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Chapter 8

The Potential for 'Next-Generation', Microalgae-Based Feed Ingredients for Salmonid Aquaculture in Context of the Blue Revolution

Sean Michael Tibbetts

Additional information is available at the end of the chapter

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Abstract

Microalgae-based ingredients have potential to ensure continued growth of salmonid aquaculture for global sustainable food security in the blue economy. Algal biorefineries must valorize the entire crop to grow profitable microalgae-based economies. With massive growth and demand for novel sustainable ingredients, farmed salmonid feed sectors are highly promising areas to focus on. Microalgae-based ingredients for salmonid feeds may have market advantages in terms of lower input costs, aerial footprint, wastewater remediation benefits and carbon credits for industrial CO_2 conversion. A handful of microalgae-based ingredients have been proposed as candidates to supply well-balanced nutrients and immunostimulatory compounds. However, technical gaps exist and need addressing before the industry could economically incorporate microalgae-based ingredients into commercial feeds. Current knowledge on comprehensive biochemical composition is incomplete, highly heterogeneous, and information on their nutritional value is scattered and/or inconsistent. The aim of this chapter is to consolidate relatively fragmented data on biochemical composition and nutritional value of microalgae-based ingredients focusing on farmed salmonid feeds. Presented are discussions on the potential for such 'next-generation' ingredients, opportunities/challenges for their use and a compendium of studies evaluating their performance in feeds for economically relevant farmed salmonids, including rainbow trout (*Oncorhynchus mykiss*), Arctic charr (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*).

Keywords: microalgae, salmonids, aquaculture feeds, composition, nutritional value

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1. Importance of aquaculture in the blue revolution

Most developed countries are nearing their terrestrial agricultural output capacity. Terrestrial agriculture will be highly challenged to meet the demands for a growing human population. Food production requires an epic shift towards leveraging intrinsic competitive advantages from our aquatic environment. As such, we have now entered the blue revolution where dietary protein and essential nutrients are increasingly derived from aquatic environments. However, most traditional capture fisheries are depleted or harvested at their biological limits. As stated a half century ago by famous marine explorer and ecologist Jacques Cousteau "We must farm the sea" in order to foster strong global food security. This was reiterated in 2012 by former UN Secretary General Kofi Annan who stated "Aquaculture is crucial for supplying the world's food needs for the next 50 years". Recently, aquaculture has grown annually at 7.8%; far exceeding that of terrestrial farming systems like poultry (4.6%), pork (2.2%), dairy (1.4%), beef (1.0%) and grains (1.4%) [1]. As the appetite for seafoods outpaces what capture fisheries can supply, global farmed seafood supplies in 2009 matched wild-caught seafood and this proportion is projected to rise to 62% of all seafood supplies by 2030. This firmly secures aquaculture's position in the blue economy as the most efficient use of resources for global food production. Gentry et al. [2] reported that a small fraction of coastal ocean waters (0.015%), about the size of Lake Michigan, specifically selected for sustainable aquaculture (excluding areas that interfere with shipping lanes, ocean oil extraction or marine protected areas) is required to exceed current demand for seafood by 100-fold. For the first time in history, global aquaculture production exceeded beef production in 2011 and in 2014 farmed aquatic production was valued at $160 billion USD (74 million metric tons [mmt]) and will exceed $240 billion USD by 2022. Indeed, as global economist and Nobel Laureate Dr. Peter Drucker recently stated "Aquaculture, not the internet, represents the most promising investment opportunity of the 21st century".

2. Formulated compound aquaculture feeds

2.1. The aquafeeds dilemma

Of the 74 mmt of global farmed seafood produced annually, the majority (57 mmt or 77% of total) is from finfish and crustaceans, which are considered 'fed' aquaculture species. This means they require mass-produced formulated complete feeds (aquafeeds) and the production of aquafeeds will exceed 87 mmt by 2025. As a result, modern aquaculture is a major consumer of world fish meal and fish oil supplies, which has placed an unsustainable burden on traditional capture fisheries in South Pacific, South-East Asia and North Atlantic countries. This scenario represents a dramatic shift in use of these finite marine resources during the past half century. Regarding fish meal; feeds for terrestrial animals have traditionally demanded virtually all global supplies and aquafeeds consumed <1% of supply only a few decades ago, while today aquafeeds consume a staggering 73%. The situation is the same for fish oil where in 1960 virtually all supplies were used as hardened edible fats or refined industrial oils and aquafeeds

used <1% of supply, while today aquafeeds consume 71%. Aside from very real ecological issues, this tremendous demand has had a direct and highly consequential economic result of tripling the cost of fish meals and oils. While farmed salmonids represent a marginal contribution (3%) to total global farmed seafood supplies, they consume a disproportionate amount of these finite resources.

2.2. Industrial farming of salmonids

Farming of salmonids (e.g., salmon, trout, charr) uses feed inputs more efficiently than terrestrial animal protein production systems (e.g., beef, poultry and pork). Typical feed conversion ratio (FCR) for salmonids is 1.2 g feed g gain^{-1} compared to 1.8–6.3 g feed g gain^{-1} for livestock. This is due to higher dietary protein and energy retention efficiency in salmonid fish (23–31%) compared to terrestrial farm animals (5–21%). Also, since fish are poikilothermic and expend less energy maintaining their position in the water column, edible yields of farmed salmonids are higher (68%) than terrestrial livestock (38–52%). Salmonid farming occupies low carbon footprints and those farmed in Norway, Chile and Canada may, in fact, be the most ecologically sustainable meat products on the global food protein market. Greenhouse gas (GHG) emissions of 2.2 kg CO_2 eq. kg^{-1} of edible meat produced are reported in contrast to 2.7–30.0 kg CO_2 eq. kg^{-1} for chicken, pork and beef. However, it's important to note that salmonids are highly piscivorous and the industry remains greatly dependent upon global ocean resources; albeit to a far lower degree than previous decades. Most commercial salmonid feeds in 1995 contained ~53% fish meal, ~31% fish oil and ~16% alternative proteins and grains, while today most feeds contain ~27% fish meal, ~15% fish oil, ~43% alternative proteins and grains and ~15% alternative oils. In Norway, total dietary composition of wild marine-based ingredients has dropped from 90 to 30% between 1990 and 2013. Nevertheless, global demand for aquafeeds is less than 40 mmt but is expected to rise dramatically to 87 mmt which will continue to exacerbate the aquafeeds dilemma. Fish meal and fish oil obtained from reduction of wild-capture pelagic fish is beyond maximum sustainable limits, is becoming cost-prohibitive and could/should be better-used for direct human consumption. These wild populations may be even more pressured by global climate change and supplies will be insufficient to meet growing aquafeed demands and thus constrain aquaculture growth. This is particularly true in emerging economies like China where production accounts for 61% of global aquaculture and continues to grow rapidly.

2.3. Alternative feed ingredients—microalgae?

The aquafeeds dilemma is not new and herculean efforts were made over three decades to identify a broad range of new ingredients. This developed new commodity markets and resulted in significant industrial use of animal- and plant-based feed inputs. These include high-quality rendered animal by-products (e.g., poultry meals, hydrolyzed feather meals, meat and bone meals, blood meals, etc.) and plant-based meals and protein concentrates produced from oilseeds, grains, pulses and legumes as complete or partial replacements for fish meals. Similarly, terrestrial animal fats and plant-based oils (e.g., poultry fat, beef tallow, vegetable oils, etc.) have extensively replaced fish oil in farmed salmonid feeds. However,

these 'second-generation' ingredients are not without limitations. Most lack certain functional properties, palatability and nutritional profiles, and many have lower digestibility and may be limited by specific antinutritional factors (ANFs) which can impair feed intake, growth performance and fish health. Some may alter final product quality for the consumer and they are also becoming increasingly costly and ecologically unsustainable. Of critical importance is that increased use of these ingredients has forced farmed salmonid production to shift alignment to terrestrial agriculture which occupies large aerial footprints, is heavily dependent on fossil fuel-based fertilizers, chemical pesticides and freshwater irrigation. Additionally, these products are grown for our own consumption; so it is of key importance to reduce competition with human food resources for sustainable production of aquafeeds. Ecological and socioeconomic issues aside, the health benefits of consuming fatty fish like farmed salmonids have become serious concerns for human nutrition with the rising use of plant-based ingredients in salmonid feeds. Uncoupling of this scenario is desperately needed to effectively minimize environmental impacts and social inequities; however, it is not simple from technological, ecological or socioeconomic viewpoints and will require economic and political incentives from governments and substantial 'buy-in' from industry and private investors.

