevant for juvenile fish (Table 11). It is important to take into consideration that commercial multivitamin complexes are generally provided within the diet; however, there is a need to explore mineral requirements when formulating a diet for broodstock.

Table 11. Macro- and micromineral requirements for various juvenile fish.

Species	Mineral	Requirement (mg)	Reference
Acanthopagrus schlegeli	Calcium	Non-essential	[99]
Gadus morhua	Calcium	Non-essential	[100]
Dicentrarchus labrax	Phosphorus	8600	[101]
Seriola quinqueradiata	Phosphorus	6500	[102]
S. quinqueradiata	Phosphorus	6700	[103]
Pagrus major	Potassium	Non-essential	[97]
G. morhua	Potassium	Essential	[100]
P. major	Sodium	Non-essential	[97]
Salmo salar	Copper	5–10	[104]
P. major	Iron	15	[105]
Sciaenops ocellatus	Zinc	Non-essential	[106]
Oncorhynchus tshawytscha	Iodine	0.6–1.1 mg	[107]
G. morhua	Magnesium	Non-essential	[100]
P. major	Magnesium	12	[108]

4.6. Carbohydrates as Functional Additives

To meet the increased demand for animal protein in aquaculture, new research has emerged to exploit plant-based protein sources. The resulting feeds carry high levels of carbohydrates. Although fish possess the enzymatic capacity to digest and absorb carbohydrates, this function depends on the species, life stage, water temperature, carbohydrate concentration and type, and the degree of processing necessary [109].

Carbohydrates are known to be used by all organisms as sources of energy. Nonetheless, there is insufficient scientific evidence to demonstrate the requirements for these macronutrients in marine broodstock, and there is no specific need for carbohydrates in fish diets. In nature, fish food usually contains small amounts of carbohydrates [16,110]. Providing energy to fish by feeding with carbohydrate supplementation can cause a reduction in the energy expenditure of other nutrients, such as protein and lipids, while ensuring maximum utilization for tissue growth and maintenance [109,110].

Dietary carbohydrate intake varies among species; for example, fish living in warmer waters may consume higher amounts of dietary carbohydrate than fish inhabiting colder waters [111]. Starch has been used in the diet of *S. quinqueradiata*, where it was shown that 10–20% dietary carbohydrate is required for optimal growth [112,113]. However, in the research that was carried out, no scientific contribution was found to support the use of carbohydrates in diets for marine broodstock fish.

5. Conclusions and Recommendations

The reviewed literature shows the current state of knowledge on the nutritional needs of broodstock fish for improving their reproductive performance, and contributes to a general understanding of the proteins, amino acids, lipids, vitamins, and carotenoids required by these organisms. Within this review, the three most investigated species were *Seriola* sp., *Sparus aurata*, and *Pagrus major*, and the three most frequently used functional additives were fatty acids, which contributed 40% of the research, followed by proteins with 24%, and carotenoids with 14%. Therefore, further research should be focused on the use of other functional additives, such as vitamins and amino acids, to elucidate different species' specific needs for these additives in their reproductive stages, as species may react differently to supplementation.

Evidence suggests that the use of functional additives is an excellent option to enhance reproductive performance. However, the source or origin of the functional additive should be considered, because the results depend on it. For example, the quality of protein varies greatly depending on the source. Thus, frozen fish should be used primarily, with animal meal constituting the second most prominent ingredient, together accounting for at least 50% of the diet. Soybean meal is not recommended as a substitute for animal protein, because it may decrease reproductive performance. Similarly, oil of animal origin (e.g., sardine) should be used in minimum concentrations of 20% as a source of fatty acids. For the vitamins, the following quantities are recommended: vitamin A at 6 $mg{\cdot}kg^{-1}$, E at 200 $mg{\cdot}kg^{-1}$, and C at 100 $mg{\cdot}kg^{-1}$; and finally, carotenes at a concentration of 30 ppm.

The dietary addition of amino acids such as histidine and taurine should be considered, using either commercial or experimental products, because these can improve reproductive performance as well as promoting health by preventing green liver. Thus, diets should contain taurine concentrations from 1.5 to 2%. Further research is essential on specific requirements for different amino acids, as well as investigation of the effects they may have on fish progeny.

Questions remain regarding optimal quantities of vitamins, amino acids, and carotenoids for broodstock diets. Despite promising results, the concentrations of these compounds within diets need to be optimized, since the amounts reported have been very variable and can benefit or negatively affect the quality of spawning. Meanwhile, the use of other vitamin sources in broodstock, such as vitamin K, has been observed to help skeletal and neuronal development as well as lipid metabolism, among other functions that need to be expanded on and better understood. In addition, biochemical analysis of eggs and larvae are recommended for understanding and evaluating the importance of these additives in broodstock diets.

Based on the information discussed above, pelleted feeds (dry or wet) are an easy method of providing all the necessary nutrients in a single product, making them an ideal tool for studying the effects of certain nutrients on the quality of oocytes, eggs, and larvae. It is important to continue increasing knowledge about broodstock nutrition. One important area for development is the provision of wet diets that can completely replace frozen foods, such as fish or squid, which involve high costs and do not guarantee optimal nutrition or improved reproductive performance. As our understanding of the nutritional needs of broodstock improves, our ability to design artificial diets should ensure consistent reproductive performance.

Future research should include determining the most effective ways to add the supplement or additive to the diet, as well as finding the appropriate concentrations and duration of the supplementation to obtain optimal response from the broodstock. It is necessary to generate more information about the role played by growth conditions, and a better understanding of fish health can improve knowledge of other factors that could alter reproductive performance and progeny. Finally, the research reported in this review focused mostly on broodstock females, and future studies should also consider the nutritional needs of males for optimal spermatogenesis development and high sperm quality.

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