3. Microalgae-based products for salmonid feeds

3.1. Opportunities

To ensure continued growth of the sustainable salmonid aquaculture sector in ways that do not deplete important terrestrial and aquatic resources, a 'third-generation' of feed inputs is urgently needed and it is generally agreed that they must come from lower trophic levels. Microalgae such as *Chlorophyceae* (green algae), *Bacillariophyceae* (diatomaceous algae) and *Chrysophyceae* (golden algae) and prokaryotic microorganisms such as *Cyanophyceae* (blue-green cyanobacteria) are among the first lifeforms on earth; having appeared ~3.5 billion years ago. Many are amenable to cultivation under photoautotrophy (e.g., inorganic CO_2, nutrients and light), heterotrophy (e.g., organic carbon and nutrients) or mixotrophy (e.g., combined strategies) and cultivation technologies exist for growth in open or closed ponds, enclosed photobioreactors and fermenters. While microalgae as feedstocks for renewable bioenergies has driven technological advances recently, they remain far from economical viability and are uncompetitive with terrestrial oilseed crops and conventional fossil fuels. In the absence of high-value compounds, algal biorefineries should take a holistic approach that valorizes the entire algal crop as an attractive path towards a viable microalgae-based industry, and the feed sectors are promising areas to focus on. There is tremendous potential for microalgae cultivation (e.g., algaculture) to be co-located with industrial point-source emitters of waste 'outputs' (e.g., CO_2, nutrients, heat) which are essential 'inputs' for rapid microalgae growth and accumulation of nutrient-rich biomass. Microalgae-based ingredients produced for aquafeeds could have competitive market advantages over terrestrial crops in terms of input costs, lower aerial foot-print, and potential for wastewater remediation and carbon credits from CO_2 conversion. Recent search efforts for strains for bioenergy purposes has sparked great interest

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from aquaculture nutritionists in terms of the biochemical composition of many microalgae and it is clear that some may be promising candidates for salmonid feeds based on their supply of well-balanced amino acids, essential omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), vitamins, minerals, carotenoids and bioactive compounds. While large-scale algaculture is a commercial reality in some parts of the world (e.g., Australia, China, Germany, India, Israel, Japan, Myanmar, Taiwan, United States), the sector is dominated by a handful of species with relatively insignificant annual production: *Arthrospira* (3,000 t), *Chlorella* (2,000 t), *Dunaliella* (1,200 t), *Nostoc* (600 t), *Aphanizomenon* (500 t), *Haematococcus* (300 t), *Crypthecodinium* (240 t) and *Schizochytrium* (10 t) and estimated dry biomass price is $8,000–300,000 USD per t. Most is presently destined for human health food markets but many producers have keen interest in penetrating the massive salmonid aquafeed sector if production tonnage can be increased and the price made more economical.

3.2. Challenges

As a cautionary note, some proponents of microalgae biotechnologies suggest that they are 'super-foods' and feeding microalgae to farmed salmonids makes perfect sense since that is what their wild counterparts would naturally consume. This thinking encourages development of lower-trophic, ecologically-sustainable salmonid feed ingredients but the notion is, unfortunately, flawed. While it's true many essential dietary nutrients for wild salmonids originate in aquatic phytoplankton (microalgae) and other single-celled organisms, they are delivered through 'indirect' passage of nutrients up the aquatic food chain and rarely via 'direct' intake; as salmonids do not actively seek to consume microalgae. The notion that wild, highly piscivorous salmonid fish derive nutrients from direct ingestion of microalgae is akin to the notion that wild, highly carnivorous lions derive nutrients from direct consumption of grass. On the contrary, higher trophic predators like salmonids evolved to rely on a progression of intermediary organisms (e.g., grazing phytoplankton, zooplankton, forage fish, etc.) to extract nutrients from complex food matrices that make up 'base-of-the-food chain' organisms (e.g., phytoplankton). This upward passage and trophic accumulation of essential nutrients, referred to as food-chain amplification, transforms them into forms that the relatively simple monogastric digestive system of salmonids can assimilate and use for productive purposes like protein synthesis, growth, tissue repair, metabolic energy and reproduction. The practical implication is that, in the absence of food-chain amplification, reliance on transformative intermediary organisms represents a nutritional barrier for direct feeding of microalgae to most monogastric animals, especially coldwater farmed salmonids. This is because their capacity to extract and utilize microalgal nutrients directly is limited by the highly recalcitrant cell walls of most microalgae, combined with the relatively short gastric (acidic) digestion phase in salmonid fishes. Some industrial downstream processing is almost certainly required in order for nutrient-rich microalgae to realize its potential as a much-needed next-generation ingredient. Like other ingredients once regarded as 'alternatives' but now established mainstream ingredients (e.g., corn, soy, wheat, canola, etc.), cost-effective processing technologies must be developed for microalgae to rupture cell walls, concentrate target nutrient levels, reduce/eliminate indigestible fibers, inactivate ANFs and increase nutrient digestibility for monogastric cold-water fish. With each processing step, nutritional value is increased but so is the cost of production and

ultimately the market price. To further attenuate this situation, unlike terrestrial crops, microalgae cultivations must begin with dewatering the highly dilute cells (typically by centrifugation) down to a dry biomass (typically by spray-drying) and usually some means of mechanical, chemical or enzymatic cell wall rupture is required, and all these processes are currently highly energy intensive and costly. Optimizing the balance between the types and extent of downstream processing and their associated costs to determine the 'point of diminishing returns' that yield algal ingredients of the highest nutritional value in a cost-effective manner for least-cost salmonid ration formulations will undoubtedly occur with innovation. However, very few microalgae-based salmonid feed ingredients have yet to reach the marketplace.

3.3. Nutrient composition of microalgae in relation to their use in salmonid feeds

Beyond high production costs and relatively high prices for microalgae for aquafeeds, several broad issues must be resolved before the salmonid aquaculture feed industry can adopt microalgae-based ingredients for routine use. First, microalgae are a widely diverse class of microorganisms and many complex issues exist around their highly variable nutrient composition. This chapter is a culmination of data collected from the literature on the relevant biochemical composition of ~50 genera of microalgae from the past century. Suffice to say that the sheer size of data tables and associated >150 references preclude inclusion within the confines of this chapter. For a relatively complete compendium of biochemical composition, readers are referred to Becker [3]. Generally, proximate composition of dry microalgae is extreme for ash (<1–53%), protein (2–73%), lipid (<1–83%), carbohydrate (1–64%) and energy (4–30 MJ kg^{-1}). This highly variable trend is predictably the same for genera that have been specifically evaluated for salmonid feeds (**Table 1**) for ash (1–53%), protein (3–73%), lipid (1–83%), carbohydrate (3–55%) and energy (6–30 MJ kg^{-1}). This variability is related to the extensive biological diversity of microalgae (e.g., >100,000 documented species) and the complexities associated with their use as biological factories, large variations in cultivation strategies, variable harvesting and downstream processing methods and under-developed and inconsistent nutrient characterization analytics. Also, in contrast to agricultural crop production, large-scale algaculture is still in its embryonic stage and production tonnage needs to dramatically rise to industrial levels to realize the benefits of economies of scale that will ensure reliable supply, consistent nutrient profile, high nutrient quality and cost-competitiveness that the massive salmonid aquafeed sector will require. Lessons could be learned from the relatively niche, poorly regulated natural health food market for microalgae such as *Chlorella*. Görs et al. [4] reported that quality control is poor for almost all *Chlorella*-based products on the global marketplace. For example, most are contaminated with bacteria, cyanobacteria and other unlisted algal species, contain highly variable levels of chlorophyll and/or its breakdown products and were greatly heterogeneous in biochemical and nutritional composition. It is also observed that *Chlorella* supplements are being marketed as 'super-foods' in part because they contain CGF (*Chlorella* Growth Factor). However, this is an ill-defined term and poorly understood consortium of various nitrogen-containing compounds that are not supported with scientific validation. This lack of quality control and nutritional 'proofing' cannot be tolerated in salmonid aquafeeds and quality assurance must be made a priority.

Genera	Ash (%)	Protein (%)	Lipid (%)	Carbohydrate (%)	Energy (MJ kg^{-1})
Arthrospira	3–13	42–73	2–16	8–25	6–23
Chlamydomonas	—	43–56	14–22	3–17	—
Chlorella	2–8	14–67	2–63	7–34	15–27
Crypthecodinium	4	15–23	20–56	—	29
Desmodesmus	16	21–27	1	—	17
Haematococcus	1–15	3–48	7–67	26–55	24
Isochrysis	13–31	20–45	16–53	13–18	—
Nanofrustulum	53	12	3	—	—
Nannochloropsis	7–23	18–48	2–68	8–36	19–27
Phaeodactylum	16–17	30–49	7–57	8–25	20
Schizochytrium	4–12	12–39	15–71	32–39	26
Scenedesmus	3–14	8–56	1–58	10–52	20–23
Tetraselmis	11–20	27–52	3–45	15–45	18–20
Thraustochytrium	8–11	12–21	8–83	39	18–30

Table 1. General proximate composition and energy content of various genera of microalgae evaluated for use in salmonid feeds (dry weight basis).

3.3.1. Protein and lipid composition

Contrary to popular belief, most industrialized microalgae species do not accumulate high-value essential n-3 LC-PUFA (e.g., those in the 20 and 22 carbon chain lengths). This essential lipid deficiency may relegate these species as poor nutritional value for use in salmonid feeds when, in fact, it's their potential for high protein accumulation that is of interest. While total protein content varies widely in the literature (often by several magnitudes) the essential amino acid (EAA) profile of that protein generally remains rather conserved among species, regardless of growth phase and/or cultivation conditions. **Table 2** shows the EAA composition of microalgae genera that have been evaluated for salmonid feeds. Leucine, arginine and lysine are generally predominant in microalgal protein (on average 7 g 100 g $protein^{-1}$), methionine, histidine and tryptophan are typically most limiting (on average 2 g 100 g $protein^{-1}$) and isoleucine, phenylalanine, threonine and valine are mid-range (on average 4 g 100 g $protein^{-1}$). An important factor when evaluating the protein quality of microalgae-based ingredients for nutrition is their concentrations of nucleic acids (RNA and DNA), which are sources of purines. It is known in primates that excessive consumption can elevate plasma uric acid, which may result in inflammatory arthritis (gout) and renal calculus (kidney stones) and this is related to the lack of digestive uricase enzyme in primates. Fortunately, farmed monogastric animals like swine, poultry and fish have different metabolic pathways which minimize accumulation of uric acid in the blood stream, such as excretion via allantoic acid, urea and ammonia. Additionally, microalgae typically contain lower levels of nucleic acids and purines (4–6%) than other single-cell proteins like yeast and bacteria (8–20%). Like other macronutrients, lipid content of microalgae varies widely and fatty acid (FA) composition is also highly

	Arthrospira	*Chlorella*	*Entomoneis*	*Haematococcus*	*Isochrysis*	*Nanofrustulum*	*Nannochloropsis*	*Phaeodactylum*	*Scenedesmus*	*Schizochytrium*	*Tetraselmis*	*Thraustochytrium*
Essential amino acid (g 100 g protein^{-1})												
Arginine	4–8	3–14	—	6–8	2–6	6	2–8	6	6–7	1–12	6–9	7
Histidine	1–5	1–6	—	<1–1	1–3	1	<1–3	2	2	<1–3	1–2	3
Isoleucine	<1–7	<1–4	—	2–5	1–5	4	<1–6	5	4–5	<1–3	3–4	4
Leucine	5–14	3–9	—	5–9	3–9	7	5–11	7	9	1–6	7	8
Lysine	3–8	2–10	—	4–6	2–6	7	3–8	6	5–6	<1–4	6–7	6
Methionine	1–5	<1–2	—	1	1–3	2	1–3	3	2	<1–10	2	3
Phenylalanine	3–7	2–8	—	2–5	2–6	4	2–6	5	5–7	<1–3	5	5
Threonine	3–7	<1–6	—	4–6	2–5	5	4–6	5	6	1–3	4–5	5
Tryptophan	<1–3	1–10	—	—	1–3	1	<1–4	3	<1–2	<1–2	1–2	1
Valine	3–7	2–7	—	3–5	2–6	5	3–7	5	6	<1–5	5	10
Fatty acid (% of total FAME)												
14:0	—	—	23	<1–1	17	7	1–8	4–7	—	1–4	2–4	1–12
16:0	26–45	14	17	12–29	12	26	11–43	11–32	15–16	16–38	14–25	14–46
18:0	2	1	<1	1–3	1	—	1–11	1–2	1	1–2	3	<1–9
16:1n-7	—	2	28	<1–1	3	38	2–31	19–43	2–3	<1	1–26	<1–13
17:1	—	4	—	<1–5	—	—	<1–10	—	4–5	—	—	—
18:1n-6	10–17	—	—	—	—	—	—	—	—	—	—	—
18:1n-9	—	45–47	1	5–44	7	—	1–12	3–9	24–30	<1–27	4–7	<1–43
18:1n-7	—	1	1	—	1	—	—	<1	—	<1	1–2	<1–10
16:2n-6	—	3–4	—	—	—	—	—	—	2	—	1	—
16:2n-7	—	—	—	—	2	—	—	1–2	—	—	—	—
16:3n-4	—	—	—	—	—	—	—	1–4	—	—	—	—
16:4n-3	—	<1	—	—	—	—	—	—	9–12	—	16–18	—
18:2n-6	11–12	21	1	20–33	<1–4	3	<1–19	1–6	13	<1–2	4–7	<1–10
18:3n-6	17–40	—	1	1–15	<1–1	—	<1–2	—	—	<1	—	<1–1
18:3n-3	—	6–7	<1	<1–40	1–6	1	<1–32	<1–3	18–23	<1	5–22	—

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	Arthrospira	*Chlorella*	*Entomoneis*	*Haematococcus*	*Isochrysis*	*Nanofrustulum*	*Nannochloropsis*	*Phaeodactylum*	*Scenedesmus*	*Schizochytrium*	*Tetraselmis*	*Thraustochytrium*
18:4n-3	—	1	<1	1–6	4–19	—	<1–3	<1–1	2–3	<1	2–8	<1–1
20:4n-6	—	—	6	<1–7	<1	4	1–6	<1–1	—	1	<1–4	<1–15
20:5n-3	—	—	17	<1–1	<1–28	9	<1–28	8–35	—	1–16	2–8	1–20
22:5n-6	—	—	—	—	2	—	—	—	—	1–7	—	<1–21
22:6n-3	—	—	1	—	5–14	—	<1–3	<1–2	—	18–44	<1	3–68
Mineral (%)												
Calcium	0.1–1.4	<0.1–0.6	—	—	0.6	—	0.1	0.3	0.1–0.2	—	3.0	—
Magnesium	0.2–0.3	0.1–0.8	—	—	1.0	—	0.3	0.7	0.1–0.2	—	0.4	—
Phosphorous	0.1–1.3	0.3–1.8	—	—	<0.1–2.6	—	0.7	1.2	0.5–0.7	—	1.5	—
Potassium	0.6–2.6	<0.1–2.1	—	—	1.2	—	1.5	2.4	0.6–0.7	—	1.9	—
Sodium	0.4–2.2	<0.1–1.3	—	—	1.6	—	1.0	2.7	0.1	—	0.9	—
Sulfur	—	—	—	—	—	—	0.6	1.4	—	—	1.4	—
Trace element (mg kg^{-1})												
Copper	4	22–1900	—	—	—	—	18	55	15–25	—	102	—
Iron	539–1800	198–6800	—	—	15	—	1395	4773	1081–1777	—	1774	—
Manganese	19–37	20–4000	—	—	801	—	151	45	74–119	—	191	—
Selenium	2	1	—	—	—	—	<1	<1	<1–1	—	<1	—
Zinc	14–40	6–5500	—	—	19	—	32	50	38–63	—	64	—
Heavy metal (mg kg^{-1})												
Arsenic	<0.1–2.9	0.1–0.5	—	—	—	—	—	—	<0.1–2.4	—	—	—
Cadmium	<0.1–1.0	<0.1–0.1	—	—	—	—	—	—	<0.1–1.7	—	—	—
Mercury	<0.1–0.5	<0.1–0.1	—	—	—	—	—	—	<0.1–0.4	—	—	—
Lead	0.1–5.1	<0.1–2.0	—	—	—	—	—	—	0.6–6.0	—	—	—

Table 2. Biochemical composition of various genera of microalgae evaluated for use in salmonid feeds (dry weight basis).

heterogeneous. **Table 2** shows the FA composition of microalgae genera that have been evaluated for salmonid feeds. The only discreet trend is that the lipid fraction of most species is dominated by the saturated FA (SFA) palmitic acid (16:0) and the monounsaturated FA (MUFA) oleic acid (18:1n-9); which combined generally account for about 40% of total FAs. Many marine and freshwater species, particularly *Scenedesmus* and *Tetraselmis*, produce significant levels (~10% of total FAs) of the n-3 PUFA α-linolenic acid (18:3n-3; ALA) which, once in the body, can be desaturated and elongated as a metabolic precursor for endogenous cellular biosynthesis of the essential n-3 LC-PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, while this is the case for most monogastric animals (including humans and salmonid fish), the extent to which this occurs in animals is limited and dependent upon activity levels of elongase and Δ5 and Δ6 desaturase enzymes in their tissue cells. In fact, this endogenous biosynthesis of n-3 LC-PUFA from ALA is rate-limiting in salmonids such as rainbow trout to a relatively low efficiency of 12–27% depending on various other dietary and farming conditions and thus essential n-3 LC-PUFA must still be added to salmonid diets. There are several, almost exclusively marine, photoautotrophic microalgae (reviewed by Colombo et al. [5]) that are good accumulators of EPA (up to 53% of total FAs), namely the marine genera *Chromophyte*, *Dunaliella*, *Isochrysis*, *Nannochloropsis*, *Pavlova*, *Phaeodactylum* and *Skeletonema*. However, the only ones evaluated in salmonid feeds are *Isochrysis* and *Nannochloropsis* (up to 28% of total FAs). The only known marine microalgae genera currently capable of industrial production of DHA at high levels are the *Thraustochytrids*, such as *Schizochytrium* and the dinoflagellates *Crypthecodinium*; all of which may accumulate up to 68% of total FAs as DHA. However, these species do not perform at high efficiency under photoautotrophic cultivation. As such, they are now cultivated heterotrophically in the absence of light in large-scale fermentation systems using organic carbon sources for industrial production of food-grade DHA and are available commercially in various processed forms (e.g., whole-cell lipid-rich powders, extracted oil emulsions, etc.). The aquaculture feed market is in desperate need of these DHA-rich oils as the Global Salmon Initiative (GSI) has indicated that its members could immediately take up 200,000 t annually of this novel alternative lipid source if it were available [6].

3.3.2. Elemental composition

There are limited data on elemental composition of microalgae and this is in contrast to macroalgae (seaweeds) where numerous species have been well characterized. This is not overly surprising as it is well-documented that most microalgae (excluding some diatoms) typically contain far less inorganic (ash) content (generally <20%) than seaweeds (22–64%). **Table 2** shows the mineral and trace element composition of microalgae genera that have been evaluated for salmonid feeds. With regard to the minerals most often required by farmed salmonids and therefore routinely supplemented in aquafeeds, calcium and magnesium levels in algal biomass are generally around 0.4% each while *Tetraselmis* appears to contain far higher levels of calcium (3%). Phosphorous levels in microalgae evaluated with salmonids are in the range from <0.1 to 2.6% but on average are around 1%. Potassium, sodium and sulfur levels are around 1–2% but appear more highly variable in the literature at <0.1–2.6%, <0.1–2.7% and 0.6–1.4%, respectively. For farmed salmonids, phosphorous (P) is the most limiting macromineral and is therefore

routinely supplemented in formulated feeds in various inorganic forms (e.g., calcium phosphates, sodium phosphates, potassium phosphates, ammonium phosphate and defluorinated rock phosphate) which are highly digestible by salmonid fish. One of the reasons for the high dietary demand for P by farmed salmonids is related to its critical role, along with calcium (Ca) and vitamin D, in the development and maintenance of the skeletal system and maintaining acid-base homeostasis in rapidly growing farmed fish. In salmonids, dietary P and body Ca pools become complexed together to form the principle component of their bone structure, known as hydroxyapatite ($Ca_5(PO_4)_3(OH)$). Fortunately, farmed salmonids are able to obtain the majority of their Ca needs from the surrounding water via direct absorption through their skin, scales and gills. However, fresh and marine culture water is generally low in P, so its requirement in the feed is highest of all macrominerals. Because of the importance of hydroxyapatite formation to healthy fish, it is not only the individual body pools of Ca and P that are important, but also their relative proportions to each other. As a result, the so-called Ca:P ratio is one of the most important considerations for mineral nutrition of farmed salmonids as it can influence their bioavailability, metabolism and physiological utilization and can also increase under-utilized P discharge into the aquatic environment and a ratio of 2:1 or less is recommended. A substantial imbalance in this ratio, especially if compounded by vitamin D deficiency, can result in poor growth performance, inferior feed conversion efficiency, anorexia and, in severe cases, skeletal deformations. The literature data for Ca:P ratio in microalgae that have been evaluated for salmonid feed applications is highly variable; ranging from 0.1:1 to 2:1. However, other common ingredients used in commercial salmonid feeds are also highly variable with lower ranges for terrestrial plant-based sources like conventional biofuel by-products (0.2–0.6:1), oilseeds (0.1–0.5:1) and grains (0.1–0.2:1) and far higher ranges for typical marine-based sources such as marine fish and crustacean by-products (1.3–9.1:1) and kelps (7.5:1). There are several likely reasons for the variations in Ca and P levels in microalgae including species differences, time of harvest and post-harvest downstream processing conditions. Historically, a large percentage of P in farmed salmonid feeds came from the mineral fractions of animal-based protein sources such as rendered animal by-products and fish meals, which are generally well digested (typically >50%) by salmonids. However, as these ingredients have become increasingly replaced by terrestrial plant-based protein sources in modern farmed salmonid feeds, the requirement for costly inorganic P supplementation has increased. This is because, unlike animal-based sources, total P levels in most plants are lower and, of that P, most is stored in the form of inositol polyphosphate, also known as phytic acid. This compound, when chelated with other minerals and trace elements such as divalent cations like Ca^{2+}, Mg^{2+} and Fe^{2+} in the feed, forms poorly digestible phytate. So, in addition to these plant-based ingredients supplying lower total levels of P to salmonid diets, phytate is also poorly digested; thus the availability of phytate-bound P is poor (generally <50%) for salmonids and can also act antagonistically to reduce the digestibility of protein and other essential minerals. In this regard, microalgae-based ingredients (although also plant-based) could potentially offer a great benefit for use in farmed salmonid feeds since it is believed that microalgae cells predominantly store inorganic P in vacuoles as polyphosphate granules, which may be more bioavailable for gastric liberation and intestinal digestion and absorption. Indeed, Tibbetts et al. [7] recently demonstrated in juvenile Atlantic salmon that dietary P digestibility was significantly higher in feeds containing more than 18% *Chlorella vulgaris* meals compared to an algae-free control diet based on fish meal and plant-based protein

ingredients, despite the fact that total dietary P levels were similar. Trace element composition of microalgae evaluated for use in salmonid feeds is highly heterogeneous for copper (4–1900 mg kg^{-1}), iron (15–6800 mg kg^{-1}), manganese (19–4000 mg kg^{-1}) and zinc (14–5500 mg kg^{-1}) while that of selenium is rather consistent (1 mg kg^{-1}). In general, the mineral and trace element composition of microalgae does not appear particularly unique relative to other common terrestrial plant-based salmonid feed ingredients, with the exception of iron (Fe). According to the literature, the Fe content of microalgae-based ingredients used in salmonid feed experiments is particularly rich at up to 0.7% of the biomass; which is high for a trace element. Fe is a key essential trace element required by salmonids and is associated with its critical role in cellular respiration, oxygen transport, acid-base balance and energy metabolism. As such, adequate Fe levels are required in the diet of salmonids as it forms a vital component of the red blood cells (erythrocytes) hemoglobin and plasma-transported circulatory system enzymes. Studies have shown that when dietary Fe is limited farmed salmonids generally become anemic so their feeds are typically supplemented with Fe at 30–60 mg kg^{-1} of diet. As companies producing salmonids feeds continue to search for natural sources of key nutrients to replace expensive chemically-synthesized feedstocks, these high levels of Fe may provide a unique and highly-marketable property for certain microalgae-based products. The high Fe content of many microalgae-based ingredients, relative to other common terrestrial plant-based salmonid feed ingredients, is likely due to the fact that most microalgae products generally contain the entire dried organism, including their chloroplast proteins responsible for photosynthesis, whereas other plant-based ingredients are produced from only the seeds which are non-photosynthetic. It is well documented that Fe is a principle component within the photosynthetically active cytochrome proteins (such as ferredoxin) in microalgal cells, responsible for electron transport to produce energy-rich components such as $NADPH_2$. Rather surprisingly, despite the fact that many phytoplankton are able to bioaccumulate environmental contaminants, there is a scarcity of information on the heavy metal contents of microalgae in the literature. **Table 2** shows the heavy metal composition of three microalgae genera that have been evaluated for salmonid feeds. Reported values for *Arthrospira* (formerly *Spirulina*), *Chlorella* and *Scenedesmus* for the key heavy metals of interest are arsenic (<0.1–2.9 mg kg^{-1}), cadmium (<0.1–1.7 mg kg^{-1}), mercury (<0.1–0.5 mg kg^{-1}) and lead (<0.1–6.0 mg kg^{-1}). Nearly all of these levels are several magnitudes lower than the proposed upper limits for safe consumption as animal feeds. However, most microalgae studied have been cultivated under pristine laboratory conditions using clean water, chemically-defined nutrient media and pure CO_2; whereas, industrial farming of microalgae is highly likely to utilize industrial flue-gas emissions and/or municipal or agro-industrial wastewaters as more cost-effective crop inputs. As such, safety and efficacy evaluation of microalgae-based ingredients for salmonids feeds must be made a priority consideration in the future, both by producers and regulatory bodies, as reviewed by Shah et al. [8]. As a starting point, several safety standards for microalgae consumption by humans was recently summarized by Matos [9]; including microbiological and insect contamination limits, and these standards could be reviewed and verified for their suitability for salmonid aquafeed applications.

3.3.3. Vitamin and carotenoid composition

Despite commercial claims of microalgae being vitamin-rich, there are minimal data in the literature on vitamin concentrations for a small number of species; namely *Arthorspira* (*Spirulina*),

Chlorella and *Scenedesmus*. Of the fat-soluble vitamins, values range widely for retinol (vitamin A; 8–84 mg 100 g^{-1}) and tocopherol (vitamin E; <1–2787 mg 100 g^{-1}) while menadione (vitamin K) concentrations are consistent (1 mg 100 g^{-1}). Reports for cholecalciferol (vitamin D) could not be found. Of the water-soluble vitamins, microalgae (based solely on *Chlorella*) appear richest in biotin (vitamin B_7; 192 mg 100 g^{-1}) but highly variable in both cobalamin (vitamin B_{12}; <1–126 mg 100 g^{-1}) and ascorbic acid (vitamin C; 8–100 mg 100 g^{-1}). Lower, and generally more consistent, concentrations are reported for thiamine (vitamin B_1; <1–5 mg 100 g^{-1}), riboflavin (vitamin B_2; 3–6 mg 100 g^{-1}) and pyridoxine (vitamin B_6; <1–5 mg 100 g^{-1}) while intermediate levels are reported for folic acid (vitamin B_9; <1–27 mg 100 g^{-1}), niacin (vitamin B_3; 11–32 mg 100 g^{-1}) and pantothenic acid (vitamin B_5; 1–22 mg 100 g^{-1}). Since many natural carotenoids display antioxidant-like properties in the body, there has been interest in their characterization in many microorganisms in recent years. In salmonid feeds, carotenoids generally represent high-value components when added either as dietary pigments (namely astaxanthin and/or canthaxanthin) or as biological antioxidants. However, in the former case, almost all commercial astaxanthin and canthaxanthin used in commercial salmonid feeds is synthetically produced and the industry is encouraged to replace these additives with more natural sources. Of the studies that have evaluated microalgae for salmonid feeds, very few reported their carotenoid composition. Based on limited data, chlorophyll content is in a fairly narrow range of 5–37 mg g^{-1} (average, 13 mg g^{-1}) and the samples appeared virtually devoid (generally <1 mg g^{-1}) of α-carotene, fucoxanthin, lycopene and zeaxanthin. Certain species may contain trace amounts of β-carotene (<12 mg g^{-1}) and lutein (<4 mg g^{-1}). While reported ranges are vast, some genera (e.g., *Chlorella* and *Haematococcus*) cultivated under optimized conditions have good potential for accumulation of astaxanthin (up to 550 mg g^{-1}) and canthaxanthin (up to 362 mg g^{-1}). Indeed, there are now commercially-available 'natural-source' astaxanthin products on the market for salmonid feeds that are produced from *Haematococcus* microalgae. However, the vast majority of natural-source astaxanthin used in salmonid feeds (mostly for organic certification) are produced from the bacteria *Paracoccus carotinfaciens* and the yeast *Phaffia rhodozyma*. Nonetheless, several companies globally are ramping up production of 'natural-source' astaxanthin from *Haematococcus* microalgae as the global salmonid feed sector continues to grow. Additionally, several workers are optimizing production of various strains of *Scenedesmus* for high accumulation of lutein, which is used as a high-value additive in poultry and fish feeds, cosmetics, drugs and health foods (~$300 million USD annually) and currently only comes from commercially farmed marigold petals.

3.4. Nutritional evaluation of microalgae for use in salmonid feeds

When evaluating the nutritional quality of potential novel ingredients for aquaculture feeds, nutritionists take a logical step-wise approach which generally involves: (1) comprehensive characterization of their major biochemical components, trace elements, possible anti-nutritional factors (ANFs) and contaminants; (2) assessment of the palatability of diets containing these novel ingredients to estimate their potential effects on feed consumption/feed refusal; (3) estimations of their nutrient digestibility through *in vitro* simulated enzymatic assays or measurement of nutrient digestibility using 'species-specific' digestive enzymes from the target animal species, which may be *in vitro* or *in vivo* (or a combination of both) and finally (4) validation of nutritional quality through *in vivo* studies with the target species to assess

various biological metrics (e.g., growth performance, nutrient utilization, expression of genes related to nutrient metabolism, intestinal and general animal health, product quality, etc.). Engle [10] appropriately points out other important logistical considerations that are often overlooked when evaluating new aquafeed ingredients, such as those based on microalgae. These include the importance of considering what impact(s) dietary inclusion of the novel ingredient might have on the functional and rheological properties of combined diet mixtures, finished pellet quality, product shelf-life and how it fits into established complex ingredient distribution and feed processing infrastructure within aquafeed production facilities. While there are estimates that up to 30% of the annual global microalgae supply is sold for animal feeds, the reality is that many of the aforementioned nutritional evaluation steps are incomplete or totally lacking for most microalgae-based aquafeed ingredients. Despite the encouraging trend towards microalgae-based ingredients for salmonid aquaculture, many of the nutritional claims lack scientific evidence because their required biochemical profiles, nutrient digestibility data, effect on the physical properties of compound aquafeeds and their effects on farmed salmonid performance are at best inadequate and typically non-existent. We can take *Chlorella* as an example, which are some of the most biotechnologically relevant microalgae for industrial applications. While these microalgae have long been proposed for large-scale cultivation for bioremediation, renewable energy feedstocks, health food supplements and sustainable animal and aquaculture feeds, there has never been a full and adequate strategic assessment of their nutritional quality as feed ingredients for salmonids; which are likely the most widely farmed coldwater fishes globally. This is also the typical case for virtually all other microalgae species under consideration for industrial mass algaculture for use in aquafeeds. While the present state of knowledge on the use of microalgae-based ingredients in salmonid feeds is still relatively scarce, the available literature has been summarized in this chapter (**Tables 3**–**6**) and discussed in the next sections.

3.4.1. Microalgae-based ingredients as protein sources

When evaluated as dietary protein sources for salmonid aquafeeds (**Tables 3**, **4**), studies have been conducted using various freshwater and marine microalgae genera with rainbow trout (*Arthrospira*, *Chlamydomonas*, *Nannochloropsis* and *Scenedesmus*), Artic charr (*Arthrospira*), Atlantic salmon (*Arthrospira*, *Chlorella*, *Desmodesmus*, *Entomoneis*, *Nannochloropsis*, *Nanofrustulum*, *Phaeodactylum* and *Tetraselmis*) and mink, *Mustela vison* (*Isochrysis*, *Nannochloropsis* and *Phaeodactylum*) as a proxy for Atlantic salmon. With rare exception, the microalgae cells tested were not cell-ruptured or their processing was left unspecified. This immediately puts into question the digestibility of these ingredients as most are known to possess highly recalcitrant cell walls and digestibility represents the first bottleneck for nutrient assimilation by an animal after consumption. Depending upon the microalgae species tested, salmonid species under investigation, the extent of downstream processing (e.g., drying, de-fatting, cell-rupture) and the methodologies applied, apparent digestibility coefficients (ADCs) for the various microalgae studied with salmonids are in a large, highly variable range of 32–85% (dry matter), 19–87% (protein), 55–94% (lipid), 51–83% (energy), 24–85% (carbohydrate), 27–99% (phosphorous), 81–102% (EAAs) and 59–93% (FAs). Based on feed intake, digestibility, growth performance, feed and nutrient utilization efficiency, whole-body and muscle composition,

Genera	Form	Inclusion levels	Main findings	Ref.
Arthrospira	Whole-cell meal	0–9%	Can be included at 7% for rainbow trout without adverse effects on growth and body composition.	[11]
Arthrospira	Whole-cell meal	0–10%	Rainbow trout fed diets with 10% *A. platensis* lost 50% less weight during a short-term fast.	[12]
Arthrospira	Whole-cell meal	0–10%	Rainbow trout fed up to 10% *A. platensis* had higher plasma red and white blood cell counts, plasma hemoglobin, serum protein, albumin and high-density lipoprotein cholesterol, reduced serum low-density lipoprotein, cholesterol, cortisol and glucose and levels were unchanged for hematocrit, serum total cholesterol, triglycerides and lactate.	[13]
Arthrospira	Whole-cell meal	0–30%	Digestibilities of *A. platensis* for Arctic charr were: organic matter (80%), dry matter (78%), protein (82%), energy (83%), phosphorous (99%) and EAAs (81–102%).	[14]
Nannochloropsis	Whole-cell meal	100%	Protein digestibilities of 79–87% were estimated for *N. granulata* by *in vitro* pH-Stat using rainbow trout stomach and pyloric caeca enzymes.	[15]
Scenedesmus / *Chlamydomonas*	Whole-cell meal	0–50%	*Scenedesmus* sp. / *Chlamydomonas* sp. blend can be included at 12.5% for rainbow trout without affecting growth and body composition.	[16]

Table 3. Present state of knowledge on dietary protein replacement with microalgae in farmed rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*) feeds.

blood histochemistry, intestinal health and gene expression, it appears that salmonids can only tolerate low inclusion levels (<10% of the diet) of whole-cell *Arthrospira*, *Chlorella*, *Entomoneis*, *Isochrysis*, *Nannochloropsis*, *Phaeodactylum* and *Tetraselmis*. On the other hand, salmonids appear to tolerate higher inclusion levels (up to 20% of the diet) of whole-cell *Scenedesmus*/*Chlamydomonas* blend, de-fatted *Desmodesmus* and *Nanofrustulum* and cell-ruptured *Chlorella*. Commercially-produced microalgae-based ingredients presently available on the market are almost exclusively produced from *Arthrospira* (*Spirulina*), *Chlorella* and *Nannochloropsis*, while a few products are produced from *Isochrysis*, *Staurosira* and *Euglena*.

3.4.2. Microalgae-based ingredients as lipid sources

The dietary essential n-3 LC-PUFAs, EPA and DHA, required by farmed salmonids have traditionally been supplied by fish oil, which is manufactured from wild-caught pelagic fish deemed unsuitable for direct human consumption, and this practice is no longer ecologically or economically sustainable. Historically, consumption of fatty fish like salmonids was the best means at achieving the recommended daily intake of 500–1000 mg of EPA and DHA for support of cardiovascular and neuronal health. However, partial or total replacement of fish oils in farmed salmonid feeds with terrestrial lipid sources has started to diminish the content of these essential n-3 LC-PUFAs. While rendered animal fats and vegetable oils commonly used in modern salmonid feeds provide excellent sources of digestible energy (calories) for farmed fish, they lack essential n-3 LC-PUFA that are responsible for dietary health benefits

Genera	Form	Inclusion levels	Main findings	Ref.
Arthrospira	Whole-cell meal	0–30%	Digestibilities of *A. platensis* for Atlantic salmon were: organic matter (85%), dry matter (82%), protein (85%), energy (83%), phosphorous (27%) and EAAs (83–101%).	[14]
Arthrospira	Whole-cell meal	0–11%	*A. platensis* can be included at 11% for Atlantic salmon without affecting growth performance and feed utilization.	[17]
Chlorella	Whole-cell and cell-ruptured meals	0–30%	EAA indices are high (0.9) for *C. vulgaris*. Average digestibilities of whole-cell and cell-ruptured *C. vulgaris*, respectively, for Atlantic salmon were: protein (77 and 87%), EAAs (84 and 91%), carbohydrate (38 and 81%), starch (40 and 80%), energy (55 and 76%), lipid (67 and 85%), SFAs (61 and 62%), MUFAs (59 and 88%) and PUFAs (63 and 93%).	[7]
Chlorella	Cell-ruptured meal	0–20%	20% *C. vulgaris* combined with 20% soybean meal counteracted the negative effects of soybean meal induced enteropathy (SBMIE) in Atlantic salmon, however growth was reduced and digestibility was not measured.	[18]
Desmodesmus	Lipid-extracted meal	0–20%	Defatted *Desmodesmus* sp. can be included at 20% for Atlantic salmon without effects on growth, feed utilization, body/muscle composition and intestinal health and digestibilities were: protein (84%), lipid (94%) and energy (80%).	[19]
Desmodesmus	Lipid-extracted meal	0–30%	Digestibilities of *Desmodesmus* for Atlantic salmon were: dry matter (32–47%), protein (54–67%), ash (41–73%) and energy (51%) and extrusion processing can increase the digestibility compared to cold-pelleting.	[20]
Entomoneis	Whole-cell meal	0–5%	*Entomoneis* can be included at 5% for Atlantic salmon without affecting growth performance and body n-3 LC-PUFA was increased. Digestibilities were: dry matter (69–70%), protein (83–85%), lipid (87–88%) and nitrogen-free extract (24–31%).	[21]
Isochrysis	Whole-cell meal	0–24%	*I. galbana* cannot be included at any level without reducing digestibility in mink[1] (estimated protein digestibility was 19%).	[22]
Nannochloropsis	Lipid-extracted meal	0–30%	Digestibilities of *Nannochloropsis* for Atlantic salmon were: dry matter (48–63%), protein (72–73%), ash (36–80%) and energy (60%) and extrusion processing can increase digestibility compared to cold-pelleting.	[20]
Nannochloropsis	Whole-cell meal	0–24%	*N. oceanica* cannot be included at any level without reducing digestibility in mink[1] (estimated protein digestibility was 35%).	[22]
Nanofrustulum	Lipid-extracted meal	0–17%	Defatted *Nanofrustulum* can be included at 17% for Atlantic salmon without affecting growth, feed utilization, body and muscle composition.	[23]
Phaeodactylum	Whole-cell meal	0–12%	*P. tricornutum* can be included at 6% for Atlantic salmon without affecting digestibility, feed utilization and growth performance.	[24]
Phaeodactylum	Whole-cell meal	0–24%	*P. tricornutum* can be included at 6–12% without affecting digestibility in mink[1] (estimated protein digestibility was 80%).	[22]
Tetraselmis	Whole-cell meal	0–7%	*Tetraselmis* can be included at 7% for Atlantic salmon without affecting growth, feed utilization, body and muscle composition.	[23]

[1]As a proxy for Atlantic salmon.

Table 4. Present state of knowledge on dietary protein replacement with microalgae in farmed Atlantic salmon (*Salmo salar*) feeds.

Genera	Form	Inclusion levels	Main findings	Ref.
Crypthecodinium	Whole-cell meal	0–9%	*C. cohnii* can be included at 6% to restore muscle DHA levels of rainbow trout fed plant oil only diets.	[25]
Schizochytrium	Whole-cell meal	0–20%	*Schizochytrium* included at 20% for rainbow trout did not affect digestibilities of protein, EAAs or ash but levels above 13% reduced those for dry matter, energy, lipid and FAs.	[26]
Schizochytrium	Whole-cell meal	0–5%	*Schizochytrium* included at 5% for rainbow trout improved growth rates and condition factors (although not statistically) and distal intestinal 'global' microbiome was not negatively affected. Lactic acid bacterial (LAB) community (considered beneficial to healthy intestinal epithelium) were elevated.	[27]
Schizochytrium	Whole-cell meal	0–20%	When included at 13% for Atlantic salmon, *Schizochytrium* did not affect digestibilities of dry matter and protein but levels above 7% reduced those for lipid and most FAs.	[26]
Schizochytrium	Whole-cell meal	0–11%	*Schizochytrium* inclusion at 11% for Atlantic salmon effectively reduced harmful persistent organic pollutants in diets and muscle tissues, restored muscle DHA levels but muscle EPA levels were reduced. Growth performance was compromised above 5.5% inclusion.	[28]
Schizochytrium / Yeast extract	Whole-cell meal	0–15%	*Schizochytrium* / Yeast blend can be included at 6% for Atlantic salmon to partially replace fish oil without affecting growth, feed utilization, digestibility, product quality or intestinal health.	[29]
Schizochytrium	Whole-cell meal	0–10%	*Schizochytrium* can be included at 10% for Atlantic salmon without affecting growth performance, biological and biochemical parameters and immune response, however, after a disease challenge, cumulative fish mortality was higher than the control fish.	[30]

Table 5. Present state of knowledge on dietary lipid replacement with microalgae in farmed rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) feeds.

associated with fatty seafood consumption. Terrestrial based oils and fats in salmonid feeds has come at the expense of EPA and DHA levels in the end product for the consumer. As a result, there is tremendous interest and forward momentum for the partial or total replacement of conventional fish and plant oils and animal fats in salmonid feeds with high n-3 LC-PUFA products of microalgal origin. The most suitable candidates are predominantly strains of *Schizochytrium* and *Crypthecodinium*. In fact, this area is presently the most advanced and first 'out-of-the-gate' in terms of making a real difference in salmonid feeds, with several products now on the market that are rapidly being added to the feedstock portfolios of global salmon aquafeed manufacturers. In addition to their ecological role in reducing pressures on wild stocks for reduction to fish meal and oil, there appear to be additional health benefits as well, which are currently being explored. Since heterotrophic cultivation of these strains is conducted under highly controlled fermentation conditions, the resulting ingredients are generally free of environmental contaminants like heavy metals, dioxins and PCBs; for which the conventional fish oil industry has received criticism. When evaluated as dietary lipid sources for salmonid aquafeeds (**Table 5**), studies have been conducted using these marine microalgae with rainbow trout (*Crypthecodinium* and *Schizochytrium*) and Atlantic salmon (*Schizochytrium*). In all cases, the ingredients tested were in a whole-cell (e.g., not cell-ruptured) dry powder form at levels of up to 20% of the diet and it was found that inclusion levels higher than

Genera	Form	Inclusion levels	Main findings	Ref.
Arthrospira	Whole-cell meal	0–10%	Inclusion of 7.5% *A. platensis* for rainbow trout resulted in suitable growth and skin/muscle carotenoid deposition and pigmentation.	[31]
Arthrospira	Whole-cell meal	0–10%	Inclusion of 10% *A. platensis* for rainbow trout resulted in high serum carotenoid levels which were positively correlated with growth, feed utilization, muscle carotenoid levels and muscle color. Serum carotenoid levels can be used to predict post-harvest fillet pigmentation levels.	[32]
Chlorella	Whole-cell meal	0–64 mg Ax^1/Cx^2 blend kg $diet^{-1}$	Muscle pigment levels of rainbow trout fed *C. vulgaris* were 1.5 times higher than those fed the control diet containing synthetic pigments; however, the control diet contained less than half of the dietary pigment; so the study was confounded.	[33]
Chlorella	Whole-cell meal	0–64 mg Ax/Cx blend kg $diet^{-1}$	Inclusion of *C. vulgaris* had no effects on feed intake or growth performance of rainbow trout but muscle pigment levels were reduced and carotenoid retention less efficient than synthetic pigments.	[34]
Haematococcus	Cell-ruptured meal	0–73 mg Ax kg $diet^{-1}$	All measured parameters were inferior when Ax was supplied by *H. pluvialis* in rainbow trout diets compared to synthetic Ax.	[35]
Haematococcus	Cell-ruptured meal	0–60 mg Ax kg $diet^{-1}$	*H. pluvialis* Ax is mostly (~88%) of the 3*S*,3′*S* optical stereoisomer, which was also reflected in rainbow trout muscle tissues and fillet color scores were the same as fish fed synthetic Ax. Coefficient of distance is useful to distinguish fish muscles tissues fed natural or synthetic Ax but is not sensitive enough to distinguish between various natural sources.	[36]
Haematococcus	Whole-cell meal	0–6% of diet (42 mg Ax / 44 mg Cx blend kg $diet^{-1}$)	Muscle carotenoid retention of rainbow trout fed 6% *H. pluvialis* was less than half that of those fed synthetic carotenoids and was attributed to the lack of cell-rupture and the small fish size used.	[37]
Haematococcus	Whole-cell meal	0–1%	Inclusion of 0.3% *H. pluvialis* for rainbow trout enhanced the antioxidant system and modulation of lipid and glucose metabolism, however, 1% raised serum aspartate aminotransferase (ASTA) activity indicating impaired liver function.	[38]
Haematococcus	Cell-ruptured meal	0–74 mg Ax kg $diet^{-1}$	Scalable high-pressure processing of *H. pluvialis* followed by spray-drying was effective at cell rupture without damaging carotenoid composition. *H. pluvialis* Ax optical isomer composition reflected that of rainbow trout muscle tissues but not skin. Growth and feed efficiency were not affected compared to those fed synthetic Ax but digestibility reduced.	[39]
Haematococcus	Cell-ruptured meal	0–50 mg Ax kg $diet^{-1}$	Serum Ax levels were reduced in rainbow trout fed *H. pluvialis* (esterified form) compared to synthetic (free form). Ax absorption is greater in the anterior intestine than the posterior, irregardless of form.	[40]
Haematococcus	Extracted oil	0–40 mg Ax kg $diet^{-1}$	Inclusion of Ax-rich oil extracts from *H. pluvialis* had no effects on rainbow trout growth. Natural esterified Ax is as efficiently utilized as synthetic free-form Ax.	[41]

Genera	Form	Inclusion levels	Main findings	Ref.
Haematococcus	Whole-cell meal	0–30 mg Ax kg diet^{-1}	Inclusion of *H. pluvialis* had no effects on female rainbow trout reproductive performance or egg protein and triglyceride content. Small (albeit inconsistent) improvements in egg lipid peroxidation and glutathione peroxidase activities noted.	[42]
Haematococcus	Whole-cell and cell-ruptured meals	0–40 mg Ax kg diet^{-1}	Inclusion of *H. pluvialis* in any form had no effect on growth performance of rainbow trout. Muscle and skin pigmentation was highest in fish fed synthetic Ax, followed by cell-ruptured *H. pluvialis* and the lowest was whole (intact) *H. pluvialis*. Unfortunately, the rate of cell wall breakage for cell-ruptured *H. pluvialis* was low (~60%).	[43]
Haematococcus	Cell-ruptured meal	0–80 mg Ax kg diet^{-1}	Weight gain of rainbow trout fed *H. pluvialis* equivalent to 40–80 mg Ax kg^{-1} was the same as those fed a diet with 80 mg Ax kg^{-1} synthetic Ax, however muscle and skin Ax deposition was less efficient than with synthetic Ax. As with the previous study, the rate of cell wall breakage for cell-ruptured *H. pluvialis* was low (~60%). Muscle tissues of fish fed diets with *H. Pluvialis* at any level contained significantly higher adonirubin, which may explain lower fillet color scores.	[44]

[1]Ax = astaxanthin.
[2]Cx = canthaxanthin.

Table 6. Present state of knowledge on dietary carotenoid replacement with microalgae in farmed rainbow trout (*Oncorhynchus mykiss*) feeds.

10–13% reduced nutrient digestibility for rainbow trout and Atlantic salmon. Moderately low dietary inclusion levels (5–7%) may enhance the beneficial microbiome of salmonids and reduce the concentrations of harmful persistent organic pollutants (POPs) in feeds and fish muscle tissues. Commercially-produced microalgae-based ingredients presently available on the market to supply n-3 LC-PUFA are almost exclusively produced from *Crypthecodinium* and *Schizochytrium* while a few products are produced from *Isochrysis*, *Nannochloropsis*, *Odentella*, *Tetraselmis* and *Ulkenia*.

3.4.3. Microalgae-based ingredients as carotenoid sources

In addition to microalgae as sources of essential nutrients, energy and LC-PUFAs, many also synthesize carotenoids and phycobiliproteins. Of particular interest is astaxanthin, which has become a rapidly growing area of study for the farmed salmonid aquafeed industry. The three predominant sources of commercially-available astaxanthin are chemical synthesis, yeast fermentation and algal induction. The cost of each are estimated at: synthetic (~$2,000 kg^{-1}) < *Phaffia* yeast (~$2,500 kg^{-1}) < *Haematococcus* microalgae (~$7,000 kg^{-1}), so it is clear that production costs must be greatly reduced before for the salmonid aquaculture industry is likely to shift to the wide use of astaxanthin derived from *Haematococcus* algae. However, the industry is feeling ever-growing pressure to reduce their reliance on synthetic astaxanthin, which is presently dominated by the commercial products Carophyll® Pink (DSM Nutritional Products)

and Lucantin® Pink (BASF Corporation). This represents an environmental and societal-driven opportunity for *Haematococcus*-based ingredients as 'natural-source' astaxanthin. In fact, the high oxygen radical-scavenging absorbance capacity (ORAC) antioxidant potential reported for *Haematococcus pluvialis*-derived astaxanthin and the fact that it is predominantly esterified (~94%), indicates its higher oxidative stability than synthetic astaxanthin, which is in a non-esterified (free) form. Additionally, *Haematococcus pluvialis*-derived astaxanthin has been certified as safe for human, animal and fish consumption, unlike synthetic astaxanthin. When evaluated as dietary carotenoid sources for salmonid aquafeeds (**Table 6**), studies have been conducted using various freshwater and marine microalgae genera with rainbow trout (*Arthrospira*, *Chlorella* and *Haematococcus*). The ingredients tested were inconsistent in their form, where some studies confirmed it to be a cell-ruptured dry powder while others used whole-cell (intact) powders, one study used an astaxanthin-rich oil emulsion and others did not specify its form or degree of processing. While *Chlorella vulgaris* has typically been evaluated as a protein source, some isolates cultivated under optimized conditions can accumulate natural astaxanthin and canthaxanthin. As such, a small number of studies were conducted with rainbow trout fed diets supplemented with *Chlorella vulgaris* to achieve dietary concentrations of 64 mg kg^{-1} of an astaxanthin/canthaxanthin blend. They showed that feed intake, growth performance and nutrient digestibilities were not affected, but they were inconsistent on flesh pigmentation efficiency. One study suggested that muscle carotenoid levels and overall pigmentation efficiency was lower than synthetic pigments while the other study observed muscle pigment levels 1.5 times higher than those fed synthetic pigments. However, it is important to note that since the control diet used in the latter study contained less than half the pigment than the *Chlorella vulgaris*-supplemented test diets, the imbalance confounds the study and makes the higher pigmentation efficiency questionable. In a similar manner, *Arthrospira platensis* (*Spirulina*) has typically been evaluated as a protein source for salmonids but it also synthesizes natural carotenoids. Two studies indicated that feeding rainbow trout on diets containing 5–10% *Spirulina* meal supported good growth and feed utilization and significantly increased serum, skin and muscle carotenoid deposition. This occurred despite the fact that the algal cells were not ruptured; providing further evidence of the less recalcitrant nature of the cell walls of cyanobacteria like *Arthrospira platensis* compared to chlorophytic microalgae like *Chlorella vulgaris*. By far, the most studied microalgae as a dietary carotenoid source for salmonid feeds is *Haematococcus pluvialis* with ~10 evaluations with rainbow trout. Of these studies, half used a cell-ruptured dry powder, one used an extracted astaxanthin-rich oil emulsion and the rest either used a whole-cell (un-ruptured) dry powder or did not specify the form. Studies using cell-ruptured *Haematococcus pluvialis* meal incorporated the ingredients at rates that achieved dietary astaxanthin concentrations of 40–73 mg kg^{-1} of diet and balanced those of the control diets containing the same astaxanthin concentration supplied in the synthetic form. A key finding from these studies was that natural-source astaxanthin from *Haematococcus pluvialis* was predominantly (~88%) made up of the 3*S*,3'*S* optical stereoisomer and that this was the same form subsequently incorporated into the muscle tissues of rainbow trout. Additionally, fillet color scores were the same as those fed an equivalent dietary concentration of synthetic astaxanthin (60 mg kg^{-1}). However, this latter finding contradicts other similar studies using cell-ruptured *Haematococcus pluvialis* meal in rainbow trout diets at similar astaxanthin levels (40–74 mg kg^{-1}) where pigmentation efficiency (measured as serum astaxanthin levels, muscle astaxanthin retention and fillet color) was inferior to synthetic astaxanthin. As might be expected, the use of whole-cell (intact) *Haematococcus pluvialis* meal in

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rainbow trout diets generally reduced nutrient digestibility and pigmentation efficiency compared to synthetic astaxanthin and, in some cases, other negative effects were observed such as elevated levels of serum aspartate aminotransferase (ASAT) enzyme activity; an indication of possible liver damage. On the other hand, when an unspecified *Haematococcus pluvialis* meal was used at 30 mg kg^{-1} for rainbow trout broodstock diets, there appears to be a slight improvement in the lipid peroxidation status of fertilized eggs. However, overall reproductive performance of gravid female fish fed this diet was not significantly affected. The most encouraging results for the use of natural astaxanthin derived from *Haematococcus pluvialis* is when an extracted astaxanthin-rich oil emulsion was used in rainbow trout diets to provide 40 mg kg^{-1}. In this case, digestibility of the cell wall or broken cell wall fragments would not have been a concern and this was reflected in equal growth as fish fed the control diet. The study also found that, based on muscle and skin astaxanthin concentrations, diets containing the natural-source 'esterified' astaxanthin from *Haematococcus pluvialis* were equally as well utilized as those containing an equal supply of synthetic 'free-form' astaxanthin. Commercially-produced microalgae-based ingredients presently available on the market as sources of carotenoids are almost exclusively produced from *Haematococcus* while a small handful of products are produced from *Arthrospira* (*Spirulina*), *Dunaliella*, *Isochrysis*, *Nannochloropsis*, *Phaeodactylum* and *Tetraselmis*.

4. Concluding perspectives

While microalgae-based products have tremendous potential as 'next-generation' feed ingredients for sustainable salmonid aquaculture, few have yet to successfully be commercialized and reach the marketplace. Strains of *Schizochytrium* and *Crypthecodinium* as source ingredients for essential n-3 LC-PUFA and *Haematococcus* that effectively accumulates natural-source astaxanthin are promising high-value replacements for conventional fish oils and synthetic astaxanthin, respectively. As such, these products are rapidly becoming added to the feedstock portfolios of global salmonid aquafeed producers. However, substitution of protein-rich fish meals and terrestrial plant-based commodities presently used in salmonid feeds with protein-rich microalgae-based ingredients remains a challenge as a result of the fragmented and inconsistent information on their biochemical composition, inconsistent nutrient characterization analytics, variable digestibility related to recalcitrant cell walls and general scarcity of adequate nutritional investigations. More research is required to further evaluate the salmonid species-specific safety and efficacy of many microalgae-based products including their effects on growth performance, nutrient utilization, fish health and product quality. Further industrial research is needed to assess what effects they may have on the functional and rheological properties of combined feed mixtures, finished pellet quality, product shelf-life and how they fit into established feed ingredient distribution and feed processing infrastructure and value chains. For the further development and commercial adoption of microalgae-based ingredients for farmed salmonid feeds there is a need for additional technological advancements in the areas of industrial algaculture scale-up, standardization of cultivation strategies and down-stream processing methods to concentrate nutrient levels and increase their nutrient bioavailability. These advancements should enable the industry to provide nutrient-dense, highly digestible microalgae-based ingredients at cost-competitive prices.

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Conflict of interest

The author declares no conflicts of interest.

Author details

Sean Michael Tibbetts

Address all correspondence to: sean.tibbetts@nrc-cnrc.gc.ca

National Research Council of Canada, Halifax, Nova Scotia, Canada

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